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Preface

The research reported in this issue of the Journal of Aquatic Plant Management was presented at the 32nd annual meeting of the Aquatic Plant Management Society and International Symposium on the Biology and Management of Aquatic Plants held in Daytona Beach, Florida, July 12-16, 1992. This meeting was attended by over 300 people representing over 30 countries in the world, probably one of the largest meetings ever held on the biology and management of aquatic plants. This symposium would not have been possible without the financial and other services donated by the Symposium sponsors. Individuals, too numerous to mention, devoted much time and expertise in program planning, local arrangements, reviewing papers and conducting other tasks that permitted such an informative meeting.

The production of this "Special Edition" was only possible through the volunteer efforts of Ms. Jessica Ruff, Technology Transfer Specialist, and Mr. J. Lewis Decell, Manager, Corps Aquatic Plant Control Research Program, of the U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

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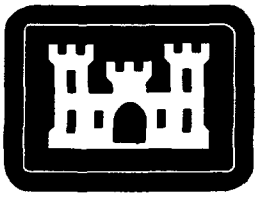
Dedication

It has been widely accepted that the waterhyacinth, the world's most widespread aquatic weed problem, was introduced into the United States at the 1884 Cotton States Exposition in New Orleans. A century ago, by 1892-93, the waterhyacinth was spreading its green growth and purple flowers through the critical navigational waterways of the Southeast United States, including the St. Johns River, some 40 km west of the symposium venue. The problem with waterhyacinth in navigation areas was severe in many locations by the mid to late 1890s, finally prompting Congress to enact the River and Harbors Act of 1899 which authorized the U.S. Army Corps of Engineers to do everything possible to eradicate the waterhyacinth and thus maintain navigation and commerce. Further modifications of the original Act through the present time authorized, in addition to Corps operational programs, federal cost sharing, research and technology transfer programs. The initial battle against the waterhyacinth required imaginative and sometimes dangerous application of mechanical and chemical control methods. Aquatic weeds have been removed from navigable waters with cargo nets pulled by tractors, giant draglines and steam shovels, and various conveyors. Plants were chopped, sawed, crushed, and sprayed with steam, various salts and acids, and even burned with propane torches. These early experiences led to the development of the current national, state, and local aquatic plant management programs in herbicidal, mechanical, and biological control.

Since 1899 many people of the U.S. Army Corps of Engineers have devoted their professional lives to research and operational programs in aquatic plant management. In recognition of these activities, the Aquatic Plant Management Society, Inc., dedicates this "Special Edition" to the men and women of the Corps of Engineers and their achievements over the last century toward the management of nuisance aquatic vegetation.



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PRESIDENTIAL ADDRESS

RANDALL K. STOCKER[1]

It is a genuine pleasure to come before you today, at our Aquatic Plant Management Society's first truly international meeting. This meeting was first suggested by George Bowes of the University of Florida. At several Board meetings we discussed the pros and cons of this large-scale endeavor, and concluded that few single actions by APMS could so clearly fit the mandate adopted by our founding members. We are joined to "assist in promoting management of aquatic vegetation, to encourage scientific research, to promote university scholarships and to extend and develop public interest in the movement."

Many of you remember our "bi-national" meeting in Vancouver, British Columbia, Canada, in 1985, but this is the first serious global effort that our organization has conducted. The 1982 European Weed Research Society (EWRS) meeting in Novi Sad is the only other international meeting that I have been able to attend, but I assure you it made quite an impression, and I have been talking about it since.

This meeting also continues a trend that has not occurred without some anguish. During the past 31 years, APMS has broadened its base from the good folks in Florida and the Southeast to include representatives from states all over the nation, and nations all over the world. Moving some meeting sites out of the southeastern United States has meant many local Florida residents have not been able to attend. However, we have watched the growth of six regional APMS chapters to fulfill local needs. Twelve years ago, the President of APMS stood before the assembly and noted that moving the meeting locations around the country, or even into different countries, would result in "some individual hardships from time to time." "But," he noted, "if we move about enough we will enhance our stature of being international in scope by making the annual meetings more accessible to more interested potential members. I believe that this is what we must do." I concur, and add that if we can't get to the rest of the world, perhaps periodically we can bring the rest of the world to us. Possibly this meeting will provide sufficient energy to move aquatic plant management into some kind of joint international format.

ECONOMIC CONCERNS

I want to come back to the international appeal of this gathering, but before I do, I want to share some concerns I have about the current status of aquatic plant management.

Consider all the changes that have taken place in the past 12 to 24 months. The Berlin Wall is gone, the two Germany's reunited; massive changes in political organization and economy in the former Union of Soviet Socialist Republics, paralleling other changes in many nations of central and southern Europe; and even today, as we speak, our hosts at the Novi Sad EWRS meetings live in a country with political and economic questions far from settled.

Indeed, we have international guests at this meeting that in the past could not have attended, at least not as easily as they may now. Unfortunately, we also have individuals that would have given anything to have been able to attend, but could not. The turmoil and economic hardships that exist in many places today are a very real part of international meetings.

Like all processes of change, the very fact that things are different seems to provide opportunity for new and improved working relations among many countries. New freedoms and less philosophically antagonistic economies should mean better chances for us; chances to learn more about aquatic plant management in different countries, chances to meet people with different experiences than our own, chances to put cooperative programs into place and truly internationalize aquatic plant management.

But therein lies the rub; at the very time when the wealth of information matches up with the wealth of opportunity, the wealth of our country is getting harder to find. Today's tough economic conditions are not conducive to expansion of research, development of new equipment, new products, or even of new contacts at international meetings.

TWO BIGGEST PROBLEMS

I see our two challenges being a shortage of non-federal program dollars and the continuing need to educate the public about aquatic plant management tools and benefits.

The profitability of our business will to a large extent be determined by how we handle state and national economic problems. California is currently $11 billion dollars in debt, and has been operating without a budget since 1 July (first IOUs since Depression, greater current debt than total budget

[1]1991-92 President of the Aquatic Plant Management Society. Manager, Planning and Technical Service Department, Imperial Irrigation District, P.O. Box 937, Imperial, CA, USA 92251.

25 years ago); the State of Florida has cut $150 million in funds available to the University of Florida in the past two years. These are just two examples; there are, unfortunately, many more.

While we appear to be universally short of money, public perception of the benefits of aquatic plant management is not as evenly distributed.

Much of publicly funded, operational aquatic plant management is "secondary" to the public's stated concerns. Now please don't overreact; I'm not saying we are any kind of second-class act, it's just that many of us in this room are in the business of reducing negative impacts caused in some manner by aquatic plants, and these actions are not usually taken unless some "primary" resource is being affected. In other words, we manage vegetation to enhance some other activity: to improve fishing access, to increase drinking water supplies, to improve drainage efficiency, to reduce discomfort to swimmers, to improve fishing, *etc.* The public's attention is on what they "perceive" to be the primary activity; we frequently operate in the background.

Our business does include some "primary" activities, but they have not been a major focus of our organization. Aquatic plant production and sales for water gardens and habitat improvement are closer to "primary" activities in the public's view, although habitat restoration is also often a secondary consideration, secondary to the primary goal of increasing biodiversity or populations of waterfowl or something similar.

I am very pleased that Mr. Don Bryne, a commercial provider of aquatic plants, will be talking at 4:00 pm today about "The Aquatic Plant Industry, an Opportunity for International Cooperation." With our organization's historic focus on reducing populations of aquatic plants, we have had little motivation to expand into areas of more "primary" interest. Indeed, our Society's original name was the Hyacinth Control Society, expanded in 1976 to reflect expanding interests.

I believe this society should expand into some of these areas of primary interest. But I didn't make the distinction between "primary" and "secondary" interest to increase membership of the society, I made it to try to simplify in my mind the relationships between global changes in social structure and economics and the field of aquatic plant management.

I would expect impacts to primary aquatic plant activities from our nation's and many of our state's economic woes to be fairly straightforward. Purchase of aquatic plants for water garden use most probably comes from discretionary income, and discretionary income appears to be what will most likely be decreased, if the growing U.S. deficit is to be brought under control.

What about secondary activities of aquatic plant management? This, I think, is much tougher to figure out. On the one hand, increasing populations put more and more pressure on drinking water supplies, recreation resources, and agricultural production, with its contribution of fertilizer and other compounds in return flows to rivers and lakes. All these increase the pressure to effectively manage aquatic resources.

On the other hand, weak economies and increasing regulatory restrictions reduce our ability to meet the population-dependent challenges just listed. Regulatory action alone could merit an international meeting. I wanted to give our international visitors a brief review of the resource laws we operate under, but it takes too long, it isn't that much fun, and there are plenty of people that can do a better job than I can. Instead, I will simply state that this nation has not yet invented the economic mechanism to provide for scientifically justified and politically acceptable social use of shared resources. We know that the benefits are not in simple proportion to the mountains of legislation and regulation being produced, and we all know of resources receiving too little protection.

But where is the money to implement the federal and state requirements? It has been a painful lesson, and its not very popular to agree with anything that comes out of Washington, DC, but it appears that most publicly funded programs will depend on either a healthy economy or continually increasing taxes. Long-term success will depend on enough money to implement successful programs. In other words, long-term ecological welfare depends, to some extent, on long-term economic welfare.

Now, I couldn't possibly close without mentioning how ironic it is that I find myself as President of the Aquatic Plant Management Society, giving the Presidential Address at our Society's largest gathering of the various entities and individuals interested in aquatic plant biology and management. The irony stems from my place in the structure of things aquatic.

I represent an irrigation district in the driest agricultural area in North America, and possibly the driest agricultural area in the western hemisphere. Our average rainfall is under 3 in. (76 mm). We are somewhat better known for our wildflowers than for our wetlands.

Our second principal unique feature is that the Imperial Valley is located below sea level, and yet we are a gravity flow irrigation district.

Our third unique feature is that all domestic, agricultural, and industrial water in the Imperial Valley is supplied by canals from the Colorado River. We use about 20% of the entire flow of the Colorado River, the single biggest user of the most heavily allocated river in the western U.S.

And if that combination wasn't bizarre enough, we have the largest, biological-control-based, flowing-water, hydrilla eradication program in the world.

The California Department of Food and Agriculture, U.S. Department of Agriculture, Imperial County, and my

organization, Imperial Irrigation District, are in the sixth year of an operational hydrilla eradication program in the irrigated desert region of southern California. The introduction of over 120,000 triploid grass carp over six years, together with chemical applications in small ponds, concrete lining for water management efficiency, and a tremendous amount of physical labor, has resulted in reduction of hydrilla from 192 km (120 miles) of canal to a current level of 4.5 km (2.8 miles) of canal.

The cost of the program has been $5,300,000 to date, but the Imperial Valley produces a billion dollars worth of agricultural products each year and restriction of water flow has serious economic consequences.

Well, what does all this mean to you? We have an eradication program, and eradication programs are not in vogue for most of the world's aquatic plant management efforts. And, we have an eradication program based substantially on biological control, not a typical use of biological control.

If all these features are so atypical of aquatic plant management projects throughout the world, what's going on in California that the rest of you can learn from? I think we are a microcosm, or perhaps a megacosm, of what will happen in other locations as pressure on water resources continues to increase.

Water resources are scarce in the western U.S. and more money will be made available to ensure that these resources are free of aquatic plant problems. As unusual as my type of aquatic plant management program used to be, I think it will become more common in the future.

WESTERN U.S. PATTERNS

There are other aspects of what we do in the western U.S. that may have a larger impact on other parts of this country than you might expect. Our irrigation systems, water delivery systems, state and federal projects are quite different than water projects in the eastern U.S., right? Well, not always. Part of the battle over allocation of water for irrigated agriculture in California stems from a prevalent voting system for state and federal irrigation districts; instead of one person-one vote, they have one acre-one vote, or 0.4 ha-one vote for those of you from progressive nations that use the metric system. The largest land-holding economic interests are free to make decisions that can be catastrophic to people at the other end of the economic scale.

Peculiar to California? Not quite. I'm now reading from a 1991 paper titled Property and Water Institutions in California:

> "...the property-weighted electoral system has spread eastward across the country. It has caught on in Florida, most interestingly in the example of the Reedy Creek Improvement District. Property, not people, votes in Reedy Creek, which has exactly the same boundaries as Walt Disney World. The five supervisors who run the district are elected on a one acre-one vote basis; since the charter specifies that all directors must be landowners, chosen nominees are deeded five acres of land by Disney. RCID was created in 1967 by an act of the Florida legislature. The board may issue municipal bonds, contract with the Federal government, build roads, operate an airport, exercise the power of eminent domain. The Reedy Creek Improvement District is listed in the telephone book under 'Walt Disney World'. It also appears on Form 10-K of the Securities Exchange Commission as a 'governmental unit of the State of Florida'. It is one governmental unit that is listed in the assets category on a corporate financial statement." (Goodall, Merrill, Property and Water Institutions in California, 1991)

So you see, it's not just Californians that you get showing up here in Florida every so often. More and more you may get to live with our peculiar water management institutions. They were designed for limited water supplies and they seem to be in vogue.

CLOSING POINTS

Let me close by summarizing what I think this meeting means. I mentioned the economic difficulty faced by many international friends that wanted badly to attend this meeting. The Aquatic Plant Management Society has done what we felt we could to help where possible and where the need was obvious. Know then, that as small an organization as we are, and as insignificant a role as we may play in the global perspective of world relations, we nevertheless chose to act as if we were significant, as if we could make a difference, as if the individual relations could be cornerstones to international bridges.

Like never before in the history of our business, our economies are tied together, our futures are tied together, and we are all sitting here together. Your officers are doing everything possible to provide these historic opportunities, but you are the opportunities—take advantage of them, and one day you'll look back on this and know you played a part.

Well, here's an opportunity to learn and grow. You came to this meeting because you had specific interests, ranging from the scientific to the sublime. The memories I carry with me from the international meeting I attended are of the people, and the places, and of course, the papers. But of these three, I believe the people come first. Next year in Charleston, SC,

we can go back to our comfortable relationships with the folks we know the best. This year let's shake it up a bit. Be bold, be daring, try something you've never done before. If nothing else comes to mind, take a Californian to lunch!

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PLENARY ADDRESS

Aquatic Weeds and Fisheries Production in Developing Regions of the World

TOMISLAV PETR[1]

INTRODUCTION

It is a great honor to be invited by the Aquatic Plant Management Society to take part in your Annual Meeting and the International Symposium on the Biology and Management of Aquatic Plants. I would like to thank you for giving me the opportunity to come to your beautiful Florida and say a few words about aquatic plants and aquatic weed management which are a problem and challenge for developing countries in tropical and subtropical regions.

Perhaps you will excuse my bias toward fish and fisheries in my considerations of aquatic plants. As I note from the meeting agenda, not many fishery biologists attend your meetings. This is quite understandable, as the topic of the meeting is aquatic plant biology and management. Not being a botanist, perhaps you will also excuse me for occasionally getting lost in my fisheries thoughts as I consider myself more at home with aquatic animals than plants. I work for the Fisheries Department of the Food and Agricultural Organization (FAO) of the United Nations, and my bias will be toward applied aspects, and how to solve problems we face in our, if not daily, then perhaps weekly or monthly contacts with governments all over the world. I shall also concern myself briefly with the problem of aquatic plants in relation to waterborne diseases. For a number of years, through a joint World Health Organization - FAO Panel of Experts on Environmental Management for Vector Control (PEEM), much information has been collated, summarized, and disseminated to member countries on this and related topics.

If we have a complex problem involving water storage, water release, channelization, eutrophication, and health, we try to incorporate fisheries so that it fits in the multiple-use of land and water resources. We try to make the best use of natural conditions, including aquatic plants, to accomplish our major task of developing sustainable fish production in the great diversity of water bodies of the world.

Since biblical times, fish have provided and continue to provide a significant source of protein for the world's population. I shall attempt to describe how we attain this objective and I shall also identify the major areas on which we concentrate advisory, management and research activities.

WHAT ARE AND WHERE ARE THE AQUATIC PLANTS?

Where there are fish, there are usually also aquatic plants. Plants serve as shelter, food, and refugia from predators, and provide substrate for periphyton on which especially some of the young fish feed. Interrelationships between fish and plants are not simple, and are much more understood in temperate regions than in tropical and subtropical areas. Submersed plants are more appreciated in fisheries management than the emergent and floating species, and dense mats are less appreciated than loose aggregates of plants. Multispecies plant communities are better for fish than monospecific ones. In this respect, fish management and aquatic plant management share a common objective.

Then there is the diversity of water bodies. There are natural lakes and floodplain lakes, rivers and oxbows, reservoirs, irrigation canals and drains, lakes receiving drains from irrigation systems, and seepage lakes. We have smaller water bodies usually maintained by small rural earthen dams called tanks in India and Sri Lanka.

Aquatic plant managers can be employed by a hydropower-producing company such as the Tennessee Valley Authority or by WAPDA (Water and Power Authority in Pakistan) to monitor unwanted accumulations of aquatic plants, which we then call aquatic weeds, and which may cause difficulties in water intakes and in irrigation systems of their command areas. Irrigation companies and government irrigation departments will offer us the job of keeping their canals clear of weeds to prevent water losses and reduction of water flow. Health Departments will ask us to clear aquatic plants to prevent the spread of vectors of waterborne diseases such as malaria, arboviral diseases and schistosomiasis. Fisheries departments will request advice on how to make the best use of aquatic plants, but will also ask us to do something about excessive concentrations of aquatic weeds. Your job in the United States may be supported by sport fishermen's

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contributions, and you will then keep open for them fishing canals in Lake Okeechobee using large, and expensive, plant harvesters. Such behemoths may be found even in developing countries. The Government of India decided some years ago to import and use a harvester to improve boating for tourists in Srinagar, the capital of Kashmir, where Lake Dal, with its famous anchored boats, is infested with hydrilla (*Hydrilla verticillata*). Macrophytes can be used for secondary and tertiary sewage treatment, such as in Uganda, where the capital city of Kampala sewage treatment works discharge their effluents into the papyrus rim of Lake Victoria, or into special tertiary treatment ponds covered with water hyacinth like those studied a few years ago in Orlando, Florida. One can also be offered a job by a mining company which may ask us to remove heavy metals from tailings using floating aquatic plants.

As a fishery manager, I am interested in the use of plants in fish production or how to prevent a decline in fish production resulting from invasion by aquatic weeds. It is logical that I combine my fisheries interests with those of aquatic plant managers, engineers, and researchers to find the most appropriate means of solving each particular situation.

AQUATIC WEED PROBLEMS IN DEVELOPING COUNTRIES

The two major nuisance aquatic weeds in developing regions of Africa and Asia are salvinia (*Salvinia molesta*) and water hyacinth (*Eichhornia crassipes*). These species originated in Latin America where they do not cause major problems, at least not in the areas of their origin.

Salvinia molesta was described by Mitchell only in 1973 after it invaded Lake Kariba in Zimbabwe/Zambia. Zaire experienced a massive expansion of water hyacinth in the 1940s, and from there it spread to Sudan in the early 1950s. This was followed by a gradual spread of both plants through Africa, with the most recent invasions by water hyacinth being reported from West Africa. In East Africa the source of water hyacinth entering Lake Victoria is the Kagera River, whose origin is in Rwanda/Burundi. The Niger River in Niger is also "exporting" water hyacinth downstream to Nigeria.

In Asia, water hyacinth is a common occurrence on many village ponds in India and it grows rapidly, especially in heavily polluted ponds. Island states of the Indo-Pacific have not escaped aquatic weeds either, with Sri Lankan tanks being infested with salvinia, and with water hyacinth being a common plant in the Philippines. Papua New Guinea experienced a massive and explosive growth of salvinia on the Sepik River system in the late 1970s and the first half of the 1980s, which was brought under control by an introduced insect, and now water hyacinth is invading the same system. To control the salvinia problem on the Sepik River, Australian Commonwealth Scientific and Industrial Research Organization (CSIRO) scientists collaborated with FAO in providing the insect *Cyrtobagous salviniae* and monitoring its spread to over some 200 km^2 of backwaters soon after its release. Within two years, there was a 99% reduction in salvinia, and concern was expressed that if the success were 100%, there would be no inoculum (insects) left to keep pressure on this weed species.

In the Philippines, water hyacinth is a major nuisance on Laguna de Bay, a lake near Manila, which has fish cage and pen culture. During the typhoon season, strong winds may blow masses of water hyacinth against these structures and cause their destruction. This act of God is, however, appreciated by capture fishermen, whose stocks are replenished by the escapees from cages and pens.

The reasons for the sudden occurrence of these two plants in various geographic areas are usually obscure, but it is frequently the result of ignorance. Salvinia, in its smallest phenotype, is an attractive floating aquarium plant and most aquarists do not know anything about the third phenotype, which causes large-scale infestations. Water hyacinth has beautiful flowers and can be found in flower shops in many countries. In Uganda the plant is still being sold as a decorative plant, although the government is publicizing it as a nuisance and raising public awareness to the problem and potential danger of the plant.

When salvinia appears upstream from its original point of infestation, it is evident that this is the result of a deliberate action. Indeed, in Papua New Guinea, local fishermen and villagers on infested lagoons decided to use the plant as a biological weapon to infest the upstream waters to make a point in their quarrel with neighbors.

On large areas, manual, mechanical, or chemical control methods cannot be applied. The cost of such measures is prohibitive and mechanical methods are slow compared to the regrowth rates. Biological control is usually less expensive, long term, and less harmful to the aquatic environment, but until recently its use on large-scale infestations was limited. The first major breakthrough in Asia was perhaps the control of the very large Sepik infestation of salvinia by *Cyrtobagous*. Since then, this control method has been applied in a number of other countries, including Sri Lanka, Botswana, Namibia, South Africa, and others. An equally efficient control of water hyacinth has not been found, although there exist several potential organisms, of which perhaps the beetles *Neochetina* spp. have the greatest potential, but are comparatively slow and less efficient than *Cyrtobagous*.

AQUATIC PLANT-FISH RELATIONSHIPS IN NATURAL SITUATIONS

A number of good reviews are available on this topic for the developed countries, such as that prepared by Canfield and Hoyer (1992) and by de Nie (1987). Reviews for developing countries are rare (*e.g.* Petr 1987).

To illustrate the situation for developing countries, I have selected several examples. The first one concerns floating meadows on floodplains of the Amazon river system. These rivers are of two types: those which carry so-called white waters, and are rich in dissolved nutrients as well as in aquatic plants, and those which are nutrient poor and are characterized by the dark color of water given to them by the presence of humic acids. The differences in their productivity are also reflected in the large differences in fish production.

The white waters of Solimões/Amazonas are rich in fish stocks and are an important source of protein to the region. The migratory characins (Family Characidae in the order Cypriniformes is a large family of economically important tropical food fishes) in these systems spawn at the beginning of floods and their fry/fingerlings grow and develop in the "varzea" floodplains, whose margins consist of dense vegetation, mainly *Paspalum repens* and *Echinochloa polystachia*. These and many other macrophytes of the "varzea" have floating root clusters which support rich invertebrate fauna, and these, together with detritus, provide an important food for the migratory characin fry and fingerlings. Other fish, such as *Colossoma*, feed mainly on filamentous algae and *Oryza* seed found in the floating meadows (Araujo-Lima, Portugal and Ferreira 1986).

In black and clear water regions of the Amazon basin, such as the Rio Negro, the aquatic floating meadows of grasses are less developed and diverse. Fishermen know of the significance of floating aquatic plants for holding or concentrating fish and use this knowledge to capture them.

Then there are the floodplain trees. In some countries tropical forest trees are resistant to long-term flooding. Such forests are present in the Amazon, but also in Cambodia, Indonesia, and the United States. During high water levels the flooded trees provide habitat and submersed surfaces for periphyton production. In Lake Tonle Sap in Cambodia, periodically flooded forests surrounding the lake contribute much to the fisheries production of the lake. Recent deforestation, followed by reclamation of the shallows for agriculture, has caused a sharp decline in fish catches. The Cambodian fisheries authorities have recognized this problem and have begun replacing trees on a small scale to return some of the lost production to the lake. Habitat restoration to increase fish production through planting water-tolerant trees, such as *Salix*, *Taxodium*, and *Eucalyptus* spp. were suggested for some U.S. reservoirs with water-level fluctuation. On the middle Kapuas in Indonesia, there are numerous black water lakes poor in nutrients, but with flood forests. In Lake Luar, the largest lake situated close to the Sarawak border, the presence of such forests is believed to sustain a high fish production.

Minimal clearing occurs in large tropical reservoirs constructed in savanna-woodland or in tropical forests because it is usually too costly and/or manpower is not available. The result is a direct benefit in the form of higher fish production, which persists for a considerable period of time, usually until the submersed trees eventually die and decay. The surfaces of the trees function as a source of food and shelter, and when aquatic plants become associated with them, they represent suitable spawning areas for many tropical fish species. In Volta Lake, Ghana, water lettuce (*Pistia stratiotes*) became widespread in areas of the lake with submersed or semi-submersed tropical forest trees. Elsewhere in the same reservoir a wide belt of the emergent grass *Vossia* developed along the water margins. Both plants had considerable significance for fisheries, but also served as vectors of waterborne diseases, such as larvae of mosquitoes and the snail *Bulinus*, the vector of schistosomes. It was determined that *Ceratophyllum* was largely responsible for the almost 100 percent incidence of schistosomiasis among children living along the shores of this reservoir. The dead trees also enhance the formation of sudd or floating islands in shallow waters. FAO evaluated the significance of flooded trees for fisheries and produced a publication by Ploskey (1985), which summarized the effects of flooded timber on fishery production in North America. Also, several studies are available on the significance of flooded trees for invertebrate and fish production in the Volta (Petr 1970) and Kariba (McLachlan 1970) reservoirs in Africa.

Managing lakes, reservoirs, and lagoons for increased fish production may be assisted by the provision of brushparks. Tree plantations may provide the material which, when submersed, will provide surfaces for periphyton and function as fish-attracting devices. The method, first described in Benin, West Africa, under the name acadja (Welcomme 1972) is now widespread. Sometimes aquatic plants are added. Annual fish yields vary from 2 t/ha, with fishing intervals of 3 to 4 days, to up to 17 t/ha with an interval of 70 days (Welcomme and Kapetsky 1981). But the requirement of wood for acadjas is about 10 t/year and this may be detrimental to the shoreline brushes and trees. The German Technical Cooperative Office in Benin identified seven tree species with a reasonable resistance to underwater decay and proposed to grow them on plantations.

USE OF AQUATIC WEEDS IN FISHERIES

The major importance of aquatic weeds for fish is to provide shelter, food and, to a lesser extent, to serve as a substrate for egg deposition. Relationships between aquatic plants and the centrarchids in North American lakes have been studied in great detail and they are now fairly well understood. This makes it possible to apply certain management measures if there is a demand for especially sport fish, or for the prey fish on which the sport fish feed. In Europe such relationships have been reviewed by de Nie (1987), and in some lakes fish have been manipulated for the benefit of fishermen. The use of such relationships in fisheries management in tropical and subtropical water bodies is less common, largely because they are unknown. The high demand for fish in Asia and Africa and the easy marketability of virtually all fish captured, even the small ones, have not made such research a priority. In Asia, the freshwater fish fauna west of the Wallace's line (a hypothetical line in the western Pacific Ocean separating the Oriental and Australian regions) is rich in species, and a single fish catch often consists of 30 or more species. Many species come from the same location as they feed on the same type of food. Cyprinids, the dominant group, are not very food selective, have high reproduction rates and grow fast. Studies of interrelationships of a large number of fish species are difficult. On the islands east of the Wallace's line, the poverty of fish species makes detailed studies easier and provides better opportunities for rational management.

Aquatic weeds or plants, depending upon one's definition, are used in some inland capture and culture fisheries in Asia. Lake Rawa Pening in Java, Indonesia, is large but very shallow. Since the introduction of water hyacinth into this lake, the plant coverage has varied over time. When the cover was excessive, the fishery declined. When water hyacinth receded, the fishery recovered and reached very high yields which is unusual for natural lakes. Water hyacinth has been accumulating in the form of peat at the bottom of the lake which is now "mined" for compost and gardening soils. In numerous places floating plants are herded behind bamboo stakes and left to attract fish which after a few days are captured by nets. Other methods of fishing are also applied including Chinese-type lift nets, gill nets, scoop nets, and dredges for prawns. Cage culture is also established, and around the cages water hyacinth accumulates, attracting even more fish.

Water hyacinth is in short supply on Lake Tempe in Central Sulawesi, but in great demand for structures called "bungka." To attract fish, water hyacinth and other floating plants are gathered behind sticks which are pushed into the lake or river bottom. Tree branches are often added as well. In Kalimantan, such systems are used on inland lakes of the Mahakam River in the east. On the Kapuas River in the west, water hyacinth is rarely found as it does not grow well in the black waters of these lakes. Any water hyacinth found is treasured and used as shade to protect fish in cages from direct sunshine, but no "bungka" are constructed there largely due to the lack of floating vegetation.

FISH IN THE MANAGEMENT OF AQUATIC PLANTS

In a 1989 report to FAO, Hartley (CSIRO, Brisbane, Australia) estimated the number of people to be negatively affected by floating aquatic weeds (water hyacinth, salvinia and water lettuce) in Africa. He estimated two million in Nigeria, including more than 24,000 fishermen. Approximately 100,000 persons living in riverine communities in Benin, West Africa, who rely solely on fishing for their livelihood, are affected. Additionally fishermen in Malawi and Ghana may be hampered by water lettuce. In the Niger, the recent explosive growth of water hyacinth has interfered with fishing on floodplains of the Niger River. In Lake Kyoga in Uganda, thousands of fishermen may not have access to landing sites and to fishing areas blocked by water hyacinth.

Biological control of aquatic weeds is the only hope for weed management in large water expanses. Recall, however, that the natural spread of biocontrol agents can have a detrimental effect on fish production in areas that rely upon aquatic weeds to increase production and on fishermen who use aquatic weeds as fish attractants. On a smaller scale, fish can be used to control some plants, especially submersed species. Grass carp is presently the most suitable species, but tilapia (*T. rendalli*) and tawes (*Puntius gonionotus*) have also been successful in some environments. They are food selective, preferring submersed weeds, and to achieve fast results the weeded areas may have to be overstocked. Overstocking also is required to overcome the exceptional abilities of fishermen to utilize nets to non-selectively harvest fish that are stocked to control aquatic weeds.

An advantage of using fish for aquatic plant control is that plant destruction is gradual and thus relatively safe for the environment as there is less danger of rapid deoxygenation of water such as that resulting from chemical control which kills plants suddenly. The side effect of biocontrol with fish is sometimes the eutrophication of water which may lead to algal blooms. This can be used to advantage by stocking plankton-feeding fish or a polyculture-type approach. Grass carp has been successfully used in irrigation systems in Egypt (van Zon et al. 1982) and in Turkmenistan (Charyev 1984).

The Kara Kum in Turkmenistan is an artificial canal 1,000 km long, which branches off the Amu-Darya River. The flow at the canal headworks is 400 m^3/sec. The muddy waters of the Amu-Darya then enter the Kelif lakes where the suspended

sediment gradually settles. In the last of these lakes, the Secchi disc transparency is 2.3 m, which encourages growth of macrophytes. Only parts of lakes and canals deeper than 5 m are free of aquatic weeds, and even the smallest distributaries are invaded.

To sort out the problem of aquatic plants in the system, grass carp was introduced in 1958 and released in large numbers in 1960 and 1961. By the mid-1960s, large-scale natural reproduction of grass carp took place and within a few years most of the aquatic macrophytes disappeared. Some problems, however, remain. Selective feeding of the grass carp has led to succession in macrophytes, with *Myriophyllum spicatum* being replaced by *Ranunculus*, which is considered toxic to grass carp. Charyev (1984) summarized the experience with the introduction of grass, silver, and bighead carps into the Kara Kum canal and emphasized the need to protect the higher aquatic vegetation, particularly in cases where macrophytes represent spawning substrate for other fish, such as common carp. In excessive numbers the grass carp can cause great damage to a body of water as an ecosystem, destroying existing food-chain relationships and threatening the spawning grounds of commercial fishes.

Grass carp, through its intensive grazing activity, also contributes to the eutrophication of water bodies. The deterioration of water quality, which is undesirable in deserts where alternative potable water supplies are rare, was successfully countered by silver and bighead carps which live on phytoplankton, zooplankton and detritus. These two species prevented phytoplankton blooms and deterioration of water quality. In the system of canals and reservoirs of the Kara Kum canal, the three carp species constitute 75 to 80 percent of the total catch and yield 45 kg/ha/year (Charyev 1984).

Environmental impact of grass carp on the aquatic ecosystem cannot be disputed: by suppressing some species of macrophytes other species may increase; by grazing off some plants the spawning substrata of important fish may disappear; without a counterbalance of phytoplankton-feeding fish, water quality deteriorates. Aquatic systems function best with a moderate abundance of aquatic macrophytes and introduction of grass carp could assist in reaching such equilibrium. But as Charyev (1984) stated, without grass carp, the irrigation system of canals and reservoirs in the Karakum desert would be much worse than with it.

BIOMANIPULATION

In 1989 I attended an international conference on biomanipulation of water quality in Amsterdam. Ozimek *et al.* (1990) described an example where the stocking of planktivorous fish resulted in the restoration of submersed aquatic plants due to improved transparency of water for light. Biomanipulation for restoration of aquatic ecosystems has focused mainly on inland lakes in temperate latitudes. Modeling such situations requires considerable data of good quality, something we still lack for most similar situations in developing countries. Standard models of relationships for temperate waters are not necessarily applicable for warm waters, where reactions are faster and the number of relationships greater. Fish have been used for biomanipulation on a large scale in Lake Kinneret in Israel, largely for the purpose of maintaining good water quality (Leventer 1981). This biomanipulation targeted especially heavy blooms of *Peridinium*. However, finding the right management strategy has been difficult. For some 20 years stocks of fish have been manipulated including the introduction of exotics, and trying various stocking rates. The results show the difficulty of using the biomanipulation approach in a large water body, but the Israeli experience has also provided a wealth of data which would not be obtained otherwise. Much flexibility on the part of managers and on-going research are two basic conditions for using this approach in warm-water bodies, particularly those used as a potable water supply.

AQUATIC PLANTS, VECTORS OF WATERBORNE DISEASES AND FISH

Allow me to deviate to rice, the most common aquatic or semi-aquatic plant in the world. Ricefields are considered wetlands or semi-wetlands and are frequently associated with other aquatic plants. There are over 150 million hectares of ricefields worldwide, of which about 80 million hectares are irrigated rice areas, representing some 35 percent of the total area of irrigated crops. Ricefields harbour a number of vectors of parasites or viruses causing diseases such as Japanese encephalitis (*Culex* mosquito), malaria (*Anopheles* mosquito), yellow fever (*Mansonia* mosquito), filariasis (*Culex* mosquito), and schistosomiasis (snails *Bulinus*, *Biomphalaria*). *Anopheles gambiae*, the main vector of malaria in Africa, is often found in high densities in ricefields. The same vectors are found in many aquatic plants including water lettuce and water hyacinth. *Bulinus* is common in *Ceratophyllum*, and blackflies, the vectors of a parasite causing river blindness, are common on submersed plants in rivers, streams and canals.

In large ricefields, mechanization may lead to mosquito outbreaks which are much less common in more traditional agroecosystems. Such systems usually combine rice production with production of vegetables, edible molluscs and fish, and fodder for cattle. Ideally, although many vectors can exist, the complexity of animal populations and predator pressure (including fish) limits productivity of any one vector (Bradley 1988). In Nepal and Afghanistan, biological control

of mosquitos using larvivorous fish has been successful and a similar success was achieved in ricefields in Java. Control of snails harboring schistosomes is also possible, especially in a pond situation, using haplochromine fish. In strict rice monoculture, such as that practiced in California, larvivorous fish may fail to control the vectors (Blaustein 1972). Observations showed that introducing larvivores triggered reactions which no modeling could predict.

Large-scale irrigation projects in Turkey, some already completed, urgently need advice on how to deal with the combined problems of aquatic weeds and disease vector control. The use of fish has been proposed as one of the alternatives.

AQUATIC PLANTS AS FISH FOOD

Grass carp is not only an aquatic plant control agent but also an important fish of semi-intensive and intensive pond culture where it is daily fed terrestrial grasses and vegetable waste.

A more sophisticated system has been developed in China and Vietnam where the duckweeds (*Lemna*, *Wolffia* and *Spirodela*) are grown to supply feed for pond and ricefield-raised fish. In Taiwan a system using *Wolffia* and *Lemna* for feeding young fish is well developed for Nile tilapia and common and grass carp. In China, grass and common carp in ricefields are grown to fingerling size, providing a yield of 225 to 300 kg/ha or even higher, depending on which system (single or double cropping) is applied. Such fingerlings are then used for stocking ponds where they are grown to marketable size. Duckweeds can be grown on septage, then fed to tilapia. In Thailand, Edwards, Polprasert and Wee (1987) reported yields of *Spirodela* in septage-fed ponds of approximately 9 t dry weight/ha/year in long-term experiments. In family ponds, 7.4 t/ha/yr was possible. While *Spirodela* can be used only as supplementary feed for large Nile tilapia, grass carp, and silver barb (*Puntius gonionotus*), *Lemna* and *Wolffia* were readily eaten. These plants can assist in solving the often difficult task of disposing, but also utilizing, domestic sewage in tropical countries and have the side benefit of producing feed for fish.

The fern *Azolla* is another useful plant. The tilapia *Oreochromis niloticus* derives 50 to 80 percent of its body weight from *Azolla* in the rice-*Azolla*-fish system, and the rate of *Azolla* digestion is 59.7 percent (Liu Chung-Chu 1987). In their feces, these fish excrete 40 percent of the nitrogen, which means that they assimilate 60 percent of *Azolla* nitrogen.

In the fish-rice-*Azolla* system *Azolla* also controls aquatic weeds. In India *Azolla* reduced growth of the emergent grass *Echinochloa* in rice paddies and increased the grain yield. Under suitable conditions *Azolla* can supply the entire nitrogen requirements for a high yielding rice crop in 10 to 20 days. *Azolla* is used for feeding not only fish, but also livestock and poultry, especially ducks. *A. filiculoides* tolerates up to 0.7 percent salt water and can be used for reclamation of coastal saline soils. If cultivated for two years, the salt content may decrease from 0.35 to 0.1 percent (Shang *et al.* 1987). This could perhaps be used in swamp and coastal pond fisheries, where acid sulfate soils are a major obstacle to achieving good prawn and fish production. The disadvantage of growing *Azolla* is the demand for labor which is not readily available in some countries. Also, intensive rice culture on large fields does not allow the use of *Azolla*.

EQUILIBRIUM, SUSTAINABILITY, MANAGEMENT, NEEDS

Having provided a number of examples of problems caused by aquatic plants, and of their benefits, especially for inland fisheries in developing countries, I shall try to identify some future needs. Before answers can be given, it is necessary to ask a few questions.

One of the spinoffs of the rising environmental consciousness is the wish to maintain natural equilibriums. There is a feeling that exploitation of resources should be replaced by sustainability, *i.e.* by managing natural resources (or exploiting them) on a sustainable basis. Can this be done? It cannot be done with minerals, but it can be done with biological resources. With an ever-increasing human population and demand for improved living standards, sustainable growth seems to be the only solution. How do aquatic plants fit in? In the developed as well as less developed world we wish to preserve species diversity, healthy growth, and nice flowers. But we also need aquatic plants for management and we have been looking at how to use them as management tools in the removal of pollutants, as fodder for cattle, as a medium for fish spawning, provision of shelter, and substrates for grazing. In some countries they provide cellulose for manufacture of paper. To sustain these functions of aquatic plants requires management. Sensible management means that we must understand the principles determining equilibriums and that we can identify the upper and lower limits of management. This is still a difficult task, requiring research, something which may be available in developed countries, but not usually present in the rest of the world.

Aquatic plant management research needs to be both basic and applied. The cost of basic research is high, and in most developing countries such research is still a luxury, although an increasing number of laboratories there now receive financial support, laboratory equipment and professional advice with training on the job or outside the developing country. There exist centers of excellence such as the

Asian Institute of Technology in Bangkok and the International Rice Research Institute in Los Banos in the Philippines. Some international organizations also coordinate local or regional research, such as the Wetlands Bureau in Bogor, Indonesia. Time is now ripe for networking among such organizations, which still largely work in isolation.

Transfer of knowledge from developed (often temperate) to developing countries (often tropical) is possible, but such knowledge is not always applicable. A good example is the tremendous amount of basic and applied research conducted on centrarchid and salmonid fisheries in the temperate zones, very little of which is useful to developing countries. Therefore, there is a need for research and training. To find a correct approach, training on a technical level may be more important than training resulting in a higher university degree. Developing countries may have western university graduates of excellence, but few with home country experience. Often these graduates may not wish to descend, in their opinion, to research or applied science on problems similar to those solved in other countries half a century ago.

Dissemination of results from research and applied science of local scientists also presents a problem. They lack peer reviews of their work as there are few scientists who work in the same field in the same country. Their contact with laboratories and researchers outside are limited. The English language is now the widely accepted medium for scientific communications and this still represents a problem for sizable groups of good scientists in non-English speaking countries, making the results of their work poorly known in the outside world. There is also a financial barrier between the developed and developing world, which prevents purchase of new publications and subscriptions to scientific journals. While computerized information retrieval systems may be accessible, the information obtained from them is good for reviewers, but not always of much use to scientists in the field.

International organizations have assisted with the production of manuals and guidelines and with their translations into local languages. Such manuals, often produced in collaboration with local scientists or entirely by them, not only address the problems, but also advise on their solution. Videotapes addressing specific problems are also becoming available and reach many developing countries.

COORDINATION, FEEDBACK

As noxious aquatic weeds such as salvinia and water hyacinth often transcend borders, their control may require trans-national coordination. Water hyacinth arriving in Lake Victoria from Rwanda through the Kagera River, or the same plant reaching Nigeria through the Niger, are two examples. Solving the problem requires international collaboration and coordination of activities as proposed by CSIRO, the International Institute for Biological Control (IIBC) and a few other organizations. Monitoring of the global spread of water hyacinth in many Anglophone countries was undertaken in the early 1970s by the Commonwealth Secretariat, but there is a need to review the situation again.

An integral part of the coordination for control of aquatic weeds is education of people through mass media about the hazards of aquatic weeds. When the Sepik River people in Papua New Guinea started transporting salvinia in their canoes to surreptitiously introduce this plant to lagoons of neighboring villages, they had little idea that they were speeding up the process of a complete blockade and isolation of their own villages.

Coordination also implies that aquatic plant managers look beyond their own problem, placing it in a broader context of the environment and watershed development. They need to know about watershed manipulation, water storage and diversion, irrigation, rice production, cotton production, pesticide application, etc. Shallowing of lakes by siltation from deforestation and excessive inputs of sewage will impact aquatic plants. A good manager will attempt to solve complex problems in close collaboration with other interested parties and to their mutual benefit.

Finally there is the need for monitoring and feedback. Many activities in the field terminate after a certain period of time, deemed sufficient for their implementation. In the case of aquatic plant management we know that it is not possible to leave such a dynamic biological system without monitoring, especially in a situation with potential for renewed explosive growth. Monitoring the introduced control agents is especially needed in remote parts of some countries which could become focal points for future invasions. Regular monitoring of the post-project situation should be included in management plans. Few governments realize that the cost of the monitoring may be less than launching similar projects again in the future.

CONCLUSIONS

What is the solution to an aquatic plant management problem? The answer will be in a good description of the problem.

Describing a problem may not always be easy. A problem for one interested party may not be a problem for others. In multipurpose use of water and land resources, aquatic plants or weeds are only one item in a complex system. A hydropower dam engineer may not necessarily insist on good water quality, but will require destruction or removal of floating aquatic weeds. A water supply manager will tolerate aquatic plants which do not decay on a large scale, and will

accept a reasonable growth of submersed plants. The manager may collaborate with a fisheries manager, who will advise on how to use fish to maintain good water quality. Sport fishermen, rare in developing countries, will request good sport fish to catch, usually a predator. But the introduction of a predator to some water bodies may be controversial and detrimental. We have heard a lot of criticism about the introduction of the Nile perch into Lake Victoria, which substantially changed the lake aquatic environment, but on the other hand has led to a flourishing fishery. Fish production managers may accept eutrophication and may therefore not be interested in water quality improvement. In summary, advice will not always satisfy everybody and may even involve some risk.

In developing countries we have to weigh the economic, social, and environmental costs of an action. Some of you may disagree with the order in which I list the above costs, but it is indeed still largely the economics which determines whether to cut littoral weeds, to buy a weed harvester, or to invest in chemicals to spray. Technical advisory agencies provide advice, but the enforcement of a particular approach is in the hands of the government. If the government has not enough money to introduce the best control method (or to allocate several specialists to deal with the task, or to establish a research laboratory), our advice may not be implemented.

For some time to come, developed countries will remain the vanguard of in-depth, sound, and applicable research and of devising the best ways for aquatic plant and weed control and management and assisting with the transfer of experience and technologies to developing countries.

In conclusion, I have prepared a short list of aquatic plant management priorities compiled in response to requests for assistance addressed to FAO. The list reflects two basic demands of people in developing countries: food and health.

1) Control of exotic aquatic macrophytes with mass distribution in open waters such as in the Niger River, Lake Kyoga and Lake Victoria, reservoirs in Cuba and Bolivia, and the Sepik river system. The major reason given for such requests is the damage aquatic weeds cause to fisheries.
2) Control of weeds and aquatic plants in numerous irrigation canals and drains. The major reasons given for requests include water loss, slowed distribution, control and eradication of parasitic and arboviral disease vectors.
3) Rehabilitation of aquatic plants in lakes under the impact of eutrophication. The major reason for this request is to re-establish the original fish, crab, and prawn fauna such as in lowland lakes of the Yang-tse river in China.
4) Assessment of the significance of wetlands for fishery. Apart from providing an inventory and program for fishery management, such surveys are to assist in the protection of wetlands against encroaching agriculture (*e.g.* in Nigeria).

This symposium has provided me the opportunity to ask that you consider the vast importance of your work to many millions of people in developing nations and further consider your activities with the respect to the four priority areas I have described above.

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PLENARY ADDRESS

Ecological Crisis in Post-Communist Central Europe

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INTRODUCTION

The countries of Central Europe which liberated themselves from Communist rule in 1989 (Czechoslovakia, Hungary, Poland and former East Germany) have inherited a weak economy and a severely deteriorated environment. The poor state of these countries is the result of the wasteful Marxists' attitude toward natural resources and of their small respect for environmental protection and nature conservation. The "new democracies" of Central and Eastern Europe are now facing a serious ecological crisis which is coupled with their economic difficulties. I will confine my presentation of this situation to the aforementioned four post-Communist countries situated at the heart of Europe while most examples will be drawn from my home country, now divided into the Czech and the Slovak Republics.

SYMPTOMS OF THE ENVIRONMENTAL CRISIS AND ITS CAUSES

In all four countries, the ecological crisis shows similar symptoms. Among the most conspicuous ones are drastic changes in the hydrological balance of whole regions, especially increased instant discharges caused by large-scale drainage of agricultural land, high levels of air and water pollution, heavy eutrophication of many standing and running waters and soils, and the acidification of others. Further symptoms, often associated with the previous ones, are a deterioration of soil quality (soil degradation), especially reduced soil humus content and waterholding capacity, and damage to forests leading even to forest dieback. The severe damage to some forest-tree species [especially conifers such as Norway Spruce (*Picea excelsa*) and Silver Fir (*Abies alba*) is parallelled by an equally severe damage to some species of aquatic and wetland plants such as some water lilies (*e.g., Nymphaea candida, Nuphar pumila*), bulrushes (*e.g., Scirpus lacustris*) and reeds (*Phragmites australis*). The generally raised nutrient concentrations in rainwater, soils, and surface and ground water bring about an impoverished species composition of biotic communities, due to the enhancement of just a few species populations that can make use of increased nutrient inputs and outcompete other species populations. Acidification has a similar effect: it is only the acid-tolerant species that can relatively thrive in areas which are strongly affected by acid rain and snow fall. These are areas with shallow podzolic or semi-podzolic soils on acidic geological substrates, mostly in the highlands or mountains. A decline in biodiversity and dying out of some species of green plants and associated fungi and animals are thus the result of both eutrophication and acidification of soils and waters. On top of that come the direct effects of environmental pollution, with all three main biosphere compartments (atmosphere, hydrosphere and pedosphere) acting as its vectors.

Human health is also adversely affected by the unfavorable environment. In most post-Communist countries, the average life expectancy is shorter by several years than in other comparable countries of Europe. The direct effects on human health are largely due to air pollution; the indirect effects are still rather obscure, but high concentrations of nitrate in many sources of drinking water and residues of agricultural chemicals and maybe PCB's are among the likely reasons, though bad food habits of the majority of Central European people can also be blamed (Figure 1). The environmental awareness of the general public is still relatively low although it is growing slowly. However, at present most people seem to be most interested in improving their economic situation, and environmental considerations play a secondary role in their decisions (perhaps with the exception of the people living in regions with the most severely deteriorated environment). Systematic environmental education, especially of the young people and children, is essential for improving the situation. A consumers' society should not be our goal!

In short, it may be stated that under Communism we lived at the expense of our children and grandchildren. In the new

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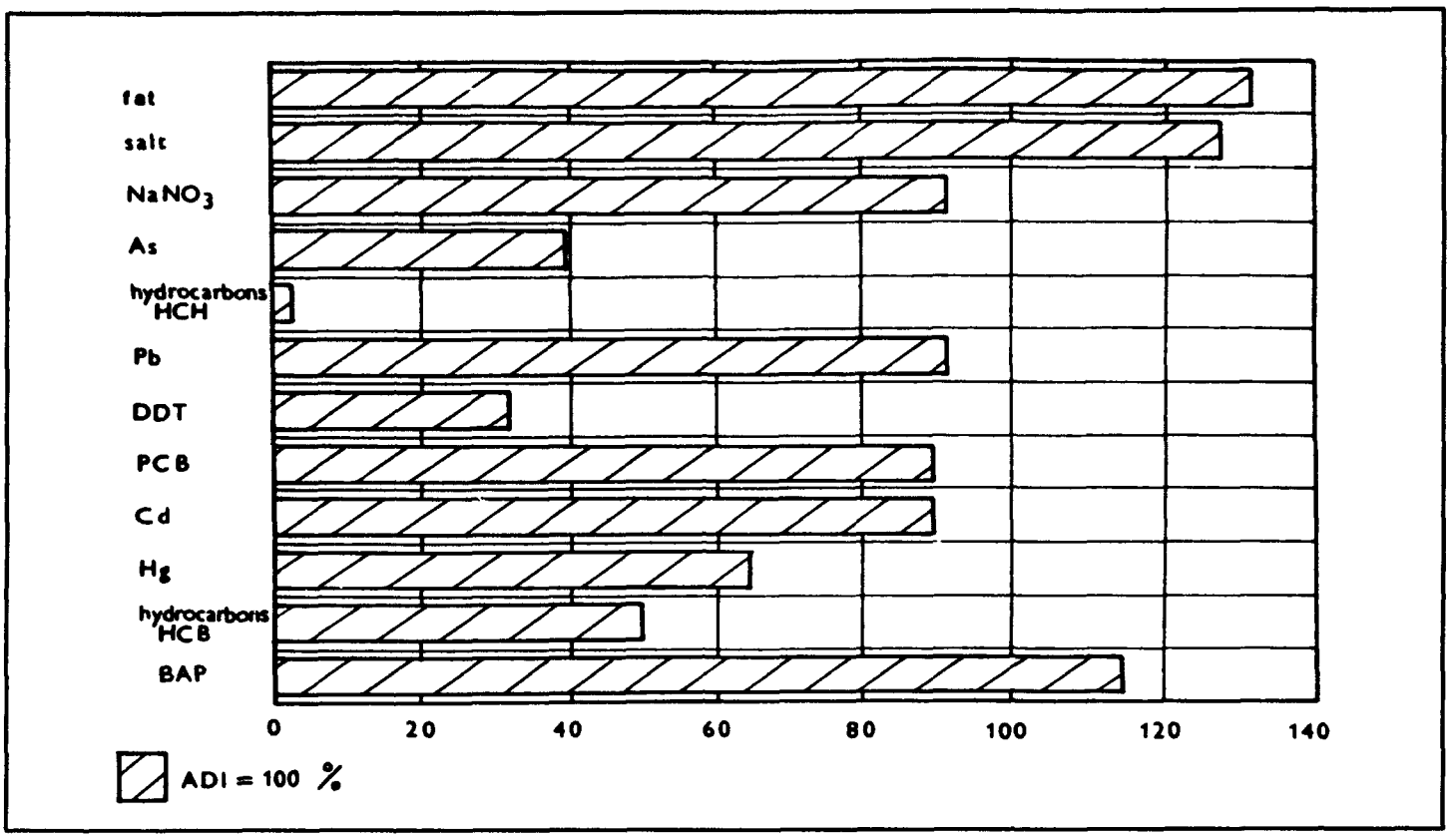

Figure 1. Contents of certain components and pollutants in the food of an average inhabitant of Czechoslovakia in 1989, expressed as percentages of the acceptable daily intake (ADI = 100%) of the respective components as defined by the World Health organization (after "Environment in the ČSFR," 1991).

political and social climate, this absurd situation should change as quickly as possible. But the implementation of a profound ecological reform together with the undoubtedly necessary economic reform and restructuring of the "new democracies'" production potential will be neither simple nor easy. A good many of the new politicians are inclined to favor technocratic rather than ecological thinking and "sustainable development" means hardly anything to them (not to speak about the more radical concept of "sustainable life"). The politicians must also resist the temptation to satisfy the people's material and social demands, however justified they may be, at the expense of the environment. This is difficult to achieve in stabilized democracies and seems hardly possible in the still unstable "new democracies." But the postponement of the solutions to an unspecified "future" would be much more costly and hazardous.

The above brief description of the new democracies' environmental problems shows that their ecological crisis is accompanied by a crisis of moral values: no unquestionable moral rules seem to be valid in these countries' transitional situation between a Party-controlled and a free society. The quickest remedy might seem to be a rapid restoration of traditional values of the pre-Communist "good old times" in which, however, ecological ethics was an unknown term. That is why it is so difficult to convince the majority of both lay people and politicians of the necessity to incorporate ecological considerations in the rules for the present political, economic, and social reforms of the post-Communist societies. For example, a long-lasting effort to have an "ecological" chapter in the new constitution of the Czech Republic has been unsuccessful although Czechoslovakia has probably adopted the greatest number of "environmental" laws of all European post-Communist countries. Nevertheless, the unfavorable environment in which people and other creatures have to live can be improved only if the causes of the environmental crisis are understood and practical lessons are drawn from this understanding.

WAYS OF SOLVING THE ECOLOGICAL CRISIS

A complicating and somewhat obscuring circumstance is that the ecological crisis caused by the Communist system has occurred against the background of a global or continental environmental deterioration (greenhouse effect, overall

atmospheric pollution, acid rain, increased atmospheric inputs of nitrogen and mineral nutrients, *etc.*). This circumstance is often misused to weaken the ecologists' critical evaluation of causes of the ecological crisis in the post-Communist countries. But it is clear that the transformation of industry, agriculture, transport and the tertiary sphere toward the use of cleaner and/or environmentally less hazardous and more energy-saving or energy-efficient technologies did not occur under the Communist rule while it has occurred (at least to a significant extent) in most economically advanced countries of Europe. The Communist countries' seemingly strict environmental regulations had numerous weak spots and gaps and were never consequently implemented, being a part of the Communist "window-dressing" toward the rest of the world. The present democratically elected parliaments of the post-Communist countries and their governments hold several keys to an environmental improvement in their hands. They must use them wisely.

The first key is the improvement of environmental legislation which, indeed, has made substantial progress in Czechoslovakia and its constituent Republics as well as in Hungary and Poland. Former East Germany (DDR) has adopted West German (FRG) laws and each new "Land" has adjusted its own regional laws to the federal ones. Czechoslovakia, for example, has got new laws on environmental protection, nature and landscape conservation, environmental impact assessment, waste management, and air pollution as well as stricter standards of water quality. In general, the environmental standards of the Central European post-Communist countries are being adjusted to those of the European Communities. Some countries, *e.g.*, the Czech and the Slovak Republics, have created, by law, special funds to support projects aimed at environmental improvement.

The next key, of which full use has not yet been made (mainly out of fear of social unrest), is the introduction of realistic prices of energy and raw materials. Yet, these changes are now gradually taking place though different and often greatly contrasting concepts of energy policy (non-nuclear against nuclear electricity; modernization of thermal power plants; centralized against decentralized management of energy supplies; electricity and gas imports and exports; different prices of oil, etc.) are still debated among specialists as well as politicians. All parties concerned, however, acknowledge the importance of energy savings in industry, transport and agriculture, to be achieved through their modernization and increased energy efficiency (Figure 2). It is also acknowledged that the present prevalent dependence of Czechoslovakia, Poland, former East Germany and to some extent Hungary on energy from brown coal with a high sulphur content contributes significantly to air pollution and acidification in Europe (Figure 3). Remedies are being sought but they are neither quick nor cheap. A large-scale change to predominantly nuclear electricity would, in addition to its known hazards, consume the money which could be used for modernizing the thermal power plants. On the other hand, brown coal is a valuable chemical raw material: burning it all would not therefore be wise either.

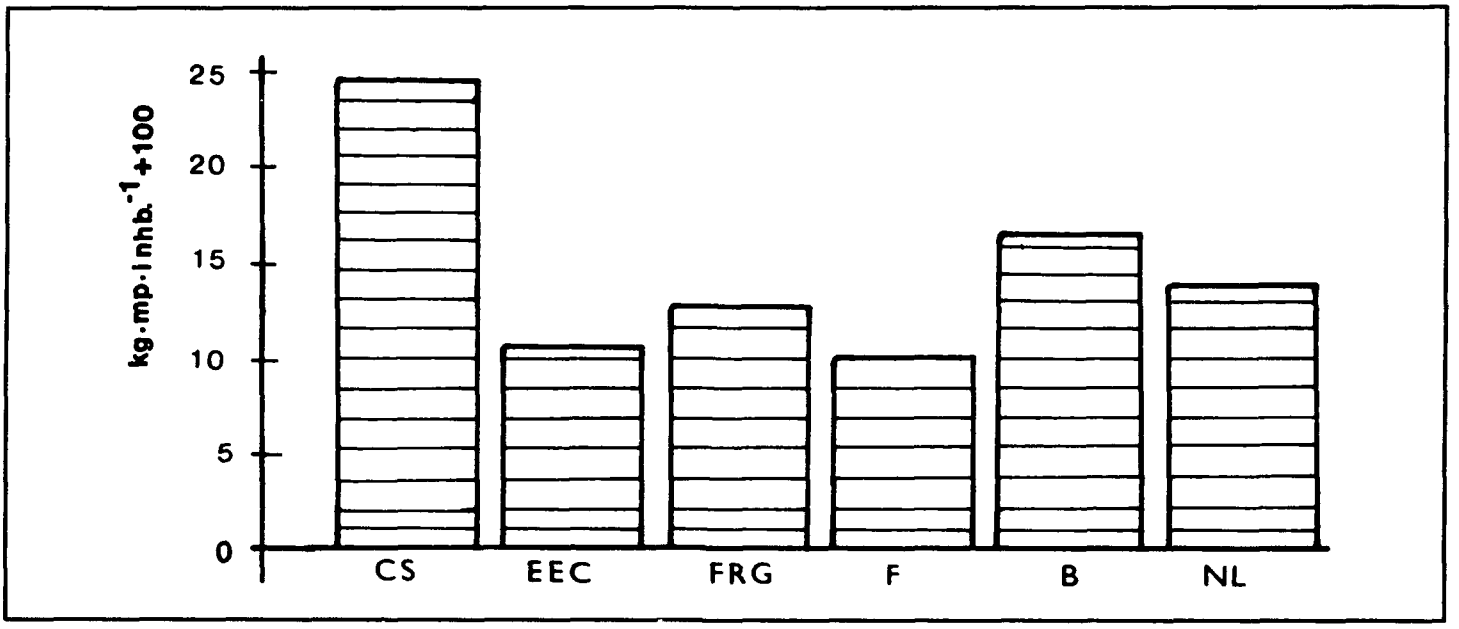

Figure 2. Per capita consumption of electric power by the industry in Czechoslovakia (CS), the whole European Economic Community (EEC), former West Germany (FRG), France (F), Belgium (B) and the Netherlands (NL) in 1988 (after "Environment in the ČSFR," 1991).

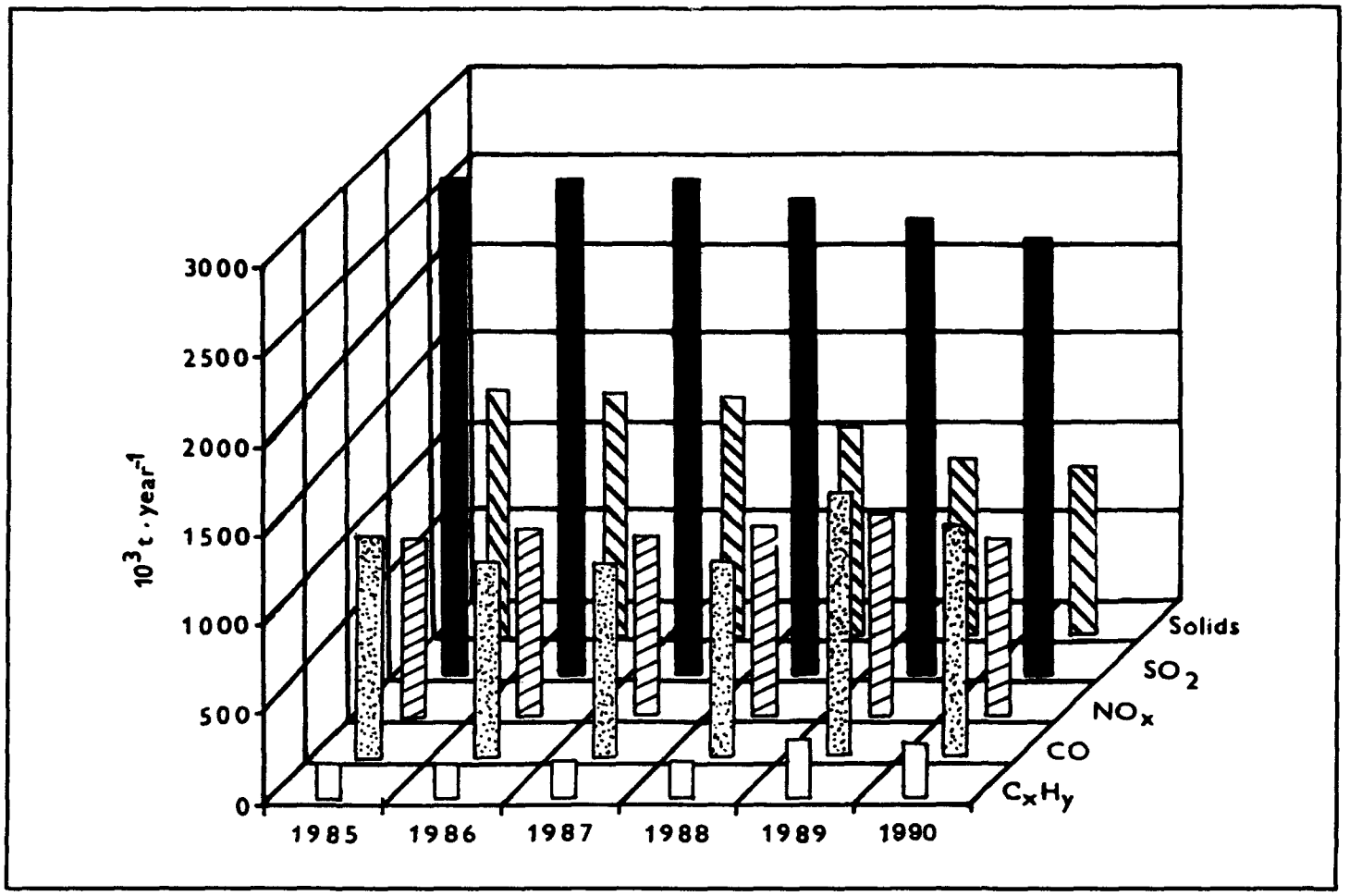

Figure 3. Czechoslovakia's total annual emissions of the main atmospheric pollutants in 1985 to 1990 (after "Environment in the ČSFR," 1991).

The third key, closely linked with the previous one, is the wide introduction of recycling technologies minimizing the production of residual wastes, for whose processing modern and ecologically safe technologies have to be introduced. These measures must be coupled with a modernization of transport systems, shortening of transport pathways from raw materials to final products, and an economic stimulation to public transport and ecologically less harmful means of transport such as waterways and railways. The tendency to favor road transport is still strong, but it is short-sighted and can bring only short-term benefits.

The fourth key involves the adoption of ecologically sound land-use planning and the elaboration of ecologically sustainable regional economic policies. A return to a greater variety in land use and town development, linked with more variety in the processing of local and regional resources, can help restore a healthier, more diverse (in terms of species richness), and more beautiful countryside and nicer human settlements, in contrast to blocks of prefabricated apartments which were commonly built in all Communist countries on a large scale. Larger parts of the countryside should be reserved for nature conservation and recreation without substantially limiting agriculture or industry. Such land-use decisions leading to the restoration of the "network of ecological stability," and the support of "soft" tourism can also bring good economic returns.

Some elements of a far-sighted environmental policy, based on the strategy of sustainable development, have already been adopted by the post-Communist countries. In Czechoslovakia, for example, the subsidies to gasoline and oil prices have been largely removed. Already in 1990, higher oil and gasoline prices have resulted in a 15% drop of oil imports. Agriculture is gradually returning to a higher share of grassland within the total cultivated area. Higher fees for waste disposal stimulate waste recycling. On the whole, however, the adoption of all the above and other desirable measures leading directly or indirectly to environmental improvement is limited by lack of funds in the post-Communist countries. The difference between the costs of the most urgent measures and the funds available is enormous in all of

them. In the Czech Republic alone, the 1992 budget has set aside some 13 billion crowns for environment-saving measures, but at least three times as much money would be needed to bring about a sufficiently fast and visible improvement. This example shows that even the most economically developed regions of post-Communist Central Europe cannot make a breakthrough in environmental improvement without substantial financial and technical assistance from abroad. This should preferably be in the form of long-term credits with low interest rates for the purchase or development of modern technologies and machinery, and for the restoration of damaged ecosystems. Lecturing, transfer of know-how and elaboration of management plans by Western firms are useful, but expensive, and thus little effective. Often local firms or specialists could accomplish most of these tasks more cheaply and equally well, and the money could be spent directly on the accomplishment of projects for environmental improvement.

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PLENARY ADDRESS

Processes of Aquatic Weed Invasions: The New Zealand Example

CLIVE HOWARD-WILLIAMS[1]

ABSTRACT

Aquatic weed problems in New Zealand are caused by introduced submerged species, particularly those of the family Hydrocharitaceae. Introduced submerged species have successfully dominated the native flora in the depth range of 1 to 6 m and spectacular invasions are still occurring. Their growth is particularly marked in clear oligotrophic lakes where weed bed heights of > 4 m and biomass values of up to 3000 g m^2 (dry mass) have been recorded. Turbulence due to wave action appears to be the major factor controlling the upper limits in natural growth for some species, but low nutrient level is a barrier for others. Weed movement between lakes is facilitated by interlake recreational boat movements. Native plants can re-establish in oligotrophic lakes if the invading species are controlled (*e.g.* by mechanical harvesting). Published literature shows that well-planned scientific experiments on weed management strategies (with adequate experimental controls) are not common. For instance, data show that large-scale natural declines in weed populations can occur which complicate the interpretation of management methods.

Key words: mechanical harvesting, dispersal, *Hydrilla, Hydrodictyon, Ceratophyllum, Rorippa, Lagarosiphon, Elodea.*

INTRODUCTION

New Zealand has all the world's worst aquatic weeds, and hence has a long history of aquatic weed management. Almost 20% of the country's aquatic and wetland flora are introduced (Johnson and Brooke 1989), and invasions by these species have had a major impact on the fresh waters of New Zealand. The most significant problems are caused by submerged species, in particular coontail (*Ceratophyllum demersum* L.) and members of the Hydrocharitaceae, notably lagarosiphon (*Lagarosiphon major* (Ridl.) Moss), elodea (*Elodea canadensis* L.) and egeria (*Egeria densa* Planch).

Aquatic weed problems in New Zealand lakes are largely manifested as commercial losses to hydropower stations and threats to recreational waters. Some herbicidal control is used by applying Diquat (the only herbicide registered for use in New Zealand's natural waters) from barges with spray booms, and large-scale experiments using triploid grass carp are underway (Clayton et al. 1992, Clayton 1992). However, most of the practical weed control work is done by mechanical means. This includes harvesting and routine control by:

a. Floating booms at an angle across the current to collect floating weed masses and concentrate them at a single site on the shore for removal (Johnstone 1982).
b. Mechanical screen cleaners which rake the penstock intake screens to hydropower stations pulling off vegetation as it accumulates. Johnstone (1981) estimated that partial blockage of screen intakes can result in losses of up to 60,000 MW hr^{-1}

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each year in the hydro stations on the Waikato River alone.

c. Lake drawdowns in summer to desiccate weed masses (Johnstone 1982) and in winter to freeze-kill weed masses in the 1- to 4-m depth zone (Howard-Williams *et al.* 1989).

Useful reviews of the control options for New Zealand's aquatic weeds have been published (Johnstone 1982, 1986, and Clayton 1992). This paper takes a general theme of invasion and concentrates on the ecological aspects of the weed invasions themselves using New Zealand lakes as an illustrative case.

INVASIONS

For some reason, when aquatic plants first arrive in New Zealand (and probably many other countries) they do spectacularly well. This is illustrated by two species which are not normally associated with large-scale weed infestations, watercress (*Rorippa nasturtium-aquaticum* (L.) Hayek) and waternet (*Hydrodictyon reticulatum* (L.) Lagerheim).

Watercress was introduced to New Zealand by the French in 1840 as a food plant (Healy 1969) and within a few years, plants which grew to a size unknown in Europe were causing problems to boat traffic on the Avon River. In 1857 a reward was offered for a plan to eradicate the weed, and by 1864 the Provincial Government in Canterbury passed what was called the "Watercress Ordinance" to prevent further obstruction of rivers by watercress and related weeds. The reward, by the way, has never been collected. The plant has long ceased to be a major weed problem and has now assumed the role of a valued food item. Although it frequently covers pasture streams, its high growth rate (RGR = 5% day^{-1}) and high nutrient requirement (*ca.* 700 mg N m^{-2} day^{-1}) mean that it is very effective at nutrient stripping from enriched pasture stream waters (Howard-Williams *et al.* 1982), thus performing a doubly valuable function. This is a case where a problem invasion has resolved itself into a beneficial introduction.

A more recent but similar invasion has been the recent arrival of waternet in field situations in New Zealand (Hawes *et al.* 1991). Waternet was first reported in the field in 1988 (Coffey and Miller 1988) where it occurred in the ponds of a tropical fish importer's property. Within two years it had spread to a wide range of waters throughout the central North Island where it has proliferated to an extent unrecorded in the international literature in large lakes as unialgal stands (Hawes *et al.* 1991). For instance, in late summer (1990) it covered approximately 200 ha of Lake Rotorua, a large wind-swept lake of 80 km^2, in a major tourist area. Beach fronts were smothered, tourist boats became inoperable, and there are still well-founded fears that the plant could spread further to block the intake screens of some of the hydropower stations.

SUCCESSION VERSUS INVASION

In classical Clemensian succession a plant community gradually alters in a more or less predictable way. The direction of change is dictated by the environmental conditions which are created by the preceding vegetation. Thus new species may gradually "invade a community" to replace the existing ones as the environment gradually changes.

However, rapid and large-scale plant invasions, such as the two I have just referred to, are clearly non-successional. Explanations for such events noted in the ecological literature are: low ecological diversity of the community being invaded; poor adaptation of the original flora to its environment in comparison with the invader; lack of predators of the invader; lower reproductive potential of the native flora relative to the invader and/or environment undergoing rapid man-made change (*e.g.* eutrophication or physical disturbance) which may promote the invader.

Frequently, none of these reasons are adequate to explain a particular aquatic plant invasion and we should look to a more general model to explain invader success. The concept of "the Safe Site" proposed by Harper (1977) may be such a model. A Safe Site is a location in which a plant can invade, grow *and* reproduce. Plants can invade a site to grow and reproduce only in the *absence* of environmental barriers (Johnstone 1985). These barriers may be biotic and/or abiotic.

For instance, watercress only invaded New Zealand streams when the native forests were cut to make way for pasture and the major abiotic barrier (shade) was removed. The reason for its success was that when the forests were cleared there were no botanical barriers preventing its spread. This is because New Zealand is the only significant non-polar land mass in the world that is without native rheophytes (emergent, flood-resistant stream plants) (van Steenis 1981). Watercress has never proliferated in the sunlit margins of New Zealand wetlands in the presence of a dense native vegetation.

In the case of waternet, in spite of its successful invasion of eutrophic lakes, this plant has not been able to invade oligotrophic lake systems. For instance, oligotrophic Lake Taupo (70 km from Lake Rotorua) was inoculated by waternet at the same time as Lake Rotorua. It occurred at one locality in Lake Taupo for approximately one year (1990-91) where it dominated the native vegetation to a depth of 20 m. It has subsequently declined and fragments are now rarely found. The barrier to invasion in this case was abiotic, namely the low nutrient status of the waters of Lake Taupo. Dissolved inorganic nitrogen (DIN) in the epilimnion in summer is

frequently below 5 mg m^{-3} and dissolved reactive phosphorus (DRP) levels are *ca.* 1 mg m^{-3} (White and Payne 1977). These concentrations are well below threshold limiting concentrations for growth of the plant of 45 mg m^{-3} DIN and 6 mg m^{-3} DRP and even below the Half Saturation Constants (K_s) of 18 and 2 for NO_3-N and DRP (Dr. I. Hawes, unpublished data).

An abiotic barrier may also be the reason why coontail has also never proliferated in oligotrophic Lake Taupo. For instance, the first patch of coontail to invade the northern end of the lake was mapped in a depth of 9 m adjacent to a boat ramp in 1981. Ten years later this small patch was still present as the only colony and has not extended in area.

The first submerged invader to have a major impact in Lake Taupo was elodea in the 1950's. This plant occupied sheltered sites throughout the lake in the depth zone of 1 to 11 m impacting on the native flora of that depth zone. In the 1960's elodea was replaced by lagarosiphon, an exotic species from Southern Africa. This pattern of invasion has been typical of a large number of oligo- and mesotrophic New Zealand lakes. In the last two decades coontail has successfully competed with lagarosiphon in all nutrient rich lakes, and in the last decade egeria has grown at the expense of all the other adventive submerged weeds in meso-eutrophic conditions. However, the last two species have not been as successful in oligotrophic conditions. A schematic diagram of invader sequences in oligotrophic vs meso-eutrophic North Island lakes (Figure 1) shows quite different sequences which relate to species specific abiotic barriers to the invasions.

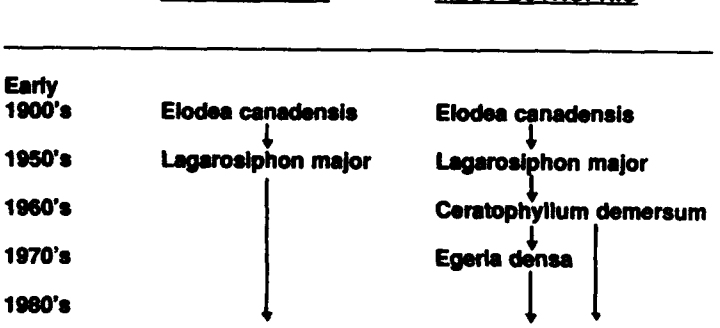

Figure 1. Sequence of dominant aquatic weed species in the invasions of oligotrophic and meso-eutrophic lakes of New Zealand's North Island.

While there may be a relationship of dominant species with eutrophication, there is no relationship between the biomass of submerged species and lake trophic status (Johnstone *et al.* 1985, Howard-Williams *et al.* 1987). In fact, the evidence indicates that submerged weed biomass declines when strongly eutrophic conditions persist. The factor which controls the upper limit of biomass in oligotrophic and mesotrophic lakes is wind and consequent wave action. An excellent example is provided by Lake Taupo which is a large lake with fetches of almost 40 km, but with many sheltered bays. The impact of wave action can be assessed from calculations of effective fetch (U.S. Army Corps of Engineers 1977). A plot of lagarosiphon height *vs* fetch (Figure 2) for Lake Taupo shows that the upper limits of height decline steadily with increasing fetch. Biomass data (Howard-Williams and Davies 1988) show a similar trend. The highest values for mean stand height (> 1 m) were found where effective fetch was less than 2 km. Nuisance growths only occurred at effective fetches of < 2 km. At fetches in excess of 10 km no lagarosiphon was recorded.

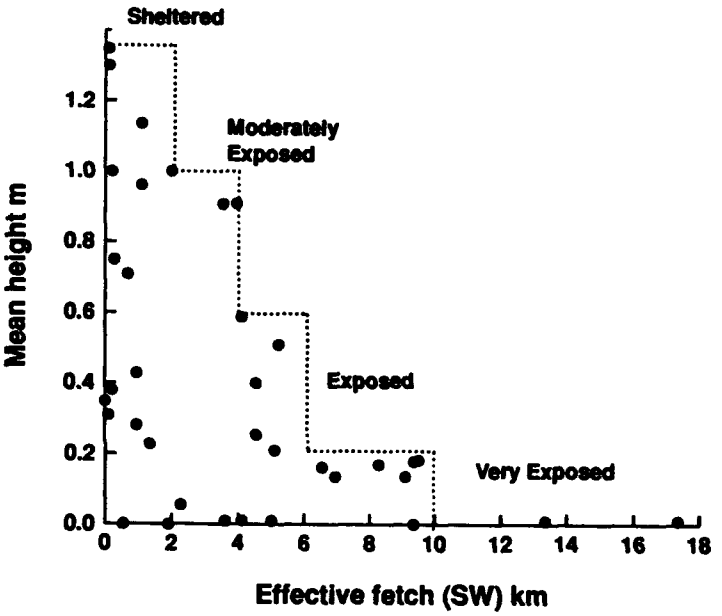

Figure 2. Mean stand height of lagarosiphon in Lake Taupo in a range of effective fetches exposed to the prevailing southwest winds.

BARRIERS TO MOVEMENT

An effective means of transport between lakes is critical if an invading species is to spread from its original infestation site. The Hydrocharitaceae in New Zealand do not produce seed, so dispersal is only by vegetative fragments. There are two aspects which need to be considered for an analysis of between-lake transport: minimum size of a vegetative fragment to be transported, and survival time of a fragment. A vegetative fragment can only germinate if there is a bud. Lateral stem buds were found to be the most resistant to desiccation so the minimum size of stem fragment that would guarantee the presence of a lateral bud can be calculated. The data for the New Zealand Hydrocharitaceae are given in Table 1 and show that for hydrilla and egeria, a stem fragment needs to be over 30 cm in length to ensure the inclusion of a

TABLE 1. MORPHOLOGICAL AND SURVIVAL CHARACTERISTICS FOR DISPERSAL PROPAGULES OF FIVE AQUATIC WEEDS IN NEW ZEALAND. LATERAL BUD FREQUENCY, MINIMUM STEM LENGTH REQUIRED TO ENSURE PRESENCE OF A LATERAL BUD, MINIMUM/FATAL WEIGHT LOSS AND SURVIVAL TIME. (DATA FROM JOHNSTONE *et al.* 1985.)

Species	Nodes/bud	Mean internode length (mm)	Minimum stem length (mm) to include one bud	Minimum fatal weight loss (%)	Time in air to reach fatal weight loss (hours)
L. major	7	34	238	70	20
E. canadensis	7	7	49	56	9
C. demersum	1	59	59	74	36
H. verticillata	5	65	325	46	5
E. densa	11	32	352	65	10

lateral bud (Table 1). Hydrilla is the most sensitive species to desiccation, with a time of only 5 hr before minimum fatal weight loss occurs.

Interlake movement of boats has been implicated almost exclusively in the transfer of aquatic weeds in New Zealand. For instance, in an analysis of 88 lakes in the North Island (Johnstone *et al.* 1985) there were 38 lakes which did not have adventive Hydrocharitaceae or coontail. In 27 of these there was no boating or fishing. Boating and/or fishing took place in the remaining 61 lakes, of which in 50 of these there was at least one of the submerged adventives. The presence of these weeds was significantly ($P < 0.01$) related to human activity (Table 2). In addition to boating and fishing, aquatic weeds have been transferred by float plane and even by weed harvesters! Water birds have not been shown to be effective vectors for the transfer of viable vegetative fragments of vascular plants. All this is of particular significance to the invasion of New Zealand by hydrilla.

TABLE 2. THE RELATIONSHIP BETWEEN THE PRESENCE OF ANY OF FIVE ADVENTIVE WEED SPECIES AND HUMAN ACTIVITY IN 88 LAKES (FROM JOHNSTONE *et al.* 1985).

Boating and/or fishing activity	Total number of lakes	Weed presence	
		Present	Absent
Absent	27	0	27
Present	61	50	11
Total	88	50	38

The dense canopy production by hydrilla, its tolerance of a wide range of habitat conditions (Bowes *et al.* 1977) and its prolific reproductive capacity have allowed hydrilla to displace not only native plants in many countries but even compete successfully with large adventive relatives such as egeria (de Kozlowski 1991) and vallisneria (Haller and Sutton 1975). Hydrilla is not a major problem in New Zealand at present as it is restricted to a small group of four lakes remote from other water bodies of the North Island. It has been there since at least 1963 (Clayton *et al.* 1992), and has not spread. If boats are the principal means of interlake transport, then the sensitivity of the plant to desiccation and the remoteness of the four lakes where it occurs from other water bodies (*ca.* 120 km) means that the chances of its accidental spread are very low (Figure 3).

While large vegetative fragments of vascular aquatic weeds are required for dispersal, this is not the case with algae such as waternet, where even a few cells may be sufficient for interlake dispersal. Procedures for cleaning a large commercial aquatic weed harvester which had been working in a waternet-infested lake provide an example of the ease with which filamentous algae can be transported. The cleaning procedure adopted before the harvester could be transported to another catchment was as follows: thorough water blasting at the haul-out boat ramp with high pressure hoses, followed by a spray of the whole machine with 56 L of quaternary ammonium algicide (QAC) applied by a hand pressure pump with flexible nozzle to reach into all visible cracks and under the machine conveyors. The harvester was then transported to the new location, but a four-day drying out was requested and then a final check before launching. After all this, viable fragments of the weed were found under a conveyor. The machine had to be resprayed with QAC again before the harvester could be used.

EFFECT ON NATIVE SPECIES

In almost every instance where an invasion has occurred the native vegetation has declined markedly. New Zealand has no native canopy-forming submerged aquatic plants. Canopy formations are a characteristic of the Hydrocharitaceae which have successfully dominated in almost every lake they have invaded. Closed stands of 3 to 5 m in height are formed by lagarosiphon and elodea in sheltered areas and stands up to 5 m have been recorded for egeria.

It is generally recognized that there are three "typical" native community types in a depth transect of New Zealand lakes: a low mixed community in the upper littoral zone, a

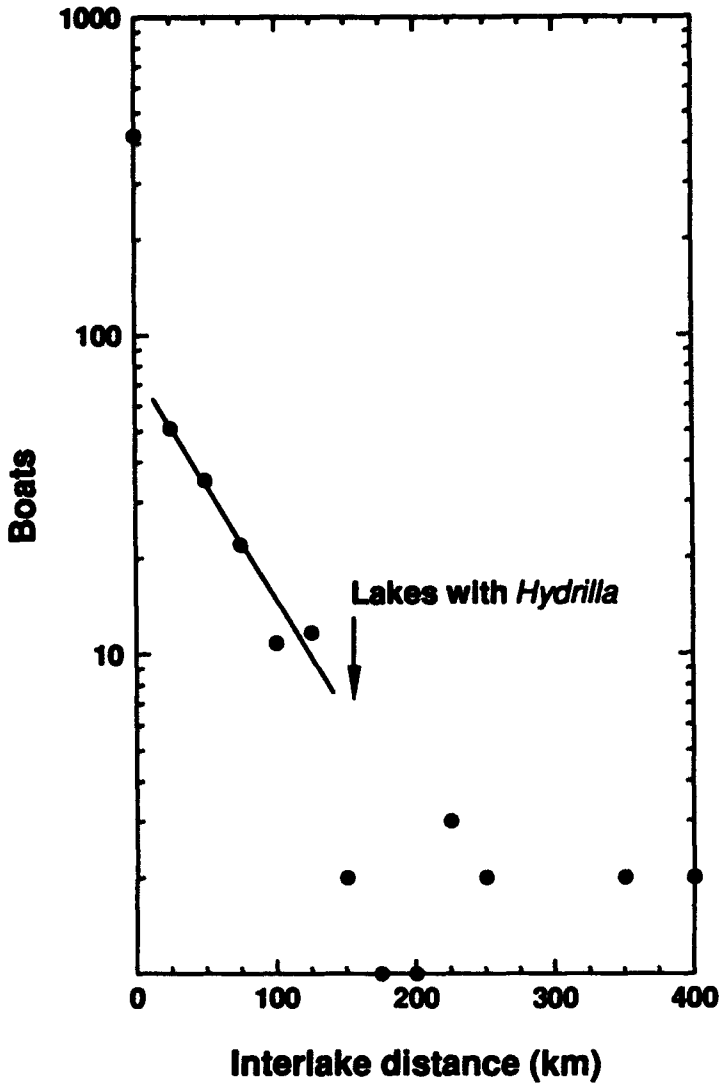

Figure 3. Frequency distribution of interlake distances traveled by boats in New Zealand (n = 564) based on boat ramp surveys. Note: only 14 (2.5%) had come from a lake >125 km distant and only a small proportion of those are likely to carry any weed away from the lakes. (Data from Johnstone *et al.* 1985.)

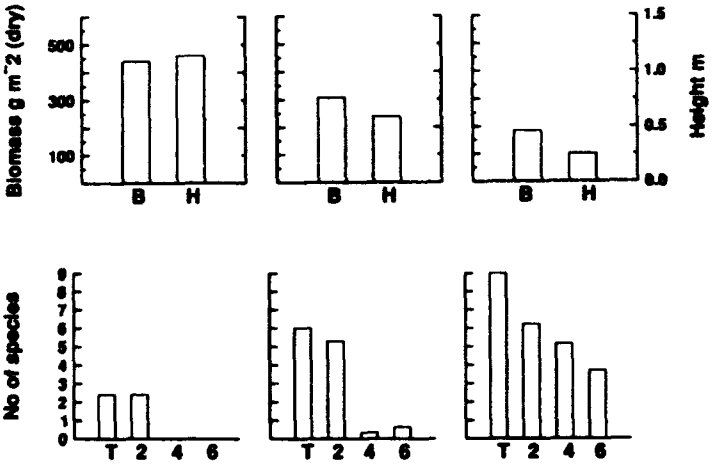

Figure 4. Relationship between biomass (B) and height(H) of the aquatic weed lagarosiphon (top) and the number of native species (bottom) at 2-, 4- and 6-m depth. T = total number of species recorded over all depths. (Data from Howard-Williams and Davies 1988.)

tall vascular community in the mid-depth range (2 to 8 m) and a characean meadow community to the bottom limit of the littoral zone. Invasion by canopy-forming plants has had a major impact on the tall vascular community. This can be shown quantitatively for data from Lake Taupo by plotting the relationships of number of native species *vs* lagarosiphon height and biomass (Figure 4). As lagarosiphon biomass and height increase, the number of native species declines, and especially so in the 4-m depth zone which is the optimal depth for the tall vascular community.

If the native community is dense, why should it not form a biotic barrier to invasion? Madsen *et al.* (1991) described how Eurasian water-milfoil (*Myriophyllum spicatum* L.) in Lake George (NY) was able to invade healthy native communities in the absence of disturbance. This is also frequently the case with lagarosiphon invasions in New Zealand with the general pattern described as follows: A viable shoot of lagarosiphon (with lateral buds) may settle on a short 'low mixed community' some 15 to 150 cm tall in shallow water (*e.g.* 2 m). Long roots grow to the sediment, bypassing the short-stature native plants. Once the invader begins to grow, side branches fall and produce more roots (*e.g.* Coffey 1980) producing small clumps of lagarisophon. This is known as 'guerilla' invasion (*sensu* Harper and Bell 1979). If these clumps coalesce a wall or 'phalanx' invasion occurs with a downward extension of a tall mass of lagarosiphon smothering the native community. Where wind fetches exceed 4 km (moderate fetch) shoots do not grow tall enough to form a phalanx and lagarosiphon and native species may co-exist.

REVERSING AN INVASION

Aquatic weed control can reverse the invasion sequence and there are examples where this has occurred. In Hamilton Lake (NZ) selective herbicide treatments with gel-formulated diquat (Clayton and Tanner 1988) were successful in removing lagarosiphon and egeria and consequently maintaining a bottom cover of desirable characeae.

Mechanical harvesting trials in Lake Aratiatia, an oligotrophic New Zealand hydro-lake, illustrate this process. Figure 5 compares a control (unharvested) site and a harvested site in a lagarosiphon-dominated section of the lake. Regrowth after harvesting was patchy and slow, and also quite substantial natural collapses in unharvested areas occurred,

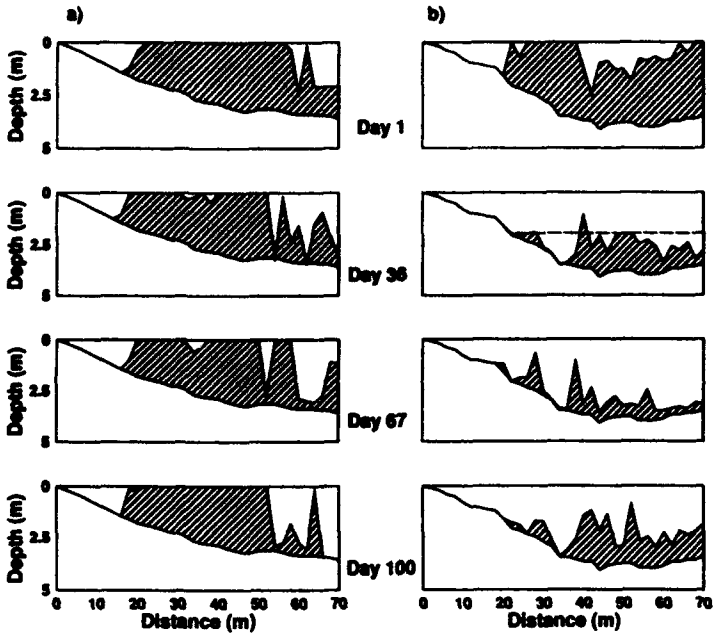

Figure 5. Profiles of weed bed height in two sites in Lake Aratiatia before harvesting (day 1) and after 36, 67 and 100 days harvesting. (a) Control site, unharvested. (b) Site cut by mechanical harvester. Dotted line = depth of cut.

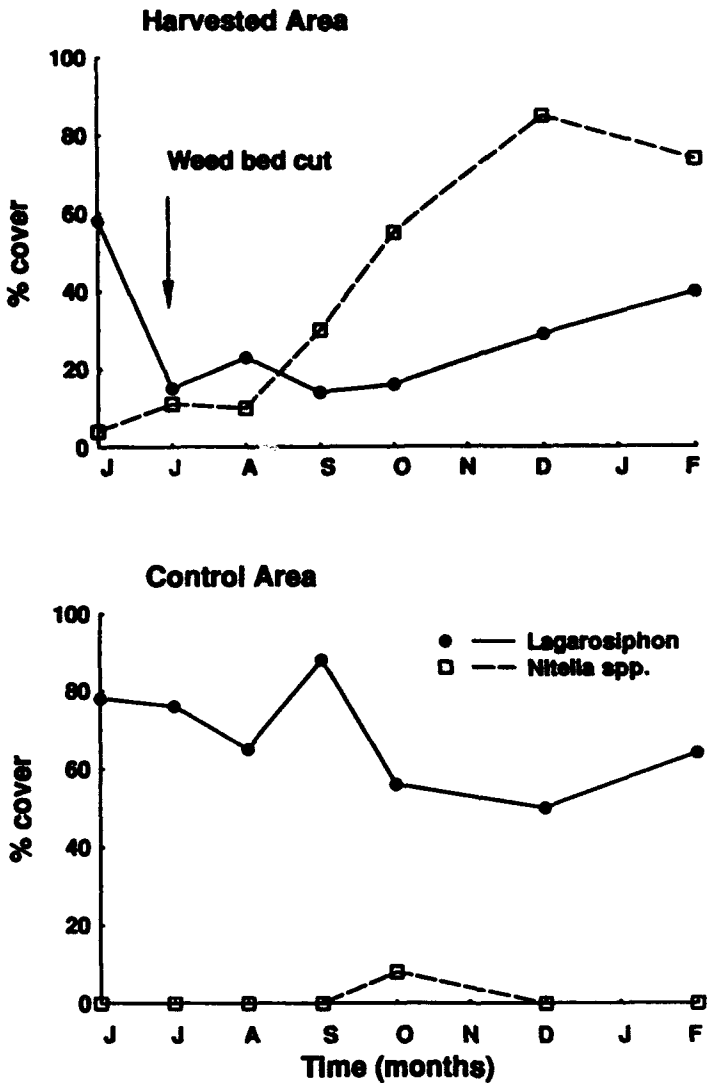

Figure 6. Percent cover of lagarosiphon and native characeae over time in a harvested and non-harvested control area in Lake Aratiatia.

reflecting natural cycles in growth. Patchy regrowth in the harvested area resulted in a rapid recolonization of sections of the transect by native characeae (*Nitella* spp) as illustrated in Figure 6.

Submerged aquatic weeds in particular are prone to marked natural fluctuations in biomass (Loucks *et al.* 1979), and reports in the literature show that such cycles in density can occur over short time scales (weeks), seasonal time scales or years.

In the now classic studies on the Eurasian water-milfoil invasion of Lake Wingra in the 1970s, Carpenter (1979) pointed out that following the natural collapse of the milfoil populations, two of the few remaining robust stands of the weed were those which were regularly harvested and conversely many of the larger stands which collapsed had never been treated with either herbicides or harvested.

The question we need to ask is "How long after an invasion can we afford to wait before being certain that the invader and its problems are here to stay, and that we need to spend big money on its control?" After all, watercress in New Zealand changed from a serious problem weed to a beneficial species.

David Sutton, in his presidential address to this society's 30th Annual Meeting in 1991 stated, "Unless we understand better the factors that contribute to causing exotic plants to get out of hand in the first place, there is little likelihood that we are going to make much progress on eliminating or reducing some of our major weed problems."

In this context the example of the recent invasion of waternet to lakes in New Zealand is a good example. Why have native filamentous green algae such as *Cladophora* and *Enteromorpha*, which naturally occur in Lake Rotorua, not proliferated to the same extent as waternet in that lake. Both these genera cause problems elsewhere but the success of waternet at the expense of the other potential competitors is a salutary reminder that we still do not fully understand species specific events of this nature.

ACKNOWLEDGMENTS

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PHYSIOLOGY

Oxygen Exchange by Entire Root Systems of *Cyperus involucratus* and *Eleocharis sphacelata.*

BRIAN K. SORRELL,[1] H. BRIX[2] AND P.T. ORR[3]

ABSTRACT

Net oxygen exchange between entire root systems of the sedges *Cyperus involucratus* Rottb. and *Eleocharis sphacelata* R. Br. was measured in a bi-compartment apparatus, fitted with a polarographic oxygen electrode and a platinum wire electrode in the root chamber. The roots of both species consumed oxygen from water in the root chamber, with no net exchange when the oxygen partial pressure (pO_2) in the chamber was zero. Rates of oxygen uptake by roots of intact plants were always lower than those of excised roots, suggesting a contribution by oxygen transport from the shoots to the root respiratory demand. The contribution of oxygen transported from the shoots increased with diminishing pO_2 in the root medium, approaching the total oxygen demand as pO_2 fell to zero. The roots released oxygen when titanium (III) citrate redox buffer (E_H = -350 mV) was used in the root chamber to mimic the redox potential of natural sediments. Rates of oxygen release into the reduced solutions were 21 ± 5 and 55 ± 7 μmol O_2 hr^{-1} g^{-1} root dry weight from *C. involucratus*and *E. sphacelata*, respectively, in the light, and 16 ± 3 and 9 ± 3 μmol O_2 hr^{-1} g^{-1} root dry weight in the dark (mean values ± 1 standard deviation). These results suggest that an agitated body of water alone is not a suitable medium for measuring root oxygen release by entire root systems. A solution with a high oxygen demand is more appropriate.

Key words: rhizosphere oxidation, waterlogging, redox, respiration, lacunar transport.

INTRODUCTION

Oxygen release from roots, as a consequence of internal gas transport in the lacunar system, has been documented for many aquatic plant species (Armstrong 1982). Root oxygen release establishes an oxidized rhizosphere, which may reduce the assimilation of reduced phytotoxins by roots (Reddy *et al.* 1989). Information on oxygen transport by aquatic plants is of interest for their management in natural wetlands, where their ability to aerate underground organs and oxidize the rhizosphere is essential for growth in deep water and reducing sediments (Armstrong 1982, Chen and Barko 1988). Root oxygen release is also an important process in constructed wetlands used for wastewater treatment, as it apparently enhances nitrification and reduces the biological and chemical oxygen demand in effluents (Reddy *et al.* 1989).

Plants differ considerably in the ability to oxidize their rhizospheres. Only some aquatic species are capable of significantly increasing the sediment redox potential above that of adjacent unvegetated sites (Chen and Barko 1988, Boon and Sorrell 1991). These differences between species must be largely due to the density and biomass of roots produced, since rates of oxygen release by individual roots are remarkably similar across a wide range of submerged and emergent taxa (Sorrell and Dromgoole 1987). Oxygen release from single roots can be estimated *in vitro* (Armstrong 1982, Caffrey and Kemp 1991). However, it is difficult to measure the total oxygen exchange by root systems of large plants, which may be more relevant to rhizosphere oxidation *in situ*. The large volumes of solution needed to immerse whole root systems in oxygen exchange chambers can dilute gas exchange processes. Furthermore, the redox potential (E_H) of wetland sediments is much lower than that of anoxic water (DeLaune *et al.* 1990).

In this study, we measured oxygen exchange by entire root systems, using an *in vitro* method that mimics reducing sediments. Our objectives were (i) to measure the net oxygen exchange by entire root systems, by using traditional polarographic methods in oxygen-depleted water; (ii) to measure the net oxygen exchange in a reduced redox buffer (DeLaune et al. 1990), and compare this with results obtained in water; and (iii) to use these methods to compare root oxygen exchange between two emergent plants. *Cyperus involucratus* Rottb. is

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a marginal species, while *Eleocharis sphacelata* R. Br. grows in water up to 2 m deep.

MATERIALS AND METHODS

Plant material. Seeds of *C. involucratus* and *E. sphacelata* were germinated in sand trays in a mist propagator. Seedlings were grown in waterlogged sand in individual polyethylene bags, and sprayed weekly with a commercial nutrient solution (Aquasol, Hortico, Sydney) until they were 4 to 6 months old. Plants were removed from the bags and used in experiments when the shoots were about 300 mm long, with mature root systems but only short young rhizomes. There were 50 to 70 adventitious roots on each plant, up to 120 mm in length, with frequent development of lateral roots < 25 mm long. Any broken, bent or dead roots were removed. The volume of roots was always < 15% of the root chamber described below.

Experimental chamber. We measured root oxygen exchange by enclosing intact plants in an acrylic bi-compartment apparatus, with the shoots and roots isolated in separate chambers. Air was pumped continuously (1 L min^{-1}) through the shoot chamber (length 0.8 m, diameter 0.1 m) and mixed with a small electric fan. The root chamber (length 0.17 m, diameter 0.07 m) was covered with foil to prevent light penetration. The total volume of distilled water in this chamber, including a side-arm for the electrodes (see below), was 744 ml. It was cooled by a thermostatically controlled 20C water jacket. The two chambers were separated by an opaque PVC plate which prevented light penetrating into the root chamber. The plants were held in position by sealing the root-shoot junction in the plate with a flexible sealing compound ("Blu-Tak"). The seal was made gas-tight with a thin film of petroleum jelly, and a 5-mm layer of water. In preliminary tests we found no oxygen leakage through this seal into de-oxygenated water in the root chamber.

The oxygen partial pressure (pO_2) in the root chamber was measured with a Clark-type oxygen electrode (Orion 97-08) and recorded on a strip-chart recorder. The electrode was calibrated in the chamber in air-saturated and de-oxygenated water. A magnetically coupled impeller provided rapid mixing in the chamber and a flow-independent electrode response. A mesh barrier above the impeller prevented it from damaging the roots. The temperature in the root chamber was monitored with a temperature probe. A circular bank of twelve 36-W fluorescent tubes and two 60-W incandescent bulbs gave a photon irradiance within the shoot chamber of 220 µmol m^{-2} s^{-1} (PAR). The root chamber was shielded from the lights with an aluminum foil cover, painted black on the inside. With the lights on, the temperature in the root chamber remained stable at 23 ± 0.5C. For dark treatments, the shoot chamber was covered with a black cloth and foil, leaving the lights on to minimize temperature changes.

Oxygen exchange experiments. Experiments were begun with either pO_2 = 20 kPa or 1 kPa in the root chamber. In some experiments, the air in the darkened shoot chamber was replaced with oxygen-free nitrogen, to demonstrate the role of oxygen transport in root oxygen exchange. At the end of experiments, the roots were excised and their oxygen uptake rates measured in the closed root chamber. Dry weights of roots were measured after drying for 24 hr at 70C. Rates of oxygen exchange were calculated by differentiation of the slope of the recorder trace (Sorrell and Dromgoole 1987), after data were transferred to a spreadsheet file, to enable smooth curves of the oxygen responses to be produced.

In a preliminary series of experiments, the significance of epiphytic microbial respiration on the measured oxygen exchange was tested. There was no measurable effect on the rates of root oxygen exchange when antibiotics (20 mg L^{-1} nalidixic acid and streptomycin) were added to the chamber. Hence, microbial growth on the roots of these plants is an insignificant component of their oxygen exchange.

Effects on redox conditions. We measured oxygen transport in reducing conditions by adjusting the redox potential (E_H) in the root chamber with titanium (III) citrate buffer. Ti^{3+} is a nonphytotoxic reductant, and the buffer was prepared as described by DeLaune *et al.* (1990), except that we used 1.0 M Tris buffer to maintain pH 6.5 in the chamber, rather than saturated sodium carbonate. Titanium (III) citrate was injected into the chamber through a self-sealing septum. E_H was measured with a platinum wire electrode, which was cleaned and calibrated as described elsewhere (Boon and Sorrell 1991). The reference electrode was a saturated calomel electrode (Radiometer K4040), and 244 mV was added to readings to give E_H values. Readings were not corrected for pH and temperature differences between runs, as these were not significant (< 0.1 pH units and < 0.5C). E_H was +420 mV in air-saturated water, and +360 mV in de-oxygenated water. The Ti^{3+} buffer reduced E_H to < -300 mV at the beginning of each experiment.

Since the oxidation of Ti^{3+} is stoichiometric (DeLaune *et al.* 1990), rates of oxygen release by the roots can be calculated from the rate of change of E_H. We established the relationship between E_H and oxygen release by titrating Ti^{3+} solutions in the chamber with oxygen-saturated water.

RESULTS AND DISCUSSION

Oxygen exchange. Time courses of pO_2 changes in the chamber show that the root systems of intact plants consumed oxygen from the root chamber (Figure 1) until the water was totally oxygen-depleted. Net oxygen uptake by the roots was

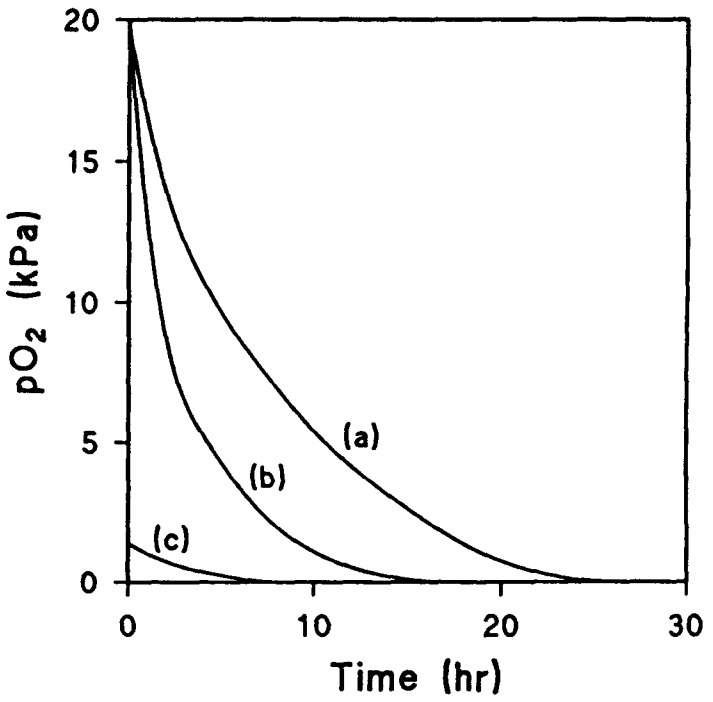

Figure 1. Chart traces of oxygen partial pressure (pO_2) in the root solution showing oxygen uptake by the roots of a *Cyperus involucratus* plant. (a) Intact plant, shoots in the dark with air in the shoot chamber; (b) Excised roots of the same plant sealed in closed root chamber; (c) As for (a), but initial pO_2 = 1 kPa in the root chamber. Root dry weight: 0.866 g.

evident for both species, irrespective of whether the shoot chamber was illuminated or darkened, or contained air or nitrogen. The rate of oxygen depletion was always lower for roots of intact plants than for the same root system when excised (Figure 1). In addition, root oxygen uptake by intact plants from the root chamber was lower when the shoot chamber contained air than when it contained nitrogen (Figure 2A, B), and was higher when the shoots were darkened than when they were illuminated (Figure 2C, D). It is clear that some of the oxygen consumed by the roots comes from the shoots. These data are all consistent with a light-enhanced oxygen transport from shoots to roots contributing oxygen to the respiratory demand of the roots.

The saturating pO_2 values for oxygen uptake were highly variable; many responses were not fully saturated over the range of pO_2 values examined (Figure 2). The contribution of lacunar oxygen transport from the shoot to the root oxygen demand varied with the pO_2 in the root chamber. Plots of the fraction of the root oxygen demand satisfied by oxygen transport from the shoot against pO_2 (Figure 2) demonstrate that the contribution of oxygen transport to the root oxygen demand increases with decreasing pO_2 in the root chamber. At pO_2 = 10 kPa, the fractions were often < 0.5, but they increased steadily to 1.0 as pO_2 approached zero. The root systems never had a net oxygen release, and hence oxygen transport from shoots to roots can only be inferred indirectly from oxygen exchange measurements in the root chamber, especially at $pO_2 = 0$. Similar results have been obtained for other emergent macrophytes when oxygen transport is measured in oxygen-depleted water (Bedford *et al.* 1991). Both plant species responded similarly to light, dark, root excision and shoot nitrogen treatments.

When attempting to measure root oxygen release, one must recognize that the immersion of entire root systems in large volumes of water does not closely mimic their behavior in nature. The roots will be supported by axial oxygen transport during growth in waterlogged substrates, but may be subject to quite different radial gradients once plants are transferred to experimental chambers. The consequence of this initial growth of roots beyond the length that internal transport can support in the chamber will be an excess of root oxygen demand over supply, and hence failure to detect any root oxygen release. Such problems are evident in other studies where entire root systems are bathed in large volumes of water or nutrient solution (*e.g.* Bedford *et al.* 1991). Even if some roots are adequately aerated and release oxygen into

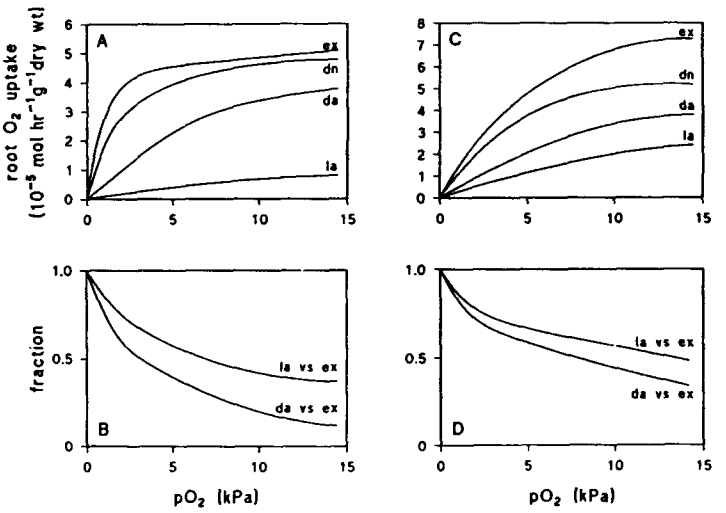

Figure 2. Examples of the response of root oxygen uptake rate to oxygen partial pressure (pO_2) in the root solution. Each graph presents curves derived from continuous oxygen electrode traces obtained from a single plant: A. *Cyperus involucratus*, root oxygen uptake response to pO_2; B. Fractions of root oxygen demand in A satisfied by oxygen transport from the aerial tissues; C. *Eleocharis sphacelata*, root oxygen uptake response to pO_2; D. Fractions of root oxygen demand in C satisfied by oxygen transport from the aerial tissues. Key: ex = oxygen uptake by excised roots; dn = root oxygen uptake with shoots in N_2 in the dark; da = root oxygen uptake with shoots in air in the dark; la = root oxygen uptake with shoots in air in the light; la vs ex = root oxygen uptake with shoots in air in the light (la) as a fraction of oxygen uptake by excised roots (ex); da vs ex = root oxygen uptake with shoots in air in the dark (da) as a fraction of oxygen uptake by excised roots (ex).

the water, dissolved oxygen in the root chamber is more readily available to respiring root tissue than the gaseous oxygen in the shoot chamber, and any oxygen released from the permeable root surfaces would be quickly resorbed.

Therefore, it is doubtful that low oxygen release rates are characterisitic of roots in the field. Rates of oxygen transport and root oxygen release are stimulated by increasing external oxygen demand (Caffrey and Kemp 1991). Hence, we believe that realistic estimates of root oxygen release, and by implication internal oxygen transport, are unlikely to be obtained by experimental designs in which large amounts of root tissue are enclosed in oxygen-depleted water. Such designs differ greatly from studies where oxygen transport is measured on single roots with sleeving microelectrodes or in small-volume chambers (Armstrong 1982, Sorrell and Dromgoole 1987, Caffrey and Kemp 1991). In such cases the re-absorption problem is avoided, and the oxygen exchange rates are quite similar to those that occur *in situ* (Caffrey and Kemp 1991).

Ti^{3+} oxidation. In these experiments, the root medium was titanium (III) citrate buffer, which has a high oxygen demand and provided E_H values as low as those that occur naturally in sediments. These E_H values remained stable during control experiments without plants in the chamber. Excised root systems of both species lowered E_H to a minimum of approximately -400 mV (Figure 3). This can only be explained by the generation of a reductant, presumably ethanol, by the excised roots. In contrast, the roots of intact plants raised E_H. For *C. involucratus*, the rate of increase was only marginally greater in the light than in the dark, whereas rates of increase were much more light-dependent in *E. sphacelata*. With nitrogen in the shoot chamber, the roots of both species failed to raise E_H. Data on rates of oxygen release into these solutions (Table 1) emphasize (i) that roots of intact plants raise E_H, whereas excised roots or roots of plants with shoots in nitrogen reduce E_H, (ii) that the rates of root oxygen release by *C. involucratus* are scarcely affected by light/dark treatments on the shoots, (iii) that root oxygen release by *E. sphacelata* is much higher in the light than in the dark. We conclude from (iii) that there is a mechanism accelerating root oxygen release by *E. sphacelata* in the light. Possible explanations include differences in stomatal behavior between the species, or that internal pressurization and mass flow of gases in the shoots, which is significant in *E. sphacelata* but not in *C. involucratus* (Brix *et al.* 1992), increases the pO_2 at the rhizome-root junctions of *E. sphacelata*.

While oxygen transport in the shoots and rhizomes of emergent aquatic plants can occur by pressurized flow (Brix *et al.* 1992), diffusion is the only significant mechanism

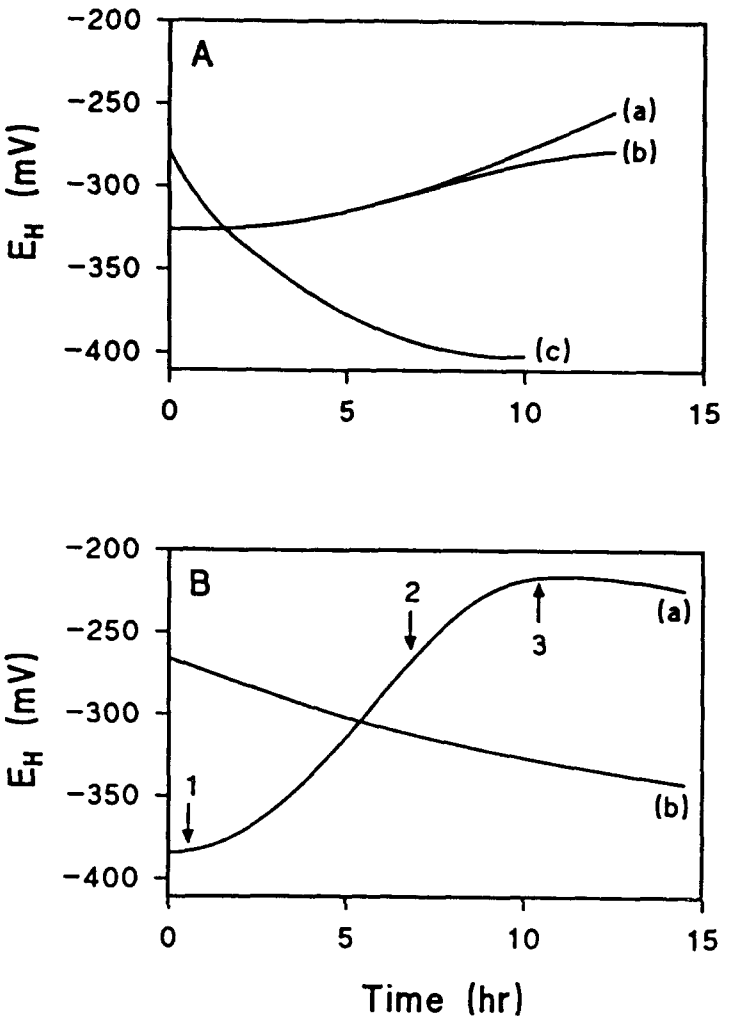

Figure 3. Examples of chart traces showing effect of root oxygen exchange on redox potential (E_H) in titanium citrate buffer. A. *Cyperus involucratus:* (a) shoots in the light; (b) shoots in the dark; (c) excised root system. B. *Eleocharis sphacelata:* (a) intact plant; (b) excised root system. Treatments: (1) Shoots in the light; (2) Shoots in the dark, and (3) nitrogen in the shoot chamber.

TABLE 1. RATES OF OXYGEN RELEASE BY ROOTS OF *CYPERUS INVOLUCRATUS* AND *ELEOCHARIS SPHACELATA* INTO TITANIUM CITRATE BUFFER AT E_H = -350 mV. RATES ARE GIVEN FOR EXCISED ROOTS AND ROOTS WITH THE AERIAL TISSUES IN THE DARK AND LIGHT, RESPECTIVELY. DATA ARE MEANS ± 1 STANDARD DEVIATION (n=3).

Sedge	Excised	Dark	Light
Cyperus involucratus	-12 ± 2	16 ± 3	21 ± 5
Eleocharis sphacelata	-37 ± 5	9 ± 3	55 ± 7

Rates are μmol O_2 hr^{-1} g^{-1} root dry weight.

within roots and into the rhizosphere (Beckett *et al.* 1988). The rate of transport within the roots and across the root wall depends upon the relative resistances of radial and axial pathways, as well as the pO_2 gradients, which can be modified by the external oxygen demand (Armstrong *et al.* 1990). When titanium citrate is used the oxygen released by aerobic parts of the root system can be detected, because Ti^{3+} oxidation is not easily reversible. This explains the release of oxygen from entire root systems of plants into titanium (III) citrate buffer, but not into oxygen-depleted water. Rates of oxygen transport and release by plants are therefore not fixed, but vary with external pO_2 and oxygen demand (Sorrell and Dromgoole 1987, Caffrey and Kemp 1991). Low rates of release into water are evident in recent data from Bedford *et al.* (1991), who found < 2.5 μmol O_2 hr^{-1} $plant^{-1}$ released by the roots of several wetland species. In contrast, *E. sphacelata* and *C. involucratus* released no oxygen into water, but their rates of release into titanium citrate (Table 1) were as high as 50 μmol O_2 hr^{-1} $plant^{-1}$ (*E. sphacelata* in the light).

Internal oxygen transport in aquatic plants allows aerobic metabolism and growth of roots in reducing sediments. Most species also release a substantial amount of oxygen from the roots, and significant sediment oxidation has been unequivocally demonstrated in several studies for species with high root biomass (Tessenow and Baynes 1975, Chen and Barko 1988, Boon and Sorrell 1991). The actual rates of oxygen release by roots depend on factors such as plant size and growth stage, season, root length, and temperature (Reddy *et al.* 1989, Caffrey and Kemp 1991). In this study we have demonstrated that root oxygen release by entire root systems is minimized when they are enclosed in solutions with no external oxygen demand. With this experimental design the roots are the only sink, so any oxygen that is released will most likely be resorbed by the respiring root tissue. Oxygen-depleted water is therefore not a suitable medium for estimating root oxygen release by entire root systems; a solution such as the titanium citrate buffer, which to some extent mimics the sediment oxygen demand, is more appropriate.

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Effect of Hexavalent Chromium on Photosynthetic Rates and Petiole Growth in *Nelumbo lutea* Seedlings

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ABSTRACT

Petiolar photosynthetic carbon assimilation (PCA) rates, seedling petiole length, and petiolar elongation rates were measured in *Nelumbo lutea* (Willd.) Pers. (American lotus) grown in aseptic liquid culture at pH 5.6 and 8.2 and exposed for 4, 48 and 96 hr to hexavalent chromium in initial concentrations varying from 0.5 to 100 mg/L. Within several hours considerable chromium partitioned into a phase removable from solution by filtration through 0.6-µm Nuclepore filtration. Exposure for 48 hr to soluble chromium levels as low as 0.02 mg/l in pH 5.6 media significantly stimulated PCA rates, but at pH 8.2 only plants exposed to the highest chromium concentrations for 96 hr exhibited enhanced PCA rates. The solubilizer method used for routine PCA analyses produced values that markedly overestimated net carbon fixation. Soluble hexavalent chromium concentrations of as little as 0.6 mg/l in growth media reduced mean petiole lengths in 48- and 96-hr experiments. Petiolar elongation rates over the course of 48- and 96-hr exposure experiments conducted at pH 5.6 and pH 8.2 were dose-specifically reduced by 0.1 to 4.7 mg/l of soluble chromium. In seedlings exposed for 96 hr to an initial chromium dose of 100 mg/l (3.4 mg/l as soluble chromium at T96 hr), petiole elongation was reduced to approximately 10% of zero-added-chromium control rates.

Key words: aquatic macrophyte, heavy metal, toxicity, bioassay.

INTRODUCTION

Chromium concentrations in aquatic systems receiving industrial wastewaters can reach levels of 1 to 20 mg/l, a concentration commensurate with LC50 and EC50 values in bioassay systems developed for fish, fathead minnow, aquatic invertebrate, and algal species (reviewed by Staves and Knaus 1985 and Guilizzoni 1991). Few studies have focused on the short-term (*i.e.*, a few hours to days) effects of chromium exposure on aquatic vascular plant ecophysiology.

Guilizzoni *et al.* (1984) found that chromium enhanced shoot growth in *Myriophyllum spicatum* up to a medium concentration of 50 µg/l. Higher concentrations of up to 1 mg/l caused an almost linear reduction in shoot length and weight and photosynthetic rates. Chromium concentrations exceeding 1.0 mg/l resulted in 8-day growth rate reductions in the duckweed genera *Lemna* and *Spirodela* (Staves and Knaus 1985).

Several studies examined leaf disk/shoot section bioassays as alternatives to whole-plant systems for evaluating responses of submerged macrophytes to environmental stimuli. Beer and Wetzel (1981) and Beer *et al.* (1982) developed a ^{14}C-photosynthetic carbon assimilation (PCA) rate assay to investigate carbon uptake and fixation dynamics in submerged macrophytes, including the bulrush *Scirpus subterminalis* Torr. Porter and Francko (1991) used leaf disks from *Potamogeton amplifolius* Tuckerm. to investigate the effect of chromium and copper on short-term (minutes to hours) PCA rates.

Francko (1986a,b) reported a liquid culture technique for germination and axenic cultivation of *Nelumbo lutea* (Willd.) Pers. (American lotus) seedlings and a petiole section PCA assay modeled after Beer and Wetzel (1981). Lotus petioles contained high concentrations of chlorophyll *a* (*ca.* 3% of fresh weight biomass) and exhibited PCA rates of approximately 500 to 30 µmol C mg^{-1} Chl *a* hr^{-1} over a pH range of 6.5 to 8.5. This PCA rate was comparable to that observed in leaves of bicarbonate-using submerged aquatic angiosperms (reviewed by Spence and Maberly 1985). In further work with lotus seedlings, Al-Hamdani and Francko (1992) characterized petiolar PCA and elongation rates in seedlings exposed to a variety of photon flux densities and temperatures and demonstrated that petiolar PCA may contribute to seedling growth and elongation, even under relatively cool, low- light conditions near the sediment-water interface.

The objectives of this study were: 1) to determine the effects of exposure to hexavalent chromium on PCA rates and growth of lotus seedlings and 2) to assess the potential for using lotus seedlings as a bioassay for chromium in fresh waters.

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MATERIALS AND METHODS

Lotus seeds were collected from plants growing at the margin of Sangre Isle Lake, a small eutrophic reservoir in north-central Oklahoma (Francko 1987). Seeds were surface disinfected using sequential rinses in detergent, 70% ethanol, distilled water, 5% sodium hypochlorite and distilled water, then scarified, and cultured aseptically by the method of Francko (1986a) in modified Medium II (Forsberg 1965) at pH 8.2 with 1.96 μmol l^{-1} total inorganic carbon and double-strength Hepes as a buffer. Alternatively, cultures were maintained at pH 5.6 using Mes in place of Hepes as the buffer system. Neither buffer system alters lotus growth or PCA capacity (Francko 1986b, Al-Hamdani and Francko 1992). Aseptic culture techniques were used to eliminate interference from algal, bacterial or fungal contaminants in subsequent assays. Culture flasks containing 700 ml Medium II and 10 seeds each were incubated in an environmental chamber at 23C, using cool white fluorescent illumination to produce a photon flux density of 100 μmol m^{-2} s^{-1} and a 12-hr photoperiod.

Two petioles of unequal length differentiate upon seed germination (2 to 3 days after placement in liquid media). When the longer petioles bearing the developing floating leaf reached 4 to 5 cm in length, seedlings were withdrawn and randomly placed in fresh Medium II (N = 6 seedlings per flask) containing no added chromium or 0.5, 5, 25, 50 or 100 mg/l of hexavalent chromium (added as potassium dichromate; N = 3 replicates for control and each chromium concentration).

Individual seedlings then were labeled for later identification with a waterproof mark on the seed coat, and the length (cm) of each marked seedling's long petiole was measured. Cultures were placed randomly back into environmental chambers under the conditions described above.

A control culture and one culture at each chromium concentration were randomly withdrawn after 4, 48, and 96 hr of incubation. Seedlings were removed and blotted dry with Kim-Wipes and the length of the longer petiole of each seedling was remeasured. These petioles were then excised for use in PCA assays.

Culture media samples were withdrawn from each flask at each sampling iteration and filtered through 0.6-μm Nuclepore filters. Replicate subsamples of each filtrate were then assayed for soluble chromium via a flame AA technique using EPA reference standards. The analytical error of these measurements was approximately 16% over the range (0.5 to 50 mg/l) of reference standards. All chromium concentrations in this paper represent measured soluble chromium available at the time of assay rather than total chromium added at zero time. For consistency in data presentation, however, data are rank ordered on the basis of chromium added at time zero.

PCA rates were determined by a ^{14}C-bicarbonate assimilation/tissue solubilizer digestion method that facilitates complete digestion of plant material and incorporated radioactivity (Beer *et al.* 1982, Francko 1986b, Al-Hamdani and Francko 1992). After measurement of individual petiole lengths petioles were cut into 1-cm sections (24 ± 0.5 mg fresh wt). In order to assay petioles of relatively uniform diameter, petiole sections within 2 cm of the crown and apical meristem were discarded.

Randomly selected sections (N = 5) were placed in 50-ml glass beakers containing 20 ml of fresh sterile Medium II at either pH 5.6 or pH 8.2. Hexavalent chromium was added at concentrations of 0.5 to 100 mg/l and beakers were placed in a fume hood at the same illumination and temperature conditions noted above. At time zero, 100 μl of a ^{14}C-bicarbonate solution was added to each beaker to yield 1.5 x 10^4 Bq ml^{-1} of total radioactivity. The ^{14}C-addition did not alter significantly the initial inorganic carbon concentration of the media. PCA rates are linear for up to 45 min under these conditions (Francko 1986b).

After 15-min incubation periods, individual petiole sections were withdrawn and rinsed for 15 sec in acidified distilled water to remove unincorporated radioactivity. Petioles were then placed in glass scintillation vials and digested overnight with 0.5 ml of a quaternary ammonium tissue solubilizer (BTS-450, Beckman Co.).

Scintillation cocktail was then added and incorporated radioactivity was assayed by liquid scintillation spectroscopy using an automatic quench control program. Solubilized petiole sections that had not been incubated with radiolabel and sections incubated with radiolabel in total darkness were used to compute background-corrected mean radiolabel incorporation values (N = 5 sections for each treatment). Aliquots of solubilized petiole sections were also used for chlorophyll *a* analyses by a fluorometric method (Francko 1986b). Radiolabel uptake rates were then used to calculate carbon assimilation rates as μmol C mg^{-1} Chl *a* hr^{-1} by the ^{12}C:^{14}C ratio method of Wetzel and Likens (1979).

The PCA method we employed may overestimate net photosynthesis rates since unincorporated ^{14}C that has exchanged with the internal dissolved inorganic carbon pool may not be removed quantitatively by a brief rinse in low-pH water. To determine the size of this error component, we conducted a replicate series of experiments in which sections were incubated at pH 5.6 or 8.2, fixed by immersion into liquid nitrogen, ground in 0.5 N perchloric acid, and then digested with solubilizer. Resultant PCA rates were then compared with those in sections treated only with tissue solubilizer as described earlier.

RESULTS AND DISCUSSION

Data on the relationship between medium chromium content and pH, exposure time period, and PCA rates are shown in Figure 1. At pH 5.6, significant increases in PCA rates ($P < 0.05$; analysis of variance (ANOVA) and Newman-Kuels multiple range test) were noted only in 48-hr exposure experiments, but no clear dose-response relationship could be determined. Plants incubated in media containing 0.02 to 2.2 mg/l of soluble chromium for 48 hr produced statistically similar effects on PCA. PCA rates were also significantly higher than those in chromium-free controls after 96 hr in media containing 0.1 mg/l of soluble chromium, and significantly reduced after 4 hr of incubation in media containing the highest titer of chromium employed.

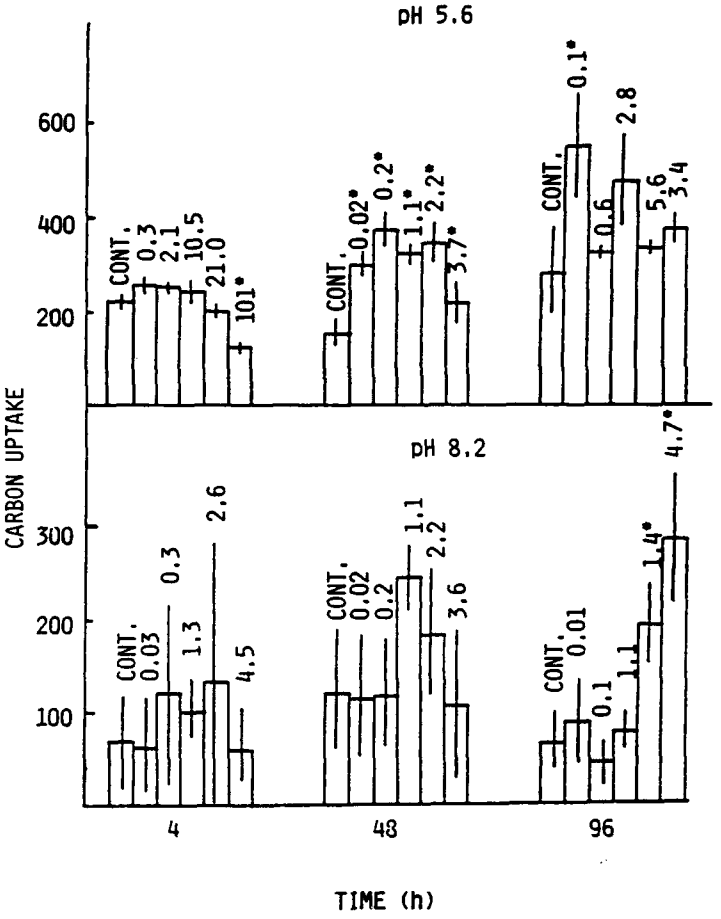

Figure 1. PCA rates in lotus petiole sections as a function of chromium concentration, exposure time, and pH. Chromium values within the bars denote soluble chromium present in culture filtrates at the time of analysis, but histograms are presented in increasing magnitude of chromium addition at time zero. Values shown as mean C-uptake rate (µmol C mg^{-1} Chl *a* hr^{-1}). Error bars denote SE (N = 6 sections per treatment variant). Asterisks denote treatments significantly different ($P < 0.05$; ANOVA and Newman-Kuels multiple range test) from control containing no added chromium (< 4 µg/l chromium present in media lacking additional chromium addition).

In pH 8.2 media, no statistically significant relationships between chromium content and PCA rates were determined in 4- and 48-hr experiments. Sections from plants incubated for 96 hr in media containing 1.4 and 4.7 mg/l of soluble chromium at the time plants were removed and assayed exhibited PCA rates 2- to 3-fold higher than the control value.

The PCA rates we measured in plants from control cultures lacking added chromium agree closely with rates previously reported for lotus seedlings assayed at the same temperature and photon flux density (approximately 220 µmol C assimilated mg^{-1} Chl *a* hr^{-1} at pH 5.6 (Al-Hamdani and Francko 1992) and 60 µmol C assimilated mg^{-1} Chl *a* hr^{-1} at pH 8.2 (Francko 1986b)). However, the data in Table 1 suggest that the method we employed more closely approximates inorganic carbon uptake into the petioles and overestimates carbon fixation rates. PCA rates in petiole sections incubated with radiolabel at pH 5.6 and treated with liquid nitrogen, acid digestion, and basic tissue solubilizer were about 7-fold smaller than PCA rates derived from petioles digested with solubilizer alone. At pH 8.2 the discrepancy between methods was 3- to 4-fold.

TABLE 1. PCA RATES IN *NELUMBO* PETIOLE SECTIONS DIGESTED WITH TISSUE SOLUBILIZER ALONE VERSUS SECTIONS FIXED IN LIQUID NITROGEN (NL_2) GROUND IN 0.5N PERCHLORIC ACID, THEN DIGESTED WITH TISSUE SOLUBILIZER. VALUES SHOWN AS MEAN µmol C fixed mg^{-1} Chl *a* hr^{-1} (SE); N=6.

	pH 5.6 Media		pH 8.2 Media	
	Trial 1	Trial 2	Trial 1	Trial 2
Solubilizer alone	239(10)	211(14)	33(3)	33(3)
LN2, Acid, Solubilizer	37(1)	36(3)	8(2)	10(1)

Accordingly, the collective data in Table 1 and Figure 1 support the view that chromium concentrations between 0.02 and 4.7 mg/l may enhance the rate of carbon uptake into lotus petioles exposed to the metal for 48 or 96 hr. Although heavy metals are typically associated with inhibition of metabolic activity, the apparent PCA stimulation we noted is not without precedent. In addition to the work on chromium and *Myriophyllum* by Guilizzoni *et al.* (1984) described in the Introduction of this paper, Jana and Choudhuri (1981) reported that copper (10 mg/l to 10 g/l) stimulated glycolate metabolism in *Potamogeton pectinatus*. Porter and Francko (1991) found that chromium and copper were capable of stimulating or repressing PCA rates in leaf disks from *Potamogeton amplifolius*, although both metals repressed PCA at or above 0.5 mg/l.

In contrast to the modest stimulatory effect of chromium on PCA, seedling growth and petiolar elongation were more markedly altered by chromium. The mean length of seedling petioles placed into various chromium treatments was statistically similar at the beginning of 48- or 96-hr incubation experiments (Table 2). Plants exposed to the two highest chromium levels for 48 hr at pH 5.6 had significantly shorter petioles than control plants, and petioles were significantly shorter in all but the smallest chromium addition after 96 hr. At pH 8.2, only the highest chromium addition employed yielded a significant decrease in mean petiole length after either 48 hr or 96 hr of exposure.

The rate of petiole elongation (change in length of individual petioles over the incubation period) was strongly and dose-specifically reduced by increasing doses of soluble chromium (Figure 2). After a 48-hr exposure period at pH 5.6, a significant decrease in petiole elongation rate was noted when as little as 0.2 mg/l of soluble chromium was present in media (5 mg/l initial dose). The magnitude of decrease increased up to 3.7 mg/l of soluble chromium (100 mg/l initial dose), at which point petiole elongation was reduced to about 20% of the control rate. A 96-hr exposure period at the same pH reduced the apparent chromium toxicity threshold to 0.1 mg/l and the maximum inhibition to approximately 10% of the control value.

In pH 8.2 media, low doses of chromium were associated with a slight increase in elongation rates after 48 hr; only the highest chromium addition yielded a decrease in elongation.

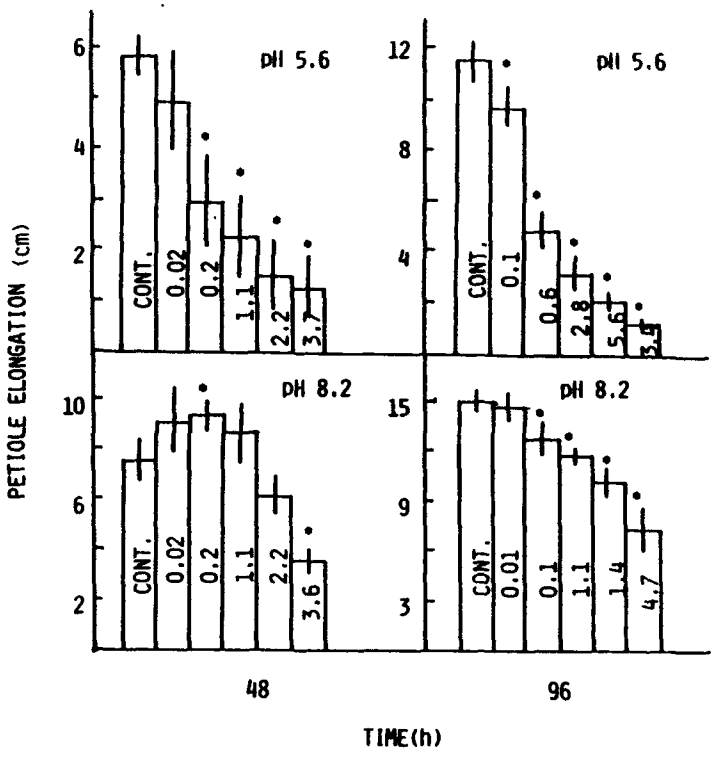

Figure 2. Petiolar elongation rates (change in cm from time zero to time 48 or 96 hr) in lotus seedlings as a function of chromium concentration, exposure time, and pH. Soluble chromium concentrations in growth media at the time of assay shown within histograms. Error bars denote SE (N = 6 seedlings per treatment). Asterisks denote treatment means significantly different from controls ($P < 0.05$; ANOVA and Newman-Kuels multiple range test).

TABLE 2. EFFECT OF 48-HR AND 96-HR CHROMIUM EXPOSURE ON PETIOLAR LENGTH IN *NELUMBO* SEEDLINGS. VALUES SHOWN AS MEAN (SD); N = 6. ASTERISKS DENOTE TREATMENTS SIGNIFICANTLY DIFFERENT FROM ZERO CHROMIUM CONTROL ($P < 0.05$; ANOVA and Newman-Kuels multiple range test).

Chromium (mg/l)	48 hr, Mean Petiole Length (cm)		Chromium (mg/l)	96 hr, Mean Petiole Length (cm)	
	Initial	Final		Initial	Final
			pH 5.6		
0	4.4(1.5)	10.2(1.3)	0	4.0(0.9)	15.4(2.0)
0.02	5.2(0.4)	10.2(2.2)	0.1	4.4(0.5)	14.0(2.5)
0.2	5.0(1.1)	7.9(1.8)	0.6	5.7(0.5)	10.1(2.2)*
1.1	5.6(0.9)	7.8(1.8)	2.8	4.4(1.1)	7.1(2.4)*
2.2	4.5(1.0)	5.9(0.6)*	5.6	5.1(1.1)	7.0(1.6)*
3.7	4.8(1.0)	5.9(1.6)*	3.4	4.7(0.8)	5.9(0.6)*
			pH 8.2		
0	11.1(2.2)	18.4(1.3)	0	11.0(0.9)	25.9(1.6)
0.02	12.2(1.3)	21.1(2.2)	0.01	10.9(2.2)	25.7(2.6)
0.2	11.3(0.9)	20.3(2.2)	0.1	11.8(3.1)	24.2(1.8)
1.1	12.3(3.2)	20.6(4.8)	1.1	10.7(2.5)	22.8(3.6)
2.2	11.7(2.5)	17.7(3.9)	1.4	11.3(2.0)	21.2(3.4)
3.6	10.9(1.3)	14.5(2.3)*	4.7	11.6(1.5)	18.9(4.6)*

After 96 hr of exposure at pH 8.2, elongation rate reductions with increasing soluble chromium resembled pH 5.6 values, although the maximum inhibitory response was considerably smaller.

The collective data suggest that 1) soluble chromium may have a slight stimulatory effect on carbon uptake rates in excised lotus petioles and 2) that the rate of petiole elongation is markedly and dose-specifically reduced by increasing concentrations of soluble chromium. Both effects were more pronounced at pH 5.6 than at pH 8.2.

Although the sensitivity and dose-responsiveness of lotus petiolar elongation support the possible utility of this technique as a bioassay system, several important points require further investigation. Our observation that the solubilizer method overestimates carbon fixation dictates the need for additional clarification on whether chromium alters carbon uptake, carbon fixation, or both processes. Petiolar elongation assays based on soluble hexavalent chromium present in media need to be coupled to tissue chromium uptake assays. Finally, petiolar elongation must be differentiated into physiological processes that reflect true growth (*i.e.*, increase in biomass) with those resulting in elongation due to cell expansion and etiolation (increase in length without an increase in biomass). Such research may shed light on the apparent contradiction between stimulation of PCA rates and reduction of petiolar elongation rates noted here. Al-Hamdani and Francko (1992) provided evidence that changing rates of elongation in lotus petioles exposed to differential environmental stimuli may result from a change in both biomass accumulation and etiolation rates.

ACKNOWLEDGMENTS

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Morphological and Photosynthetic Characteristics of *Potamogeton obtusifolius* from Different Depths

S. C. MABERLY[1]

ABSTRACT

Plants rooted between 0.5 m and 3.6 m were collected from Esthwaite Water, UK. Specific leaf area increased and leaf dry weight decreased with depth. Shoot length varied 5-fold over the depth range, largely because of variable mean internode length rather than internode number. Shoots close to the depth limit invested nearly 7-times less biomass in the production of a unit length of shoot than did shoots at 0.5 m. Shoot dry weight varied 14-fold with depth and was least at 3.6 m. Seed heads were not produced on plants rooted at 3.5 or 3.6 m, and side shoots were largely absent from shoots at 3.6 m. This and a very low average dry weight suggest that plants at this the maximum colonized depth are derived from turions produced in shallower water. Analysis of the response of photosynthesis to light at saturating inorganic carbon showed that the saturating rate per unit dry weight declined slightly with depth. The initial slope of photosynthesis to light increased 2.6-fold, the light compensation point decreased 1.4-fold and I_K decreased 3.4-fold with depth. The underwater light climate at any time and depth was modeled from field data in order to assess the relative effects of the measured characteristics on plant production. Calculations suggest that shoot elongation increased production 2.5-fold and superior photosynthesis characteristics increased production 2.8-fold for shoots rooted at 3.5 m. For shoots rooted at 1.5 m, shoot elongation was the major factor allowing increased production. Discrepancies between growth rate calculated from the model and estimated from biomass changes were particularly marked in shallow water and are consistent with carbon-limitation of growth.

Key words: specific leaf area, shoot elongation, light, production model, carbon-limitation.

INTRODUCTION

Differences between the characteristics of freshwater macrophytes from different depths within a lake are usually interpreted as being a response to the quality and, more frequently, the quantity of light to which they are exposed, although other factors such as different nutrient availability in the sediment (Chambers and Kalff 1987), concentration of inorganic carbon (Maberly 1985b), temperature (Moeller 1980), or the direct effect of pressure (Hutchinson 1975) at great depth could also be involved. Nevertheless, the roughly 10% reduction in above-surface radiation caused by reflection at the air-water interface and the marked attenuation with depth which occurs within the water column mean that all but the most shallow waters are shade environments (Spence 1981). Two types of response to low light are typically recognized. The first includes changed leaf and shoot morphology such as increased shoot length, increased specific leaf area and changes in the content of photosynthetic pigments. The second involves changes in the photosynthetic apparatus which lead to increased rates at low light. However, in very few cases have both types of response been measured (but see Titus and Adams 1979) and so their relative contribution to performance has not been assessed: this is the aim of the present study on the grassy pondweed, *Potamogeton obtusifolius* Mert. and Koch. This species is non-rhizomatous and dies back to overwinter either as turions or potentially as seeds, and so the biomass of a shoot is largely the result of photosynthesis and growth in the current season.

MATERIAL AND METHODS

Collection site. *P. obtusifolius* was collected from the North Bay in Esthwaite Water, English Lake District, toward the end of the growing season between 1 and 14 September 1982. A grab operated from a boat was used to collect whole shoots from five rooting depths: 0.5, 1.5, 2.5, 3.5 and 3.6 m. The last depth was the maximum colonizable by this or any other macrophyte in this site. Attenuation of photosynthetically available radiation (PAR, 400 to 700 nm) was measured with a cosine-corrected submersible quantum sensor (Lambda Q 221-0174).

Morphology. Ten shoots from each depth were scored for shoot length, number of internodes on the main stem, number of branches, number of seed heads and also shoot dry weight after drying at 80C for 24 hr. From each rooting depth the length and breadth of ten fully expanded leaves (6 for 3.6-m depth) from the apex of the shoots were measured. Leaf area (1-sided) was estimated from a comparison of the weight

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of photocopied leaves and graph paper of known area. Leaf weight was measured after drying at 80C for 24 hr.

Photosynthesis and respiration. Net photosynthesis and respiration were measured on healthy 5-cm apical shoots with duplicate measurements for each depth studied: 0.5, 1.5 and 3.5 m. Changes in O_2 concentration were measured under well-stirred conditions in a $KHCO_3$ solution of 0.4 mequiv l^{-1}, which is similar to the alkalinity of the site (Sutcliffe *et al.* 1982), using a Clark-type electrode (Radiometer E5046) in a perspex chamber at 14C, the ambient temperature at the time of collection. A pH value of 6.0, maintained by automatic injection of CO_2-enriched $KHCO_3$ solution controlled by a pH-stat system, produced a CO_2 concentration of 1.04 mmol l^{-1} which was probably at or close to saturating. The chamber was illuminated from above by a 1000-W tungsten-halogen lamp whose light was filtered through a 16-cm-deep running water bath. Different photon irradiances, produced by interposing neutral-density filters between the lamp and the chamber, were measured with the sensor used in the field studies, placed in the position of the chamber. A dark respiration rate was measured at the end of the exposure to light. Full details of the equipment and procedure are given in Maberly (1985a). A non-linear regression was used to obtain estimates of the maximum gross rate of photosynthesis (P_{max}^{gross}) and the initial slope of photosynthesis versus photon irradiance (α_I) using the equations of Smith (1936) as a model. The rate of dark respiration (R) was calculated from the mean of two measurements.

RESULTS AND DISCUSSION

Leaves. There was a progressive decline in average leaf dry weight with depth, and leaves at 0.5 m were nearly 1.5 times heavier than leaves at 3.6 m (Figure 1). Average leaf area was greatest between 1.5 and 3.5 m with slightly smaller leaves at the two extreme depths, but area only varied 1.13-fold. The average 1-sided specific leaf area (SLA) derived from these two measures followed the converse pattern to that for dry weight with leaves from 0.5 m having a low SLA of 0.72 cm^2 mg^{-1} increasing 1.51-fold to 1.09 cm^2 mg^{-1} at 3.5 m (Figure 1). The increase in SLA with depth is in broad agreement with the results of Spence and Chrystal (1970), Spence *et al.* (1973) and Spence and Dale (1978) for this and other species of *Potamogeton*. Average leaf length varied in a similar way to leaf area since the mean breadth at the different depths varied by only 0.15 mm. A linear regression between leaf length/mm (y) as a function of 1-sided area/cm^2 (x) yielded y = 30.5 + 22.1*x. The adjusted variance

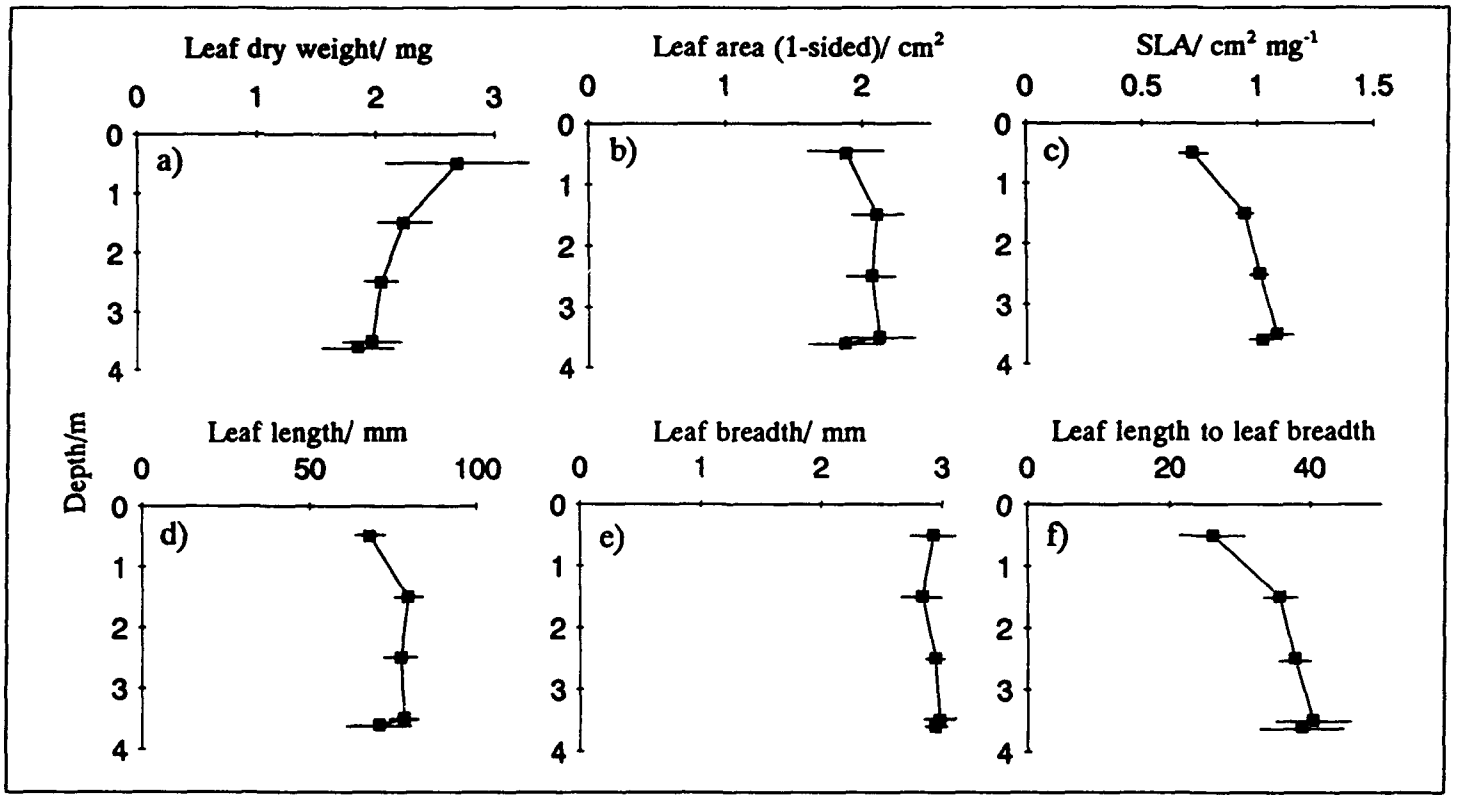

Figure 1. Average leaf characteristics at different depths; a) dry weight, b) 1-sided area, c) 1-sided SLA, d) length, e) breadth, f) length-to-breadth ratio. Error bars represent one standard deviation.

accounted for, r^2, was 0.60 and an analysis of variance showed this to be statistically significant with $p < 0.001$. The average ratio of length to breadth varied 1.2-fold and was least at 0.5 m, but showed little trend unlike *P. richardsonii* where the length-to-breadth ratio increased at low light (Spence and Dale 1978).

Shoots. Average shoot dry weight varied 14.5-fold from a maximum of 591 mg at 1.5 m and a minimum of 41 mg at 3.6 m (Figure 2). Shoot length varied 5.16-fold and was greatest at 2.5 m and least at 0.5 m. The variation in shoot length with depth was mainly a result of the mean internode length (MIL) which varied 3.92-fold, while the number of internodes on the main stem varied only 1.76-fold (Figure 2). A similar increase in shoot length and internode length at low light has been found in a laboratory study with *P. crispus* (Tobiessen and Snow 1984). In *P. obtusifolius*, shoot length per unit dry weight increased monotonically with depth, so that shoots close to the depth limit invested nearly 7-times less biomass in the production of a unit length of shoot than did shoots at 0.5 m (Figure 2). This was achieved by plants at a depth of 3.6 m nearly eliminating side branches and producing leaves of low weight at each node. The average MIL of these plants, however, was only 2 cm which is less than that at all depths except in the shallowest water and plants at 2.5 m produced internodes with an average length of nearly 4 cm. The major morphological responses to growth at depth noted here, namely increased SLA, increased shoot length and increased MIL, are likely to be caused in large part by the reduced light level at depth, although other factors such as temperature (Spence and Dale 1978; Barko *et al.* 1981) may also be involved.

Reproduction. Since *P. obtusifolius* is neither rhizomatous nor evergreen, next year's plants will derive either from seeds or from turions which, in this species, are starch-filled axial shoots with unextended internodes. A measure of the ability of plants from each rooting depth to contribute to next year's population was estimated from the number of seed heads and the number of side branches, which will determine the upper limit to the number of turions which can be produced. The number of seed heads produced per shoot at each depth was very variable, with a maximum of 7 for one plant at 1.5 m, the depth with the maximum average seed-head production and where 8 of the 10 shoots examined had flowered (Figure 2). Plants at 3.5 and 3.6 m failed to produce seed heads and only two of the plants at 0.5 m produced seed heads. A linear regression on all the data between number of seed heads per shoot (y) and shoot dry weight/mg (x) yielded: $y = -0.435 + 0.0041*x$; $r^2 = 0.56$, $p < 0.001$. The number of side branches, providing sites for turion production, in addition to the apex, was greatest on shoots at 1.5 and 2.5 m. At 3.6 m, only 1 of the 10 shoots had a side branch (Figure 2). A linear regression on all the data, except for three with high numbers of branches, between number of side branches per shoot (y) and shoot dry weight/mg (x) yielded: $y = 1.66 + 0.0289*x$; $r^2 = 0.62$, $p < 0.001$. The slope of this regression indicates that on average one side branch is produced for every 34.6 mg dry weight. This value is nearly identical to the average reported turion dry weight in this species of 33.8 mg (Webster 1975,

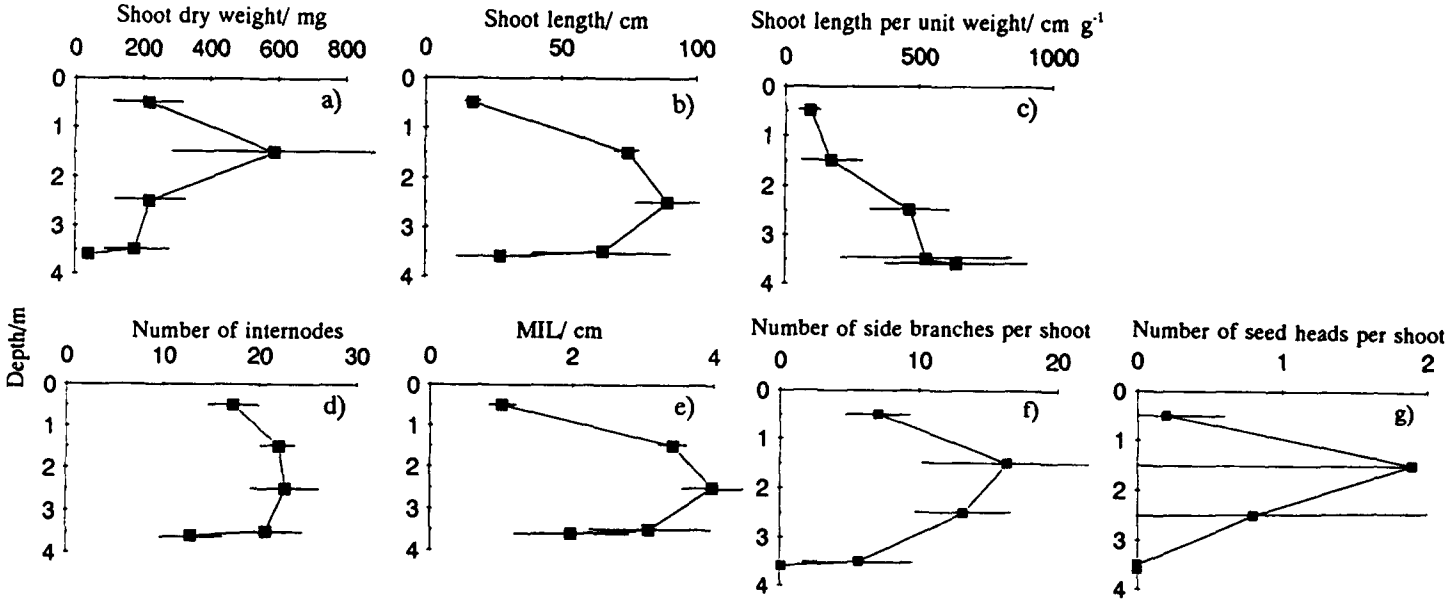

Figure 2. Average shoot characteristics at different depths; a) dry weight, b) length, c) length per unit dry weight, d) number of internodes on the main stem, e) MIL, f) number of side branches, g) number of seed heads. Error bars represent one standard deviation.

unpublished Ph.D. thesis, St. Andrews University, U.K.), which indicates that side branches are produced in relation to the capacity of the shoot to support turions. Shoots at the depth-limit of 3.6 m at the time of collection near the end of the growing season had no seed heads, and their average dry weight of 41 mg suggests that on average only 1 turion will be produced per plant, indicating no net population growth. If possible losses are taken into account then plants at this depth are probably maintained by transport of turions from shallower water.

Photosynthesis. The Smith (1936) model of the effect of light on rate of photosynthesis provided an adequate description of the data with the adjusted percentage variance accounted for varying between 87 and 99% (Table 1). The gross rate of photosynthesis at light- and CO_2-saturation was higher for plants rooted at 0.5 m than for those at the two other depths, but only varied 1.4-fold. If these rates are divided by the average specific leaf area (Figure 1) for each depth to *estimate* rates on an area basis, there is a 2-fold range with calculated rates of 2.52, 1.37 and 1.25 μmol O_2 m^{-2} s^{-1} (both sides) for shoots rooted at 0.5, 1.5 and 3.5 m, respectively. Surprisingly, the rate of dark respiration was greatest at 3.5 m (Table 1). The reason for this is unclear, although a similar effect has been described for *Hydrilla verticillata* (Barko and Smart 1981). The effect of the higher rate of dark respiration on rates of net photosynthesis at low light is largely offset by the high value of α_I which was about 2.6-fold higher at 3.5 m than at 0.5 m. As a result, the calculated light compensation point (I_c) for shoots from 3.5 m was 1.4-fold lower than for shoots from 0.5 and 1.5 m. The calculated value I_K, which expresses the onset of light saturation, varied 3.4-fold and decreased with rooting depth (Table 1).

Modeled production. In order to assess the relative effect of the various morphological and physiological responses to growth at depth outlined above, a model was constructed to predict rates of photosynthesis using the photosynthetic responses in Table 1, and calculations of underwater photon irradiance following the approach in Maberly (1985b). The photon irradiance at a given depth and time, I_z^t, (μmol m^{-2} s^{-1}) was estimated from

$$I_z^t = \left\{ \text{antilog}_e \left[\log_e (0.9 I_0^{\Sigma}) - (Kz) \right] / \lambda \right\} \left[1 + \cos\left(\frac{2\pi}{\lambda t}\right) \right] * 10^6 \quad (1)$$

where I_0^{Σ} is the daily photon irradiance (PAR) at the surface (mol m^{-2}), K is the attenuation coefficient for PAR ($\log_e$ m^{-1}), z is the depth below the water surface (m), λ is the day length (s), and t is the time (s) from noon. Production over 24 hr was calculated for the weekly average values of I_0^{Σ}, K, z and λ for 15 wk between the end of April and the end of September 1982 where data were available. As will be discussed later, no account was taken of the effects of temperature and concentration of CO_2 on rates of photosynthesis.

The light climate and photosynthesis characteristics allowed shoot apices at 0.33 m to be 3.8-fold more productive than shoot apices at 2.84 m although the average daily photon irradiance was 6.1-fold higher (Table 2A). Shoot elongation was responsible for a small increase in light availability and productivity to plants rooted at 0.5 m. Shoots rooted at 1.5 m received 1.7-fold more light as a result of shoot elongation, causing a commensurate gain in production. Shoots rooted at 3.5 m received 1.55 times more light as a result of elongation, and this allowed production to increase by 2.5-fold (Table 2A).

The photosynthetic characteristics of shoots rooted at 3.5 m gave greater modeled production at all depths because of the high α_I value. However, differences between the different photosynthesis characteristics were relatively minor at the surface whereas at 2.84 m, the depth of the 3.5-m apices, there was a 2.8-fold and 1.4-fold greater production for the photosynthesis characteristics of shoots rooted at 3.5 m compared to 0.5 m and 1.5 m, respectively (Table 2B).

TABLE 1. CHARACTERISTICS OF THE RESPONSE OF PHOTOSYNTHESIS TO LIGHT AT 0.4 mmol $HCO_3^- l^{-1}$, SATURATING CONCENTRATION OF 1.0 mmol CO_2 l^{-1}, AND 14C. R DETERMINED AS THE MEAN OF TWO VALUES, P_{max}^{gross} AND α_I DETERMINED BY NONLINEAR REGRESSION, SEE TEXT. STANDARD DEVIATIONS GIVEN IN PARENTHESES.

Rooting depth (m)	P_{max}^{gross} (μmol O_2 g^{-1} DW h^{-1})	R (μmol O_2 g^{-1} DW h^{-1})	α_I (μmol O_2 g^{-1} DW h^{-1} / μmol photon m^{-2} s^{-1})	I_c (μmol photon m^{-2} s^{-1})	I_K (μmol photon m^{-2} s^{-1})	r^2
0.5	1304 (66)	-39.9 (5.3)	3.08 (0.22)	12.9	423	0.97
1.5	933 (75)	-32.9 (1.2)	3.35 (0.49)	9.8	278	0.87
3.5	972 (16)	-74-5 (1.2)	7.89 (0.33)	9.5	123	0.99

TABLE 2. COMPARISONS OF MODELED AVERAGE PRODUCTION OVER 24 HR (mmol O_2 g^{-1} DW d^{-1}) AND AVERAGE DAILY PHOTON IRRADIANCE AT DEPTH (mol m^{-2} d^{-1}) FOR DIFFERENT STATED CONDITIONS IN RESPONSE TO A. SHOOT ELONGATION, B. PHOTOSYNTHESIS CHARACTERISTICS, AND C. THE RELATIVE EFFECT OF SHOOT ELONGATION AND PHOTOSYNTHETIC CHARACTERISTICS

A. Effect of shoot elongation from the rooting depth

Depth (m)		Average I_z^{Σ}		24-hr production		Top/bottom	
Bottom	Top	Bottom	Top	Bottom	Top	Average I_z^{Σ}	24-hr production
0.5	0.33	10.73	12.27	6.42	7.15	1.14	1.11
1.5	0.75	5.07	8.84	3.71	5.46	1.74	1.47
3.5	2.84	1.29	2.00	0.77	1.90	1.55	2.47

B. Effect of photosynthesis characteristics of named rooting depths

Depth of shoot top (m)	24-hr production			24-hr production stated depth/0.5 m	
	0.5 m	1.5 m	3.5 m	1.5 m	3.5 m
0.33	7.15	6.86	8.65	0.96	1.21
0.75	5.41	5.46	7.55	1.01	1.40
2.84	0.68	0.94	1.90	1.38	2.79

C. The relative effect of shoot elongation and photosynthesis characteristics

Rooting depth (m)	Features characteristic of named rooting depths				24-hr production		% contribution to increased prodcution	
	Unadapted		Adapted					
	Length	P vs I	Length	P vs I	Unadapted	Adapted	Length	P vs I
1.5	0.5	0.5	1.5	1.5	3.46	5.46	88.5	11.5
1.5	0.5	0.5	1.5	3.5	3.46	7.55	48.1	51.9
3.5	0.5	0.5	3.5	3.5	0.22	1.90	36.1	63.9

The relative contribution of shoot elongation and photosynthetic characteristics to the greater modeled production at depth by the shoots rooted at 1.5 m and 3.5 m compared to those at 0.5 m was assessed by modeling production when only one of these two variables was optimal. The results suggest (Table 2C) that for shoots rooted at 3.5 m, 64% of the increased production is a result of "shade" photosynthesis characteristics. For shoots rooted at 1.5 m, shoot elongation was responsible for 89% of the increased production. If these shoots had the photosynthesis characteristics of the 3.5-m shoots, however, then production would have increased by 1.4-fold and shoot elongation and "shade" photosynthesis charactersitics would have had approximately equal influence in allowing increased production. The production data in Table 2 can be used to calculate relative growth rates using the assumptions that 1 mol O_2 produced is equivalent to 1 mol CO_2 fixed, and that carbon forms 0.45 g of each gram dry weight. These calculations yield average relative growth rates of 0.167, 0.111 and 0.024 $\log_e$ d^{-1} for shoots rooted at 0.5, 1.5 and 3.5 m, respectively. Biomass-based growth rates were calculated from the measured average shoot biomass, an assumed initial turion biomass of 35 mg dry weight and an assumed growing season of 130 days. These growth rates were lower than those predicted by the model at 0.014, 0.022 and 0.012 $\log_e$ d^{-1}, by 11.9, 5.1 and 2.0-fold for 0.5, 1.5 and 3.5 m, respectively. The generally lower growth rates based on biomass changes could be caused by several factors such as loss of dry weight during the growing season and a shorter growing season than assumed. However, the fact that the difference between modeled and biomass based growth rates decreases with depth strongly suggests that a major cause of the difference is likely to be limitation of rates of photosynthesis

by availability of inorganic carbon. Depth profiles of CO_2 concentration made during the season show increased concentration at depth (see for example Maberly 1985b, Figures 1 and 2), which is in accord with the suggestion that carbon-limitation is involved.

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The Mechanism of Action of Bensulfuron-Methyl on Hydrilla

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ABSTRACT

Bensulfuron-methyl has been reported to specifically inhibit acetohydroxyacid synthase (AHAS, E.C. 4.1.3.18) in susceptible terrestrial plants. AHAS is the first enzyme unique to the synthesis of the branched-chain amino acids leucine, isoleucine and valine. Extraction of AHAS from the submersed aquatic plant hydrilla (*Hydrilla verticillata* (L.f.) Royle) and the assay conditions used are described. *In vitro* inhibition of AHAS by bensulfuron-methyl was significant. The addition of 1 n M of herbicide resulted in a 25% inhibition of enzyme activity. Increases in herbicide concentration caused significant increases in inhibition (1 mM = 93% inhibition). The I_{50} was calculated to be 22 nM. The level of inhibition was also shown to be time-dependent. *In vivo* inhibition of hydrilla by bensulfuron-methyl was also investigated and assessed as growth inhibition (I_{50} = 110 nM). These results suggest that the inhibition of hydrilla growth by bensulfuron-methyl is due to the inhibition of AHAS.

Key words: acetohydroxyacid synthase, AHAS, sulfonylurea.

INTRODUCTION

The aquatic herbicide bensulfuron-methyl (methyl 2-[[[[[(4,6 dimethoxypyrimidin-2-yl)amino]carbonyl] amino]sulfonyl] methyl]benzoate): Mariner®) is currently being tested for its effectiveness in controlling nuisance aquatic plants. Bensulfuron-methyl is a member of the class of herbicides known as the sulfonylureas which includes such herbicides as chlorsulfuron (Glean®) and chlorimuron-ethyl

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(Classic®). Sulfonylureas exhibit extremely high levels of herbicidal activity (as low as 1 g ha^{-1}) and very low mammalian toxicity. As herbicides, they are very potent inhibitors of plant growth with visual symptoms often occurring within 1 or 2 days of treatment in some broadleaf species. A wide range of secondary plant responses often develop depending on the species being treated and the environmental conditions.

The mechanism-of-action of the sulfonylurea herbicides involves the inhibition of the enzyme acetohydroxyacid synthase (AHAS, EC4.1.3.18: also known as acetolactate synthase, ALS). AHAS is the first enzyme unique to the biosynthesis of the branched chain amino acids leucine, valine and isoleucine. AHAS catalyzes (a) the condensation of two pyruvate molecules to form CO_2 and α-acetolactate which leads to the production of valine and leucine and (b) the condensation of one molecule of pyruvate with α-ketobutyrate to form CO_2 and α-aceto-α-hydroxybutyrate which leads to isoleucine biosynthesis. Several publications have recently discussed the methodologies involved in assaying AHAS and the effects of inhibitors such as the sulfonylurea herbicides on the activity of this enzyme (Singh *et al.* 1988, Ray 1984, Schloss 1990, Landstein *et al.* 1990, Durner *et al.* 1991 and Shaner and Little 1989).

In this paper, the effect of bensulfuron-methyl on the growth of hydrilla and the activity of AHAS extracted from hydrilla will be discussed.

METHODS AND MATERIALS.

In vivo study. Hydrilla was established from 10-cm apical stem segments into 40-L aquaria under greenhouse conditions (16:8 h light/dark, 1000 μmol m^{-2} s^{-1} PAR, 30/20C day/night). Plants were grown for 18 days prior to the onset of the treatment. Bensulfuron-methyl was added as a one-time addition to bring the initial water concentrations to 0, 0.12, 1.2, 2.4, 12, 24, 122 and 244 nM. Five plants from each treatment were then removed for growth analysis after 3 days and 1, 2, 3, 4 and 6 weeks. At each harvest, shoot length, shoot number and fresh weight were recorded. Shoot tissue was dried for 3 days at 70C and the dry weights were then determined. The amount of growth or change (increase) in growth was also determined by subtracting the growth at each harvest time from the initial growth determined at the time of treatment. Regression analysis ($P < 0.10$) was used to determine the effect of rate and time for each growth parameter. These equations were then used to derive I_{50} values for the change in shoot length, number of shoots, and fresh and dry weights. I_{50} values will only be reported for the final harvest.

In vitro study. AHAS was extracted from hydrilla following the procedure of Ray (1984). The extraction solution consisted of 100 mM potassium phosphate (pH 7.5), 1 mM sodium pyruvate, 1 mM $MgCl_2$, 0.5 mM thiamine pyrophosphate (TPP), 10 μM flavin adenine dinucleotide (FAD), and 10% (w/v) glycerol. Plant material (10 to 20 g of 1- to 2-cm apical shoots) was ground in a mortar with a pestle and the homogenate filtered through 8 layers of cheesecloth. The filtrate was then centrifuged at 25000 g for 20 min. $(NH_4)_2SO_4$ (50% w/v) was then added to the supernatant. This mixture was then centrifuged at 25000 g for 20 min and the supernatant discarded. The pellet was dissolved in 50 mM potassium phosphate (pH 7.0), 20 mM sodium pyruvate, and 0.5 mM $MgCl_2$. This was then desalted through a Sephadex G25 column prior to assaying for enzyme activity.

The assay method used for AHAS was based on the procedure of Singh *et al.* (1988). AHAS activity was measured by estimating the amount of acetolactate produced, after conversion by decarboxylation in the presence of acid to acetoin. Standard reaction mixture (500 μl total volume) contained the enzyme in 25 mM potassium phosphate buffer (pH 7.0), 200 mM sodium pyruvate, 0.5 mM TPP, 20 mM $MgCl_2$, and 25 μM FAD. Various concentrations of bensulfuron-methyl were also added where appropriate. The reaction mixture was incubated at 37C for 60 min after which the reaction was stopped with the addition of 50 μl 6N H_2SO_4. The reaction product was then decarboxylated at 60C for 15 min. The acetoin formed was determined by incubating with creatine (0.17%) and 1-α naphthol (1.7%) by the method of Westerfield (1945). Maximum color formation was achieved by incubation at 60C for 15 min and a further 15 min at room temperature. The absorption of the color complex was measured at 530 nm. Protein concentrations were determined following the procedure of Bradford (1976).

RESULTS AND DISCUSSION

Bensulfuron-methyl has been shown to inhibit the growth of hydrilla (Van and Vandiver 1992, Haller *et al.* 1992) and other aquatic plants (Anderson and Dechoretz 1988). However, to date, there has been little research into the actual mechanism-of-action of bensulfuron-methyl on aquatic plants. In this study, we have investigated both the inhibition of vegetative growth in hydrilla and the inhibition of AHAS, a key enzyme in branched chain amino acid biosynthesis.

Based on shoot length, the effect of bensulfuron-methyl at 12 nM or higher significantly reduced growth (Figure 1). Growth was inhibited at 12 and 24 nM by approximately 40 and 50% of the control, respectively. At the two highest concentrations no increases in shoot length were recorded. Six weeks after initial treatment I_{50} values based on shoot length, number of shoots and fresh and dry weights ranged from 85 to 183 nM (Table 1). In several previous studies (Anderson and Dechoretz 1988, Haller *et al.* 1992, Van and

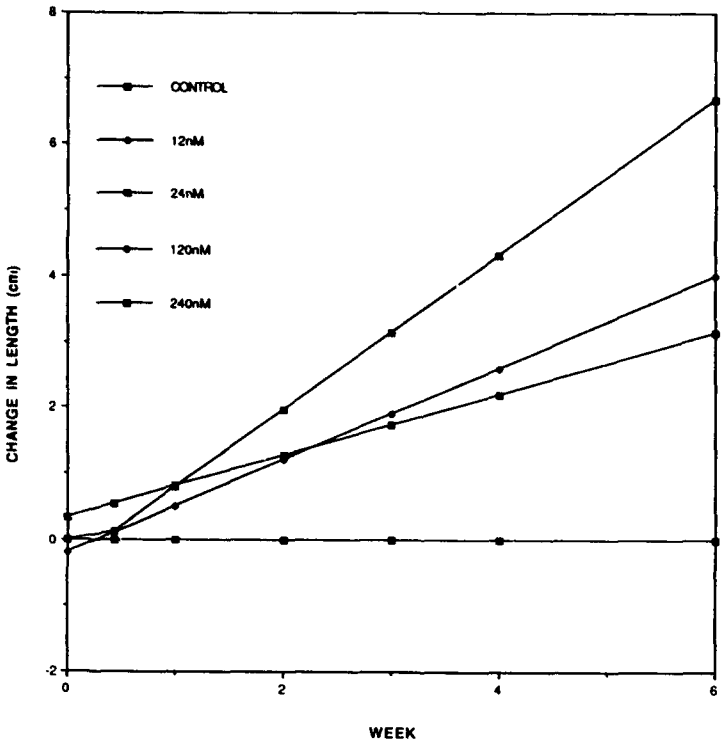

Figure 1. Effect of increasing concentrations of bensulfuron-methyl on the shoot growth of hydrilla.

TABLE 1. COMPARISON OF INHIBITION (50%) VALUES CALCULATED FOR HYDRILLA BASED ON GROWTH AND AHAS ACTIVITY, AND FOR SEVERAL TERRESTRIAL SPECIES BASED ON AHAS ACTIVITY (FROM RAY 1984)

Species		Treatment	I_{50} (nM)
Hydrilla			
(a) Growth	-Shoot length	Bensulfuron-methyl	95.3
	-Shoot number	Bensulfuron-methyl	101.6
	-Fresh weight	Bensulfuron-methyl	85.3
	-Dry weight	Bensulfuron-methyl	182.7
(b) AHAS activity		Bensulfuron-methyl	22.0
Pea		Chlorsulfuron	21.0
Wheat		Chlorsulfuron	18.5
Soybean		Chlorsulfuron	23.0
Tobacco		Chlorsulfuron	28.3
Green Foxtail		Chlorsulfuron	25.8
Johnsongrass		Chlorsulfuron	35.9
Morning Glory		Chlorsulfuron	24.4

Vandiver 1992), vegetative growth of hydrilla as well as other aquatic plants was shown to be inhibited by a minimum of 24 nM. However, to this date no I_{50} values for bensulfuron-methyl have been published. However, it is important to point out that the I_{50} values calculated for this herbicide will be very dependent upon the rate of growth of the treated plant and the duration of exposure. I_{50} values determined at the shorter time intervals were higher than those presented (data not shown). The susceptibilty of plants to sulfonylurea herbicides also varies greatly depending on various factors, including growth stage (Beyer *et al.* 1988). In hydrilla, Haller *et al.* (1992) noted that newly sprouted hydrilla plants were more susceptible to bensulfuron-methyl than mature plants. This would result in variable I_{50} values depending on the growth phase of the plants.

One interesting symptom observed during this experiment was the apparent "hardening" of tissue when hydrilla is treated with this compound. Initial measurements suggest that there is a significant increase in the percent dry matter content in treated hydrilla relative to control plants (data not shown). The cause of such an increase is unclear but will be investigated as part of this ongoing project. Another important point related to this "hardening" is the need to be careful when selecting which growth parameter is suitable to use as an estimate of growth for the calculation of I_{50} values. We concluded from the experiments described in this paper that by only using dry weight data, the activity of bensulfuron-methyl on hydrilla growth would be underestimated. In this instance, shoot length provided the most consistent measurement and appeared to provide the best estimate of herbicidal efficacy.

Secondly, we investigated the *in vitro* effect of bensulfuron-methyl on the activity of AHAS extracted from hydrilla. Bensulfuron-methyl inhibited AHAS activity with the addition of as little as 1 nM and the inhibition was dose dependent (Figure 2). At 1 µM, enzyme activity was 93% inhibited after a 60-min incubation period. An I_{50} of 22 nM was calculated from these data and it is similar to values previously published for this class of herbicides with terrestrial plants (Ray 1984; Table 1). However, a comparison of the I_{50} values calculated for hydrilla growth and hydrilla AHAS activity indicated that the value is five-fold higher in the whole plant system (Table 1). This difference was not unexpected and the relative difference, as stated earlier, will be dependent upon the growth stage of the plants treated. In addition, the I_{50} values for growth parameters reflect virtually all factors influencing herbicidal activity. Factors such as microbial decomposition, plant metabolism, and adsorption to soil colloids would tend to decrease the concentration of the herbicide at the active site thus increasing the I_{50}.

The inhibition of AHAS activity was also shown to be time dependent; inhibition increased with increasing time in the presence of herbicide (Figure 3). This suggests that this enzyme may be even more sensitive to inhibition since even very low concentrations of bensulfuron-methyl might eventually cause some level of inhibition. The results obtained in this part of the study are consistent with the known mode-of-

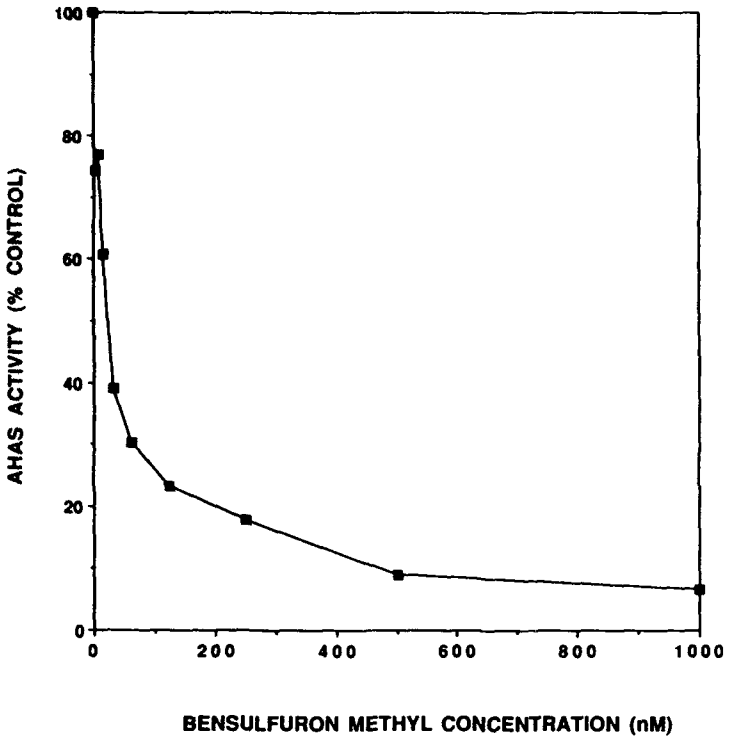

Figure 2. Effect of increasing concentrations of bensulfuron-methyl on AHAS activity following a 60-min incubation period. AHAS was extracted from hydrilla.

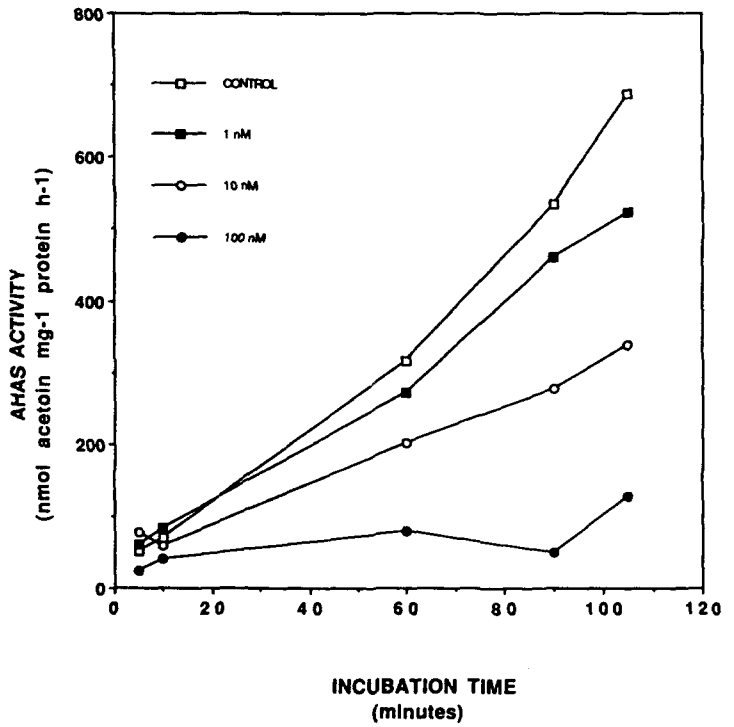

Figure 3. Effect of time on the inhibition of AHAS activity in hydrilla shoot tips by bensulfuron-methyl

action of the sulfonylurea herbicides, namely the inhibition of AHAS (Ray 1984, Singh *et al.* 1988, Schloss 1990, Durner *et al.* 1991). In conclusion, the results presented in this paper demonstrate that the herbicidal effect of bensulfuron-methyl on hydrilla growth appears to be due to the inhibition of AHAS, a key enzyme in branched-chain amino acid biosynthesis.

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J. Aquat. Plant Manage. 31: 43-45

Comparisons of Herbicide Toxicity Using *In Vitro* Cultures of *Myriophyllum spicatum*

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ABSTRACT

In vitro cultures of the aquatic plant *Myriophyllum spicatum* L. were used to determine the effects of the herbicides 2,4–D (dichlorophenoxyacetic acid), atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) and glyphosate (N-[phosphonomethyl] glycine) and the leaf defoliant thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) on plant development. The developmental response measured percent reduction of new branches relative to controls. Linear regressions of percent branch number reduction (BNR) as a function of the log (toxin concentration+1) were highly correlated and statistically significant. The plant growth regulator 2,4-D had the greatest effects on development of branches with a 50% BNR of 0.04 mg/l, followed by glyphosate (1.6 mg/l), atrazine (3.7 mg/l), and thidiazuron (9.8 mg/l). The short 5-day time period required for these assays and ability to determine dose–response relationships supports the use of *in vitro* culture of *Myriophyllum spicatum* as a bioassay system.

Key words: atrazine, 2,4-D, glyphosate, thidiazuron, bioassays.

INTRODUCTION

Bioassays play a significant role in toxicological research. Bioassay data may be used to determine effects of environmental pollutants or the design of application protocols for aquatic weed control. Bioassay organisms are particularly useful if they grow rapidly, are easy to culture and are important in the environments of interest (Rand and Petrocelli 1985).

In vitro culture of aquatic plants offers advantages for bioassays. Methods have now been developed for culture of a number of aquatic plants *in vitro* (Kane *et al.* 1991, Jenks *et al.* 1990, Kane and Gilman 1991). Such cultures are axenic, hence microflora do not affect toxin chemistry or concentrations. Growth under *in vitro* culture is rapid. Aquatic plant cultures can be easily maintained in the laboratory.

Recently, Kane and Gilman (1991) demonstrated that *in vitro* cultures of *Myriophyllum* spp. show decreases in growth as a function of Cycocel concentrations, a growth retardant. They used shoot length and percent dry weight as measures of growth. We examined the effects of several herbicides and thidiazuron on organogenesis in nodal cultures of *Myriophyllum spicatum* L. grown in liquid medium (Christopher and Bird 1992). The development of new branches appeared to be a reliable indicator of toxin effects over a short period of 5 days. In this paper, I examine whether branch development can be modeled to determine dose–response relationships of several herbicides and a defoliant.

MATERIALS AND METHODS

Liquid stock cultures of *Myriophyllum spicatum* were propagated in a Murashige and Skoog salt-based medium (Kane and Gilman 1991). Axenic stock cultures of *M. spicatum* were obtained from M. Kane, University of Florida. Bioassays were performed on three node segments in which the foliar portions of the middle node were excised. Each segment was cultured in 10 ml of the bioassay medium and the nominal concentration of herbicide (see Christopher and Bird 1992 for details). The three herbicides were 2,4-D, atrazine and glyphosate. The defoliant was thidiazuron. Atrazine was tested over a concentration range of 0.1 to 100 mg/l, 2,4-D over 0.02 to 0.1 mg/l, glyphosate from 0.5 to 10 mg/l and thidiazuron from 1.0 to 50 mg/l. Controls containing no toxin were included in each experimental run. After 5 days, the segments were removed and the number of new branches at the middle node tabulated. For statistical purposes, three experimental runs were performed for each type of toxin and five replicates were used at each concentration.

Statistical analyses were run on the mean values of the data from the three replicate experiments for each toxin. The number of branches produced in the controls was compared with the numbers produced at each concentration of toxin to determine the percent of branch number reduction (BNR). The concentrations of the toxins were transformed using the log of concentration+1 (conc+1). Probability plots of the percent of BNR were examined to determine whether data were normally distributed using Fastat (Systat 1989). The data were checked for homogeneity for variance (Steel and Torrie 1960). Regressions and 95% confidence intervals were determined for each toxin using Fastat (Systat 1989). Regression

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equations were used to determine the concentration at which a 50% reduction in new branches occurred.

RESULTS

There were strong correlations between the log (conc+1) transformed concentrations of the toxins and percent of branch number reduction (Figure 1). The herbicide 2,4-D caused branch number reduction (BNR) over a concentration range of 0.02 to 0.1 mg/ml. The percent of BNR was linear as a function of the transformed concentrations (log [conc+1]). Analysis of variance (ANOVA) indicated that the regression was highly significant ($p = 0.002$, $r = 0.96$) with a 50% BNR at a concentration of 0.04 mg/l of 2,4-D.

Atrazine also resulted in significant percent BNR, although there was more variability in the data than for the other toxins. Despite some variability, the regression was highly significant as determined by ANOVA ($p = 0.024$, $r = 0.82$) with a 50% BNR of 3.7 mg/l.

Glyphosate treatment resulted in a significant BNR, particularly at concentrations of 1.0 mg/l and higher. The regression was highly significant as determined by ANOVA ($p = 0.003$, $r = 0.93$). A 50% BNR of 1.6 mg/l was determined from the regression equation.

Thidiazuron caused a reduction in branch number in concentrations ranging from 1 to 50 mg/l. The regression of percent BNR on log (conc+1) was linear and highly significant as determined by ANOVA ($p = 0.003$, $r = 0.92$). The regression equation was used to calculate a 50% BNR of 9.8 mg/l.

DISCUSSION

In our prior paper (Christopher and Bird 1992), comparisons of concentration effects for these toxins were made using empirical data and statistical comparisons of means. In that paper, we suggested that the order of greatest developmental inhibition of *Myriophyllum spicatum* was 2,4-D

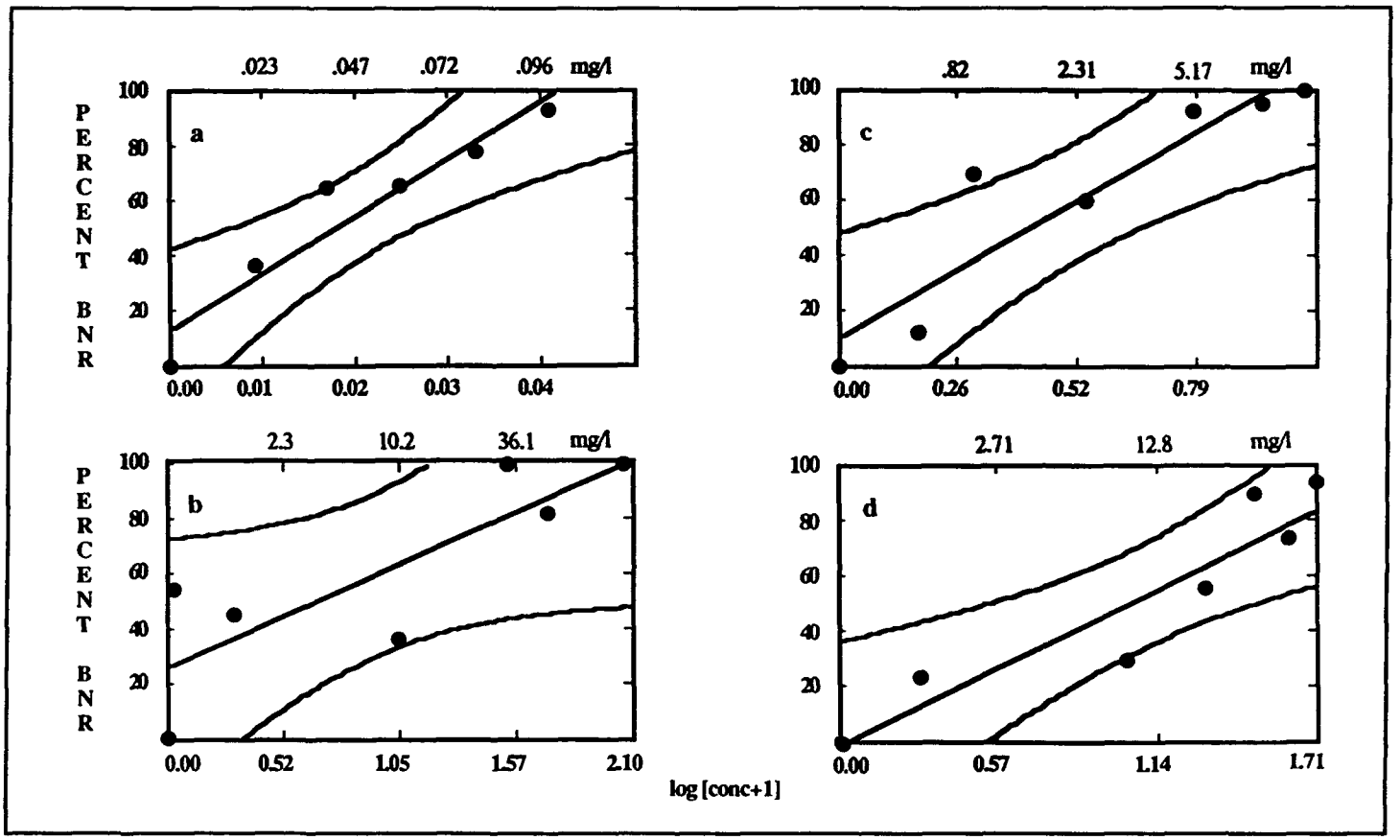

Figure 1. Linear regressions of percent branch number reduction (BNR) of *Myriophyllum spicatum* determined as a function of the log (concentration+1) of a) 2,4-D, b) atrazine, c) glyphosate and d) thidiazuron. Lower axes show log [conc+1] and upper axes show untransformed concentrations of these toxins (in mg/l) for comparison. The regression equations are: a) percent BNR = 12.4+2095.9(log[conc+1]), $r = 0.96$, $p = 0.002$; b) percent BNR = 25.6+36.2(log[conc+1]), $r = 0.82$, $p = 0.024$; c) percent BNR = 9.8+95.1(log[conc+1]), $r = 0.93$, $p = 0.003$; d) percent BNR = 1.7+50.0(log[conc+1]), $r = 0.92$, $p = 0.003$. Each datum point represents 15 replicates.

>atrazine >glyphosate >thidiazuron. Use of regression analyses for prediction of 50% BNR suggests that 2,4–D >glyphosate >atrazine >thidiazuron.

Myriophyllum spicatum appears to be extremely sensitive to 2,4–D. When the concentrations which caused a 50% BNR are compared, the amount of 2,4-D required (0.04 mg/l) was approximately 100 times less than the amounts of the other toxins, which ranged from 1.6 to 9.8 mg/l. Van *et al.* (1986) found that a concentration of 0.08 mg/l was low enough to control *M. spicatum* growth in a flow-through growth system. This concentration is close to that reported for effective control of *M. spicatum* in Kitty Hawk Bay, North Carolina (Getsinger *et al.* 1982). Similarly, Bergquist (1971) found that levels of 1 mg/l 2,4-D caused significant morphological changes. The effective concentration of 0.04 mg/l 2,4-D for 50% BNR in this analysis is in close agreement with these other studies.

This bioassay system was rapid and based on developmental parameters (numbers of branches). Such a system may be more sensitive than measuring mortality, changes in weight or growth. In addition, use of branch number reduction as a parameter provided data that could be used to develop concentration dependent linear regression analyses. Such analyses are key components of bioassay systems (Finney 1978). These analyses support the suggestion of Kane and Gilman (1991) that *in vitro* cultures of *M. spicatum* provide useful systems for toxicity bioassays. *In vitro* cultures of other aquatic plant species should also be useful in toxicological research.

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Peroxidase Changes as Indicators of Herbicide-Induced Stress in Aquatic Plants

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ABSTRACT

Increase in peroxidase enzyme activity and change in number of isozymes expressed have been associated with reactions to environmental stress in terrestrial and aquatic plants. Such alterations may be useful in monitoring herbicide efficacy in submersed weeds. Two aquatic species, Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* (L.f.) Royle), showed a two- and three-fold increase in peroxidase activity, respectively, when exposed to 12- to 48-µg/l fluridone for 30 days.

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Peroxidase levels varied among tissues in untreated watermilfoil, with roots and leaves having higher activity than stems and apical tips. Fluridone-treated hydrilla showed relatively greater increase of this enzyme in tips than in shoots. Extracts from untreated plants of both species were electrophoresed in starch gels, and activity staining revealed peroxidase isozymes.

Key words: enzyme analysis, efficacy, tissue specificity, *Myriophyllum spicatum*, *Hydrilla verticillata*, fluridone.

INTRODUCTION

An early and reliably quantifiable signal of herbicide effect would have value for weed control in the aquatic environment by indicating sufficient contact time, or obviating re-application. Here we present a brief review of peroxidase (PRX) as a general indicator of stress in plants, and summarize some preliminary work on monitoring levels of this enzyme in hydrilla and Eurasian watermilfoil (hereafter called milfoil) following treatment with fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone).

The group of enzymes that have peroxidase (PRX) activity (donor: H_2O_2 oxidoreductases, EC 1.11.1.7) occur in almost all plant tissues. These glycosylated heme proteins, which are monomers of approximately 40 to 50 kD, oxidize certain substrates at the expense of hydrogen peroxide, and rid the cell of excess peroxide produced by metabolism under both normal and stress conditions (10).

PRX analysis is frequently encountered in plant studies due to the enzyme's broad involvement in metabolism and ease of measurement. Changes in PRX activity have been associated with a wide array of physiological processes involved with auxin function and cell wall synthesis, and are readily monitored in crude extracts via the colored products formed by the enzyme as it reacts with a variety of substrates *in vitro* (10, 16, 20, 22, 23).

PRX activity in plant tissue tends to be negatively correlated with auxin level and growth rate, and positively correlated to lignin synthesis (19, 20). Consequently, many normal processes of the plant life cycle are marked by significant fluctuations in PRX levels: abscission, aging and senescence, apical dominance, cold tolerance, dormancy, fruit development and ripening, seed development, germination and early growth ·x expression, and tuberization (1, 20, 22, 24). The association with auxin and lignification has made PRX analysis informative in the study of plant response to external stimuli such as light, temperature, irritation and wounding, parasites and pathogens, and variation in ion status (3, 6, 7, 13, 21).

Although poststress elevation in PRX levels may be limited because of its binding to cell wall components, or to the iron-dependent availability of heme (14), changes in PRX activity have been used to monitor stresses imposed by cold, drought, abscissic acid, gamma irradiation, salt, hypoxia, ion deficiency or toxicity, and pollutants (6, 10). In an evaluation of heavy metal and pesticide contaminants in sediment, Byl and Klaine (5) showed that increasing concentrations from 10 mg/l of the herbicide sulfometuron methyl (methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl] benzoate) produced a dose-dependent elevation in PRX activity in unrooted apical portions of hydrilla.

Instances of stress-induced response suggested that alterations in PRX activity and isozyme expression following exposure to certain herbicides could be used to monitor stress or indicate lethal dose in submersed aquatic plants. To assess PRX response to fluridone, a slow-acting herbicide which suppresses carotenoid synthesis, we evaluated protocols for activity and isozyme analysis in hydrilla and milfoil, and monitored enzyme levels in untreated and treated tissues.

MATERIALS AND METHODS

Extraction. Crude extracts of plant tissue contain levels of PRX activity that readily support spectrometric and electrophoretic investigation. In the average plant cell the majority of peroxidases are cytoplasmic, with approximately 55% characterized as freely soluble, and 30% as less freely soluble. Of the remaining 15% which are complexed to cell walls and membranes, the ionically bound 10% can be extracted with salt solutions (13). The rest are covalently bound and insoluble, but able to be extracted following cellulase and pectinase treatments (20).

Acetate and phosphate solutions between 4.5 and 7.0 in pH are common extraction buffers for soluble PRX (*e.g.* 3, 15), with salt solutions such as $CaCl_2$ and KCl being used to release ionically bound enzyme (5, 13, 21). Buffer additives for enzyme stabilization include EDTA, mannitol, insoluble polyvinylpyrolidone, ascorbate, *etc.* (1, 22). While mercaptoethanol is often used to protect activity of other enzyme systems during extraction for electrophoretic analysis, it interferes with measurement of PRX function.

Substrates. A variety of phenolic or aromatic compounds which are converted to a colored end-product in the presence of H_2O_2 are used as substrates for PRX analysis. Guaiacol, ferulic acid, *o*-dianisidine, pyrogallol, phenol, and benzidine are commonly used in kinetic studies (16, 20, 22). For isozyme development following electrophoresis, an insoluble end-product which will precipitate in the matrix at the location of enzyme activity is required. Suitable substrates include 3-amino-9-ethyl carbazole, 4-chloro-1-naphthol and 3,3'-diamino-benzidine (12).

Some PRX isozymes are capable of using a variety of cellular substrates (11), while others are substrate-specific. When guaiacol is converted to tetraguaiacol in the presence of the plant extract, the analysis is said to test for "guaiacol peroxidase" (6). Use of ascorbate will monitor the activity of those isoforms with a high preference for this compound as a reductant; these are termed "ascorbate peroxidases" (1, 16).

Spectrophotometric assays. Once plant extract is added to a suitable reaction solution containing substrate, H_2O_2, and buffer, activity is characterized by monitoring change in absorbance (optical density) at a specific wavelength over a suitable linear portion of the kinetic curve, usually during the first 5 min of the reaction. The rate of change is reported for a unit time, either per weight of tissue or per total protein concentration in the crude plant extract, quantified with methods such as the Lowry or Bradford tests, usually with the bovine serum albumin standard (4, 11). Activity is also reported as absorbance units based on defined changes in optical density; or as units of enzyme activity, calculated from a standard curve based on commercially quantified horseradish PRX (5, 18). Other definitions of activity include rate of conversion of substrate, based on the extinction coefficient of the product (24), or rate of H_2O_2 reduction (1). Optimal wavelength for absorbance measurement differs with the color properties of the reaction product. For a guaiacol substrate, readings are usually taken at 470 nm; for pyrogallol, at 430 nm. The reaction with ascorbate produces a compound which decreases absorbance of the mixture, and it is monitored in the UV range at 290 or 265 nm (1, 16).

Electrophoresis. PRX occurs in multiple molecular forms (isozymes) which can represent allelic differences at the level of the gene, or post-translational modifications of the same transcript. Application of crude plant extracts to starch gels allows the separation of differently charged PRX isozymes. The cationic forms tend to be associated with auxin-mediated growth regulation, and the more numerous anionic ones with cell wall formation (9, 14). The number of isozymes resolved and their direction of migration depend partly on the pH of the solutions used; many common buffer systems resolve clear tissue-specific profiles for PRX. Analysis of various plant parts with different reaction substrates can show whether particular isozymes are induced or suppressed in response to herbicide treatment.

Current protocols. Material assayed for PRX in this study was taken from plantings initiated in October 1991 and March 1992. Apical portions of milfoil or hydrilla were placed into sediment and grown in 55-L aquaria in a controlled-environment chamber, following previously described regimes for establishment, radiation, day length, and fertilization (17). Untreated milfoil was analyzed from the first planting. In the second, established hydrilla and milfoil were exposed to fluridone concentrations of 0, 12, 24 and 48 µg/l for 30 or 60 days. Following treatment, herbicide was flushed from the aquaria and replaced with fresh water. PRX activity was monitored pretreatment and at 8, 30 and 60 days after treatment (DAT) was initiated. Biomass data were collected and reported elsewhere (Netherland, Getsinger and Turner, in press).

For PRX analysis, plant parts were harvested immediately before extraction and placed on ice. Tissue was blotted, and a 1.0-, 0.5-, or 0.25-g sample was macerated with a chilled mortar and pestle using 0.1 M $NaPO_4$ buffer, pH 6.1 (22) or 0.5 M $CaCl_2$ (5). The extract was poured into a chilled test tube and centrifuged at 2000 g for 5 min. The supernatant was removed and the pellet washed with buffer in a repeat centrifugation. Supernatants were then pooled and filtered through one layer of Miracloth (Calbiochem). A ratio of either 1:10 or 1:20 g fresh weight of tissue to total milliliter volume of extraction buffer was maintained.

Plants were sorted by tissue before analysis. The uppermost 5 cm of apical or axillary growing points were designated as tips; the portion of plant from 5 to 30 cm below apices was used as shoot (stem plus leaves) or separated into leaves and stem. Roots were cleaned of sediment before use.

A 200-µl aliquot of supernatant was combined with 2.8 ml of a reaction solution, consisting of either 0.1 M $NaPO_4$, 4 mM guaiacol, and 3 mM H_2O_2 (22), or 5 mM MES, pH 6.0, 80 mM phenol, 44 mM H_2O_2, and 2 mM aminoantipyrine (5). Absorbance was monitored at 470 nm (guaiacol) or 510 nm (phenol), with readings taken at the end of the first and third minutes after mixing the plant extract and reaction solution together; the rate of activity was reported as change in absorbance per minute per fresh weight of tissue. Three reactions from each extract were run to produce a mean activity per sample. Analysis of variance was done, and treatment means were separated with a Bayesian LSD at the 95% confidence level.

For the horizontal starch gel electrophoresis, the tank (bridge) buffer used was 0.03 M lithium hydroxide (monohydrate) and 0.19 M boric acid, pH 8.1. The same solution was combined with a buffer of 0.05 M TRIS and 6 mM citric acid, pH 8.4, in a ratio of 1:10, and heated with 10 to 12% hydrolyzed starch to produce the gel matrix. Crude plant extract was applied to the solidified gel using paper wicks. The apparatus was placed inside a refrigerator at 4C, and a constant current was applied to the gel for 4 hr before developing in a 0.1 M Na acetate, pH 5.0, solution containing 4 mM 3-amino-9-ethyl carbazole, 10% N,N-dimethyl formamide, and 15 mM H_2O_2.

RESULTS AND DISCUSSION

The protocols described were successful in monitoring activity and isozymes of PRX in crude extracts produced from aquarium-grown hydrilla and milfoil following treatment with various aquatic herbicides. They have also been found applicable to egeria (*Egeria densa* Planch.; data not shown).

Tests of the extraction procedure showed that little additional PRX activity (0.9% of the total found) was retrieved by washing the pellet a second time, and the single pellet wash method was used for this work. Many herbicide compounds have ring structures, and compounds to be used for treatment are evaluated to ensure that they do not act as PRX substrates when substituted for phenol or guaiacol in reaction solutions. No herbicide-generated activity has been seen.

The different extraction buffers and reaction substrates used in this preliminary work produced varying levels of PRX activity from the same plant material (data not presented). From this we assume that the protocols monitor soluble (PO_4-extracted) and ionically bound ($CaCl_2$-extracted) isozymes, which can differ in activity and substrate specificity. By extracting both types of isozymes from a range of tissues, and reacting them with more than one substrate, it may be possible to describe activity of the various forms of PRX more fully.

Tissue specificity of PRX isozymes in plants is well-established (10), and initial electrophoretic examination of untreated material showed variation in isozyme profiles between leaves and roots in milfoil and among leaves, roots and tubers in hydrilla (data not presented). Analyzing plant parts separately for enzyme activity provided information about PRX levels in untreated tissues and revealed differential changes following herbicide contact.

The low activity measured in apical tips and stem of untreated milfoil from the first planting, compared to leaves and root (Figure 1), is consistent with relative levels of PRX found in other species and with the negative correlation found between PRX and auxin in plant tissues in general (2, 8, 14). Pretreatment levels of PRX in milfoil and hydrilla leaves from the second planting were approximately twice those of shoots or of apical portions (data not shown). Differential increase in enzyme activity was seen in fluridone-treated hydrilla, where apical tips treated with 12 and 24 µg/l were significantly higher in activity than shoots or reference material at 8 DAT (Figure 2). (Milfoil was not analyzed at this time.)

Pre- and posttreatment tissue differences in PRX levels require that well-defined samples be used when activity is reported relative to weight of plant material. The nonparallel increase in PRX between treated tips and shoots shows the desirability of monitoring enzyme changes in more than one tissue. One plant part may be more indicative of stress than

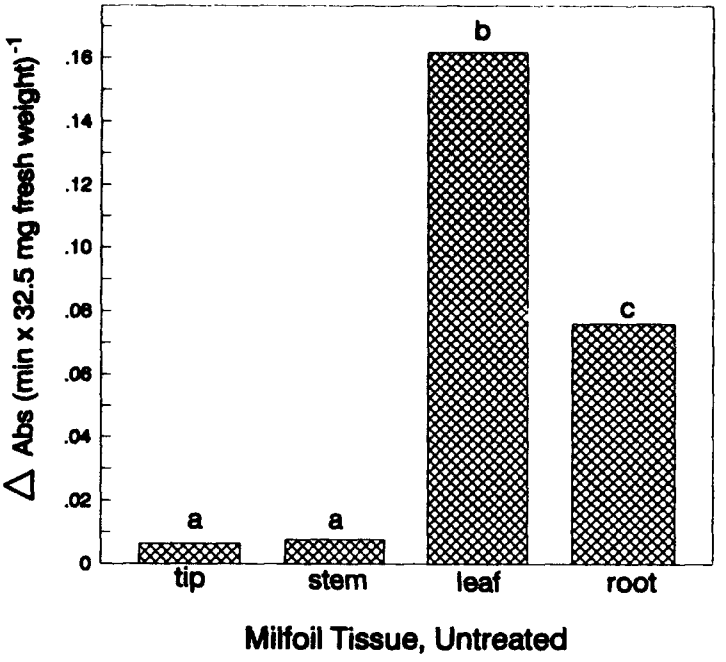

Figure 1. Levels of PRX activity in Eurasian watermilfoil tissue taken from untreated aquarium-grown plants, following reaction with guaiacol substrate. Different letters indicate significant differences between tissue means at the 5% level according to a Bayesian LSD test.

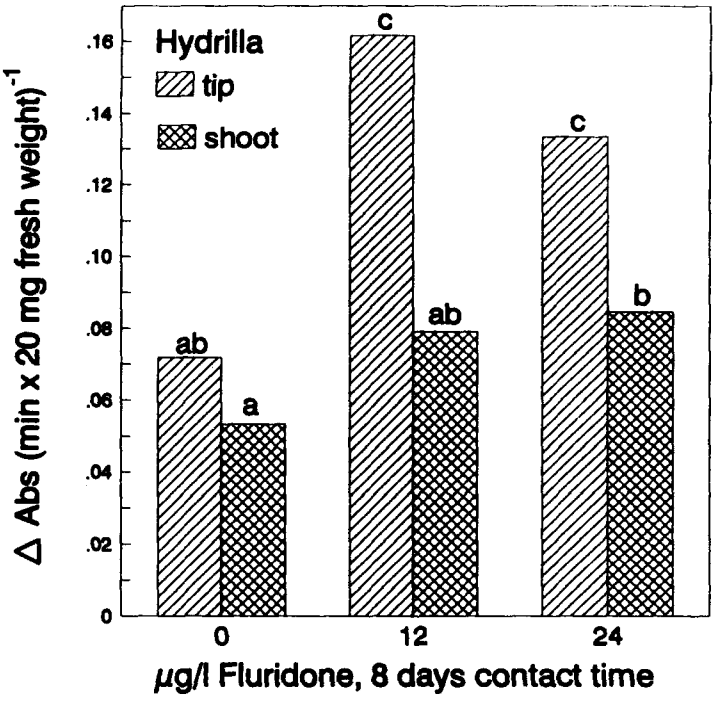

Figure 2. Levels of PRX activity in tips (apical 5 cm) and shoots (stems and leaves below 5 cm) of aquarium-grown hydrilla following 8 days contact time with fluridone at 0, 12 and 24 µg/l. Activity reported following reaction with phenol substrate. Different letters indicate significant differences between treatment means at the 5% level according to a Bayesian LSD test.

another, depending on the herbicide's mode of action. With fluridone treatment, stem apices in hydrilla and milfoil become bleached as chlorophyll is reduced. The elevated PRX seen in hydrilla tips suggests a localization of stress response.

Elevated PRX activity with fluridone application was seen in both species. At the end of a 30-day exposure time, milfoil treated with 12, 24 and 48 µg/l was approximately three times higher in activity than reference material, although differences among concentrations were not significant (Figure 3). Shoots from hydrilla treated with 48 µg/l had significantly higher levels than those from plants exposed to lesser concentrations or from untreated material (Figure 3). At 60 DAT, PRX levels in apical tips and shoots of milfoil given 30-day exposure and 30-day recovery did not differ significantly from untreated material, while activity in plants treated continuously for 60 days at 12 and 24 µg/l remained significantly elevated in both tissues (data not shown).

Strong correlations between increase in PRX and herbicide efficacy were not shown here. The 30-day exposure to fluridone resulted in reduced shoot biomass by 90 DAT only in hydrilla treated at 48 µg/l (Netherland, Getsinger and Turner, in press), whereas elevated PRX had been found in both species at 8 and 30 DAT. Milfoil treated for 60 days, which had elevated shoot and tip PRX at that time, did remain reduced in biomass at 90 DAT.

These initial results suggest that PRX monitoring of various tissues may help describe the chronology and severity of herbicide stress in aquaria-grown plants, and be applicable to use in mesocosm systems and the field. By relating PRX activity to other stress indicators, such as phenolic compounds or enzymes such as superoxide dismutase, catalase, and polyphenoloxidase (13, 24), more informative descriptions of metabolic status in both target and nontarget plant species following herbicide treatment may be produced.

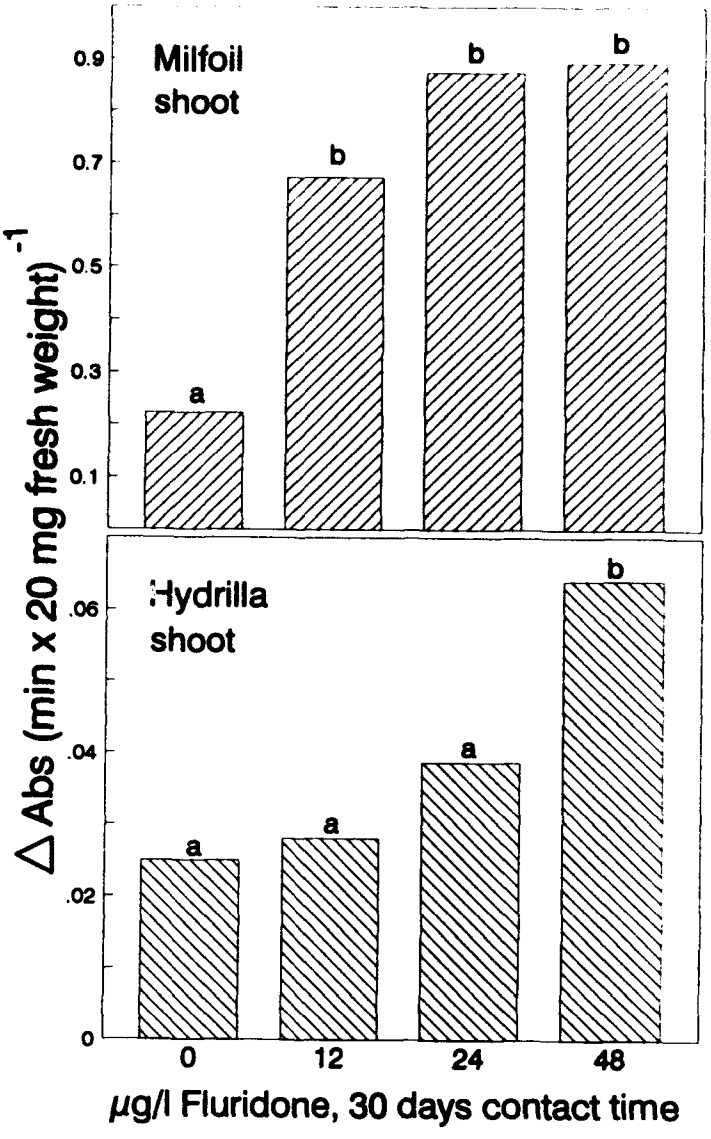

Figure 3. PRX activity in shoots of aquarium-grown Eurasian watermilfoil and hydrilla following 30 days contact time with fluridone at 0, 12, 24 and 48 µg/l. Activity reported following reaction with guaiacol (milfoil) or phenol (hydrilla) substrate. Different letters indicate significant differences between treatment means at the 5% level according to a Bayesian LSD test.

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Effects of Endothall and Other Aquatic Herbicides on Chlorophyll Fluorescence, Respiration and Cellular Integrity[1]

G. E. MACDONALD, D. G. SHILLING AND T. A. BEWICK[2]

ABSTRACT

Part of the mode of action of several aquatic herbicides is cellular disruption which can be caused by the generation of oxygen radicals or loss of adenosine triphosphate (ATP) needed to maintain cellular integrity. In an attempt to distinguish between the varying mechanisms by which certain compounds cause cellular disruption, ion leakage (light and dark regimes), chlorophyll fluorescence, and oxygen consumption (a normal consequence of respiration) were monitored over time from leaf tissue exposed to endothall, simazine, dinoseb, diquat and gramicidin. All compounds, except simazine, caused high ion leakage in both light and dark. Diquat caused more rapid leakage in light, while endothall and gramicidin caused more rapid leakage in the dark. Diquat, dinoseb and simazine increased chlorophyll fluorescence, but endothall and gramicidin did not. Oxygen consumption was stimulated by gramicidin and diquat but inhibited by endothall and dinoseb. Comparing the effects of compounds with known mechanisms-of-actions on ion leakage, chlorophyll fluorescence and oxygen consumption suggest that endothall acts to inhibit respiration.

Key words: dinoseb, diquat, simazine, gramicidin, conductivity, oxygen radical, mode-of-action, cucumber.

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INTRODUCTION

Endothall (7-oxabicyclo[2,2,1]heptane-2,3-dicarboxylic acid) has been used in aquatic plant management since the early 1960's (7) and provides good control of many submersed species (19,25). In addition, endothall is or has been used as a defoliant in cotton (17) and other crops (18,26), as a potato-vine dessicant (5), and for selective weed control in sugar beets (27) and turf (24). Endothall is classified as a phthallic acid herbicide (1) and is a derivative of cantharidin (23) which is a natural compound produced by the blister beetle (*Epicauta* spp.) that causes burning and blistering of the skin (3).

Several formulations of endothall (mainly salts) have been or are presently registered for various weed management uses. The halflife of the potassium and sodium salt formulations in the aquatic environment is 2 to 3 days under normal conditions, while the alkylamine salts are more persistent (14 to 21 days). Microbial degradation is the major mechanism of dissipation (15,16). Although accumulation of endothall is limited due to its short halflife, this compound in concentrated form is highly toxic: LD_{50} (rat) for technical endothall acid is 38 to 51 mg/kg, 182 to 198 mg/kg for Na and K salts, and 206 mg/kg for the amine salt formulation (9).

When applied to the soil, endothall is taken up by plant roots and translocated via the transpiration stream. Endothall is not phloem mobile (10). In contrast, movement in aquatic plants is limited to the symplast (20) and uptake in hydrilla [*Hydrilla verticillata* (L.f.) Royle] is enhanced by high temperatures and low light levels (6). Endothall affects several plant processes including lipid (11) and protein synthesis (12) and dipeptidase and proteinase activities (21). Furthermore, Penner and Ashton (14) found that endothall decreased proteolytic activity and was similar to that caused by actinomycin D. From this they postulated that endothall interferred with mRNA metabolism.

Endothall is considered to be a membrane-active compound, causing cellular disruption of plant tissue within 2 to 5 days (10). Herbicides that cause rapid cellular disruption are not mobile in plants because cellular integrity is essential for translocation. This may help to explain the limited phloem mobility of endothall. Herbicides that interfere with protein, lipid, or amino acid synthesis often require 2 to 4 wk to cause plant death after initial application. The symptomology of plants treated with these types of herbicides includes discoloration and stunting. However, endothall produces necrotic lesions and an overall browning of the tissue, characteristic of an oxygen radical generating compound. Therefore, the reported mechanisms-of-action do not appear to adequately explain the symptoms of plants treated with endothall. The objective of this research was to determine a more plausible mechanism-of-action for endothall. This was accomplished by utilizing compounds with known mechanisms of action and comparing their effects on chlorophyll fluorescence, oxygen consumption and cellular integrity to those effects caused by endothall.

MATERIALS AND METHODS

Plant material for all experiments was obtained from approximately 10-day-old cucumber (*Cucumis sativa* L.) seedlings ('Poinsett 76') which were grown in potting soil (Metro-mix 200) in a growth chamber under the following conditions: 14 hr light/10 hr dark photoperiod with an average light intensity of 350 $\mu mol\ m^{-2}\ sec^{-1}$ at 25C. Leaf disks (0.9 cm^2) were excised from the cotyledons and utilized similarily in all experiments.

Ion leakage. Four leaf disks were placed in distilled water (control) or 4 ml of solutions containing one of the following compounds: herbicides; endothall, simazine (6-chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine), dinoseb[3] (2-sec butyl-4,6-dinitrophenol), diquat (6,7-dihydrodipyrido[1,2-α:2',1'-c]pyrazinediium ion); respiratory poisons; gramicidin S[3]. Treated tissue was maintained under continuous light (400 $\mu mol\ m^{-2}\ sec^{-1}$) or in continuous dark conditions at 25C. Conductivity (μmhos/cm) was measured utilizing a conductivity bridge[4] at 6, 12, 18, 24, 36, and 48 hr after initial exposure. Dark-adapted tissue was assayed at the same times as those under light conditions, with an additional 72-hr measurement. Initial measurements of conductivity were taken on the solutions alone. Following completion of the experiment, the tissue was frozen and thawed twice to release all ions, providing a measurement of total conductivity. Treatment concentrations of compounds in all studies were: 10 mM endothall, 0.1 mM dinoseb, 0.1 mM diquat, 0.1 mM gramicidin, and 0.1 mM simazine. These concentrations were the approximate I_{50} concentrations based on preliminary studies (data not shown). However, for simazine, an accurate I_{50} value could not be determined due to limited activity under the described experimental conditions. Data are presented as percent conductivity derived from the following equation: % conductivity = ((measured-initial)/(total-initial))*100; where measured equaled the amount of conductivity at each time of measurement.

Fluorescence. Leaf disks were exposed to the same compounds (with the exception of gramicidin) and I_{50} concentrations used in the ion leakage study, but were placed under continuous light (400 $\mu mol\ m^{-2}\ sec^{-1}$) for 2, 4, and 6 hr

[3]Sigma Chemical Company, St. Louis, MO 63178.

[4]Model 31, Yellow Springs Instrument Co. Inc., Yellow Springs, OH 45387.

after initial exposure. Before determining chlorophyll *a* fluorescence treated tissue was equilibrated for 10 min in complete darkness. Induction curves were recorded and data analyzed using initial (after 5 ms), peak (after 1 sec) and steady-state (after 50 sec) time intervals. Data are presented as the ratio of peak/terminal fluorescence as follows: (peak-initial)/(terminal-initial).

Oxygen consumption. Twenty-five leaf disks were exposed in the dark to treatment solutions with the exception of simazine which was omitted. Leaf disks were vacuum infiltrated with the treatment solution for 20 min to eliminate possible diffusion differences between the compounds. Since vacuum infiltration removes dissolved oxygen, leaf disks were transferred to fresh solutions. The oxygen probe was then placed in the solutions and containers were sealed to prevent exposure to outside air. Oxygen concentrations (mg/l) were monitored 0.0 (initial), 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 hr after initial exposure using an oxygen probe[5]. Data are presented as oxygen consumption, derived from the formula: Oxygen consumption (mg/l) = (oxygen initial - oxygen measured), where oxygen measured represents levels at time of measurement.

Data were subjected to analysis of variance to determine if the effects from herbicides were significant. Because the responses as a function of time and herbicides were consistent for both experiments ($P < 0.05$) data are presented averaged over experiments. However, time of evaluation and herbicides differentially influenced the responses ($P < 0.05$) and data are presented accordingly. Standard errors of the mean (four replications) are present for ion leakage and fluorescence whereas oxygen values were regressed to obtain a best-fit model ($P < 0.05$).

RESULTS

Ion leakage. Following exposure, simazine caused less than a 20% increase in conductivity in the light (Figure 1), while gramicidin caused increases of 30 and 55% after 6 and 48 hr, respectively. Diquat and dinoseb also caused dramatic increases in conductivity with diquat causing a slightly larger increase than dinoseb. However, by 48 hr both compounds produced nearly a 100% increase in conductivity. The effect from endothall was more gradual, with a 50% increase after 24 hr. However, by 48 hr endothall also caused nearly a 100% increase in conductivity.

Gramicidin caused more rapid ion leakage in the dark than in the light and resulted in almost 90% ion leakage after 72 hr (Figure 2). The effect from diquat closely mirrored that of gramicidin in the dark, but was slighty less until 72 hr, when

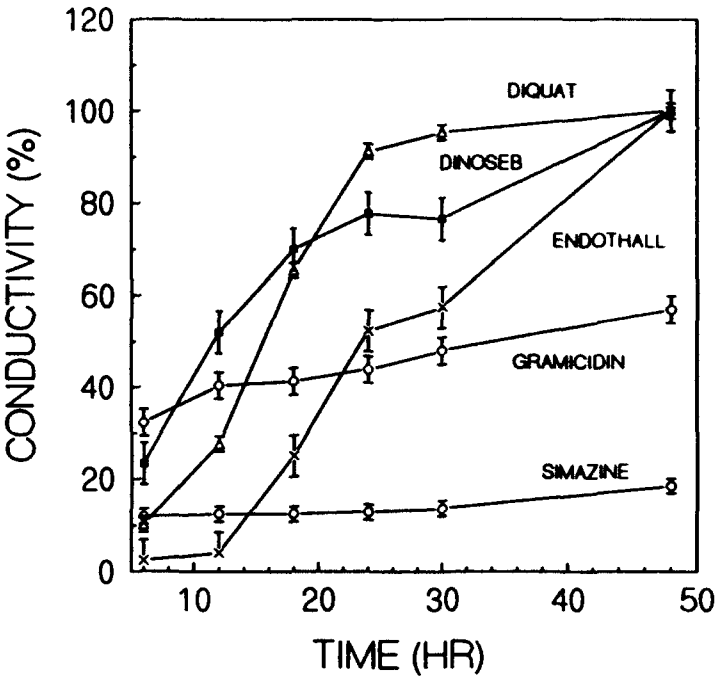

Figure 1. The effect of simazine, gramicidin, dinoseb, diquat and endothall on ion leakage from cucumber leaf disks in the light.

it also caused 90% leakage. The effect of diquat in the dark was markedly reduced from that produced in the light. Dinoseb produced similar results in the dark compared to the light, while the effect from endothall in the dark was greatly enhanced. Endothall caused a greater than 30% increase in conductivity after 6 hr and nearly 90% after 24 hr in the dark. The effect caused by simazine in the dark was neglible.

Fluorescence. Dinoseb, simazine, and diquat lowered the peak/terminal ratio, indicating increased chlorophyll *a* fluorescence relative to the control 2, 4, and 6 hr after treatment (Figure 3). Endothall did not affect fluorescence until 6 hr after treatment when fluorescence decreased below that of the control, probably due to indirect effects caused by membrane disruption. However, endothall caused minimal effects on chlorophyll fluorescence compared to the other compounds tested. Gramicidin did not affect fluorescence regardless of time (data not shown).

Oxygen consumption. Gramicidin and diquat caused a rapid increase in oxygen consumption (Figure 4) in the first 2 hr of exposure. After 2 hr, the rate of oxygen consumption caused by diquat slowed but gramicidin-treated tissue continued to consume oxygen at a rapid rate. Dinoseb and endothall reduced the ability of the tissue to use oxygen, with the effect from dinoseb being slightly greater than that of endothall.

[5] Monitor II, Beckman Instruments, Inc., Irvine, CA 92713.

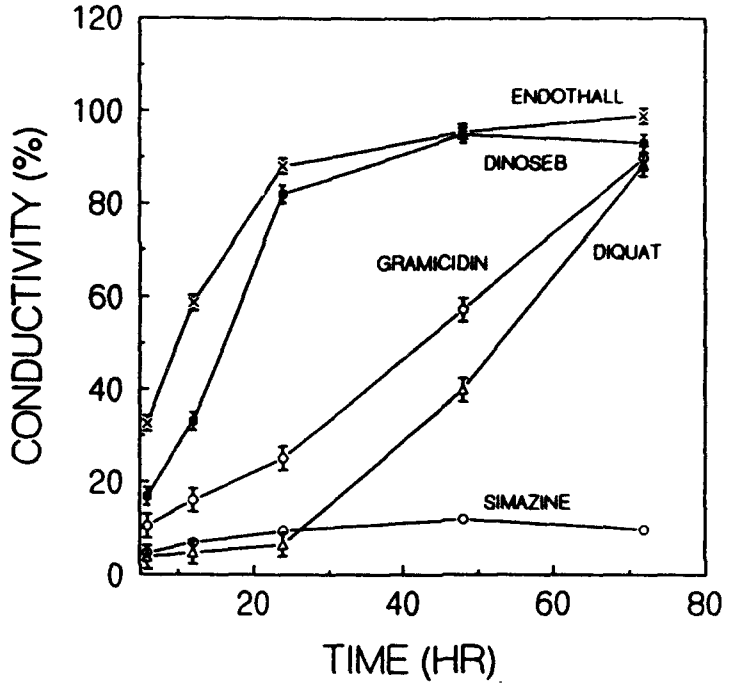

Figure 2. The effect of simazine, gramicidin, dinoseb, diquat and endothall on ion leakage from cucumber leaf disks in the dark.

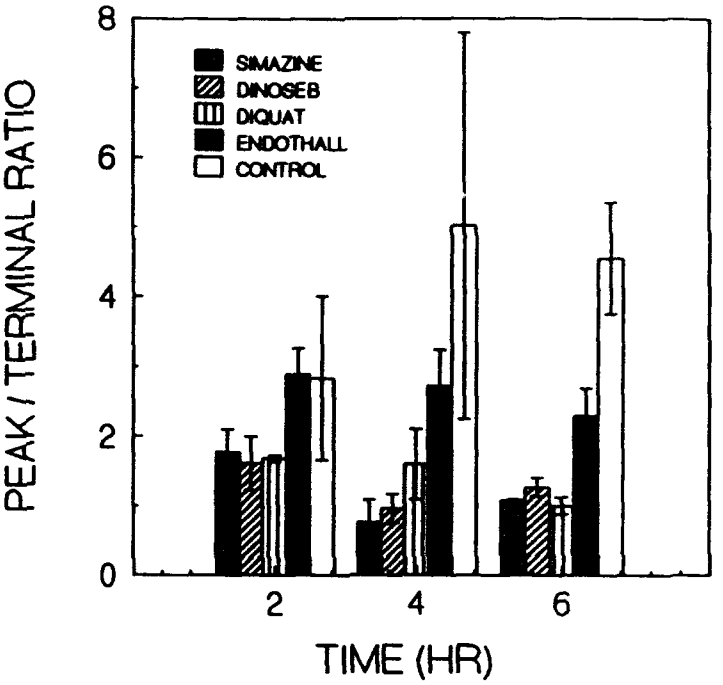

Figure 3. The effect of simazine, dinoseb, diquat and endothall on chlorophyll *a* fluorescence in cucumber leaf disks.

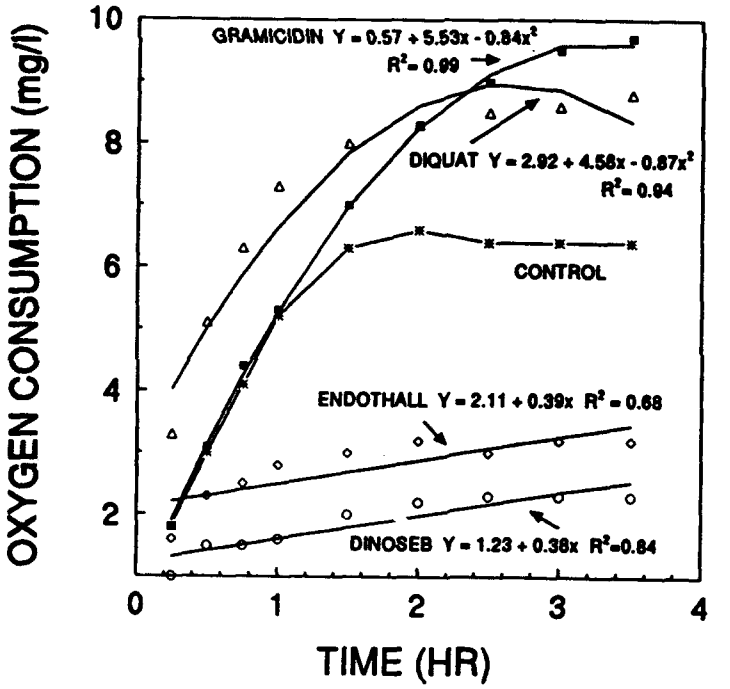

Figure 4. The effect of gramicidin, dinoseb, diquat and endothall on oxygen consumption (mg/l) in cucumber leaf disks.

DISCUSSION

Rapid ion leakage and cellular disruption caused by endothall occurred under both light and dark conditions. However, the effect from this compound was greater in the dark, indicating the mechanism-of-action of endothall is not light dependent. Since all energy under dark conditions is produced via respiration, these data implicate respiratory inhibition by endothall.

Dinoseb and diquat are known to affect both photosynthesis and respiration, injuring the cell through two separate mechanisms (2,13). Therefore, in the light these compounds cause injury through a disruption of photosynthesis (as evidenced by change in fluorescence) and respiration, whereas in the dark they only affect respiration. This is evident from elevated ion leakage in the light, whereas both these herbicides caused slower rates of leakage in the dark. Therefore, the activity of these compounds in the light was additive. These compounds are known respiratory poisons, acting directly on the cell's ability to produce adenosine triphosphate (ATP)(4). The loss of ATP causes the cell to 'leak' because its ability to maintain electro-chemical gradients is diminished. Although some oxidative stress is induced by inhibition or diversion of oxidative electron flow, the major cause of

membrane disruption is the collapse of the membrane gradient due to a lack of energy. Endothall appears to be acting similarly to the respiratory poisons in the dark; however, the increase in activity under dark conditions correlates with a compound that only inhibits respiration. This is because under light conditions, photosynthesis would provide some energy for respiration, thus diminishing the activity of endothall.

Gramicidin directly affects respiration but has little influence on photosynthesis (22). This compound also caused greater ion leakage in the dark. The similarity of endothall to gramicidin provides additional evidence that endothall affects respiration.

Chlorophyll fluorescence is often measured to determine the effect of various compounds on the light reactions of photosynthesis (15). In normal light reactions, light is absorbed by chlorophylls and other pigments and transmitted to reaction centers where light energy is converted to chemical energy through the donation of electrons. However, not all of the energy absorbed by chlorophyll molecules can be utilized and some is re-radiated as fluorescence (2). Normal fluorescence values for the ratio of peak to terminal for our study ranged from 3 to 5. This ratio indicates the ability of the plant to utilize light energy with higher ratios corresponding to more efficient light use, while low ratios (near 1.00) indicate that most of the energy is being lost to fluorescence.

Both simazine and dinoseb produced very low peak to terminal fluorescence ratios, which is characteristic of their known mechanisms-of-action. These compounds block electron flow at photosystem II, causing a feed-back effect (2). Chlorophyll molecules continue to absorb light energy, and must re-radiate most of this energy as fluorescence to avoid photo-oxidation. Diquat also produced low ratios, but this was probably due to the degradation of the photosynthetic apparatus by oxygen radicals. This is characteristic of diquat's mechanism-of-action. Endothall had virtually no effect on chlorophyll *a* fluorescence but lower peak/terminal ratios occurred after 6 hr in conjunction with significant ion leakage. Once ion leakage increases, the disruption of the photosynthetic apparatus can occur and the use of chlorophyll fluorescence to determine the mechanism of action is diminished.

In this study, endothall and dinoseb severely reduced the ability of cucumber tissue to utilize oxygen for respiration, while diquat and gramicidin increased oxygen consumption. Gramicidin acts directly on the mitochondrial membrane by dissipating the pH and charge gradient that allows ATP production (22). The cell increases its respiration rate and consumes more oxygen. Diquat, on the other hand, acts as an alternative reductant, diverting electrons away from the electron transport chain and utimately to oxygen. This also decreases the gradient, increasing respiratory rate, and creates a greater demand for oxygen.

Dinoseb inhibits respiration at site IV in the oxidative electron transport chain, blocking the flow of electrons to oxygen (13). Oxygen consumption can also be inhibited by compounds that block phosphorylation, causing an increase of the gradient and feedback inhibition of respiration, and resulting in a decrease in oxygen consumption. Endothall may be acting at either of these two sites to inhibit respiration.

In conclusion, higher ion leakage in the dark, minimal effects on fluorescence, and a reduction in oxygen consumption collectively indicate that endothall is a respiratory poison. In addition, endothall has been suggested to affect the mitochondria of animals (8) and the mode-of-action in plants may be similar.

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Effect of Fluridone on Chlorophyll, Carotenoid and Anthocyanin Content of Hydrilla[1]

R. L. DOONG, G. E. MACDONALD AND D. G. SHILLING[2]

ABSTRACT

Hydrilla (mature and young plants) were exposed to 0.05, 0.5, 5.0, and 50 ppb of fluridone for 2, 4, 6, 8 and 12 wk and monitored for changes in chlorophyll, carotenoid and anthocyanin content. Fluridone decreased carotenoid and chlorophyll content of mature hydrilla plants. As fluridone exposure times and rates increased, chlorophyll and carotenoid content decreased concomitantly. Regardless of time, 50 ppb fluridone reduced carotenoid and chlorophyll content by 80 to 95%. In younger plants, 50 ppb fluridone lowered carotenoids and chlorophyll by at least 50 and 65%, regardless of time, respectively. Fluridone at 50 ppb caused an increase in anthocyanin content (5X the control) in mature hydrilla but did not affect anthocyanin content in young hydrilla. However, both plant types became pink in color after exposure to fluridone. Apparently, anthocyanins were simply unmasked after chlorophyll photooxidation in young hydrilla while an increase in anthocyanin biosynthesis occurred in the mature plants. The differential response in anthocyanin content of hydrilla to fluridone could be related to physiological stage of development and/or light intensity.

Key words: plant pigments, stress, herbicide, photooxidation.

INTRODUCTION

Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone) is often used in Florida to control hydrilla (*Hydrilla verticillata* (L.f.) Royle)(21), a submersed aquatic macrophyte that is a major problem throughout Florida and many other areas (7,9). Fluridone is frequently referred to as a "bleaching herbicide," due to the characteristic white coloration of treated tissue (17). Fluridone blocks the synthesis of carotenoids which are pigments essential for normal plant growth (16). Carotenoids function to protect the photosynthetic system from photodynamic damage by quenching triplet-state chlorophyll and singlet oxygen (1,5,6). In the absence of carotenoids, chlorophyll would photooxidize resulting in bleached, white tissue.

Plants grown under high light conditions will normally produce more carotenoids to offset the increase in light-

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generated oxidative stress. Therefore, the effect of a carotenoid-inhibiting compound will be exacerbated under high light intensity due to a higher rate of photooxidation (1,5,15) and growth (*i.e.*, higher demand for *de novo* synthesis of carotenoids). However, hydrilla and many other submersed aquatic macrophytes persist in areas of very low light intensity yet fluridone provides good control.

Generally, apical meristems and new growth are the first to display the characteristic changes in pigmentation resulting from fluridone treatment. These symptoms are followed by a deterioration of the tissue and eventual plant death. However, hydrilla and certain other aquatic plants produce a pink coloration at the growing tips after exposure to fluridone. Pink coloration in plant tissue is generally attributed to anthocyanins (10), but little research has been conducted on the effect of bleaching herbicides on these pigments.

Control of hydrilla with fluridone is highly dependent upon adequate exposure time and concentration (8). Pigment levels are a measure of plant viability, and knowledge concerning the effect of fluridone on these levels could be used to improve management. Furthermore, the effects of fluridone on chlorophyll and carotenoid content in hydrilla have never been fully documented. Therefore, the objective of this study was to evaluate the effect of fluridone on the chlorophyll, carotenoid and anthocyanin content in hydrilla over time.

MATERIALS AND METHODS

Plant culture. Hydrilla was planted (2/22/91) from apical stem segments in 10- by 10-cm^2 pots (4 segments/pot) filled with organic potting medium. The medium was amended with a slow-release fertilizer and a 1-cm-thick sand cap added to prevent floating. The plants were allowed to grow under greenhouse conditions (16 hr light/8 hr dark photoperiod, 1000 $\mu mol \cdot m^{-2} sec^{-1}$ (PAR) average light intensity at noon, 30C day/20C night) for approximately 7.5 months in a 900-L plastic-lined tank (150 by 100 by 60 cm). Hydrilla rapidly formed a dense mat at the water surface which persisted until the experiment was initiated.

Plants (15 pots/vault - 5 harvest dates x 3 replications) were transferred to 900-L concrete vaults (217 by 76 by 55 cm) on 10/11/91. At the same time 10-cm apical stem segments (young plants) were established in a manner similar to that described for mature plants (2 segments/pot) and grown concomitantly with the mature plants. On 10/16/91 the following concentrations of fluridone were established in the vaults: 0.0, 0.05, 0.5, 5.0, or 50 ppb. The plants were maintained outdoors under natural conditions (short-day photoperiod). After 2, 4, 6, 8 or 12 wk of exposure to fluridone, two apical shoot segments from each treatment and age group (approximately 0.1 g fresh weight each) were excised and analyzed for chlorophyll, carotenoid and anthocyanin content.

Chlorophyll/carotenoid analysis. Apical meristems were homogenized in 15 ml of chloroform/methanol (2:1, v/v) for 3 min on ice. The homogenate was filtered and the crude extract then dried under a stream of nitrogen at room temperature under dim light (<5 $\mu mol \cdot m^{-2} \cdot sec\text{-}1$). The residue was resuspended in 2 ml of 80% acetone and absorbances at 663, 646 and 470 nm were measured spectrophotometrically. Total chlorophyll concentration was calculated utilizing the following formula: ($17.32 \times A_{646} - 7.18 \times A_{663}$). Total carotenoid content was calculated using the following formula: ($1000 \times A_{470} - 3.27 \times Ca - 104 \times Cb$)/229, where $Ca = 12.21 \times A_{663} - 2.81 \times A_{646}$ and $Cb = 20.13 \times A_{646} - 5.03 \times A_{663}$. Ca and Cb are the concentrations of chlorophyll *a* and *b* in µg/ml (13).

Anthocyanin analysis. Apical meristems were homogenized in 15 ml of methanol containing 1% HCl (v/v) for 3 min on ice. The homogenate was then filtered with suction and the absorbance of the extract determined at 530 and 657 nm, spectrophotometrically. Chlorophyll has some absorbance at 530 nm in acidic methanol. Therefore, corrections were made by subtracting the absorbance contributed by chlorophyll under acidic conditions at 657 nm using the formula $A_{530} - 0.25 \times A_{657}$ as described by Mancinelli (14) which assumes an extinction coefficient of 34,000 $M^{-1}cm^{-1}$ (11).

Statistical analysis. Data were initially analyzed by analysis of variance to test for treatment effects and interactions. Age by rate interactions were significant ($P < 0.05$) and data are presented accordingly. Treatment means within a harvest interval were compared to the untreated control utilizing Dunnett's 'T' test at the 0.05 level of significance.

RESULTS

Mature plants. Carotenoid concentration in the mature plants was significantly reduced by fluridone with higher rates causing a greater decrease (Table 1). Carotenoid content was reduced to near zero by 50 ppb fluridone at 2, 6 and 12 wk. Fluridone at 5.0 ppb caused a 75% reduction at 2 and 6 wk, and by 12 wk carotenoid levels were near zero.

Fluridone at 5.0 and 50 ppb reduced chlorophyll by at least 65 and 80%, respectively, at all weeks (Table 1). Between 6 and 12 wk after treatment 0.5 ppb fluridone caused reductions of 44 to 57%. Conversely, chlorophyll content was significantly increased at 0.05 ppb fluridone 2, 8 and 12 wk after initial exposure.

Fluridone at the 50-ppb concentration increased anthocyanin content 322, 293, 275, 132, and 40% after 2, 4, 6, 8, and 12 wk of treatment, respectively. Concentrations of 0.05 and

TABLE 1. EFFECT OF FLURIDONE (ppb) ON CAROTENOID, CHLOROPHYLL AND ANTHOCYANIN CONTENT IN MATURE HYDRILLA. PIGMENT VALUES ARE PRESENTED AS μg/g FRESH WEIGHT.

Pigment	Weeks after Treatment	Fluridone				
		0	0.05	0.5	5.0	50
Carotenoid	2	207	363	241	46*[1]	0*
	4	—	—	—	—	—
	6	201	188	130	50*	0*
	8	—	—	—	—	—
	12	194	85*	0*	7*	5*
Chlorophyll	2	1571	3014*	2082	523*	304*
	4	1053	970	900	329*	186*
	6	1183	1094	660*	376*	148*
	8	1311	1340*	709*	446*	186*
	12	1178	1380*	504*	423*	356*
Anthocyanin	2	116	166	171	236	490*
	4	111	119	151	156	436*
	6	138	100	119	111	517*
	8	148	51	65	248	344*
	12	124	195*	196*	147	171*

[1]Values within a week followed by * are significantly different from the control (Dunnett's 't' test at the 0.05 level).

0.5 ppb fluridone also increased anthocyanin content but only after 12 wk of treatment.

Young plants. Carotenoid content in young, untreated plants was about 50% that of the mature plants, and the drop in carotenoid content in response to fluridone was much less than that of the mature plants (Tables 1 and 2). Concentrations of 0.5, 5.0 and 50 ppb fluridone significantly reduced carotenoid content 8 wk after treatment (Table 2). Chlorophyll content was also much lower (40%) in the young hydrilla plants as compared to the mature hydrilla and was reduced by 50 ppb of fluridone to about 65% of the control for all times of treatment (Table 2). Fluridone at 5.0 ppb reduced chlorophyll content by 54 to 68% for exposure times longer than 2 wk. The 0.5-ppb concentration caused a reduction in chlorophyll content 4 and 8 wk after treatment. A significant increase in chlorophyll at 6 and 8 wk was observed at 0.05 ppb fluridone. Anthocyanins in young plants did not significantly change in response to fluridone.

DISCUSSION

Carotenoid content of the mature plants declined rapidly followed by a decrease in chlorophyll. Chlorophyll content was lower in the younger plants, similar to the findings of Van et al. (20). They suggested that this was due to the greater amount of stem versus leaf tissue at the lower depths. Carotenoids were also lower, presumably due to the lower chlorophyll content. Interestingly, in the young plants fluridone caused less decrease in carotenoid content than in the mature plants (*i.e.* 50% or more relative to the control). Chlorophyll content also decreased in response to fluridone to a lesser extent in the young plants. Both Devlin *et al.* (5), and Bartels and Watson (1) demonstrated that a reduction in chlorophyll was dependent on light intensity, with a greater reduction under high light. The younger plants in this study were approximately 0.5 m below the surface of the water and probably received less light than the mature plants which were on the surface.

The younger hydrilla plants were exposed to a lower light intensity, decreasing the level chlorophyll photooxidation as carotenoid content decreased in response to fluridone. This decrease in carotenoid content in response to fluridone could have resulted from lower biosynthetic rates at the lower light intensity, ultimately lowering susceptibility.

When hydrilla was treated with fluridone at 5.0 or 50 ppb the apical meristems became pink in color. Further characterization indicated the pink pigments were anthocyanin as characterized by an absorption peak at 530 nm and a colormetric change at higher pH values (3). In the mature plants treated with 50 ppb fluridone, anthocyanin content was four to five times higher. There was also a significant increase in anthocyanin content in response to fluridone at the 0.5-ppb rate 12 wk after initial exposure. Anthocyanin content did not change in response to fluridone in the younger plants.

Many aquatic plants possess anthocyanins (18,19). These pigments are produced in response to various stress-

TABLE 2. EFFECT OF FLURIDONE (ppb) ON CAROTENOID, CHLOROPHYLL AND ANTHOCYANIN CONTENT IN YOUNG HYDRILLA. PIGMENT VALUES ARE PRESENTED AS μg/g FRESH WEIGHT.

Pigment	Weeks after Treatment	Fluridone				
		0	0.05	0.5	5.0	50
Carotenoid	2	84	84	89	98	46
	4	101	93	69	57	61
	6	84	122	114	65	59
	8	126	121	84*[1]	79*	64*
	12	106	87	55	55	49
Chlorophyll	2	688	733	700	683	194*
	4	693	739	511*	222*	233*
	6	604	889*	691*	277*	293*
	8	845	894*	540*	354*	300*
	12	801	668	494	267*	220*
Anthocyanin	2	202	130	83	182	176
	4	146	160	161	140	161
	6	129	107	108	166	98
	8	325	86	72	96	110
	12	100	110	85	133	96

[1]Values within a week followed by * are significantly different from the control (Dunnett's 't' test at the 0.05 level).

related factors including high light, low water temperature, and nutrient limitation (10,18). Work by Spencer and Ksander (19) showed that a decrease in chlorophyll content (due to high light intensities) in *Potamogeton gramineus* L. was responsible for the apparent anthocyanin increase. Although they suggested this phenomenon was an unmasking due to the loss of chlorophyll, they did report a slight increase in anthocyanin pigment production under high light intensities. Hydrilla is known to lose chlorophyll (12) and produce anthocyanins (2) during the fall as a result of leaf senescence. Therefore, the increase in anthocyanins in hydrilla treated with fluridone could have been due to a loss of chlorophyll. Young plants did not respond to fluridone by increasing anthocyanin content but a pink coloration was still evident. This could have resulted from an unmasking of the anthocyanins as was the case in *P. gramineus*. In addition, anthocyanin production in some plants has been correlated with high carbohydrate status (4) and the younger developing shoots could be deficient in reserves required for anthocyanin production.

Fluridone causes many changes in the pigment composition of hydrilla. As expected, carotenoid and chlorophyll levels were dramatically reduced, ultimately causing the death of the plant. Fluridone also stimulated the production of anthocyanins, albeit in mature plants only, but the exact mechanism and cause(s) remains equivocal.

ACKNOWLEDGMENTS

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Growth Regulator Effects on *in Vitro* Shoot Regeneration of *Crassula helmsii*[1]

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ABSTRACT

Effects of cytokinin type (N^6-benzylaminopurine [BA], 2-isopentenyladenine [2iP] or 6-[4-hydroxy-3-methylbut-2-enyamino]purine [zeatin]) and concentration (0-10 µM) on *in vitro* shoot regeneration of *Crassula helmsii* (T. Kirk) Cockayne (swamp stonecrop) from single-node explants were examined. The influence of either BA, 2iP, or zeatin (0-25 µM) in factorial combination with 0 or 1.0 µM α-naphthaleneacetic acid (NAA) on the capacity to form adventitious shoots *in vitro* from leaf blade and internode explants was also examined. Axillary shoot production from nodal explants was promoted in media supplemented with cytokinin. Maximum shoot regeneration from nodal explants occurred in liquid medium consisting of full-strength Murashige & Skoog mineral salts, 0.56 mM myo-inositol and 1.2 µM thiamine-HCL and 58.4 mM sucrose supplemented with 5.0 µM BA. Zeatin and 2iP were ineffective in promoting lateral branching from single node explants. Cultured internode explants produced adventitious shoots in the absence of exogenous growth regulators. Both leaf blade and internode explants exhibited rapid adventitious shoot development (ASD) when cultured on agar-solidified medium supplemented with a cytokinin and 1.0 µM NAA. These results suggest *in vitro* culture techniques may be used to rapidly screen aquatic plant growth potential.

Key words: aquatic plants, adventitious shoot development, cytokinins, growth potential.

INTRODUCTION

Most weedy aquatic angiosperms expand within their range primarily through effective vegetative reproduction (Cook 1987). Rapid colonization of water bodies is attributed, in part, to the high capacity of these plants to regenerate and grow from stem fragments and specialized hibernacula including tubers or turions (Sculthorpe 1967, Madsen *et al.* 1988, Sutton *et al.* 1992).

It is well documented that aquatic plant growth and development *in situ* is influenced by many abiotic and biotic factors acting in concert (Spencer and Bowes 1985). However, it is plant genotype which ultimately determines maximum

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proliferation *under nonlimiting growth conditions*. Therefore, it may be feasible to determine the relative growth potential of aquatic species by comparing their capacity for growth and regeneration under nonlimiting culture conditions of light and temperature as well as nutrient and carbon availability. Such conditions can be provided by using *in vitro* whole plant and tissue culture techniques. This approach involves the sterile culture of whole plants or tissues under nonlimiting conditions of nutrient, carbon, and growth regulator enrichment under controlled conditions of light and temperature.

In vitro whole plant and tissue culture systems have proven useful to precisely study the physiological factors controlling development in certain aquatic plants (Mohan Ram and Kapoor 1976, Mohan Ram and Kakkar 1983, Kane and Albert 1989a). Studies in our laboratory suggest that aggressive species such as *Myriophyllum heterophyllum* and *M. aquaticum* possess inherently high capacities for rapid axillary branching and adventitious shoot production from isolated tissues cultured *in vitro* (Kane and Albert 1989b, Kane and Gilman 1991, Kane *et al.* 1991). Preliminary data suggest that a close relationship may exist between the cellular capacity for shoot regeneration and growth *in vitro* and growth potential *in situ*. Comparative studies of *in vitro* growth and regeneration of designated known weedy and putative nonweedy species could provide valuable baseline information with which to evaluate aquatic plant growth potential. However, the *in vitro* growth performance of diverse aquatic plant genera must be screened to verify this relationship.

Crassula helmsii (swamp stonecrop), a succulent perennial aquatic angiosperm native to Australasia, has become established in Europe and naturalized in over 140 sites in Britain (Dawson and Warman 1987). The rapid spread of this species has been attributed to its enormous potential to regenerate from small stem fragments (Dawson and Warman 1987). In the present study, we examined the influence of growth regulators on the regenerative capacity of swamp stonecrop in vitro from single-node, leaf blade and internode segments.

MATERIALS AND METHODS

Initial establishment of in vitro shoot cultures. Shoots of swamp stonecrop were kindly provided by Dr. F. H. Dawson, Freshwater Biological Association River Laboratory, Great Britain. Defoliated stem segments (consisting of two to three nodes) were rinsed for 30 min in tap water and then surface sterilized in aqueous 1.05% (w/v) NaClO containing 0.01% (v/v) Tween-20 for 12 min, followed by three 5-min rinses in sterile deionized water. Stem segments were transferred into 500-ml aluminum-foil-capped Erlenmeyer flasks containing 250 ml of sterile liquid basal medium (BM) consisting of half-strength Murashige and Skoog mineral salts (1962) supplemented with 0.56 mM myo-inositol, 1.2 μM thiamine-HCl and 58.4 mM sucrose. The medium was adjusted to pH 5.7 with 0.1 N KOH before autoclaving at 1.2 $kg \cdot cm^{-2}$ for 20 min at 121C. Stock cultures and experiments were maintained at 25 ± 2C under a 16-hr photoperiod provided by cool-white fluorescent tubes (Sylvania F96T12/CW) at a photosynthetic flux density of 38 $\mu mol \cdot s^{-1} \cdot m^{-2}$ as measured at culture level. Stock plant cultures were further increased by aseptically subculturing the branch shoot tips produced at 4-wk intervals (Figure 1A).

Cytokinin effects on regeneration from nodal explants. The effects of cytokinin enrichment on shoot regeneration from single node segments (explants) cultured in liquid BM were examined. Single node explants 5 to 7 mm long (Figure 1A) were transferred into 150- by 25-mm culture tubes containing 12 ml of liquid BM supplemented with 1-10 μM of either N^6-benzylaminopurine (BA), 2-isopentenyladenine (2iP), or 6-[4-hydroxy-3-methylbut-2-enyamino]purine (zeatin). A tube containing a single node explant represented the experimental unit. Each treatment was replicated nine times. Shoot number, length and node number were determined after 28 days in culture. Treatment effects were statistically analyzed using the General Linear Models (GLM) procedures developed by Statistical Analyses System (SAS 1985). Mean separation was determined using Tukey's (HSD) studentized range test ($\alpha = 0.05$). For brevity, only the optimal responses at the 5-μM cytokinin level are described.

Capacity for adventitious shoot formation from leaf and internode explants. The capacities of 3.0-mm-long leaf blade (Figure 1A) and 5- to 8-mm-long internode (Figure 1A) explants to form adventitious shoots *in vitro* were evaluated on a modified BM (MBM) supplemented with either BA, 2iP, or zeatin (0-25 μM) in factorial combination with 0 or 1.0 μM α-naphthaleneacetic acid (NAA). The modified BM components were the same as previously described except that 87.6 mM sucrose was substituted and the medium was solidified with 0.8% (w/v) TC® agar (JRH Biosciences, Lenexa, KS). Media were dispensed as 10-ml aliquots into 60- by 15-mm Falcon® #1007 polystyrene petri dishes (Becton Dickerson, Lincoln Park, NJ). Each replicate consisted of a petri dish inoculated with a leaf blade and internode explant placed horizontally on the surface of the medium. Treatments were replicated nine times. Treatment effects on adventitious shoot number were determined after 28 days in culture. For brevity, effects of medium supplementation with only 20 μM cytokinin and 1.0 μM NAA are described.

Histological sectioning. For histological observations of adventitious shoot development, internode explants were cul-

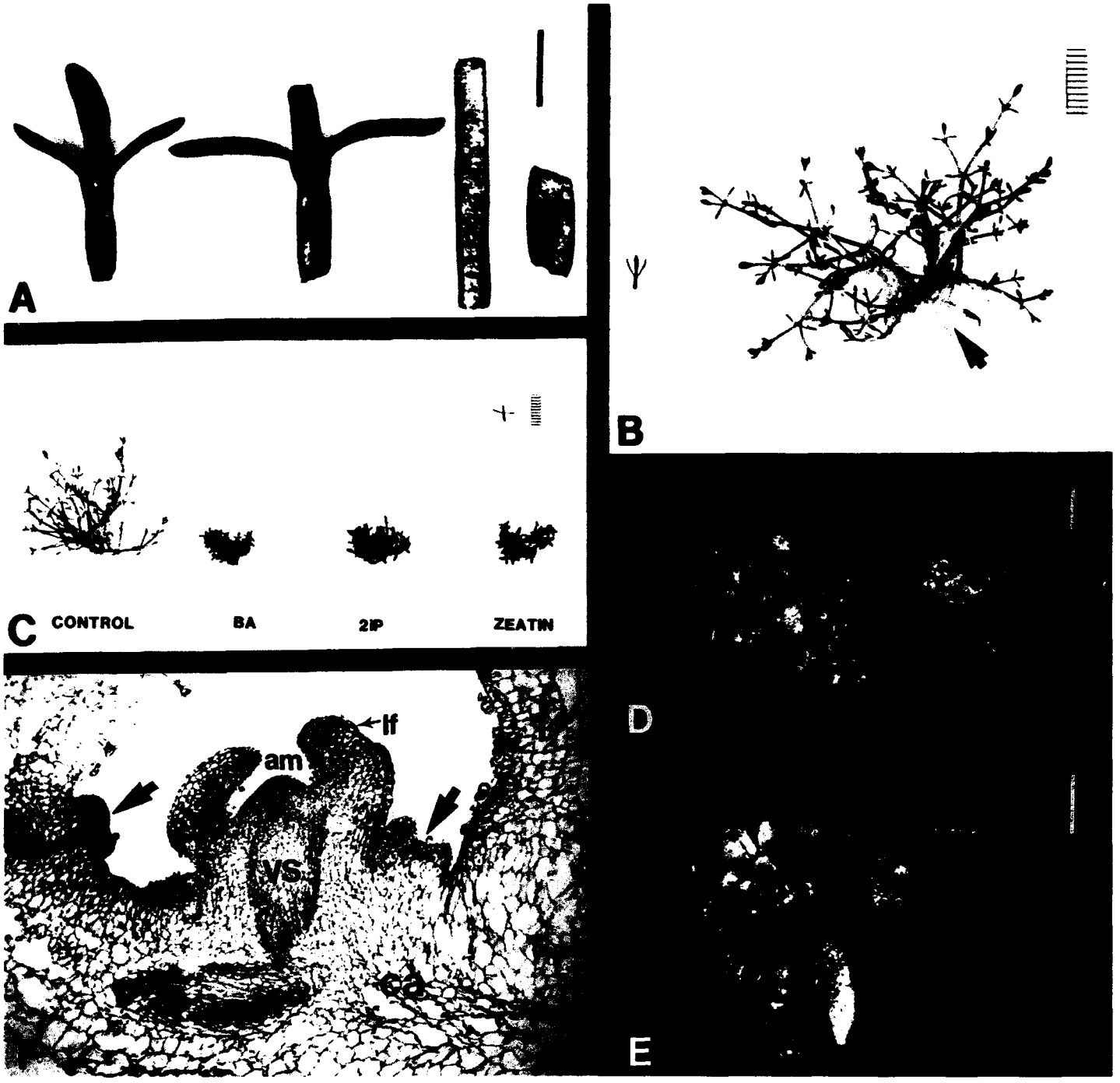

Figure 1A-F. Effect of explant and cytokinin type on *in vitro* shoot regeneration from shoot tip, single node, internode and leaf blade explants of *Crassula helmsii*. Figure 1A. Explant types (left to right): shoot tip, single node, internode and leaf blade. Scale bar = 3.0 mm. Figure 1B. Rooted (arrow) shoot mass produced from a single shoot tip (left) cultured in basal medium for 28 days. Scale bar = 10 mm. Figure 1C. Effect of cytokinin type (5 μM) on shoot regeneration from a nodal explant (upper right) cultured in liquid medium for 28 days. Control = basal medium without cytokinin; BA = N^6-benzylaminopurine; 2iP = 2-isopentenyladenine; zeatin = 6-[4-hydroxy-3-methylbut-2-enyamino]purine. Scale bar = 10 mm. Figure 1D. Multiple adventitious shoot formation from internode explant cultured on agar-solidified medium supplemented with 10 μM 2iP and 1.0 μM NAA for 28 days. Scale bar = 1.0 mm. Figure 1E. Adventitious shoot formation from leaf blade explant cultured on agar-solidified medium supplemented with 10 μM 2iP and 1.0 μM NAA for 28 days. Scale bar = 1.0 mm. Figure 1F. Multiple adventitious shoot development (arrows) from callus (ca) produced on internode explant after 28 days culture on agar-solidified medium supplemented with 10 μM 2iP and 1.0 μM NAA. Mature adventitious shoot bud consists of apical meristem (am) and leaf primordia (lf). Note provascular strand (vs) development. Scale bar = 100 μm.

tured on modified BM supplemented with 10 µM 2iP and 1.0 µM NAA for 28 days and then fixed in formalin-acetic-alcohol (FAA) under vacuum, dehydrated through a graded ethanol-tertiary butyl alcohol series and embedded in Paraplast Plus™ (mp: 56 C, Monojet Scientific, St. Louis, MO). Embedded tissues were sectioned at 10 µm and stained with 0.05% toluidine blue (w/v) in citrate phosphate buffer (pH 6.0) for 25 sec (Sakai 1973).

RESULTS AND DISCUSSION

In outdoor experimental conditions, swamp stonecrop exhibits the capacity for rapid regeneration (1.4 to 2 shoots per node) from single node fragments (Dawson and Warman 1987). This regrowth potential is further accentuated *in vitro*. Both shoot tip and single node explants exhibit similar but extraordinarily high capacities to form densely branched and rooted shoot masses when cultured in liquid BM for 28 days in the absence of plant growth regulators (Figure 1B; 1C control). A shoot mass comprised of 62 shoots and a total of 127 rooted nodes and 254 lateral buds is regenerated from a single shoot tip in 28 days (Figure 1B and 2). The *in vitro* shoot regeneration rate of swamp stonecrop in basal medium is approximately five times greater than that observed for *M. aquaticum* (Kane *et al.* 1991).

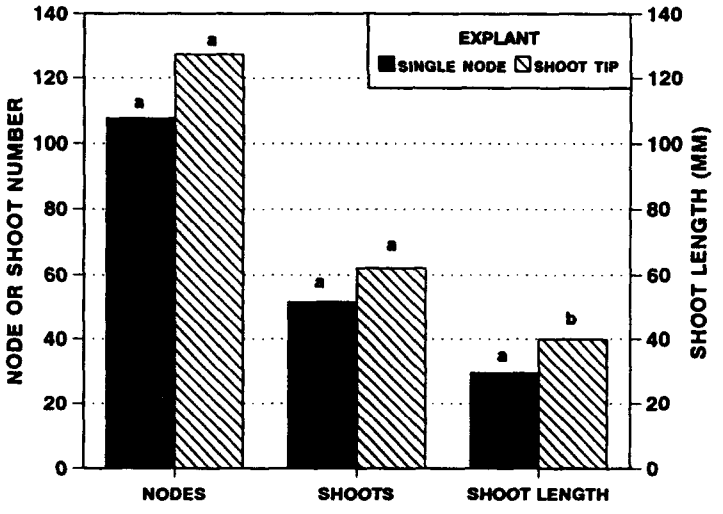

Figure 2. Comparative shoot regeneration from single node and shoot tip explants cultured in liquid basal medium for 28 days. Each histobar represents the mean response of 9 explants. For each response, histobars with the same letter are not significantly different; 5% level.

Over the concentration range used (0 to 10 µM) only the synthetic cytokinin BA promoted shoot regeneration and node number over that observed from single node explants cultured in BM only (5-µM treatment responses shown; Figure 3). Medium supplementation with cytokinins inhibited

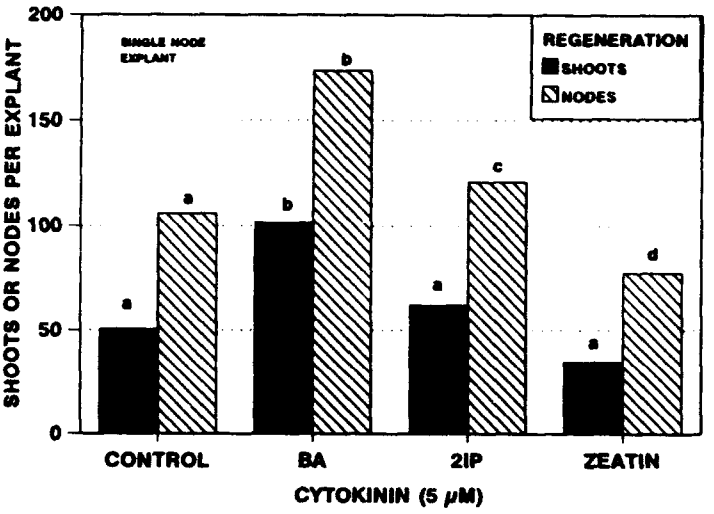

Figure 3. Effect of cytokinin type on axillary shoot and node regeneration from single node explants cultured in liquid basal medium for 28 days. Each histobar represents the mean response of 9 explants. For each specific response, histobars with the same letter are not significantly different; 5% level.

shoot elongation resulting in the production of compact shoot masses (Figure 1C). We have observed similar inhibition of shoot elongation in other aquatic species (Kane *et al.* 1991). The ineffectiveness of the naturally occurring cytokinins 2iP and zeatin to further promote shoot production suggests that axillary shoot production in swamp stonecrop is not limited by endogenous cytokinins. Conceivably, the multiple roots produced at most nodes (see Figure 1B) serve as sites of endogenous cytokinin biosynthesis (Davies 1987). This would explain the extremely highly branched growth habit of plants observed *in situ* (Dawson and Warman 1987). Conversely, the lack of multiple nodal roots in *Myriophyllum aquaticum* could account for the significant promotion of axillary shoot production we have observed following cytokinin supplementation (Kane *et al.* 1991). Cytokinin treatments only slightly enhance axillary shoot production in *Hydrilla verticillata* (Anderson 1985). However, in hydrilla, apical meristem and nodal explants exhibit dissimilar responses to both exogenous cytokinin type and level.

Numerous aquatic plants exhibit the capacity to regenerate through formation of adventitious shoots from fragmented tissues (Hagemann 1932; Sculthorpe 1967). In swamp stonecrop, both leaf blade and internode explants exhibited the capacity to produce adventive shoots. No ASD occurred on leaf blade explants cultured on MBM. In contrast, internode tissues exhibited the capacity (11% responsive explants) for ASD (mean: 1.5 shoots/explant) when cultured on agar-solidified MBM without cytokinin supplementation. Dissimilar regenerative capacities may be related to the difference in the initial size of the leaf blade and internode explants. Medium

supplementation with up to 20 μM cytokinin only slightly enhanced ASD on internode explants in the absence of NAA (data not shown). However, ASD from both leaf blade and internode explants was significantly promoted with increased cytokinin level in the presence of 1.0 μM NAA. Comparative maximum ASD responses for leaf blade and internode explants in the presence of 20 μM cytokinin and 1.0 μM NAA are depicted in Figure 4. The promotive effects of the three cytokinins on ASD were not significantly different. Rost and Paterson (1976) reported a similar requirement for medium supplementation with both cytokinin (2iP) and auxin for optimal ASD from leaf explants of the terrestrial species *Crassula argentea*.

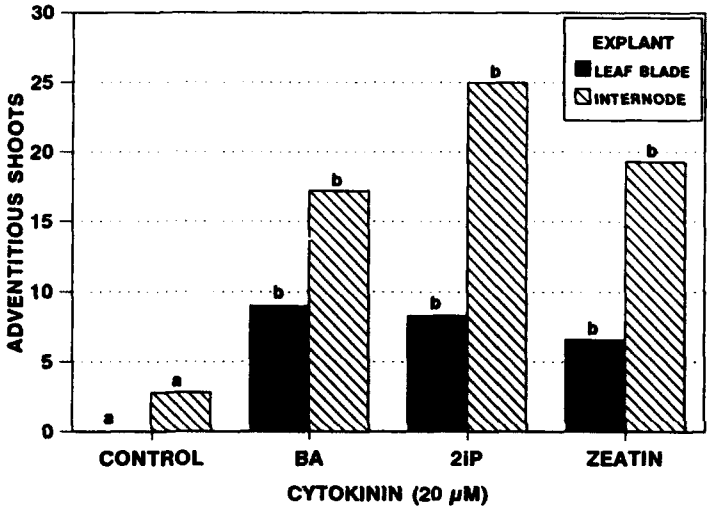

Figure 4. Effects of explant and cytokinin type on adventitious shoot development from leaf blade and internode explants cultured on agar-solidified media supplemented with 1.0 μM α-naphthaleneacetic acid (NAA). Each histobar represents the mean response of 9 explants. For a given explant type, histobars with the same letter are not significantly different; 5% level.

Multiple adventitious shoot meristems formed secondarily from callus which first developed on the basipetal cut surfaces of both explants types (Figure 1D-F). By day 28, multiple adventitious shoots covered both cut ends of the internode explants (Figure 1D). Members of the Crassulaceae are noted for their capacity for ASD from stem and leaf segments. The developmental pattern of ASD observed in *Crassula helmsii* is similar to that described for other *Crassula* species (Rost and Paterson 1976). Given that adventitious shoot regeneration from internode tissues arises *in vitro* on basal medium, the possibility arises that regrowth from fragmented stem segments without pre-existing buds probably occurs under field conditions as observed in other aquatic plants (Hagemann 1932, Sculthorpe 1967).

The regenerative capacity exhibited by *Crassula helmsii in situ* is clearly reflected in its growth *in vitro*. Our results suggest that a close correlation exists between regenerative capacity *in vitro* and the capacity for prolific shoot regeneration and growth *in situ* such as observed following the naturalization of *Crassula* in Britain (Dawson and Warman 1987). Although typically not considered weedy in its native range in Australia, *Crassula* has recently become obstructive in flowing water channels (L. Anderson pers. comm.). This implies that abiotic factors act in concert to modulate expression of the inherently high growth potential in this species.

Our results indicate that *in vitro* culture responses, particularly shoot regeneration and growth, may prove useful for screening aquatic plant growth potential in other species. However, care must be taken in making broad generalizations. The occurrence of minimal shoot regeneration from cultured hydrilla 2-node explants (Anderson 1985) suggests that shoot regeneration alone may not be a reliable indicator of weed potential in all species. Consequently, in addition to *in vitro* shoot regeneration, concurrent consideration of other biotic and abiotic parameters may be necessary for reliable assessment of overall weed potential.

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Injection of Nutrients Into Sand Rooting Media for Culture of Dioecious Hydrilla[1]

DAVID L. SUTTON[2]

ABSTRACT

Growth studies were conducted outdoors with dioecious hydrilla (*Hydrilla verticillata* (L.f.) Royle) cultured in concrete tanks filled with flowing pond water. Nutrients were supplied to the root zone of hydrilla plants either by injecting Hoagland's nutrient solution from the surface of the water through tubing connected to a silica glass air diffuser located in a sand rooting media, or by placing a layer of fertilizer in the sand. The fertilizer layer consisted of either Vigoro or a combination of Osmocote, Esmigran and dolomite. Each culture container was surrounded with a large-mesh plastic netting and window screening to form a water column 80 cm in height by 380 cm in surface area which enclosed growing hydrilla plants. Growth of hydrilla was similar for plants cultured for 8 wk with Vigoro or Osmocote plus Esmigran and dolomite. High amounts of nitrogen in Hoagland's nutrient solution, 187.50 mg per injection, severely reduced growth of hydrilla but growth improved with reduced amounts of nitrogen. This study shows the potential of an injection system to evaluate various nutrients or other chemicals placed in the root zone on growth of hydrilla.

Key words: aquatic plants, fertilizer, hydrosoil, sediments, propagules, tubers.

INTRODUCTION

Hydrilla causes serious submersed weed problems in many tropical and subtropical areas (Cook and Lüönd 1982, Pieterse 1981). Its ability to conduct photosynthesis under low light (Van *et al.* 1976), produce new plants from each node (Langeland and Sutton 1980) in addition to those that can form from turions and tubers (Haller 1967), and form a canopy just below the surface of the water that can shade other submersed plants (Haller and Sutton 1975) are some of the characteristics that allow hydrilla to colonize and grow as a monoculture by replacing indigenous plants in a variety of aquatic habitats. However, the influence of macronutrients and micronutrients in water and substrate on growth of hydrilla is not clearly understood.

Sutton (1986) found dry weight of hydrilla cultured in sand plus fertilizers to be dependent on the concentration of fertilizer in the root zone and was from 6 to 14 times that of plants cultured in sand alone. Of nine nutrients measured in plant tissue from hydrilla cultured in sand amended with fertilizers (Sutton 1986), only phosphorus in both shoots and roots was dependent on the level of fertilizer in the root zone.

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Water surrounding hydrilla shoots was found to be the primary source for potassium (Barko 1982), but sediments were the primary source for two other macronutrients, nitrogen and phosphorus (Barko 1982, Barko *et al.* 1991, Steward 1984). Studies by Barko (1982) and Steward (1984) stressed the need for research to evaluate the influence of sediment composition and sampling procedures on the growth of submersed plants in general.

A method for injecting nutrients in the root zone of hydrilla plants was developed to provide additional information on the influence of various nutrients on growth of hydrilla. The method employed an air diffuser placed in the root zone that supplied Hoagland's nutrient solution to the sand rooting media by means of a syringe and tubing. Dry weight of plants cultured by this method was compared with hydrilla plants grown with commercially available fertilizers known to promote growth of hydrilla.

MATERIALS AND METHODS

Hydrilla was cultured outdoors at the Fort Lauderdale Research and Education Center (FLREC), which is located 26°05'N and 80°14'W, in concrete tanks (6.2 m in length by 3.1 m in width) filled with pond water. Pond water, from the same source as described by Steward (1984), flowed into the tanks at the surface of one end and out from bottom drains at the other at a rate which allowed for a complete exchange of water every 24 hr. Nutrient treatments were arranged in rows perpendicular to the flow of water. Treatments were assigned at random within a row, and four rows were used for each culture period.

For all culture periods, nutrients were supplied to the root zone of hydrilla plants by either placing a layer of fertilizer 7.6 cm below the surface of the sand or injecting nutrient solution (Figure 1). The top of the air diffuser was placed the

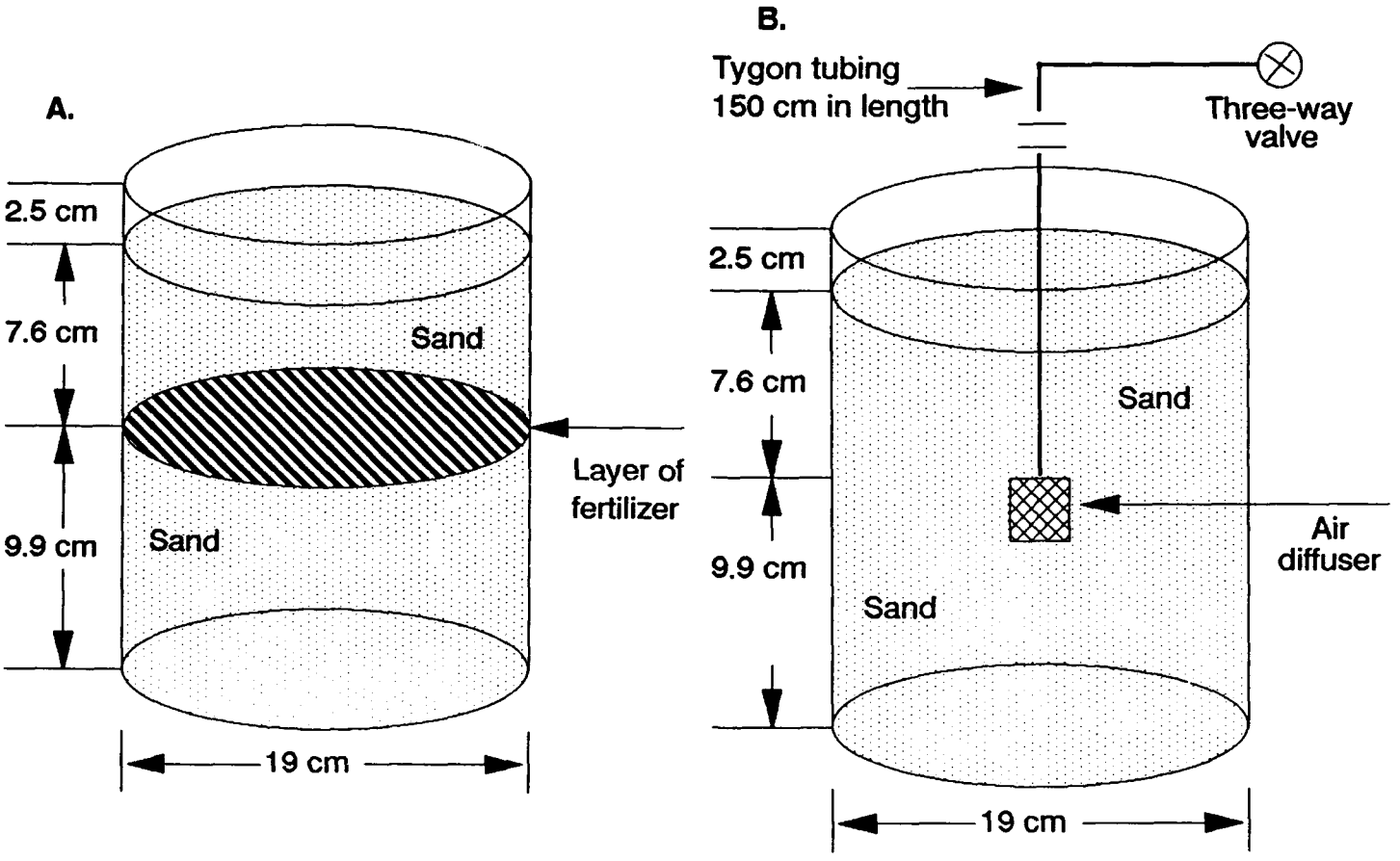

Figure 1. Diagrammatic representation of culture containers showing placement of nutrients in sand rooting media used for culture of hydrilla in outdoor tanks filled with flowing pond water. A. (Left) Location for layer of Osmocote or Vigoro fertilizers. B. (Right) Location of air diffuser for injection of Hoagland's nutrient solution.

same distance from the surface of the sand as the layer of fertilizer. The air diffuser was constructed of silica glass with dimensions of 1.5 cm in length by 1.5 cm in width by 1.5 cm in height. A three-way valve at the top of the tubing allowed for a solution of nutrients to be injected without air being pushed in the root zone.

Nutrient solutions were injected with a syringe three times each week on Monday, Wednesday, and Friday. This resulted in a total of 24 injections during an 8-wk culture period. Each nutrient solution injection consisted of 50 ml of distilled water which contained either 15.63 mg, 31.25 mg, 62.50 mg, or 125.00 mg of nitrogen; 27.25 mg of phosphorus; 0.6 mg of iron (Fe) supplied by Sequestrene 330Fe[3]; and the amounts of other nutrients normally contained in 50 ml of full-strength Hoagland's nutrient solution (Hoagland and Arnon 1950). Injections of 15.63 mg, 31.25 mg, 62.50 mg, or 125.00 mg of nitrogen resulted in total amounts of 375 mg, 750 mg, 1,500 mg, or 3,000 mg of nitrogen, respectively, added to the sand in the culture containers during the hydrilla growth period. After the nutrient solution was injected, 50 ml of distilled water was used to flush the tubing and air diffuser. Four containers (Figure 1) were injected with each different nutrient treatment of nitrogen for each culture period. Commercially available fertilizers were supplied by adding either 75 g of Vigoro[4], an all-purpose 6-10-4 granular formulation with micronutrients, or a combination of 25 g of Osmocote[5] (18-6-12), 0.7 g of Esmigran (micronutrients in sustained-release form) and 4.2 g of dolomite (mined material containing 55% calcium as $CaCO_3$ and 30% magnesium as $MgCO_3$) (hereinafter referred to as Osmocote) to each of four containers for each culture period.

In a preliminary study, when hydrilla plants were injected three times a week for 8 wk with each injection consisting of 187.50 mg of nitrogen and 27.25 mg of phosphorus, sprouted tubers of hydrilla did not grow well. Thus, the starting concentration for Experiment 1 was reduced to 125 mg of nitrogen per injection.

Tubers were collected from stock hydrilla plants grown at the FLREC. Stock plants were originally collected from Lake Okeechobee. Tubers were sprouted in pond water until shoots reached approximately 12 cm in length and root initiation had begun. For each culture period, four sprouted tubers were placed with three to four nodes below the surface of the sand of each container.

After planting sprouted tubers, each container was surrounded with large mesh plastic netting and window screening to form a water column 80 cm in height by 380 cm^2 in surface area to enclose growing hydrilla plants. Screening enclosed each container with no excess space between the container and screen.

The study consisted of two separate culture periods: (Experiment 1) 11 September to 6 November 1990 and (Experiment 2) 26 November 1990 to 21 January 1991. At the end of each culture period, hydrilla plants were removed from the tanks and hydrilla shoots were cut at the sand surface and washed with pond water to remove algae, sand, and other debris, then dried to a constant weight in a forced-air drying oven at 60C. Below-ground biomass including roots, rhizomes, stem fragments (hereinafter referred to as roots) and tubers in each culture container was washed with pond water to remove sand, fertilizer, and any other adhering debris, and dried at 60C. Tubers were counted when present.

Water temperatures were recorded during each culture period. A maximum/minimum thermometer was placed 30 cm below the surface of the water and temperature was recorded 5 days a week, generally at 4:00 p.m. Water temperature for each culture period was calculated as a daily mean of maximum and minimum values obtained.

Once hydrilla plants reached the surface of the water, an emulsifiable concentrate of malathion (0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate) was added to achieve a concentration of 1.0 ppm in the tank as necessary to control feeding activity of the herbivorous moth *Parapoynix diminutalis* Snellen.

The Statistical Analyses System (SAS)[6] software designed for use on personal computers was used to analyze plant dry weight following Analysis of Variance (ANOVA) procedures. For analysis purposes, hydrilla dry weights were converted to natural logs and tuber count data were transformed by using the square root of the count plus one (Steel and Torrie 1960), but the nontranformed values are presented. The Waller-Duncan Bayesian LSD procedure was used for mean separation.

RESULTS AND DISCUSSION

Daily water temperature values averaged 28.7C with a maximum of 32.0C and a minimum of 21.0C for the 11 September to 6 November 1991 culture period. For the 26 November

[3]Sequestrene 330Fe is manufactured by CIBA/GEIGY Corp., Greensboro, NC 27419. Mention of a trademark or a proprietary product does not constitute a guarantee or warranty of the product by the University of Florida or the USDA and does not imply its approval to the exclusion of other products that also may be suitable.

[4]Vigoro® All-Purpose (6-10-4) is manufactured by Vigoro Industries, Inc., Fairview Heights, IL 62208.

[5]Osmocote with an 8- to 9-month release time and Esmigran are manufactured by Grace Sierra Horticulture Products Company, Milpitas, CA 95035; and Dolomite (Soil Doctor) by Soil Doctor, Inc., Crystal River, FL 32629.

[6]SAS Institute Inc., Cary, NC 27512-8000.

1990 to 21 January 1991 culture period, daily water temperature values averaged 24.2C with a maximum of 28.0C and a minimum of 18.5C. Mean water temperatures for both culture periods are in the range for good growth of hydrilla as suggested in the study by Van *et al.* (1978).

Dry weight of hydrilla was similar for plants cultured with Osmocote or Vigoro fertilizers, but the magnitude of dry weight varied with culture period (Tables 1 to 2). Total dry weight of pooled values for Osmocote and Vigoro for Experiment 1 was 64% higher than for Experiment 2. This ranking is in the same order as that for mean water temperatures for the two culture periods.

In one of the first studies using Osmocote, a commercially available controlled release fertilizer, for culture of hydrilla in sand rooting media, Sutton (1986) reported that growth of hydrilla under south Florida conditions with this fertilizer depended on temperature and concentration of the fertilizer. Based on findings of Harbaugh and Wilfret 1981, Sutton (1986) concluded that temperature, as related to rate of release of nitrogen and phosphorus in the Osmocote prills, was probably a major factor in the observed differences in hydrilla growth. However in the experiments with Osmocote and Vigoro, a granular material not formulated for nutrient release related to temperature, growth of hydrilla was similar. These results indicate that water temperature differences are influencing metabolic processes related to hydrilla growth rather than influencing the rate of nutrient release from Osmocote.

Photoperiod was conducive for tuber formation for both culture periods (Van *et al.* 1978 and Sutton *et al.* 1992). Interestingly, no tubers were produced by plants cultured with Vigoro fertilizer. It is not known why plants cultured with Vigoro fertilizer did not produce tubers during these periods in contrast to tubers produced by plants cultured with Osmocote.

Although concentrations of nitrogen for the Vigoro and Osmocote fertilizers were the same, the amounts of phosphorus, potassium, and micronutrients were not present in the same amounts due to differences in formulations of these fertilizers.

In general, dry weight of hydrilla cultured with Osmocote or Vigoro was higher than plants injected with Hoagland's nutrient solution with various amounts of nitrogen (Tables 1 to 2). Since only nitrogen, supplied by KNO^3 and $Ca(NO_3)_2 4H_2O$, varied in the Hoagland's nutrient solution treatments, it appears that the amounts of nitrogen added to the root zone was a major factor influencing growth of hydrilla under these conditions. Although other nutrients in the Hoagland's nutrient solution were not varied, their interaction with the various amounts of nitrogen may have influenced growth of hydrilla. Additional studies will be needed to evaluate the influence of these other nutrients on growth of hydrilla.

The preliminary study showed that when hydrilla plants were injected with Hoagland's nutrient solution at a rate

TABLE 1. DRY WEIGHT OF HYDRILLA AFTER GROWTH OUTDOORS IN SAND AMENDED WITH FERTILIZER OR INJECTED WITH HOAGLAND'S NUTRIENT SOLUTION. SPROUTED TUBERS WERE PLANTED 11 SEPTEMBER 1990, AND PLANTS WERE ALLOWED TO GROW UNTIL 6 NOVEMBER 1990.

Total amount of nutrient (mg)[a]		Plant dry weight (g)[b]			Number of tubers
Nitrogen	Phosphorus	Shoots	Roots	Total[c]	
Osmocote					
4,500	654	12.26 a	1.35 a	13.61 a	0 a
Vigoro					
4,500	3,273	12.45 a	1.18 a	13.63 a	0 a
Hoagland's nutrient solution					
3,000	654	5.36 c	0.68 b	6.05 c	1 a
1,500	654	8.10 b	1.10 a	9.26 bc	1 a
750	654	9.78 ab	1.16 a	11.05 ab	3 a

[a] Total amounts of nitrogen and phosphorus in Osmocote or Vigoro applied as a layer in sand rooting media prior to planting. For Hoagland's nutrient solution, one twenty-fourth of the total amounts of nitrogen and phosphorus were injected three times each week during the culture period.
[b] Values for plant dry weight in a column followed by the same letter are not significantly different at the 5% level according to Waller-Duncan Bayesian LSD Procedure. Each value is the mean of plants from four culture containers.
[c] Includes weight of shoots, roots, and tubers.

TABLE 2. DRY WEIGHT OF HYDRILLA AFTER GROWTH OUTDOORS IN SAND AMENDED WITH FERTILIZER OR INJECTED WITH HOAGLAND'S NUTRIENT SOLUTION. SPROUTED TUBERS WERE PLANTED 26 NOVEMBER 1990, AND PLANTS WERE ALLOWED TO GROW UNTIL 21 JANUARY 1991.

Total amount of nutrient (mg)[a]		Plant dry weight (g)[b]			Number of tubers
Nitrogen	Phosphorus	Shoots	Roots	Total[c]	
Osmocote					
4,500	654	7.47 a	1.00 a	8.71 a	8 a
Vigoro					
4,500	3,273	7.30 ab	0.56 bc	7.86 ab	0 c
Hoagland's nutrient solution					
3,000	654	2.09 d	0.42 c	2.52 d	1 c
1,500	654	3.72 c	0.68 ab	4.47 b	3 b
750	654	5.43 b	0.88 a	6.53 ab	7 a
375	654	5.35 b	0.75 ab	6.22 b	3 b

[a] Total amounts of nitrogen and phosphorus in Osmocote or Vigoro applied as a layer in sand rooting media prior to planting. For Hoagland's nutrient solution, one twenty-fourth of the total amounts of nitrogen and phosphorus were injected three times each week during the culture period.
[b] Values for plant dry weight in a column followed by the same letter are not significantly different at the 5% level according to Waller-Duncan Bayesian LSD Procedure. Each value is the mean of plants from four culture containers.
[c] Includes weight of shoots, roots, and tubers.

which resulted in a total of 4500 mg of nitrogen being added to the root zone, the same amount as that contained in the Osmocote or Vigoro fertilizers, sprouted tubers grew very little, and roots at time of harvest were heavily encrusted with a salt- appearing material. When hydrilla was harvested after 8 wk of growth, plants treated with 24 injections of 187.50 mg of nitrogen for a total of 4500 mg of nitrogen averaged 86% less in dry weight than plants cultured with Osmocote or Vigoro.

A decrease in amounts of nitrogen from 3000 to 750 mg injected in the sand root media resulted in an increase in hydrilla dry weight (Table 1). Also, dry weight of plants injected with 750 mg of nitrogen was similar to plants cultured with Vigoro or Osmocote. In Experiment 2, dry weight of plants cultured with a total of 375 mg of nitrogen was similar to hydrilla plants for the 750-mg rate of nitrogen (Table 2).

These data show a method to inject nutrients in the root zone of hydrilla. Additional studies are needed to inject nutrients in the root zone of hydrilla based on measurements of nutrients in lake sediments. In this way it may be possible to predict potential hydrilla growth based on nutrient content of various sediments.

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J. Aquat. Plant Manage. 31: 70-76

ECOLOGY

The Distribution, Ecology and Conservation of *Luronium natans* (L.) Raf. in Britain

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ABSTRACT

Despite a wide ecological amplitude, *Luronium natans* remains a rare aquatic plant endemic to Europe. The main populations occur in Britain, where *Luronium* is found in clear water lakes, navigation canals and ponds with widely ranging water chemistry and associated plant communities. The spread of the plant this century from upland to lowland sites via an interconnecting system of waterways is chronicled. *Luronium* is a permanent member of the flora in lakes where various combinations of oligotrophy and disturbance by waves produce a stable low biomass, open-structured vegetation. It also occurs prolifically in more productive waters as an early colonist of recently disturbed, often artificial habitats, but in later stages of succession is out-competed by more vigorous species. *Luronium* is, therefore, transient or erratic in these artificial habitats, because it depends on sustained human disturbance to arrest hydroseral succession and maintain its niche. Dealing primarily with canal sites, the problems of habitat management and conservation of *Luronium* are discussed.

Key words: oligotrophic, canal, disturbance, management.

INTRODUCTION

Luronium natans (L.) Raf., (Alismataceae) the Floating Water-plantain, is a rare aquatic plant, endemic to Europe, whose distribution is decreasing in most areas. In Great Britain its natural habitat is mostly oligotrophic, upland lakes, but since the late nineteenth century it has shown a notable spread down into the apparently quite different habitat of eutrophic, lowland navigable canals, where its main populations are now located. This change raises interesting questions for the aquatic botanist. What features have permitted this extension of the plant's distribution? How can they be sustained to conserve the species in its new artificial habitat? This paper offers a preliminary attempt to answer these questions.

Luronium natans is a small, perennial, stoloniferous aquatic plant with a heterophyllous growth form. The floating-leaved form (f. *repens* Buch.), found in shallow water or occasionally along channel margins (Hanspach and Krausch 1987) and on exposed wet mud (pers. obs.), has small, ovate leaves carried on ascending petioles arising either from a submerged basal rosette or, in emergent plants, directly from the stolon. The three-petalled flowers, borne usually at the water surface, are small (12 to 16 mm across), white, hermaphrodite and normally solitary. The fruits (achenes) comprise small, buoyant, single-seeded capsules which are beaked and finely ribbed and are probably dispersed by both drift and waterfowl.

The submerged form (f. *submersum* Glück) consists of shallow-rooting rosettes of narrow tapering leaves. It is wholly vegetative and generally occurs in faster flowing water or where light intensities are reduced by shading, turbidity, dystrophy or as depth increases (up to 2 m). Vegetative propagules in the form of buoyant, viable plants are yielded by fragmentation of the stolons and are dispersed by drift.

Luronium has a distribution centered on Belgium, France, Great Britain, the Netherlands and northern Germany (Hanspach and Krausch 1987). Outlying populations have been reported from Scandinavia (southern Sweden, Fritz 1989; west Denmark and south Norway, Björkqvist 1961). Tutin *et al.* (1980) mention occurrences in Spain, Italy and Yugoslavia, extending eastwards into Bulgaria, Poland, and the regions of southwest and possibly Baltic Russia. They consider it to be extinct in Czechoslovakia and Romania. Its recorded habitats include lakes, reservoirs, ponds, bog pools, ditches, canals and slowly flowing rivers.

Throughout its continental range, *Luronium* is reportedly extremely scarce (Hanspach and Krausch 1987) and many remaining sites are now endangered (Wittig and Pott 1982, Fritz 1989, Meriaux 1982). Serious declines of *Luronium* or its phytosociological grouping have been documented in the Netherlands (*e.g.* Arts *et al.* 1990) and Germany (Wittig and Pott 1982, Hanspach and Krausch 1987) as a result of

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acidification or eutrophication of formerly oligotrophic waters (Wittig 1982, Roelofs 1983, Arts *et al.* 1990). This has led to its inclusion in the IUCN list of Rare, Threatened and Endemic Plants as a species vulnerable to extinction (Lucas and Walters 1976). Recently *Luronium* was also added to Annexe I of the Berne Convention on the Conservation of European Wildlife and Natural Habitats, which affords special protection for listed plants and their habitats.

In Britain *Luronium* is scarce, but not sufficiently rare to justify inclusion in the Red Data Book for Vascular Plants (Perring and Farrell 1983), and it seems probable that Britain supports the bulk of the remaining world population. However, to honor its obligation to the Berne Convention, the British government has added *Luronium natans* to Schedule 8 of the Wildlife and Countryside Act 1981, making picking, uprooting or willful destruction of the plant illegal.

To date, an ecological appraisal of the plant and comprehensive statement of its current status in Britain are lacking. As these are prerequisites for informed conservation action, this paper aims to offer information on both these aspects.

METHODS

Changes in the distribution of *Luronium* in Great Britain were analyzed from the British Biological Records Centre data, complemented by Country Floras and other records from reports of botanical excursions, the general ecological literature, details of herbarium specimens, unpublished data from canal surveys and personal observations made during extensive surveys of canal vegetation since 1989. These sources were also used to define the plant communities associated with *Luronium* in its two principal habitats, *viz.* lakes and canals. For sites where *Luronium* no longer occurs, County Floras were used to reconstruct the flora at or around the time it was present. Seddon's (1972) extensive work on Welsh lakes in the early 1960s provided additional information on communities containing *Luronium* and on associated water chemistry. Analyses of Cumbrian lake waters since 1953 (Carrick and Sutcliffe 1982) and of canal waters (regional water authorities and original data) were also used.

RESULTS

Ecology in Main Habitats

i. Lakes

Luronium has a long recorded history in some lakes, particularly those in the uplands of North and Mid-Wales. It occurred at Llanberis in Snowdonia from at least as early as 1729 and there are nineteenth century records from 12 other lakes in this region. Since 1960 *Luronium* has been reported from at least 20 lakes in upland Wales. Until the end of the last century it was also known from several lakes in Cumbria.

Seddon (1972) studied 70 Welsh lakes varying in area from 0.001 to 1.53 km^2 and at altitudes from 4 to 701 m. *Luronium* was found in 11 of these water bodies (area 0.08 to 1.01 km^2; altitude 91 to 454 m), being notably almost confined to larger lakes in the high altitude part of its range, with smaller water bodies becoming colonized only in lower lying areas. This trend also applied to the former Cumbrian populations (lake area 0.64 to 8.94 km^2; altitude 44 to 145 m).

Seddon (1972) also provides analyses of water chemistry for six of the lakes in which *Luronium* was found. These data are summarized in Table 1. Combined with other descriptive evidence, they support the general view in the botanical literature that *Luronium* is a plant of soft, slightly acid waters with low nutrient concentrations.

TABLE 1. COMPARISON OF SELECTED WATER CHEMISTRY PARAMETERS BETWEEN WELSH LAKES[1] AND THREE CANAL SITES[2].

Parameter	Welsh lakes	Canals		
		Mont.	Roch.	Ash.
Conductivity (μmhos)	53 (33-70)	147 (66-270)	383 (318-485)	210 (145-200)
pH	6.5 (5.5-7.6)	7.3 (6.7-8.8)	7.3 (6.9-9.2)	7.5 (6.7-8.3)
Ca^{2+} (mg l^{-1})	2.7 (1.3-4.5)	22.5 (10.7-58.8)	17.7 (12.7-24.6)	38.5 (12-65)

[1]Mean of 6 sites, from Seddon (1972); Ca^{2+} estimated from total hardness values.
[2]Mont. = Montgomery; (3 to 10 replicate samples from 19 sites) from Briggs (1988); Roch. = Rochdale; (spot samples from 38 sites) from Shimwell (1984); Ash. = Ashton Canal plus Huddersfield Narrow Canal to Stalybridge and Peak Forest Canal to Hyde (3 to 7 replicate samples from 10 sites). Upper number is the mean, bracketed numbers are the range.

The vegetation in lakes in which *Luronium* is found (Table 2) normally includes *Lobelia dortmanna* L., *Littorella uniflora* (L.) Aschers., *Isoetes lacustris* L. and *Callitriche hamulata* Kutz. ex Koch, a core group characteristic of oligotrophic waters and often accompanied by *Juncus bulbosus* L., *Potamogeton polygonifolius* Pourr., *P. natans* L., *Myriophyllum alterniflorum* DC., *Nuphar lutea* (L.) Sm. and *Sparganium angustifolium* Michx. The emergent vegetation at these sites is also typical of upland, nutrient-poor waters and includes *Equisetum fluviatile* L., *Carex rostrata* Stokes and *Menyanthes trifoliata* L. Although this analysis is confined to recent or current sites in Wales, historical evidence

TABLE 2. INCIDENCE OF PRINCIPAL MACROPHYTES IN LAKES (14 sites) OR IN CANAL SECTIONS CONTAINING *Luronium*[1].

Lake species	Frequency	Canal Species	Frequency		
			Mont.[a]	Roch.[b]	Ash.[c]
*Callitriche hamulata**	3	*Acorus calamus*	2		
Carex rostrata	2	*Alisma plantago-aquatica*	2	1	1
Elatine hexandra	1	*Butomus umbellatus*	1		
Eleocharis palustris	1	*Callitriche hamulata**	3		1
Equisetum fluviatile	3	*C. hermaphroditica*	1	1	
*Glyceria fluitans**	1	*C. stagnalis*	2	1	
Hydrocotyle vulgaris	1	*Ceratophyllum demersum*	3		
Isoetes lacustris	3	*Elodea canadensis*	3	3	
I. setacea	1	*E. nuttallii*	3	2	4
Juncus bulbosus	2	*Glyceria fluitans**		1	
Littorella uniflora	3	*G. maxima*	4	4	4
Lobelia dortmanna	3	*Hottonia palustris*		1	
Menyanthes trifoliata	2	*Hydrocharis morsus-ranae*	[1]		
*Myriophyllum alterniflorum**	2	*Lemna minor*	4	4	4
*Nuphar lutea**	2	*L. trisulca*	2		2
Nymphaea alba	1	*Myosotis scorpioides*	2		1
*Potamogeton berchtoldii**	1	*Myriophyllum alterniflorum**	1		
*P. natans**	2	*M. spicatum*	2		
P. polygonifolius	2	*Nitella sp.*	3		
Eleogiton fluitans	1	*Nuphar lutea**	2		
Sparganium angustifolium	2	*Polygonum amphibium*	1		
Subularia aquatica	1	*Potamogeton alpinus*	[2]		1
		*P. berchtoldii**	2	2	1
		P. compressus	3		3
		P. crispus	2	1	1
		P. epihydrus		2	
		*P. natans**	4		4
		P. obtusifolius	4		
		P. pectinatus	[2]		1
		P. perfoliatus	[2]		1
		P. praelongus	[1]		
		P. trichoides			1
		Ranunculus circinatus	1		
		Sagittaria sagittifolia			4
		Sparganium emersum	4	3	3
		S. erectum	4		
		Typha latifolia	1		

[1](a = maxima during 1985 to 1987, based on 21 to 31 sections of 1 km; b = 39 bridge lengths (mean length 550 m) surveyed 1983 to 1984; c = 10 bridge lengths (mean length 500 m) surveyed 1990 to 1992.) 1 = 20-39% of sites; 2 = 40-59%; 3 = 60-79%; 4 = >80%. Bracketed values are historical records for species formerly of more widespread distribution. * = principal species common to both habitats. Site details and sources as for Table 1.

indicates that the former Cumbrian populations had similar associations of species.

ii. Canals

Luronium first appeared on the canal system near Llangollen, N. Wales, about 1860. The Llangollen Canal receives water from the River Dee, which drains a number of upland catchments containing *Luronium*. There followed a period of rapid expansion along the canals of Cheshire, Shropshire and the Welsh Border counties coinciding with a decline in the use of these canals by freight boats, as competition with the railways intensified (Murphy *et al.* 1982). *Luronium* then continued to spread until by the late 1930s it had colonized much of the Macclesfield Canal and part of the Manchester canal system (including water thermally polluted by mill effluents, Shaw 1963). It subsequently became established at various locations on canals in Staffordshire, the Midlands and Leicestershire and extended northward from Manchester along the Rochdale Canal into West Yorkshire, while consolidating existing populations. This pattern of colonization is consistent with the suggestion by Lousley (1970) that

Luronium spread eastward into lowland Britain from an original focus of distribution in the Welsh mountains via the interconnecting canal system (Figure 1). Today it remains well represented in the floras of several canals, although its overall range has contracted since 1960, following a rapid increase in propeller-driven recreational boat traffic on some of the waterways.

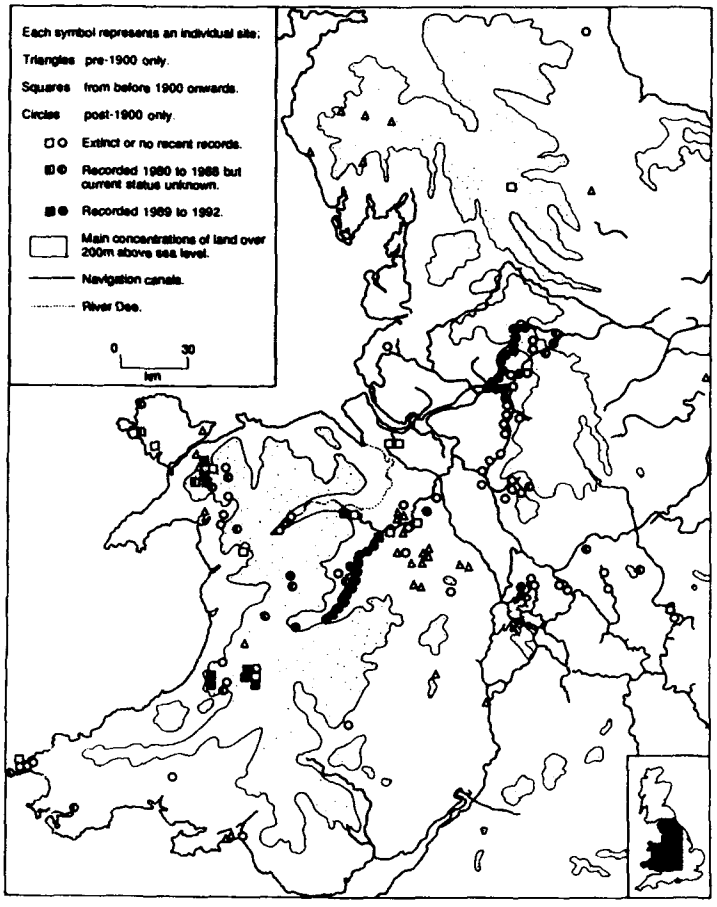

Figure 1. Diagrammatic representation of changes in the distribution of native *Luronium natans* populations in England and Wales since 1800 in relation to relief and the navigation canal system.

Compared to upland lakes (Table 1), canals have harder, more alkaline and calcium-rich waters with much higher major ion concentrations. Some populations occur at conductivities up to 800 µmho, far exceeding the range in Table 1, but are not listed there as information on the other environmental factors is not yet available.

Table 2 lists some of the principal species with which *Luronium* often coexists in canals, based on the three largest existing canal populations. This illustrates the diverse composition of the associated lowland floras and their complete contrast in character to upland lake communities, as is evident from the small number of species common to both lists.

Fringes of *Glyceria maxima* (Hartm.) Holmberg and a partial surface covering of *Lemna minor* L. are ubiquitous and *Elodea nuttallii* (Planch.) St. John and *Sparganium emersum* Rehm. are widespread. The canal communities are phytosociologically characteristic of the Potametea classes, with *Potamogeton compressus* L., *P. obtusifolius* Mert. and Koch, *P. crispus* L., *P. natans* and *P. berchtoldii* Fieb. the species most frequently encountered. In the Rochdale Canal, where *P. natans* and *P. compressus* are very scarce or absent, the morphologically similar *P. epihydrus* Raf., an alien species which naturalized from cotton mill waste in the early 1950s (Shaw 1963), may occupy an equivalent niche.

iii. Other Habitats

Luronium has been recorded from a variety of other freshwater habitats in Britain, including slow flowing rivers and streams, ditches, ponds, small lowland lakes or reservoirs and open water pools associated with fens or lowland raised bogs, for example on the Shropshire-Cheshire Plain. These records are concentrated in Wales or bordering counties and involve outlying sites, remote from the main center of distribition or originate from the mid-1800s, prior to the expansion of *Luronium* into lowland districts via the canal system.

Existing sites in this class are often situated on sandy peat and have clear, shallow, neutral or mildly acid water of variable base status. Many are poorly documented but it is known from historic records that most supported a flora characteristic of mesotrophic conditions at the time *Luronium* was present. The most distinctive and commonly associated species are *Echinodorus ranunculoides* (L.) Parl., *Eleogiton fluitans* (L.) Link, and *Apium inundatum* (L.) Reichb.f., with *Pilularia globularia* L., *Hypericum elodes* L., *Veronica scutellata* L., *Potamogeton gramineus* L. and *Sparganium minimum* Wallr. often also present. These species are occasionally found intermixed with others more characteristic of base-rich environments, such as *Potamogeton lucens* L. and *Hippurus vulgaris* L. The persistence of the isoetids *L. dormanna* and *Littorella* was, by contrast, a temporary feature of sites in the Shropshire meres. Running water sites are additionally characterised by the presence of *P. natans*, *N. lutea* and *M. alterniflorum*, while *M. trifoliata*, *E. fluviatile* and *C. rostrata* are of general occurrence.

In terms of their physical, chemical and vegetational characteristics, these sites are more typical of contemporary continental *Luronium* habitats, as described, for example, by Wittig and Pott (1982), Arts *et al.* (1990), than the two previous groups. Heavy losses during this century, due in part to drainage, agricultural improvements, nutrient enrichment and successional infilling are also reminiscent of the fate of

Luronium in continental Europe, although acidification, a widely noted factor in the decline of Littorellion communities on the continent, is not implicated in the loss of *Luronium* from equivalent habitats in Britain.

DISCUSSION

The ability to persist in plant communities, water types and local climates of very contrasting character proves *Luronium* to be a remarkably catholic species. Despite being traditionally regarded as a plant of soft, acid waters (Libbert 1940), it is clear that *Luronium* can thrive in the circumneutral to mildly alkaline, relatively base-rich waters found in canals and some other lowland habitats. Perhaps the only real significance of water chemistry to a plant of such wide ecological amplitude is as an influence on the composition and productivity of the vegetation with which it must coexist.

Initially, therefore, such a localized distribution seems anomalous. The one factor which unifies these diverse habitats, however, appears to be a permanent or temporary low abundance of competitively dominant macrophytes such as tall growing, but easily damaged, elodeids and marginal reedswamp species.

Upland oligotrophic lakes offer a chronically unproductive, periodically wave-disturbed habitat, the intermittent severity of which is often revealed only by a strandline accumulation of uprooted isoetid plants which may appear after gales. Low nutrient and inorganic carbon availability also require efficient nutrient harvesting and conservation mechanisms and cannot support the heavy demands of high biomass-density, competitive species for growth and repair of plant tissue. Here *Luronium* forms part of a disturbance and/or stress-tolerant vegetation, dominated by short, compact, robust, slow growing, evergreen plants with high belowground:aboveground biomass ratios (Hutchinson 1975, Spence 1982).

Shallow, lightly trafficked canals, a niche rare in continental Europe, are species rich, productive habitats where the stresses imposed by competitive species are curbed by the moderate disturbance of the boat movements (Murphy and Eaton 1983). Slowly flowing, unnavigated rivers subject to occasional scouring floods represent a comparable niche more often occupied by continental populations (*e.g.* Weigleb 1983).

In disused canals undergoing restoration, or more rarely in ponds and ditches, *Luronium* occurs as a prolific opportunistic colonist in the wake of severe disturbance by dredging or other clearance operations. Hanspach and Krausch (1987) describe the widespread occurrence of *Luronium* in recently cleaned or newly constructed drainage ditches in southeast Germany.

Small, sheltered or isolated sites may support a low biomass of aquatic vegetation due to a fluctuating water level, infertile or poorly structured sediment, nutrient-poor or low pH water, shading by an overhanging tree canopy or simply due to chance dispersal effects allied to water area or remoteness from a pool of suitable propagules.

In lowland areas outside canals, a high natural turnover of *Luronium* populations should be anticipated due to the ephemeral nature of the post-disturbance, pioneer niche (unless this is renewable) and the susceptibility of small isolated populations to extinction simply through stochastic fluctuations.

The absence of *Luronium* from habitats occupied by more aggressive species, or its presence only as a relic pioneer species, indicates a weak competitive ability. In growth-strategy terminology (Grime 1977), *Luronium* habitats lie on a gradient of stress and disturbance. Selection appears to favor a ruderal strategy, but tolerance of the stresses imposed by low-nutrient concentrations, low pH or partial shading from trees or more competitive aquatic species confers a wide ecological amplitude. Heterophylly may provide an added dimension to phenotypic plasticity and may partly account for the broad adaptability of *Luronium*.

CONSERVATION AND MANAGEMENT

Between 1900 and 1970, *Luronium* expanded its distribution in Britain. Upland populations, some with a recorded history of over 200 years, were largely stable and despite their vulnerability seem to be continuing to resist the insidious threat of acidification via acid deposition (*cf.* Wade 1983). Early losses from lowland ponds and ditches, often due to habitat destruction (*e.g.* Sinker *et al.* 1985), were offset by expansion along canals. Despite suggestions in the popular botanical literature of an ongoing expansion (*e.g.* Rose 1981, Blamey and Grey-Wilson 1989), this trend has more recently reversed due to the decline of *Luronium* on canals where recreational boat traffic has increased, often greatly, over the last 30 years (*e.g.* Macclesfield Canal; Newton 1990). Nevertheless *Luronium* retains a relatively healthy status in Britain compared to continental Europe, and surviving lowland canal populations are clearly of increasing international importance (Briggs 1988). Several canals and lakes where *Luronium* occurs are now notified as Sites of Special Scientific Interest (SSSIs) and therefore protected by statute under the Wildlife and Countryside Act 1981.

In Wales some lake populations have persisted for centuries without specific protection. Geographical isolation in a sparsely populated region with little intensive agriculture has probably contributed to the stability of these populations. An underlying historical trend toward cultural acidification (*e.g.*

Fritz *et al.* 1990) threatens their long-term security, but is outside the scope of current legislation and its effects are often hard to predict or detect. Sites in lowland Britain where *Luronium* populations are less stable must therefore be considered a priority for conservation.

Effective policies depend here on fully recognizing the crucial importance of controlled disturbance, often from artificial sources. This in itself will present a challenge to many conservationists. Meanwhile there are the parallel dangers of complacency arising from a belief that *Luronium* is still increasing and, paradoxically, from new legislation which will risk losses through an over-protectionist lack of disturbance. The decline or displacement of *Luronium* from a number of lowland SSSI's over the last 15 years, due to excessive disturbance or successional overgrowth of more competitive species in the absence of management, is a reminder that statutory protection alone does not guarantee survival.

The main management dilemma, therefore, is to create and maintain a disturbance regime which errs neither toward neglect nor toward excessive damage. On navigable canals, regulated, light recreational boat traffic may be the most effective means of providing low intensity disturbance, because direct physical intervention is not required. In steep-sided channels this is especially true, perhaps because reflective scour from boat-wash and an unfavorable bank profile suppress the establishment and overgrowth of reed swamp (notably *G. maxima*) and so retain an open marginal zone for less aggressive species. With this conservation strategy, *Luronium* can persist indefinitely at low abundance. Furthermore, water-plant communities then tend to be at their most species-rich and often contain additional species which are scarce in Britain, such as *P. trichoides* Cham. and Schlecht and *P. compressus*.

However, some prime canal habitats (*e.g.* Montgomery and Rochdale Canals) remain largely unnavigable. Natural infilling processes demand periodic channel dredging or control of emergent marginal vegetation to maintain water supply or land drainage functions, although these operations may also support a longer term objective of renewed navigation. Channel engineering works have inadvertently encouraged growth of *Luronium*, sometimes evident one or two seasons later as extensive monodominant stands. Without further intervention these populations are eventually displaced by more competitive elodeid species and reedswamp. Conservation by routine channel clearance may therefore offer an alternative to light boat traffic, but with less lasting results. Dredging must be repeated regularly to restore the open water phase and arrest succession. This could be on a rotational basis to provide a gradation of recovery states and accelerate recolonization from adjacent populated lengths.

Renewed boat traffic on restored canals presents a threat to *Luronium* if it exceeds critical levels. Sole reliance on the passive management effects of either low traffic or periodic channel clearance as outlined above may not be possible. The more difficult options of actively developing and managing offline refuges (BWB and NCC 1986), transplanting material, or regulating boat traffic on the main channel may have to be considered.

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Temporal Variation in the Biomass of Submersed Macrophytes in Lake Okeechobee, Florida

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ABSTRACT

Random aboveground biomass samples of submersed vascular macrophytes were collected to document the relative effects of selected biotic and abiotic factors on temporal variation in abundance and community composition in three macrophyte communities in Lake Okeechobee, Florida. Submersed taxa included southern naiad (*Najas guadelupensis* (Spreng.) Magnus), Illinois pondweed (*Potamogeton illinoensis* Merong), vallisneria (*Vallisneria americana* Michx.), and hydrilla (*Hydrilla verticillata* (L.f.) Royle). Summer rainfall increased mean water column station depth by *ca.* 1.5 m. Biomass was negatively correlated to Secchi depth ($r = 0.74$, $p < 0.05$). Naiad was most negatively affected by changes in water transparency. Secchi depth, water depth, and subsurface light penetration (PAR) were the most consistent factors in the regression models, although macrophyte response to environmental factors is species specific.

Key words: algal epiphytes, depth, vallisneria, hydrilla, naiad, pondweed.

INTRODUCTION

Submersed vascular macrophytes are important ecological components of aquatic systems (Wetzel 1985). These primary producers provide habitat for invertebrates (Soszka 1975), epiphytes (Cattaneo and Kalff 1980), fish (Wiley *et al.* 1984) and a variety of other aquatic organisms (van der Velde 1987). Aquatic macrophytes can play a critical role in the nutrient dynamics of aquatic systems (Carpenter and Lodge 1986). Macrophyte distribution and biomass are influenced by a variety of environmental factors including water transparency and depth, light, and nutrient availability (Spence 1967, Canfield *et al.* 1983, Chambers and Kalff 1987), as well as biotic factors such as the degree of colonization by epiphytes

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(Sand-Jensen and Borum 1984, Stevenson 1988, Sand-Jensen 1990).

A review of the literature indicates that little research has been done on macrophyte dynamics in shallow tropical systems. In addition, much of the work that has been done on lakes occurring in tropical latitudes has been in deepwater oligotrophic systems located at high elevations (Denny 1972, Denny 1973, Harper 1992). Shallow lakes often possess extensive littoral zones relative to deeper water systems. Additionally, sampling regimes in previous investigations of submersed macrophytes in lacustrine systems typically consist of one or two samplings during the months of peak biomass (Langeland 1982, Duarte *et al.* 1986, Canfield and Duarte 1988, Chambers and Prepas 1990). Few studies have addressed temporal variation in the species composition of submersed aquatic macrophyte communities throughout the entire growing season. The purpose of this research was to observe changes in plant community composition and abundance within individual beds of submersed macrophytes in Lake Okeechobee, a subtropical-tropical lake located in south central Florida. Repeated point sampling during a 13 month period facilitated examination of the effects of temporal variation in water quality, lake stage, and epiphyte abundance on macrophyte biomass and species composition.

MATERIALS AND METHODS

Study site. Lake Okeechobee (26°56.00'N, 80°55.00'W) is a large, shallow subtropical-tropical lake (surface area *ca.* 1805 km^2, mean depth of *ca.* 2.7 m) with a littoral zone that occupies *ca.* 21% of the total surface area (Schelske 1989). The lake is a highly managed system serving as a reservoir, fishery, recreation site, and floodwater control. Lake Okeechobee is eutrophic to hypereutrophic (Canfield and Hoyer 1988).

Sample collection. Three stations were established in December 1990 in selected macrophyte beds (all greater than 275 m on the shortest axis). Stations were located by triangulation with fixed landmarks and Loran coordinates. All sites were protected from wind and wave action by nearby emergent vegetation and dense surrounding submersed vegetation.

Random aboveground biomass samples (0.25 m^2) were collected monthly in triplicate and transported to the laboratory for processing. Individual plants were also collected in triplicate to measure epiphytic chlorophyll. Samples were hand-collected (by snorkeling) in order to avoid the sampling error associated with mechanical collection methods which can either overestimate or underestimate plant biomass (Downing and Anderson 1985).

Coincident water column measurements were made at each station. A Hydrolab unit, model Surveyor II was used to measure water temperature, pH, conductivity, and dissolved oxygen concentration. Light penetration was measured as water transparency using a Secchi disk and as photosynthetically active radiation (PAR) using a LiCor data logger model LI-1000 equipped with a LI 193 SA spherical light probe. A depth pole marked at 0.1-m intervals was used to measure station depth. Surface water grab samples were collected to determine water column chlorophyll *a*, alkalinity, and total nitrogen, phosphorus, and silica. Samples were analyzed for chlorophyll *a* and nutrient concentrations according to APHA (1989) standard procedures. Water samples were stored on ice in the dark until processed.

Sample processing. Each aboveground biomass sample was cleaned of contaminants and partitioned by species. Each sample was then dried at 60C for a minimum of 48 hr and weighed.

A modification of the mechanical removal method of Gough and Woelkerling (1976) was used to separate the epiphytic algae from the individual macrophytes (Zimba and Hopson in prep). Plants were placed in separate 1-L plastic bottles containing 100 mL of distilled water. Each bottle was then agitated by hand at approximately 180 revolutions per minute. A subsample of the resultant epiphyte suspension was filtered through glass fiber filters (0.7-µm porosity). Epiphyte chlorophyll samples were processed and chlorophyll concentrations calculated in accordance with APHA (1989) guidelines and equations. Epiphyte chlorophyll concentrations were normalized to dry weight of host plant. The monthly mean for epiphyte chlorophyll was then used to calculate algal biomass per square meter of macrophytes at each station per sample date.

Data analyses. The data were initially tested for normalcy by analysis of mean:standard deviation ratios. Log+1 transformations were used to reduce variance (Ricker 1973). Data were then analyzed using stepwise multiple regression analysis (SAS Institute, Inc. 1988). Macrophyte biomass was used as the dependent variable and epiphyte chlorophyll and field measurements were used as the independent variables in each of the macrophyte regression models. Forward independent variable selection was halted when the addition of new variables explained less than 10% more of the total variance. Use of regression analysis was deemed more appropriate to identify forcing variables than path analysis; regression techniques typically result in models containing more significant components than causal relationship models (Asher 1976, Zimba 1985).

RESULTS AND DISCUSSION

Total submersed plant biomass ranged from 27 to 1593 g/m^2 dry weight, with a mean value of 759 g/m^2 (Figure 1). This mean value is three times the maximum biomass reported by Langeland (1982) for several Florida lakes. Southern naiad was the dominant species, accounting for 70 to 99% of the total submersed plant dry weight at each station during at least 9 of the 13 months of the study (Figure 2). Community structure at the more northern stations (depicted in Figures 2a and 2b) had higher concentrations of hydrilla relative to the southern site, especially during the winter. The southern site was dominated by naiad throughout the study period.

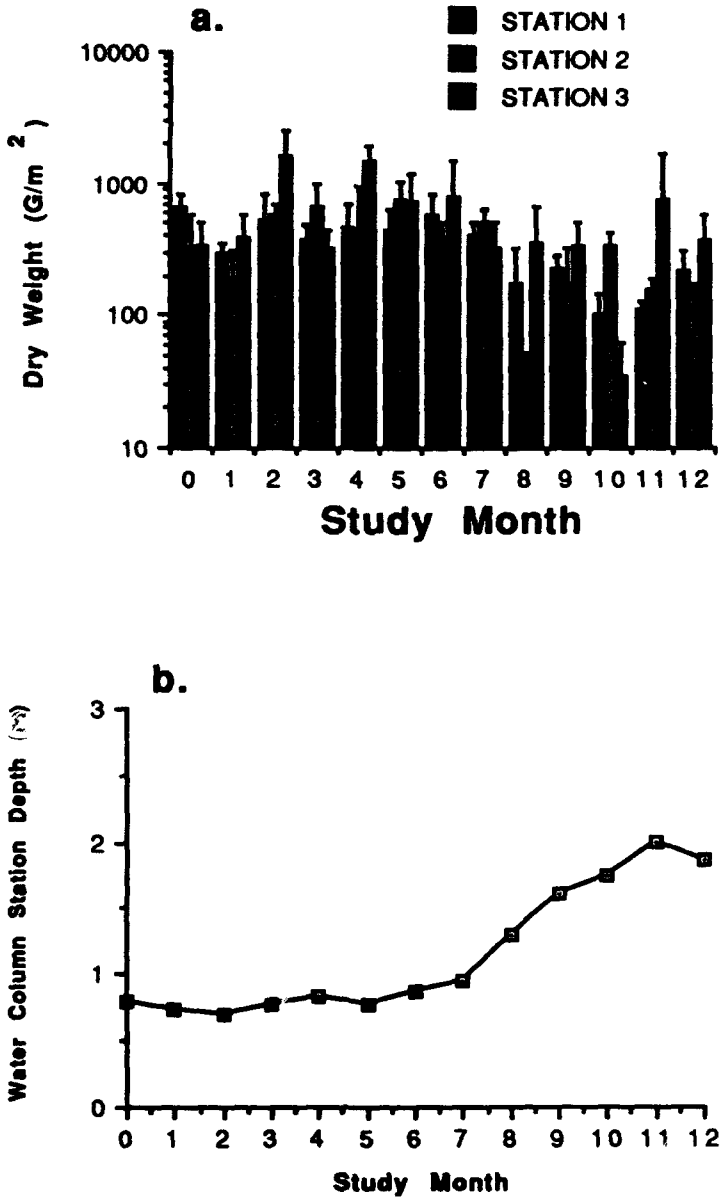

Figure 1. (a) Temporal variation in total mean biomass (g/m^2 dry weight) at three sites in Lake Okeechobee, Florida. Monthly mean values (n = 3) were determined for December 1990 through December 1991. (b) Mean water column depth at the three sampling sites.

Secchi depth was identified by multiple regression analysis as the factor explaining more than 54% of the variation in total submersed plant biomass (Table 1). Addition of subsurface light to the total biomass model explained an additional 10.15% of the variation. Water transparency (measured as Secchi depth) also accounted for the greatest amount of the variation in southern naiad biomass. The results indicate an inverse relationship between water transparency and macrophyte biomass whereas subsurface light and biomass were positively related. In each model where epiphyte chlorophyll was a significant factor, epiphyte chlorophyll and macrophyte biomass were directly related. This positive relationship contrasts with the conclusions of Sand-Jensen and Borum (1984), Stevensen (1988), and Sand-Jensen (1990) that increases in epiphyte biomass negatively influence macrophytes. It is possible that the enhanced productivity levels of macrophyte communities in tropical habitats (Stevenson 1988, Duarte 1989) allow macrophytes to "outcompete" epiphytic floras by rapid growth. Macrophyte success would be limited to environments where macrophyte:epiphyte competition for limiting resources such as light, nutrients, and gaseous exchange (*cf.* Allen 1971, Sand-Jensen and Borum 1984, Sand-Jensen 1990) does not significantly decrease maximal growth rates (μmax) of the macrophytes.

Our results may reflect fluctuation in water levels caused by heavy rainfall in the watershed during the summer months of the study. Higher water levels would result in increased total fetch and the increased water turbulence and mixing would increase the frequency of sediment resuspension and seiche events.

Such events result in an increase in the quantity of suspended solids in the water column and in turn a decrease in water transparency. As indicated by the results of the regression analyses, we would expect decreased light availability to have the greatest effect upon plants with a decumbent habit such as naiad. The results indicate that, ultimately, each of these interrelated factors, water transparency, water depth, and subsurface light penetration, represent factors which play a significant role in determining the growth of submersed macrophytes. However, the differential weights of the independent variables in each respective regression model indicate the species specific nature of macrophyte response to environmental conditions.

In summary, our results indicate that total biomass in submersed macrophyte communities in Lake Okeechobee is most influenced by fluctuations in water transparency and subsurface light penetration (PAR). The response of component taxa to environmental conditions is species specific.

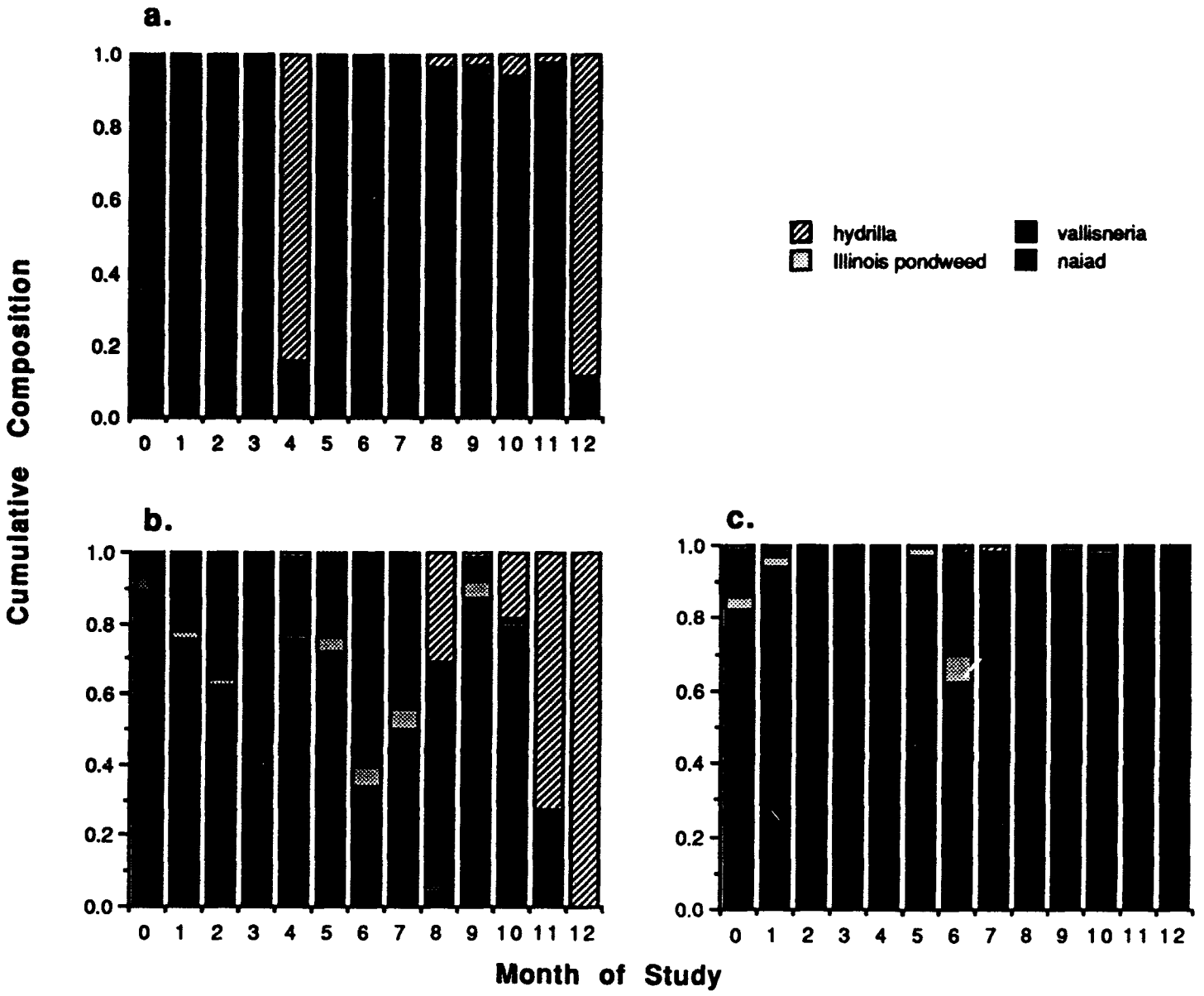

Figure 2. Community structure of macrophytes at stations 1 (a), 2 (b) and 3 (c) in Lake Okeechobee, Florida. Monthly mean values (n = 3) were determined for December 1990 through December 1991.

This conclusion agrees with findings reported by Scheffer *et al.* (1992) in which two species of pondweed exhibited differential responses to changes in water depth. Chambers (1987) concluded that the composition and relative abundance of macrophyte species are determined by differential physiological responses of plant species to environmental factors. We also agree with Canfield and Duarte's (1988) conclusion that generalizations about the response of macrophytes to different environmental conditions should be made with extreme caution. Species specific response to environmental conditions is of special importance in the management of lacustrine systems because fish appear to be differentially attracted to individual species of SAM (D. Fox, Florida Game and Freshwater Fish Commission pers comm., Chick 1992). Our predictive models may be useful in the development of strategies for lake management in tropical systems which promote more "desirable" species of macrophytes. Additional study in this field is required, however, before any predictive models such as the ones developed by Duarte *et al.* (1986) can be designed to facilitate management of other systems with similar limnological features.

TABLE 1. MULTIPLE REGRESSION ANALYSIS RESULTS USING PLANT BIOMASS AS THE DEPENDENT VARIABLE AND PHYSICAL/CHEMICAL STATION MEASURES AND EPIPHYTE CHLOROPHYLL AS THE INDEPENDENT VARIABLES. MACROPHYTE BIOMASS, SUBSURFACE LIGHT, EPIPHYTE CHLOROPHYLL, AND CONDUCTIVITY VALUES WERE LOG TRANSFORMED PRIOR TO ANALYSIS.

Dependent variables	Independent variables	Cumulative R^2	F value	Prob. > F
Total biomass	-0.898*Secchi	54.66	33.77	0.0001
	0.252*log (sub I_o)[a]	64.81	5.48	0.0303
Naiad biomass	-1.134*Secchi	47.66	18.21	0.0004
Pondweed biomass	-0.013*alkalinity	17.82	8.95	0.0086
	-1.080*depth	30.62	12.33	0.0029
	0.141*bottemp[b]	49.32	11.06	0.0043
Vallisneria biomass	-1.303*depth	32.22	17.92	0.0004
	-0.503*pH	53.20	8.52	0.0088
Hydrilla biomass	5.737*TP[c]	19.31	5.15	0.0352

[a]Submersed light insolation values (as PAR).
[b]Temperature measured at the bottom of the water column (C).
[c]Total water column phosphorus (ppm).

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Submersed Aquatic Plant Communities In Western New York: 50 Years Of Change

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ABSTRACT

Chautauqua Lake and the Cassadaga Lakes in western New York have shown substantial changes in the relative abundance of various species in the submersed macrophyte communities over the last 50 years. Many of the *Potamogetons* species, in particular, have declined dramatically and currently two exotic submersed macrophytes, Eurasian watermilfoil (*Myriophyllum spicatum* L.) and (*Potamogeton crispus* L.) are the dominant species. The two lake systems have been subjected to only minor changes in ecological factors during this time period, with the exception that Chautauqua Lake has had extensive aquatic plant management programs for the last 25 years. It has been suggested that these herbicide and mechanical harvesting practices are the major factor causing the submersed plant community changes in Chautauqua Lake and more recent changes are compatible with that hypothesis. However, the Cassadaga Lakes system has shown equally large changes in the plant communities, even though aquatic plant management programs have been much less extensive. It thus appears that aquatic plant management programs, while possibly a contributing factor, are not the major cause of the observed declines in relative abundance of many aquatic plant species.

Key words: macrophyte, relative abundance, herbicides, harvesting.

INTRODUCTION

Changes in the macrophyte communities of many lakes in the Great Lakes region (and throughout the world) have been documented (*e.g.* Nichols and Mori 1971, Stuckey 1971), but little evidence as to the cause of these changes has been cited. Invasion by two exotics, *Potamogeton crispus* L. and *Myriophyllum spicatum* L, appears to coincide with a sharp decrease in species richness and the relative abundance of species in many temperate lakes of North America (Nicholson 1981). Nicholson indicates that management practices (herbicides and mechanical harvesting) are the most likely cause for aquatic plant community changes in Chautauqua Lake, New York, but evidence presented in this paper suggests that this may be too simple an explanation.

METHODS AND MATERIALS

The study areas consist of two natural lake systems of glacial origin in western New York. Chautauqua Lake is a large (57 km^2) mesotrophic/eutrophic lake which is heavily used for recreational purposes. It consists of two distinct basins, each rather long and narrow. The northern basin is deeper (avg. depth 7.8 m) and is mesotrophic while the southern basin (avg. depth 3.5 m) is eutrophic. There is a large body of information concerning Chautauqua Lake water chemistry, phytoplankton and macrophytes dating from the 1930s, 1970s and early 1980s (extensively reviewed by Mayer *et. al.* 1978).

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The Cassadaga Lakes, actually three small, interconnected lakes (40 ha, 10 ha and 40 ha), likely formed as glacial kettle holes and are naturally eutrophic. There are year-round homes and some summer cottages on the lower lake, but swampy areas have minimized shoreline development on both the middle and upper lakes. Population on the watershed has been stable or declining over the past 50 years.

Both lake systems are surrounded by farmland or abandoned farmland reverting to a beech/maple deciduous forest. There is no industrial activity in the watershed of either lake. Population levels and available water chemistry data suggest that overall nutrient loading and other environmental parameters have changed little in the Chautauqua Lake system since the 1930s (Nicholson 1981). There are fewer data available for the Cassadaga Lakes, but alkalinities and pH values have not changed and a severe oxycline at about 3 m which was reported in 1937 still occurs at that depth (Mantai, unpublished data). Water chemistry and geomorphology data from both lake systems indicate that they are very similar in overall characteristics, although the northern basin of Chautauqua Lake is more mesotrophic than the Cassadaga Lakes.

Nicholson (1981) noted that aquatic weed management practices, including herbicides such as diquat and endothall (applied yearly in late June at certain sites) and mechanical harvesting (continuous from late June through September over most of the lake), have been extensively used since the 1950s on Chautauqua Lake and he suggests that weed harvesting is a major factor causing changes in the species composition of aquatic plant communities in this lake.

The Cassadaga Lakes, however, have had little macrophyte management until recently, and on a much more limited scale (Thorp, personal communication). The treatments consisted of diquat or endothall applied in late June to only the most heavily used areas, mostly in the lower lake, or a single mechanical harvesting performed in July or August on areas near cottages or swimming beaches. Large areas of the lakes, particularly the upper lake, have never received any aquatic plant management. Thus the Cassadaga Lakes system can serve as a reasonable comparison to test the hypothesis that weed management practices have a major impact on the relative abundance of aquatic plant species in these lakes.

McVaugh (1938) utilized manual techniques to sample the macrophyte communities on Chautauqua Lake and the Cassadaga Lakes (the time of the year when sampling occurred was not indicated in the paper) while Nicholson (1981) performed very extensive studies on Chautauqua Lake measuring cover, frequency and biomass using hand-picking in shallow water and SCUBA and grappling in water >1 m deep. Numerous quadrats and transects at many sites were sampled from April to October in the years 1972 to 1975. In the study reported in this paper, available resources limited us on Chautauqua Lake to sampling biomass with random 25-cm^2 quadrats only in the shallow water (<1 m) communities. Visual observations from <1 m to 2-3 m in depth were made with SCUBA along transects perpendicular to the shoreline (one at each site). A total of 9 sites (2-4 quadrats per site with a total of 30 quadrats) were sampled in late June and early July and these were among those also sampled in 1972-1975 and in the 1937 study. On the Cassadaga Lakes, visual observations from a boat were made in late May when water clarity is at a maximum and aquatic plant growth was still at a point where individual plants could be discerned. The lakes are small enough so that the macrophyte beds of the entire lake system could be observed. Individuals of each plant species were collected for positive identification. The observations included depths from <1 m to the limit of plant growth (about 3 m). Data are reported as relative abundance (according to McVaugh 1938).

RESULTS AND DISCUSSION

Decreases in relative abundance of some species of aquatic macrophytes were evident in 1991 compared to 1937 and 1972-1975, with several species apparently disappearing entirely from the study sites on Chautauqua Lake (Table 1). Because fewer sites were sampled in 1991 it is not certain that these species are no longer in the lake. Some of the *Potamogeton* species, however, are now totally absent from sites where they formerly were abundant. At some sites where *P. amplifolius* was reported to be common in 1937 and infrequent in the 1970's it is now absent.

The dominant species in the upper basin of Chautauqua Lake are clearly *Myriophyllum spicatum* and *P. crispus*. However, there is a conspicuous zonation of submersed macrophytes in Chautauqua Lake with both *M. spicatum* and *P. crispus* tending to be found in the deeper waters. Our shallow water sampling (≤1 m) thus may have undersampled these species, although the SCUBA transects extended to 2-3 m in depth.

Najas flexilis seems to be increasing in abundance, based on recent work by Storch (personal communication). *Najas*, being a low-growing species, is less susceptible to removal by mechanical harvesters than tall species such as the *Potamogeton* species. Biomass data on shallow sites (1 m) show a virtual absence of *Najas* in the 1970s (Nicholson 1981) but an average of 26% of the biomass in the 30 quadrats sampled in 1991.

Nicholson (1981) reported that in Chautauqua lake in 1972 to 1975 the species richness was essentially unchanged

TABLE 1. RELATIVE ABUNDANCE OF SUBMERSED AQUATIC MACROPHYTES IN CHAUTAUQUA LAKE, NY IN 1937, 1972-5 AND 1991.

Species	Relative abundance[1]		
	1937[2]	1972-5[3]	1991
Potamogeton diversifolius	+	-	-
P. epihydrus	+	-	-
P. vaseyi	P	-	-
P. amplifolius	++++	++	-
P. zosteriformis	++++	+	-
P. gramineus	+++	+	-
P. praelongus	++++	+++	++
P. illinoensis	++	+	-
P. Robbinsii	++	+	+
P. crispus	++++	++++	++++
P. pusillus	++++	++++	+
P. foliosus	++	++	-
P. natans	+	+	+
P. richardsonii	P	++++	+
Heteranthera dubia	++++	+++	++++
Najas flexilis	++++	+++	++++
Megladonta beckii	++	+	-
Elodea sp.	++++	++++	++++
Najas guadalupensis	-	++++	-
Ranunculus trichophyllous	-	+++	-
Myriophyllum spicatum	+++	++++	++++
Ceratophyllum demersum	++++	++++	++++
Valisneria americana	++++	++++	++++
Myriophyllum tenellum	P	++	-

[1]After definitions used by McVaugh (1938): ++++ = common, widespread wherever suitable; +++ = frequent, in 25-50% of characteristic habitats; ++ = infrequent, in <25% of characteristic habitats; + = rare, seen only once or twice; P = abundance not given; - = not found.
[2]McVaugh 1938.
[3]Nicholson 1981.

from 1937, although five species recorded in 1937 were not found and three new species were added. Relative abundance, however, decreased substantially, with 14 species showing declines and only 4 increasing.

Nicholson concluded from his studies that plant management techniques (herbicides and mechanical harvesting) were the primary causes of the observed changes in the relative abundance of macrophyte species in Chautauqua Lake. Data reported in this paper show continued declines in species reported by Nicholson to have low resistance to plant management practices.

Similar plant abundance studies were performed on the Cassadaga Lakes in 1990. In spite of much less human intervention, dramatic changes have also occurred in the aquatic plant communities as indicated in Table 2. Most of the species reported in the Cassadaga Lakes in 1937 to be "common" or "frequent" are now "infrequent," "rare" or were not seen at all. Most of the *Potamogeton* species which were observed in 1990, or in a spot-check in 1992, were seen only as widely scattered individual plants. In almost no cases were there "beds" of plants. The submersed plant community of the lakes is totally dominated by *M. spicatum*, with an understory of *Ceratophyllum demersum*. Unfortunately, we do not know when *M. spicatum* displaced *M. exalbescens* in either Chautauqua Lake or the Cassadaga Lakes as identification is difficult (Aiken, Newroth and Wile 1979) and herbarium specimens from the earlier studies are not available.

McVaugh (1938) in his narrative on the Cassadaga Lakes noted that the lakes "had the most diversified plant life of any lake studied except Chautauqua Lake" (in the Allegheny River watershed), although he also noted that "milfoil" was "particularly abundant" in the lakes. The verbal descriptions of the sampling sites on Chautauqua Lake by McVaugh also provide a useful means to compare the "relative abundance" definitions used in Tables 1 and 2. While there is a certain subjectiveness in describing plant communities in relative terms, a careful comparison of the data obtained in this study with verbal descriptions of the sites in 1937 suggest that the comparisons made among the three studies are valid.

TABLE 2. RELATIVE ABUNDANCE OF SUBMERSED AQUATIC MACROPHYTES IN CASSADAGA LAKES, NY IN 1937 AND 1990.

Species	Relative abundance[1]	
	1937	1990
Potamogeton amplifolius	C	-
P. crispus	I	I
P. epihydrous	F	R
P. gramineus	I	-
P. natans	I	-
P. pusilus	C	R
P. praelongus	C	R
P. robbinsii	F	-
P. vaseyi	F	R
P. zosteroformis	C	R
P. illinoesis	-	R
Najas flexilis	C	-
Elodea canadensis	C	R
Valisneria americana	§	-
Heteranthera dubia	C	R
Ceratophyllum demersum	C	C
Myriophylum spicatum	C	C
Utricularia vulgaris	C	-

[1]After definitions used by McVaugh (1938): C = common, widespread wherever suitable; F = frequent, in 25-50% of characteristic habitats; I = infrequent, in <25% of characteristic habitats; R = rare, seen only once or twice; - = not found; § = reported by Zenkert (1934).

It is clear that large changes in the relative abundance of the various species of submersed aquatic macrophytes have occurred in both Chautauqua Lake and the Cassadaga Lakes over the last 50 years. The *Potamogetons*, in particular, have declined dramatically and some of the large-leafed species which were formerly abundant may now be totally absent from these lakes. In contrast to most other American lakes, these two lake systems have been subjected to surprisingly little increases in human intervention during this time period, except that aquatic plant management practices, both herbicides and mechanical harvesting, have been widespread on Chautauqua Lake for at least the last 30 years. These facts, in part, led Nicholson (1981) to conclude that aquatic plant management practices were the major cause of the changes in relative abundance of aquatic plants in Chautauqua Lake.

The Cassadaga Lakes, however, have not been subjected to aquatic plant management to nearly the extent nor for nearly as long as Chautauqua Lake and yet show an even greater change in the structure of the plant community. Unfortunately, we do not have yearly data for these lakes which would provide a "rate constant" for the changes which have occurred. The data from Chautauqua Lake are compatible with the hypothesis that aquatic plant management practices are a contributing factor in modifying the structure of aquatic plant communities. The Cassadaga Lakes data, however,suggest that other factors, as yet undetermined, have an even greater impact, unless macrophyte control practices produce very large changes in species abundance in a short time and extend to areas of a lake which have not been directly subjected to management. Clearly a great deal more work needs to be done before the effects of herbicides and harvesting on aquatic plant communities can be determined.

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Seasonal Relationship Between Southern Naiad and Associated Periphyton

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ABSTRACT

Periphyton growing on southern naiad (*Najas guadalupensis* (Sprengel) Magnus) were collected monthly to determine the relationship between southern naiad and its associated periphyton in south Florida. Periphyton biomass was determined indirectly by measuring the chlorophyll *a* of cells after separation from apical portions of the macrophyte and reported as mg chlorophyll *a* per g DW of macrophyte. The periphyton biomass, composed mainly of Cyanophyceae and Bacillariophyceae, changed seasonally depending on light intensity ($r = -0.72$, $p < 0.01$), temperature ($r = 0.54$, $p = 0.05$) and water nutrients ($O\text{-}PO_4$ $r = 0.58$, $p < 0.05$). A significant inverse relationship between periphyton and southern naiad biomass ($r = -0.77$, $p < 0.01$) was found. The shading caused by periphyton appears to promote the seasonal senescence of southern naiad, which releases nutrients and subsequently stimulates further periphyton growth.

Key words: epiphytes, *Najas guadalupensis*, senescence, seasonality, light attenuation.

INTRODUCTION

The native submerged macrophyte southern naiad causes restricted recreational use and water flow in many of southwest Florida lakes and canals (Blackburn and Weldon 1964,

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Lawson 1991). Ecological studies of southern naiad are lacking and the information that is available is largely restricted to its distribution and taxonomy (Haynes 1977, 1979; Lowden 1986). Martin *et al.* (1970) studied the relationship of nutritional and environmental factors that affect the growth of macrophytes of the genus *Najas* but little information concerning the life history and seasonal growth requirements of southern naiad has been published.

The relationship between macrophytes and associated periphyton has been studied intensively in species other than *Najas* spp. (Allanson 1973, Cattaneo and Kalff 1978, Blindow 1987). The effect that periphyton have on macrophytes is controversial. Two such controversial ideas include periphyton inhibitory effect on macrophytes and periphyton proliferation as a result of metabolite "leakage" from macrophytes (Rejmankova 1989).

Light intensity, temperature (Barko *et al.* 1984) and nutrient availability (Landers 1982) play an important role in regulating macrophyte seasonality. We believe these environmental factors affect periphyton as well.

The objective of this study is to describe the seasonal periodicity and community composition of a periphyton community associated with southern naiad and the potential significance of this periphyton community on the seasonality of the host macrophyte.

MATERIAL AND METHODS

The study area was located in a sparsely populated area of the city of Cape Coral, FL, latitude 26°37'N and longitude 81°57'W. The study site was a man-made freshwater reservoir with a surface area of 6.5 ha and a maximum depth of 3 m.

Periphyton associated with southern naiad were collected monthly from April 1991 to May 1992. Six samples were taken from randomly selected sites using SCUBA equipment. Apical portions (approx. 25 cm) of southern naiad were carefully placed inside 2-L wide-mouth glass containers under water. Samples were analyzed the same day of collection at the laboratory. Jars containing the samples were slowly drained by siphoning with plastic tubing. FAA solution (500 ml of ethanol, 350 ml of water, 100 ml of formalin and 50 ml of glacial acetic acid) was added (approx. 250 ml) to the glass container. The loss of cell metabolites, due to the use of FAA solution, can occur and reported values for chlorophyll *a* may be conservative. This was inevitable in order to remove and to preserve the material for enumeration and identification. Removal of the periphyton was accomplished by a combination of agitation and acid hydrolysis of the mucilage-like adhering structures (Gough and Woelkerling 1976). After 1 min of vigorous shaking, "clean" macrophyte material was removed, dried in an oven for 24 hr at 105C and weighed on an analytical balance. Microscopic inspection of southern naiad leaves and stems revealed less than an estimated 1% of the periphyton remaining after the extraction process. Loose periphyton in solution was collected in a graduated cylinder and diluted to 500 ml with deionized water. Aliquots of 100 ml were used to determine chlorophyll *a*, following APHA (1989) methodology. Periphyton biomass was expressed as mg of chlorophyll *a* per g of dry macrophyte (mg Chl *a*/g DW). Additional aliquots of 30 ml were kept in vials for enumeration and identification. A Palmer counting cell was used as a counting chamber to determine percentages of the following algal groups: coccoid blue-green, filamentous blue-green, coccoid green, filamentous green, centric diatoms and pennate diatoms.

Southern naiad biomass was estimated monthly from April 1991 to May 1992 by means of a 0.1-m^2 drop-and-cut-type sampler. Thirty samples were collected from random locations, sorted according to species, rinsed of adhering periphyton and oven-dried at 105C for dry weight determination.

The water column was sampled with a Kemmerer water sampler at two deep-water stations and the results of these analyses were averaged. Samples were analyzed for turbidity, chlorophyll *a*, ortho-phosphate and nitrates following the methodologies described in APHA (1989). Turbidity was measured using a HF Scientific Inc., Model DRT-100B turbidimeter and expressed as Nephelometric Turbidity Units (NTU). Chlorophyll *a* was determined by spectrophotometry after acetone extraction. Ortho-phosphates were determined by the ascorbic acid method and nitrates were determined by the cadmium reduction method. Irradiance, measured at 0.5-m intervals with a LI-COR, Model LI-1000 Data Logger, was averaged and expressed as $\mu E/m^2/s$.

Multiple regression analysis (SPSS) (Norusis 1986) was used to compare potential statistical relationships between the dependent variable periphyton biomass and the independent variables southern naiad biomass, light intensity, temperature and ortho-phosphate.

RESULTS AND DISCUSSION

Monthly estimates of southern naiad biomass indicate a seasonal pattern, having the highest value in June (98.0 g/m^2 DW) and declining to the lowest value in August (0.3 g/m^2 DW) (Figure 1). Seasonal changes in associated periphyton biomass demonstrate a significant inverse relationship ($r = -0.77$, $p < 0.01$) with southern naiad biomass.

Periphyton community composition was dominated numerically by filamentous blue-green algae (Cyanophyceae) from July through October when periphyton biomass reached

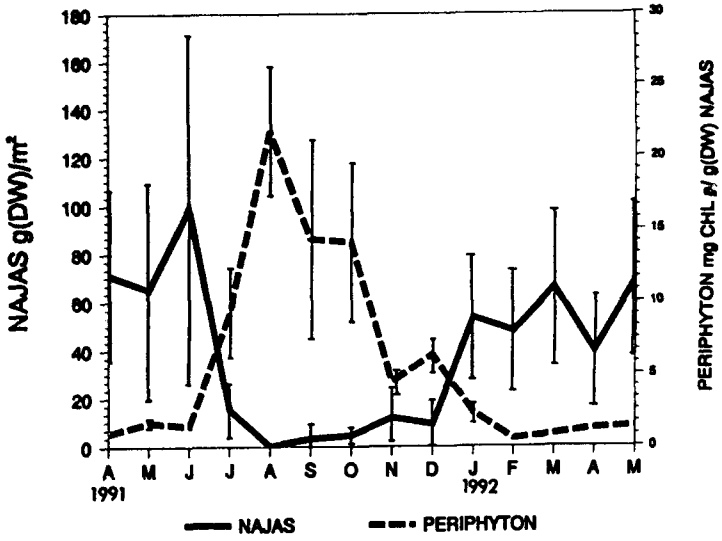

Figure 1. Average southern naiad biomass (n = 30) and associated periphyton biomass (n = 6) collected monthly from April 1991 to May 1992.

its maximum density (21.8 mg Chl *a*/g DW Najas) (Figure 2). A subsequent shift to dominance by diatoms (Bacillariophyceae) occurred from November through February after periphyton biomass declined. Dominance by diatoms also occurred during June 1991 (56%) although periphyton biomass is lower than when filamentous blue-green algae were dominant. Barko *et al.* (1988) reported similar algal succession where species composition shifted from diatoms and green algae in the early summer to blue-green and green filamentous algae in late summer and autumn. In our study,

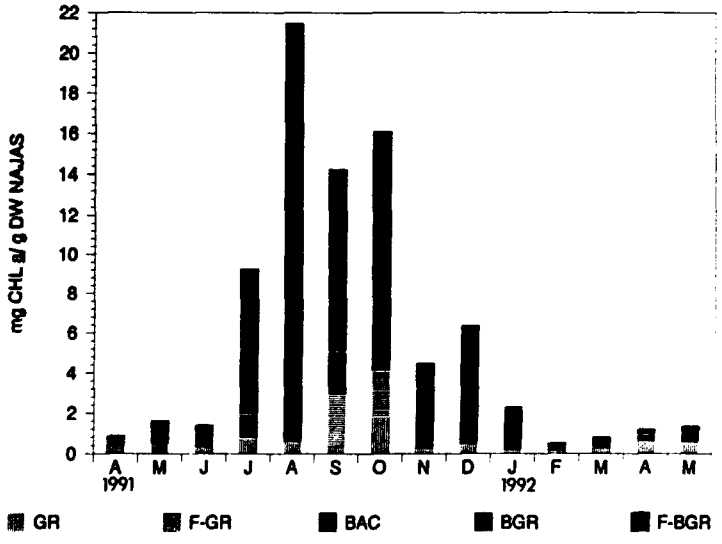

Figure 2. Periphyton community composition associated with southern naiad. GR = coccoid green algae, F-GR = filamentous green algae, BAC = Bacillariophyceae, BGR = coccoid blue-green algae, F-BGR = filamentous blue-green algae.

the period of dominance by filamentous blue-green algae could be characterized by relatively low water transparency and warmer temperatures (Table 1). Average light intensity was inversely related to periphyton biomass ($r = -0.72$, $p < 0.01$). Water column phytoplankton, as measured by chlorophyll *a*, and turbidity were primary factors regulating light availability (Table 1). Beer *et al.* (1986) found that at reduced light and high temperature, blue-green algae tend to compete more effectively than other algal groups.

The periphyton proliferation could be interpreted as the result of the natural senescence of the macrophyte, thereby increasing nutrient availability from the decaying material to the periphyton (Burkholder and Wetzel 1990). Another interpretation is that periphyton promotes senescence of the macrophyte by reducing light availability (Sand-Jensen and Sondergaard 1981, Bulthuis and Woelkerling 1983).

Rejmankova (1989) reviewed several studies suggesting two prevailing theories relating the effect of light attenuation on macrophyte photosynthesis. In the first theory, the density of periphyton causes a shading effect suppressing macrophyte photosynthesis. However, in the second theory, the shading effect does not suppress macrophytes substantially because older leaves, which developed the most dense periphyton, are not as photosynthetically active as new leaves. Periphyton biomass, as reported in our study, was collected from apical portions and was considered relatively dense. For this reason, we believe periphyton may have a significant shading effect on southern naiad.

Periphyton extract nutrients from the water column in contrast to most macrophytes that derive their nitrogen and phosphorus primarily from the sediments (Barko and Smart 1980, 1981). As a result, periphyton and macrophytes are not competing for nitrogen and phosphorus from the same source. In general, relatively high phosphorus concentrations promote phytoplankton proliferation which decreases water transparency and subsequently reduces light available to macrophytes. Similarly, ortho-phosphate was present in relatively high concentrations (May 1991) before the macrophyte decline (Table 1), which may have accounted for the increase in phytoplankton and the decrease in light. Consequently, southern naiad biomass starts to decline and phosphorus levels increase further in August stimulating periphyton growth. Landers (1982) reported that the annual dieback of eurasian water milfoil (*Myriophyllum spicatum* L.) results in a seasonal increase of water column nutrients with subsequent increases in periphyton and phytoplankton.

A somewhat different temporal pattern for nitrate concentrations was evident. When periphyton biomass declined (October through November 1991), nitrate concentrations in the water column increased (Table 1). The increase of nitrates may be related to the release of inorganic nitrogen compounds

TABLE 1. AVERAGE PHYSICAL AND CHEMICAL WATER QUALITY PARAMETERS DETERMINED AT THE STUDY SITE, AN ARTIFICIAL RESERVOIR IN CAPE CORAL, FL.

Month	Light intensity ($\mu E/m^2/s$)	Turbidity (NTU)	mg Chl a/m^3	μg O-PO_4/l	μg NO_3-N/l	Temperature C
Apr 1991	337	0.6	3.0	1	1	26
May	394	1.1	6.2	6	3	28
Jun	295	1.2	5.6	2	5	29
Jul	132	2.0	22.6	5	1	29
Aug	163	0.8	3.7	6	3	30
Sep	288	1.0	4.7	4	4	29
Oct	268	0.7	3.0	2	18	26
Nov	262	0.8	3.3	2	55	21
Dec	314	1.0	2.8	4	46	19
Jan 1992	331	0.8	2.9	2	2	18
Feb	377	0.9	1.6	2	1	19
Mar	442	0.5	1.5	1	0	21
Apr	459	0.8	3.0	2	0	22
May	424	0.7	1.9	1	2	26

from senescing periphyton, especially the blue-green algae component, which was declining during this period (Figure 2). It has been reported that filamentous blue-green algae can release inorganic nitrogen compounds, such as ammonium, under unfavorable conditions (Zimmermann 1989). During this same period, average water temperatures declined from 25.6C to 21.1C (Table 1), and may have contributed to the decline of periphyton biomass, as indicated by the statistical relationship between temperature and periphyton ($r = 0.54$, $p = 0.05$). Bushong and Bachmann (1989) reported that water temperature was an important factor in controlling periphyton growth rates and that nutrients seldom limit the growth of attached algal communities.

The grazing effect on periphyton by gastropods has been reported (Hunter 1980) to significantly affect the productivity of "aufwuchs." However, we suspect that in this case, macroinvertebrates had an insignificant effect on periphyton abundance since none were observed on the leaves and stems of southern naiad when sampled.

In conclusion, it appears that a combination of two cause-and-effect interactions contribute to southern naiad seasonality. Increasing periphyton abundance, responding to seasonal patterns in light availability, water temperature and nutrients, may be largely responsible for declines in associated southern naiad through shading. Concomitantly, nutrients in the water column increase as southern naiad tissue senesces, stimulating further periphyton growth.

ACKNOWLEDGMENTS

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Physiological Plasticity in *Elodea nuttallii* (Planch.) St. John

J. IWAN JONES, J.W. EATON AND K. HARDWICK[1]

ABSTRACT

Elodea nuttallii, a problem weed in Britain, has been found to adapt rapidly to high pH and low CO_2, conditions which are normally associated with low growth rates. This adaptation has consequences with respect to modelling plant growth and predicting weed problems in the field. Investigations indicate that *E. nuttallii* is able to utilize bicarbonate by active transport, pumping H^+ to the lower leaf surface and OH^- to the upper surface, as proposed by Prins *et al.* (1982). For much of the time this mechanism does not operate in the field, but laboratory experiments have shown that it is switched on within a few days, over a very small pH range, as carbon dioxide supply becomes limiting. Evidence is presented that bicarbonate uptake does occur in the field and its significance to the plant's growth is discussed. These results are compared with physiological plasticity in the closely related *Elodea canadensis*.

Key words: marl, bicarbonate use, photosynthesis, water plant.

INTRODUCTION

Dissolved inorganic carbon (DIC) is present in natural waters in different ionic forms, which are freely interconvertible. These consist of free CO_2 (dissolved CO_2 and H_2CO_3, hereafter referred to as CO_2*), HCO_3^- and CO_3^{2-}, in proportions largely determined by pH, with the equilibrium shifting toward CO_3^{2-} with increasing pH. Many productive waters have pH values in the range 7 to 8, where concentrations of CO_2* are low and can be limiting as a photosynthetic carbon source, especially in still conditions where replenishment is retarded by the slow diffusion of dissolved gases in the aquatic environment (Smith and Walker 1980, Black *et al.* 1981). Plants growing in such waters often experience adverse conditions as photosynthesis raises oxygen concentrations and

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reduces CO_2* to very low concentrations, severely restricting further photosynthetic assimilation and inducing conditions for photorespiration (Simpson *et al.* 1980). This can occur both as rapidly changing diurnal cycles (Goulder 1970, Van *et al.* 1976) and as much longer term seasonal changes (Bindloss 1976, Talling 1976, Frodge *et al.* 1990). Any mechanism which enables a plant to overcome such limiting conditions will confer a competitive advantage, reducing photorespiration and maintaining net photosynthesis and growth when other species are under stress. One such mechanism is the ability to use bicarbonate as a carbon source. This ability has been described for many species common in eutrophic and hard waters (Maberly and Spence 1983, Madsen and Sand-Jensen 1991) and has been used to explain the field distribution of some species (Kadono 1982, Adams 1985). Other species typical of such waters, which lack the ability to use bicarbonate, avoid the problem of low CO_2* supply in the water by using other sources, *e.g.* by having floating or emergent leaves and thereby using atmospheric CO_2 (Salvucci and Bowes 1982, Maberly and Spence 1989, Madsen and Sand-Jensen 1991) or CO_2* produced by the sediment (Wium-Andersen 1971, Sondergaard and Sand-Jensen 1979, Maberly 1985a, b, Boston *et al.* 1989) or by growing in flowing waters (Sand-Jensen 1983).

To take full advantage of changing conditions, a plant must be able to adapt its physiology accordingly, using HCO_3^- by active uptake only when necessary, since it involves energy usage in order to gain carbon and the uptake of CO_2* by simple diffusion does not. Such a capability has previously been reported for Canadian pondweed (*Elodea canadensis* Michx.) by Sand-Jensen and Gordon (1986) and suggested for common water-crowfoot (*Ranunculus peltatus* Schrank) by Madsen (unpublished, referred to in Madsen and Maberly 1991), with the former reporting that an increase of HCO_3^- use took 56 days to develop (though loss of HCO_3^- use occurred over a shorter period, see Figure 2). This extended time scale is only sufficient to allow the plant to adapt to seasonal changes or to movements between water bodies as a result of vegetative spread. It will not allow adaptation to short-term changes, such as those which might arise during periods of hot, calm weather or algal blooms. The present work was undertaken to investigate the ability of Nuttall's pondweed (*Elodea nuttallii* (Planch.) St. John), a fast-growing, dominant species, typical of eutrophic waters, to utilize HCO_3^- as a carbon source and the rate at which it can adapt its carbon uptake characteristics to changing conditions. These abilities could be crucial to the success of this submerged plant in the changeable conditions of lentic eutrophic waters.

In charophytes the deposition of marl (crystalline $CaCO_3$ precipitated at high pH) is generally considered to be a result of the HCO_3^- uptake mechanism, being associated with the alkaline bands involved in the process (Raven, Smith and Walker 1986), the formation of which may be more an integral part of the process than a byproduct (McConnaughey 1991, McConnaughey and Falk 1991). For submerged angiosperms it has been suggested variously that i) as photosynthesis raises the pH of the water column, marl forms and precipitates onto the leaf surfaces, ii) that marl is a byproduct of epiphyton photosynthesis, or iii) that marl is a consequence of HCO_3^- uptake by a polar leaf mechanism, through which the pH at the adaxial surface (facing toward the growing tip) is raised as a result of net OH^- efflux, while that at the abaxial surface is lowered by efflux of H^+ (Prins *et al.* 1982). An investigation was made to determine whether marl is deposited on the leaves in a polar fashion, which would indicate that HCO_3^- is taken up by the polar leaf mechanism in Nuttall's pondweed.

The second part of this work was an investigation into the rate at which bicarbonate utilization is induced, as an assessment of the plant's ability to adapt to short-term changes in environmental conditions.

MATERIALS AND METHODS

Mechanism of marl deposition. Twelve 10-cm-long healthy shoots of Nuttall's pondweed, collected in June 1991 from the calcium-rich Leeds and Liverpool Canal, Merseyside, UK (53°28'N, 2°57'W), were gently brushed clean of filamentous algae and loosely tied to glass rods using cotton thread. The plants were then arranged randomly, six in normal, vertical orientation and six inverted through 180° (upside down), in a glass tank with blackened sides, containing approximately 30 l of canal water filtered twice through fine plankton netting (25 TI 35, 40 by 40 µm mesh), 2.4 mM DIC, pH 8.1, $[Ca^{2+}]$ = 1.5 mM. The rods were supported by acid-washed sand. These plants were then grown for 10 days at 15 ± 2C, 100 µmol m^{-2} s^{-1} PAR, by which time a substantial layer of marl had developed on the leaves. The water was neither aerated nor mixed during this period. The plants were subsequently examined using light microscopy. Some were also prepared for examination with a Phillips 501B scanning electron microscope (SEM), by dehydration in cold (-10C) alcohol and critical point drying, before being attached to aluminium stubs with glue and sputter-coated with gold.

Induction of bicarbonate utilization. Brown plastic beakers, height 7.5 cm, diameter 7 cm, were filled with canal sediment and covered with disks of black plastic. Into each beaker five 10 cm long shoots, collected in July 1991 from the canal, were inserted through small slits in the plastic, thus providing the plants with access to natural substrate, but largely isolating the CO_2-producing mud from the water body. Five plants were used to ensure an adequate supply of material for the physiological determinations. Each planted

pot was carefully placed in a glass jar containing 2 l of twice-filtered canal water and 24 such jars were incubated at each of the two temperatures, 25 and 15C, illuminated with 100 µmol m^{-2} s^{-1} PAR in a 16 hr light 8 hr dark cycle. The water was aerated by bubbling with air adjusted in one of the following three ways, eight jars per treatment;

i) Low CO_2* - air previously passed through soda lime at a rate which reduced the CO_2* in the water to about half ambient,
ii) Ambient CO_2* - untreated air,
iii) High CO_2* - untreated air, with small quantities of dilute hydrochloric acid added each evening, as required, to the water to reduce the pH to about 7.5. The amount of acid added had a negligible effect on the conductivity of the water.

Jars were randomly removed at different times and the ability of four of the five plants in each to use bicarbonate as a carbon source was determined as below. Conductivity and pH were measured daily each morning, and a water sample was taken from each jar to determine total alkalinity by titration to pH 4.5 with 0.01 M HCl on the day it was sampled. Total DIC and the proportions of its constituent species in the water samples were calculated according to Mackereth, Heron and Talling (1978). Bicarbonate utilization by eight plants freshly collected from the field was also determined at the start of the experiment to establish a baseline.

In order to assess bicarbonate utilization, the photosynthetic rate of leaves was measured successively in media adjusted to pH 6.5 and pH 9 and bicarbonate utilization was expressed as the ratio of photosynthesis in these two media. At pH 6.5, CO_2* is plentiful, being about 40% total DIC (=0.98 mM) and any limited change in pH produces little change in photosynthetic rate. At pH 9, CO_2* is only 0.3% total DIC (=5 µM) and the plant can therefore only carry out significant photosynthesis if it can utilize HCO_3^-. Use of the ratio removes effects due to variations in absolute rates between plants.

Photosynthetic rates were measured as oxygen evolution at 20C using a Clarke-type oxygen electrode (Hansatech, King's Lynn, UK), with a tungsten slide projector bulb providing saturating incident light of 290 µmol m^{-2} s^{-1} PAR. For determinations, three leaves from a whorl 3 cm from the tip of the plant were carefully excised with a scalpel, brushed clean of epiphytes and marl with a soft paintbrush and placed in the electrode reaction chamber containing 1.5 ml of Forsberg (1965) medium, modified by omission of Na_2SiO_3 and carbon sources and adjusted to the appropriate pH. In each case photosynthesis was initiated by injection of 0.1 ml of $NaHCO_3$ solution to give a final concentration of 2.4 mM DIC. Preliminary studies showed that no significant pH change occurred on addition of $NaHCO_3$.

To reduce the effects of photorespiration on photosynthesis (Simpson *et al.* 1980), all photosynthetic rates were determined within 1 mg O_2 l^{-1} amplitude change, in solutions containing 9 mg O_2 l^{-1} (approximately 100% saturation), being sparged when necessary with either O_2 or N_2 to achieve this concentration before measurements began. Photosynthetic rates were determined from the measured rate of change in oxygen concentration in the reaction chamber and calculated as rate of oxygen change per unit chlorophyll. The chlorophyll content of leaves was determined by the method of Arnon (1949).

To minimize diurnal influences on photosynthetic rates, the determinations were all made within the middle 8 hr of the photoperiod.

RESULTS AND DISCUSSION

The results of the first experiment (Table 1) showed that inversion of plants had no effect on the pattern of marl deposition. The consistent development of marl on adaxial rather than abaxial leaf surfaces of the inverted plants is evidence that its production is not simply a general precipitation from the water above the leaves. Neither is marl a product of epiphyton photosynthesis, since SEM examination of leaves from the inverted plants showed heavy epiphytic development on abaxial surfaces, with no marl, whereas marl on the adaxial surfaces had no epiphytes amongst it (Figure 1a). Instead it confirms that marl is produced by the leaves in a

TABLE 1. EFFECT OF ORIENTATION ON THE DISTRIBUTION OF MARL DEPOSITS ON THE LEAVES OF *Elodea nuttallii*. The Mann-Whitney U test showed there was no difference (0.05 sig.) between the pattern of marl deposition on the abaxial surface of normal and inverted plants. All leaves looked at had marl deposits on the adaxial surface. Results: U'=25.5, $U_{0.05,6,6}$=31.

Orientation of shoots	No. leaves counted	Leaves marled on abaxial surface	
		No.	(%)
Normal	32	3	(9.4)
	45	5	(11.1)
	14	3	(21.4)
	31	2	(6.5)
	20	2	(10.0)
	47	0	(0.0)
Inverted	68	3	(4.4)
	69	2	(2.9)
	60	8	(13.3)
	38	1	(2.6)
	22	0	(0.0)
	56	5	(8.9)

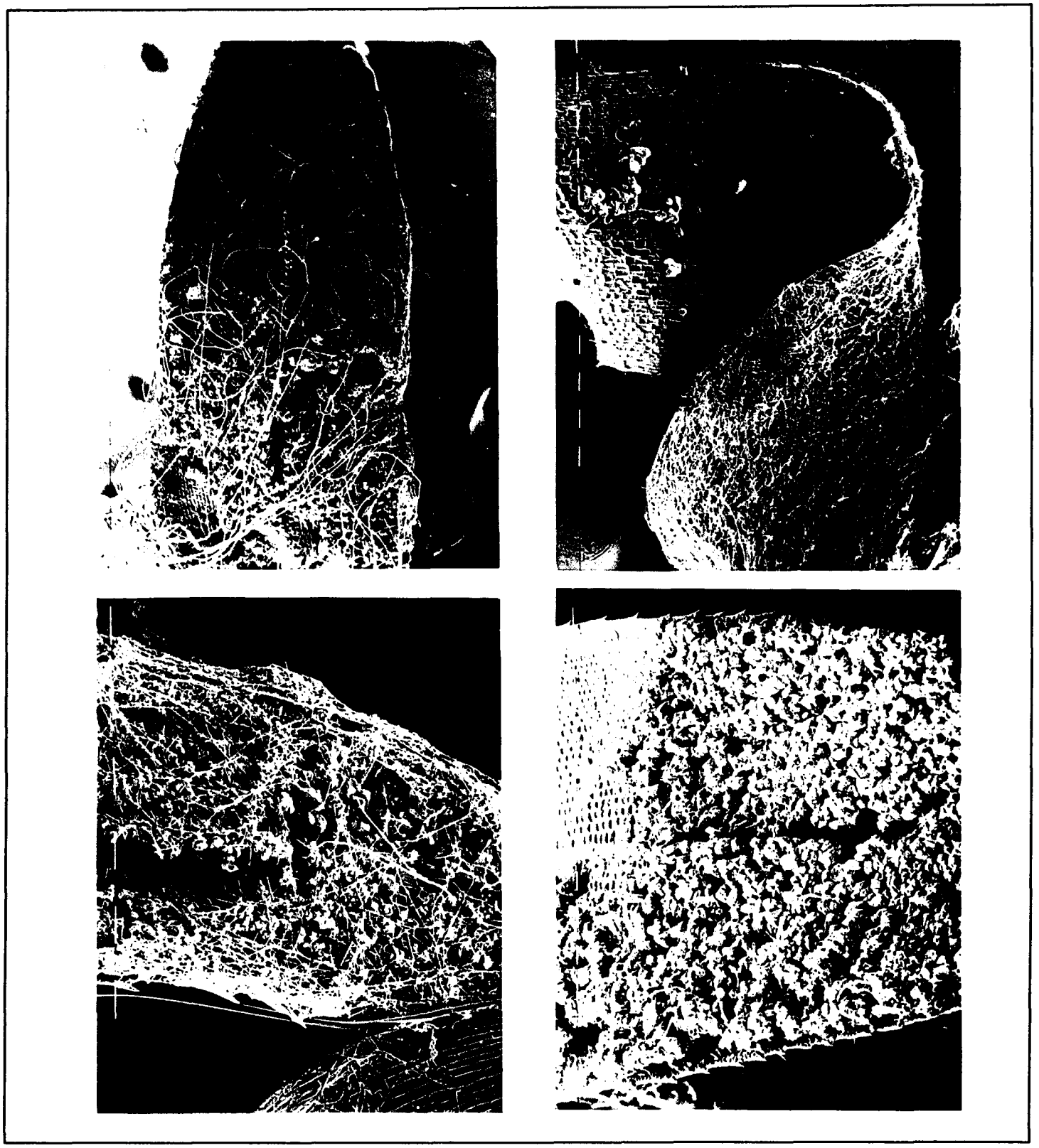

Figure 1. a) (top right) A leaf from an inverted *Elodea nuttalli* shoot showing growth of epiphyton on the abaxial surface (away from the growing point) and marl on the adaxial surface. b) (bottom right) E*lodea canadensis* leaf showing marl deposits; note absence of deposits from the midrib. c) (top left) Another leaf from an inverted *E. nuttallii* leaf, again midrib marl-free. d) (bottom left) *E. nuttallii* leaf from the field, showing similar patterning. Scale bar = 100 μm.

polar fashion and that this polarity is retained after inversion. The detailed arrangement of the marl crystals on the leaf surface, closely associated with the photosynthesizing cells of the leaf blade, but absent from the transport cells of the midrib (Figure 1b and c), is further circumstantial evidence that leaf photosynthesis is the cause of marl accumulation. In the few cases where marl was found on the abaxial surface, in both normally orientated and inverted plants, its presence could be explained easily by dislodgement from the upper surface of the next leaf during handling, or by interaction between the leaves where they were positioned very close together.

The second part of the work concerned the rate of initiation of HCO_3^- utilization, measured as the ratio of photosynthesis at pH 9 relative to that at pH 6.5. This ratio slowly decreased in plants grown under both ambient and increased CO_2* conditions, but increased in those grown under reduced CO_2 conditions (Figure 2a and b), with a statistically significant increase in HCO_3^- use detectable after only 5 days at 25C, [CO_2*] 16 µM, and 8 days at 15C, [CO_2*] 23 µM. The least significant difference used is the SE of the mean from an analysis of variance of the data (for 25C SE = 0.05 and for 15C SE = 0.085). The pH range over which this switch occurred was very small, the difference between the low and ambient treatments being about half a pH unit (Table 2). When compared with the 56 days reported by Sand-Jensen and Gordon (1986) for an increase in bicarbonate use (Figure 2c), the rates reported here are rapid, and are clearly sufficient to allow plants to adapt to short-term changes in the water body. A further, similar experiment using both Nuttall's and Canadian pondweeds collected from another site, the Lancaster Canal (54°15'N, 2°44'W), where the species grow together (results not presented here), showed that bicarbonate utilization increased very rapidly in both species, demonstrating that the faster induction rate found here compared to that found by Sand-Jensen and Gordon (1986) is not due to a specific or a clonal difference. This faster induction rate could be due to differences in the physiological states of the plants at the start of the experiments. At the time of collection, the plants used here had very little affinity for HCO_3^-, whereas the Danish ones were already utilizing HCO_3^- at a substantial rate and any further increase above this could take a considerable time to develop. The [CO_2*] of the low treatment used here (16 µM at 25C, 23 µM at 15C) is similar to that used by Sand-Jensen and Gordon (14.8 µM) to produce an increase in HCO_3^- use, and it is likely to be this low CO_2 concentration, not the pH of the water, that triggers the switch to HCO_3^- utilization in both cases.

The results presented here indicate that marl is produced in a polar fashion by Nuttall's pondweed, as a result of the physiology of the leaf, a finding consistent with the polar leaf

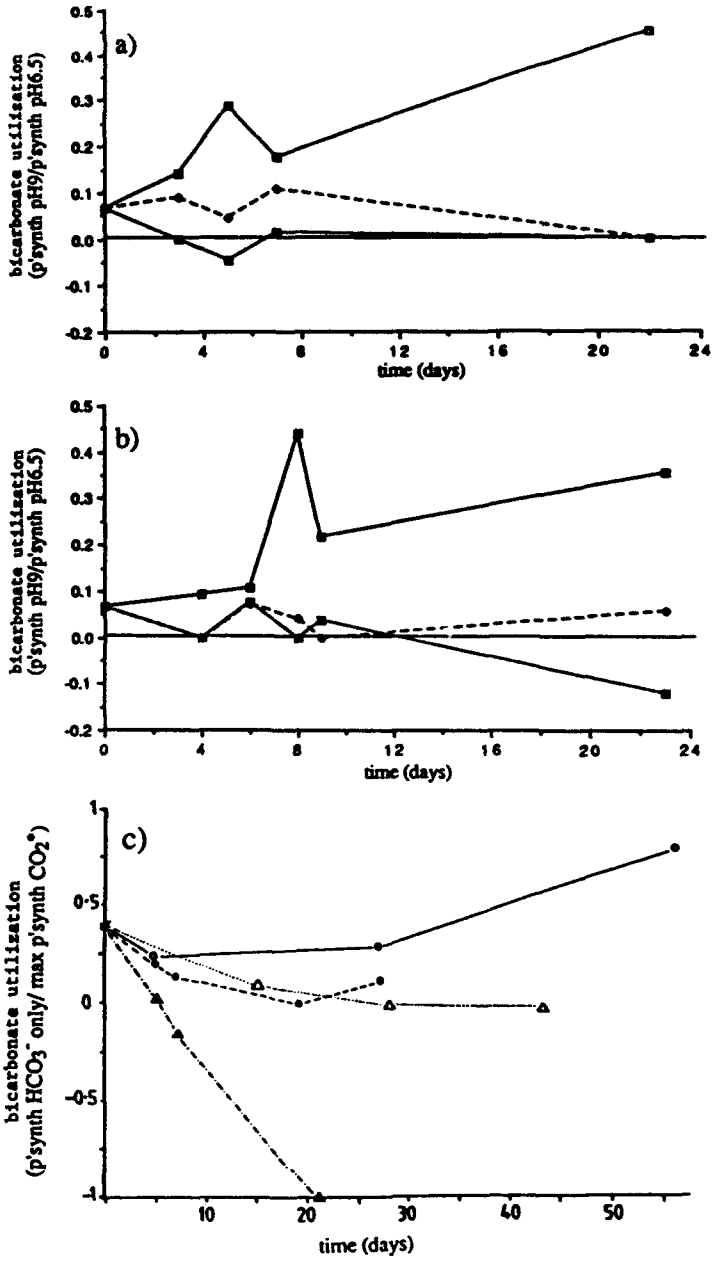

Figure 2. Changes in bicarbonate utilization of *Elodea nuttallii* when grown under different conditions, SE from ANOVA a) 25C SE = 0.05, b) 15C SE = 0.085, low CO_2* —■—, ambient CO_2* —●—, increased CO_2* —□—, (see Table 2 for concentrations). Absolute values of photosynthesis were calculated as rate of oxygen evolution per unit chlorophyll. c) *Elodea canadensis* grown at 15C, 14.8 µM CO_2* ○——○, 37 µM CO_2* ●——●, 1,000 µM CO_2*△·····△, 2,200 µM CO_2* ▲--▲ (redrawn from Sand-Jensen and Gordon 1986).

mechanism of HCO_3^- utilization proposed by Prins *et al.* (1982). These findings do not support the acid-alkali banding theory of HCO_3^- uptake suggested by Eighmy *et al.* (1987), though no investigation was made into whether or not HCO_3^- uptake is an energy-requiring process utilizing a proton pump mechanism, as they concluded.

TABLE 2. MEAN CONCENTRATIONS OF DISSOLVED INORGANIC CARBON AND CO_2* (means of six measurements taken on the day the vessel was sampled), AND pH (means from daily measurements) DURING THE EXPERIMENT. ALSO SHOWN ARE MEAN SUMMER VALUES FOR THE LEEDS AND LIVERPOOL CANAL (Saednia 1980).

Temp (C)	Treatment	CO_2*(μM)	DIC(mM)	pH
25	Low	16	2.08	8.42
	Ambient	39	2.27	7.90
	Increased	147	2.06	7.42
15	Low	23	2.35	8.38
	Ambient	49	2.29	7.87
	Increased	111	1.69	7.40
	Canal water	44.6	2.40	8.10

The Leeds and Liverpool Canal varies from pH 7 to pH 9, with CO_2* fluctuating accordingly (Saednia 1980), a situation typical of productive waters, so it would be expected that the plants would at times use HCO_3^- as a carbon source. SEM investigation of fresh material from the canal showed the pattern of marl deposition typical of plants using HCO_3^- (Figure 1d), indicating that the plants do indeed utilize HCO_3^- at times in the field situation.

Although uptake of HCO_3^- by active processes involves some energy costs and hence it is preferable for the plant to take up CO_2 by passive diffusion when available in sufficient amounts, the ability to switch rapidly to HCO_3^-utilization confers a large advantage on plants growing in changeable aquatic environments, by allowing growth to continue when conditions of high pH and low CO_2 develop. The rates of induction reported here are sufficiently rapid to allow adaptation to short-term changes in the water column, such as those which might arise during periods of hot, calm weather or algal blooms. If this ability to adapt rapidly to changing environmental conditions is widespread among aquatic plants, then it will have to be taken into consideration in the construction of models predicting weed growth in relation to resource availability.

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J. Aquat. Plant Manage. 31: 94-98

Rhizome Longevity in Two Floating-leaved Aquatic Macrophytes, *Nymphaea tetragona* and *Brasenia schreberi*

HIDENOBU KUNII[1]

ABSTRACT

Long-term observations on the fate and functioning of the underground parts in two floating-leaved macrophytes, *Nymphaea tetragona* Georgi and *Brasenia schreberi* J. F. Gmel., under field and seminatural conditions were carried out. While the individual tuberous rhizomes in *N. tetragona* never branched nor proliferated and persisted for a long time (>5 yr) at the suitable safe-site, runners in *B. schreberi* performed short-distance dispersal and more than 60% of the current-year rhizomes died off within a year in the field and their life expectancies averaged *ca.* 1.5 yr. It was also assumed that *N. tetragona* maintained an equilibrium rhizome volume by annual turnover of 20 to 30% of its mass.

Key words: biomass turnover, growth, underground part, water lily, water shield.

INTRODUCTION

The underground parts of aquatic macrophytes cannot be ignored because they form a large part of the biomass (Westlake 1982). They play an important role for the maintenance of populations, and many investigations on the production and biomass of helophytes have been made (Good *et al.* 1978, Whigham and Simpson 1978, Brinson *et al.* 1981, Westlake 1982, Sjörs 1991). However, there is still a general lack of information concerning the longevity and production of underground parts in floating-leaved macrophytes, except for particular species such as *Nuphar luteum* (L.) Sibth. & Smith (Twilley *et al.* 1985) and *Nymphoides peltata* (Gmel.) O. Kuntze (Van der Velde *et al.* 1979, Brock *et al.* 1983).

Nymphaea tetragona Georgi and *Brasenia schreberi* J. F. Gmel. are both perennial floating-leaved aquatic macrophytes and often occur together in irrigation ponds in Japan (Shimoda 1985, Kunii 1991). While *N. tetragona* has a short, erect tuberous rhizome, *B. schreberi* bears prostrate stoloniferous rhizomes (or runners). When these two species coexist within a single water body, *N. tetragona* usually occurs in shallower water than *B. schreberi*. Kunii and Aramaki (1987, 1992) have documented the life span of floating leaves of these macrophytes. However, it is clear that in order to understand the functioning of the plants within natural communities or to estimate true production, it is necessary to examine the persistence and growth of underground parts as well as aboveground parts (*cf.* Fitter 1987). Thus, the objective of the present study was to clarify the dynamics of underground parts in these two nymphaeid aquatic macrophytes.

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MATERIALS AND METHODS

Field observations. Field observations were made in two adjacent irrigation ponds (Engi-ike and Ryuzo-ike), located in Matsue, Shimane Prefecture, Japan (Kunii and Tsubaki 1987). To monitor the annual growth and longevity of rhizomes in *B. schreberi*, short shoots and tips and nodes of rhizomes were tagged with bamboo stakes. The short shoot is a part of the rhizome where the length of the internode is quite small (refer Figures 2 and 3 in Van der Velde *et al.* 1979). Marking was first done in December 1986 and the first observations were made in November 1987. After judging whether the marked parts were dead or alive, the length of the newly produced rhizome was measured. All growth beyond the stake, within the year, was regarded as annual growth. The marking and observations continued until November 1990 and 1991, respectively.

The individual rhizomes in *N. tetragona* were marked only once, in 1986, and their subsequent survival was determined by leaf development. Since the short rhizomes in *N. tetragona* were firmly rooted deep in the sediment, it was difficult under field conditions to excavate and examine their growth repeatedly without damaging them. Therefore, no effort was made to evaluate their annual growth.

Observations under seminatural conditions. To estimate the annual growth and/or loss of rhizomes in *N. tetragona*, many seeds were collected from Pond Ryuzo-ike during October 1986. In April 1987, 96 seedlings were planted outdoors in a tall cylindrical pot (70 cm in height). After a year, the 33 surviving plants were transplanted in two containers (60 cm in width by 40 cm in length by 30 cm in height in 10 cm of sediment taken from Pond Engi-ike) and then placed into an outdoor concrete pond (1.5 m deep). All plants were freely grown during the growth period and excavated annually during the resting period (late December to early March). Rhizome length from the lowest part to the upper part where buds sprouted and diameter (thickness) at the uppermost position where the roots developed were measured using a micrometer.

The growth and/or loss of rhizomes in *B. schreberi* was also monitored under the same seminatural conditions as described above. Thirty shoot apices (winter buds) were taken from Pond Engi-ike in 1987, marked individually, and planted in two containers (the same as described above). All rhizomes were uprooted during the resting period and current-year elongation of runners was measured. The newly produced runners were marked by loosely coiling flexible, thin wires around them and then buried again.

These observations under seminatural conditions are still ongoing.

RESULTS AND DISCUSSION

More than 60% of the current-year rhizomes of *B. schreberi* died off within 1 yr (Figure 1). No short shoots or nodes lived longer than 3 yr. Life expectancy of rhizomes (including apex, short shoot and node) averaged *ca.* 1.5 yr. Similar results were obtained from the plants grown under seminatural conditions (Figure 2): 49.2% and 46.3%, respectively, of the new runners produced in 1988 and 1989 survived until the end of the next growing seasons. No 2-yr old runners were found.

Figure 2 also shows that most of the runners in *B. schreberi* are the current ones, younger than a year, and only 29.6% and 29.0% are those of the previous years, 1989 and 1990, respectively. Thus mean runner age can be estimated as 1.3 yr both in 1989 and in 1990. The total length increased gradually through 1989 (21.6 m/m^2) and 1990 (24.3 m/m^2). These values compare with the maximum value in total length obtained from the natural habitat (18.5 m/m^2, unpublished data). However, the total suddenly dropped to the minimum value (4.6 m/m^2) in 1991. This phenomenon may reflect the detrimental effect of the continuous culture within a restricted place.

It is probable that the plants with smaller rhizomes in *N. tetragona* cultivated under seminatural conditions died earlier than those with larger ones (Figure 3). The plants seemed to have established stable states 4 to 5 yr after germination when the rhizomes became thick. In addition, field observation on

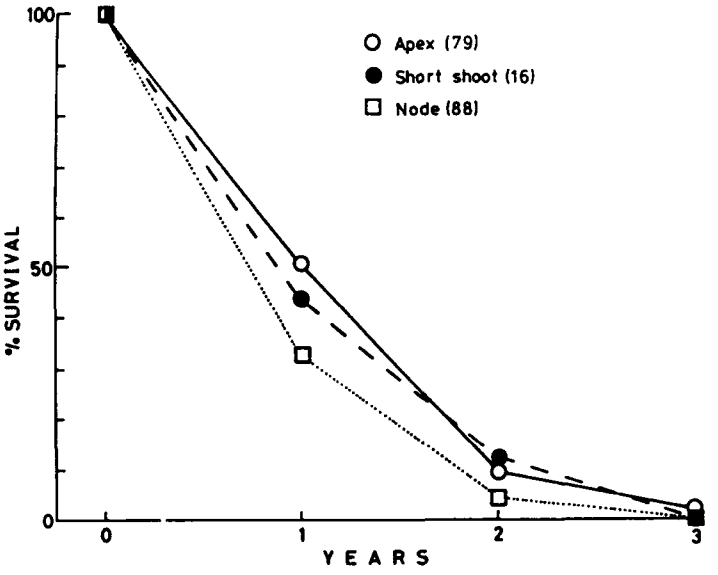

Figure 1. Time trend in percent survival of each organ of *B. schreberi* in the irrigation ponds. Results are shown as percent of the total marked in 1987 and 1988. Marked and observed numbers are shown in parentheses.

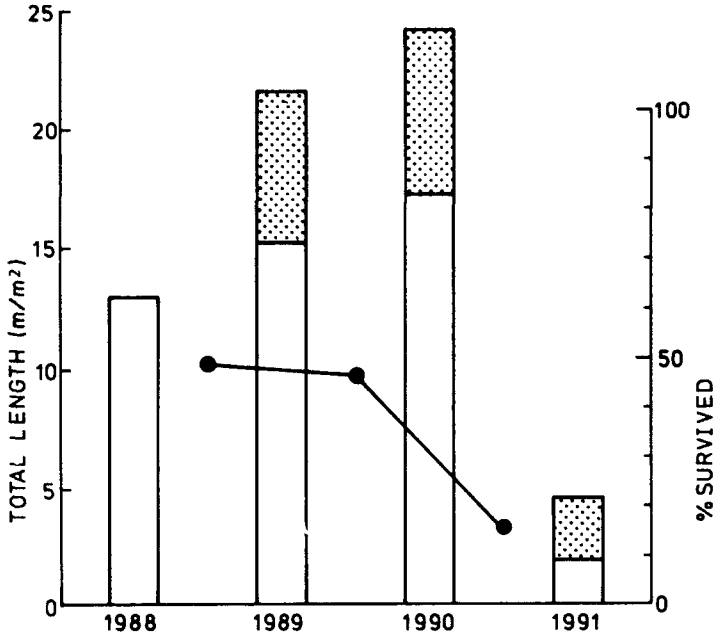

Figure 2. Temporal changes in total length of rhizomes of *B. schreberi* grown under seminatural conditions. Open and hatched columns indicate current year and previous year rhizomes, respectively. Percentage survival (in terms of length) of previous year rhizomes is shown as solid circles connected by solid lines. The results are shown as a total of two pots.

the survival of *N. tetragona* rhizomes showed that the rhizomes marked in 1986 were all still alive in 1991. These facts suggest that the rhizomes persist for a long time once they become larger than some critical size (*cf.* Heslop-Harrison 1955).

Annual growth of *N. tetragona* rhizome is shown in Figure 4. Because the rhizome shape is considered to be cylindrical, rhizome volume can be computed from length and diameter. The rhizomes attained their peak mean size in 1990 (26.2 ± 5.9 mm in length and 17.8 ± 3.5 mm in diameter) and then slightly decreased. It must be noted here that a recognizable necromass was found in 1991. The basal part of the rhizome was blackish and often without roots. The mean rhizome length in 1991 was 30.6 ± 8.3 mm and 23.4 ± 5.6 mm with and without necromass, respectively. Although there was a significant difference in diameter (F-test, $0.01 < P < 0.05$), no significant differences were found in both length and volume between the rhizomes in 1991 (without necromass) and those from Pond Ryuzo-ike. These facts imply that there is an equilibrium rhizome volume and the thickened rhizome maintains this volume by annual turnover of 20% to 30% of its mass. Further study is needed to confirm this estimation.

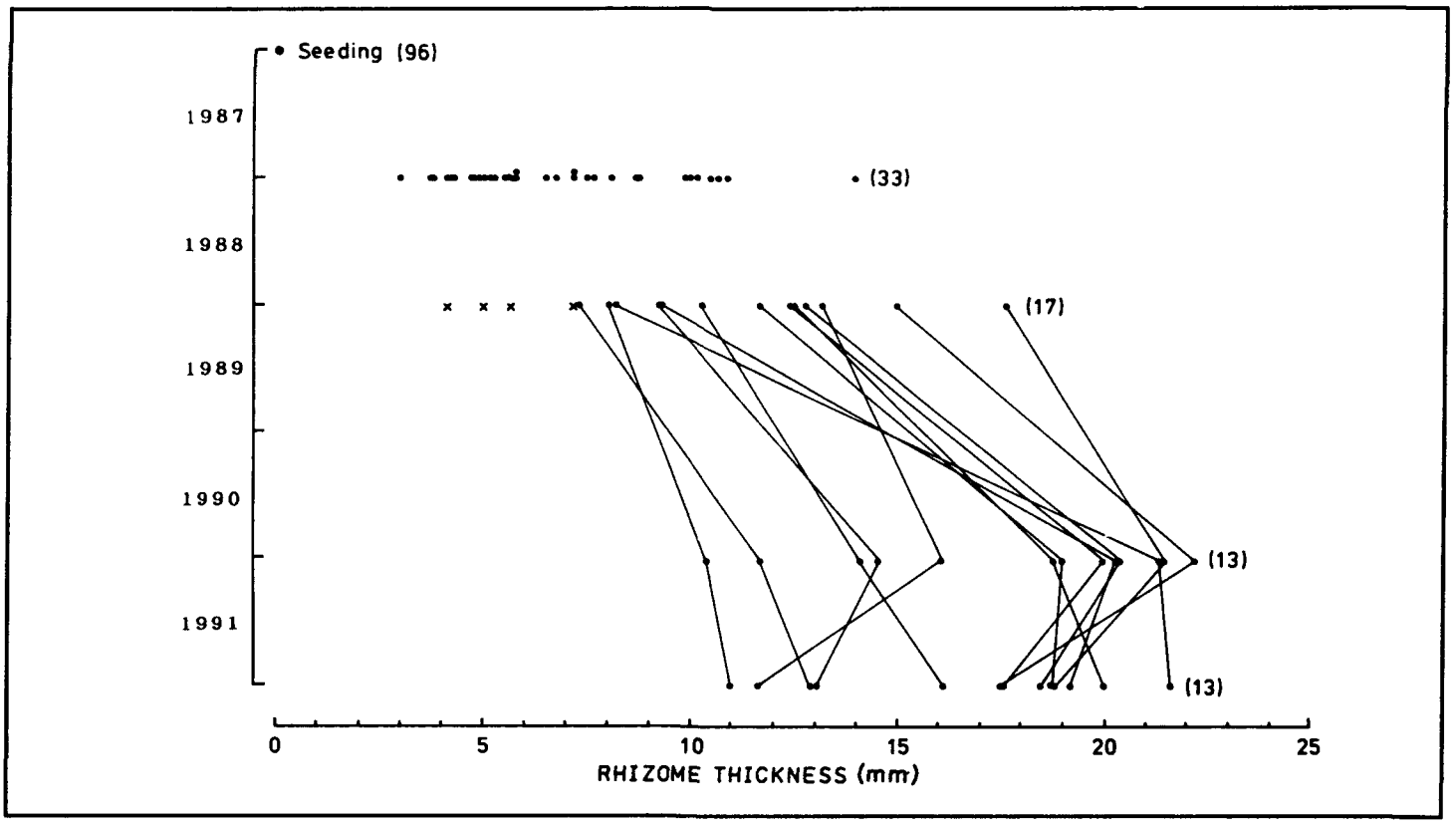

Figure 3. Temporal changes in rhizome thickness (diameter) of *N. tetragona* grown under seminatural conditions. Each rhizome was marked individually since December 1988. Figures in parentheses show number of surviving plants at each observation date.

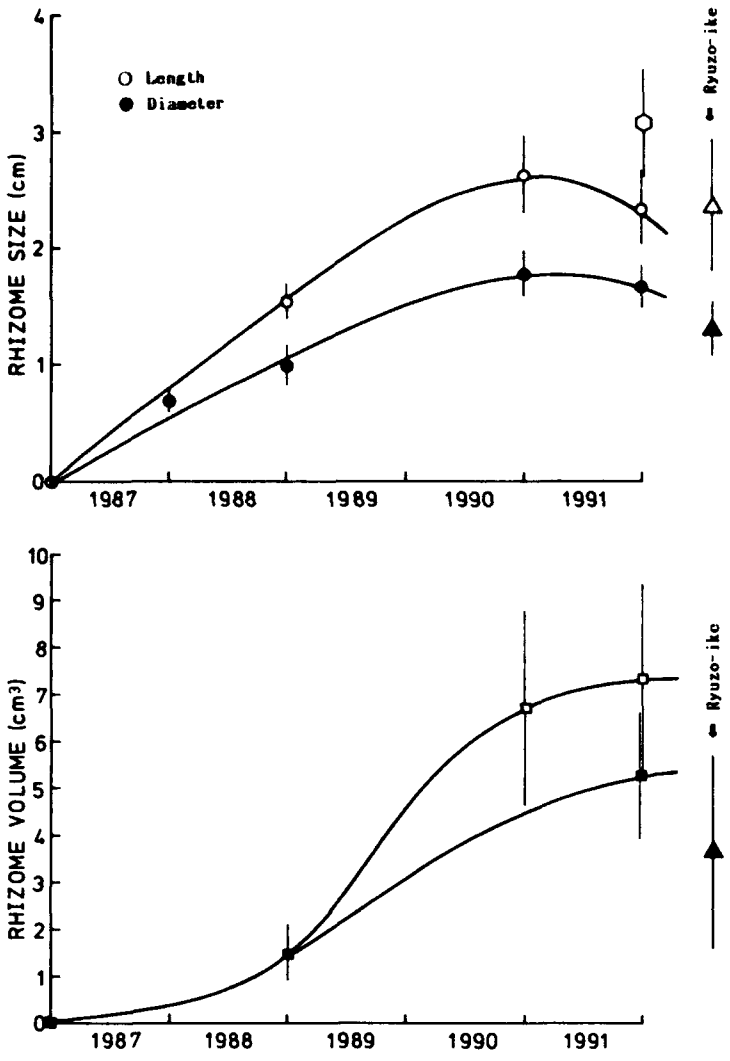

Figure 4. *Top*: Temporal changes in mean rhizome length (open circles) and diameter (solid circles) of *N. tetragona* grown under seminatural conditions. Open hexagon denotes mean rhizome length including decaying necromass and open and solid triangles show, respectively, length and diameter of rhizomes sampled from Pond Ryuzo-ike in 1985 (n = 7). *Bottom*: Temporal changes in mean rhizome volume with (open squares) and without (solid squares) necromass. Solid triangle shows mean volume of rhizomes sampled from Pond Ryuzo-ike. Vertical bars indicate 95% confidence intervals.

In general, rhizomes function as reproductive organs as well as storage organs. Short and erect tuberous rhizomes of *N. tetragona*, however, never branch nor proliferate, and only function as storage organs. They are buried at a relatively deeper site (3 to 5 cm below the sediment surface) and thus are protected from drought which often occurs in the shallow littoral zone. The plants, therefore, are enabled to persist for a long time at suitable sites and produce many seeds annually. Dispersion of this species thus depends entirely on seed. In contrast, rhizomes of *B. schreberi* act mainly as reproductive organs. The prostrate rhizomes or runners elongate just beneath/above the sediment and are suited for short-dispersal. Their longevity is quite short but they can seek the safe-sites successfully during the growing period. Shoot apices of this plant are also effective vegetative organs but somewhat less reliable than rhizomes (Adams 1969). Hard seeds seem to contribute little to the dispersal within a pond but they may allow between-pond long-distance dispersal or long periods of dormancy (Madsen 1991). The functioning of these species within natural communities will be discussed fully, coupled with information concerning the dynamics of aboveground parts in Kunii and Aramaki (1987, 1992).

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Seed Germination of *Typha subulata* in Relation to Weed Management

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ABSTRACT

The effects of light and temperature on germination of *Typha subulata* were examined under a combination of various temperature and light regimes. The influence of different storage environments was studied by placing the seeds in the dark under air-dry conditions or immersed in water at 4 ± 1 and 21 ± 3C and tested for germination after 6 and 14 months. The significance of salinity was assayed by incubating the seeds in sodium chloride solutions. The seeds of *T. subulata* exhibited a high potential for germination (88 to 98%) in a wide range (10 to 35C) of continuous or alternate temperatures. After the 6-month-storage pretreatment, germination was close to 100% in all treatments. The 14-month-old samples showed a germination rate higher than 96% when they were maintained under dry conditions at both temperatures. However, germination decreased to 64% at 4C and 0% at 21C under wet storage. The germination was affected by salinity only at high concentrations. These results indicate that in the irrigation districts of southern Argentina, the infesting potential of this weed could be minimized by disrupting the sexual reproductive cycle and by maintaining wet channel beds during the period of irrigation recess.

Key words: southern Argentina, irrigation districts, germination conditions.

INTRODUCTION

The study area is located in a temperate irrigation area in Argentina, the Valle Inferior del Rio Colorado (62°37'W, 39°23'S), where approximately 90,000 ha of land are irrigated. The average temperature during the coldest month (July) is 1.8C and during the hottest month (January) is 29.6C. The absolute minimum and maximum were -9.5 and 42.9C, respectively. Water comes from the Colorado River and is distributed by a network of 331 km of main irrigation channels and more than 3000 km of secondary and subsidiary farm channels. Water conductivity in the irrigation channels is between 0.5 and 1.5 mS cm^{-1}, and maximum and minimum salinities (mainly sodium chloride) are recorded in winter and late spring, respectively. According to Peinemann *et al.* (1979), water salinity in the area is between 1.0 and 1.5 g l^{-1} in irrigation channels and between 3 and 20 g l^{-1} in drainage channels.

In Argentina, the genus *Typha* is represented by *T. latifolia*, *T. domingensis*, *T. angustifolia* and *T. subulata*. One of the main aquatic weed problems in the irrigation network, particularly in the smaller channels, is infestation by *T. subulata* Crespo and Pérez Moreau. No publications are available on the biology and ecology of the genus *Typha* in Argentina. The taxonomy of *Typha* species in Argentina has been reported by Crespo and Pérez Moreau (1967); however, most of the international literature refers mainly to species other than *T. subulata*.

In *Typha*, the spread of an existing stand is sustained largely by vegetative means, but introduction to uninfested areas usually occurs by seeds, which may remain viable for several years (Spencer and Bowes 1990). Knowledge of the mechanisms which enable the propagules of the species to infest and reinfest the channels will assist in obtaining more

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appropriate control strategies. Therefore, the aim of the present paper was to study the seed germination characteristics of *T. subulata*.

MATERIALS AND METHODS

Mature female spikes were randomly harvested at the study site. As in all the taxa the seeds of *T. subulata* are very small, ranging from 1.5 to 2 mm in length. Seeds were separated from other floral parts by decantation in water, the number of seeds was counted in subsamples and total per spike estimated by weighting.

The effect of light and temperature on germination was examined by placing freshly ripened seeds under a combination of various light and thermoperiod conditions. The influence of different storage environments on seed germination capability was studied by placing the seeds in the dark in paper bags under air-dry conditions (dry) or immersed in water (wet) at 4 ± 1 and 21 ± 3C. After 6 and 14 months they were tested for germination under the optimum conditions found from the above experiment: 20C (dark) - 30C (light) daily thermoperiod and a 10-hr photoperiod. Under the same germination conditions, the significance of salinity was also assayed by incubating freshly harvested seeds in the presence of sodium chloride solutions ranging from 4 g l^{-1} to 20 g l^{-1}.

Five replicates of 50 seeds each were used for each germination trial. The seeds were placed in small (1.4 by 0.8 cm) polyethylene boxes containing 0.5 ml of distilled water, except in the last experiment where water was replaced by the saline solution. Seeds were left to germinate for 15 days; none germinated after the first 10 days. Light was provided by a panel of 20-W fluorescent tubes supplemented with 40-W incandescent bulbs. No damage on seed coats nor fungi or bacterial effects were observed during the experiments.

RESULTS

The estimated number of seeds per inflorescence was 56,000 (± 6,000). There were no differences in the rate of germination when the propagules were exposed in the light to a wide range of continuous or alternate temperatures (Table 1). Light was essential since virtually no germination was observed in its absence. After a 6-month storage pretreatment, germination was close to 100% independent of the storage conditions. The 14-month-old samples maintained under dry conditions showed a germination rate higher than 96% at both temperatures. However, when they were stored under wet conditions their germination decreased to 64 (±2.9) % at 4C and 0% at 21C.

Germination was affected by salinity only at relatively high salt concentrations, above 12 g l^{-1} (Table 2).

TABLE 1. THE EFFECT OF TEMPERATURE AND LIGHT ON THE GERMINATION PERCENT OF FRESHLY HARVESTED SEEDS OF *T. subulata*. VALUES ARE MEAN ± SE.

Daily temperatues during			Daily temperatures during		
10 hr dark (C)	14 hr dark (C)	Germination (%)	10 hr light (C)	14 hr dark (C)	Germination (%)
10	10	0	10	10	92 (±1.7)
25	25	0	25	25	97 (±1.8)
35	35	0	35	35	88 (±2.8)
25	10	0.4 (±0.4)	25	10	97 (±1.8)
30	20	0.8 (±0.9)	30	20	98 (±1.1)
35	10	0.4 (±0.4)	35	10	98 (±1.0)

TABLE 2. GERMINATION OF FRESHLY HARVESTED SEEDS OF *T. subulata* UNDER DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE. PHOTOPERIOD 10 HR (light) AND THERMOPERIOD 30C (light) AND 20C (darkness). VALUES ARE MEAN ±SE.

Treatments g l^{-1}	Germination (%)
0	99(±1.8)
4	98(±1.7)
8	96(±0.7)
12	95(±3.2)
15	8(±0.5)
20	0

DISCUSSION

Seed production in *T. subulata* is typical of the highly prolific character of the genus, as shown for other species (Yeo 1964, Linde *et al.* 1976). Light was essential for the germination, and this requirement was not partially replaced by alternating temperatures, as was reported for *T. latifolia* (Morinaga 1926). The seeds of *T. subulata* exhibited a high potential for germination (88 to 98%) in a wide range (10 to 35C) of continuous or alternate temperatures. In contrast, germination in seeds of *T. latifolia* was clearly reduced at temperatures below 20C (Sifton 1959).

The salinity of the Colorado River water, sometimes as high as 1.5 g l^{-1}, frequently affects the production of crops. Galinato and Van der Valk (1986) reported a significative germination reduction in *T. glauca* in waters with more than 1 g l^{-1} NaCl concentrations. However, water salinity in the channels is below the concentration that appears likely to constrain the germination of *T. subulata* seeds (Table 2). Tolerance of some *Typha* species to salinity is a known feature (McMillan 1959, Von Oertzen and Max Finlayson 1984).

The number of germinating seeds of *T. subulata* can be influenced by their environment during storage. Wet storage affected the germination capability and the intensity of the effect was temperature dependent: a marked reduction was observed at 4C and no germination was recorded at 21C. Similarly, Comes *et al.* (1978) found higher germination after dry than after wet storages in *T. latifolia* during the first year. Several authors have indicated that the germination ability of *Typha* tends to diminish with age ranging from several months up to more than 5 yr (Croker 1938, Bedish 1967, Smith 1967, Comes *et al.* 1978, Grace 1983).

Colonization of new habitats in the irrigation channels of the area by *T. subulata* is initiated by the germination of seeds which have been most likely transported by the flowing water. This study has shown that the infesting potential of *T. subulata* into new territories may be strongly emphasized by their high seed production, longevity and germination capability in a wide range of environmental conditions. Sobrero (1991) found that a single plant of *T. subulata* initiated from seed germination is able to reproduce sexually and vegetatively during its first year growth cycle (7 to 8 months). The perennation of the genotype in a given location, its expansion year after year, and the annual production of sexual propagules will be secured since the underground structures are persistent.

One management strategy to minimize the seed infesting capacity of this weed would be to interfere with its sexual reproduction, for example by mechanical or chemical procedures that prevent flowering or seed formation. Since fructification is concentrated during a short period in the summer (Sobrero *et al.* 1991), it is not necessary to repeat the treatment during the growing season.

A weed control procedure currently in use is the elimination of patches of *T. subulata* by physical methods during the winter, when irrigation is suspended. During this time the channel beds frequently remain dry. In clearing the channel bed by dredging, seeds previously buried may be brought closer to surface, where they may be exposed to light. In the following growth season, reinfestation with *T. subulata* in the same site will be dependent on the growth of reproductive underground structures not properly eliminated, or by the germination of seeds stored in the seed bank near the surface. New seedling growth in these areas will be free from competition with adult plants. The differences in germination observed in this work when the seeds were stored under dry or wet conditions suggest the possibility of reducing the potential of new infestation in weed-free areas or reinfestation in others previously invaded if the channel beds remain permanently wet even during the interval when they are not used.

ACKNOWLEDGMENTS

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Turion Production by Dioecious Hydrilla in North Florida[1]

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ABSTRACT

A 14-month study was conducted to determine the effects of photoperiod, plant density, temperature, and herbicides on turion production by floating apical stems of dioecious hydrilla [*Hydrilla verticillata* (L.f.) Royle]. Turion production begins under short day conditions in September, decreases during the cold months of December and January, increases again in the late spring and essentially ceases during June through August. Free-floating (non-rooted) hydrilla stems produced more turions than stems planted in sand. Short daylength (<12 hr light) increased turion production and high plant density decreased production. Exposure to 2.5, 5.0 and 10.0 µg/l bensulfuron methyl or fluridone significantly reduced turion production.

Key words: bensulfuron methyl, fluridone, herbicide, photoperiod, plant density, temperature.

INTRODUCTION

Dioecious hydrilla is the most widespread submersed aquatic weed in Florida. Its spread and reproduction is limited to asexual means including fragmentation and the production of two specialized reproductive propagules, subterranean turions and axillary turions. Subterranean turions have also been called "tubers" in several papers (Haller *et al.* 1976, Van *et al.* 1978, Van and Steward 1990) and the term "tuber" will be used in this paper to avoid possible confusion. Axillary turions will be called simply, turions.

Tubers are produced in the soil at the ends of rhizomes in response to short day conditions (Van *et al.* 1978). Turions, or winter buds (Mitra 1955, 1964), are condensed shoots (axes) of 12 to 15 compact internodes surrounded by fleshy leaves arranged in alternating whorls (Lakashmanan 1951). They are oval to oblong in shape, 3 to 12 mm long and 2 to 3.5 mm wide, green in color, are produced in the axils of the leaves, and are filled with reserve food material in the form of starch (Lakashmanan 1951). Turions can be distinguished from vegetative buds by the lack of spines on the midrib of their leaves (Mitra 1964).

Tubers are thought to be the more important of the two propagules for reproduction (Haller *et al.* 1976, Van *et al.* 1978, Sutton and Portier 1985), consequently, much research into tuber production has occurred while the biology of turions is not well documented.

The objective of this study was to determine the influence of environmental conditions and herbicide exposure on turion production by hydrilla.

MATERIALS AND METHODS

Five experiments were conducted to examine aspects of annual turion production, production by floating or rooted stems, effects of photoperiod, influence of plant density, and the effects of treatment with selected aquatic herbicides. Apical stem sections collected for all the experiments were examined for the presence of turions, and any turions found were discarded. All experiments, except the production experiment, were begun in March 1992, and ended in May 1992 using hydrilla from Newnan's Lake, Florida. Results were expressed as numbers of turions per kilogram of hydrilla fresh weight for both initial and final weights. Hydrilla was weighed after all excess water was removed by blotting with paper towels.

The outdoor tanks used in most of the experiments were 217 cm long by 75 cm wide by 64 cm deep. Water depth was maintained at 56 cm using a standpipe, giving a water volume of 911 L.

All data were subjected to an analysis of variance (ANOVA). Numbers of turions per kilogram (kg) fresh weight were transformed by taking square roots in order to satisfy the homogeneity of variance assumption of ANOVA (Snedecor 1940).

Production. The production experiment was begun in the first week of August 1991 and continued until October 1992. Five hundred grams of hydrilla apical stem sections (approximately 20 cm long) from Jumper Creek, Florida, were allowed to float on the water surface in each of three sections of each of two tanks (tanks A and B) containing well water and exposed to ambient climatic conditions. Each tank was divided into thirds with window screen to allow free water movement between the sections but to contain the hydrilla within each section. During the first week of September (after 1 month) the hydrilla from tank B was harvested, processed, and 500 g of freshly collected hydrilla was put in each section

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of tank B. At the same time 500 g of hydrilla was started floating in each of the three sections in a third tank (tank C). During the first week of October, the hydrilla in tank A, into which hydrilla had been placed in August (2 months prior), and that in tank C, into which hydrilla had been placed in September (1 month prior), were harvested, the number of turions determined, and new hydrilla started. During the first week of November, hydrilla in tank B (2 months) and in either of tank A or C (1 month) were harvested, processed, and restarted with new floating hydrilla. Each month thereafter the hydrilla from two of the tanks was harvested, processed, and restarted. One of the tanks contained hydrilla that had been floating for 2 months and the other tank contained hydrilla that had been floating for 1 month. The pseudoreplication (tanks divided into thirds) was used in order to minimize the amount of hydrilla which had to be processed both initially (to be discarded) and at harvest for the collection of turions, yet maintain a plant density (1.0 kg fresh wt/m^2) typical of a hydrilla population. At harvest each pseudoreplicate was weighed and the number of turions was counted. Beginning in April 1992, hydrilla from Wacissa River, Florida, was used to continue this experiment as Jumper Creek was inaccessible due to low water.

Floating/Rooted Production. Twenty apical sections were allowed to float in each of five 24-L buckets submerged in a tank and covered with 20 cm of water. The buckets had cylinders of plastic screen placed in them to contain the hydrilla. Another 20 apical sections were planted in washed builders sand (no nutrients added) in each of another five 24-L buckets with screens extending above the water surface to contain the hydrilla. In addition to containing the hydrilla, the purpose of the buckets and the screens was to collect any turions that would fall from the free floating or rooted hydrilla.

Photoperiod. Six 100-g replicates of hydrilla apical sections were floated in 24-L buckets and kept outdoors under ambient light conditions of a gradually lengthening day (from approximately 11 hr in March to 13 hr in May). Another six 100-g replicates of hydrilla were floated in 24-L buckets outdoors using 2 floodlamps and a timer to provide a 16-hr photoperiod.

Density. Four weights (100 g, 250 g, 500 g and 1000 g) of hydrilla apical stem sections were placed in 24-L buckets with cylinders of plastic screen in each and submerged in tanks of water. The calculated densities of the hydrilla in the buckets were 2.5, 6.25, 12.5 and 25.0 kg fresh weight/m^3. Two tanks were used with three replicates of each density per tank. In addition to the ANOVA, regression analysis was used to determine the relationship between the square root of the number of turions/kg and the number of kg/m^3 of hydrilla.

Herbicides. The 2-month herbicide study was performed twice, once beginning in December 1991 and ending in February 1992 and again beginning in March 1992 and ending in May 1992. A total of seven tanks were used in this experiment to determine the effect of bensulfuron methyl (methyl 2-[[[[[(4,6 dimethoxy pyrimidin-2-yl)amino]-carbonyl]-amino]sulfonyl]methyl]benzoate) and fluridone (1-methyl-3-phenyl-5-[3-(trifluromethyl)phenyl]-4(1H)-pyridinone) on turion production. Bensulfuron methyl was chosen for this experiment because previous studies showed a reduction in tuber formation in hydrilla (Haller *et al.* 1992). Fluridone has been successfully used to control hydrilla in many locations but its effects on hydrilla turion production are unknown. Each tank was divided into three sections (pseudoreplicates) and three rates of herbicide (one per tank) were used: 2.5, 5 and 10 µg a.i./L with one tank as the control. Pseudoreplication in this experiment was used for the reasons previously stated for the production experiment as well as to minimize measurement error in the herbicide application.

Five hundred grams of hydrilla apical stem sections were placed in each of the divided areas of the tanks immediately after applying and thoroughly mixing the herbicides with the water.

An orthogonal polynomial contrast was used to compare the control to all rates for each herbicide after the ANOVA was done.

RESULTS AND DISCUSSION

Production. Turion production (Table 1) in the hydrilla held for 1 month was highest in October and November, declined from December to February, and increased again in March. Turion production in the hydrilla held for 2 months showed the same general trend with a significant increase in March-April followed by a large decline in April-May. This bimodal activity is very similar to that reported for tuber production during cold winters (Van *et al.* 1978). The decline in turion production through the winter months coincides with the decline in the average temperature and photoperiod which occurs naturally with the change of seasons. Turions were not produced in significant numbers in the fall until October when daylength was 12 hr or less. Although daylengths were less than 12 hr in December through February, few turions were formed, most likely because of low temperatures (≤15C). A 14C temperature was previously noted as the minimum temperature at which this tropical species becomes physiologically active (Haller *et al.* 1976). In the spring, turions formed under daylengths greater than 12 hr in March and April. Plants at this time of the year are exposed to gradually lengthening days. Photoperiods ≤12 hr probably induce turion formation and turion production continues for a period of time beyond the spring equinox (March 21). Relatively few turions were produced by plants floated April 1; consequently, the natural induction

TABLE 1. TURION PRODUCTION BY HYDRILLA APICAL STEM SECTIONS (turions/kg freshwt/month) FLOATED FOR 1-MONTH AND 2-MONTH DURATIONS WITH AVERAGE DAYLENGTH AND TEMPERATURE FOR THE PERIOD.

1-month duration					2-month duration				
Month of growth	No. turions		Daylength (hr)	Temp (C)	Month(s) of growth	No. turions		Daylength (hr)	Temp (C)
	Mean	SD				Mean	SD		
Aug[1]	0	0	13.3	27	Aug-Sep[1]	2	0.3	12.9	26
Sep	0.3	0.6	12.4	26	Sep-Oct	179	48	12.0	23
Oct	179	16	11.5	21	Oct-Nov	221	39	11.2	18
Nov	141	87	10.9	15	Nov-Dec	33	7	10.7	15
Dec	3	3	10.5	15	Dec-Jan	18	10	10.8	13
Jan	32	6	10.9	11	Jan-Feb	4	2	11.3	13
Feb	32	6	11.6	15	Feb-Mar	83	40	12.0	16
Mar	157	66	12.5	16	Mar-Apr	400	100	12.9	18
Apr	1	1	13.4	19	Apr-May	16	15	13.7	20
May	11	7	14.1	22	May-Jun	7	4	14.2	24
Jun	13	3	14.4	26	Jun-Jul	4	2	14.2	27
Jul	0.7	1	14.1	27	Jul-Aug	0.4	0.6	13.7	27
Aug	0.7	1	13.3	26	Aug-Sep	2	0.7	12.9	26
Sep	0	0	12.4	26	Sep-Oct	73	76	12.0	23
Oct	861	280	11.5	20					

[1]Hydrilla apical stem sections were floated the first week of the month indicated and harvested the first week of the following month, or second month as indicated. Each value is the mean of three replicates followed by the standard deviation (SD).

that occurs in March does not continue for a long period of time. These data are very similar to tuber formation data reported previously (Haller *et al.* 1976). These data also suggest that there is an interaction between temperature and photoperiod on turion production which has been reported on tuber formation by hydrilla (McFarland and Barko 1990).

Floating/Rooted. Turions (±1 SD) were found to be significantly more likely to be produced on floating plants (75.6 ± 37.4 turions/kg) than on rooted plants (28.6 ± 7.8 turions/kg). Haller *et al.* (1976) postulated that turions were produced more often on floating plants and Anderson (1985) showed that tubers were formed more readily on rooted plants than were turions. This is also consistent with speculation that turions are produced by plants which have broken away from a stand, an adaptation allowing hydrilla to become established in new areas (Haller *et al.* 1976, Thullen 1990). Tubers (±1 SD) were produced (33 ± 34 tubers/kg) on some of the rooted plants in this study, but none were produced on the floating plants.

Photoperiod. Significantly greater ($p < 0.0007$) numbers of turions (±1 SD) were produced by hydrilla which was maintained in an ambient (11.8 to 12.9 hr) photoperiod (512 ± 277 turions/kg) compared to the number produced under long day (16 hr) photoperiods (63 ± 63 turions/kg). Production of the 63 turions/kg fresh wt in the 16-hr photoperiod likely results from the pre-induction of the plants that were collected during March with short day ambient conditions and subsequently placed in the artificial long day conditions. Van der Zweerde (1982) found that short days favored turion production under laboratory conditions in accordance with Haller *et al.* (1976) and Van *et al.* (1978) and also suggested that light intensity may increase turion production. Mitra (1955) also postulated that high light intensity promotes the formation of turions, which would seem reasonable, as detached floating stems would receive high light intensities at the water surface.

Density. Hydrilla density had an inverse linear relationship with turion production (Figure 1). Regression of all six replicates of turion production against density indicated that 70% of the variation in the production of turions can be explained by the variation in the density of hydrilla (Figure 1). As density increased it is likely that self-shading reduced light intensity to the plants that were closer to the bottom of the buckets, thereby reducing turion production.

Herbicide. Both bensulfuron methyl and fluridone were shown to suppress turion production in both studies conducted in December to February and in March to May. At harvest in May, the average numbers of turions in the control were much higher than in February. This is consistent with the findings of the production experiment where turion production is low from November and December to February and increases in March and April. Figure 2 shows the results of the March to May study. When the orthogonal polynomial contrast was used to compare the control against all rates, each rate was

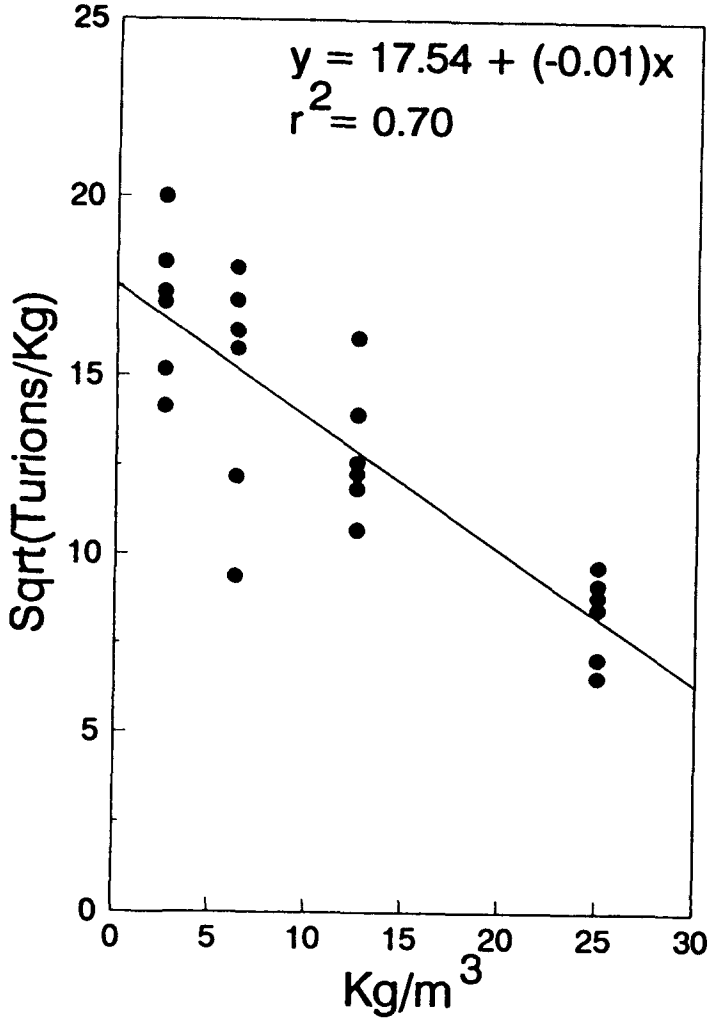

Figure 1. The effects of hydrilla density (kg/m^3) on turion production. Data for turions are expressed as the square root of the numbers produced per kilogram fresh weight.

significantly different from the control indicating that turion production was reduced across all rates. There were no significant differences in turion production between the rates. There was a significant decrease in turions produced across all rates of fluridone when compared to the control, but fluridone does not seem as effective as bensulfuron methyl as a turion inhibitor at the lower rates. The contrast showed that the concentration of fluridone has a definite linear effect. Had these plants not been pre-induced by short day conditions in the field, it is likely that turion production would have been much less, particularly by the hydrilla treated with bensulfuron methyl.

These studies have documented or verified previous hypotheses or experimental results that hydrilla reproduction by turions occurs primarily under short day conditions (photoperiod ≤ 12 hr, September 21 through March 21) and is much more likely to occur on floating plants than on rooted plants. Turion production is greater when temperatures are 15C or higher and decreases as plant density increases. Bensulfuron methyl and fluridone both reduce turion production at application rates of 2.5, 5 and 10 µg/L. Further research is needed to determine the length of pre-induction period required to initiate turion production and to examine the possible interaction between temperature and photoperiod. The importance of light intensity also needs to be determined.

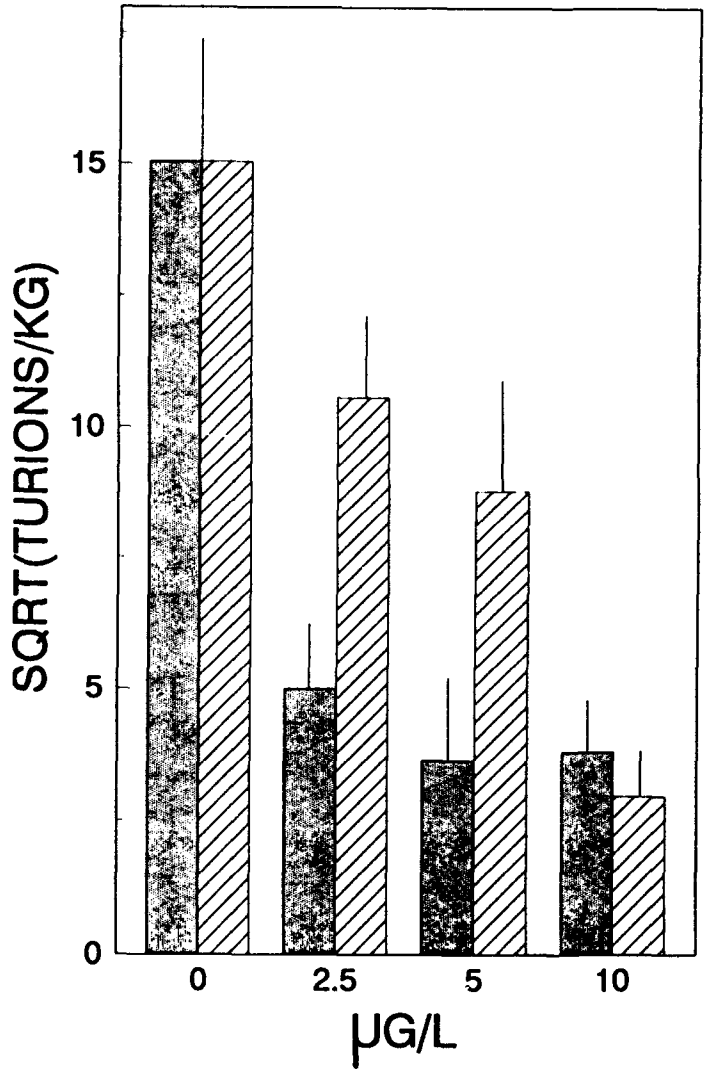

Figure 2. The effect of bensulfuron methyl (solid bar) and fluridone (hatched bar) on turion production by hydrilla. Data, from the March-May study, are expressed as the square root of the numbers of turions produced per kilogram fresh weight of hydrilla. Extended lines are one standard deviation.

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Distribution of Hydrilla in Northern China: Implications on Future Spread in North America

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ABSTRACT

Hydrilla (*Hydrilla verticillata* L.f. Royle) has greatly expanded its range in the USA since it "escaped" cultivation in Florida streams in the early 1950s. It now occurs in all the southern border states, as well as along the eastern seaboard as far north as Delaware, but further northward expansion by hydrilla in the U.S. appears controversial. We have recently been collecting potential biological control agents for hydrilla in China, and present our observations on the density and distribution of hydrilla in northern China. These are supplemented by data from hydrilla specimens in the herbaria of various Chinese scientific institutions, as well as literature records from northern Asia. These collections, along with comparisons of climatic data, indicate that hydrilla has the potential to grow in aquatic habitats almost anywhere in North America, including Canada and parts of Alaska.

Key words: *Hydrilla verticillata*, range expansion, Harbin, Sino-American Biological Control Laboratory, Manchuria.

INTRODUCTION

Hydrilla is a submersed Hydrocharitaceae, native to Australia (Swarbrick *et al.* 1981) and to Asia and Central Africa (Cook and Luond 1982). Hydrilla was introduced into the United States in 1951 or 1952 by an aquarium fish and plant dealer who released six bundles of hydrilla from Sri Lanka

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(then Ceylon) into a canal near his business in Tampa, FL (Schmitz *et al.* 1991). Hydrilla's spread in Florida was rapid, but it was incorrectly identified as *Elodea canadensis* Rich., or sometimes as *Egeria densa* Planch., until 1965 (Blackburn *et al.* 1969). The hydrilla in Florida is dioecious, with only the pistillate (female) form being present. Despite the lack of sexual reproduction (and therefore seeds), by the early 1980s, pistillate hydrilla had spread westward across the southern states into California, while simultaneously moving northward into Georgia, Alabama and North Carolina (Steward *et al.* 1984). Around this time, hydrilla was discovered in Washington, D.C. (Haller 1982). This infestation, along with those in Delaware, Maryland, North Carolina and Virginia, consisted of the monecious (both male and female flowers on the same plant) form, and was apparently the result of a new introduction from an unknown foreign source (Steward *et al.* 1984).

Forty years after its introduction, hydrilla infestations in the U.S. continue to expand. In Florida during 1991, hydrilla infested over 26,000 Ha, the most ever recorded (Schardt 1992) in state-sponsored surveys, and appears to be becoming more troublesome in some of the other states where it was introduced more recently. Several scientists (Balciunas 1985, Steward and Van 1987) have noted that, based on its distribution in northern Europe, the potential range of hydrilla in North America could include all of the U.S. as well as southern Canada. However, in the past decade few new states have been added to the list of those infested by hydrilla, encouraging some people to hope that hydrilla's geographical expansion in the U.S. has ceased.

We believe that our recent investigations into hydrilla's distribution in the Peoples Republic of China have relevance to this weed's future spread in the U.S. In 1989, the U.S. Dept. of Agriculture, in cooperation with the Chinese Academy of Agricultural Sciences, jointly established the Sino-American Biological Control Laboratory (SABCL). One of the initial (and still major) projects at the SABCL was the search for biological control agents for hydrilla and Eurasian watermilfoil, *Myriophyllum spicatum* L. (Balciunas 1990). While the SABCL is physically based in Beijing, with proper permits and contacts, we had access to sites throughout China. We also gained access to aquatic plant specimens in the herbaria of various Chinese scientific institutions. This paper presents some of our observations, based on our own collecting and collections by other SABCL staff and cooperators, as well as data from hydrilla specimens at various Chinese scientific institutions.

METHODS AND MATERIALS

Between August 1989 and the end of 1991 we collected hydrilla and other aquatic plants at numerous sites throughout China. Our collections were supplemented by those made by other SABCL staff and cooperating scientists. In addition, we examined hydrilla specimens deposited in Academica Sinica's herbarium at Fragrant Hills, in Beijing's northwest suburbs. Dr. Diao Zhengsu, Yuzhou University in Chongqing, provided a listing of the hydrilla specimens which he had collected. We also examined aquatic plant specimens in the herbaria at Inner Mongolia University and at Xinjiang August 1st Agricultural College. A report by the Fisheries Institute of Jilin Province provided us with a few hydrilla records for that province.

RESULTS AND DISCUSSION

Hydrilla is widespread throughout China and a complete listing of our hydrilla collections, as well as those from the herbaria, is being prepared for later publication. The map in Figure 1 shows the provinces in China at which we collected hydrilla and/or from where hydrilla specimens in various herbaria were collected. This map also shows the three northernmost cities (Harbin, Shenyang and Beijing) near which we personally collected hydrilla. Hydrilla was not uncommon at these three locations, and we found hydrilla in at least several sites within and near each of these three cities.

Harbin, the capital of Heilongjiang Province (previously part of Manchuria) is the northernmost of our collecting sites. It lies near the 46th parallel which in North America passes through Portland, OR, north of Minneapolis, MN, and Montreal, Canada, and above Bangor, ME, on the east coast. Thus, based only on latitude of Harbin China, hydrilla could easily occur in most of the continental USA.

Even more troubling is that hydrilla is known from numerous locations in the former U.S.S.R. (Cook and Luond 1982). Dr. C.D.K. Cook supplied the senior author with a partial listing (unpublished) of the specimens used in preparing the maps shown in the above publication. A specimen from the Angara River, north of Irkutsk, in the Kraysnoyarskiy Kray region of Siberia, at a latitude of 58°30'N, appears to be the northernmost specimen on Cook's list. This is only 9° below the Arctic Circle, and the corresponding latitude in North America would include all of Canada below the Yukon and Northwest Territories, as well as the southeastern peninsula of Alaska.

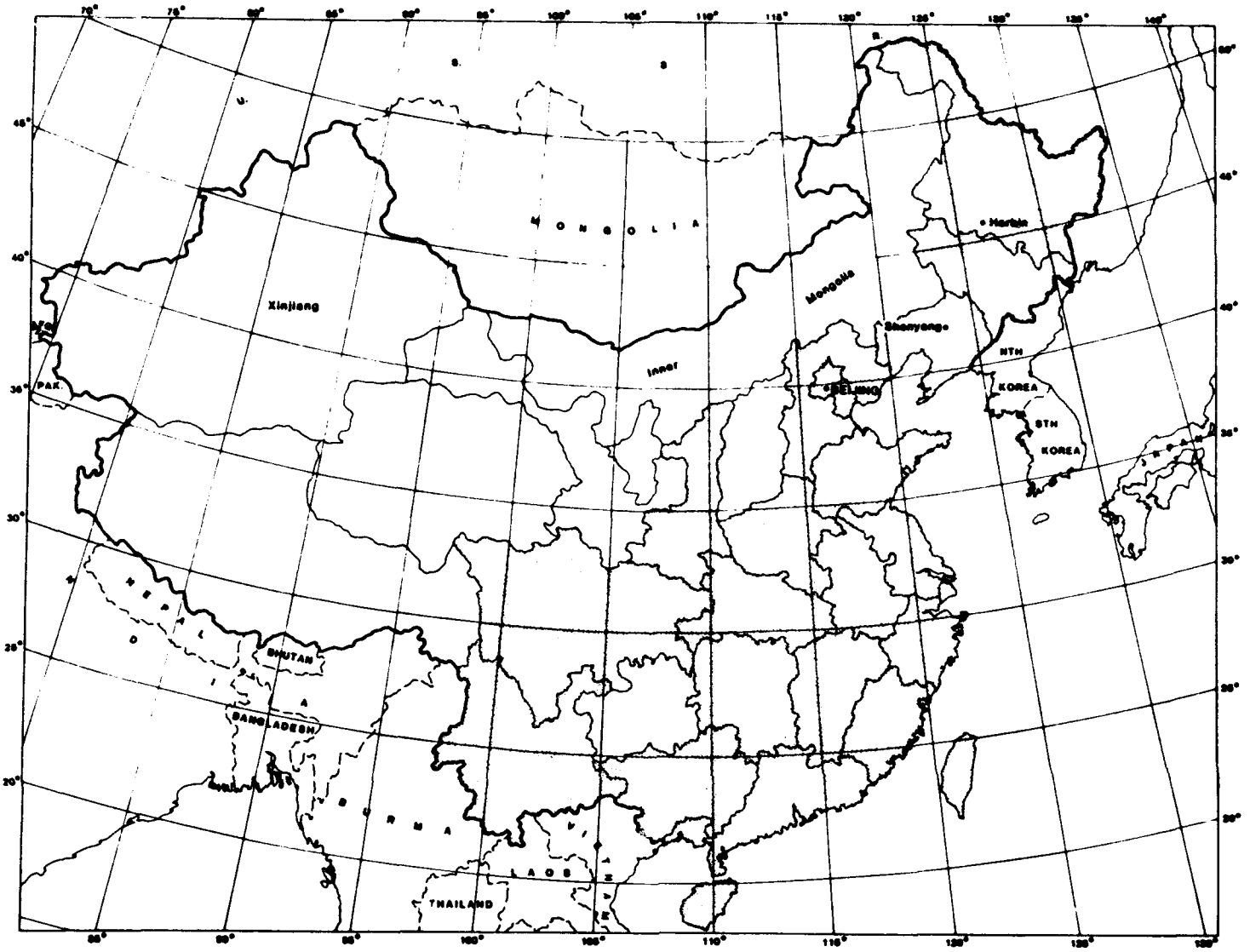

Figure 1. Shaded areas on the map of China indicate provinces where the authors collected hydrilla or where herbarium specimens of hydrilla were collected. Collection sites near the cities of Beijing, Schenyang and Harbin were the northernmost sites at which the authors found hydrilla. Hydrilla was absent in Xinjiang and Inner Mongolia provinces.

While latitude, because of its extremely strong correlation with insolation (solar energy per unit area) and day length, plays a major role in the distribution of plant species, other factors must also be considered. We feel that the amount of rainfall, which is critical to many terrestrial plants, plays only an indirect role in hydrilla's distribution. While aquatic habitats are more common in wet areas, the few aquatic habitats in dry areas may contain hydrilla. This is the case in Australia, where the few aquatic habitats in the arid interior, when they contain water, also frequently contain hydrilla (Balciunas, pers. observation).

Since hydrilla occurs much more commonly in tropical and near-tropical climates, low temperature probably restricts its distribution. The average January temperatures in Beijing, where hydrilla is a common submersed plant, are 0 to -10C, and are similar to those in New York, Chicago and Vancouver (see Figure 2), where hydrilla has (as yet) not been recorded. Hydrilla occurs frequently in Shengyang and Harbin, although not as abundantly as in Beijing. Both of these cities lie in a temperate belt which experiences January temperatures similar to Quebec and Edmonton, Canada, as well as a significant portion of Alaska. Harbin lies on the edge of an even colder temperature belt, which dominates most of the former U.S.S.R. Since Cook and Luond (1982) show over a half-dozen hydrilla sites from this portion of the U.S.S.R., it is clear that hydrilla can survive in climates similar to northern Alaska and Canada.

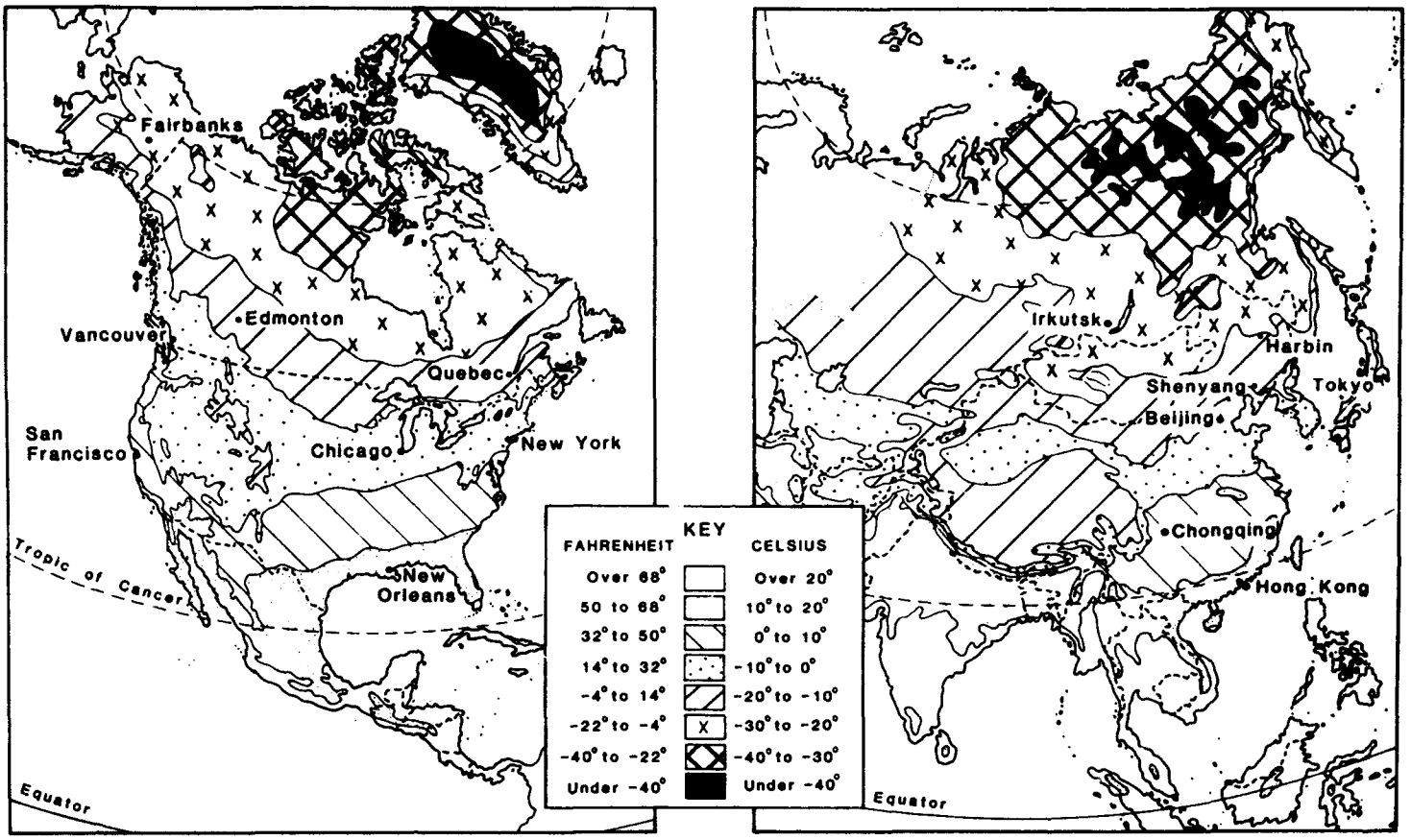

Figure 2. Average January temperatures in North America and Asia (modified and redrawn from Hammond Publication Advisory Board 1991). Winter temperatures in the U.S. and Canada are generally milder than at the same latitudes in China and northern Asia.

Thus, based on both latitudinal and temperature comparisons with China and central Asia, hydrilla has the potential to invade not only all of the continental U.S., but most of Alaska and Canada. However, if hydrilla does eventually occur in northern U.S. or Canada, the infestations are not likely to resemble those in Florida. The hydrilla populations in Ireland, Poland and Lithuania do not seem to be spreading (Cook and Luond 1982). Hydrilla in Beijing does occasionally reach levels that might be considered problematic, but hydrilla in Harbin seldom occurs in pure stands and is usually mixed with *M. spicatum*, *Ceratophyllum* sp. and emergent aquatic plants. However, we did find aquatic weevil larvae associated with hydrilla at one of our Harbin sites (Buckingham 1992), so insect herbivores may be reducing hydrilla growth even in cold climates. Arid areas in high elevations with low rainfall may be relatively "safe" from hydrilla. We have looked fairly carefully in Inner Mongolia which occupies most of China's northern border. Much of Inner Mongolia consists of a dry (precipitation less than 200 mm/yr) and high (over 1000 m) plateau (Sivin *et al.* 1988). Searches of herbarium records, including those at the University of Inner Mongolia, confirm our field observations, and it appears that hydrilla does not occur there. The same holds true for Xinjiang Province. China's most authorative book on aquatic plants, The Illustrated Atlas of Aquatic Plants of China (Wuhan Institute of Botany 1980), upholds our observations about the absence of hydrilla from these regions.

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Effects of Metabolic Products of Cellulose-Utilizing Organisms on Hydrilla

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ABSTRACT

Organic-rich sediments from lakes where growth of hydrilla (*Hydrilla verticillata* (L.f.) Royle) appeared to be inhibited were extracted previously, and the extracts were shown to inhibit growth of hydrilla cultured under laboratory conditions. The same sediments were used to isolate organisms that may utilize cellulosic material with production of metabolic products that inhibit growth of hydrilla. A cellobiose-based medium was used, and metabolic products were isolated by filtering the medium and autoclaving the filtrate. A 3-day growth period produced the maximum yield of hydrilla-inhibiting material, as measured by changes in fresh weight of hydrilla and by changes in chlorophyll content. High performance liquid chromatograms for the metabolic product and for the hydrilla-inhibiting extract provided an indication of the similarity of the inhibitors.

Key words: inhibitors, fungi, bacteria, cellobiose, sediment, cellulose degradation.

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INTRODUCTION

Dooris and Martin (1980) suggested that the hydrilla-inhibiting organic material(s) found in certain lakes may be a precursor or an intermediate in lignin synthesis and is the result of microbial degradation of lignified tissue. Though the lakes seem to be unique because of associated cypress (*Taxodium distichum*), aqueous extracts of the trees (bark or leaves) did not inhibit hydrilla growth.

The presence of this hydrilla inhibitor was determined in various natural waters using HPLC (high performance liquid chromatography). The intensity of a diagnostic peak (*i.e.*, one characteristic of the inhibitor) in the HPLC was directly related to the relative concentration of the inhibitor, and was inversely related to the relative abundance of hydrilla (Martin *et al.* 1986).

Additional studies provided more information about the hydrilla inhibitor(s). Rate studies indicated the inhibitor suppressed photosynthesis and increased respiration rates (Barltrop and Martin 1983, 1984). In addition, evidence was obtained that indicated the inhibitor served as a sensitizer for singlet oxygen. Finally, the effect of the inhibitor on the ultrastructure of hydrilla was examined through the use of

electron microscopy (Dooris *et al.* 1988). This study revealed that the inhibitor caused an increase in starch accumulation and a distortion of chloroplasts.

The production of the inhibitor by organisms acting on organic substrate has remained unstudied until recently. The present study was concerned with production of hydrilla inhibitors in the laboratory. The strategy involved inducing growth of microorganisms present in lake sediments (Lake Starvation and White Trout Lake, Hillsborough County, Florida) by providing a suitable organic substrate (a cellobiose-based medium).

MATERIALS AND METHODS

Hydrilla samples were obtained from a retention pond located northeast of Fowler Avenue and Bruce B. Downs Boulevard, north of University Square Mall in Tampa. Other samples were obtained from the Hillsborough River under Morris Bridge west of I-75 and from an area south of the 40th Street underpass. Hydrilla samples were rinsed, cleaned of debris in tap water, then stored in Floridan aquifer well water in aquaria with lighting provided by cool white fluorescent lamps (12 hr light: 12 hr dark); 80 μmol/m^2/sec at 23C.

Lake sediments were taken from stored samples whose source has been described previously (Dooris and Martin 1980, Martin *et al.* 1986, Dooris *et al.* 1988). The White Trout Lake sample had been stored dry at room temperature for several years in a sealed container; Lake Starvation samples had been stored in a freezer at -17C.

Hydrilla inhibitor preparations were made as follows: Sediment (5.0 g dry weight basis) was added to each of eight 250-ml Erlenmeyer flasks containing sterilized growth medium, containing (g/L): cellobiose (Sigma), 10; ammonium sulfate, 0.5; $MgSO_4 \cdot 7\ H_20$, 0.2; calcium chloride, 0.1; yeast extract (Difco), 0.5; distilled water (to 1 liter). A control culture was prepared using the same ingredients, except dextrose (10.0 g), an alternative carbon source, was substituted for the cellobiose. Flasks (with gauze or foam stoppers) were placed in a gyrorotary shaker bath (New Brunswick Scientific, model G76, 60 rpm) at 27C for a known period, typically 10 days, by which time all bubbling had ceased. The pH was monitored daily with a pH meter (Chemtrix, Type 40 E). After 10 days pH was constant at 3.0.

The extracts from 3-, 6-, and 10-day cultures were collected by filtering through Whatman #1 filter paper, then the filtrates were autoclaved at 120C for 20 min. [The filter paper was saved and stored in a sealed container for further culturing of the spores on growth media.]

The procedure of Dooris and Martin (1980) was used for bioassays. Specifically, 1.0-g hydrilla sprigs were weighed and placed into 500-ml Erlenmeyer flasks containing varying volumes of filtrate duplicate samples (0 to 400 ml) and diluted with sterilized 10% Hoagland's medium (Steward and Elliston 1974) until the flasks were filled. The flasks were sealed with rubber stoppers, inverted, and exposed to 40-W cool white fluorescent lights on both sides (12 hr light: 12 hr dark) with an intensity of 80 μEinsteins/m^2/sec for 10 days, and then the fresh weight was again determined, and the percent change was calculated.

Bioassays were also done by measuring the change in chlorophyll content. Samples of hydrilla were treated as before, but at the end of the bioassay, the sprigs were collected from each flask, and homogenized in a Waring blender. Chlorophylls were extracted into 80% (v/v) aqueous acetone, and the absorbance was measured at 647 and 664 nm, using a Shimadzu recording spectrophotometer. Chlorophyll *a* and *b* and total chlorophyll were calculated using standard equations (Combes *et al.* 1985).

HPLC chromatograms were obtained using a Beckman (Altex) model 110 liquid chromatograph equipped with model 160 solvent programmer, and a LKB (model 2238) multiwavelength detector set at 254 nm for these experiments. A Dupont preparative scale Zorbax™ (21.2 x 350 mm) column was used, and in all analyses a linear gradient was used during a 20-min analysis starting with 60:40 water-methanol, and ending with 100% water. Prior to injection, the sample was passed through a C-18 Bond-elut® cartridge (Analytichem International) by centrifuging for 3 to 5 min on a clinical centrifuge. Inorganic and total carbon were measured using a Beckman model 915 carbon analyzer.

RESULTS AND DISCUSSION

The extracts obtained from culturing the soil samples had certain characteristics in common, regardless of the replicate or source. The pH of the medium decreased from an initial value of about 6.0 to a minimum (pH 2.9 to 3.5). The extract was malodorous.

Additions of filtrates from the extract were deleterious to the hydrilla: within 72 hr, tissue became chlorotic and soft. Roots disintegrated and detached from the plant. Turions wilted and became flaccid. In contrast, control samples in 10% Hoaglands at pH 7, had a fresh-weight increase of 10 to 20% in a 10-day period, and the sprigs were green. Other controls were prepared. One cellobiose control consisted of the initial medium pH 6.0 (without sediment) with the pH adjusted to 3.0. The change in fresh weight was 11.0 ± 4.1% (mean ± SE) for 10 to 100 ml of filtrate (Table 1).

Another cellobiose control was used to check the effect of acidity. The extract from the cellobiose culture (pH 3.0) was neutralized with 0.25 *M* NaOH prior to dilution with Hoagland's solution (final pH 6.7) and compared with the

TABLE 1. SUMMARY OF CONTROL SYSTEMS TESTING THE EFFECT OF pH AND MEDIA ON CHANGES IN THE FRESH WEIGHT OF HYDRILLA OVER A 10-DAY PERIOD.

Sample	N	% change[1]
10% Hoagland's at different initial pH		
pH 6.6	10	11.4 ± 2.3
pH 6.6	10	12.0 ± 2.8
pH 5.7	20	9.0 ± 1.3
Celloboise extract, pH 3.0 adjusted to pH 7.0, different volumes of extract (10 to 100 ml) diluted to 500 ml with 10% Hoaglands with final pH 6.7	10	12.1 ± 2.7
Celloboise control, without sediment, initial pH 6.0, adjusted to pH 3.0, 10 to 100 ml diluted to 500 ml with 10% Hoaglands	10	11.0 ± 4.1

[1]Mean ± S.E.

result of not acidifying when 10 to 100 ml of filtered culture media were added. No significant differences were noted (see Table 1) indicating the cellobiose was not being degraded at pH 3.0 to produce hydrilla inhibitors.

A third cellobiose control (20 ml) consisted of the medium left uncovered in the laboratory for a 10-day period, then added to 10% Hoaglands and bioassayed. The mean change in fresh weight was 18 ± 9%, consistent with controls, *i.e.*, 10% Hoaglands at pH 7 (21 ± 8.3%).

Experiments were conducted to check for the effectiveness of related media in which dextrose was substituted for cellobiose. The extract (after 10 days) was malodorous and had a pH of 3.5. Twenty milliliters of extract was diluted to 500 ml with 10% Hoaglands to give a final pH of 5.7 and the effect on hydrilla was bioassayed. The control system (10% Hoaglands adjusted to pH of 5.7) had a growth increase of 9.0 ± 1.3% over a 10-day period, whereas the test fresh weight decreased 6.2 ± 1.1% (N=20). Each test flask, however, showed an overgrowth of microbes, and most of the stoppers were pushed open by gas bubbles. For better comparison, 20 ml of dextrose medium was added to 10% Hoagland's and the mixture (pH 6.6) was bioassayed. Both control (dextrose medium) and test (dextrose medium plus inhibitor) experienced excessive organism growth, and there was no statistically significant difference (Student's t-test) between the two systems.

The effect of re-culturing sediment samples was investigated. A previously used sample was autoclaved, then cultured, and after 10 days, the filtrate was collected. No unusual odor was noted, and addition of 20 ml of extract did not cause notable decrease in growth of hydrilla. Apparently the organisms were not airborne nor an adventitious contaminant, *i.e.*, the organisms were isolated from the sediment.

The results of 10-day incubation in cellobiose medium yielded extracts that consistently inhibited hydrilla. Extracts from three different cultures were bioassayed, and the volume of extract needed to produce zero growth or loss of biomass was 16 ± 3 ml. Possibly subsequent experience might lead to a lowering of the value of extract to produce the zero-growth effect in the bioassays. One possibility for improvement was length of culturing.

Sediments were cultured for 0, 3, 6, and 10 days, and 20 ml samples of each were used for the bioassay (10 replicates) with the 20-ml extract diluted to 500 ml with 10% Hoaglands of final pH 7. The mean percent changes in fresh weight were -40 ± 7 (3 days), -16 ± 3.5 (6 days), and -26 ± 7 (10 days) and -21 ± 7 (10 days). These results suggest that optimum yield of hydrilla inhibitor occurred at an early stage, e.g., after 3 days and that the subsequent growth inhibition was about constant within experimental error. The bioassay behavior of the three extracts (three versus 6- and 10-day incubation) was different. While chlorosis was not observed for the 3- and 6-day incubation extracts, damage was severe: leaves had disintegrated, and it was necessary to collect the sample on cheesecloth. Disintegration was also observed for 6-day extracts. Chlorosis and disintegration were observed with 10-day extracts.

Two different types of bioassays were run. Typically, change in fresh weight was studied, but for one bioassay, the change in total chlorophyll content was also studied in parallel with change in fresh weight for doubly replicated samples as a function of volume of 10-day extract (Figure 1). The agreement between the results for total chlorophyll and change in fresh weight was generally good, and supports the validity of using change in fresh weight as a means of characterizing inhibition.

However, there is a complicating factor involved in this particular bioassay: the pH was not adjusted, and the pH of solutions containing more than 30 ml of extract were less than 5.0. The effect of pH probably becomes significant only at pH of 3.5 or less (Trent *et al.* 1978, Table 1). In previous bioassays, the pH of the final solution of Hoaglands was adjusted so that the pH with extract was 6.7.

The research strategy was formulated from a general hypothesis of Dooris and Martin (1980) that a chemical inhibitor detected in water and sediment from lakes that do not support prolific growth of hydrilla is derived from microbial degradation of cellulose material. This material may derive from bald cypress, but past experience (Martin *et al.* 1986) has indicated that other sources of carbon may be involved. It was hypothesized that fungi might be the primary organisms responsible for the degradation.

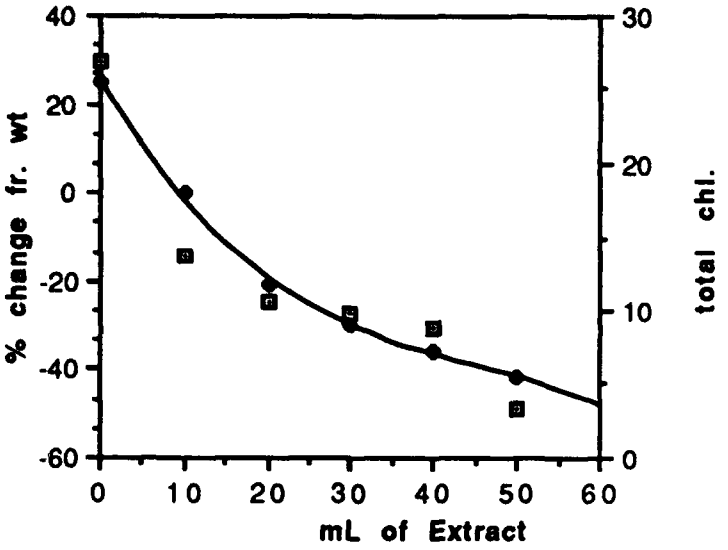

Figure 1. Percent change in fresh weight of hydrilla (closed diamonds) and total chlorophyll content (squares) as a function of extract. Total chlorophyll content was calculated from mean absorbance of chlorophyll *a* and *b*.

Previously collected sediment samples from White Trout Lake and Lake Starvation were used to inoculate media, and several considerations were involved. Cellobiose was selected because it is a smaller unit of cellulose, and it was believed that the chemical inhibitor was derived from degradation of the cellulose/lignin material. Also, the culture medium, pH = 6.0, was reported (Booth 1971) to be highly selective for fungi. The pH was selected because lakes that tend to be rich in tannins and acidic do not support rampant growth of hydrilla. In addition, a pH of 6.0 would inhibit the overpopulation of competing organisms.

Cultures were allowed a growth period of about 10 to 14 days. At the end of this time, gas evolution was at a minimum or could not be detected. The pH had reached a minimum and remained constant at 2.5 to 3.0. Presumably at this pH, competitive organisms that might initially be able to utilize cellobiose media would be eliminated. In retrospect, it appears that a shorter period of incubation (perhaps 3 to 4 days) might have the advantage of greater activity.

Previous experiments indicated that a pH of 3.5 did not affect the growth of hydrilla (Trent *et al.* 1978), but there was still concern that the low pH of the extract could be adversely affecting the growth of hydrilla in the assays. Four controls were tested: 10% Hoagland's at different initial pH, varying amounts of cellobiose extract (day 1) with initial pH 3.0 added to Hoaglands (with final pH adjusted to pH 6.7) (Table 1). We conclude from these results that neither pH, nor the volume of cellobiose medium used, affected the change in fresh weight of hydrilla under assay conditions.

Assays of extract activity indicated several features about the production of the hydrilla-inhibiting chemicals. The volume of inhibitor to reduce growth to 0% was consistent (16 ± 3 ml) for a 10-day incubation period. Probably maximum activity was reached during the first few days, and similar results were obtained whether monitoring change in fresh weight or change in total chlorophyll content (Figure 1). The pattern of behavior was consistent with that observed previously, i.e., development of significant chlorosis.

While attention was focused on a medium for fungi development, we did not neglect the possibility of other organisms. The effectiveness of dextrose utilization was compared with that of cellobiose. A control dextrose medium was evaluated, and the control sample (medium only, no sediment) clearly showed an inhibition as well as an overpopulation of organisms attached to the hydrilla sprigs. This experiment reaffirmed that opportunistic organisms living on hydrilla can become detrimental to its survival (Mansell and Silver 1974). Possibly the growth inhibitor studied here affects the change of phenolic substances produced by the plant (*cf.* Woodward *et al.* 1974).

We are not the first to be concerned about deleterious organisms on hydrilla. Charudattan (1973) has been concerned with the pathogenicity of fungi and bacteria for years. More recently, Joye and Cofrancesco (1991) described the isolation of about 200 fungal and 27 bacterial organisms from hydrilla. From their isolates, Joye and Cofrancesco (1991) determined that *Macrophomina phaseolina* isolate was detrimental to hydrilla and duck lettuce *Ottelia alissmoides* (L.) Pers.

We believe that the organisms that we are examining act in a different manner, i.e., they act on a cellulose substrate and produce an inhibitory substance that occurs in locations where there is no hydrilla.

The present study is linked to previous studies (Dooris and Martin 1980, Martin *et al.* 1986, Dooris *et al.* 1988) in a common search for the significance and identity of a naturally occurring hydrilla inhibitor. The similarity of properties between the previously studied hydrilla inhibitor and the material produced in this study has been described. One other significant similarity exists: similarity of HPLC chromatograms. For cellobiose extract, the HPLC peak appeared at 69 ± 3% of the programmed run versus 65% for the Lake White Trout extract. While the absolute location is different from previous results, the injection loop used in the HPLC unit was also different, 100 µl vs 20 µl.

Given the convenience of the method of production of hydrilla inhibitor by culturing, it should be much easier to seek out the chemical identity of the hydrilla inhibitor(s).

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Effects of Eutrophication on *Ranunculus* and *Potamogeton*

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ABSTRACT

Water crowfoot (*Ranunculus penicillatus* subsp. *pseudofluitans* (Syme) S.Webster) plants were grown in two artificial recirculating rivers, in one of which the phosphate concentration of the input was raised from 40 μgPl^{-1} to 200 μgPl^{-1}. Fennel pondweed (*Potamogeton pectinatus* L.) plants were planted as a competitor in association with 50% of the *Ranunculus* clumps. Chemical concentrations of the major elements in the water were measured weekly. Filamentous algae grew in profusion in the channel with added phosphate (0.77 T fresh weight), compared with an immeasurably low amount in the control channel. After 100 days the plants were removed, dried and weighed and the tissue concentrations of the major elements were measured. The *Ranunculus* shoots grew less in the eutrophic channel, and its roots grew less in the presence of *Potamogeton*. The *Potamogeton* showed a greater reduction in shoot and root biomass than the *Ranunculus*. Tissue phosphate concentrations were higher in both species in the eutrophic channel. The data suggested that *P. pectinatus* is a more competitive species (*sensu* Grime) than *R. penicillatus*.

Key Words: macrophytes, algae, phosphate, river, competition.

INTRODUCTION

River plants tend to be associated with particular nutrient concentrations (Holmes and Newbold 1984). In streams where there is an inflow of polluted water (for example

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sewage effluent), species such as *Ranunculus fluitans* L. and *R. penicillatus* are often replaced by *Potamogeton pectinatus* (Butcher 1933, Harding 1979, 1980). Although the pollution is sometimes intense enough to destroy the *Ranunculus* outright (Hawkes 1978), often it appears that the change in vegetation is due to a change in the competitive balance between the plant species present. However, there is a paucity of experimental data to back up conclusions which have mostly been drawn from field observations. The aim of the experiment described here was to investigate the effects of pollution by increased phosphate concentration on *R. penicillatus* subsp. *pseudofluitans* and *Potamogeton pectinatus*.

METHODS

The experiment was carried out in two artificial recirculating rivers at the Waterston Experimental Station of the Institute of Freshwater Ecology. Each artificial river consists of a 53-m-long race-track shaped fiber-glass channel, incorporating an Archimedes screw pump to circulate the water. The water velocity was, on average, 0.25 m s^{-1}, with no significant difference between the two channels. The channels were both filled with water to a depth of 0.4 m above the gravel substratum. They have a trapezoid cross-section, and the base was filled with gravel to a depth of 0.4 m. The channels were continuously topped-up with groundwater from a borehole. The input was adjusted to 100 m^3 $week^{-1}$. The volume of water in each channel was *ca.* 50 m^3 when the gravel was installed (Fox 1987). Bullhead fish (*Cottus gobio* L.) were electro-fished from a nearby stream and placed in the channels (equal numbers in each) to prevent large fluctuations in populations of invertebrate grazers.

The water supply has a constant chemical composition which is similar to the source of many chalk streams (Marker and Casey 1982, Casey and Newton 1973, Westlake *et al.* 1972). It contained adequate concentrations of all the ions necessary for plant growth, with the exception of iron (Marker and Casey 1982), so $FeCl_3$ was added (together with EDTA) at a concentration equivalent to 1 mg l^{-1} Fe^{3+} in the borehole water.

In one channel (the control) no other additional chemicals were added. In the other, phosphate was added as H_3PO_4. The input PO_4-P concentration was increased from 40 µgP l^{-1} to 200 µgP l^{-1} (see Figure 1). Concentrations of 200 to 750 µgP l^{-1} have been measured in southern English chalk streams such as the River Itchen which has abundant *R. penicillatus* subsp. *pseudofluitans* (Spink 1992). The phosphate was continuously added by a peristaltic pump from a 60-L vat. The major chemical elements in the water were analyzed weekly (Figure 1). Soluble phosphate and nitrate were measured using flow injection analysis (Ruzicka and Hansen 1981), potassium using atomic absorbtion spectrophotometry (American Public Health Association 1980) and sulphate using an ion-exchange procedure (MacKereth 1955).

On 31 March 1990 *R. penicillatus* subsp. *pseudofluitans* and *Potamogeton pectinatus* plants were planted in the two channels. Ten groups of five pots were planted with five *Ranunculus* plants in each pot and an additional four *Potamogeton* plants in 50% of the pots. The fresh weight of all the plants was measured before planting to ensure that there was no initial difference between treatments. Before planting, water was pumped for several hours between the two channels (in both directions, consecutively) to ensure that the initial algal populations were similar for both channels.

On 12 July 1990 (after 100 days) the plants were removed from the channels, dried (95C), separated into roots and shoots, weighed, and the tissue concentrations of phosphorus, nitrogen, carbon and potassium were measured. An estimate was also made of the weight of filamentous algae (*Cladophora glomerata* (L.) Kutz) in the channels. A rigid polypropylene container (*ca.* 20 l) was carefully placed in the channel and allowed to fill with water plus algae. The container was removed from the channel, the volume of water was determined, and the weight of the algae was used to estimate the total weight in the total volume of the channel (nine replicates).

RESULTS AND DISCUSSION

The control channel had an immeasurably low amount of algae growing in it, whereas the channel with added phosphate had an estimated 770 kg fresh weight (23 kg dry weight) of filamentous algae (*Cladophera glomerata*).

The channel with added phosphate had less *Ranunculus* shoot biomass, the root-to-shoot ratio was increased and there was a greater concentration of major nutrients in the shoots. There was no effect on the root biomass, but this was decreased in the pots with *Potamogeton pectinatus* present. The *Potamogeton pectinatus* root and shoot biomass was reduced in the channel with added phosphate (though the ratio remained unaltered) and there were reduced levels of other major nutrients (see Table 1 and Figure 2).

The addition of phosphate to one channel had a major effect on filamentous algal growth in that channel. A likely result of this greatly enhanced filamentous algal biomass would be to substantially reduce the quantity of light available for submerged macrophyte photosynthesis, and so would be a significant cause of stress (Turner *et al.* 1991, Marrs *et al.* 1992).

The ten pots in each channel were not true replicates but "pseudoreplicates" as they were not statistically independent (Hurlbert 1984). As only two channels were available for this

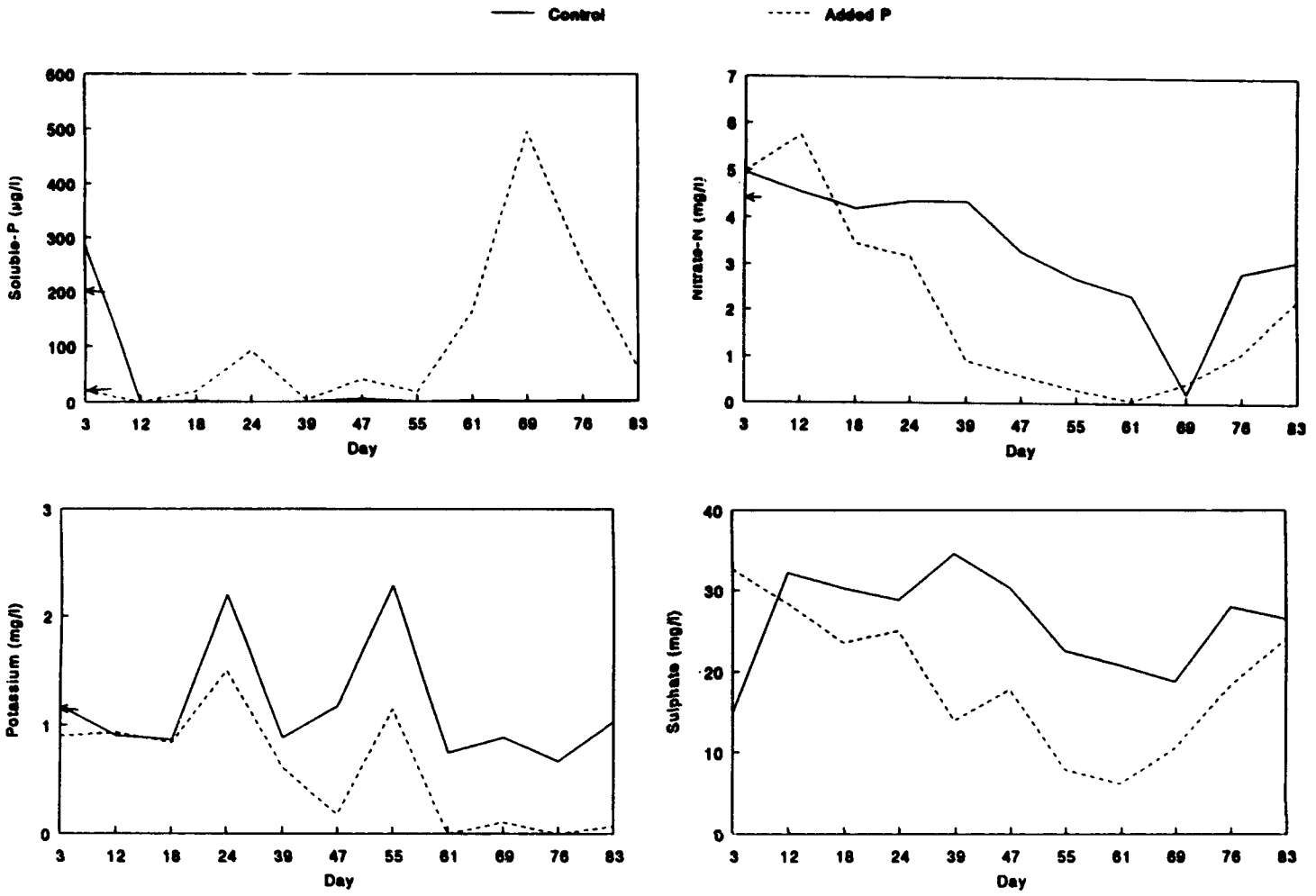

Figure 1. Chemical concentrations of soluble phosphate, nitrate, potassium and sulphate in artificial rivers. Control channel shown in solid line, channel with added phosphate in dotted line. Concentration of input shown by arrow on ordinate (not available for sulphate).

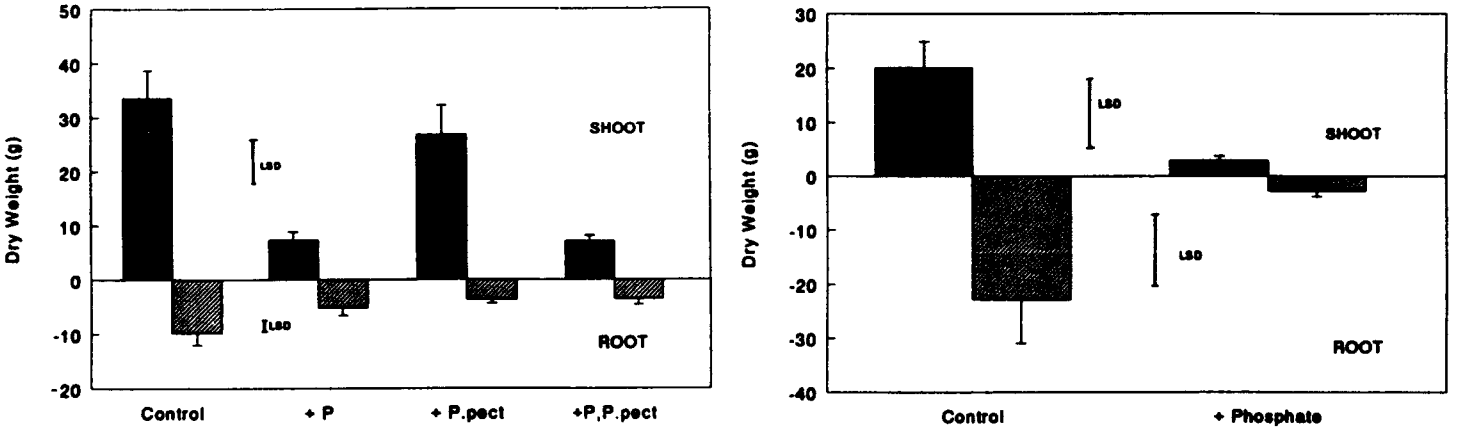

Figure 2. Effect of channel with added phosphate (+P) and presence of Potamogeton *pectinatus* (+P.pect) on *Ranunculus penicillatus* subsp. *pseudofluitans* growth (left graph), and on *P. pectinatus* growth (right graph). "Control" represents plants in channel without added phosphate, and growing without competition from *P. pectinatus*. Bars on histograms represent ± 1 standard error, separate bars represent least significant difference (LSD) ($p \leq 0.05$).

TABLE 1. SUMMARY OF ANALYSIS OF VARIANCE. "COMPETITION" INDICATES THE EFFECTS OF *POTAMOGETON PECTINATUS* PLANTS ON THE *RANUNCULUS* PLANTS. "CHANNEL" INDICATES THE DIFFERENCE BETWEEN THE CONTROL CHANNEL AND THE ONE WITH ADDED PHOSPHATE. NUTRIENT VALUES REFER TO LEVELS IN SHOOT TISSUE. IF THE VALUE IS INDICATED AS AN "INCREASE" IT IS GREATER IN THE TREATMENT THAN IN THE CONTROL. LEVELS OF SIGNIFICANCE AS FOLLOWS: n.s. = not significant, * = 95%, ** = 99%, *** = 99.9%.

Variate	Source	Significance	Direction
Ranunculus:			
Shoot dry weight	Competition	n.s	
	Channel	***	Decrease
	Interaction	n.s.	
Root dry weight	Competition	*	Decrease
	Channel	n.s.	
	Interaction	n.s	
Nitrogen	Competition	n.s.	
	Channel	***	Increase
	Interaction	n.s.	
Phosphate	Competition	n.s.	
	Channel	***	Increase
	Interaction	n.s	
Potassium	Competition	n.s.	
	Channel	***	Increase
	Interaction	n.s.	
Potamogeton:			
Shoot dry weight	Channel	**	Decrease
Root dry weight	Channel	*	Decrease
Nitrogen	Channel	**	Increase
Phosphate	Channel	***	Increase
Potassium	Channel	*	Increase

experiment it was not possible to fully replicate the treatment. The statistical comparisons are therefore comparisons between the two channels rather than between the two treatments. The question therefore arises as to whether it is a reasonable assumption that the differences observed between the channels were due to the phosphate treatment or due to another factor. The major measured differences between the channels were the relatively high phosphate concentration (Figure 1) and large algal growth in the channel with the added phosphate; both were clearly directly caused by the treatment. Conversely, the concentrations of many of the other chemical elements rose and fell in concert in each channel during the growing season (Figure 1; details in Spink 1992). This indicates that it is likely that external factors acting on the channels had similar effects on each channel. However, although it is likely that the effects on the plants observed were due to the treatment, the possibility that it was due to another factor cannot be excluded.

The data indicate that in the channel with the increased phosphate the *Ranunculus* responded with a reduction in shoot growth (but no change in the root growth). Conversely, the competition from the *Potamogeton* did not cause any reduction in shoot biomass, but it did cause a significant reduction in root growth. The reduced growth in both *Ranunculus* and *Potamogeton pectinatus* was most likely to have been caused by shading which resulted from increased filamentous algal growth. This effect is consistent with the hypothesis proposed by Phillips *et al.* (1978) to explain the disappearance of aquatic macrophytes from the Norfolk Broads.

The tissue phosphate concentrations of both the *Ranunculus* and the *Potamogeton pectinatus* were increased in the high phosphate channel. As the sediment concentrations of nitrogen and potassium were identical in both channels these data indicate that the concentration of these elements in the water is a significant factor, demonstrating that, for both *Potamogeton pectinatus* and *R. penicillatus* subsp. *pseudofluitans*, shoots as well as roots are an important pathway for nutrient (N, P and K) uptake.

A number of characteristics of the biology of *Potamogeton pectinatus* suggest that some ecotypes of this species show a strongly competitive strategy (*sensu* Grime 1979). *P. pectinatus* is frequently found in very productive eutrophic habitats and can form an extensive dense canopy (Van Wijk 1988). It shows little physiological acclimation to changes in light intensity, responding instead with changes in biomass (Hootsmans and Vermaat 1991). The species shows a strong seasonal variation in phenology and photosynthesis (Van Wijk 1988). From these characteristics it might be expected that *Potamogeton pectinatus* would be a more competitive (and so less stress-tolerant) plant than *Ranunculus penicillatus*—the data from this experiment go some way toward confirming that. A competitive plant responds to stress with relatively large changes in growth rate (Grime 1977, 1979), whereas a more stress tolerant plant will show a smaller change. The *Potamogeton* shoot biomass was seven times smaller in the added phosphate ("stress") treatment, whereas the *Ranunculus* shoot was only 4.4 times smaller (Figure 2). In addition there was a significant biomass reduction in both the shoot and root of the *Potamogeton*, whereas there was only a significant reduction in the shoot of the *Ranunculus*.

In several English chalk streams dominated by *R. penicillatus* subsp. *pseudofluitans*, the water phosphate concentrations have increased over the past few decades. For example, the River Itchen at Winchester has shown a three-fold increase in phosphate concentration during the period 1979-1989 (National Rivers Authority, unpublished data). The results from this experiment indicate that, if the concentration of phosphate continues to increase, it is likely that there may be a decline in macrophytes and an increase in filamentous algae.

In the recirculating channels the algae could not get washed away downstream and so this may have led to more algal accumulation than would occur naturally in a fast-flowing river, though algal populations apparently as dense as those in this experiment have been observed in some chalk rivers (Spink 1992). In situations where algae may not grow so abundantly it would be useful to predict what changes might occur in the balance between the species making up the macrophyte community. Data from this experiment support the hypothesis that *Potamogeton pectinatus* is a more competitive taxon than *R. penicillatus* subsp. *pseudofluitans* (it showed larger morphological changes when stressed). So it is likely that if there was a situation of increased nutrient supply without the stress caused by competition from filamentous algae, the *Potamogeton* would forage nutrients more efficiently than the *Ranunculus*, show a greater plasticity in its growth response, and so outcompete the *Ranunculus*. Indeed, there is evidence (Caffrey 1990) to suggest that this has already happened in some organically polluted rivers in the British Isles.

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J. Aquat. Plant Manage. 31: 118-122

Structure and Environmental Factors in Macrophyte Stands

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ABSTRACT

Aquatic macrophytes not only provide multistructured habitats for numerous autotrophic and heterotrophic aquatic organisms but also for spawn and juvenile stages of higher vertebrates. Two contrary growing types are distinguished: the pillar-type with a rather equal biomass distribution along the more or less parallel vertical axes of the individual plants in contrast to the canopy-type with a significant concentration of biomass in the top layers. These two types are differentiated from each other by data on the vertical distribution of biomass of *Myriophyllum spicatum* L., a pillar-type representative, and *Potamogeton pectinatus* L., a typical canopy type. The influence of vertical biomass distribution on the vertical light gradient is presented.

Key words: aquatic plants, biomass distribution.

INTRODUCTION

In aquatic ecosystems submerged macrophytes should not be considered as a biotic factor only, because their structure represents an abiotic factor as well. There are numerous interactions between structural features of weed beds and physical environmental factors, physiological functioning of the plant itself and organisms using this habitat.

The plants serve as a three-dimensional habitat for parasitic organisms (Sharma Subhasini Sharma and Mathur 1988). The surfaces of macrophytes are covered by sessile organisms like bacteria (Park-Lee 1986) or algae (Schwencke-Hofmann 1987) which are consumed by mobile grazers (Svenson and Stenson 1991, Horn 1989) that serve as a food source for predators (Hughes 1980). Temporary users are organisms which need macrophytes for attaching their spawn or those seeking refuge in the dense plant structures such as juvenile stages of fish (Hynes 1970). In this ecological context the description of structure and physical conditions within weed beds can also serve as an improved database for some limnological disciplines.

The term "structure" has been used in various ways when describing macrophytes. When using "structure" one should keep in mind the chameleon-like semantics of this term. In the terrestrial environment, structure usually refers to the morphological, geometrical or spatial information of individual plants. Architecture, a higher order of the three-dimensional description of vegetation, has also no single meaning. It is used at the level of plant communities as a means of plant sociological interpretation, using the abundance of species as architectural elements. At the level of vegetational units or single plant stands the spatial interpretation uses the true geometrical and spatial arrangements, *i.e.* the structure of single species, as architectural elements. In this paper single species populations are evaluated and the term structure is used in its "transformed" meaning, keeping the term architecture for multispecies weed beds, but not necessarily for plant communities (Ross 1981, Myneni *et al.* 1989).

According to Myneni *et al.* (1989) the vertical leaf area density function, the leaf normal orientation distribution function and the distribution function of leaf spatial dispersion are paramount structural parameters. However, less sophisticated approaches are very common. Biomass per unit area, non-stratified leaf area index, number of individual plant organs, or even the number of species in a plant association found in sociological studies have been used as more or less valid ways to describe structure. In this study the vertical stratification of biomass and the number of individual plant organs (stems, leaves) have been used as a measure for spatial information.

Frequently the whole plant cover above ground is called the plant canopy (Myneni *et al.* 1989) in the context of structure and architecture. The term "canopy" will be used in a more restricted way here, confined to a single type of structure only and the use of different terms for other types of plant cover will be discussed.

MATERIAL AND METHODS

Collection and preparation of plant material. Samples of macrophytes were taken from central parts of beds in various water bodies, among them oxbows, cutoff sidearms and even littoral reaches of an impoundment at the River Danube. Whenever necessary, scuba-diving was used to work on stands in deeper water. Biomass was assayed by the stratified-clipping method (Monsi and Saeki 1953, Fujimori 1971, Myneni *et al.* 1989), dividing the stand into 10-cm

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horizontal strata. Four replicates were made of all samples. All plant parts were cleaned, counted and then used to determine fresh and dry matter per unit area.

Biometric assay. The counting of plant organs was done on a plastic sheet with a 5-cm-square grid. A minimum of 10 individual plants from different parts of a plant stand were placed on the sheet, photographed, and then the number of plant parts and branches was recorded. The average number of plants per square meter was determined and the mean area covered per individual plant was calculated. Considering the number of plant parts per plant, mean radial distances could be computed for different strata.

Light measurements. Light attenuation was measured with a LI-COR and a SKP-200 light-meter, respectively, using submersible quantum sensors (400 to 700 nm). The sensors were attached to a 70-cm horizontal metal bar fixed to a vertical bar graded at 5-cm distance at a right angle. Misalignments from the vertical axis less than 15 deg had no influence on readings (Machata-Wenninger and Janauer 1991). By means of the horizontal bar it was possible to place the sensor correctly entering the plant stands carefully from a sideways position. If any disturbances of the spatial arrangements of the stand occurred, the sensor was removed and cautiously entered again. If any fluctuation at a single sensor position was observed, up to 10 individual readings were made. The mean value was taken as representative for this stratum of a profile. Incident light intensity was taken just above the water surface in full sunlight, and relative light intensities were calculated from readings taken in 0.2-cm depth and at each full 10-cm depth. At least four individual vertical profiles were assessed.

RESULTS AND DISCUSSION

Original data are presented from *Myriophyllum spicatum* L. and *Potamogeton pectinatus* L. In addition, data of *Berula erecta* (Hudson) Coville, *Myriophyllum verticillatum* L., *Potamogeton lucens* L. and *Potamogeton perfoliatus* L. are discussed.

Vertical distribution of biomass. The effects on light and temperature gradients in plant stands are caused by the structure of the individual plants of the plant population.

The structure of a species may be different from that of single plants, especially due to seasonal changes. Thus architecture is well-fitted to describe the structure of woody plant stands, but much less so with herbs.

In the aquatic environment synonymous use of structure, architecture and even pattern occurs because single species stands often predominate. In the case of multispecies macrophyte stands, often interpreted as plant communities in the sociological sense (which may be wrong in many cases), the horizontal extension and hardly visible vertical strata, as compared to a multistoried forest, explain why describing three-dimensional attributes fail to be well determined at present.

The vertical distribution of biomass is an uncomplicated but still meaningful way of describing structure when relations with some physical factors of the environment of the plants are desired. In Figure 1, biomass data (grams dry matter per unit area) are compared for 10-cm strata of *P. pectinatus* and *M. verticillatum*. Two structural types can be distinguished in Figure 1. The canopy-type has a significant concentration of biomass in the top layers, *e.g. P. pectinatus*, *P. lucens* and *B. erecta*. The same situation is reported for *Hydrilla verticillata* Royle (Haller and Sutton 1975). Other canopy-forming species are *Utricularia purpurea* Walt. and *Potamogeton natans* L. (Chambers 1987, Chambers and Kalff 1987). In contrast the pillar-type exhibits a rather equal biomass distribution along the more or less parallel vertical axes

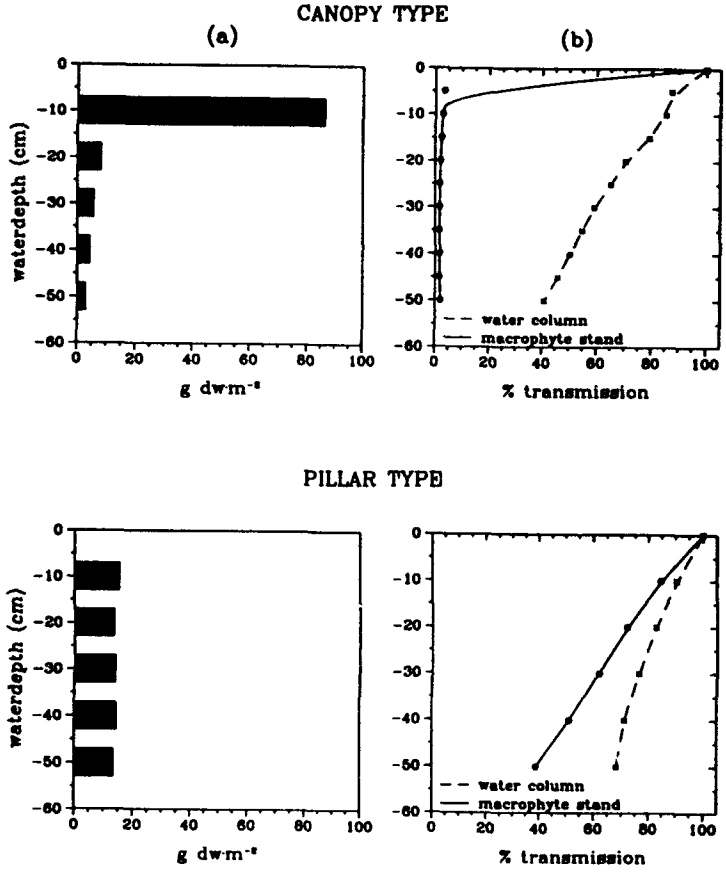

Figure 1. a. Average amount of biomass (in grams dry matter) per unit area in each stratum (in cm) of *P. pectinatus* representing the canopy-type and *M. verticillatum* the pillar-type, and b. Attenuation of light intensity in a stand representing the canopy-type (*P. pectinatus*) and a stand representing the pillar-type (*M. spicatum*), both in comparison to percent transmission in a water column without macrophytes.

of the individual plants. *M. spicatum*, *M. verticillatum* and *P. perfoliatus* are typical representatives of this structural type (Janauer 1991). *Potamogeton crispus* L. and *Elodea canadensis* Michx. are also included in this growth form by some authors (Chambers 1987).

According to our experiences, however, *Ceratophyllum demersum* L. and *M. spicatum* are not forming canopies in still waters observed in the floodplain system of the River Danube (Janauer *et al.* 1990, Janauer 1991). These divergent observations demonstrate the pronounced structural variation the growth form of some species may show under different habitat conditions.

Furthermore, this classification seems to be correct for mature macrophyte stands only. During the course of a single growth period, the development of biomass of *P. pectinatus* clearly shows that the canopy-type is reached only in late summer (Wychera 1989). Earlier in the year biomass is more evenly distributed among the strata and the structural appearance of the plant stand is more like the pillar-type.

Considering the plant organ spatial dispersion within a stratum (Figure 2), the number of leaves and stems can be counted and the mean radial distances can be calculated. It is apparent that the spatial dispersion of leaves and stems of *P. pectinatus* shows a distinct vertical variation depending on the time of the growing season.

The calculation of mean radial distances does not take into account that the leaves, leaf sheath and side branches of *P. pectinatus* are not evenly distributed in space, but will rather keep a plagiotrope position, at a certain angle, close to the main axis under natural conditions. The appearance of the plants in Figure 2 was produced by preparation of the sample for the counting, spreading the secondary axes sideways. However, the mean radial distances can be a generalized measure for the density of plant parts within a specific stratum. This is an important habitat variable for pelagic animals, *e.g.* juvenile stages of fish (Lillie and Budd 1992).

Other methods of describing the spatial dispersion (Myneni *et al.* 1989) or the total impediment to horizontal visibility (Lillie and Budd 1992) have been reported, but calculating and especially assaying the exact nature of the dispersion of plant parts, be it truly regular, providing equal distances for optimal perception of incident radiation in some species, or rather clumped around the vertical axes, is still a task for future work. New sampling techniques like video techniques or freeze coring in the plant stands might provide addtional information.

Effects of structure on the incident radiation. Light measurements have been carried out in macrophyte stands mainly for nondestructive biomass estimation (Westlake 1974). But vertical attenuation can also serve as a means for estimating the extent and type of stratification of biomass. This is of

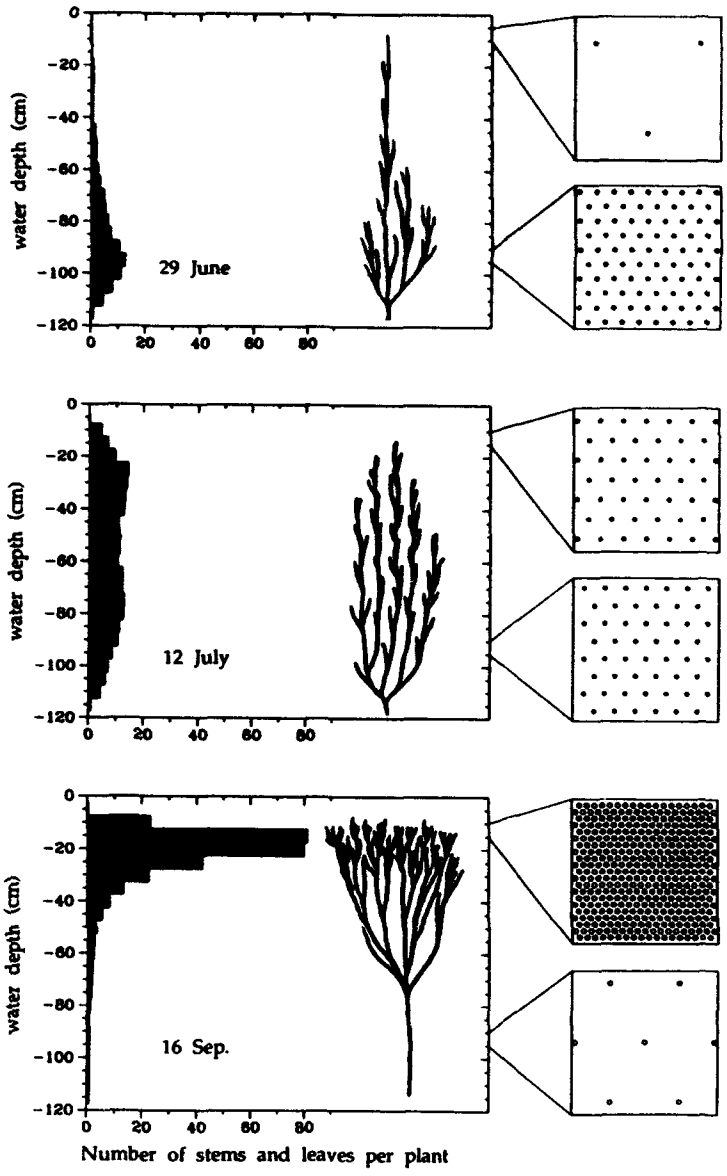

Figure 2. Changes in the plant organ (leaves and stems) number in each stratum (in cm) of *P. pectinatus* during the course of the season, right side: Mean radial distances of plant organs of *P. pectinatus* in a stratum between 10 and 15 cm, and 90 and 95 cm, respectively, during the course of the season.

importance not only for the analysis of structure, but also for discriminating euphotic from dysphotic strata within the plant stands. Effects on the composition of photosynthetic pigments influenced by the light conditions within a weed bed can be expected in strata with moderate or almost no light (Van *et al.* 1977). Figure 1 shows the light attenuation, expressed as percent transmission, in a mature canopy-type plant stand of *P. pectinatus*. The pronounced decrease in light intensity correlates well with the 70% of the total biomass which is found in the top 10-cm stratum (Wychera 1989). Only 5 cm below the surface in a canopy stand, 3.4% of incident light

could be recorded, implying that photosynthesis may be impossible in the lower layers of the leaf canopy. Rather similar information is reported by Westlake (1975), although the data were derived from plant stands in a river.

Although slight, an increase in light intensity in the strata close to the bottom was detected where the biomass consists mainly of defoliated stems. It has been reported that some diffused lateral light (Janauer 1991, see also Titus and Adams 1979) may reach the stand. However, the intensity is low (2% transmission) and no photosynthetically active plant parts are present. So far, no data have been collected by the authors on the effect of lateral diffused light on the photosynthetic efficiency of single plants or the whole stand.

Contrasting results are found in pillar-type plant stands (Figure 1b). *M. verticillatum* that had reached the water surface had 22.5% light transmission in a depth of 50 cm, and *M. spicatum* in the same depth had 38.8%, respectively (Wegleiter 1990). The absence of a dense cover of biomass at the surface, which is the characteristic attribute of the pillar-type, permits light to penetrate the plant stand much deeper and apparent photosynthesis may occur to the base of the plants.

Plant populations in flowing waters which are bent in the direction of flow show intermediate light attenuation attributes. *Ranunculus penicillatus* (Dumortier) Barbington var. *calcareus* (R.W.Butcher) C.D.K.Cook (Westlake 1975) and *Ranunculus trichophyllus* Chaix in Villars (Janauer, unpublished data) reached the 1% transmission level in approximately 40 to 60 cm of depth (counted from the top level of the stand). Other flowing water species like *Ranunculus fluitans* Lamarck, however, represent a true canopy-type (Janauer, unpublished data), which is valid also for *Groenlandia densa* (L.) Fourreau (Machata-Wenninger and Janauer 1991) as far as the characteristics of the light attenuation are concerned.

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Potential for Re-Establishment of Aquatic Plants in Lake Ellesmere (New Zealand)

P. GERBEAUX[1]

ABSTRACT

Past and present aquatic plant surveys show that *Ruppia megacarpa* R. Mason and *Potamogeton pectinatus* L. were major contributors to aquatic plant biomass in Lake Ellesmere, a shallow coastal lagoon located on the east coast of the South Island (New Zealand). Today, in response to natural and anthropogenic pressures (wind, lake openings to the sea), the lake environment appears too stressed and too disturbed to provide suitable conditions for growth of these two species, leaving only space to sparse growth of stress-tolerant ruderal species (*Ruppia polycarpa* R.Mason, *Lepilaena bilocularis* Kirk). However, research conducted in the field between 1985 and 1988 has shown that potential for re-establishment did exist. The role of water-level schedules and associated effects on salinity is discussed in relation to past and present aquatic plant strategies. Management that could foster re-establishment of submerged aquatic vegetation is then suggested.

Key words: coastal lagoon, stress, disturbance, water-level fluctuations, *Ruppia* spp., *Potamogeton pectinatus*.

INTRODUCTION

Between 1985 and 1988, an ecological research was conducted in Lake Ellesmere, a large (16,000 to 20,000 ha) brackish coastal lagoon located on the east coast of the South Island (New Zealand), in order to identify the main factors that could have affected regeneration of aquatic plant stands after these were washed away in 1968 during what is known locally as the Wahine storm. A study of the optical properties of the lake water and a glasshouse experiment showed that light availability in the water column and salinity were two factors controlling growth of *Ruppia spp.* seedlings and potentially limiting submerged vegetation biomass in the lake (Gerbeaux 1989, Gerbeaux and Ward 1991). Penetration of photosynthetic active radiation fluctuates in response to natural and anthropogenic disturbances such as wave resuspension of sediments and wind-controlled or man-controlled water-level fluctuations (seiches, artificial opening of the bar at the mouth of the lake). Lake openings existed before Europeans settled around the lake, and the lake is known to have been opened to the ocean by the Polynesians (known locally as Maoris) in the years 1852, 1854, 1856, 1861, 1863, 1865, and 1867. After and until 1875, it was opened every year by European settlers (Bray 1875). During Maori time, digging through the bar was done by hand (with sticks) to lower the water level and prevent flooding of villages. Today, openings are achieved with the use of bulldozers and draglines, and take place to prevent flooding on surrounding pastoral lands. Closings occur naturally. Lake Ellesmere is a wildlife habitat of international importance (O'Donnell 1985) and critical considerations for wildlife management are the duration of lake openings, the magnitude in the drop in water, the prescribed levels at which the lake is opened and the timing of the openings. These considerations are linked to aquatic plant management needs. A fluctuating water regime is necessary to maintain saltmarsh productivity but it can also be a source of disturbance to the submerged vegetation through sudden drops in water level. If the lake remains open for a long time, the level is so low that shallow areas dry out completely and expose submerged plants. Lake openings are equally a potential source of stress through the salinity and underwater light climate fluctuations they create. This paper relates historical data (over the last 70 years) on frequency and duration of openings to qualitative and quantitative data on aquatic vegetation and investigates what is the optimum water regime for re-establishment of submerged plants in the lake.

METHODS

Lake openings. A long record of data related to the openings (water-level fluctuations, dates and length of openings) was made available by the Canterbury Regional Council who also processed the data to produce lake-level duration curves. Water-level schedules may determine the actual vegetation response and Rorslett (1984) suggested that the probability distribution of water levels should preferably be used in place of the mean annual range of water-level variation in an analysis of response features. Thus, lake-level duration

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curves display the relationship between lake levels and the percentage of time they are exceeded. These curves were obtained for the period 1970-1987 in order to understand how they could have affected regeneration of aquatic plant stands after the 1968 storm. The values have been computed for each year, over the growth season only (September through March).

Data on salinity levels prior to 1985 were obtained in the literature (Hughes *et al.* 1974, Lineham 1983) and salinity was also measured fortnightly in the course of the present study with an YSI Model 33 salinometer (Yellow Springs Instrument Co., OH, USA) below the surface and near the bottom in order to estimate salinity fluctuations in relation to lake openings.

Plant survey and plant life-cycle observations. Prior to 1985, visual observations on macrophyte stands that grew in the lake were made by Mason (1946, 1951); other observations are reported in Hughes *et al.* (1974). Between December 1978 and May 1982, Webb (1982) made monthly records of *Ruppia megacarpa* height in four enclosures located at four sites of the eastern part of the lake. Plant harvesting was carried out in 1986, as part of the present study, in two bays of the western side during the peak biomass period (in early February). Harvesting was made along transect lines, as described in Gerbeaux and Ward (1991), and standing crops were obtained for all the species present. Resource allocation in *R. megacarpa* and *Potamogeton pectinatus* L. was also quantified. Harvesting was repeated in 1987 in one of the bays along the same 600-m-long transect line. Information on life cycles was obtained from the literature and from a glasshouse growth experiment with *Ruppia* spp. (Gerbeaux 1989). Strategy traits were assigned using classification of survival trait characteristics proposed by Murphy *et al.* (1990). Additional visual observations were made by the author until early 1992.

RESULTS

Lake openings. The frequency and length of the openings since the 1910s are summarized in Figure 1. A change in management can be seen in the figure as lake levels became more controlled from the late 1940s, with more frequent openings. New prescriptions were indeed ordered in 1947, and since then the lake is opened when levels reach 1.05 m

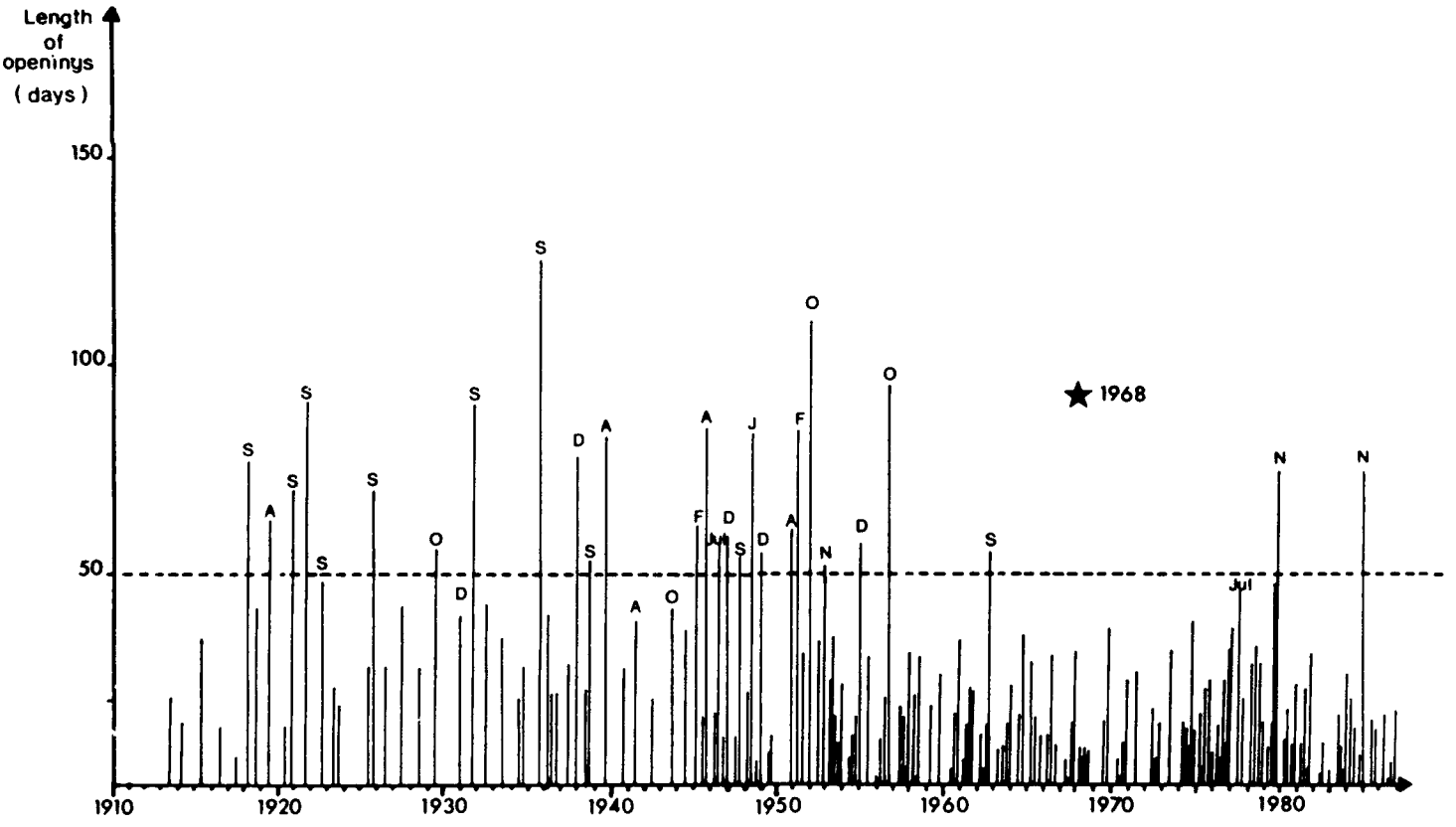

Figure 1. Frequency and length of lake openings between 1910 and 1987. (Letters relate to the starting month of openings that lasted 20 days at least and that resulted in the lake being opened during the growth season (September-March): Jul: July; A: August; S: September; O: October; N: November; D: December; J: January; F: February).

above mean sea level (msl) from September to April, and 1.13 m above msl from May to August (Hughes *et al.* 1974). The average number of openings per year increased from 1.59 between 1913 and 1947 to 3.46 between 1947 and 1986. Over the same periods, the average length of opening decreased from 42.5 days to 23 days.

While water levels continually and irregularly change with time in response to natural events (floods, seiches), the lake level drops very suddenly after an opening. The higher the lake at the time of opening, the greater the scour at the outlet. Thus, while the new policy contributed in lowering the average opening level (from 1.53 m to 1.18 m), it also resulted in increasing the average closing level (from 0.46 to 0.63 m).

The analysis of lake-level fluctuations performed over the period 1970 to 1987 for each growth season highlighted three types of water level schedules. Figure 2 illustrates one example of each type. In the first one, the lake remains at low levels for a long period with short periods at higher levels; in the second one, no particular level is prominent; and in the third type most of the time the lake is at moderate to high levels, being at lower levels for short periods. Each type depends on the timing of opening and on the rainfall pattern after the opening: type 1 corresponds to a late spring opening followed by low rainfall, type 2 to a late winter/very early spring opening followed by one or several openings over the period due to high rainfall, and type 3 to an early opening without subsequent opening (due to low to moderate rainfall). Table 1 presents some additional hydrological information over the same period. The level 0.5 m was arbitrarily chosen (from field observations) as the threshold that could expose plant to desiccation, and the percentage of time spent below this threshold is indicated in the table. The minimum level is another important parameter since it determines, along with turbidity, the maximum amount of light penetrating the water column to the bottom.

Lake openings also affect salinity. From the data obtained in the literature and from the fortnightly measurements the following conclusions could be made: (a) salinity lies most of the time within the 5 to 10 parts per thousand (ppt) range; (b) minimum values recorded were just below 3 ppt; (c) openings lasting between 20 and 30 days raise the level by 1 to 4 ppt; (d) salinity will be higher than 10 ppt if openings last more than 30 days, and more than 15 ppt if they exceed 50 days; (e) in such an event, it will take from 3 to 9 months for the level to return to normal range (5 to 10 ppt) depending on freshwater inflows; (f) long periods without opening may bring the level below that range; (g) salinity could vary from 7 ppt near the surface to 22 ppt near the bottom during opening and calm periods; (h) increase in salinities were observed following storms as a result of wave overtopping.

Macrophyte surveys before 1985. Dense weed beds of *R. megacarpa* and *P. pectinatus* with plants 6 to 8 ft long are reported to have fluctuated during the first half of the century, with a decline starting from the 1920s and being more rapid in the early 1940s, moving north from the outlet (Mason 1946, 1951). The recovery of the weed beds during the 1950s was spectacular and luxuriant growth persisted until the devastating effect of the Wahine storm in 1968 (Hughes *et al.* 1974). Webb (1982) reported that *R. megacarpa* and *P. pectinatus* were still present here and there between 1978 and 1982. He further added that there was better growth after 1980 (plants

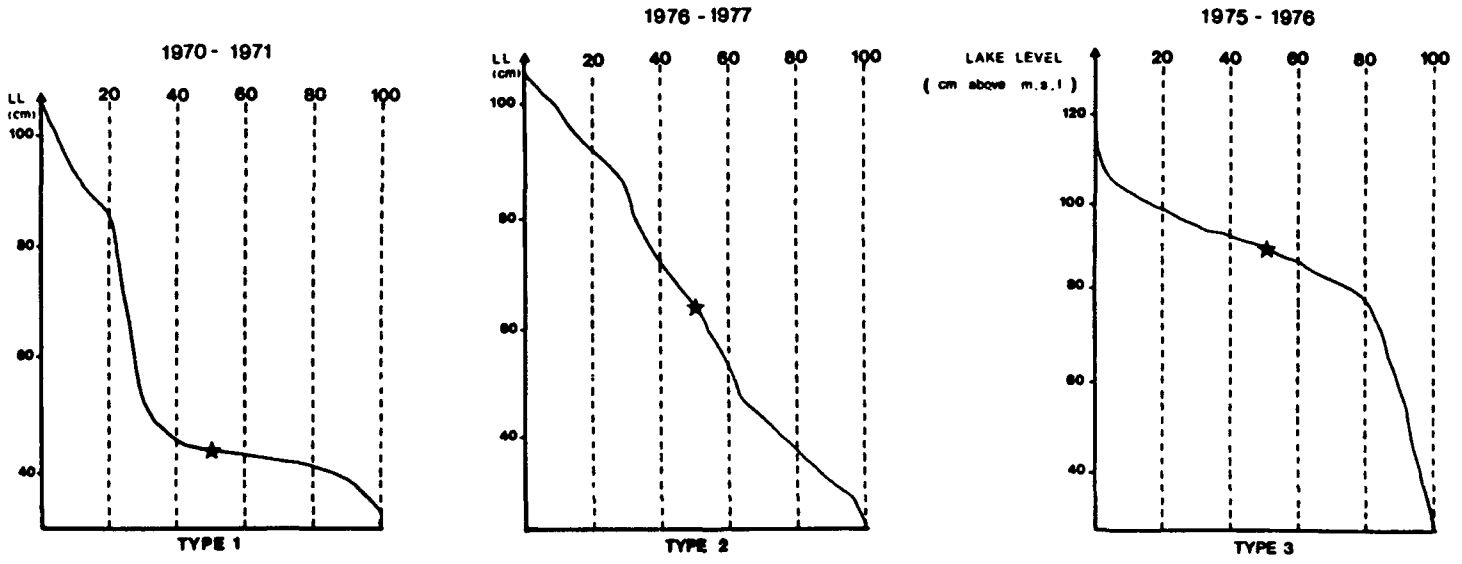

Figure 2. The different types of lake-level duration curves. The star indicates the median value.

TABLE 1. LAKE-LEVEL PARAMETERS IN LAKE ELLESMERE OVER THE AQUATIC PLANT GROWING SEASON FROM 1970-1991 (CANTERBURY REGIONAL COUNCIL DATA)

	Lake status in 19__																					
Parameter	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91
Type of duration curve	1	3	1	3	2	3	2	3	2	2	3	3	3	3	1	3	3	1	3	1	1	3
Lake level < 0.5 (% time)	70	0	3	0	15	5	38	7	12	30	10	15	0	2	50	0	4	20	0	8	0	0
Minimum level (cm ab msl)	32	62	49	58	35	30	25	19	38	16	36	25	63	31	28	51	41	40	78	42	50	76
Median level (cm ab msl)	45	82	55	78	70	91	64	83	67	63	77	67	79	90	50	91	90	58	85	61	62	91

NOTE: ab msl—above mean sea level.

observed were taller). When our first observations took place over summer 1984-1985, only sparse seedlings of *R. polycarpa*, *Lepilaena bilocularis*, and *Lamprothamnium papulosum* (Wallr.) J.Groves could be found in exposed areas due to low lake levels.

Life-cycles and reproductive patterns of macrophytes observed after 1985. The luxuriant and unusual growth (as reported by fishermen) which occurred during the study enabled observations in the glasshouse to be compared with observations in the field. Information on the main contributors to standing crop obtained from these observations and from the literature (reviewed by Madsen 1991) is summarized in Table 2. Surveys around the lake often led to the observation of young seedlings of *R. megacarpa* floating near the shore after lake openings. No such observations were made for *P. pectinatus*. Some *R. megacarpa* fruit were collected from Lake Ellesmere for a test of viability. Only 4% were found viable. The remaining ones did not contain a developed

TABLE 2. REPRODUCTIVE/LIFE CYCLE TRAITS, BIOMASS ALLOCATION AND STRATEGY TRAITS FOR SUBMERGED MACROPHYTES IN LAKE ELLESMERE.

Species	Propagule	% germination	Period of propagule formation	Propagation	Maximum standing crop in 1986/87 g DW m^2	Resource allocation in % of mean standing crop		Strategy traits[1]		
						Seeds	Vegetative underground parts	C	S	D
R. megacarpa R. Mason	Seed	27.8[2]	Jan/Feb	Horizontal then vertical (canopy)	496	14	6.1	3	3	0
R. polycarpa R. Mason	Seed	35[3]	Nov-Dec/ Jan-Feb	Horizontal	107	21	—	1	2	3
P. pectinatus L.	Tuber/seed	100/8[4]	Dec-Apr/ Jan-Feb	Horizontal and vertical (canopy)	176	7.2	11.1	6	0	2
L. binocularis Kirk	Seed	85[3]	Nov-Dec/ Mar-Apr	Horizontal	89	29	—	1	2	3
L. papulosum (Wallr.) J. Groves	Bulbil seed	Not known	Dec-Apr/ Dec-Feb	Horizontal	77	Not known	Not known	1	2	4

[1]Using classification of survival trait characteristics (Murphy et al. 1990) (C = competitive, S = stress, D = Disturbance).
[2]Brock (1982).
[3]Vollebergh and Congdon (1986).
[4]van Wijk (1983).

seed. The fruit cavity had a full-size testa but no embryo or food reserve had developed within.

DISCUSSION

Prior to the 1968 storm, high standing crops of aquatic vegetation that existed in Lake Ellesmere could have been the result, as suggested by Johnstone (1986) in other New Zealand lakes, of minimal biomass loss rather than maximal growth in a unit time. Our 1986 and 1987 records (see Table 3 in Gerbeaux and Ward 1991) support this suggestion. Assuming that the large perennial *Ruppia* beds which existed in the past had a slow t mass turnover (a stress-tolerant trait), they were able to dampen short-term and rapid oscillations in the environment such as the periods of low lake levels and high salinities recorded until the early 1950s. It should be acknowledged here, however, that Delroy (1976) and Congdon and McComb (1979) suggested from field observations in Australia that flowering of *R. megacarpa* was stimulated by increasing salinities. Light at that time was not considered to be a stress factor as the dense beds acted as baffles against wave action, reducing inorganic turbidity. There is thus evidence that decline of *R. megacarpa* occurred through a shift in resource allocation from vegetative growth into flowering under high salinities. Meanwhile, populations of *P. pectinatus* are known to produce higher number of shoots and tubers at low salinities, *i.e.*, *ca.* 3 ppt (van Wijk *et al.* 1988). Periods of long openings of the late 1930s and 1940s (see Figure 1) would have thus played a significant role in the decline that was reported then. Conversely, the low salinities that resulted from the absence of long openings in the late 1950s and in the 1960s could have both stimulated germination of seeds and tubers present in the seed bank and subsequently encouraged vegetative growth. In comparison with the effects induced by lake openings, the 1968 storm was a type of environmental oscillation which had a frequency slower than the characteristic frequency of the dominant macrophytes, resulting in the disruption of the organization in the system and the decline of macrophytes.

With no established populations of macrophytes left in the lake, the assessment and understanding of regenerative strategies is essential in the planning of future lake management. As stated by Harper (1977) "the presence or absence and the density of a seedling population depends not only on the availability of seeds but on the frequency of safe sites that provide the precise conditions required by a particular seed." First, is the number of propagules present in Lake Ellesmere sufficient to enable the regeneration of macrophytic vegetation in Lake Ellesmere? The high number of seeds produced by stress-tolerant ruderals such as *R. polycarpa*, *L. bilocularis*, or *L. papulosum* added to their life-cycle characteristics confers on these species an obvious advantage for survival under high disturbance and stress pressure (see also Brock and Canasova 1991) and explains why they were the only species found in 1984-1985. The seeds and seedlings of *R. megacarpa* found along the shoreline in some areas suggest they also are an important component of the seed bank. Their morphology with long and solid stalks may however prevent dispersal, as seeds have often been seen entangled into large balls buried in the sediment or rolled up on the shore. Wave action on the lake bottom at low lake levels encourages such phenomenon. Their low germination rate is a handicap as it prevents large-scale re-establishment; but it is also a means of maintaining a stock and increasing the chance of survival. The development of *P. pectinatus* populations in one area leads to the conclusion that it also has propagules present in the lake sediment.

Second, what conditions are required for the germination processes and seedling establishment to proceed in Lake Ellesmere? One first condition is related to salinity. Brackish aquatic macrophytes all have low salinity germination requirements (see Dubois (1968) for *L. papulosum*, Brock (1982) for *R. megacarpa*, van Wijk (1986) for *P. pectinatus* and Vollebergh and Congdon (1986) for *Lepilaena cylindrocarpa*, a species close to *L. bilocularis*). Low salinities also favored the development of *Ruppia* seedlings from Lake Ellesmere, and good illumination improved rhizome elongation, contributing to a more developed anchoring capacity, a condition necessary for successful survival of *Ruppia* seedlings as shown by Gerbeaux (1989). With regard to temperature, optimum conditions of growth are found for *P. pectinatus* and for most macrophyte species from above 17C (Spencer 1986, Barko and Smart 1981). Traits like shoot elongation and rapid canopy formation, besides the possibility to rely on its tuber reserves, appear sufficient to guarantee survival of *P. pectinatus* in turbid freshwater habitats (Hootsmans and Vermaat 1991). In fact, van Dijk and van Vierssen (1991) showed that *P. pectinatus* responded to shading by increasing the allocation of available carbohydrate to the tubers, but such response largely depended on how long the above biomass could sustain tuber production. Tubers and a well-developed rooting system also confer *P. pectinatus* a firm anchoring system likely to be an advantage in wind-exposed lakes. Other barriers for plant regeneration such as exposure to desiccation and grazing are less species selective.

It appears that regeneration windows were opened during spring 1985 in Lake Ellesmere on several potential safe sites around the lake. At this time, a low lake level provided increased light intensity and favored higher temperatures on the lake bottom on sunny days. Furthermore, salinities were

low, daily wind velocities were below average (and therefore turbidities were lower) and an extremely low number of waterfowl were grazing in the area. These regeneration windows were again opened the following year. The type 3 water-level schedule (see Figure 2) which prevailed over the growth season of both years as well as in the early 1980s (Table 1) made habitats more favorable and more predictable. It is likely (see Kautsky 1988) that the resulting low disturbance and stress pressure led to re-establishment of stress-tolerant competitors like *R. megacarpa* and competitive ruderals like *P. pectinatus* (CS and CD strategists, respectively). Such a lake schedule can only be favorable: it optimizes temperature and light conditions during early growth, and thus rapid horizontal propagation. Short low lake levels decrease the risk of desiccation and grazing by birds before completion of the life cycle. The later stabilization of the lake level at a medium to high median values also provides favorable conditions for canopy development. The impact of the long opening in 1984-1985 remains uncertain. The resulting exposure to desiccation in the areas where the luxuriant growth was recorded, followed by favorable environmental conditions, may have triggered a large-scale germination through disruption of the exocarp of achenes (see also Congdon and McComb 1981). It may also have improved the quality of the sediment. Follow-up visual observations at the same sites revealed that no luxuriant growth has taken place again after 1987. Overgrazing has undoubtedly put pressure on the macrophytes in 1987 (25 swans per hectare in the area surveyed, which is a lot higher than the 4 swans per hectare obtained by Mitchell *et al.* (1988) on the basis of a relationship between macrophyte biomass and swan number for another South Island coastal lake). However, the various water-level schedules which took place between 1987 and 1991 also provided some unfavorable conditions (20% of the growth season in 1987 below 0.5 m, low median level in 1989 and 1990, high minimum level in 1988 and 1991). Moreover, they may have represented a lack of predictability which the plants could not cope with.

In future management all causes of stress and disturbance should be addressed. It is suggested that the third type of water-level schedule reported above would reduce most of these causes and provide the best potential for aquatic vegetation re-establishment. With respect to lake management goals such as maintenance or improvement of water quality, wildlife habitat, fishing, boating and sailing conditions, it is also attractive. For instance, high summer water levels can reduce nutrient resuspension from the sediment and reduce phytoplankton growth; the proposed timing for opening the lake in spring enables inward fish migration and prevents outward migration, thereby increasing fishery value of the lake; recreational uses (boating and sailing) benefit from relatively high summer levels although excessive growth may impede some areas; and the strategy alleviates Maori grievances, especially through retaining the lake at its optimum level for eel fishing. It thus shows that management of aquatic plants may contribute to enhanced ecological, economic and recreational values and balance conflicts between user-groups.

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Modeling Resource Allocation in *Potamogeton pectinatus* L.

GERDA M. VAN DIJK AND J. H. JANSE[1]

ABSTRACT

In the present study, a simulation model of the life cycle of *Potamogeton pectinatus* L. (sago pondweed) is presented to analyze the implications of resource allocation for long-term survival. The model is calibrated on field observations of *P. pectinatus*. The model is simple and can be used as a tool to improve understanding of the dominant mechanisms in resource allocation and long-term survival. The model is used to test the hypothesis that the resource allocation to the tubers at lower photon flux density increases and that, consequently, sloughing of the vegetation is enhanced. Simulations indicate that the increased resource allocation to the tubers under low photon flux density can explain the observed biomass development and tuber bank size adequately at various light conditions in Lake Veluwe in The Netherlands. Only at the highest shading was the calculated production much lower than the actual production. Apparently, other mechanisms, such as an increase in photosynthesis with increased tuber formation, are involved. An increase in the resource allocation to tuber production under low photon flux density led to a lower biomass of the vegetation during the growing season, but seems an appropriate strategy in terms of reproductive output and long-term survival of the population.

Key words: simulation model, submerged macrophytes, tubers, light climate, shallow lake, sago pondweed.

INTRODUCTION

Studies on life cycles and controlling factors are of particular importance in managing submerged aquatic vegetation, for example, by revealing parts of a life cycle vulnerable to management techniques, or critical in establishment and distribution of species. The life cycle of many submerged macrophytes is characterized by the annual production of generative and vegetative propagules to survive periods unsuitable for vegetative growth. The production of propagules requires allocation of resources. Resource allocation studies are well represented in terrestrial-plant ecological literature, while such studies for submerged macrophytes have received

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little attention so far (Madsen 1991, Titus and Hoover 1991). In terrestrial plant ecology, it is generally accepted that reproduction, growth, and maintenance interact within the individual and compete for limited resources (Bazzaz *et al.* 1987). Recent studies (Spencer and Anderson 1987, Van Dijk and Van Vierssen 1991) indicate that also in the aquatic submerged macrophyte *Potamogeton pectinatus* L., a trade-off between growth, maintenance, and reproduction occurs. The latter study revealed that at low photon flux density, the number of tubers per gram aboveground and below ground biomass produced was higher. The reproductive output at the end of the growing season was, however, lower. It was hypothesized that increased production of tubers at low photon flux density would lead to a higher demand on the carbon sink, resulting in exhaustion of resources early in the growing season.

This paper presents a simple simulation model, 'FLORA,' to simulate and test the hypothesis that increased tuber production per gram below ground biomass at lower photon flux density increases the resource allocation to the tubers and, consequently, enhances sloughing of the vegetation. Simple models are considered very useful to improving insight into the processes that may play a role under actual field conditions (Best 1991, Scheffer *et al.* 1992). The present study aims at improving the theoretical framework for understanding biomass development, reproduction and population dynamics of *P. pectinatus* under various light conditions through simple mathematical modeling.

EXPERIMENTAL OBSERVATIONS

In a shallow eutrophic lake (Lake Veluwe, The Netherlands, latitude 52°20'N) a long-term shading experiment was conducted (1986-1988) in an existing, homogeneous *P. pectinatus* vegetation. Stands were subjected to three different levels of shading; nets were extended above the water surface, reducing the photon flux density just above the water surface by 23, 45 and 73%, respectively. In this way four different light climate conditions in the vegetation were created: (1) a control (without shading) and (2 to 4) three shading levels (23, 45 and 73% reduction in photon flux density). More detailed information on this experiment is given by Van Dijk and Van Vierssen (1991) and Van Dijk *et al.* (1992).

The present paper uses data on biomass and tuber bank development of *P. pectinatus* in the control for the 1986 growing season to calibrate the FLORA model (Table 1). It can be seen from Table 1 that in Lake Veluwe, *P. pectinatus* is characterized as a perennial herbaceous plant according to the definitions of Madsen (1991). It overwinters through vegetative propagules (tubers) and in spring vegetation is re-established by germination of tubers. The production of new tubers starts in June. At the end of the growing season,

TABLE 1. SUMMARY OF THE SEASONAL VARIATION IN TOTAL BIOMASS (aboveground and root/rhizome complex), ROOT:SHOOT RATIO, NUMBER OF NEWLY PRODUCED TUBERS, BIOMASS OF NEWLY PRODUCED TUBERS, AND THE NUMBER OF NEWLY PRODUCED TUBERS PER AMOUNT OF BIOMASS ROOT/RHIZOME COMPLEX IN THE CONTROL SITUATION DURING THE 1986 PERIOD.

	Total biomass (g AFDW m^{-2})		Root:shoot ratio	Tubers[1] (n m^{-2})		Tuber biomass[1] (g AFDW·m^{-2})		No. tubers[1] per g AFDW root (n g^{-1} AFDW)	
Date	mean	sd	mean	mean	sd	mean	sd	mean	sd
13 May	1	1	0.36	—[3]	—	—	—	—	—
26 May	9	2	0.28	—	—	—	—	—	—
9 Jun	12	1	0.36	—	—	—	—	—	—
23 Jun	27	3	0.21	18	28	0.6	0.6	3	6
7 Jul	27	11	0.18	70	45	1.6	1.5	16	8
21 Jul	46	4	0.14	85	65	2.7	1.9	15	11
4 Aug	57	9	0.09	73	6	2.3	1.2	17	2
18 Aug	59	15	0.06	155	39	5.9	1.3	44	1
12 Sep	0	0	x[2]	180	65	6.9	2.9	x	x
2 Oct	0	0	x	167	56	5.0	0.8	x	x
3 Nov	0	0	x	241	98	7.9	4.0	x	x

Note: Values are calculated as grand means of three replicate experimental areas. The mean per experimental area is estimated as the mean of four to five subsamples.

[1]From 23 Jun onward, the density of newly produced tubers is estimated by subtracting the number of hibernated and tubers not germinated as measured on 9 Jun from the total number of tubers measured per experimental area.

[2]Biomass was too low to calculate a reliable mean.

[3]Number of newly produced tubers was zero by definition.

the aboveground biomass and the root/rhizome complex slough away completely (Van Wijk 1988, Van Dijk and Van Vierssen 1991, Van Dijk *et al.* 1992).

MODEL

The FLORA model has been developed to simulate the seasonal cycle of vegetation biomass and tuber bank. The model consisting of three state variables was kept as simple as possible: the total vegetation biomass *DVeg* [g AFDW m^{-2}], the biomass of the tuber bank *DProp* [g AFDW m^{-2}], and the size of the tuber bank *NrProp* [n m^{-2}] (Figure 1). The vegetation biomass includes both aboveground plant parts and the root/rhizome complex. For simplicity, the root:shoot ratio is fixed (default: 0.176). Tuber bank size and biomass were modeled separately to allow for dynamics in mean individual tuber size. The vegetation growth was modeled roughly according to the model MEGAPLANT (Scheffer *et al.* 1992), whereas aspects of the tuber development were derived from the complex model SAGA1 (Hootsmans 1991) and incorporated in FLORA in a simplified manner.

The vegetation is re-established by germination of tubers and subsequent initial growth as soon as the water temperature rises above a certain limit (10C) in spring. A certain fraction (20%) of the hibernated tubers remains dormant (Van Dijk *et al.* 1992). Further growth of the vegetation is made possible by photosynthesis. Daily irradiance and temperature follow a sine function over the year, according to average measurements in The Netherlands. The fraction of photosynthetically active irradiance (PAR) of the total global irradiance is set at 0.48; 10% of the light is reflected at the water surface (Kirk 1983). Light attenuation in the water column is modeled according to Beer's law. The extinction coefficient is the sum of the contribution of water with dissolved and particulate substances, and of the vegetation itself. The extinction coefficient of the water is fixed at 2 m^{-1} (Van Dijk and Achterberg 1992), the self-shading coefficient of the vegetation at 0.02 m^2 g^{-1} AFDW (Van der Bijl *et al.* 1989). For simplicity, the vegetation is assumed to be homogeneously distributed over depth.

Total daily production is modeled according to a Monod-type production *versus* irradiance function (a so-called P/I function), integrated over both time and depth of water. Both the maximum asymptotic value of P, P_{max} (7.5 mg O_2 g^{-1} AFDW h^{-1} at 20C) and the half-saturation constant, k_m (100 µE m^{-2} s^{-1}) are dependent on temperature according to a Gaussian equation, approximating a Q_{10} of 2.0 in the observed temperature range. Maintenance respiration is modeled as a first-order process at a rate of 1.75 mg O_2 g^{-1} AFDW h^{-1}. Respiration in light is not modeled separately because it is already included in the production data. It is

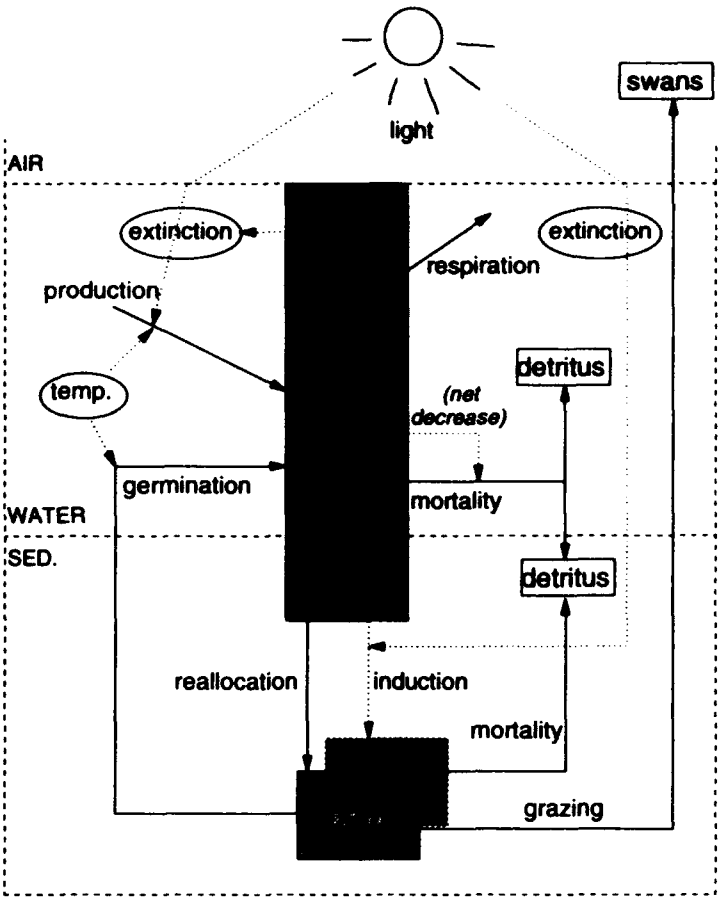

Figure 1. Conceptual diagram of the FLORA model, with the main processes determining the life cycle and resource allocation of *P. pectinatus*. State variables are: vegetation biomass (including the aboveground plant parts and the root/rhizome complex, *Dveg*), tuber bank biomass (*DProp*) and size of the tuber bank (*NrProp*).

assumed that production is not limited by nutrients. The mortality rate of the vegetation is assumed to be very low during the growing season (0.001 d^{-1}).

Tuber induction starts when the daily photoperiod exceeds 16 hr, a value reached in early June. The number of tubers induced per gram root/rhizome complex is defined as being dependent on the light conditions, the so-called relative photosynthetic period. This parameter has been defined as the fraction of the photoperiod during which at least 200 µE m^{-2} s^{-1} reaches the top of the vegetation (Hootsmans 1991). In the model the number of tubers per amount of roots and rhizomes is negatively linear related to the relative photoperiod: the lower the relative photoperiod, the more tubers are induced per gram root/rhizome complex (increasing from 18 under control conditions with a relative photosynthetic period of 0.8 or higher to, for example, 78 when the relative photosynthetic period drops to 0.5; unpublished data).

Re-allocation of biomass from the vegetation to the induced propagules is modeled as a first-order propagule

growth (with a maximum rate of 0.05 d^{-1}) with a fixed efficiency, until all tubers have reached the pre-defined maximum biomass per tuber (0.03 g AFDW). The re-allocation is also made dependent on the vegetation biomass available.

The decline of the vegetation at the end of the growing season is modeled as a sharp increase in the mortality rate as soon as the production is no longer sufficient to sustain both maintenance respiration and re-allocation to the tubers.

In the model's definition, tubers can get lost due to natural mortality (taken as 0.001 d^{-1}) or as a result of grazing by Bewick's swans (*Cygnus bewickii*) in winter (set at 15 October to 15 March). This is estimated as 250 g AFDW per animal per day assuming a swan density of 0.4 ha^{-1} vegetated lake (Hootsmans and Vermaat 1991b).

Simulations have investigated the hypothesis that the increase in the amount of tubers formed per unit plant biomass with lower photosynthetic period affects the biomass development and reproductive output. The model is calibrated on the basis of control conditions and is applied according to data of the *P. pectinatus* biomass development at the three shading levels (2 to 4).

RESULTS AND DISCUSSION

After calibration, the calculated biomass development of the vegetation and the tuber bank size agreed well with actual data in the control situation (Figure 2) ignoring that the simulated vegetation biomass started to slough later in the season and thus reached a higher maximum value than the actual vegetation. In such large water bodies as Lake Veluwe, sloughing of the vegetation at the end of the growing season is largely enhanced by autumn storms (Van Wijk 1988). To ensure no masking of any possible resource allocation effects at the end of the growing season, a tentative storm factor was not included in the model. The results of the simulations of the vegetation biomass at the shading levels 2 and 3 (25 and 50%) fitted the actual data adequately, whereas at level 4 the simulation underestimated the actual data considerably during

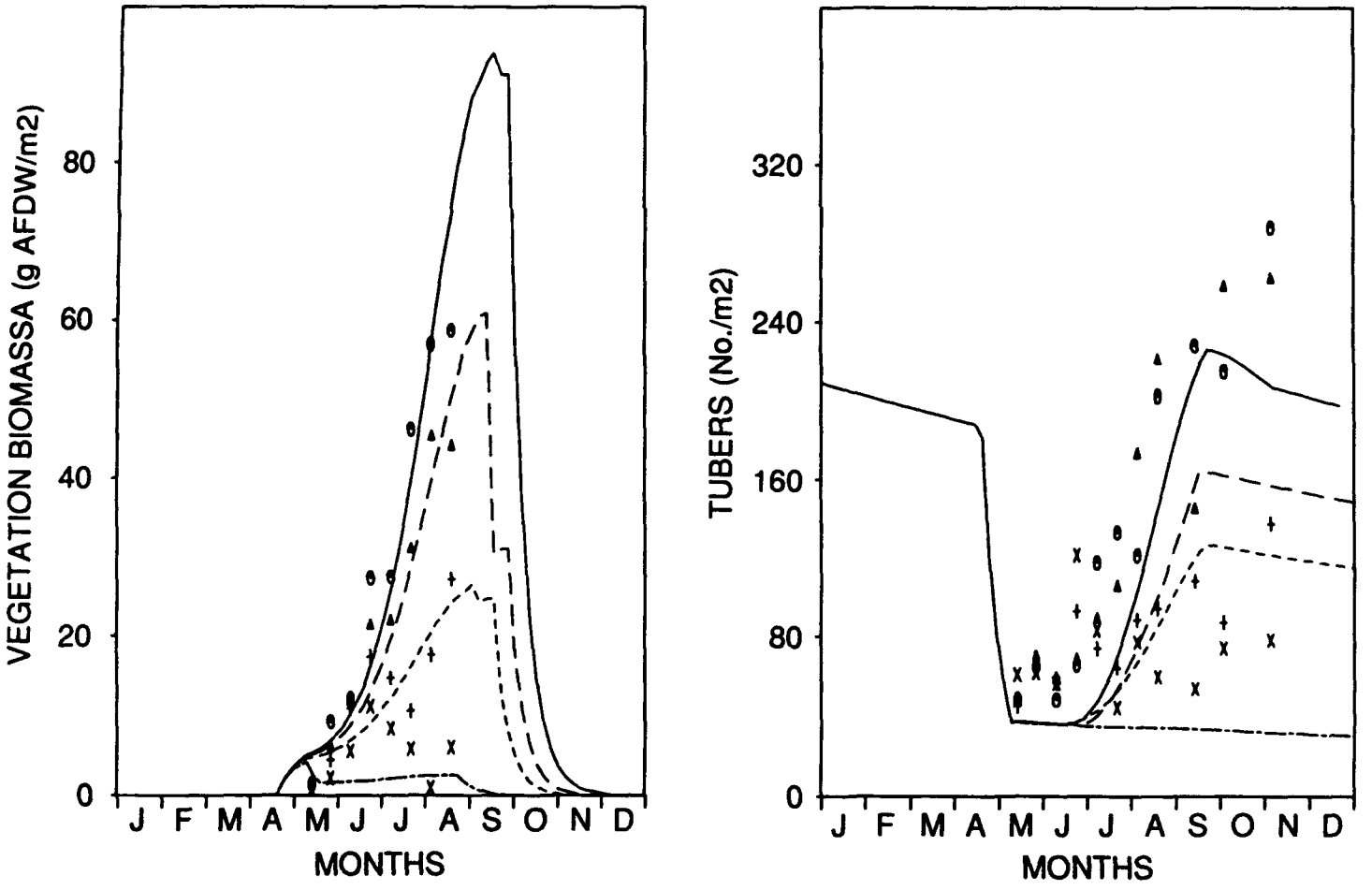

Figure 2. Results of the simulations (lines) of the vegetation biomass (left) and size of the tuber bank (right) with the FLORA model, as well as actual data of the 1986 growing season (symbols) for the control situation on the basis of which the model is calibrated (—,0), and the three shading levels (level 2: – –, Δ; level 3: - - -, +; level 4: — –, x).

almost the entire growing season. The simulation derived that with increasing shading the maximum biomass was reached earlier in the season. This agrees with the field observations and supports the idea that with increasing allocation of resources to the tubers, sloughing of the vegetation is enhanced.

The calculated tuber density at the lowest photon flux density, level 4, was much lower than the observed density (Figure 2). Obviously, the model is not able to simulate the biomass development at low photon flux densities. The calculated production at level 4 was too low to represent the actual production adequately, as both the calculated vegetation biomass and tuber density were lower than the actual data. Apparently, other mechanisms, which are not modeled are involved at these light conditions. The observed difference in calculated and real production at level 4 may result from the assumption that the biomass was equally distributed over depth. It is well accepted that *P. pectinatus* may allocate most of its biomass just below the water surface (Van Wijk 1988). However, even when most of the biomass was allocated just below the water surface, the main shading factor (the nets) remained. So far studies have not shown a response of *P. pectinatus* to low light photon flux densities by acclimation of its photosynthetic tissue (Van der Bijl *et al.* 1989, Hootsmans and Vermaat 1991a). However, in these studies no clear distinction was made between tuber-producing and non-tuber-producing plants under various light conditions. The discrepancy between the calculated and real production at level 4 may be explained by an increase in photosynthesis with tuber formation which has not been modeled. In terrestrial plant ecology, increase of photosynthesis during the production of reproductive propagules is a common phenomenon (*e.g.* Ng and Loomis 1984). The effect of reproduction on leaf photosynthesis can probably be attributed to an increase in reproductive sinks (Reekie and Bazzaz 1987). Evidence exists that an increase in photosynthesis with tuber formation occurs in *P. pectinatus* as well (Hootsmans and Vermaat 1991a).

The FLORA model enables us to simulate well the biomass development of *P. pectinatus* under various light conditions, despite its simple structure and generalizations. The present analysis revealed that the hypothesis of an increased tuber production at lower photon flux density enhancing the sloughing of the vegetation seems valuable. It is likely that photosynthesis is positively correlated with tuber formation.

Considering its relatively simple structure the FLORA model is applicable to a variety of aquatic ecosystems although calibration is a prerequisite. In its present form it is calibrated for the typical Lake Veluwe conditions with *P. pectinatus* exhibiting a wide phenotypic plasticity. Under field conditions both an annual and a perennial life cycle have been observed (Van Wijk 1988). Furthermore, allocation to reproduction varies greatly with extremes in environmental conditions ranging, for example, from 5 to 42% of the total biomass for *P. pectinatus*, depending on whether it is found in sheltered or partially exposed habitats (Kautsky 1987). Under typical environmental conditions, biomass allocation to propagation is quite consistent at about 30% of total biomass for annual and perennial submerged macrophytes (Madsen 1991, Table 3) and terrestrial plants (Fitter 1986). Compared to these observations, relatively little biomass was allocated to the tubers in Lake Veluwe. Including the non-germinated hibernated tubers, however, results in a tuber contribution of 24% (Van Dijk and Van Vierssen 1991). Van Wijk *et al.* (1988) found enhanced tuber production under reduced light conditions in a greenhouse culturing experiment (long-day conditions of 16 hr) with tubers originating from Lake Veluwe. Tuber mass (AFDW) was 26% of total biomass while this was only 7% in the control culture in Lake Veluwe tubers. As it is not always clear whether the non-germinated hibernated tubers are included in the tuber biomass, data such as given by Madsen (1991) should be interpreted with caution.

The number of tubers produced per gram AFDW root/rhizome complex may be highly variable under different conditions. Spencer and Anderson (1987) found a maximum of about 550 tubers per gram AFDW root/rhizome complex (assuming an AFDW:DW ratio of 0.60 as in Van Wijk (1988)) for plants grown under a 10-hr photoperiod. This is about seven times higher than the highest value observed in Lake Veluwe. Such short-day conditions occurred, however, in Lake Veluwe only at the very end of the growing season when most of the vegetation had already died off.

The question on what the ecological implications of an increased resource allocation at lower photon flux densities are in terms of long-term survival of the population may arise. Running the simulation for several years (Figure 3, left) revealed that in the control situation, the vegetation biomass development was stable, with a maximum biomass of about 90 g AFDW m^{-2}. At shading levels 2 and 3 the average biomass steadily decreased with the years until very low values at level 3 after five years. The highest shading was not included as the model simulations were not reliable at that level. A lower vegetation biomass thus entails the risk of too low a tuber production and, finally, extinction of the population. This extinction may be enhanced or retarded by relatively bad or good meteorological conditions, respectively. The simulation showed that although the vegetation was able to photosynthesize at shading level 3, too few tubers were formed to sustain survival in the long term. This conclusion agrees with field observations (Van Dijk *et al.* 1992).

Increased resource allocation to reproductive propagules with lower photon flux density has a negative impact on the

maximum vegetation biomass on a short term, but it seems an adequate strategy in terms of survival on a long term (Figure 3, right). Without an increased resource allocation the vegetation will become extinct more rapidly.

Many studies have been focusing on the photosynthetic production, the reproductive output of submerged macrophytes and the effects of various conditions on that. The results of the present study strongly indicate that also total resource allocation to reproduction, with its effect on vegetative growth, is a prerequisite to consider in explaining the population dynamics of submerged macrophytes under various environmental conditions.

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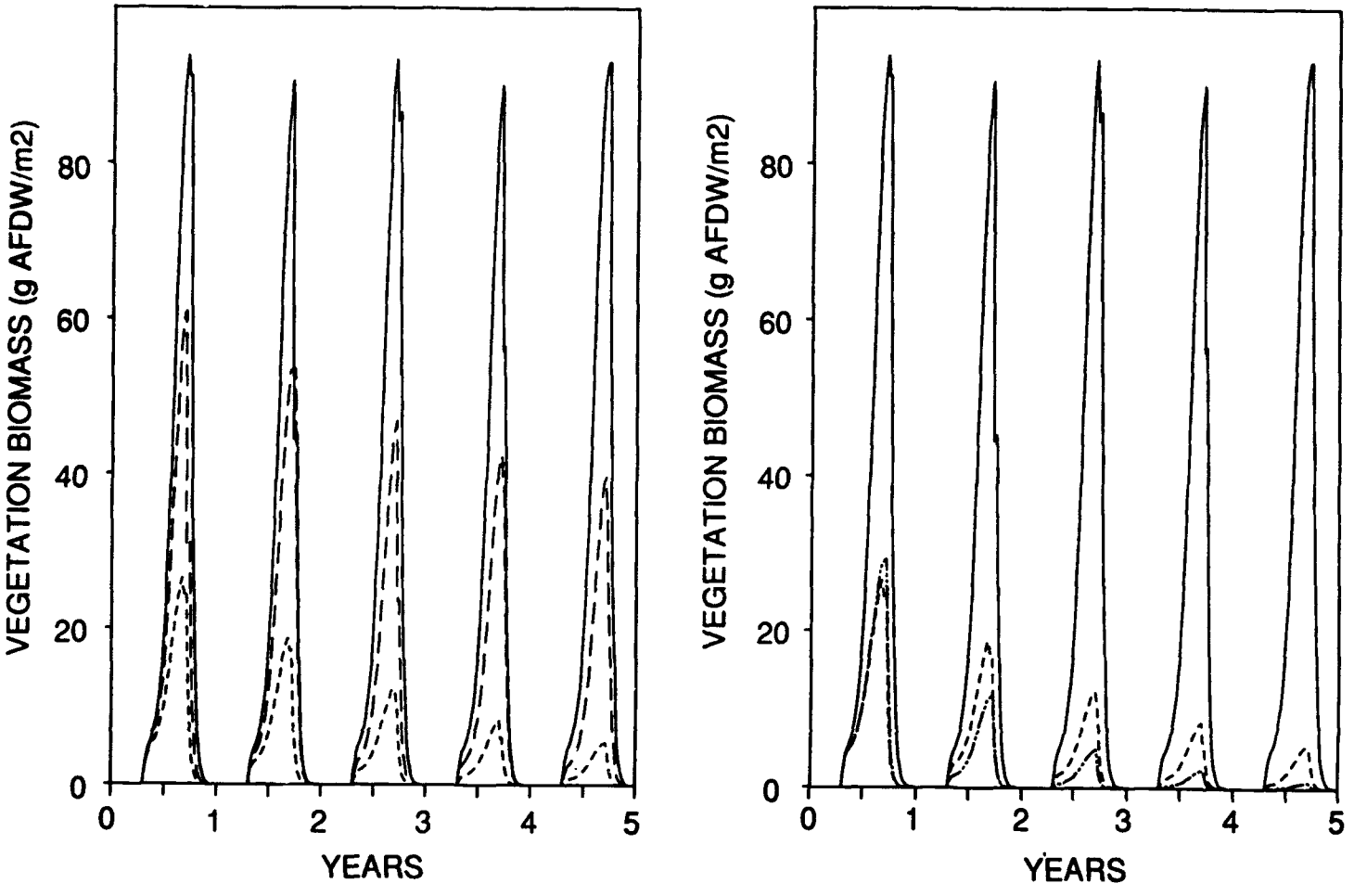

Figure 3. At the left are the results of a 5-yr simulation of the biomass of the vegetation in Lake Veluwe in the control situation: ——, shading level 2: — —, and shading level 3: - - - -. At the right are the results of a 5-year simulation of the biomass of the vegetation in Lake Veluwe in the control situation: ——, shading level 3, with an increased tuber production factor due to lower photon flux density: — - - —, and shading level 3, without an increased tuber production factor: - - - -.

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Growth and Biomass Allocation Patterns During Waterhyacinth Mat Development[1]

JOHN D. MADSEN[2]

ABSTRACT

Two experiments in ponds were conducted to determine growth rates and biomass allocation patterns of waterhyacinth during different life cycle and developmental stages and seasons. Experimental ponds were maintained below a pH of 8.5, and one pond was amended with 11.4 kg of nitrogen per week. Plants were separated into constituent parts after sampling, dried, and weighed to determine biomass. Early in development, plants allocated most production to root material, with little increase in average plant size. Once a critical density was reached, plants increased in average weight and production of daughter plants, with reduced allocation to roots. At peak density, daughter plant production was reduced, but average plant size increased rapidly, resulting in plant mortality. Waterhyacinth exhibited a positive density dependent growth pattern early in development, and switched to the negative density dependent pattern after peak density was achieved.

Key words: mat formation, density-dependence, phenology, self-thinning, *Eichhornia crassipes* (Mart.) Solms.

INTRODUCTION

Seasonal biomass allocation patterns of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) have been examined in small-scale (Luu and Getsinger 1990), pond (Madsen et al. in press), and field (Center and Spencer 1981) situations. The normal seasonal growth patterns determined from these

[1] Part of this information was previously published in the U.S. Army Corps of Engineers Aquatic Plant Control Research Program Annual Proceedings, Misc. Paper A-92-2.

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studies are useful in examining critical stages for control of waterhyacinth, but the seasonal versus developmental stage (*e.g.*, stand age) components in allocation patterns have not been differentiated. For instance, early season allocation patterns may be largely due to the small size and low density of plants at that time versus the environmental effects on biomass allocation patterns. This study aims to distinguish the variations in biomass allocation patterns observed at different developmental stages of waterhyacinth populations.

MATERIALS AND METHODS

Waterhyacinth populations were studied at the U.S. Army Engineer Waterways Experiment Station Lewisville Aquatic Ecosystem Research Facility near Lewisville, TX (Latitude 33°04'45"N, Longitude 96°57'33"W). Two experimental ponds (0.3 ha) were utilized. Both ponds were amended with hydrochloric acid and organic material (hay) to maintain appropriate pH (between 6.5 and 8.5), and Aquashade™ (1 mg l^{-1}) to reduce algal growth. One pond was also amended with 11.4 kg of nitrogen fertilizer (as ammonium sulfate) per week (+N; nitrogen pond), while the other pond was not amended with fertilizer (REF; reference pond). Two studies ("run" and "ring") were performed using each pond.

Run Study. The run study was designed to examine the spatial development of waterhyacinth mats. Eighteen runs were used in each of the two ponds, each run being 1 m wide and 4 m long. Runs were constructed of a wooden frame with wire mesh sides, with a mean side height of 0.3 m. Water depths ranged from 10 cm at the base to 30 cm at the outer edge of the run. All 18 runs in both ponds were planted with 20 small rosettes (approximately 6 leaves, 2 g DW average) of waterhyacinth on 29 May 1991. Plant samples were taken at 5, 7, 9, 11, 13, and 15 weeks after planting; at each time three randomly selected runs were sampled. A given run was sampled only once. Samples were taken at the origin (or base), the front (*e.g.*, leading), and in the middle of the mat using a 0.1-m^2 quadrat, with plants counted, separated into shoots and roots, dried and weighed to determine biomass.

Ring Study. The ring study examined the temporal development of waterhyacinth at a given location across time. Rings utilized were 1 m^2, made of wire mesh with floats to provide buoyancy, and attached to wire supports to maintain their position in the middle of the pond in approximately 0.5 to 1 m of depth. The rings were circular, with a height of 1 m total and a mean above-water height of 0.5 m. A total of 48 rings were used in each pond. This study was initiated by "planting" 5 small rosettes (approximately 6 leaves, 2 g DW) of waterhyacinth per ring. Three cohorts or time periods were used: cohort A was initiated 27 May 1991, cohort B was initiated 23 July 1991, and cohort C was initiated 24 September 1991. Three rings of plants were harvested for each sampling period for each cohort; cohorts were sampled at 0, 1, 2, 3, 5, 7, 9, 12, 15, 18, 22, and 26 weeks after the initiation of the cohort, or until the first week of December. The number of mature plants were counted, and these plants sorted to component parts, dried and weighed to determine biomass.

RESULTS AND DISCUSSION

Run Study. Run study data provided information on both the development of waterhyacinth in one location (the origin) over time, and spatially across the developing mat. A plot of average shoot weight versus density of plants in the runs for all sample periods presents both spatial and temporal developmental trends (Figure 1A). Examining just origin samples (open and filled circles), developmental trends across time are indicated (Figure 1A, 1B). "Invading" origin plants began as

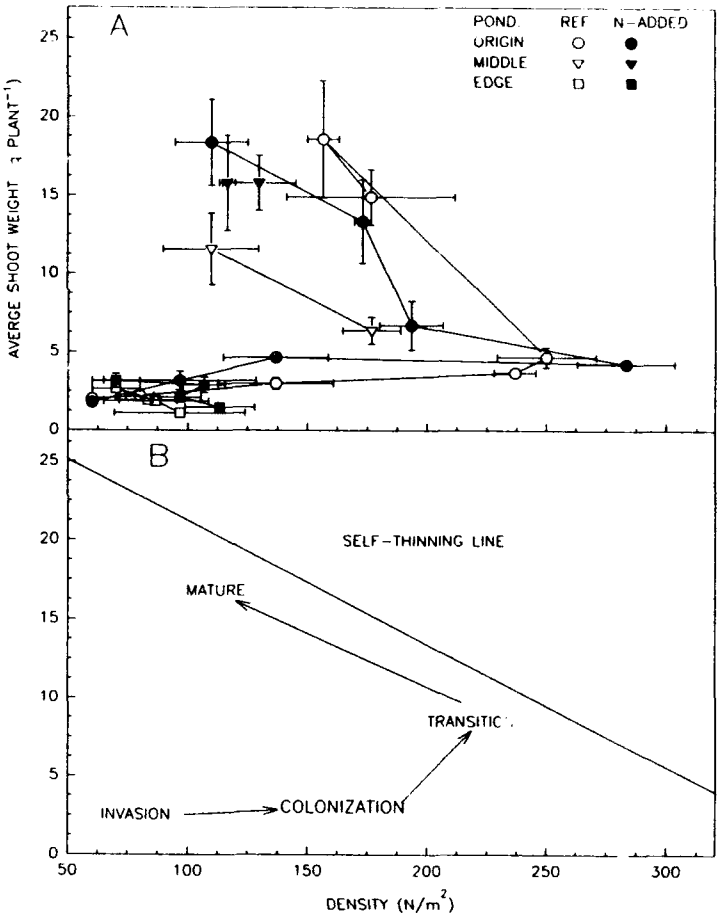

Figure 1. Developmental trends in the relationship between plant density (N m^{-2}) and average shoot weight (g) for waterhyacinth in experimental runs. A) Mean (±1 standard error) for plants from the mat edge, middle and origin of runs in the reference and nitrogen-added ponds; and B) a diagrammatic explanation of trends both temporally at one location and spatially across the mat from edge to origin.

relatively small, sparse rosettes (Figure 1B). Development occurred first through the increase in density or numbers, but all plants were small ("colonization," Figure 1B). As a critical density was reached at approximately 180 rosettes m^{-2}, plants began to increase in size (5 g DW) as well as density (colonization to transition, Figure 1B). These changes were a positive density-dependent increase: plants increased in size more rapidly as density increased. At a second critical density or inflection point of almost 250 rosettes m^{-2}, intraspecific competition began to take place, and density decreased as plants continued to increase in average size (up to 19 g DW). This change reflected a negative density-dependent relationship (transition to mature, Figure 1B). A maximum relationship of size and density followed the -3/2 self-thinning relationship (Watkinson 1986). The second inflection point, from a positive to negative density-dependent developmental relationship, was important for other aspects of population development, as will be discussed in relation to ring study data.

The spatial relationship of growth forms within a waterhyacinth mat are also exhibited in the plot of average shoot weight to density (Figure 1A,B). Edge samples (that is, from the growing edge of the mat composed of the youngest rosettes represented by open and filled circles) were all of a similar size (2 to 4 g DW), but ranged in density from 50 to 150 individuals m^{-2}. At this point, shoot growth increased dramatically, and leaves were oriented vertically rather than horizontally. Transitional plants were intermediate in density and weight to mature plants. Mature basal samples paralleled the -3/2 thinning line as adjacent plants competed for light and space.

Ring Study. Invasive stage plants were typically small, low in biomass (2 g DW each, up to 100 g m^{-2}) and low in density (up to 50 m^{-2}), with a high allocation to root growth, prostrate buoyant leaves, and low allocation to flowering (Figure 2). Plants in the colonization phase of the mat edge were very similar in form to invasive plants, but root allocation was increased over invaders and daughter production was slightly higher. Typically, little flowering occurred in these plants. Once the colonizing plants filled in empty space, vertical leaf growth began. The mass of the mat itself became sufficient to float plants without bladders on the leaves, and leaves began a transition to the mature growth form. At this time, daughter plant production was at its peak and allocation to flowering increased. Also, shoot mass allocation increased more rapidly than root allocation. Plant densities reached their peak (up to 200 m^{-2}), with intense intraspecific competition following. Density then began to decline, as self-thinning occurred. Daughter plant production decreased dramatically, while average shoot size increased markedly. However, root allocation continued to decline. Biomass

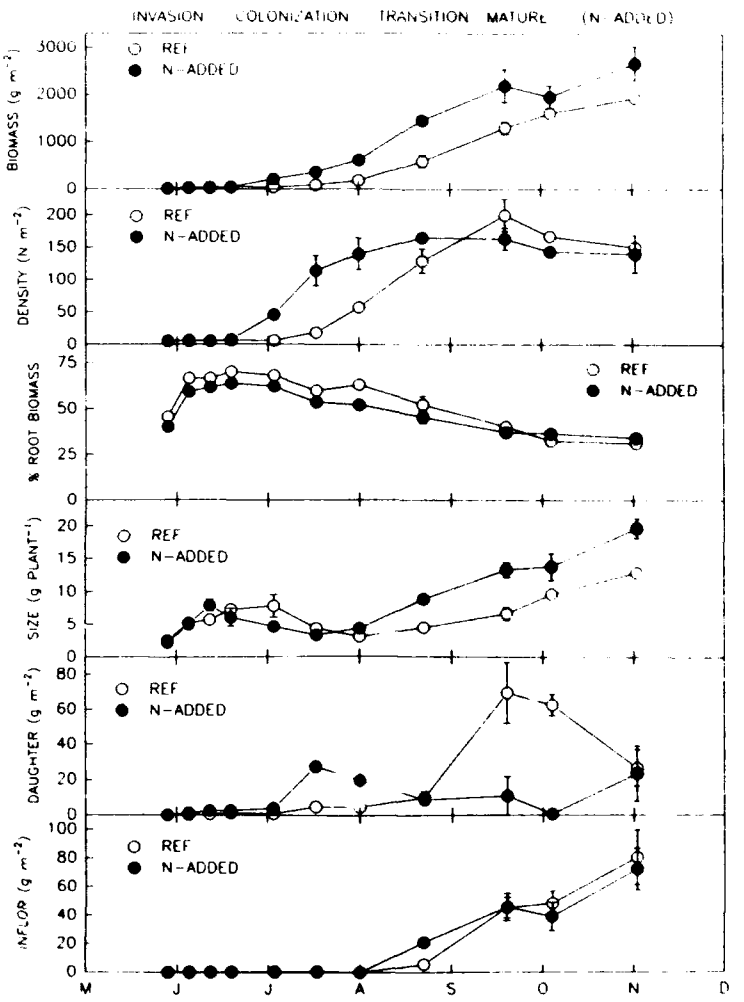

Figure 2. Developmental trends of waterhyacinth mats using data for the reference and nitrogen-added ponds, ring study (cohort A only) in the invasion, colonization, transition and mature phases for biomass, density, percent root allocation of total biomass, average plant size, daughter plant biomass, and inflorescence biomass (top to bottom). Bars indicate ±1 standard error of the mean.

reached its peak (up to 3000 g m^{-2}) in mature stands of waterhyacinth. Since biomass and production are at their peak, but little vegetative propagation can occur, energy was diverted to extensive flowering and carbohydrate storage in stem bases. This may explain in part the observed increase in starch storage in stem bases in late summer and early fall reported in other studies (Luu and Getsinger 1990; Madsen *et al.* 1993). Trends were similar for both reference and nitrogen-added ponds, with a possible lag in developmental rate observed in the less fertile pond.

Many changes that are perceived as seasonal changes in plant allocation are actually related to a population achieving a given developmental stage at a given time. As plant populations develop in density and age, morphological changes occur due to density-dependent effects. In this study,

preliminary indications of some developmental effects of biomass allocation patterns were examined. Early in population development, individual waterhyacinth rosette size was small and density was low. Initial growth patterns resulted in increased rosette density, but little change in average rosette size. As densities reached 150 to 180 rosettes m^{-2}, rosettes increased in both density and size. Once a peak density of approximately 220 to 250 rosettes m^{-2} was reached, average rosette size increased more rapidly, but density began to decrease as a mature stand developed. With these mature stands, daughter production was greatly reduced, and biomass allocation was diverted to either flowering or stem base production from daughter production.

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J. Aquat. Plant Manage. 31: 137-140

Elemental Composition of Five Submersed Aquatic Plants Collected from Lake Okeechobee, Florida

PAUL V. ZIMBA,[1] M. S. HOPSON[2] AND D. E. COLLE[1]

ABSTRACT

Chemical composition of the macrophytic alga chara (*Chara* sp.), and four angiosperms-hydrilla (*Hydrilla verticillata* (L.f.) Royle), Illinois pondweed (*Potamogeton illinoensis* Morong), southern naiad (*Najas guadalupensis* (Spreng.) Magnus), and vallisneria (*Vallisneria americana* Michx.) was determined on samples collected during August 1990 from 146 sites in Lake Okeechobee, Florida. Eleven macro- and micro-nutrients were analyzed: C, N, Ca, Mg, Mn, K, P, Zn, Na, Fe, and Cu. MANOVA was able to separate plant species on the basis of their chemical composition; micronutrients were most important in separation of species by discriminant analysis. Hydrilla and southern naiad had the highest concentration of nitrogen and phosphorus in the species tested.

Key words: chara, eelgrass, hydrilla, musk grass, southern naiad, Illinois pondweed, vallisneria.

INTRODUCTION

Submersed aquatic plants are a conspicuous feature of many aquatic systems. Submersed plants play a key role in aquatic food webs by providing substrate for epiphytes (Cattaneo and Kalff 1980) and invertebrate colonization (Soszka 1975) as well as providing a forage source and refugia for fish (Lubbers *et al.* 1990). Shardendu and Ambasht (1991) analyzed nutrient composition of four submersed macrophytes and concluded that variation in nutrient content was a function of the age of the species tested.

Little research has been conducted on tropical freshwater systems (Table 1) and most systems studied were mesotrophic to oligotrophic deepwater systems. Scheffer *et al.* (1992) for example reported that the distribution of two *Potamogeton* species was differentially affected by changes in lake stage over a 20-yr period in six interconnected shallow-water lakes in the Netherlands.

Submersed plant samples were collected along 60 transects in the northern, western, and southern littoral area of Lake Okeechobee. Individual plant taxa were separated to test whether these plant species have a unique chemical composition.

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TABLE 1. CHEMICAL COMPOSITION OF FIVE SUBMERSED PLANTS FROM LAKE OKEECHOBEE AND OTHER LOCATIONS.

Species	C	N	Ca	Mg	K	P	Zn	Na	Fe	Cu	Mn	n	Locality	Reference[1]
Chara	N.D.[2]	17.10	195.00	7.90	13.90	2.90	68	400	N.D.	25	1,620	N.L.[3]	New Jersey	1
	N.D.	14.30	260.40	N.D.	8.01	9.40	139	26,630	12,200	N.D.	N.D.	28	Poland	2
	206.92	15.03	110.06	7.20	4.68	0.77	112	12,983	13,221	90	1,155	12	Florida	3
	(69.89)	(7.26)	(53.38)	(4.90)	(7.12)	(0.49)	(66)	(11,493)	(9,765)	(61)	(936)			
Hydrilla	N.D.	20.78	269.20	4.00	38.51	2.05	N.D.	N.D.	3,300	N.D.	N.D.	74	Florida	4
	N.D.	17.80	41.60	23.40	23.42	0.53	N.D.	36,600	970	5.7	150	6	Florida	5
	N.D.	25.10	N.D.	N.D.	52.14	0.83	N.D.	N.D.	N.D.	5.8	N.D.	10	Florida	6
	N.D.	14.55	32.5	2.61	58.24	0.77	87	1,560	1,060	N.D.	60	2	Florida	7
	N.D	22.50	N.D.	N.D.	N.D.	2.20	278	N.D.	N.D.	98	N.D.	37	Australia	8
	316.58	33.24	48.13	6.52	25.78	1.87	134	26,043	12,518	84	2,904	32	Florida	3
	(66.56)	(6.15)	(36.66)	(3.74)	(12.11)	(1.05)	(58)	(15,182)	(9,801)	(179)	(2,089)			
Naiad	N.D.	N.D.	9.80	4.70	34.90	1.50	48	6,100	710	48	34,900	N.L.	South Carolina	9
	313.77	23.66	67.24	6.52	20.27	1.07	133	43,968	9,285	60		22	Florida	3
	(35.70)	(11.26)	(44.79)	(4.77)	(10.89)	(0.54)	(88)	(20,045)	(7,023)	(36)				
Illinois pondweed	N.D.	4.79	208.00	0.60	7.74	0.42	N.D.	N.D.	0.6	N.D.	N.D.	N.L.	South Carolina	4
	319.75	16.52	63.83	5.76	16.41	1.22	130	3,419	46.8	65	1,186	36	Florida	3
	(45.37)	(6.86)	(52.92)	(5.21)	(9.20)	(0.90)	(99)	(1,215)	(33.3)	(47)	(1,256)			
Vallisneria	N.D.	42.00	8.2	N.D.	N.D.	4.30	N.D.	N.D.	N.D.	N.D.	20	20	New York	10
	324.82	20.64	27.77	5.36	33.91	1.64	241	7026	1.01	77	295	59	Florida	3
	(57.47)	(11.06)	(30.16)	(1.97)	(19.32)	(1.31)	(176)	(2,924)	(1.30)	(83)	(338)			

NOTE: Macronutrients (Carbon = C, Nitrogen = N, Calcium = Ca, Magnesium = Mg, Potassium = K, Phosphorus = P) are expressed as mg/g dry weight, micronutrients (Zinc = Zn, Sodium = Na, Iron = Fe, Copper = Cu, Manganese = Mn) are expressed as µg/g dry weight. sample number indicated by n. Lake Okeechobee values are mean and (in parentheses) standard deviations.

[1]References: 1 Hutchinson 1975, 2 Bernatowicz 1969, 3 this research, 4 Langeland 1982, 5 Sutton 1985, 6 Sutton & Portier 1983, 7 Sutton & Portier 1991, 8 Finlayson *et al.* 1980, 9 Boyd 1978, 10 Grise *et al.* 1986.

[2]N.D. = Not determined.

[3]N.L. = Not listed.

MATERIAL AND METHODS

Lake Okeechobee(26°56.00'N, 80°55.00'W), located in south central Florida, is a managed reservoir with multiple uses including flood control, irrigation, and recreation, and serves as a regional source of potable water. Lake Okeechobee is the second largest freshwater lake wholly within North America and is subtropical to tropical. The lake is classified as eutrophic and has a mean water column depth of <3.0 m (Canfield and Hoyer 1988). Over 21% of the lake area consists of littoral habitat.

Submersed plant biomass samples were collected during 18 to 31 August 1990 from 60 transects located along the northern, western, and southern littoral zone. Samples were collected along these transects when species dominance changed (Canfield *et al.* 1983). Biomass samples consisted of all above-sediment vegetation in 0.25-m^2 quadrats. Samples were cleaned of sediment and debris, washed free of obvious epiphytes, separated into component species, dried at 60C, and then ground to homogeneity using a Wiley Mill equipped with a #60 (250 µm) mesh. Carbon and nitrogen analyses were made with a Carlo Erba Model NA1500 elemental analyzer using atropine as the external calibration standard. For all other elemental analyses (Ca, Mg, Mn, K, P, Zn, Na, Fe, and Cu), 1.0 g of each sample was ashed at 550C for 4 hr, then acidified (HCl) and analyzed by the IFAS Soil Testing Laboratory using an ICAP spectrophotometer (Hanlon and Devore 1989). Data were normalized (to sample weight digested) prior to statistical analyses (Statistical Analysis System 1985).

RESULTS AND DISCUSSION

A total of 146 samples were analyzed for chemical composition (Table 1). Vallisneria was the most abundant species analyzed (59 samples), whereas chara had the fewest replicate samples (12).

Generally hydrilla, southern naiad, Illinois pondweed, and vallisneria had similar chemical composition relative to that of chara. Only calcium content of chara was higher than in the other four species. High calcium content would be expected in charophytes because of the calcium carbonate cell

wall (Bold and Wynne 1985). It is surprising that the calcium value for chara is low compared to other studies as Lake Okeechobee water column calcium concentrations exceed 50 mg/l throughout the lake (Zimba pers. obs.). Perhaps previous researchers included a significantly larger proportion of the external carbonate-epiphyte outer layer, or the combustion temperature used during our ashing procedure exceeded the optimal for this single element (Hanlon, pers. comm.).

MANOVA was able to separate the five species based upon their unique chemical composition. All eleven variables were statistically different ($F = 6.57$, $p \leq 0.001$, d.f = 5,1,93). Discriminant analysis identified what elements allowed for separation of the five species (Figure 1). A plot of the group centroids (means) on canonical axes 1 + 2 suggests greatest separation of chara from the other taxa. Micronutrient concentrations, particularly iron, copper, and magnesium, were most significant in separating chara from the other four species.

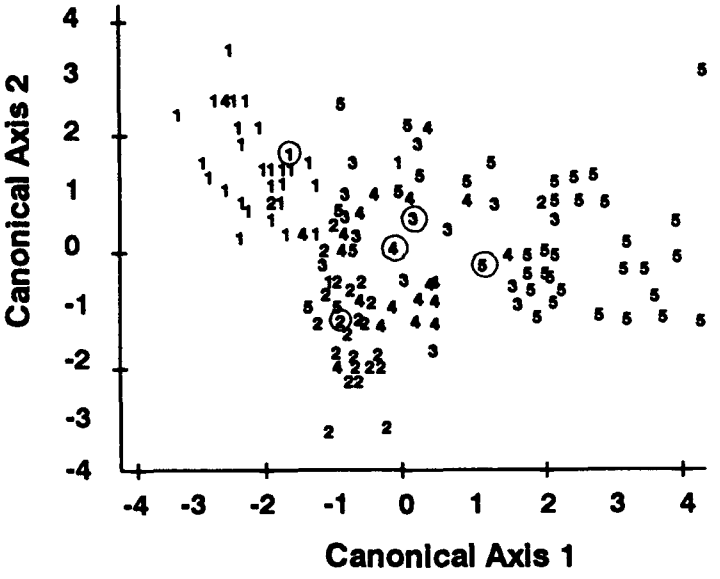

Figure 1. Plot of discriminant analysis results on canonical axes 1 and 2. Circled values are group centroids for each species (1 = chara, 2 = hydrilla, 3 = naiad, 4 = Illinois pondweed, 5 = vallisneria).

Ratios of carbon:nitrogen (C:N) or nitrogen:phosphorus (N:P) have been used to assess physiological state of aquatic vegetation (Goldman *et al.* 1979, Wheeler and Bjornsater 1992). C:N ratios for the five macrophytes averaged 14.40 (range 9.46 to 19.44), a value much higher than the Redfield ratio of 6-7 which suggests plants are nitrogen limited. N:P ratios for the macrophytes averaged 16.33 (range 12.58 to 23.55), suggesting phosphorus limitation. However, both nitrogen and phosphorus concentrations in the macrophytes are in excess of critical levels determined for five species of green and red macroalgae (Wheeler and Bjornsater 1992) or six species of submersed aquatic plants (Gerloff and Krombholz 1966). These data suggest that caution should be exercised when using elemental ratios as the sole means of judging physiological health.

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J. Aquat. Plant Manage. 31: 141-144

MANAGEMENT/UTILIZATION

The Influence of Vegetation Pre-dredging on the Post-dredging Community

P. M. WADE[1]

ABSTRACT

Vegetation present in a channel immediately before it is dredged was found to have a significant influence on the post-dredging vegetation in terms of species composition. Approximately 60% of the species recorded prior to dredging were found in the first 2 yr after dredging. Mean cover values of species indicative of the latter stages of channel hydroseral succession (*e.g.* great pond-sedge (*Carex riparia* Curtis) and common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) are much less in the post- than in the pre-dredging channel. The most important elements of post-dredging vegetation were filamentous algae and floating species such as common duckweed (*Lemna minor* L.), fat duckweed (*L. gibba* L.) and frogbit (*Hydrocharis morsus-ranae* L.). These species were present before management but only with small percentage cover. Although no submerged species was recorded pre-dredging, a number of such species did appear in channels post-dredging but they did not persist.

Key words: drainage channel, colo.i.ation, succession, Gwent Levels.

INTRODUCTION

The management of aquatic vegetation is typically dependent upon a single disruptive act such as a weed cut or the application of a herbicide designed to reduce the biomass of that vegetation for a significant period of time. The period of satisfactory control is dependent upon a number of factors, one of which is the rate of re-colonization of the habitat by aquatic vegetation. This re-colonization can be due to new species moving into the habitat or to the recovery or persistence of species present in the habitat before the management event. The movement of species into a water body has received some attention in the past beginning with the observations of plant ecologists such as Godwin (1923), though neither process has been investigated in any detail. Dredging is one of the most extreme forms of aquatic plant management, an event which is intended to remove not only all plant material but also accumulated sediments. This investigation was designed to determine whether the vegetation in a drainage channel immediately before it was dredged-out had any effect on the composition and development of the flora after dredging.

MATERIAL AND METHODS

Subsidiary channels of the Gwent Levels, South Wales, were chosen for the project, typically 2.6 m in width at water surface with a mean water depth before dredging of 23 cm, and a maximum sediment depth of 72 cm. Post-dredging, these values rose for the water depth (78 cm) and decreased for the sediment depth (19 cm). The drainage system of the Gwent Levels is described in detail by Scotter *et al.* (1977). A total of 18 subsidiary drainage channels located on the Wentooge Level of the Gwent Levels were identified as due to be dredged-out in the summer of 1984. A single 10-m sampling unit was established for each channel based on methodology described by Wade (1978) and the aquatic vegetation was assessed as percentage cover. Additionally, estimates of water depth and sediment depth were made. None of the channels had been dredged-out for at least 8 yr. Each channel was dredged-out along its entire length by a Priestman Mustang hydraulic dredger in June (5 sites), July (7 sites), September (4 sites) and October (1 site). These channel sites were revisited in June or July of the 2 yr following dredging. On both these latter occasions the aquatic vegetation was sampled as for the first visit. This first visit to the channel before it was dredged is termed the pre-dredging visit and the other two visits the post-1 dredging and post-2 dredging.

One site, the reference site, was sampled using three 10-m sampling stretches. These stretches were visited on a monthly basis for the duration of the investigation to give an indication of the spatial and temporal variation of plant species.

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RESULTS AND DISCUSSION

The dredging operation successfully cleared the vegetation and a significant depth of sediment from the channels such that immediately after dredging the sites were totally devoid of vegetation (Figure 1) except for occasional fronds of common duckweed (*Lemna minor* L.), fat duckweed (*L. gibba* L.), and filamentous algae floating on the surface. The results from the reference site indicate that a single 10-m sample stretch and two visits in the summer per annum are sufficient to accurately record the recovery of a site (Figure 1). Figure 1 also illustrates the uniformity of vegetation along a channel.

The mean number of species in the pre-dredging visits was 10.3 species. At the post-1 dredging visit a mean of 9.6 species had become established rising to 10.3 species by the post-2 dredging visit. Not only was the number of species pre-dredging similar to that recorded after dredging but the vegetation present in a channel immediately before dredging had a profound effect on the vegetation developing within a channel in at least the first 2 yr after dredging.

The post-1 dredging flora contained on average 43% of the species present pre-dredging and the post-2 dredging flora, 50%. Taking the combination of the post-1 dredging and post-2 dredging species complement, 61% of the species present before dredging were recorded in the first 2 yr after dredging.

Those species with a percentage frequency of occurrence in the 17 sites of greater than 50% are ranked in Table 1. Out of a total of 51 species recorded from all three visits from all the sites, 35 were noted in the pre-dredging visit and 38 in the post-dredging visits.

Three species are common to both lists (Table 1), and lesser water parsnip (*Berula erecta* Huds. Coville), tufted forget-me-not (*Myosotis laxa* Lehm.) and great pond-sedge (*Carex riparia* Curtis), though recorded with a less than 50% frequency in post-dredging visits were present at 40%, 40% and 47%, respectively. These recurring species fall into three categories (Table 2):

(a) Species with high percentage cover pre-dredging but with low percentage cover post-dredging, *e.g.* common reed (*Phragmites australis* (Cav.) Trin. ex Steudel), tubular water dropwort (*Oenanthe fistulosa* L.) and great pond-sedge.

(b) Species with low percentage cover pre-dredging but with high percentage cover post-dredging, *e.g.* water-plantain (*Alisma plantago-aquatica* L.).

(c) Species showing no differences between pre- and post-dredging situations, *e.g.* common duckweed, fat duckweed and tufted forget-me-not.

These findings support the description of successional processes in subsidiary drainage channels in which common reed, for example, although a frequent species in recently dredged channels, does not exist in significant stands (>15% cover) until approximately 8 to 10 years after dredging. In contrast water-plantain was present in large stands in recently dredged subsidiary channels but only occurred with low cover values in the older ditches (Wade 1978).

Certain plant species failed to reappear after dredging, for example, lesser marsh bedstraw (*Galium palustre* L.) (13 sites before and only two after dredging); hard rush (*Juncus inflexus* L.) and soft rush (*J. effusus* L.) found in seven and eight sites, respectively, pre-dredging were not subsequently found in any sites. Other similar examples were yellow iris (*Iris pseudacorus* L.) and bittersweet (*Solanum dulcamara* L.)

Taking the reverse case, the most notable plants recorded post-dredging were filamentous algae (Table 1), 12 sites, not found at all pre-dredging. Other submerged marcophytes were recorded only after dredging: *Chara vulgaris* L., rigid hornwort (or coontail) (*Ceratophyllum demersum* L.), pink water-speedwell (*Veronica catenata* Pennell) (submerged form) and lesser pondweed (*Potamogeton pusillus* L.) (Table 3). Filamentous algae and submerged macrophyte species are typically primary colonizers in the hydroseral succession though their duration in subsidiary channels is considered to be short lived, probably only 1 or 2 yr (Wade 1978). This is borne out by the absence of all these species from one or more sites on the second visit (Table 3). A persistent submerged species (*Lemna trisulca* L.) which, although present in sites before management (47% occurrence), increased to 88% in post-dredging sites, a figure sustained for the second visit. Likewise, small pondweed (*Potamogeton berchtoldii* Fieb.) was well established in the reference site 2 yr after management (Figure 1).

The most important element of re-colonization was the floating vegetation, *e.g.* common duckweed, fat duckweed and frogbit (*Hydrocharis morsus-ranae* L.) (Table 2). Other species exploiting this niche were water fern (*Azolla filiculoides* Lam.) and greater duckweed (*Lemna polyrhiza* L.).

Two questions are raised by these observations: did those species appearing apparently for the first time in the sites post-dredging come from propagules already in the sediment and released by the disturbance of management or were they introduced into the site anew? and did all the species which recurred after management survive into the third year and beyond?

Given the ability of a number of plant species as described above to survive such a destructive management regime as dredging, it would not be surprising to learn that other species observed post-dredging, although not present immediately

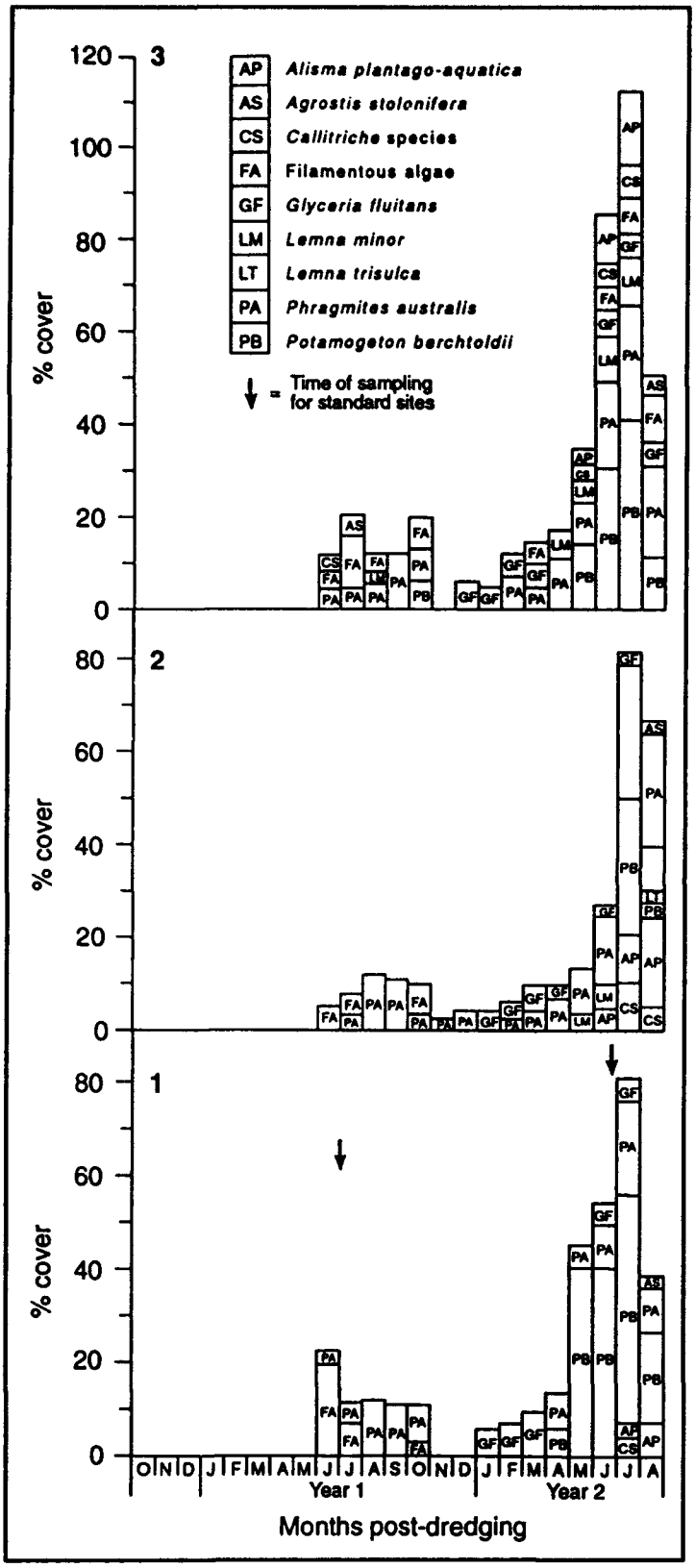

Figure 1. Cover data for species with percentage cover >5% in reference site post-dredging. 1, 2, and 3 are the three 10-m sample stretches of the reference site. Dredging occurred in September prior to Year 1.

TABLE 1. SPECIES OCCURRING WITH PERCENT FREQUENCY >50% IN CHANNELS PRE-DREDGING AND POST-DREDGING (post-1 and post-2 visits combined). (Total number of sites = 17).

Species	Pre-dredging	Species	Post-dredging
Phragmites australis	100	*Phragmites australis*	100
Galium palustre	76	*Lemna minor* [1]	94
Lemna minor	71	*L. trisulca*	88
Carex riparia	71	*Hydrocharis morsus-ranae*	76
Oenanthe fistulosa	59	Filamentous algae	71
Alisma plantago-aquatica	53	*Agrostitis stolonifera*	71
Berula erecta	53	*Oenanthe fistulosa*	59
Myosotis laxa	53	*Lemna gibba* [2]	53
		Sparganium erectum	53
		Glyceria fluitans	53

[1]Including the flat form of *L. gibba*.
[2]Excluding the flat form of *L. gibba*.

TABLE 2. MEAN PERCENTAGE COVER OF SELECTED SPECIES PRE- AND POST-DREDGING.

Species	Pre-dredging	Post-1 dredging	Post-2 dredging
Berula erecta	6.5	0.5	2.5
Oenanthe fistulosa	7.0	0.5	1.0
Alisma plantago-aquatica	1.0	8.0	13.0
Hydrocharis morsus-ranae	1.0	3.0	27.0
Lemna minor & L. gibba	15.0	16.0	37.0
Sparganium erectum	1.0	4.0	5.0
Carex riparia	12.0	0	0.5
Phragmites australis	23.0	3.0	5.0

TABLE 3. PRESENCE OF SUBMERGED MACROPHYTE SPECIES THAT WERE NOT PRESENT PRE-DREDGING IN SITES AND POST-DREDGING. (Total number of sites = 17.)

Species	Number of sites	
	Post-1 dredging	Post-2 dredging
Chara vulgaris	5	1
Ceratophyllum demersum	6	5
Veronica catenata [1]	4	2
Potamogeton pusillus	5	2

[1]Submerged form.

before dredging, had been present in a site a number of years earlier. Wade and Edwards (1980) exploring the historical ecology of the Gwent Levels developed just such a hypothesis for species of plants such as *Chara vulgaris* L., a species exhibiting similar distributional patterns elsewhere (Wade 1990).

It has already been indicated that some species, notably the submerged macrophytes, were already in decline in the second year after dredging, presumably due to the dominance of floating species. No doubt other species would also have failed to maintain themselves beyond the second year after management.

Thomas, Allen and Grose (1981) found that removal of reed sweet-grass (*Glyceria maxima* (Hartm,) Holmberg) by dredging from drainage channels on the Ouse Washes, U.K., produced a habitat suitable for a range of colonizing species. They considered that propagules dormant in the mud can be brought to the surface, where suitable conditions for growth may be more likely, thus accounting for a higher floral diversity in channels 1 to 2 yr after dredging compared with the diversity at other times.

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Suction Harvesting of Eurasian Watermilfoil and Its Effect on Native Plant Communities

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ABSTRACT

Seven sites on Lake George, New York, were selected for control of Eurasian watermilfoil by a diver-operated suction harvester. Prior to suction harvesting, ten 0.1-m^2 biomass samples were collected from each site. Samples were randomized within the area to be harvested, sorted by species, dried and weighed. A grid system of 36 contiguous 1-m^2 quadrats was also located within each of the treatment areas. The species present and their relative percent cover in each quadrat were recorded prior to harvest, shortly after and 1 year postharvest. A substantial reduction in the biomass of milfoil at all sites was noted as a result of suction harvesting. One year after harvest, the impact of harvesting on the native plant community included a greater number of species per unit area and reduced biomass and percent cover at a majority of the treated sites.

Key words: plant management, milfoil, macrophyte, plant ecology.

INTRODUCTION

Eurasian watermilfoil (*Myriophyllum spicatum* L.) was discovered in 1985 in Lake George, a large oligotrophic lake (3 km by 50 km; 110 km^2) located in northeastern New York State. Initially found in only three bays, it had spread to over 90 locations by 1991. Herbicides and mechanical cutting programs were unacceptable because the lake is used as a public water supply and the spread of the plant would be accentuated by fragmentation generated by cutting. Management targeted at milfoil was initiated in 1989 as part of a U.S. EPA Clean Lakes Phase II program and utilized physical control techniques such as hand harvesting, benthic barrier, and suction harvesting. In 1990 suction harvesting was proposed as the primary management technique at seven sites where both the effectiveness of control and evaluation of its impact on native plant communities would be documented.

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METHODS

Harvesting sites were chosen from throughout Lake George to represent a variety of sediment types, slopes, shoreline orientations, and diversity of native plant communities. Site names and milfoil location numbers are given in Table 1.

The suction harvester was a diver-operated, hydraulic vacuum system that was created by a diesel-powered venturi pump mounted on a 28-ft pontoon boat. The plant material, including vegetative stem and leaves plus roots, was pulled from the bottom by the diver and fed by hand into one of two 4-in. vacuum hoses. The harvested material was pumped via the vacuum hose and venturi system to the surface of the lake and discharged into an aluminum wet well mounted in the deck of a second pontoon boat. The wet well was perforated to allow water and sediment to drain back into the lake, while retaining the harvested plant material.

Prior to suction harvest, each milfoil colony was mapped with the perimeter of the harvest area identified by permanent submerged markers. These provided reference locations for biomass collection and for the placement of grids for percent cover measurements of the plant community. Plant biomass within each treatment site was determined prior to treatment. Within each harvest area, ten 0.1-m^2 biomass samples were collected randomly, sorted to species, and dried to constant weight. A second set of biomass samples was collected 1 year following treatment.

A grid system of contiguous 1-m^2 quadrats was established in each treatment area. Two grids of 3 m by 6 m were installed at sites with sufficient harvest area, while smaller sites received a single 3-m by 6-m grid. The species present in each grid and their relative abundance were recorded prior to harvest, shortly after harvest, and 1 yr later.

RESULTS AND DISCUSSION

Effectiveness of suction harvesting was evaluated by (a) comparison of the weight of milfoil removed during initial harvesting with the weight removed 1 yr later, that is the regrowth, (b) comparison of the number of man-days expended to harvest each site initially and the man-days expended to harvest regrowth 1 yr later, and (c) the dominance of milfoil at each site enumerated by biomass and percent cover data before and after suction harvesting. Total harvest weight, rather than harvest weight per unit area, was used because of the heterogeneity of milfoil distribution within each area harvested.

In 1990, a total dry weight of 710 kg of milfoil was removed by suction harvesting from all the evaluation sites (Table 1). Hand harvesting of regrowth of milfoil during follow-up visits in 1991 yielded 49.6 kg at six of the sites. At the remaining site (M-61), growth of milfoil within the perimeter of a beaver lodge was sufficiently dense to exceed levels which could be readily hand harvested. Discounting this site, 684 kg were removed by suction harvesting and regrowth 1 yr later (49.6 kg) represented only 7% of the initial biomass of milfoil. Stated another way, 93% of the dry weight of milfoil, on average, was removed from each site by suction harvesting. Comparing initial suction harvesting and follow-up hand harvesting 1 yr later (Table 1), removal efficiencies ranged from 86 to 94%. This removal efficiency is comparable to that previously reported for hand harvesting of milfoil in Lake George (Eichler *et al.* 1991b).

A total of 28 man-days was spent suction harvesting milfoil in 1990 (Table 1). This effort is actual time spent in plant management, and does not include man-hours spent in evaluation activities such as percent cover and biomass determinations. On a site-by-site basis, harvesting efforts for regrowth required from 64 to 89% fewer man-hours than initial harvest efforts. Removal of regrowth by hand harvesting in 1991 required 5.7 man-days or 20% of the initial harvesting effort. The use of hand harvesting to remove milfoil during follow-up visits was considerably more labor intensive than suction harvesting on a biomass-removed-per-unit-effort comparison.

Suction harvesting reduced both the biomass and percent cover of milfoil. Milfoil was the most abundant species by

TABLE 1. SUCTION HARVEST SITES, WITH MILFOIL SITE NUMBER (M number) AND AREA OF HARVEST. INITIAL PLANT HARVEST (milfoil dry weight removed and harvest effort) WAS MADE IN 1990 WITH FOLLOW-UP COLLECTIONS IN 1991.

Site	M number	Area (m^2)	Dry weight (kg)		Effort (man-days)	
			Initial	Follow-up	Initial	Follow-up
Westover Lodge	M-29	838	46	5.9	4.4	0.7
Bolton Bridge	M-44	168	51	4.1	3.5	1.2
Smith Bay	M-47	591	74	5.9	3.7	1.3
Eichlerville	M-51	354	40	5.5	3.1	1.1
S. Green Island	M-57	443	357	20.0	5.7	0.9
Camp Andrew	M-60	213	116	8.2	4.7	0.5
Harbor Island	M-61	221	26	—	2.9	—
Total		2828	710	49.6	28.0	5.7

weight in the biomass samples prior to suction harvesting (Figure 1) and declined to the fifth most abundant species after harvesting. Total biomass declined or remained the same following treatment. Substantial decreases in total biomass were observed following harvesting at three sites, M-44, M-57, and M-60.

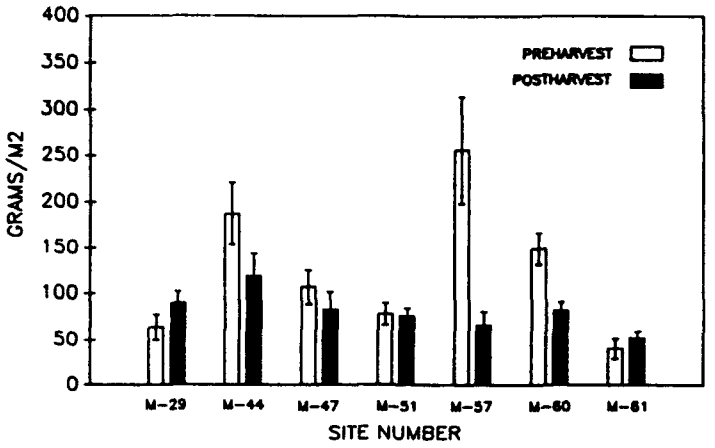

Figure 1. Comparison of aquatic plant biomass before and after suction harvesting. Error bars represent mean values + standard error, n = 10.

Site M-57 displayed the greatest differences in both the number of species present and the total biomass. The depth distribution of milfoil at this site produced plants in excess of 3 m in height. Two species, milfoil and coontail (*Ceratophyllum demersum* L.), dominated, accounting for more than 90% of the total biomass prior to suction harvesting. The dominance of these two species at this site and the tendency for them to be intermingled resulted in a greater proportion of the total plant community being removed by suction harvesting, milfoil and coontail representing 75% of the total biomass by weight. Coontail, lacking roots, is particularly susceptible to removal or relocation by harvesting activities. An increase was observed in the average number of species present in each biomass sample at this site, from 5.7 prior to suction harvesting to 10.0 1 yr postharvesting, while biomass declined from an average of 256.1 g/m^2 prior to harvesting to 66.5 g/m^2 following suction harvesting.

The substantial differences between sites in both the number of species present and total biomass can be attributed to physical characteristics of each site (*i.e.* depth, sediment type, and bottom slope) and dominance of milfoil. At the sites selected for evaluation, sediments ranged from soft silt and clay (M-47, M-57 and M-61) to sand and silt (M-29, M-44, and M-60) and detrital material (M-51). Soft sediments generally supported the greatest biomass and species diversity, harder sediments supported intermediate levels of biomass and species diversity, and detrital sediments supported the most impoverished aquatic plant populations, particularly those overlying hard bottoms.

Results for number of species and total percent cover within the grid systems are presented in Figures 2 and 3. One year postharvest, six of the seven sites had greater numbers of species present than before harvesting and one had fewer. The general increase in the number of species present following the removal of milfoil supports the conclusion that milfoil suppresses native species (Eichler *et al.* 1991a, Madsen *et al.* 1988). The increase in number of species 1 yr after harvesting also indicates that suction harvesting does not have long-lasting negative impacts on plant communities.

Total percent cover within the grid systems declined sharply between preharvest and postharvest ranging from 10 to

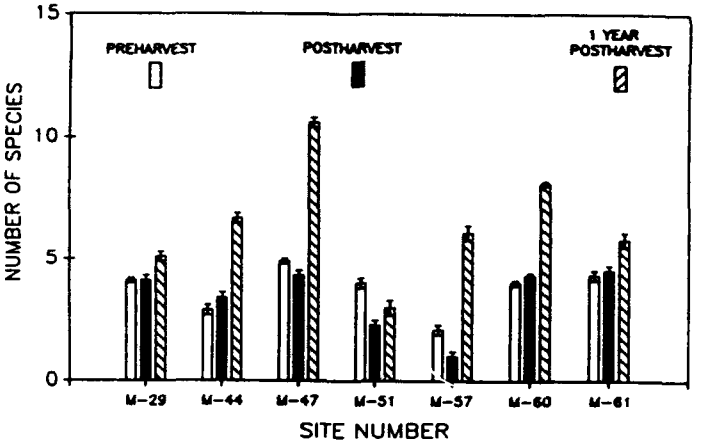

Figure 2. Comparison of number of species per site with grid quadrats inspected before (preharvest), shortly after (postharvest) and 1 yr following suction harvesting. Error bars represent mean values + standard error, n = 36.

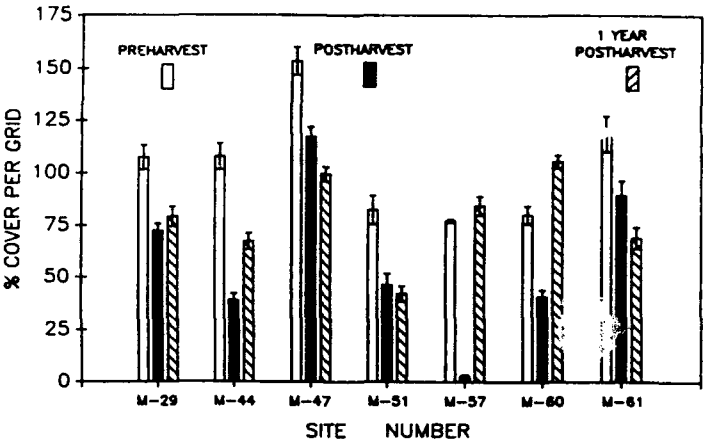

Figure 3. Comparison of relative percent cover within grid quadrats inspected before (preharvest), shortly after (postharvest) and 1 yr following suction harvesting. Error bars represent mean values + standard error, n = 36.

80% as a result of harvesting activities. The most intensively harvested site, South Green Island (M-57), showed the greatest reduction in total percent cover, with the plant community almost completely removed through suction harvesting and physical dislocation of nonmilfoil species as a result of the activities of the divers operating the harvester. Percent cover results 1 yr following harvesting were highly variable relative to percent cover prior to harvesting activities (Figure 3). Five of the seven sites had not returned to preharvest percent cover 1 yr following harvesting.

Reviewing changes in percent cover on a species-by-species basis (Figure 4), milfoil showed the greatest changes as would be expected. From an average preharvest percent cover in excess of 30% for all grids, milfoil declined to less than 5% as a result of harvesting. One year later, milfoil remained at an average of approximately 7% cover. Other species showed variable responses to suction harvesting. A decline in the percent cover of *Potamogeton amplifolius* and *Vallisneria americana* was observed while *P. robbinsii*, *Heteranthera dubia*, *Elodea canadensis*, and *P. gramineus* reflected little change in percent cover relative to harvesting. Both *V. americana* and *P. amplifolius* are perennials, and expand their populations primarily through growth of subsediment runners. The inadvertent harvesting of one plant of either of these species frequently removed a number of plants growing from the same runner. *Najas flexilis* showed substantial increases in percent cover relative to suction harvesting. This species is an annual, growing from seed each year. Following harvesting, areas of exposed bottom were present which would encourage species which spread primarily by seeds or turions.

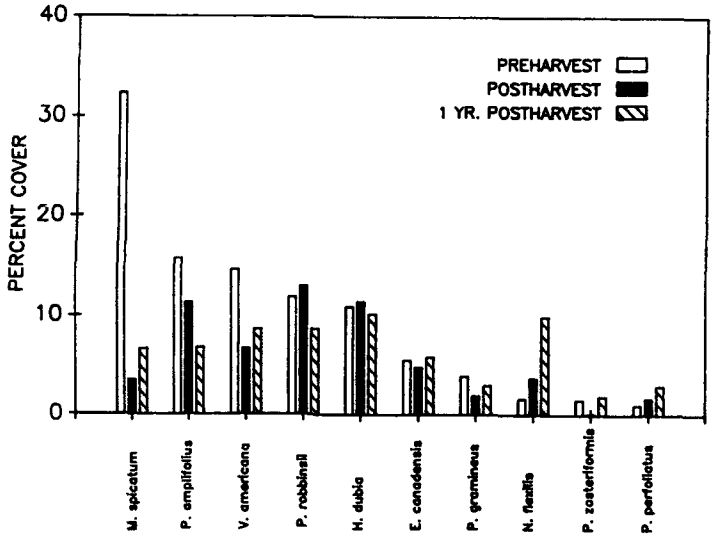

Figure 4. Comparison of relative percent cover determined for the ten most abundant species before (preharvest), shortly after (postharvest), and 1 yr following suction harvesting.

Suction harvesting was found to have a number of limitations, primarily in relation to physical characteristics of the sites harvested. Soft flocculent sediments or shallow waters were not well suited for suction harvesting. Flocculent sediments disturbed easily and reduced visibility. Shallow waters limited diver access and handling of the suction harvester intakes. Suction harvesting is best suited for areas where milfoil reaches moderate to high density infestation levels over limited geographic areas and plant density is too great for hand harvest. Transportation and setup time make this technique impractical for sparsely scattered populations. The principal limitation of this technique for large, dense milfoil populations relates to availability of equipment, manpower, and financial resources for suction harvesting. Disposal of the harvested material also requires substantially more effort for larger areas.

In comparing cost effectiveness per unit effort, one should bear in mind the selectivity of the suction harvesting technique. This technique is applied to areas where the target species (milfoil) is a major component of the total population, but less than a clear dominant. Cost per unit effort is based on an 8 hr man-day at $160 per man-day. Using this base, suction harvesting costs in this program were $6.32 per kilogram dry weight of milfoil removed. On an aerial basis, costs were $1.58 per m^2 or about $15,800 per hectare. These costs are based on labor alone and do not reflect expenses associated with equipment, transportation, survey, and evaluation.

In conclusion, results for suction harvesting indicate that while this technique did not eliminate milfoil populations in a single season of harvesting, a substantial reduction in the biomass of milfoil present and management effort necessary to maintain these locations was achieved. Impacts on the native plant community adjacent to managed areas also appeared to be relatively minor, with benefits including increased number of species and reduced percentage cover of aquatic plants observed at the majority of sites following harvesting efforts.

ACKNOWLEDGMENTS

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The Impact of Mechanical Harvesting Regimes on the Species Composition of Dutch Ditch Vegetation: A Quantitative Approach

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ABSTRACT

It was demonstrated that management regime can influence the species composition of ditch vegetation. This effect, however, is very small compared to the effects of other factors such as the within-site spatial variation and soil and water quality. Cutting late in November had the largest effect.

The vegetation was composed of 139 plant species. The semi-aquatic and aquatic species were less numerous than the terrestrial ones (52 compared with 87). The total number per vegetation type and site ranged from 2 to 49, that of persistent plant species from 1 to 22. Only 20% of the species were influenced significantly by management regime.

Significant effects inherent to within the year repeated management regimes on plant species were ascribed to (1) freeing sites for colonization of new species, (2) improving the light climate for seedlings, which had already colonized, and (3) exhaustion of carbohydrate reserves of solitary species. Management once a year had the reverse effect and caused suffocation due to its undecomposed autumn harvest.

The highest species richness was attained for the semi-aquatic and aquatic vegetation: (1) on sand and clay by cutting three times per year, (2) on peat by cutting once a year (late in November); and for the shore vegetation: (1) on sand and clay once a year (in spring) or two times per year (in spring and summer), and (2) on peat, once a year (in spring or late autumn).

Key words: wetland vegetation, ditches, species-environment relations, conservation, The Netherlands.

INTRODUCTION

The largely cultivated landscape of The Netherlands, rich in embankments, grassland and arable land, is frequently dissected by ditches which form an intricate interconnecting network. Particularly in the low-lying parts of the country the ditch length can be considerable, varying from >225 m/ha in parts of the provinces of Utrecht and Zuid-Holland to 26 to 75 m/ha in the peat-grasslands of the provinces of Noord-Holland, Zuid-Holland and Friesland (Bruinsma 1982).

The management of ditches usually falls under the jurisdiction of water management services like Water Boards and Provincial Services, cooperating with the national service, "Rijkswaterstaat." Ditch management practice was aimed in the past at securing the transport of water from areas with a surplus, involving the complete removal of vegetation mass by mechanical harvesting. Public awareness that ditches can be valuable landscape elements and may function as refugia for plant species is growing, as is the willingness of water management agencies to modify their ditch-cleaning practice to better suit nature conservation purposes. These modifications would involve not the complete but the partial removal of the vegetation mass and changes in timing and frequency of mechanical harvesting. It remains to be seen, however, if, and which, changes in ditch-cleaning practice will be sufficient to conserve or enhance natural values.

Recently attention has been given in The Netherlands to the various effects of mechanical harvesting practices (frequency, timing and equipment; Melman 1991, Van Strien *et al.* 1991, Ter Stege and Pot 1991) and to the effects of several environmental factors (Van Strien *et al.* 1989) on the vegetation of ditch banks. These studies had a more observational than experimental nature. Information on the quantitative effects of well-described management regimes on species

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composition of aquatic and shore vegetation of ditches versus the effects of environmental factors is lacking.

This study was carried out to investigate whether species composition and species richness of aquatic and shore vegetation can be influenced by mechanical harvesting regime, the issue being that if influence could be demonstrated then mechanical harvesting regime could be a useful tool for nature conservation. An experimental approach was chosen in which management regime was the factor to be tested and in which the duration of the experiment was secured. This was done, because in all other studies uncertainties regarding the exact nature and performance of management regime were obvious, and often the number of years during which the vegetation was treated varied, obscuring the evaluation of the results.

The present study is aimed at quantifying the impact of mechanical harvesting regime on species composition of ditch vegetation. Three underlying questions can be distinguished in regard to this: (1) Is it possible to demonstrate an impact of mechanical harvesting on the species composition of ditch vegetation? (2) Which environmental factor (including mechanical harvesting) affects the species composition of ditch vegetation the most? and (3) Which species are sensitive to mechanical harvesting regime and which are tolerant?

METHODS

Site characteristics. Six ditches were chosen, which at the beginning of the experiment were judged representative for ditches in low-lying agricultural areas. The total of six ditches was composed of two groups of three ditches of which one group was supposed to have eutrophic fresh water and one group eutrophic brackish (Cl-concentration>300 mg/l) water. Division into the fresh and brackish group was based on the water quality data of the preccding 5 yrs. Both ditch groups encompassed one sand, one peat and one clay bottom (soil quality according to the national soil survey map (De Bakker and Locher 1991). All ditches were similar in history (dredged longer than 1 yr ago), land use (both sides in use for agriculture), morphometry (4 to 6 m wide, 0.3 to 0.5 m deep) and exposure to irradiation (north-south orientation). The last characteristic entailed that most ditches were situated in the southwest of The Netherlands.

In the course of the experiment, however, it became clear that the actual characteristics deviated from those originally ascribed to the sites chosen. New water quality data indicated that two sites, which were expected to have brackish water (Callantsoog and Tempelpolder) in reality had fresh water most of the time. Three sites, which were supposed to have a thick monotypic topsoil, turned out to have a two-layered topsoil (Hazerswoude-Rijndijk, Callantsoog and Zonnemaire). Thus, the experiment encompassed five freshwater sites and one brackish site, and the soil for aquatic and shore vegetation differed at Callantsoog, Hazerswoude-Rijndijk and Zonnemaire (Table 1).

Cutting regimes, cutting method and experimental design. The effects of four management regimes on the species composition of ditch vegetation were investigated. The criteria for the choices of these regimes were that they are (a) currently in use in The Netherlands and (b) satisfy legal requirements to maintain the primary ditch function. On the basis of these criteria the following three regimes were chosen: (1) 1 x p.y. (May), (2) 2 x p.y. (May, July), (3) 3 x p.y. (May, July, September), abbreviated as M5; M5, 7 and M5, 7, 9. The fourth management regime chosen was carried out late in November (M11), which is later than usual (August-September). This regime was thought least detrimental for the vegetation but was required to maintain the ditches' open water zone. The regimes were imposed during three successive years (1989-1991). Ditches in The Netherlands are usually dredged once every five years, and therefore the duration of this experiment is representative for the normal "lifetime" of Dutch ditches.

One cutting method, a mowing basket, was utilized. The harvested plant material was deposited on the ditch sides; consequently, nutrients in the plant mass were removed from the water and added to the ditch banks. This method is the one most widely utilized in The Netherlands.

At each ditch a randomized block design was used to investigate the effects of management regimes. A stretch of

TABLE 1. SIGNIFICANCE TEST OF MECHANICAL HARVESTING ON SPECIES COMPOSITION. DATA ALL SITES TESTED PER YEAR (test of significance of trace static; RDA, CANOCO).

Vegetation	Year								
	1989			1990			1991		
	P-value	F-ratio	Trace	P-value	F-ratio	Trace	P-value	F-ratio	Trace
Aquatic	0.01	1.52	0.01	0.01	1.62	0.01	0.01	1.40	0.01
Shore	0.01	5.84	0.08	0.01	1.96	0.02	0.08	1.27	0.01

100 m was used for the experiment. Each stretch was divided into five sequential, 20-m-long blocks in the south-north direction. Each block was composed of a western shore portion, a ditch portion and an eastern shore portion. The four management regimes were randomized within the blocks.

Vegetation. Aquatic and shore vegetation were described separately. Aquatic vegetation grew in the permanently inundated part of the ditch, shore vegetation on the lower bank parts as far as the influence of the water on vegetation was visible.

Plant species were recorded annually (mid-July) in five plots of 5 by 4 to 6 m (aquatic vegetation) and 5 by 0.30 m (shore vegetation). Each year relevés were made of the aquatic vegetation (one per location per block per treatment) and the shore vegetation (two, one on each side of the ditch, per location per block per treatment). The actual percentage cover per species was estimated. Rare species received a cover percentage of 0.1. Nomenclature of vascular plant species follows Van der Meijden *et al.* (1990). Filamentous algae were considered as one group. Mosses were excluded.

The plant species (139) of the full dataset were classified into eight ecological groups, notably: three terrestrial groups (grasses, sedges and herbs) and five semiaquatic plus aquatic groups (pseudohydrophytes, helophytes, pleustohelophytes, reptohelophytes and hydrophytes; according to Den Hartog and Van der Velde, 1988). The terrestrial groups were subdivided into two life forms, notably annuals and perennials. The classes of the semiaquatic and aquatic plants are distinguished largely using life form as criterion. Both the terrestrial and the semiaquatic and aquatic groups were ranked according to flowering period to investigate the relationship between the timing of management regime and the cover of the species.

Species which tolerated the same management regime for three successive years at a site were termed persistent. Species absent at the beginning but present at the end of the experiment at the same management regime at a site were termed as species increase. Species present at the beginning and absent at the end of the experiment were termed as species decrease.

Sampling and analysis of soil and water. Representative soil samples were taken at the beginning of the experiment: of the ditch bottom, one sample per location per block; of the bank, two samples, one on each side of the ditch (15-cm sampling depth, core volume 295 cm^3; roots removed by hand before processing). Soil and water samples were transported to the laboratory and kept deep-frozen until analysis. The average values of five replicates were used in the statistical analyses.

Representative water samples were taken monthly at each site (1989-1991; surface water). Water temperature and transparency (Secchi disk) were recorded *in situ*. The average summer values (April-September) for each year were used in the statistical analyses.

The soil samples were analyzed for granular composition, and the contents of organic matter, $CaCO_3$, total-N and total-P. The contents were expressed on dry weight basis.

The water samples were analyzed for pH, and the concentrations of HCO_3^-, nitrogenous compounds (total N, NH_4^+ and NO_3^-), phosphorus compounds (total and $H_2PO_4^-$) and Cl^-. Determinations were according to Dutch standard methods (Best, in press).

Statistics. Aquatic vegetation and shore vegetation were analyzed separately.

Two statistical approaches were used: redundancy analysis and analysis of variance.

Redundancy analysis (RDA) was applied to analyses of relevés and both measured and nominal environmental data. Version 3.1 of the program CANOCO (Ter Braak and Prentice 1988, Jongman *et al.* 1987) was used. RDA is a technique which relates a set of multivariate data (vegetation relevés) to explanatory variables (environmental variables). The latter consisted in the analysis of soil parameters (four grain size classes, and the organic matter contents, $CaCO_3$, total-N and total-P), water parameters (temperature, pH, and the contents of HCO_3^-, NO_3^-, NH_4^+, $H_2PO_4^-$ and Cl^-), and the nominal variables management regime (1-4), site (1-6) and block (1-5).

It was tested whether the species composition of the vegetation was affected by management regime after elimination of the site and block effects (CANOCO; overall significance tested by Monte Carlo permutation test at the 1% level). All samples per vegetation type and all environmental variables were included in this ordination. The percentage cover data were transformed to the natural logarithms, after addition of 1 to accommodate zero values. The management treatments were used as environmental variables and sites and blocks as covariables.

Analysis of variance (ANOVA; Genstat V.1 Package) was used to assess the sensitivity of plant species to management regime. Only those species were tested which (1) occurred in >15% of all relevés per vegetation type (aquatic or shore) and (2) were present for three successive years at any particular site (1 of 6). Effects of sites and blocks were eliminated. Effects at confidence levels below 10% were noted as significant. This analysis unfortunately provides only information on the frequently occurring species with often high cover. So far it proved technically unjustified to analyze data of infrequent species with often low cover, usually comprising the rather rare species in The Netherlands. Therefore, analysis of this group still remains "handwork."

RESULTS AND DISCUSSION

The impact of management regimes on the species composition of ditch vegetation. Management regime influenced the species composition of the aquatic and shore vegetation only to small extent. The management effect was continuously significant on the aquatic vegetation, and only during the first and second experimental year on the shore vegetation (Table 1, $P < 0.01$). The fraction variation in the vegetation response explained, however, was very small (trace < 0.08).

From an RDA test per site (data not shown) it became clear that the variance explained by the blocks usually exceeded the variance explained by the management regimes indicating that most variation in species composition at any location was caused by block-related factors. It was, therefore, wise to carry out the experiment in blocks. Various soil and water quality parameters usually also explained a larger fraction of variance than mechanical harvesting did (Best, in press). M11 had the largest impact. M5,7 sometimes significantly affected the aquatic vegetation, and M5 and M5,7,9 the shore vegetation.

Characteristics of ditch vegetation: species composition, species richness, tolerance and sensitivity to management regime. The vegetation of the ditches investigated was composed of 139 plant species (Table 2). The semiaquatic and aquatic species were less numerous than the terrestrial ones (52 compared with 87). Most semiaquatic and aquatic species were classified as helophytes (18 species) and hydrophytes (14 species); most terrestrial species as dicotyledonous herbs (61 species). One species, *Rorippa nasturtium-aquaticum*, has been listed as potentially endangered in The Netherlands (Van der Meijden *et al.* 1990).

Only 25 species (about 20% of total) were influenced significantly by management regime (Table 2). The effects of a particular management regime on a sensitive plant species could be significant on one soil type, but not on another pointing to the importance of nutritional status of the plant at the time of cutting. M11 proved the management regime causing most extremes in plant cover: under this regime the majority of the minima but also of the maxima in plant cover occurred.

The total and the persistent number of plant species varied strongly with location (Figure 1). The total number per vegetation type and site ranged from 2 to 49; that of persistent plant species from 1 to 22. The total number was highest for Reeuwijk (peat), somewhat lower for Callantsoog (sand/clay), intermediate for Hazerwoude Dorp and Hazerswoude Rijndijk (sand/sand and clay/sand), and lowest for Zonnemaire (sand/clay, brackish).

It was attempted to relate the significant effects of management regime to plant-inherent factors such as life form and cycle. Few distinct patterns emerged, which are discussed in more detail by Best (in press). Management several times a year, on one hand, opened up the vegetation, not only freeing sites for colonization of new species but also improving the light climate for seedlings, which had already colonized; on the other hand, it also exhausted carbohydrate reserves of solitary species. Management once a year had the reverse effect and caused suffocation due to its undecomposed autumn harvest. The latter has been pointed out already by Westhoff (1971) and more recently for ditch bank vegetation in particular by Melman (1991). Van Strien (1991), however, hypothesizes that infrequent mechanical harvesting (less than once a year) yields longer succession series and, therefore, more differentiation in plant species composition. Van Striens' hypothesis is supported by Ter Stege and Pot (1991).

Management geared at enhancing species richness. In this study the behavior of ditch vegetation for certain constant management regimes has been observed and expressed in terms of species composition, species richness and sensitivity and persistence to management regime. However, it may still be difficult for water management agencies to decide which regime might be a useful tool to conserve the natural value of ditch vegetation. Reasons for this are that (1) natural value can be expressed in different ways, mostly as species richness and the number of rare species (on a regional, national or international scale), (2) species richness is not constant over time, and (3) rare species can belong to ecologically different groups and, therefore, a certain management regime can be favorable for one desired species but not for another. It has been demonstrated, however, that species richness and the natural value of ditch bank vegetation are positively correlated (Van Strien *et al.* 1991), and, therefore, a high species richness can be used synonymously with natural value. From this it can be inferred that a suitable criterion for the most favorable management regime can be to aim at the highest "net" number of species possible for a particular location, *i.e.* the number of persistent species augmented by the newly colonized ones. Using this criterion, it becomes clear that there is not one most favorable management regime for ditch vegetation, but several favorable management regimes of which the degree of success is related to vegetation type (semiaquatic and aquatic versus shore) and soil class (Table 3). Management three times per year yields most species for the semiaquatic and aquatic vegetation on sand and clay, but management once a year (late in November) on peat. Management once (in spring) or two times per year (in spring and summer) allows most species for shore vegetation on sand and clay, and once a year (in spring or late autumn) on peat.

TABLE 2. PLANT SPECIES, ARRANGED ACCORDING TO ECOLOGICAL GROUP, HEIGHT (short <50 cm; tall >50 cm), LIFE FORM (LF; A, annual; P, perennial) AND FLOWERING TIME. S, SIGNIFICANCE (ANOVA; *, P <0.1).

Ecological group of species	LF	S	Ecological group of species	LF	S	Ecological group of species	LF	S
TERRESTRIAL								
1. Grasses, short			**3. Herbs, short (Con't)**			**3. Herbs,tall (Con't)**		
Poa annua	A		*Spergularia arvensis*	A		*Polygonum persicaria*	A	
Alopecurus geniculatus	A	*	*Stellaria uliginosa*	A		*Solanum nigrum*	A	
Festuca rubra	P	*	*Geranium molle*	A		*Bidens tripartita*	A	
Agrostis stolonifera	P	*	*Matricaria recutita*	A		*Chenopodium album*	A	
Grasses, tall			*Polygonum aviculare*	A		*Polygonum hydropiper*	A	
Bromus hordeaceus	A	*	*Scutellaria galericulata*	A		*Bidens cernua*	A	
Alopecurus pratensis	P		*Juncus bufonius*	A		*Equisetum arvense*	P	
Anthoxanthum odoratum	P		*Matricaria discoidea*	A		*Ranunculus acris*	P	
Poa pratensis	P		*Scirpus setaceus*	A		*Anthriscus sylvestris*	P	
Poa trivialis	P	*	*Cerastium fontanum*	P		*Glechoma hederacea*	P	*
Holcus mollis	P		*Sonchus arvensis*	P		*Rumex acetosa*	P	
Dactylis glomerata	P		*Taraxacum officinale*	P	*	*Juncus conglomeratus*	P	
Holcus lanatus	P	*	*Cardamine pratensis*	P	*	*Lychnis floscuculi*	P	*
Catabrosa aquatica	P		*Bellis perennis*	P		*Stellaria palustris*	P	
Cynosurus cristatus	P		*Ranunculus repens*	P	*	*Rumex crispus*	P	*
Festuca arundinacea	P		*Potentilla anserrina*	P		*Cirsium palustre*	P	
Phleum pratense	P		*Sagina procumbens*	P		*Hypochaeris radicata*	P	
Elymus repens	P	*	*Plantago lanceolata*	P		*Juncus effusus*	P	
Festuca pratensis	P		*Prunella vulgaris*	P		*Lathyrus pratensis*	P	
Deschampsia cespitosa	P		*Trifolium pratense*	P		*Cirsium vulgare*	P	
Lolium perenne	P	*	*Trifolium repens*	P	*	*Cirsium arvense*	P	
2. Sedges			*Plantago major*	P		*Epilobium hirsutum*	P	
Carex nigra	P		*Rorippa sylvestris*	P		*Urtica dioica*	P	
Carex hirta	P		*Lotus corniculatus*	P		*Rumex obtusifolius*	P	
Carex cuprina	P		*Archillea millefolium*	P		*Senecio jacobea*	P	
Carex distans	P		*Leontodon autumnalis*	P		*Achillea ptarmica*	P	
Carex oederi	P		**Herbs, tall**			*Angelica sylvestris*	P	
Carex spec.	P		*Capsella bursa-pastoris*	A		*Rubus idaeus*	P	
3. Herbs, short			*Ranunculus sceleratus*	A		*Epilobium spec.*	P	
Senecio vulgaris	A		*Galium aparine*	A		*Rumex spec.*	P	*
Stellaria media	A							
SEMIAQUATIC AND AQUATIC								
1. Pseudohydrophytes, short			**2. Helophytes (Con't)**			**4. Reptohelophytes**		
Myosotis palustris		*	*Eleocharis palustre*			*Rorippa amphibia*		
Galium palustre			*Glyceria fluitans*		*	*Rorippa nasturtium-aquat.*		
Triglochin palustris			*Phalaris arundinacea*			*Berula erecta*		
Pseudohydrophytes, tall			*Scirpus maritimus*			**5. Hydrophytes**		
Veronica catenata			*Lycopus europaeus*			*Elodea nuttallii*		*
Equisetum palustre			*Sparganium erectum*			*Potamogeton crispus*		
Oenanthe fistulosa			*Butomus umbellatus*			*Stratiotes aloides*		
Oenanthe aquatica			*Juncus articulatus*			*Potamogeton pectinatus*		*
Lysimachia nummularia			*Alisma plantago aquatica*			*Zannichellia palustris*		
Lotus uliginosus			*Polygonum amphibium*			*Ceratophyllum demersum*		
Sagittaria sagittifolia		*	*Glyceria maxima*		*	*Potamogen trichoides*		*
Ranunculus flammula			*Rumex hydrolapathum*			*Hydrocharis morsus-ranae*		
Mentha aquatica			*Phragmites australis*			*Potamogeton acutifolius*		
2. Helophytes			**3. Pleustohelophytes**			*Potamogeton pusillus*		
Carex disticha			*Wolffia arrhiza*			*Ranunculus circinatus*		
Carex acutiformis			*Lemna spec.*		*	*Potamogeton natans*		
Carex paniculata			*Lemna trisulca*			*Myriophyllum spicatum*		
Equisetum fluviatile			*Spirodela polyrhiza*		*	*Callitriche spec.*		
Iris pseudacorus			*Azolla filiculoides*					

TABLE 3. MANAGEMENT REGIMES ALLOWING THE HIGHEST "NET" SPECIES RICHNESS. BETWEEN PARENTHESES: THE NET INCREASE AND DECREASE IN SPECIES NUMBER RELATIVE TO THE NUMBER OF PERSISTENT SPECIES. Man., management.

Location name	Water type	Aquatic vegetation		Shore vegetation	
		Soil type	Man. regime	Soil type	Man. regime
Hazerswoude Dorp	fresh, eutr.	sand	M5,7,9 (+100%)	sand	M5 (-22%)
Callantsoog	fresh, eutr.	sand	M5,7,9 (+2%)	clay	M5,7 (+39%)
Reeuwijk	fresh, eutr.	peat	M11 (-7%)	peat	M5 (+2%)
Tempelpolder	fresh, eutr.	peat	M11 (+17%)	peat	M11 (-5%)
Hazerswoude Rijndijk	fresh, eutr.	clay	M5,7,9 (+28%)	sand	M5,7 (-46%)
Zonnemaire	brackish, eutr.	sand	M5,7,9 (+400%)	clay	M5,7 (+150%)

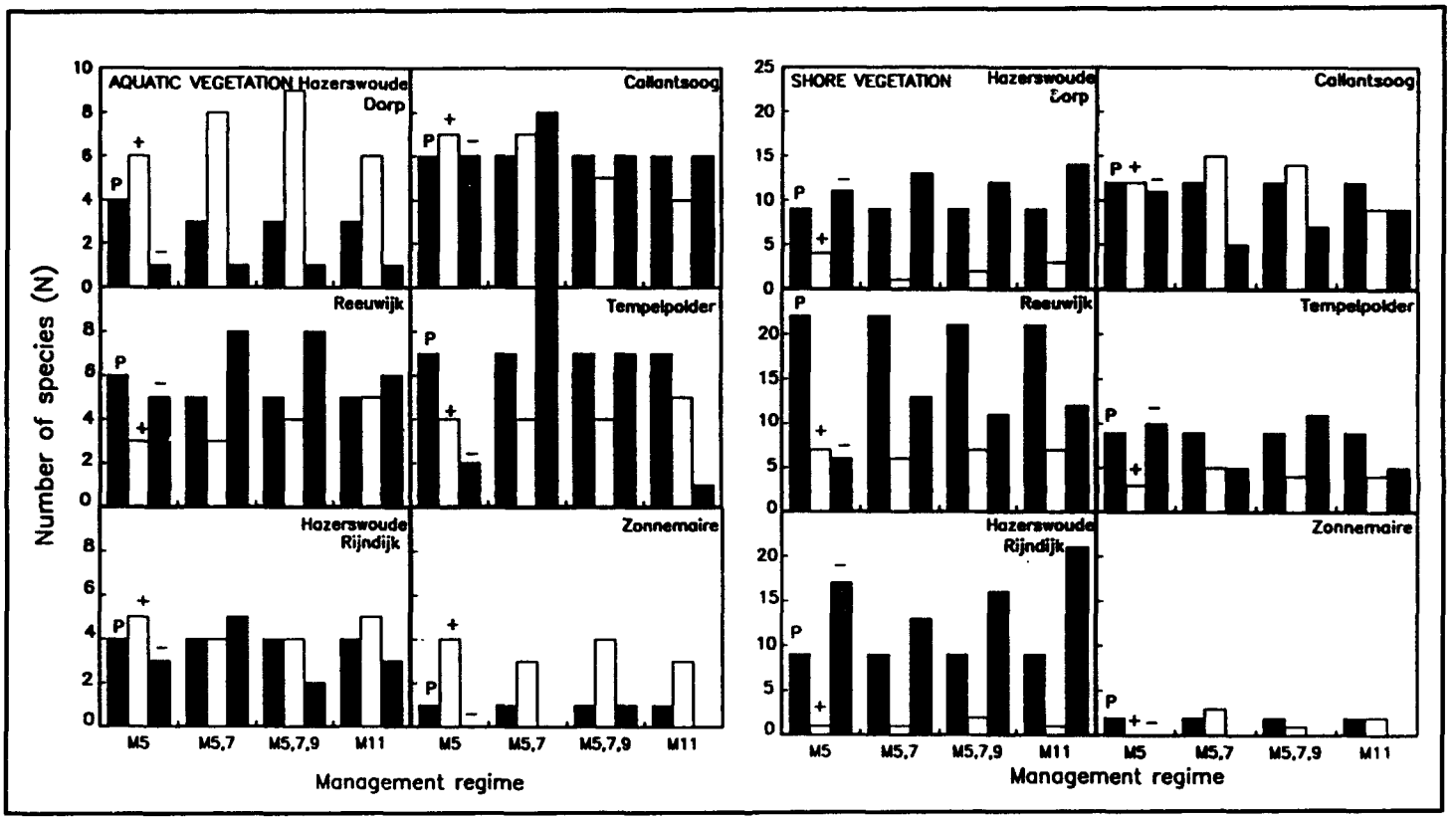

Figure 1. Numbers of plant species of the aquatic (left) and shore (right) vegetation which are persistent (P), increasing (+) or decreasing (-) due to the management regimes applied.

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Seed Production and Growth of Waterchestnut as Influenced by Cutting

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ABSTRACT

A major infestation of waterchestnut (*Trapa natans* L.) in a reservoir in New York State was studied to evaluate the efficacy of air boat cutting as a possible control measure. A measure of the status of the seed bank through sediment core analysis showed that deposition of new seeds in a treatment site was reduced to no net gain of seeds versus an average of 170 seeds/m^2 added to the seed bank in an untreated site. Results from seed-fall collection baskets placed in two untreated sites revealed new deposition of 180 seeds/m^2 and 143 seeds/m^2, respectively, which corroborated the results from sediment core data. Cut rosette fragments, produced by the cutting operation, were compared to undisturbed whole plants for vigor and seed production. Both vigor and seed production rates were less than those of intact plants.

Key words: aquatic macrophyte, water caltrop, aquatic plant control, *Trapa natans*, aquatic plant cutting.

INTRODUCTION

Waterchestnut is a floating leaf, aquatic plant introduced from Eurasia to New York State in the late 1800's and is now found throughout the Northeast (Countryman 1978). Major infestations exist along the shoreline of the Hudson and Mohawk Rivers and in Lake Champlain, as well as in numerous regional lakes and ponds.

In the Northeast, seed germination occurs in late May and by early June a dense canopy of rosettes is soon established on the water surface. Bud formation in the rosettes begins in late June followed by flowering in early July. The first fruits reach maturity by August and upon abscission, these negatively buoyant seeds fall to the sediment. Seed production continues until the senescence of the vegetative plant. As a true annual, waterchestnut overwinters entirely by seed (Smith 1955). A portion of seeds produced each year germinate the following spring while those remaining in the sediment accumulate to produce a seed bank[3]. Seeds have been shown to be viable in excess of five years (Kunii 1988).

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[3]Madsen, J. 1990. Waterchestnut (*Trapa natans* L.) Research in the Watervliet Reservoir-1989 Report. Fresh Water Institute Report #90-8. Rensselaer Polytechnic Institute, Troy, New York. 29 pp.

Waterchestnut has become a nuisance plant largely due to aggressive growth habits. Native plant populations are adversely impacted creating a loss of biodiversity. Waterchestnut has less value as food and shelter to most fish and waterfowl than native plants and has a deleterious effect on water quality (Kiviat 1987). Its narrow, flexible stem supports rosettes which impede passage of boats or people by its extensive surface growth. The fruits possess sharp spines with recurved barbs which are capable of causing painful wounds.

Until recently, control programs have centered on two management forms: (1) physical, including hand pulling or mechanical harvesting and (2) chemical, primarily use of the herbicide 2,4-D [(2,4- dichlorophenoxy) acetic acid] (Smith 1955). In New York State, a control program in the 1970s using 2,4-D was successful in nearly eradicating waterchestnut. However, concerns about adverse chemical effects on fish and aquatic invertebrates lead to a discontinuation of the program in 1977 (Countryman 1978). Since that time, waterchestnut has reestablished to nuisance proportions.

One such infestation exists in the Watervliet Reservoir. A study was begun in 1989 to examine seed production and the effect of a cutting program that utilized a specially adapted air boat. This research was developed in conjunction with an ongoing operational program with the major objectives to: (1) confirm that interruption of the life cycle at flowering and during seed set would decrease mature seed production, (2) ascertain the existence of a decreasing relationship between lower seed production and disappearance of seeds in the sediment, and (3) examine if cut rosettes contain sufficient vigor and can remain viable to produce mature seeds.

MATERIALS AND METHODS

The Watervliet Reservoir is a 175-hectare, potable water supply located in Albany County, New York. Mean water depth for the entire reservoir is 3.5 m. In the western end where all sampling took place, mean water depth was 3 m. The design of the air boat and cutting blade allowed for more rapid movement and coverage of larger surface areas per unit time than traditional harvesters. A sharp V-shaped metal blade mounted in front of the bow was lowered approximately 10 cm below the water surface during cutting operations such that rosettes, once detached from their stems, were disrupted from normal growth and seed production cycles. Under the cutting program managed by the City of Watervliet, rosettes were not removed from the water after cutting.

Sample sites included a treatment area in the main body of the reservoir with a mean depth of 1.5 m where cutting was performed. An untreated reference site characterized by a mean depth of 1.5 m was maintained throughout the growing season in an embayment approximately 200 m from the treatment area (untreated site #1). A second untreated reference site with a similar mean depth of 1.5 m and 10 m from the treated site, located in the main body of the reservoir, was also maintained (untreated site #2).

The status of the seed bank was determined by analyzing 20 sediment cores (7.6 cm *i.d.*) removed from both the treatment site and untreated site #1 in June and November. Seeds from these cores were collected, counted, dried to a constant weight, and examined for the presence of an endosperm as a measure of viability. The core data obtained after senescence (November) represent existing seeds as well as those newly deposited to the bank (previous deposition plus current deposition). The difference between the two sampling periods (November minus June) provides a measure of new seed deposition.

A second method to estimate seed production was obtained by the use of seed-fall collectors which gathered newly produced seeds. The collectors consisted of wire mesh baskets (30 cm by 60 cm) placed directly underneath sections of the canopy in untreated sites #1 and #2. All baskets (10 baskets from each set) were retrieved in November after senescence.

Two transects in untreated site #1 were made every other week to examine seed production and selected phenological traits from early June through early October. The transects consisted of 20 0.1-m^2 quadrats in a straight line, each quadrat separated by a distance of 1 m. The total number of rosettes was counted in each quadrat with two rosettes per quadrat chosen at random for further observation. Phenological traits counted included leaves, buds, flowers, pollinated flowers, and seeds.

Vigor of cut rosettes was determined by collecting and labeling 100 of them with numbered tags to follow development of individual rosettes throughout the remainder of the growing season. Phenological traits as above were measured to provide data analogous to those collected from the transects. Ten cut rosette fragments were placed into each of ten nylon mesh enclosures. The dimensions of the mesh enclosures were 45 cm by 90 cm by 60 cm with 60-cm-long posts at each of the four corners of the enclosures serving to anchor them in the sediment. The ten enclosures were grouped in the center of untreated site #1 in two rows with approximately 1 m separating each enclosure. In the first six, each cut rosette was placed in an "upright" growing position. In the remaining four enclosures, extra untagged cut rosettes were mixed haphazardly with the ten tagged ones to better simulate the "crowded" position typical of the piling of cut fragments upon one another which normally occurred after cutting.

The presence of a significant difference between new seed deposition or loss values calculated from data obtained

by the sediment coring method was determined. Calculation of the p value was made by first obtaining the sample variances of each mean data point. Next an estimate of variance between the differences (November minus June values) was determined followed by performance of the Student's t Test to obtain the actual p value. An analogous method was used to determine if the presence of significant differences existed between new seed deposition values obtained via the sediment coring and seed-fall collection techniques. All variance values calculated are 95% confidence intervals.

RESULTS AND DISCUSSION

The core data from the June sampling were obtained after germination of that year's cohort. Therefore, they represented seeds that remained in the bank from previous deposition and could potentially germinate in future years. In the untreated site #1 and the treatment site, the number of viable seeds averaged 40 seeds/m^2 and 80 seeds/m^2, respectively. This would indicate that a seed bank had accumulated at the sites through previous seed production (Table 1).

TABLE 1. AVERAGE NUMBER OF VIABLE WATERCHESTNUT SEEDS FOUND PER M^2 OF SEDIMENT AREAL COVER AND THE RESULTING SEASONAL SEED DEPOSITION OR LOSS. SEED DEPOSITION OR LOSS REPRESENTS THE DIFFERENCE IN VALUES BETWEEN NOVEMBER 1990 AND JUNE 1990.

Treatment	Sample date		Deposition or loss of new seeds/m^2/year (11/90-6/90)
	6/90	11/90	
Sediment cores			
Reference site #1	40 ± 0.2	210 ± 0.6	170 ± 2.8
Treatment	80 ± 0.3	20 ± 0.1	-60 ± 2.8
Seed-fall collectors			
Reference site #1	—	180 ± 4.1	—
Reference site #2	—	143 ± 4.3	—

In the treatment site, a net loss of 60 seeds/m^2 occurred from June to November. This is a significant loss when compared to the net deposition of 170 seeds/m^2 in untreated reference site #1 ($p < 0.001$). Consequently, less than half as many seeds were observed in the cut site after senescence than in the initial sampling. Similar loss of the number of seeds to the seed bank was observed in a treatment site from the previous year.[3] The loss of seeds in the treatment area may have resulted from several factors including seed transport due to water and sediment movement, and/or seed mortality. The disruption of the canopy by cutting could potentially shift environmental factors to favor germination of dormant seeds in the sediment, although light intensity, *per se*, is not a factor in seed germination of waterchestnut.[4]

Mean values of 180 and 143 seeds/m^2 from untreated site #1 and untreated site #2, respectively, were measured via the seed-fall collection method. No statistically significant differences were calculated when comparing the untreated site #1 value with the net deposition value of 170 seeds/m^2 obtained in that site by the sediment coring technique ($p = 0.22$). A statistically significant difference was obtained when comparing the seed-fall collection value in untreated site #2 with the sediment core value from site #1 (Table 1). This difference can be attributed to variability between the sites. However, a comparison of the seed deposition values obtained in the two untreated reference sites from each method reflects an overall homogeneity in seed production in this region of the reservoir.

The results of these experiments suggest two important conclusions. First, a significant amount of new seed production is prevented by the cutting program as evidenced by a reduction in seed production relative to untreated sites. Secondly, the use of sediment cores and seed-fall collectors to measure seed production are potentially effective methods for monitoring the success of waterchestnut management techniques.

Vigor of cut rosette fragments was less than that of whole plants growing *in situ* (Table 2). A decrease in vigor was observed in both leaf size and number, especially by fragments placed in crowded conditions. In addition, the number of buds, flowers and pollinated flowers decreased steadily throughout the experiment and were always less on fragments than those counted on plants *in situ*. Despite this, seed production by the cut rosettes did occur. At the final sampling,

TABLE 2. COMPARISON OF PHENOLOGICAL TRAITS BETWEEN WHOLE PLANTS AND CUT ROSETTE FRAGMENTS. DATA REPRESENT AVERAGES ± 95% CONFIDENCE INTERVALS OF MAXIMUM MIDSUMMER VALUES.

Phenological traits	Whole plants	Upright rosettes	Crowded rosettes
Leaves	35.5 ± 0.8	20.0 ± 1.3	15.0 ± 1.7
Buds	4.0 ± 0.4	1.4 ± 0.3	1.3 ± 0.3
Flowers	0.5 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Pollinated flowers	4.4 ± 0.4	3.5 ± 0.5	2.0 ± 0.4
Seeds	10.2 ± 0.7	3.0 ± 0.5	3.5 ± 0.6

[4]Madsen, J. D. 1990. Waterchestnut (*Trapa natans* L.) Seed Germination: Effects of Temperature and Day length. Fresh Water Institute Report #90-16. Rensselaer Polytechnic Institute, Troy, New York. 12pp.

upright cut rosettes averaged 3.0 ± 0.53 seeds/rosette while crowded cut rosettes averaged 3.5 ± 0.63 seeds/rosette (Figure 1). Rosettes attached to plants examined along transects averaged 10.2 ± 0.74 seeds/rosette (Figure 1).

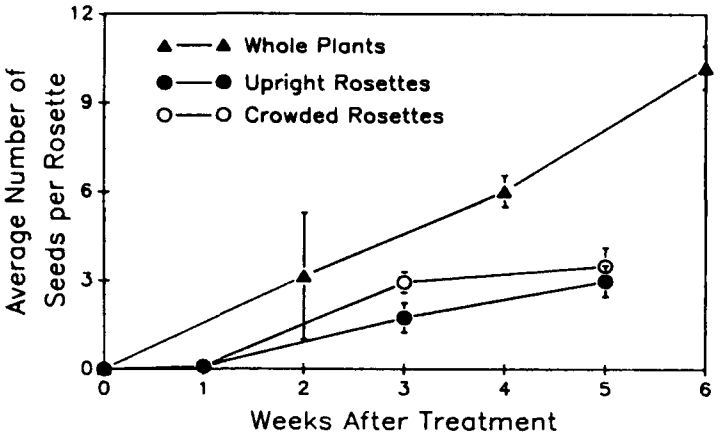

Figure 1. Average number of seeds produced per rosette along transects of uncut plants and cut rosettes arranged in upright and crowded conditions. Values represent the means of 80 repetitions for whole plants and 60 and 40 repetitions from the two cut rosette fragment studies, respectively. Error bars represent ±95% confidence intervals.

As a management practice, these results suggest that cutting minimized, but did not prohibit, seed production in waterchestnut. The final sampling showed the number of buds per fragment rosette was slightly less than one bud per cut rosette versus four buds per rosette of whole plants. Rosette fragments containing buds, flowers, and pollinated flowers present at the time of cutting were capable of continuing seed maturity. However, those rosette fragments without buds after cutting had greater difficulty producing new buds which have the potential for mature seed set. To decrease the number of seed forming components present leading to mature seed production, it would be necessary to cut original growth or regrowth as frequently and aggressively as possible, especially if rosette fragments are not being removed promptly from the water after cutting.

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Vegetation Zones along Watercourses: Inter-Relationships and Implications for Mechanical Control

ROELF POT[1]

ABSTRACT

The effect of mechanical control on vegetation structure and species composition of watercourses was studied. Samples were taken of each vegetation zone in a number of watercourses in The Netherlands for which the management regime was well-documented. In most cases a series of two to five vegetation zones could be distinguished along watercourses. Mechanical control measures take this zonation into account treating each zone differently. Classification of 1365 zone samples, using Braun-Blanquet methods, produced groups of samples which were related to differences in management practices in several ways. Both the type of machinery used and control frequency affected species

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composition in some zones. Analyses of the combinations of zone types for each watercourse by a second order classification showed relationships between these zones. The role of *Glyceria maxima* dominated zones is discussed.

Key words: gradients, classification, aquatic plants, *Glyceria*.

INTRODUCTION

The practice of vegetation control in watercourses in The Netherlands has changed greatly in recent decades. Herbicides have been used for some time but their use in watercourses is now very restricted, and will be banned completely over the next five years. Vegetation growth in 95% of the watercourses in the Netherlands is controlled mechanically. The scale of the maintenance problem has increased because many water drainage systems have been improved to make agriculture more profitable. With the planning of these drainage systems it was assumed that aquatic plants could be controlled effectively by repetitive control measures. However, when aquatic plant growth increased more than was expected, either frequency of control was increased, or more powerful machines were used. As a result there was a shift in the flora to opportunist species which create even more management problems.

One of the difficulties in research on vegetation in watercourses is describing the plant communities. There is a strong gradient on the slopes of the watercourses with species that have a range of environmental demands. The Braun-Blanquet approach of vegetation research (Westhoff and Van der Maarel 1973) is commonly used to describe vegetation types that can be related to environmental factors, but this method needs homogeneous sample plots. Sampling the whole gradient gives no practical description of the vegetation because of the heterogeneity. Mechanical control techniques in watercourses affect vegetation structure and species composition both above and below the water. An adequate description of the bank vegetation as well as the aquatic vegetation is essential for understanding the impact of the control techniques on the whole gradient.

In this study the gradient is split into zones of vegetation that are recognizable through their structure or species dominance. Within each zone, the vegetation is considered to be more or less homogeneous. The zones are analyzed separately and in combination to determine the relationships between mechanical control techniques and the vegetation in watercourses. This paper presents the method of splitting the gradient into zones and gives some results relating to differences in management practices.

METHODS

The watercourses sampled were selected on the basis of previous management practice, soil type and landscape type. They all had a drainage function, but water flow in most of them was very slow (standing or less than 5 cm/sec). Management practice was well-documented and had been constant for at least five years. Extremes in water quality were avoided. The vegetation in the watercourses was divided into zones as shown in Figure 1, but no watercourse was found in which all the zones were present. Between two and five zones could be distinguished consistently in most watercourses. When no separate bank top zone could be distinguished, that part of the profile was considered as a part of the dry bank slope zone. Likewise, the permanently wet ground zone could be part of the emergent plant species zone and the floating-leaved plant species zone could be part of the submerged plant species zone. The width of the zones varied from 0.3 to 5 m. The length of the sampled area was determined by the minimum area to cover all the variance within each zone sample but not including gradients. This length varied from 10 to 50 m. When two banks on opposite sides of the same watercourse were sampled, they were treated separately.

All macrophyte species for each of the zones were recorded. An estimation of their presence was made using the combined cover-abundance scale of Braun-Blanquet, modified by Barkman *et al.* (1964) into a scale with nine ordinal classes. Scales 1-4 concern species with cover less than 5%, the number of individuals per 10 m^2 determines the scale: 1 = 1-2, 2 = 3-20, 3 = 21-100, 4 = more than 100. Scales 5-9 concern species with cover more than 5%: 5 = 5-12%, 6 = 13-25%, 7 = 26-50%, 8 = 51-75%, 9 = 76-100%. All zone sample data were analyzed using TWINSPAN (Hill 1979) to classify the samples into groups. The indicator value of the species was weighted using their cover-abundance in the samples. Classification results were summarized into a synoptic table in which constancy classes and characteristic presence of the species for each group are indicated (Table 1). Constancy classes are: + = species found in 1-5% of the samples in the group, 1 = 6-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, 5 = 81-100%. The characteristic presence of a species is defined as the mean cover-abundance class, considering only the samples in the group in which the species actually was found.

The management techniques were described in terms of machinery and control frequency (number of times per year). These data were compared with the classification groups by calculating their constancy class for each group. Constancy

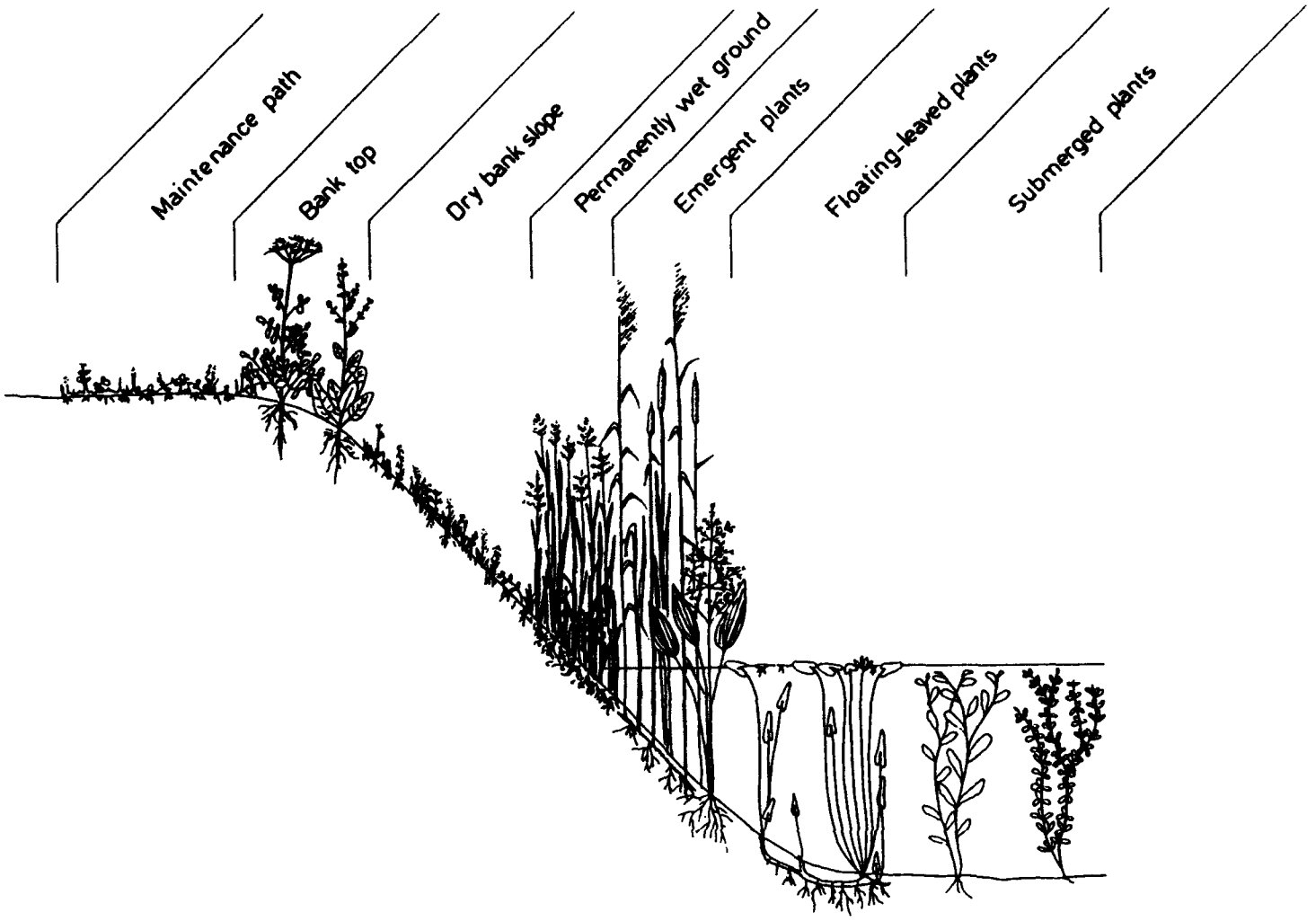

Figure 1. Zonation scheme in watercourses. Only part the zones are found in any particular watercourse.

class is defined in the same way as for species: + = machine applied in 1-5% of the samples in the group, etc.

The results of the classification were used to describe the gradients in the watercourses as a whole. A gradient sample was defined as a combination of the zone samples of a watercourse. For this, the zone samples were encoded as zone types using a symbol for the kind of zone they were taken from and the group they were classified into. For instance, when a watercourse had a bank slope zone which was classified into group 3, an emergent plant species zone which was classified into group 10 and a submerged plant species zone which was classified into group 11, then the gradient sample was defined as a combination of the zone types B-3, E-10 and S-11. The gradient samples were analyzed again with TWINSPAN, resulting in a second order classification in which similar gradient samples are clustered into groups. The results were summarized into a synoptic table in which constancy classes of the zone types for each gradient group are indicated (Table 2). Thus, the gradient groups consist of combinations of zone types that are regularly found to coexist in the same watercourse. In these combinations information can be found on the inter-relationships of the zones by using the information about the zone types from the first classification.

RESULTS AND DISCUSSION

In the primary analyses 1365 zone samples with 450 species were classified into 47 groups. Eleven of these groups were made up of only five samples or less. These latter groups were not considered any further because correlations would lack any significance with so few samples.

In general, the division into groups could be explained to a large extent on the moisture preference of their species (Ellenberg 1979). Groups with samples from dry slopes were at the opposite side in the classification to those groups

TABLE 1. CLASSIFICATION OF ZONE SAMPLES. GROUPS THAT ARE NOT DISCUSSED AND SPECIES WITH NO CONSTANCY HIGHER THAN 40% ARE OMITTED. FIGURES SEPARATED BY A COLON ARE CONSTANCY CLASS AND CHARACTERISTIC PRESENCE. CONSTANCY CLASS OF CONTROL FACTORS AND ZONES INVOLVED ARE PRESENTED AT THE BOTTOM.

Zone Group:	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of zone samples:	41	64	83	16	6	18	29	13	40	6	34	20	24
Mean number of species:	14.6	17.3	18.4	11.3	4.8	3.6	7.1	6.1	8.0	7.2	8.8	8.1	6.3
(grasses)													
Alopecurus geniculatus L.	2:4	+:2	-	-	-	-	-	-	2:5	1:3	-	-	-
Elymus repens (L.) Gould	4:6	2:3	3:5	1:5	-	-	-	-	+:5	-	-	-	+:7
Agrostis stolonifera L.	2:5	5:6	4:6	1:5	-	-	2:3	2:3	2:4	-	2:3	-	1:2
Holcus mollis L.	3:6	1:2	4:6	1:4	-	-	-	-	-	-	-	-	-
Holcus lanatus L.	4:5	5:6	4:5	1:4	-	-	-	-	+:5	-	-	-	-
Lolium perenne L.	4:5	4:5	4:5	3:6	1:4	-	-	-	-	-	-	-	1:4
Poa trivialis L.	4:5	-	+:4	1:4	-	1:2	1:4	-	3:5	-	-	-	-
Alopecurus pratensis L.	+:3	-	+:4	5:6	-	-	-	-	-	-	-	-	-
(ruderals)													
Rumex obtusifolius L.	3:2	1:1	2:2	2:2	-	-	+:1	-	-	-	-	-	-
Ranunculus repens L.	5:3	4:3	5:3	1:4	2:1	-	+:2	-	3:2	-	+:2	-	+:2
Taraxacum officinale s.s. Wiggers	5:3	4:2	4:2	2:4	-	-	-	-	+:2	-	-	-	-
Urtica dioica L.	2:2	3:2	4:3	4:5	-	1:5	+:1	-	+:2	-	-	-	-
Anthriscus sylvestris (L.) Hoffmann	1:1	1:2	1:2	4:4	-	-	-	-	-	-	-	-	+:1
(emergents)													
Phragmites australis (Cavanilles) Steudel	+:1	2:3	1:5	1:4	4:6	1:6	2:4	1:1	+:2	-	1:2	1:3	+:2
Glyceria maxima (Hartman) Holmberg	+:5	2:2	4:3	1:4	5:4	5:6	5:6	5:5	4:5	1:2	2:2	2:3	2:3
Phalaris arundinacea L.	1:4	3:4	4:4	1:2	-	2:4	1:3	3:3	4:4	2:3	1:2	2:2	2:4
Glyceria fluitans (L.) R.Br.	1:3	1:2	1:3	-	2:4	2:3	1:5	1:3	4:6	5:3	2:3	1:3	3:4
(aquatics)													
Callitriche platycarpa Kuetzing	-	+:1	+:2	-	2:3	-	+:3	2:4	3:5	4:7	1:3	1:2	2:3
Elodea nuttallii (Planchon) St.John	-	+:2	-	-	-	-	3:5	1:3	+:2	4:4	5:7	4:5	2:4
Ceratophyllum demersum L.	-	-	-	-	-	-	-	-	-	-	2:3	4:4	1:2
Hydrocharis morsus-ranae L.	-	-	-	-	-	-	2:2	1:3	-	-	2:2	4:3	-
Lemna minor L.	-	-	-	-	-	-	3:4	5:3	1:4	1:4	3:2	5:4	5:5
Spirodela polyrhiza (L.) Schleiden	-	+:1	-	-	-	-	3:4	1:4	+:4	-	3:3	5:4	4:6
(less indicative species)													
Angelica sylvestris L.	+:1	2:2	3:2	-	-	-	-	-	-	-	-	-	-
Epilobium tetragonum L.	-	2:2	3:3	-	-	-	-	-	-	-	-	-	-
Equisetum palustre L.	+:3	-	1:2	2:3	4:4	1:3	+:3	-	2:2	-	+:1	-	-
Festuca ovina L.	-	2:5	2:5	1:5	-	-	-	-	-	-	-	-	-
Festuca rubra L.	2:5	+:2	+:4	2:5	-	1:2	-	-	-	-	-	-	-
Galium aparine L.	-	+:2	1:2	3:4	-	1:6	-	-	-	-	-	-	-
Glechoma hederacea L.	2:3	3:3	3:3	1:3	-	-	-	-	+:3	-	+:4	-	-
Juncus effusus L.	1:3	3:2	2:2	2:3	1:1	1:4	1:3	-	+:3	-	1:3	1:2	-
Lamium purpureum L.	+:2	-	-	3:3	-	-	-	-	-	-	-	-	-
Lemna trisulca L.	-	-	-	-	-	-	1:5	1:5	-	-	1:3	3:3	-
Myosotis palustris (L.) L.	1:3	2:3	2:2	-	-	1:2	2:2	-	3:3	2:2	1:2	1:3	1:2
Potamogeton pusillus L.	-	-	-	-	-	-	1:4	2:3	-	4:4	3:3	1:2	+:3
Ranunculus acris L.	2:2	2:2	1:2	3:3	-	-	-	-	-	-	-	-	-
Ranunculus bulbosus L.	-	-	-	3:3	-	-	+:2	-	-	-	-	-	-
Rumex acetosa L.	3:3	4:3	4:3	1:3	-	-	-	-	+:2	-	-	-	-
Flail cutter	3	-	+	-	-	-	-	1	1	-	-	-	-
Cutter bars with rake	3	+	+	1	-	2	-	-	2	-	+	-	-
Mowing Bucket	1	5	5	5	5	2	5	4	3	4	3	4	3
Cutting boat	-	-	-	1	-	1	-	-	+	1	2	1	2
Control frequency: 1 / yr	+	3	3	-	5	1	1	2	2	4	-	1	-
Control Frequency: 2 / yr	5	3	3	5	-	3	4	3	4	-	3	3	3
Control Frequency: 3 / yr	-	+	+	-	-	-	-	-	-	-	1	-	-
Control Frequency: 4 / yr	-	-	-	-	-	1	+	-	-	-	2	1	1
Control Frequency: 5 / yr	-	-	-	-	-	-	-	-	-	1	-	-	1
sampled in maintance path zone(P)	3	2	1	1	-	-	-	-	-	-	-	-	-
sampled in bank top zone (T)	3	+	+	1	-	-	-	-	-	-	-	-	-
sampled in dry bank slope zone (B)	1	4	5	4	-	-	-	-	-	-	-	-	-
sampled in permanently wet zone (W)	-	+	+	1	-	-	-	-	+	-	-	-	-
sampled in emergent plant species zone (E)	-	-	+	-	5	5	5	4	5	-	1	+	2
sampled in floating-leaved plants zone (F)	-	-	-	-	-	-	+	1	-	4	-	-	2
sampled in submerged plant species zone (S)	-	-	-	-	-	-	+	2	-	2	5	5	2

TABLE 2. CLASSIFICATION OF GRADIENT SAMPLES: CONSTANCY CLASSES OF ZONE SAMPLE TYPES. ONLY ZONE TYPES AND GRADIENT GROUPS THAT ARE RELEVANT TO THE DISCUSSION ARE PRESENTED. FIGURES THAT ARE DISCUSSED IN THE TEXT ARE INDICATED.

Gradient group:	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
Number of gradient samples:	13	1	9	3	44	8	3	4	15	3	1	5	70	71	68	8	3	26	35	50	39	5	4
Zone type:																							
B- 2	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-
B- 3	-	-	-	-	-	-	-	-	-	-	-	-	+	5	-	-	-	-	-	-	-	-	3
B-14	-	-	-	-	-	-	-	-	-	-	-	-	+	-	5	-	-	-	-	-	-	-	-
B-15	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	5	5	-	-	-	-	-
B-16	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	1	-	-	5	-	-	4	-
B- 1	-	-	-	-	-	5	-	-	-	-	-	-	-	+	+	5	-	-	-	-	-	-	-
B-17	-	-	-	-	+	-	5	5	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-
B- 4	-	-	4	-	-	-	-	-	-	-	5	5	-	-	-	-	-	-	-	-	-	-	-
B-18	5	5	2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E- 5	4	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
E- 6	-	-	5	-	-	-	-	-	-	-	-	1	-	1	+	-	-	+	+	+	-	1	-
E- 9	-	-	-	5	4	3	4	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
E-19	-	-	-	-	-	-	-	4	-	-	-	-	-	+	+	-	-	-	+	1	+	-	-
E- 8	-	-	-	-	-	-	-	-	1	-	-	-	+	1	-	-	-	1	1	-	-	-	-
E- 7	-	5	-	-	-	-	-	-	-	5	-	2	1	2	1	-	-	-	1	+	-	-	-
E-20	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	3	-	-	-	+	1	-	-
E-21	-	-	-	-	-	1	-	-	-	-	5	-	+	+	+	-	-	-	1	1	-	-	-
S-10	-	-	2	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
S-22	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
S-23	-	-	-	-	+	2	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
S-11	-	-	-	-	+	-	5	-	-	-	-	2	2	1	1	2	-	+	1	-	-	-	-
S-12	-	-	-	-	-	-	-	-	-	-	-	-	2	1	1	-	-	+	-	-	-	-	-
S-24	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	4	-
S-25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-
S-26	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	1	-	-	-	5
F-13	-	-	3	-	-	-	-	2	-	-	-	-	-	-	-	-	5	-	1	-	+	-	-
F-27	-	-	-	2	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
F-28	-	-	-	-	+	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-

Zone types:
B = Bank slope zone type
E = Emergent plant species zone type
S = Submerged plant species zone type
F = Floating-leaved species zone type
Number: zone sample group in Table 1.

comprising only submerged species. At lower levels the division of several groups could be explained for a large part on the trophic state of the species (Ellenberg 1979). Other low level divisions could be explained mainly on vegetation structure: dominant large species versus low growing species, or submerged species versus floating-leaved or emergent species. In some cases two related groups differed merely for the one having clearly more annual species.

Four types of machines for control of the vegetation were used more often than other machines. The impact of these machines on the vegetation differs. Cutting boats only cut the plants growing in the water with their V-shaped knifes pulled along the bottom. Flail cutters used for maintenance paths and slope zones chop the plant material into very small pieces which decay very rapidly. Most of the machines which operate from the maintenance path and use cutter bars, rake the plants to the bank top or onto the maintenance path. They can reach into the water as far as the emergent zone. Mowing buckets cut the plants in the water and on the slopes and dump the cut material on the maintenance path. Machines that remove the cut plants to another zone cause a shift in trophic state from one zone to the other. Control frequency does not depend on the machinery, but in general cutting boats are used more frequently than the other machines and mowing buckets are not used more than twice a year.

Table 1 shows some of the groups in the classification of zone samples. The choice for these 13 groups out of 47 is based on the possibility to interpret the differences as effects of management techniques, both as zone groups and as part of gradient groups in the second order classification. Species that had no constancy higher than 40% in any of the presented zone groups are omitted. In the second part of the table the constancy classes of the control techniques, of the control frequency, and of the zones in which the samples were taken are presented.

Groups in which *Elodea nuttallii* and *Lemna minor* have a high constancy also have a high constancy for high frequency of control measures: groups 11 and 12 for instance. Groups with ruderal species such as *Urtica diocia, Anthriscus silvestris* and *Rumex obtusifolius* had control techniques that left the cut plants in the zone, or dumped plants from other zones into them. Group 1 is such a group. Approximately half of the 41 samples in this group are from maintenance path zones, the other half from bank top zones. In half of the samples a flail cutter was used and in the other half a cutterbar with rakes was used which transported the cut plants into the bank top zone or onto the maintenance path. Some of the species with constancy class 3 or higher in this group such as *Taraxacum officinale, Rumex obtusifolius, Ranunculus repens, Lolium perenne* and *Elymus repens* are typically known from eutrophic habitats.

There were five groups in which *Glyceria maxima* had a high constancy: groups 5, 6, 7, 8 and 9. Group 6 contains the samples in which *G. maxima* was most dominant; in the other groups other species were also important. Group 5 differs from the others having both a low control frequency and *Phragmites australis* as the next most important species.

In the second order analyses gradient samples were classified into 51 groups. Table 2 shows the groups that are discussed in this paper and other groups that show considerable constancy with the presented zone types.

Gradient groups A to K all have a high constancy for some emergent plant species zone type. They also have high constancy figures for at least one other zone type. Gradient groups D, E, F and G do not differ in emergent plant species zone type, but they do in bank slope zone type. On the other hand, gradient groups G, H, I and J do not differ in bank slope zone type, but they do in emergent plant species zone type. The only explanation for these differences that could be found was that the vegetation on these bank slope zones is more or less independent from the emergent plants species zones.

In gradient group C, a combination of zone type E-6 with zone types B-4 and either S-10 or F-13 was found. Zone type E-6 is the one with a strong dominance of *Glyceria maxima*. Zone type B-4 had both high constancy and characteristic presence of ruderal species such as *Urtica dioica*, *Anthriscus sylvestris*, *Galium aparine* and *Alopecurus pratensis*. Zone type F-13 has samples with a dense cover of *Lemna minor* and *Spirodela polyrhiza*, and can be considered as one of the most ruderal vegetation types in ditches. Zone type S-10 has amphibious species such as *Callitriche platycarpa* and *Glyceria fluitans*. Control frequency is lower in zone type S-10 than in zone type F-13. The conclusion might be that in similar eutrophic watercourses with dense stands of *Glyceria maxima*, there can be either a vegetation dominated by lemnids (duckweeds) or a vegetation with submerged or amphibious plants. Control frequency could explain the difference between the two.

There are indications that there is a relationship between those submerged plant species zone types with fast growing plants such as *Elodea nuttallii*, *Ceratophyllum demersum*, accompanied by floating species such as *Lemna minor* and *Spirodella polyrhiza*, and certain types of other zones. The submerged plant species zone type S-11 in which *E. nuttallii* is the dominant species combines best with the *Glyceria maxima* zone type E-9, the most species rich one (gradient group G). However, in this group there are only three gradient samples. The zone type in which only lemnids (duckweeds) dominate, zone type S-12, combined only poorly with other groups. Types B-2, B-3 and B-14 combine either with type S-12 or with type S-11 (gradient groups M, N and O). These bank slope zone types are dominated by short grasses that are cut once or twice a year, and in more than 80% of the gradient samples. There is no emergent plant species zone in these gradients because the plants are cut too often. From these data it appears that fast growing aquatic plants gain some advantage from the absence of emergent vegetation. This has implications for management practices. Emergent plant species zones can be useful as a natural tool for reducing the growth of the more troublesome submerged plants and duckweeds by competition.

Splitting up the vegetation gradient into zones proves to be a method that makes it possible to analyze interactions between these zones and the management techniques, although most of the relations that are found this way are indicative, and the methods used cannot be statistically tested. The role of the emergent plant species zone is one example of how these data can be interpreted to explain the effects of mechanical control on watercourse vegetation. More detailed information will be produced when more watercourses have been sampled and compared.

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Aquatic Plant Management in Relation to Irish Recreational Fisheries Development

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ABSTRACT

Aquatic plant control programs are often conducted in isolation rather than as part of an integrated habitat management strategy. The impact that this policy has on recreational fisheries in Irish canals is described. The study examined the ecological impact of aquatic weed control procedures employed in the canals and determined fish-holding capacity in relation to a range of aquatic plant species. Fish standing crop estimates were conducted using electrical fishing apparatus in canal habitats which had been subjected to different forms and intensities of weed control. Approximately 200 km of canal was surveyed during the investigation. Canals with

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weed cover values between 20 and 70% generally exhibited fish biomass levels which were between two and four times, respectively, greater than those recorded from densely (>70% cover) or sparsely (<20%) vegetated areas. Submerged plants with broad or complex leaf arrangements or mixed species assemblages had a greater fish harboring capacity than had submerged, strap-leaved or floating-leaved forms. The implications of these findings for aquatic plant management in canal fisheries are discussed.

Key words: Canals, weed control, habitat management, angling.

INTRODUCTION

Irish canals are unique aquatic ecosystems that serve a multitude of recreational pursuits including angling, navigation, canoeing, swimming, walking and nature study. Angling is the largest single recreational activity on these canals and the numbers of participants are increasing annually. Coarse fish, including pike (*Esox lucius* L.), perch (*Perca fluviatilis* L.) and cyprinid species, are the principal angling quarry in the canals.

The canals under study (Figure 1) are man-made and were originally designed specifically for boat traffic. They vary between 13 and 20 m in width, have a shallow (1.6 to 1.8 m) uniform configuration and are relatively unshaded over much of their length. Flow velocities in the canals are low, ranging between 0 and 10 c sec^{-1}. Boat traffic intensity is generally low, causing the minimum of in-channel disturbance. Water supply to the canals is provided by feeder rivers, which are largely unpolluted (Caffrey and Cooney 1992). Water quality in the three canal systems under study (Royal, Grand and Barrow) is good, with mean total phosphorus levels rarely exceeding 35 $\mu g\ l^{-1}$. While nutrient levels in the canal water are relatively low, a nutrient-rich sediment throughout the system provides suitable conditions for the proliferation of macrophytes (Caffrey 1991a).

Studies conducted in Ireland and elsewhere demonstrate that waterbodies rich in vegetation generally support productive fisheries (Whitcomb 1968, Northcott 1979, Kelsall 1981, Murphy and Eaton 1981, Wiley *et al.* 1984, Durocher *et al.* 1984, de Nie 1987, Caffrey 1986, 1990a). This reflects the cover, direct and indirect food supply and spawning substrates that macrophyte provide for fish and invertebrate species (Wright *et al.* 1992), although the influence that water fertility has on fish standing crop must also be considered (Hoyer *et al.* 1985; Hoyer and Canfield 1991). The composition or architecture of the vegetation has also been shown to influence fish stock levels, through its affect on macroinvertebrate and periphyton supply (Reynolds and Eaton 1983, den Hartog and van der Velde 1988) and by interfering with the fishes

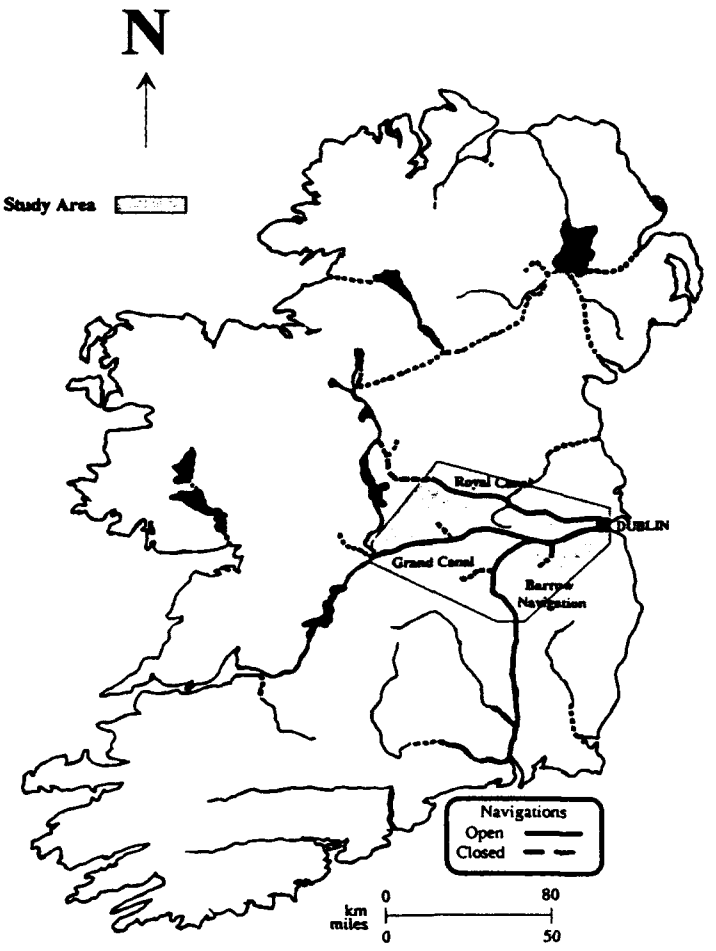

Figure 1. Map of the major inland waterways of Ireland, the study area and the watercourses which are open and closed to navigation.

freedom of movement (Bouquet 1978, Hannon 1992, Canfield and Hoyer 1992).

Prior to the introduction of the Canals Act (1986), canal management procedures focused on maintaining open, relatively weed-free channels where unobstructed boat movement would be facilitated. Since 1986, when the Office of Public Works assumed responsibility for the canals, the primacy focus has been on the development and management of these watercourses as multipurpose user resources, with a particular emphasis on optimizing their recreational angling potential.

The objectives of this paper are to demonstrate the role that aquatic plants play in recreational canal fisheries development and to determine weed management practices that will maximize the potential of this valuable resource.

MATERIALS AND METHODS

Field studies on approximately 200 km of the Royal, Grand and Barrow Canals (Figure 1) were conducted between

1987 and 1991. Fish standing crop estimates, in kilograms per hectare (kg ha^{-1}), were assessed using electrical fishing apparatus in all of the canal habitats studied. Electrical fishing operations were normally conducted between May and October each year. Fifty-seven canal sections, ranging in length between 0.5 and 5 km, were electrofished during this period. Sections were generally contiguous and, during the electrofishing operation, were separated by fine-meshed (5-mm quad) stop nets. The majority of standing crop estimates were obtained using the Zippin (1958) multiple removal technique, although the Seber and Le Cren (1967) mark-and-recapture method was occasionally employed.

At each section details relating to the habitat, and particularly the macrophyte community structure and level of abundance, were recorded. The percentage cover of predominating species and growth forms, defined as the percentage surface area of canal bed covered by the vertical projection of the aquatic plants (Best 1981), as determined by eye, by the author having walked and boated each section. In some sections dry weights (g m^{-2}) of the vegetation were determined, although these are not presented in this paper.

For the purposes of establishing relationships between fish standing crops and abundance of aquatic vegetation, the canal sections investigated were grouped into three broad categories, based on their aquatic plant regimes: densely (>70%), moderately (70 to 20%) and sparsely (<20%) vegetated. The number of sections included in the densely, moderately and sparsely vegetated sections was 17, 24 and 16, respectively.

To investigate the effect that the growth form of the predominating vegetation had on fish standing crop, sections supporting specific growth-form categories (*e.g.* submerged with broad/complex leaves, submerged with strap-shaped/streamlined leaves, floating leaved, mixed categories and filamentous algae) were examined.

RESULTS

The main fish species collected in the canals were bream (*Abramis brama* L.), roach (*Rutilus rutilus* L.), rudd (*Scardinius erythrophthalmus* L.), cyprinid hybrids, tench (*Tinca tinca* L.), carp (*Cyprinus carpio* L.), pike (*Esox lucius* L.), perch (*Perca fluviatilis* L.) and eels (*Anguilla anguilla* L.). Most prolific among these were bream and roach, although the remaining species did present significant standing crops in certain canal sections.

Standing crop estimates for combined fish species from densely, moderately and sparsely weeded canal sections are presented in Table 1. Mean crop estimates from moderately weeded sections, supporting 20% to 70% plant cover, were approximately 2.5 times higher than those recorded from densely weeded canals (>70% cover) and four times higher than those recorded from sparsely weeded areas (<20% cover).

TABLE 1. STANDING CROP ESTIMATES (kg ha^{-1}) FOR FISH IN CANALS WHICH SUPPORT DIFFERENT LEVELS OF AQUATIC PLANT GROWTH. DENSE (>70%) PLANT COVER, MODERATE (20 to 70%) PLANT COVER AND SPARSE (<20%) PLANT COVER.

Weed category	Number of sections	Fish crop estimates (kg ha^{-1})		
		Mean ± S.E.	Minimum	Maximum
Dense	17	123.8 ± 28	11	390
Moderate	24	327.8 ± 45	116	825
Sparse	16	82.3 ± 14	5	644

Most of the 16 sparsely vegetated sections that were electrically fished, totaling approximately 30 km of canal, supported between 30 and 82 kg ha^{-1} of fish. One section, however, supported a standing crop of 644 kg ha^{-1}. This atypically high value, comprising mainly bream, was recorded in a section where overhanging deciduous trees cast a heavy shade on the water. As a consequence, little light penetrated to the canal bed and few aquatic plants were present. Adjacent canal sections were unshaded and generally supported abundant and diverse macrophyte communities. It is probable that the school of bream recorded from this sparsely vegetated section were intercepted while moving from one vegetated area to another. Such feeding migrations among bream schools are well documented (Backiel and Zawisza 1968, Goldspink 1978, Whelan 1983, Connolly *et al.* 1991).

Of the 17 densely weeded (>70% plant cover) sections examined, totaling approximately 40 km of canal, only four supported fish crop values in excess of 200 kg ha^{-1}. In two of these areas, each supporting about 90% plant cover, tench was the dominant fish species. These are relatively sedentary, bottom-loving species that thrive in weeded channels (Kennedy and Fitzmaurice 1970).

The densely weeded canal section that supported the highest fish crop value (390 kg ha^{-1}) was dominated with filamentous green algae (mainly *Vaucheria* sp.), stoneworts (*Chara* spp.) and fennel pondweed (*Potamogeton pectinatus* L.). Adult bream, up to 2 kg in weight, dominated the fish community during this early-June sampling occasion. Bream of this size are uncommon in Irish canals and it is probable that these fish had migrated for spawning purposes into this section from the River Barrow, only 0.5 km distant. Local information suggests that large schools of river bream migrate to the canal in May and return, after spawning, in mid to late June each year. The fact that poor angling catches were

reported from this canal section in July and August would support these observations.

Ten of the 17 densely weeded sections examined supported fish standing crops less than 100 kg ha^{-1} and represented poor recreational fisheries. Most supported mixed fish communities although pike and perch, both piscivorous species, were well represented.

In moderately vegetated canal sections, occupying approximately 130 km of canal, the lowest recorded fish standing crop estimate was 116 kg ha^{-1}. Sixteen of the 24 sections electrically fished supported standing crops greater than 200 kg ha^{-1} and six of these sections yielded crops in excess of 400 kg ha^{-1}.

Different growth-form categories among aquatic plants support very different mean fish standing crops (Table 2). The highest mean crop estimate (451.3 kg ha^{-1}) was recorded from canal sections where submerged plants with broad leaves, *e.g.* broad-leaved pondweeds (*Potamogeton* spp.), or with complex leaf types, *e.g.* milfoil (*Myriophyllum* spp.), charophytes, and hornwort (*Ceratophyllum demersum* L.), predominated.

TABLE 2. STANDING CROP ESTIMATES (kg ha^{-1}) FOR FISH IN CANALS RELATIVE TO THE GROWTH FORM OF THE AQUATIC VEGETATION PRESENT.

Growth forms	Number of sections	Fish crop estimates (kg ha^{-1})		
		Mean ± S.E.	Minimum	Maximum
Submerged (broad/complex leaf)	7	451.3 ± 116.1	60	825
Mixed	30	201.5 ± 33.3	5	800
Filamentous algae	5	148.2 ± 57.4	25	305
Submerged (strap-shaped/streamlined leaf)	10	120.4 + 33.8	30	335
Floating leaved	5	30.5 + 19.5	11	50

A mean standing crop estimate of 201.5 kg ha^{-1} was recorded from canal sections where vegetation exhibiting a mixture of growth forms was present. Thirty such sections were electrically fished and 13 of these supported standing crops greater than 200 kg ha^{-1}. A further 11 sections supported standing crops of less than 100 kg ha^{-1}. This is unlikely to result from sampling error as every effort was made to standardize crews and equipment during these electrical fishing operations. The variation in results probably reflects the wide range of plant species and growth forms included in this category and suggests that further examination, perhaps at plant species level, might yield more definitive results.

A mean fish standing crop estimate of 148.2 kg ha^{-1} was recorded from the five sections examined where algae were the main species. This figure possibly under-represents the value of algae in canal fisheries since two of the sections were totally overgrown with algae and were virtually uninhabitable by fish. This reflects the large diurnal fluctuations in dissolved oxygen concentrations recorded during the summer months at these sites (C. Monahan personal communication). These sections supported only 25 and 35 kg ha^{-1} of fish. The remaining three sections supported 116, 260 and 305 kg ha^{-1}, respectively, of mixed fish species.

Most of the submerged strap-shaped or streamlined plants present in Irish canals, *e.g.* unbranched bur-reed (*Sparganium emersum* Rehm.), clubrush (*Scirpus lacustris* L.) and arrowhead (*Sagittaria sagittifolia* L.), are resistant to the activity of dichlobenil and commonly establish dense vegetation stands in dichlobenil-treated areas (Caffrey and Monahan 1991). The mean standing crop value for this growth-form category was 120.4 kg ha^{-1}, which contained two high estimates (298 and 335 kg ha^{-1}). The remainder of the sections supported values well below that regarded as providing good sport angling.

In the areas where floating-leaved species predominated, poor stocks were recorded from beneath the dense canopy layer (Table 2).

DISCUSSION

Studies conducted in North American lakes and streams have shown a positive correlation between trophic status, as measured by total phosphorus concentrations, and fish standing crops (Hoyer *et al.* 1985, Hoyer and Canfield 1991). Thus, eutrophic watercourses generally support larger fish crops than oligotrophic habitats. The Irish canals under study may be regarded as mesotrophic and there is little variation in nutrient levels either within or between the three systems (Caffrey and Cooney 1992). It is therefore reasonable to suggest that factors other than water quality or nutrient status of the water are primarily responsible for the variations in fish standing crops recorded in these canals. Among these factors, the aquatic plant regime must be considered an important determinant.

In British canals the level of aquatic plant growth is closely correlated with boat traffic intensity (Murphy and Eaton 1981). Where boat traffic is heavy few plants are present and where little or no traffic is recorded an abundant flora prevails. In Irish canals boat traffic intensity is light and is not sufficient to preclude aquatic plant growth. In these canals the vegetation regime is governed principally by weed control programs.

The levels of aquatic plant growth recorded during this investigation largely reflect past weed control practices. The indiscriminate use of the aquatic herbicides dichlobenil in the

canals prior to the implementation of the Canals Act (1986) probably accounted for the poor floral regimes present in most sparsely vegetated canal sections. Many of these were sprayed each year, whether plants impeded boat traffic or not. This total removal of susceptible plants facilitated the unrestricted movement of boats but also significantly reduced fish habitats, and consequently stocks, within these channels.

Fisheries research conducted on English rivers (Linfield 1981) and on Irish canals (Connolly *et al.* 1991) revealed that watercourses which support 200 kg ha^{-1} or greater of coarse fish generally represent good sport fisheries. It is, therefore, clear that good angling may be expected in moderately vegetated canals, while poorer catches may be achieved in densely or sparsely vegetated channels (cf. Table 1).

Dense and obstructive vegetation was recorded in channels where species resistant to the activity of dichlobenil predominated and in many of the sections where mechanical weed control was operated. An examination of the reaction of aquatic plant species to mechanical cutting demonstrated that the rate of regrowth among cut plants is generally rapid and may exceed that recorded among uncut plants (Caffrey 1990b, 1991b, Caffrey and Monahan 1991). If navigation channels must be maintained, therefore, it is often necessary to apply two or even three cuts in each growing season.

While densely weeded channels supported higher mean fish standing crops than sparsely vegetated sections (*cf.* Table 1), these habitats did not present ideal conditions for coarse fish. This reflects the adverse effect that large diurnal fluctuations in dissolved oxygen concentrations, commonly recorded in densely vegetated watercourses (Simpson and Eaton 1986), can have on fish populations in these areas. It might further reflect the restriction on free movement among open water fish species, *e.g.* roach, rudd, pike and perch, imposed by dense vegetation (Bouquet 1978, Canfield and Hoyer 1992).

The difference in fish standing crop levels recorded from the five growth-form categories of vegetation examined (cf. Table 2) probably relates to the architecture or spatial arrangement of the plant species involved. Submerged plants with broad or dissected leaves provide abundant cover and concealment for adult fish and nursery habitats for fry. They also provide a wide range of microhabitats for fish-food invertebrates and periphyton, and spawning substrates for fish and invertebrates. Submerged plants with narrow strap-shaped leaves offer fewer habitats and provide reduced cover for fish. Furthermore, while coarse fish will deposit their adhesive egg masses on most plant species, they exhibit a preference for submerged species with broad or complex physical forms (Kennedy and Fitzmaurice 1968, 1970, 1974).

Floating-leaved plants offer minimal cover, direct or indirect food supply or spawning substrates for fish or invertebrates. This is reflected in the low mean fish standing crop recorded among this vegetation type (*cf.* Table 2). Filamentous algae are not noted for the cover they provide to fish but they commonly harbor large numbers of invertebrates. This probably explains the good fish crops recorded in sections where moderate growths of algae occupied the channel.

In addition to plant form, another feature that probably influenced the fish standing crops recorded was the seasonality of the vegetation. This reflects the availability of plant cover and an invertebrate food supply for fish throughout the winter and spring (Reynolds and Eaton 1983). A number of aquatic plants species initiate their growth cycle relatively late in spring and die-down completely in autumn, passing the colder months as rhizomes, tubers, turions or other overwintering organs (Caffrey 1990a). Canal sections where these short-lived species dominate the flora are practically devoid of vegetation between October and March each year and, therefore, provide relatively poor habitats for fish. Murphy and Eaton (1981) observed that fish normally resident in canals dominated by short-lived plant species moved out of these sections in winter in search of weeded channels. No such movements were observed in canals where plant populations overwintered. It is noteworthy that the more common short-lived species present in Irish canals are the principal representatives of the two growth-form categories which supported the lowest fish crops: Unbranched Bur-reed, clubrush and arrowhead (submerged with strap-shaped or streamlined leaves) and broad-leaved pondweed (floating leaved).

Plants that maintain vegetation stands for most of the year provide almost continuous cover and food supply, thus ensuring favorable year-round conditions for fish. The more important plant species that exhibit this long-lived pattern include marestail (*Hippuris vulgaris* L.), Eurasian milfoil (*Myriophyllum spicatum* L.) and charophytes, all included in the submerged, broad or complex leaf growth-form category. This helps explain the high standing crop figures for fish recorded from canal sections supporting this vegetation type.

IMPLICATIONS FOR AQUATIC PLANT MANAGEMENT

Plant management programs operating in recreational fisheries should work to achieve partial rather than total weed control. The results from this study indicate that at least 20% aquatic plant cover should remain following weed treatment. Partial control may be achieved using mechanical means or using herbicides that are specifically formulated for selective control, *e.g.* granules or gels.

Where vegetation that includes a variety of growth forms occupies a channel, those that support low fish densities should be specifically targeted for treatment. This is possible

with current advances in mechanical cutting apparatus and herbicide formulations.

Weed control operations in channels dominated by submerged plants with strap-shaped or streamlined leaves, by floating-leaved plants or by species that die-down completely in winter should aim to treat this vegetation severely, thereby leaving niches available for colonization by plant species with greater fish-harboring capacities.

Water managers should keep abreast of advances in weed management technology to avail themselves of new, more efficient and cost-effective control procedures.

Integrated weed control programs, using a broad combination of techniques, generally provide more efficient, cost-effective and environmentally sensitive plant management (Mitchell 1986, Murphy *et al.* 1987, Caffrey 1990b, 1991b).

Reed fringes should be preserved since they play an important role in bank side stabilization.

Weed control operations should be suspended during and immediately after fish spawning. A knowledge of fish community structure in these watercourses will allow the manager to determine when operations can safely resume.

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Capture of Grass Carp from Vegetated Lakes

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ABSTRACT

Seven techniques were evaluated for catching grass carp (*Ctenopharyngodon idella* Val.) in five Washington lakes containing aquatic vegetation. The capture methods included angling, pop-nets, lift nets, or traps in baited areas; angling in nonbaited areas; heating the water in small areas to attract fish; and herding fish into a concentrated area and removing them with gill nets or seines. Herding was the most effective of the techniques ($P < 0.001$), followed by angling in baited areas. Herding removed 0.8% to 8.2% of the original grass carp stock in one sweep in lakes containing thick vegetation, submerged logs and other underwater obstructions. This technique may be effective for reducing numbers of fish in small (<10 ha) overstocked waters, or for capturing fish to monitor growth.

Key words: white amur, fish capture methods, angling, herding.

INTRODUCTION

Grass carp have been used for aquatic plant control in the United States for over 25 years (Allen and Wattendorf 1987). However, information about the growth and mortality of grass carp stocked in natural lakes is hard to obtain because of the difficulties associated with capturing the fish, which has also hindered removal of grass carp from overstocked lakes.

Several studies investigated methods to capture grass carp. Conventional fish capture techniques such as fyke, gill and trammel nets, and electroshocking were used to catch small numbers of fish, but were not effective for removing large populations (Cumming *et al.* 1975, Shireman and Maceina 1983, Hestand *et. al.* 1987). Grass carp are sensitive to various piscicides (Marking 1972, Henderson 1974, Cumming *et al.* 1975) and were selectively removed from certain fish populations (Cumming *et al.* 1975, Colle *et al.* 1978). Angling was used to reduce grass carp numbers in lakes denuded of vegetation (Terrell and Fox 1974, Hestand *et al.* 1987) and in lakes containing less preferred plants (Buckley and Stott 1977). Haul seining was effective in capturing some grass carp in Florida lakes where vegetation was largely eliminated (Shireman and Maceina 1983, Hestand *et al.* 1987).

These methods were only moderately successful, and even the most efficient cannot be used in all situations. Use of piscicides can be restricted, publicly unacceptable, or impractical in certain waters. Success of angling in lakes containing preferred plants species remains unproven, and active netting techniques such as haul seining are not suited for lakes containing substantial stands of aquatic vegetation, submerged obstructions, or variable basin morphometry.

We tested several new methods to capture grass carp in lakes containing significant amounts of aquatic vegetation and underwater debris, as well as other methods which were reported as successful in the literature, from telephone surveys, and on a worldwide questionnaire (Bonar 1990). A major objective of the experiment was to identify methods which could capture significant numbers of grass carp in lakes containing palatable aquatic vegetation, so the fish could be

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removed before all valuable plant communities were eliminated. Further objectives of the study were to identify which of these methods would result in the highest catch-per-unit-effort (CPUE), and identify those techniques which would allow grass carp to be removed alive from natural lakes.

MATERIALS AND METHODS

Seven techniques for catching grass carp in vegetated lakes were evaluated in 1991. Four Washington lakes (Keevies, East Pipeline, Bull South, Shincke Road) stocked with 20- to 30-cm (total length) triploid grass carp in 1987, and one lake (Big Chambers) stocked with 20- to 30-cm triploid grass carp in 1990 were used in the experiment. The lakes ranged in size from 0.8 to 24.3 ha (Table 1), in maximum depth from 2.0 to 4.0 m, and were approximately round in shape, with the exception of Big Chambers which was long and narrow. Techniques were first tested in Keevies and Big Chambers Lakes, which received the highest stocking rates, to determine if the methods were successful. Techniques that successfully captured grass carp in one of these two lakes were then tested in the other lakes.

TABLE 1. SIZE OF LAKES (ha), ORIGINAL STOCKING RATES, MEAN DAILY AIR TEMPERATURE (C) DURING HERDING, AND PERCENT OF ORIGINAL STOCKED POPULATION REMOVED WITH ONE PASS USING HERDING.

Lake	Size	No. Fish	Temp.	Percent
Keevies	2.9	1,595	13	0.8
Big Chambers	24.3	12,622	10	0.4
Bull South	0.8	183	21	8.2
Shincke Road	13.2	395	—	—
E. Pipeline	0.9	103	15	6.8

We first attempted to attract grass carp with a thermal plume. This method was based on studies which showed fish can be attracted to warm effluent when ambient water temperature falls below their preferred temperature (Neill and Magnuson 1974, Reynolds 1977, Cincotta *et al.* 1982). Six 3-kw titanium bayonet heaters powered by a 20-kw generator were suspended on a 1.6-m^2 floating platform. A 28-m^2 pop-net was submerged and placed on the lake bottom underneath the platform to capture any fish attracted to the heater. The heater was operated in late spring so the ambient water temperature of the lake (15 to 19C) would be lower than that preferred by grass carp (25.3C, Bettoli *et al.* 1985). After the heaters were started, grass carp activity over the net was monitored visually from shore at 1-hr intervals both day and night. Continuous hydroaccoustical monitoring of the site was also conducted using a 107-kHZ transducer connected to a Simrad HE-203 chart recorder.

Next, bait was used to attract fish over the unheated submerged pop-net. This study was to evaluate previous trials by Schramm and Jirka (1986) who reported that grass carp were readily attracted to unobstructed baited areas. Thirteen different baits were presented to grass carp at field sites and in 4270-L laboratory tanks. These baits were: lettuce (*Lactuca sativa*), white bread, dry cat food, hominy (*Zea mays*), cabbage (*Brassica oleracea*), saltine crackers, sunflower sprouts (*Helianthus annuus*), cheese curls, alfalfa hay (*Medicago sativa*), spinach (*Spinacia oleracea*), alfalfa cubes and blocks, soybean sprouts (*Glycine max*), and dried bread cubes. Observation of baits selected first in these initial trials identified those to be used in the subsequent capture experiment. Preferred baits were concentrated in a 1.1-m^2 bamboo frame over the pop-net and also spread over a larger area to attract fish to the site. After fish were seen taking the bait, the frame was filled with bait at dawn and dusk to train grass carp to feed at those times. The net was popped from a blind on shore to capture fish after they became accustomed to feeding at the site.

Bait was also used to attract grass carp to two types of traps. In the weir trap (Figure 1), both a leader and a 1.1-m^2 bamboo frame inside the trap were baited and the trap was checked twice daily for captured grass carp. The L-trap consisted of an area enclosed by an "L" shaped poultry wire fence with one open side (Figure 2). A net curtain, operated from a blind on shore, could be pulled across the open side to catch fish attracted to bait scattered in the trap.

Angling was attempted using nine baits and two artificial lures which were: biscuit doughballs, bread, catfish power bait (cheese flavor), cheese marshmallows, cheese curls, cornbread dough, garlic marshmallows, hominy, iceberg lettuce, crappie jigs, and artificial minnow lures. Both tank and field observations were used to determine which baits were most effective, and comparisons also were made between sites previously chummed with bait and sites not chummed. Angling was conducted from a boat in a wide range of vegetated and nonvegetated habitats at various times of the day.

A literature and telephone survey were conducted to identify methods used to capture grass carp in their native range in Eastern Asia. We then used techniques that are commonly used throughout the region. The first was a 28-m^2 lift-net attached to a counterweighted lever mounted on a 5.2-m boat. The net was suspended just below the water surface, baited, and lifted to capture fish swimming over the net to feed.

Herding techniques have assisted substantially in the harvest of Chinese carp in China (Dela Cruz 1980, Lu 1986). We modified these techniques for use in our study lakes. First, fish were frightened starting at one end of the lake using

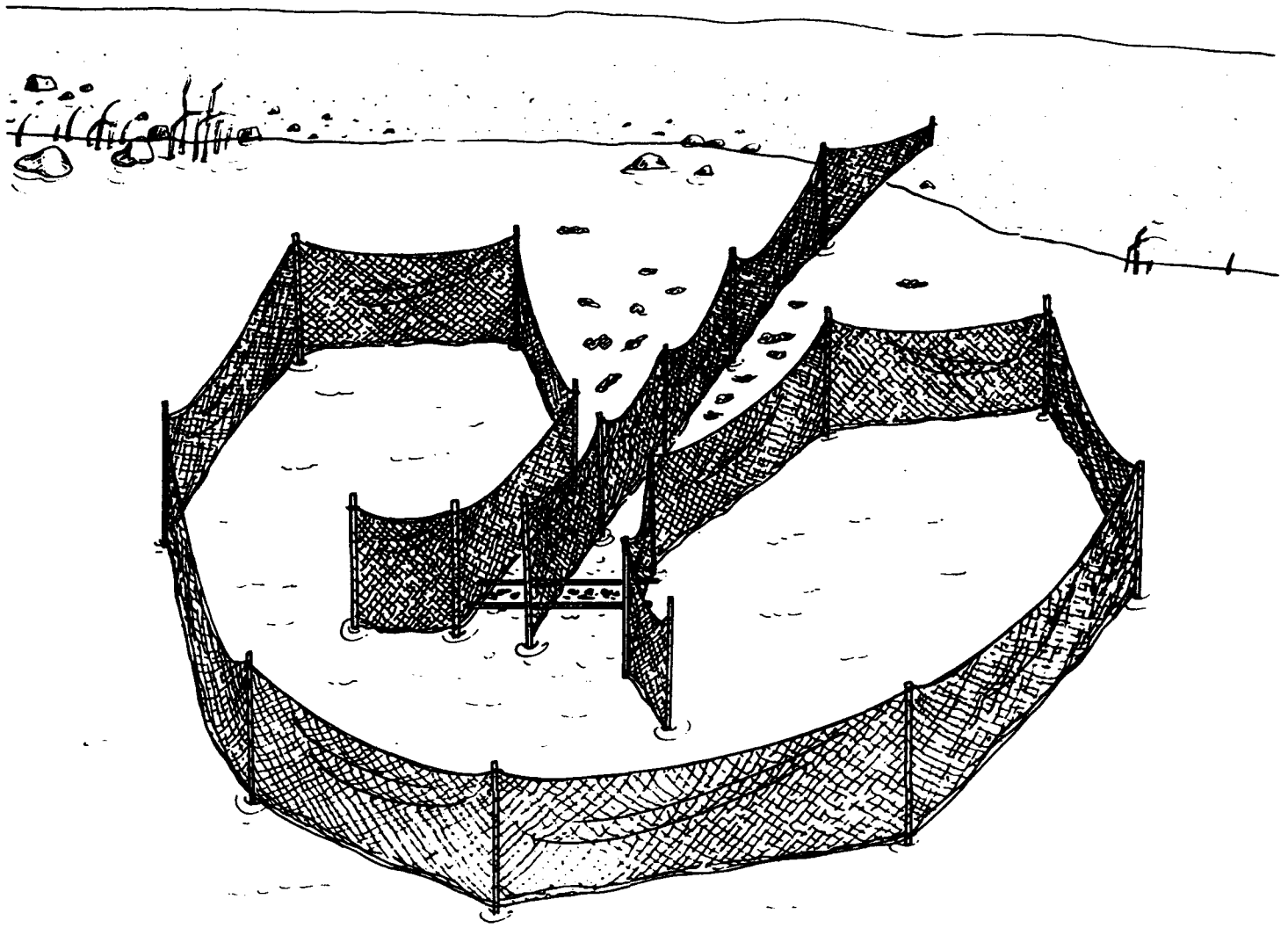

Figure 1. Diagram of the weir trap showing bamboo frame holding bait at the mouth of the trap.

various noisemakers including boat motors, plungers, scarelines (Brant 1984), boat paddles or human movement through the water (Figure 3). A 200- to 300-m long monofilament gill net, with 11.4 to 15.2-cm stretch mesh panels that reached from the lake's surface to the sediment, was used to cordon off the area after a reasonable amount of noise had been made. Barrier nets of 3.8-cm stretch nylon mesh were also used for the same purpose in some of the lakes. Noise was then made along the front of this net, again scaring the fish from the area. Another net was set out in front of the previously placed net. This process was repeated until fish were concentrated at the far end of the lake (Figure 3). Here, the monofilament gill net was elevated approximately 1 m by metal tripods floating on tire tubes to prevent the retreat of the fish by jumping back over the net (Figure 3). A combination of seining and herding the fish back into the gill net was used to capture fish. Only one pass was made through each lake using this technique. Fish were herded across the entire lake in all lakes except Big Chambers, where the method was used only in one large cove. Unlike the other techniques, this method was first attempted in Bull South Lake to determine its practicality because of the logistical problems of testing it initially in the larger Big Chambers or Keevies lakes. The method was not tested in Shincke Road Pond.

Nonparametric randomized block procedures, blocked by lake, were used to test the null hypothesis that the CPUE of the various capture methods were similar ($\alpha = 0.05$).

Effort (man-hours) for active techniques was defined as the time a crew fished multiplied by the number of people in the crew, while for passive techniques, the time required to check the trap multiplied by the number of people required to check it was used. Soak time, the amount of time a passive trap was fished, was also recorded.

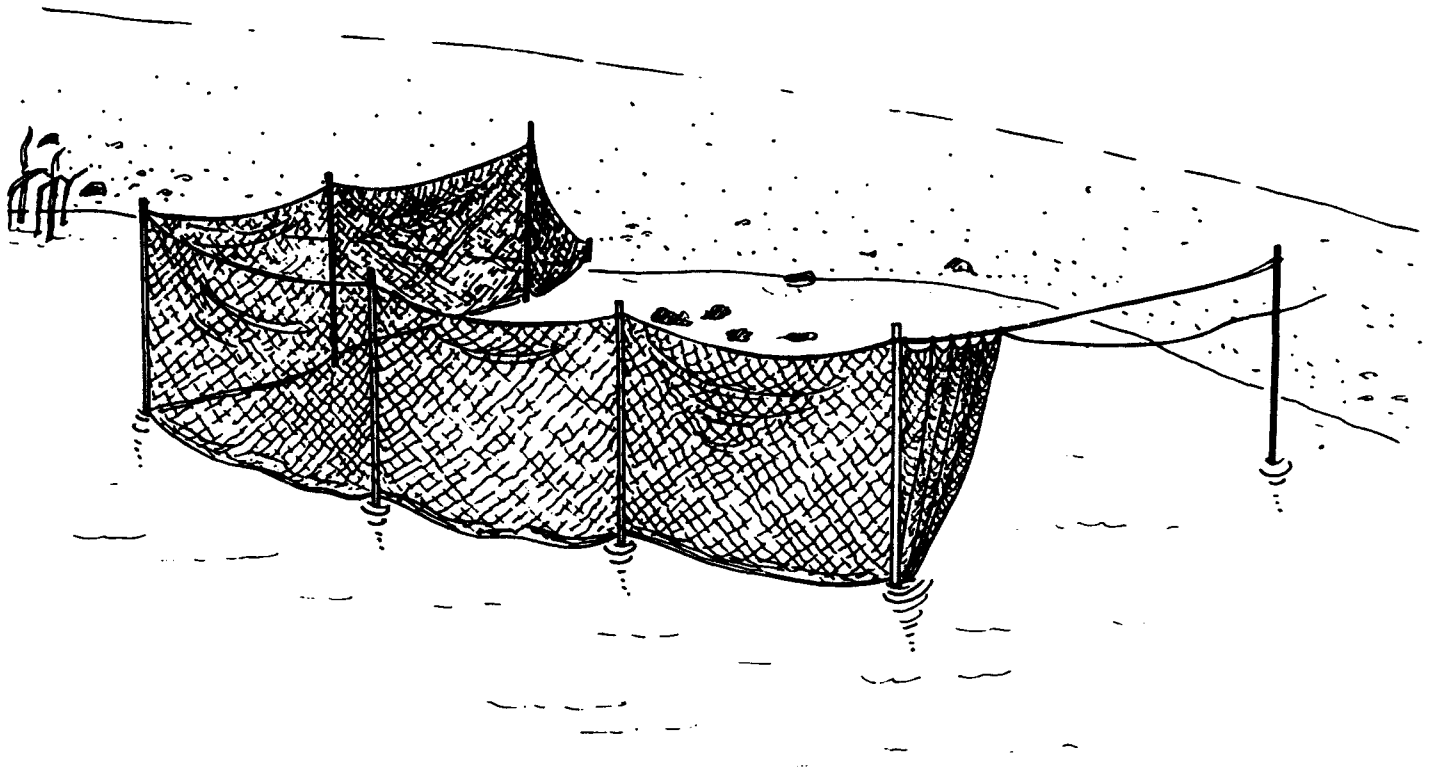

Figure 2. Diagram of the L-trap showing fenced portion of the trap and net curtain.

RESULTS AND DISCUSSION

Based upon CPUE, herding was the most effective method for catching grass carp, followed by angling ($P < 0.001$, Table 2). Time required to herd grass carp ranged from a single 6- to 10-hr day in smaller lakes (East Pipeline and Bull South) to 3 to 4 days in larger lakes (Keevies and Big Chambers).

Herding CPUE's (0.17 to 0.56 fish/man-hour) were comparable to CPUE's of the most effective methods in other studies. Ten grass carp were caught using a baited cage over a 34-day period in an agricultural canal (Schramm and Jirka 1986). Seventy-seven grass carp were removed in a 4-hr selective rotenone application in Florida with 72 personnel used in the operation, resulting in a CPUE of 0.26 fish/man-hour (Colle *et al.* 1978). Haul seining removed between 12 and 65 fish during each of six 6-hr hauls on a Florida lake (Hestand *et al.* 1987). Considering five to six fishermen were needed for each haul, CPUE ranged from 0.36 to 2.0 fish/man-hour. Different standing crops of grass carp, water temperatures, basin morphometry, and density of underwater obstructions in the various sites allow only the most general comparison of these CPUE's.

Several modifications may improve the success of herding. Only one pass was made on each lake during this study and multiple passes could result in an increase in the total number of fish caught. Herding was conducted in the fall when water temperatures were low compared to those preferred by grass carp (Bettoli *et al.* 1985). No water temperature data were taken during herding, and more points were needed to establish a significant correlation. However CPUE increased with mean daily air temperature (Tables 1 and 2). When temperatures were low, fish moved sluggishly, and would often not be scared out of an area toward the far end of the lake. When temperatures were warmer, fish moved quickly and were herded successfully across the lake. Herding fish at mid-summer when water temperatures are highest may improve CPUE.

In small lakes, such as Bull South and East Pipeline, a higher proportion of the original stock of grass carp was removed than in larger lakes such as Keevies and Big Chambers (Table 1). Not including the initial costs for nets (approximately $1,000 to $3,000) and assuming labor costs of $8.00 per person per hour and a constant CPUE, herding costs would range from $1,471 to remove 50% of the original stock of fish from East Pipeline Lake to $90,157 to remove

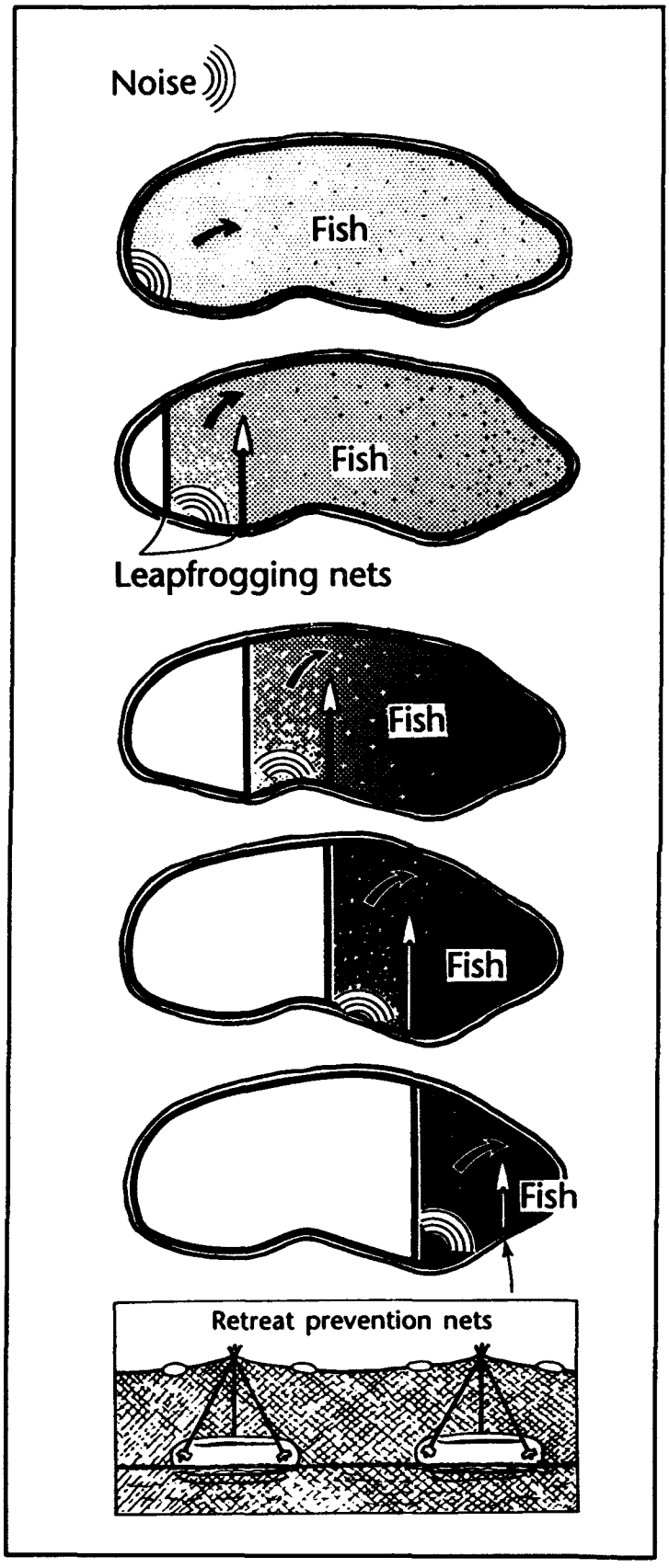

Figure 3. Diagram of herding technique and retreat prevention nets (insert)

TABLE 2. CATCH RATES OF GRASS CARP FROM KEEVIES LAKE (KV), BIG CHAMBERS LAKE (CH), BULL SOUTH LAKE (BS), EAST PIPELINE LAKE (EP), AND SHINCKE ROAD POND (SR), WASHINGTON, USING DIFFERENT CAPTURE METHODS.

Lake	Method	Effort (man-hr)	Fish caught	CPUE (fish/ man-hr)
KV	Herding	78.3	13	0.17
	Angling, baited area	65.3	7	0.09
	Thermal plume	31.4	0	0.00
	Weir	12.0 (193.3)[1]	0	0.00
	Baited pop-net	24.3	0	0.00
CH	Herding	79.8	45	0.56
	Angling, baited area	25.2	7	0.14
	Angling, no baited area	72.1	0	0.00
	Lift-net	27.2	0	0.00
	L-trap	15.8	0	0.00
BS	Herding	65.3	15	0.23
	Angling, baited area	68.5	0	0.00
EP	Herding	24.7	7	0.28
	Angling, baited area	4.0	0	0.00
SR	Angling, baited area	16.8	0	0.00
	L-trap	11.4	0	0.00

[1]Soak time (hr).

the same proportion from Chambers Lake. Herding techniques need to be improved to be cost effective for large-scale removal in larger lakes. In mainland China, herding has substantially increased fish recovery from larger lakes (Dela Cruz 1980). Fishermen harvested 500,000 kg of various Chinese carp species in one operation from a 1,466-ha lake stocked originally with 3,000 fingerlings (>15 cm) per hectare. However, 1 to 2 dozen motor boats, 32 pieces of 2,000-m-long gill nets and at least 70 people were used in the proceedings, which lasted from 25 to 30 days (Dela Cruz 1980). Such resources would probably not be available for many grass carp removal projects in the United States. Simply making more than one pass on a lake will likely capture fish that escaped the first time. Placing nets closer together in larger lakes would result in smaller areas from which to herd the grass carp, but this would substantially increase the amount of time to complete the process. Developing better methods to drive grass carp may be a more effective means of raising CPUE. Schramm and Jirka (1986) reported that grass carp were driven by electroshocking more effectively than by surface disturbances. Qi-Wen (1990) reported manpower requirements were reduced 45% and man-days reduced 60% to 85% by using an electric driving system as opposed to traditional net-driving systems. Use of electroshock

systems to drive fish may lower the number of fish returning through the source of disturbance, and decrease the amount of manpower needed to create effective disturbances.

The retreat prevention devices elevated the net and blocked escape of several fish which tried to jump back over the net. However, a slight wind would capsize them, and they were difficult to place in the net. Some other simpler device to elevate the net when fish are crowded could lower the effort required for herding.

Angling was considerably less successful than herding. The most effective angling technique was to attract fish to an area baited with iceberg lettuce (Lactuca sativa L.), and fish using lettuce tied to a #8 hook. Of all of the baits tried in the experiments, iceberg lettuce was the most effective. No fish were caught with bread, but it was successfully used to attract fish into an area. Other baits were not successful in either respect.

Greater than 9-kg test line was most effective for catching the fish, which ranged in size from 0.7 to 6.8 kg. Lighter line frequently broke, and of a total of 14 fish hooked in the experiments, 8 were lost because of line failure.

Greatest angling success was obtained in the evening or on calm days. It was difficult using the method on windy days because the bait used to attract the fish would be blown out of the area, and the feeding activity of the grass carp could not be easily observed.

In most previous studies where angling was reportedly successful, there was a lack of preferred plants in the lake. CPUE's ranged from 0.04 to 1.56 fish/man-hour in ponds devoid of vegetation (Terrell and Fox 1974, Hestand *et al.* 1987). In a northern British lake containing stands of less-preferred *Myriophyllum spicatum* L. and *Ranunculus cicinatus*, CPUE was 0.13 to 0.35 fish/man-hour (Buckley and Stott 1977). Fish that did not have anything to eat may have been readily attracted into baited areas and captured, which is consistent with the findings of our study where grass carp were attracted to bait in Keevies and Big Chambers Lakes which contained only less-preferred plants such as *Nuphar* sp., *Nymphaea* sp., *Brasenia schreberi* Gmel., and *Typha* sp. (Bonar 1990).

Bull South Lake, East Pipeline Lake and Shincke Road Pond contained preferred plants such as *Elodea canadensis* (Planch.) Casp., *Potamogeton pectinatus* L. and other thin-leaved pondweeds (Bonar 1990). Grass carp were not attracted to baits at these sites, probably because of the abundance of preferred foods. Wilson and Cottrell (1979) reported an angling CPUE of only 0.0046 fish/man-hour in a pond that contained sufficient amounts of algae and rooted vegetation to provide an adequate food supply for the grass carp. However, they did not describe the species of plants present. Their baits were earthworms, artificial minnow lures, spinners, and aquatic and terrestrial vegetation. While some of their baits may not have been effective, their results and ours suggest that grass carp may be effectively angled or attracted to baited areas only in lakes containing less-preferred plants or devoid of aquatic vegetation.

The baited pop-net, the weir, the L-trap, and the lift net did not catch any fish. Small groups of three to five fish were attracted to baits over the pop-net and into the L-trap. However, the grass carp were able to escape by jumping over the sides of the pop-net after it was popped, or between the net curtain and the fencing of the L-trap as the curtain was being pulled across the opening. A roller-net was installed on the pop-net to cover the top when the net was popped, but fish were able to find gaps along the side of the roller-net to escape. Fish fed around the edges of the lift net, but could not be induced to swim over it. Fish fed on bait placed in the leader of the weir, but did not go into the trap.

The heating unit did not create a thermal plume sufficient to attract fish. With all heaters working, water temperatures were only raised a fraction of a degree next to the heating units. Further calculations revealed that a prohibitive power requirement would be necessary to create an effective thermal plume.

This study did not investigate the efficiency of electroshocking to capture grass carp. However, electroshocking was used on the lakes in the spring of 1990 and 1991 to survey all fish species. No grass carp were caught in Keevies, East Pipeline or Shincke Road Pond, but a CPUE of 1.52 fish/man-hour was obtained in Big Chambers. Hestand *et al.* (1987) reported catch rates of 5.0 to 7.0 fish/man-hour using electroshocking, but expressed doubt about its feasibility for removing large numbers of fish. Their reasons were the substantial manpower requirements of this method coupled with a reduced catch rate of continued electroshocking due to gear avoidance and a diminishing population. The majority of respondents from a worldwide survey that included questions on grass carp capture methodology rated electroshocking as "poor" for capturing grass carp (Bonar 1990). Electroshocking is effective as a survey tool, has potential as a herding device, and may be effective for reducing grass carp populations in some lakes. However, use of this technique for large-scale removal of grass carp is apparently limited.

We conclude that herding and angling are effective for removing small numbers of grass carp. Herding, with further modifications, might be successful for removing large numbers of fish, especially from small (<10 ha) lakes and ponds. Herding would be effective in shallow lakes containing any combination of plant species. It would be most effective in narrow lakes, or those containing coves that could be cordoned off easily. Herding would probably not work well in deep lakes where nets would not reach to the bottom, or large, round lakes where areas could not be cordoned off.

Although angling did not result in the capture of many fish, there are advantages to using the technique when conditions are favorable. It requires less gear than any of the other techniques, and could easily be used by lakefront homeowners or the general public to aid in the removal of fish. The most effective terminal gear we found to catch fish is simple, and could be used quite easily at low cost. A disadvantage to this technique would be that anglers may move captured grass carp to unstocked lakes.

It is unlikely that modifying the pop-net, L-trap, weir, or lift net to capture the fish attracted to them would result in CPUE higher than that associated with herding or angling. Since only small numbers of fish were over or in the netted areas at any one time, they could probably be removed as effectively by angling as by the more gear-intensive techniques. Additionally, once the pop-net was popped or the net curtain pulled on the L-trap, fish would not return to the site for several days. These baited traps also would not work well in lakes containing preferred plant species.

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Hydrilla Response to Mariner[1] Applied to Lakes[2]

K. A. LANGELAND[3]

ABSTRACT

Mariner, which contains the active ingredient bensulfuron methyl, was applied at different times and concentrations to four lakes in Florida to determine its effect on hydrilla growth and reproduction. In one lake, four sequential applications every 44 days of sufficient Mariner to result in 25 ppb bensulfuron methyl resulted in no observable above-sediment hydrilla biomass 1 yr after application of the product. Hydrilla was also eliminated 1 yr after application from another lake treated once with 100 ppb bensulfuron methyl, and lake volume occupied by hydrilla remained less than 5% of pretreatment levels in this lake 2 yr after application. In a third lake, 40-ppb bensulfulron methyl eliminated hydrilla biomass for the period of a growing season. In the fourth lake application of 25 ppb bensulfuron methyl resulted in a small reduction in biomass, and vigorous growth was evident 11 months after application. Mariner was applied to this lake again to target 50 ppb bensulfuron methyl, and hydrilla biomass approached zero 1 yr following the second application. Hydrilla tuber density was reduced in all lakes where Mariner was applied and tuber density was measured. However, even after large reductions in tuber numbers, high tuber density (up to 300/sq m) remained in the hydrosoil of two lakes and tubers were not eliminated from any of the lakes.

Key words: bensulfuron methyl, biomass, Florida, tubers, growth, aquatic weed control.

INTRODUCTION

Mariner, which contains the active ingredient bensulfuron methyl (methyl2-[[[[[(4,6-dimethoxy-2-pyrimidinyl]amino] carbonyl]amino]solfonyl]methyl]benzoate), was registered for experimental use in aquatic sites by E. I. du Pont de Nemours & Co., Inc. from 1989 through 1991. Several laboratory studies have demonstrated that hydrilla (*Hydrilla verticillata* (L.f.) Royle) growth is sensitive to this compound. Growth was reduced by exposure to 1 ppb, and 50 ppb resulted in 60% reduction in growth (Anderson and Dechoretz 1988). Van and Vandiver (1992) exposed hydrilla to 50, 100, and 200 ppb bensulfuron methyl for 4 weeks and reduced dry weight accrual by 90% after 2 months with all concentrations. Langeland and Laroche (1992) observed cessation of growth when hydrilla was exposed to 200 ppb for 192 hr. Inability of hydrilla to produce tubers has also been observed under laboratory conditions (Anderson 1988, Van and Vandiver 1992, Haller *et al.* 1992, Langeland and Laroche 1992) when the plants were exposed to bensulfuron methyl.

In a field study, where bensulfuron methyl was applied to the bottoms of dewatered irrigation channels, Bowmer *et al.* (1992) observed fair control of elodea (*Elodea canadensis* Rich.) and moderate control of ribbonweed (*Vallisneria gigantea* Graebner), which are closely related to hydrilla.

The purpose of this study was to determine the response of hydrilla growth and tuber production to application of Mariner in operational settings in lakes. This information is necessary to develop use patterns for the product if it is registered for aquatic use.

MATERIALS AND METHODS

Sufficient Mariner was applied to Johnny's Lake (Brevard Co., FL) to result in 25 ppb bensulfuron methyl (all concentrations are nominal) on May 17, June 28, August 8, and October 10, 1990. The lake was 2.2 ha in surface area and 5 m in average depth at all application times except October when the average depth was 4 m. Total alkalinity was 104 ppm as $CaCO_3$ and pH was 8.0. Hydrilla presence in the water column was recorded routinely with a recording fathometer (Raytheon DE-719). Five biomass samples were collected with the "Waterways Experiment Station's Hydraulically Operated Submersed Aquatic Plant Sampler" (Sabol 1984), along each of five transects, prior to the first Mariner application and 1, 2, 5, 7, 12, 14, and 16 months after application. Biomass samples were returned, on ice, to Gainesville, FL, where they were dried to constant weight in a forced air drying oven and weighed. Tuber density was not measured in this lake because of the hard rocky bottom and depth.

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[1]Mariner is a registered trade name of E. I. du Pont de Nemours & Co., Inc.

[2]Published with the approval of the Florida Agricultural Experiment Station as J. Series No. R-02816. This material is based upon research supported in part by IFAS/ARS cooperative agreement No. 58-43YX-9-001. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture. Mention of trade names is not intended to recommend the use of one product over another.

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Sufficient Mariner was applied to Catfish Lake (Pasco Co., FL) on July 10, 1990, to result in 100 ppb bensulfuron methyl. The lake was 10.4 ha in surface area and 2.3 m in average depth at the time of application. Total alkalinity was 31 ppm as $CaCO_3$ and pH was 7.4. Hydrilla presence in the water column was measured routinely with a recording fathometer (Raytheon DE-719). Tuber density was measured routinely by collecting 15 samples in each of three locations within the lake with an 81-cm^2-diameter core sampler similar to that described by Sutton (1982) and compositing the samples within each sample site.

Sufficient Mariner was applied to Palmer Ranch Lake (Sarasota Co., FL) to result in 25 ppb bensulfuron methyl on August 30, 1990 and 50 ppb on August 13, 1991. The lake was 3.4 ha in surface area and 2.8 m in average depth at the time of the first herbicide application and 3.5 m in depth at the time of the second application. Total alkalinity and pH were 108 ppm as $CaCO_3$ and 8.2, respectively. Hydrilla presence in the water column was measured with a recording fathometer (Raytheon DE-719). Hydrilla biomass was measured by hand collecting all hydrilla from within four 0.24-sq-m quadrants at each of three locations in the lake, and determining dry weight. Tuber density was determined as previously described.

Sufficient Mariner was applied to Lake Wastena (Pasco Co., FL) on August 29, 1991, to result in a bensulfuron methyl concentration of 40 ppb. At the time of application, the lake was 10 ha in surface area and average water depth was 3.7 m. Rains occurred during the week following application that increased water depth by approximately 0.7 m. Total alkalinity and pH were 2 ppm as $CaCO_3$ and 6.2, respectively. Hydrilla presence in the water column was measured with a recording fathometer (Raytheon DE-719). Twenty-six biomass samples were collected with the "Waterways Experiment Station's Hydraulically Operated Submersed Aquatic Plant Sampler" (Sabol 1984) along two transects and dry weights and tuber density were determined.

All Mariner was applied in 187-L water/ha (20 gal/acre) with 3.7-m long hoses trailed from the bow of an airboat.

Biomass and tuber density were averaged over all samples collected in each lake, at each collection time. Hydrilla response to Mariner applications is presented as the regression of biomass or tuber density as the dependent variable and time after application as the independent variable. Lake volume occupied by hydrilla was determined in Catfish Lake as the proportion of dots that contacted hydrilla images on fathometer tracings (Maceina and Shireman 1980) compared to the number of dots occupied by the entire cross section of the tracing, using an Eros Data Center (March 1977) Area Dot Grid (100 dots/sq in.). Hydrilla response in Catfish Lake is presented as the regression of percent lake volume occupied by hydrilla as the dependent variable and time after herbicide application as the independent variable.

RESULTS

Presence of hydrilla in the water column of Johnny's Lake declined 3 months after the initial Mariner application (two 25-ppb applications had been made by this time), and occupied less than the bottom 3 ft of the water column 6 months after application (four 25-ppb applications had been made by this time), as determined by recording fathometer tracings (data not presented). Hydrilla biomass had increased slightly 2 months following the initial 25-ppb Mariner application and then began to decline approximately 1 month later (Figure 1). Biomass then declined rapidly and no observable biomass was present after 1 yr. Sprouting hydrilla tubers were observed 16 months after application, which suggests that the herbicide was no longer impacting hydrilla growth in the lake.

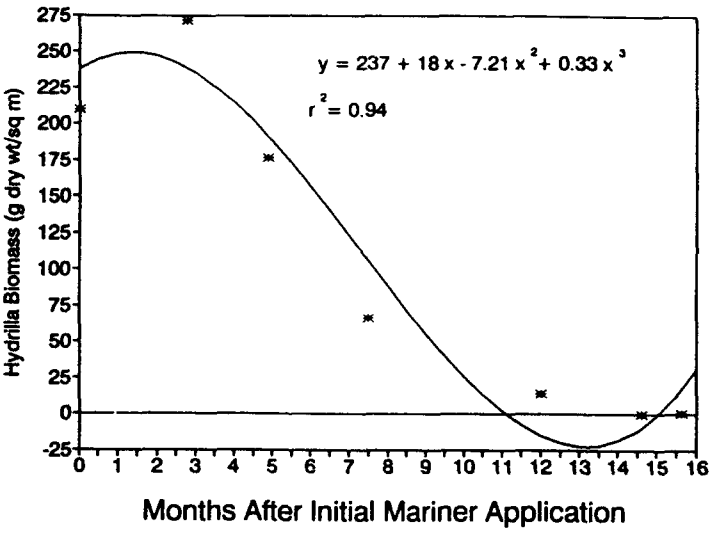

Figure 1. Hydrilla biomass in Johnny's Lake after four sequential applications, every 44 days (May , June, August, October 1990), of sufficient Mariner to result in 25 ppb bensulfuron methyl each (each asterisk represents an average of 25 observed values).

Lake volume occupied by hydrilla in Catfish Lake declined rapidly after Mariner application and was unmeasurable less than 1 yr after application (Figure 2). Almost 2 yr after application, presence remained very low, although sprouting hydrilla tubers could be observed along the lake margin. Tuber density in Catfish Lake decreased from over 200/sq m at the time of Mariner application to approximately 25/sq m 28 months after application (Figure 2).

Hydrilla was reduced to less than 1 ft in height, as determined by fathometer tracings, 8 months after application of 25 ppb bensulfuron methyl (August 1990) to Palmer Ranch

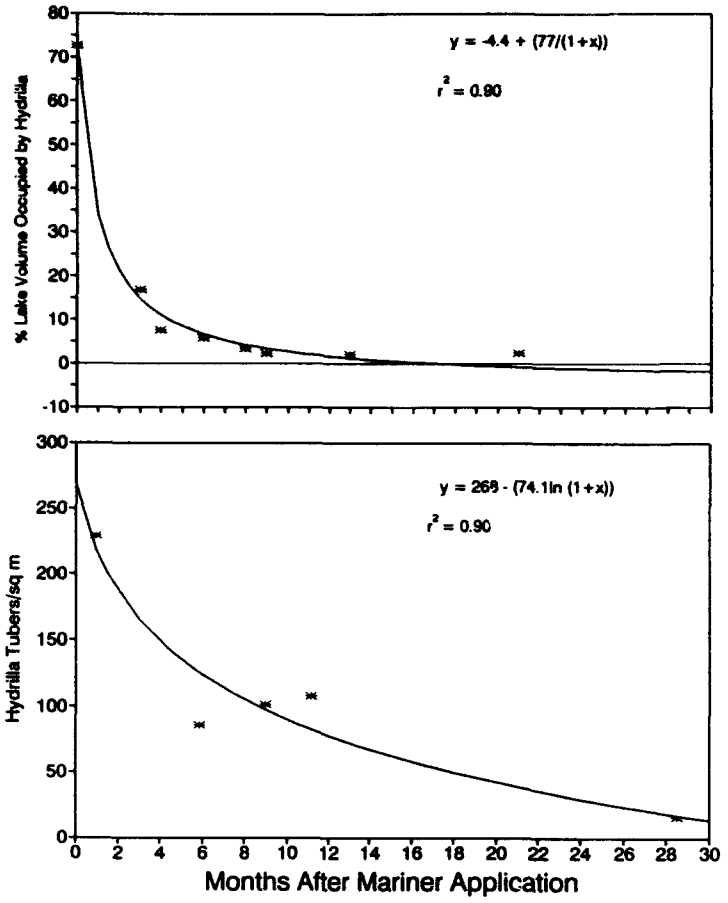

Figure 2. Lake volume occupied by hydrilla and tuber density in Catfish Lake after application of sufficient Mariner to result in 100 ppb bensulfuron methyl, in July 1990 (each asterisk represents an average of five observed biomass values or 3 tubers/sq m values).

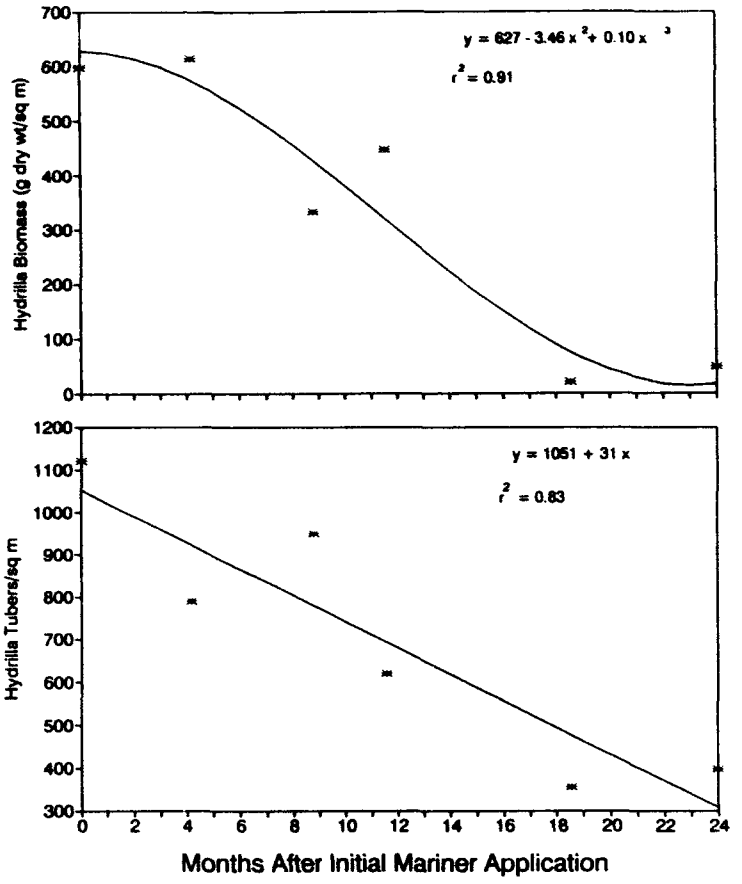

Figure 3. Hydrilla biomass and tuber density in Palmer Ranch Lake after application of sufficient Mariner to result in 25 ppb bensulfuron methyl, in August 1990, followed by 50 ppb 1 yr later (each asterisk represents an average of 12 observed biomass values or 3 tubers/sq m values).

Lake (data not presented). However, 11 months after application, hydrilla was actively regrowing. By 1-1/2 months after 50 ppb bensulfuron methyl was applied (11 months after the initial application) hydrilla had again fallen to the bottom. Biomass declined slowly during the 8 months following the initial application (Figure 3). Biomass then appeared to increase 1 yr following application, as indicated by the observed average biomass shown in Figure 3. However, application of 50 ppb in August 1991 resulted in continued decline, with biomass approaching zero by 2 yr after the initial Mariner application (Figure 3). Tuber density decreased linearly from 1,000 to 400 per sq m following Mariner applications in Palmer Ranch Lake.

Hydrilla in Lake Wastena was falling to the bottom, as it had in the other lakes of this study, by 53 days after application of 40 ppb bensulfuron methyl. Hydrilla biomass and tuber density decreased linearly during the year following Mariner application (Figure 4). Hydrilla was only recently introduced into this lake; therefore, biomass and tuber density were initially low compared to the other lakes in the study. One year following application, hydrilla biomass had declined to an unobservable level and tuber density to less than 10/sq m.

DISCUSSION

In Palmer Ranch Lake, 25 ppb bensulfuron methyl was not sufficient to control hydrilla but appeared to act as a growth regulator. Hydrilla was not killed but biomass was reduced and the decrease in tuber density suggests that tubers were not formed during that growing season. Therefore, Mariner may have potential use for hydrilla growth regulation. All applications of 40 ppb or greater (sequential applications in Johnny's Lake) resulted in at least season-long control and up to 2 yr of control. All hydrilla tissue exposed to the treated lake water appeared to die. Therefore, Mariner also has potential as a herbicide for hydrilla control.

The herbicidal affect of Mariner, when applied at bensulfuron methyl concentrations of 40 to 100 ppb, is somewhat unexpected based upon previous laboratory studies because hydrilla did not die when Langeland and Laroche

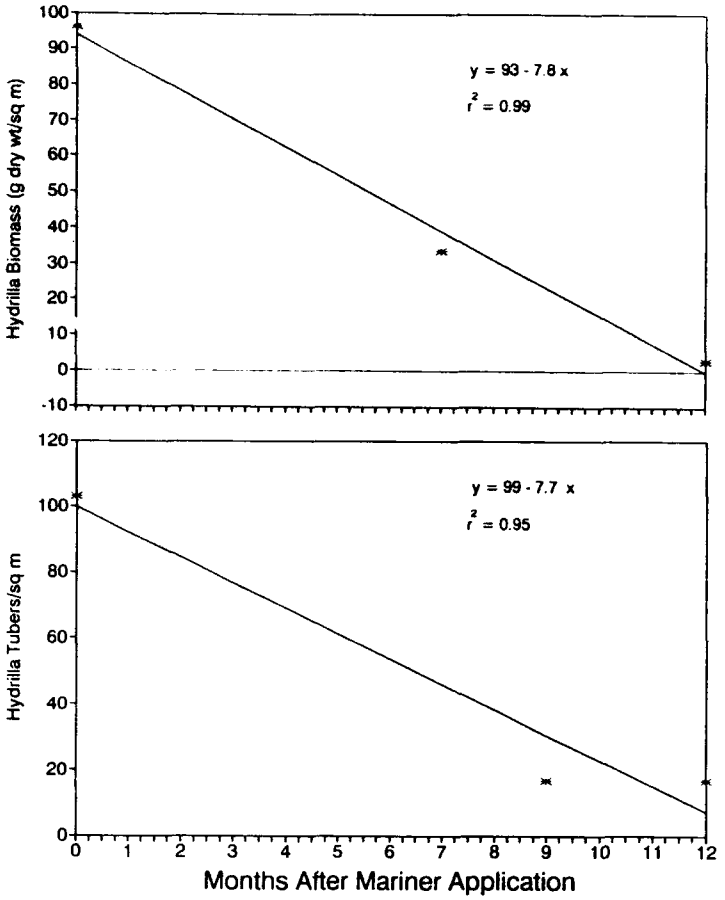

Figure 4. Hydrilla biomass and tuber density in Lake Wastena after application of sufficient Mariner to result in 40 ppb bensulfuron methyl in August 1991 (each asterisk represents an average of 26 observed biomass values or 3 tubers/sq m values).

(1992) exposed plants to concentrations up to 100 ppb for up to 192 hr or when Van and Vandiver (1992) exposed plants to concentrations up to 200 ppb for 4 weeks. In these studies, plants renewed growth after initial growth reduction. A possible explanation for the difference is that whole lake application to these enclosed lakes (little water exchange) resulted in longer exposure to low bensulfuron methyl concentrations, relative to the laboratory studies. The importance of exposure time suggests that special techniques may be necessary for the compound to be effective in flowing water or for partial application in large lakes.

Inhibition of hydrilla tuber production and subsequent decrease in tuber density in these lakes following Mariner application suggest the potential for use of the product to reduce the year-to-year regrowth potential of hydrilla. Sequential applications of Mariner could be used for this purpose, or Mariner could be applied sequentially with other herbicides. However, the large numbers of tubers that were present, even after large reductions, suggests that elimination of hydrilla tubers from lakes will be a long-term process. Van and Steward (1990) demonstrated that 4 years were necessary to deplete monoecious hydrilla tuber populations under experimental conditions due to "environmentally-imposed forced dormancy." Therefore (assuming similar longevity of dioecious hydrilla tubers), several annual sequential applications to inhibit tuber production would be necessary to eliminate potential hydrilla regrowth from tubers in lakes.

ACKNOWLEDGMENTS

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Bensulfuron Methyl Activity on Eurasian Watermilfoil[1]

L. S. NELSON, M. D. NETHERLAND AND K. D. GETSINGER[2]

ABSTRACT

The efficacy of bensulfuron methyl (BSM) at 40 concentration and exposure time combinations was evaluated on Eurasian watermilfoil (*Myriophyllum spicatum* L.) under controlled-environment conditions. BSM concentrations ranged from 0 to 4600 µg/l; exposure times ranged from 7 to 42 days. Efficacy was based on shoot and root biomass harvested at the conclusion of each experiment. Herbicide injury was evident 1 week after application on all treatments, and symptoms included leaf chlorosis, deformed leaves on shoot tips, downward bending of leaves at upper nodes, stem necrosis, and formation of axillary buds. Following 7-, 14-, 21-, 28-, 35-, and 42-day exposure periods at concentrations ranging from 10 to 4600 µg/l, biomass was reduced 10 to 90% compared to untreated plants. Increasing exposure time was more efficacious than increasing concentration. BSM concentrations <10 µg/l did not significantly reduce growth. Effects on roots were variable depending on concentration and plant age. Only treatments of 230 µg/l and higher inhibited root growth on plants grown for 3 weeks prior to treatment. Regrowth emerged from rootcrowns, axillary buds, and injured shoot apices, and was evident 1 to 2 weeks following completion of the exposure period and removal of the BSM-treated water. Plant death was not achieved at the concentrations and exposure times tested in these studies.

Key words: herbicide, sulfonylurea, exposure time, *Myriophyllum spicatum* L.

INTRODUCTION

As nuisance aquatic plant infestations continue to increase throughout the United States, so does the need for developing additional management tools, such as new herbicides and plant growth regulators. Bensulfuron methyl (methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl] methyl]benzoate) is currently registered as Londax® herbicide for use in rice production. However, recent studies have demonstrated efficacy on several aquatic plant species including hydrilla (*Hydrilla verticillata* Royle), Eurasian watermilfoil, and several species of *Potamogeton* (Anderson 1988, Haller *et al.* 1992, Van and Vandiver 1992). Large-scale evaluations for use in aquatic systems have been conducted under an Experimental Use Permit issued by the U.S. Environmental Protection Agency (Getsinger *et al.* 1992b, Langeland 1992, Pringle and Sisneros 1992).

Bensulfuron methyl (BSM) is a member of the sulfonylurea herbicide group which is characterized by high levels of activity at application rates as low as 0.002 kg/ha (0.03 oz/acre). Plant uptake of BSM occurs readily through roots and foliage, and once inside the plant, is translocated via the xylem and phloem. The mode of action of BSM is inhibition of the plant enzyme acetolactate synthase (ALS), which is necessary for the synthesis of two essential amino acids, valine and isoleucine (Beyer *et al.* 1988). Visual symptoms of plant injury (chlorosis, leaf bending and curling, leaf discoloration due to enhanced anthocyanin production, and necrosis) usually appear within 1 to 2 days posttreatment (Du Pont 1988). Although growth cessation immediately follows treatment with BSM, plant death occurs gradually as plants utilize and eventually deplete internal carbohydrate reserves.

The fact that BSM affects a plant enzyme system that is nonexistent in animals helps explain its low toxicity to nontarget organisms. Although the ALS enzyme is present in all plants, not all species are highly susceptible indicating some degree of selectivity to BSM. Tolerant plants (*e.g.*, rice), can quickly metabolize the active ingredient to herbicidally inactive compounds, whereas susceptible species cannot. In addition, the range in sensitivity to BSM among plants is wide. Beyer *et al.* (1988) reported that the differential sensitivity of plants to sulfonylurea herbicides can be over 1000-fold. As a potentially selective herbicide, BSM could benefit management strategies in which the objective of the treatment is to control a target species with minimal harm to desirable native species. Furthermore, sulfonylureas exhibit growth-regulating effects on plants when applied at sublethal concentrations. The potential benefits of growth regulation versus the complete removal of plant biomass (as with a herbicide) in an aquatic system have been suggested by several researchers (Anderson 1988, Klaine 1988, Lembi and Netherland 1990).

Understanding the relationship between rate of application and length of time a chemical is in contact with a target plant species is also important to achieve desired plant control. This is especially critical in systems where water flow and thermal- and wind-induced circulation patterns influence

[1]Portions of this manuscript have been previously published in U.S. Government project reports.

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herbicide dispersion and, consequently, treatment performance (Getsinger *et al.* 1990 and Fox *et al.* 1991). Concentration/exposure time (CET) relationships have been described for several aquatic herbicides and can be helpful in predicting treatment success under field conditions (Hall *et al.* 1984, Van and Conant 1988, Green and Westerdahl 1990, Netherland *et al.* 1991, Netherland and Getsinger 1992).

To date, most of the BSM research conducted on aquatic plants has focused on hydrilla. Several investigators have observed reduced shoot growth and tuber formation of hydrilla following treatment with BSM (Anderson 1988, Haller *et al.* 1992, Van and Vandiver 1992). Reduced hydrilla reproduction by germinating tubers and turions was also observed under field conditions (Haller *et al.* 1992).

Investigations concerning the effectiveness of BSM on Eurasian watermilfoil (hereafter referred to as milfoil) are limited. Therefore, the objective of the following studies was to determine the effects of selected concentrations and exposure times of BSM on the growth of milfoil.

MATERIALS AND METHODS

Three separate experiments were conducted in two similar laboratory systems at the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS. The system used for Studies 1 and 2 consisted of twenty-four, 55-L aquaria (0.75 m tall by 0.09 m^2) located in a controlled-environment room. Overhead lighting was provided by a combination of 400-W mercury vapor lamps and 250-W high-pressure sodium lamps. The mean photosynthetically active radiation (PAR) was 450 ± 50 $\mu E/m^2/sec$, with a photoperiod of 13:11 hr. Water temperature was maintained at 25 ± 3C throughout both experiments.

Study 3 was conducted in a controlled-environment growth chamber equipped with thirty-six 55-L aquaria. Overhead lighting was provided by lamps as previously described, with a mean PAR measured at the water surface of 510 ± 45 $\mu E/m^2/sec$ and a light:dark cycle of 13:11 hr. Water temperature was maintained at 24 ± 2 C.

Sediment for all studies was collected from Brown's Lake at the WES, and was amended with commercially available fertilizers (Ra-pid-gro, 20:15:15, and Osmocote, 15:15:15) to avoid possible nutrient deficiencies or limitations during the course of each study. Milfoil was supplied by the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX. Beakers (300 ml) were filled with sediment, and four 10- to 15-cm apical shoots of milfoil were planted (5 cm deep) into each beaker. A thin layer of silica sand was added to the sediment surface of each beaker to prevent suspension of sediment during water exchange periods. Aquaria were independently supplied with a simulated hard water solution (Smart and Barko 1984) via peristaltic pumps that were calibrated to provide a complete water volume exchange every 72 hr. Air was bubbled through each aquarium to provide a source of carbon dioxide and thorough mixing of the water column.

BSM stock solutions used for all treatments were prepared from the commercial formulation Londax® (dry flowable, 60% active ingredient). All treatment concentrations are reported as µg/l (ppb) of the active ingredient. At the time of treatment, the flow-through water system was deactivated (peristaltic pumps turned off) and calculated volumes of the BSM stock solution were added to aquaria to provide desired treatment concentrations. At the end of the assigned exposure times, each aquarium was drained and refilled with fresh water three times to remove chemical residues, after which the peristaltic pumps were reactivated, providing water exchange for the duration of the experiment. Water samples were collected and analyzed for chemical residues following the rinse cycle. Results from these analyses indicated that >99% of BSM residues were removed following the drain procedure (data not presented).

Treatments (concentration x exposure time) evaluated in these studies are summarized in Table 1. Studies 1 and 2 each

TABLE 1. BENSULFURON METHYL TREATMENT RATES (µg/l), EXPOSURE TIME (days), AND CONCENTRATIONS (µg/l) IN EXPERIMENTAL TANKS AT 7 DAYS POSTTREATMENT.

Study	Target rate concentration	Exposure	Concentration at 7 days posttreatment
1	0 (untreated)	0	0.0
	50	14	52.1
	75	14	78.4
	5	21	ND[1]
	10	21	ND[1]
	25	21	—[2]
	50	21	—[2]
	5	28	—[2]
2	0	0	0.0
	230	7	210.3
	1150	7	1090.1
	1730	7	1611.0
	2300	7	2401.7
	4600	7	4560.3
	1150	14	—[2]
	2300	14	—[2]
3	0	7, 14, 21, 28, 35, 42	0.0
	50	same as above	52.9
	75	same as above	79.3
	100	same as above	102.5
	125	same as above	118.3
	150	same as above	148.0

[1]ND = not detected, both the 5- and 10-µg/l treatment concentration were below analytical detection limits by 7 days after treatment.
[2]No sample.

consisted of eight BSM CET treatments, ranging from very low to extremely high application rates. Each treatment was replicated three times and randomly assigned to a test aquarium. Beakers planted with milfoil were placed in each aquarium (11 beakers/aquarium in Study 1 and 9 beakers/aquarium in Study 2) and allowed to grow for 2 weeks to establish new shoot and root growth. After 2 weeks of growth, rapidly elongating shoots were trimmed back to a height of 20 cm; 1 week thereafter, chemical treatments were applied. Shoots were trimmed back to a uniform height to facilitate evaluation of the growth-regulating potential of BSM on small shoots supported by a healthy root system.

Immediately prior to treatment, one randomly selected beaker of plant material was removed from each aquarium. Mean shoot and root dry weights (DW) were measured, and these values were multiplied by the number of beakers remaining in each aquarium to provide an estimate of pretreatment biomass (Mean ± SD).

Milfoil was harvested at 5 weeks posttreatment in Study 1 and at 6 weeks in Study 2. Harvested plants were separated into viable roots and shoots, and oven-dried (70C for 48 hr) to a constant weight. Shoot and root biomass data were subjected to analysis of variance (ANOVA) and treatment effects separated using Waller-Duncan *k*-ratio *t* Test. Weekly visual observations were also recorded to characterize the initial plant response to BSM treatment, the progression of injury symptoms, and the initiation of regrowth.

Study 3 consisted of five BSM concentrations ranging from 50 to 150 µg/l, subjected to a series of exposure times ranging from 0 to 42 days. Treatments were not replicated; however, 36 different CET combinations were evaluated. Eight beakers containing milfoil were placed in each aquarium and given a 3-week pretreatment growth period. Plant growth was vigorous and many shoots had reached the water surface by the time of treatment.

At 8 weeks posttreatment, plants were harvested, and roots and shoots were separated and dried. Linear regression procedures were used to relate plant biomass to increased exposure times at each BSM treatment rate tested. Visual ratings of plant injury were recorded weekly.

RESULTS AND DISCUSSION

Study 1. Five treatments significantly reduced milfoil shoot biomass in Study 1 (Figure 1). Reductions ranged from 26 to 69% when compared to untreated plants, with the most effective treatment being a 21-day exposure to 50 µg/l BSM. Higher concentrations at shorter exposure periods were less effective, suggesting that contact time is an important factor in determining treatment success.

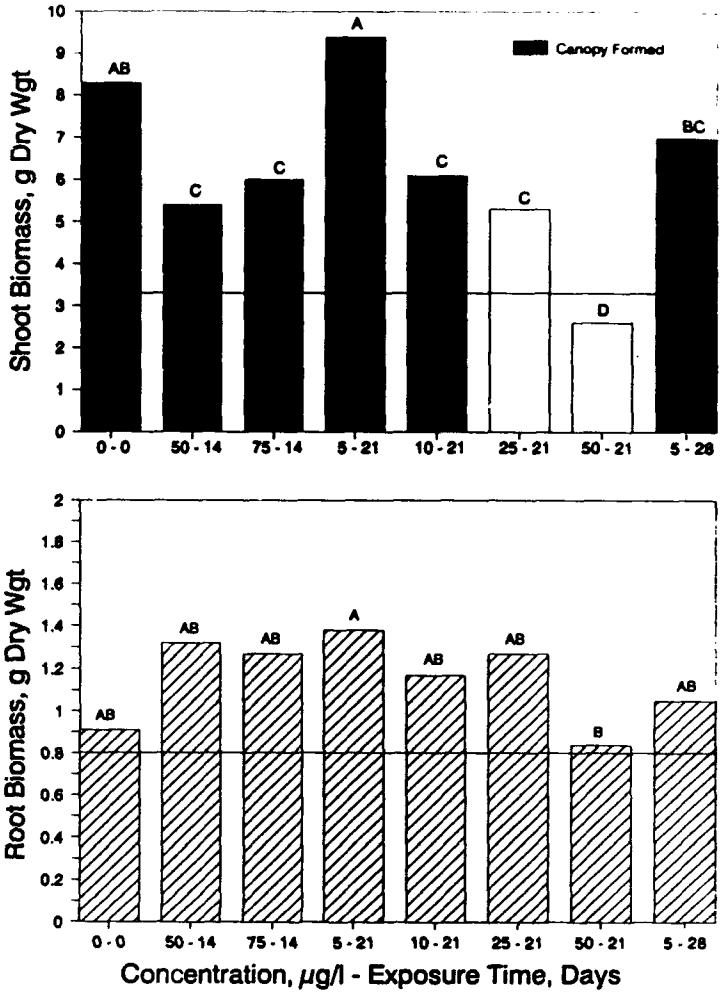

Figure 1. Effects of bensulfuron methyl on shoot and root biomass of Eurasian watermilfoil harvested at 5 weeks posttreatment. Horizontal lines represent pretreatment biomass; shoots = 3.4 ± 1.5 g DW, roots = 0.8 ± 0.3 g DW. Different letters among treatments indicate significant differences at the 5% level according to Waller-Duncan *k*-ratio *t* Test. For shoot biomass, unfilled bars represent those treatments in which a vegetative canopy did not form.

Two treatments, 21- and 28-day exposures to 5 µg/l, showed no significant difference in biomass production from that of untreated plants. Plants subjected to these treatments showed initial injury symptoms (leaves of shoot apices appeared compressed and slightly chlorotic) but continued to grow during the exposure period, indicating that under these experimental conditions, milfoil was tolerant to low doses of BSM. Similarly, milfoil treated with 10 µg/l and exposed for 21 days, exhibited active growth while in contact with BSM; however, final biomass was significantly reduced by 26%. Active growth during treatment further suggests that at low concentrations (<10 µg/l), milfoil can metabolize BSM quickly enough to prevent complete inhibition of the ALS

enzyme system. In all other treatments, substantial regrowth of milfoil was evident only after the chemically treated water was removed following the designated exposure period. Regrowth emerged from root crowns, lateral buds along stem nodes, and injured apical shoots, and was evident 1 to 2 weeks following removal of BSM from the water column.

Initial injury symptoms observed on milfoil treated with BSM concentrations of 25 µg/l and higher were described as a chlorosis and/or browning of apical shoots, with some upper leaf drop and/or downward bending of foliage. These symptoms were evident 1 week following treatment. The appearance of injury symptoms at active growing points (shoot tips) was expected, given the mode of action of BSM.

Formation of small, axillary buds along the nodes of most stems was also noted at these higher concentrations, but buds did not further develop during the chemical exposure period. Plant stems were also affected with necrotic lesions visible on lower stems 2 weeks following chemical application. In some instances new foliage showed morphological differences, such as reduced leaf area and/or a lobed leaf shape. Despite a three-fold difference in chemical concentration, the degree of injury varied little between plants treated with 25 µg/l and those treated with 75 µg/l.

By the end of 5 weeks, regrowth was observed in all treatments, and plants had grown to the water surface in all but two treatments (25 and 50 µg/l at 21-day exposures). Although the final data showed significant reductions in biomass, canopy formation showed strong regrowth potential indicating an inadequate treatment. Despite slight increases in root biomass with several treatments, no significant differences in root growth were observed compared to untreated plants (Figure 1).

Results of this study differ from studies by Anderson (1988), in which milfoil shoot and root DW were reduced by 50 to 70% and 40 to 77%, respectively, after a 4-week exposure to BSM concentrations of 1 to 20 µg/l. Variation in response may be due, in part, to the difference in age of plant material used in experimentation (1-week-old plants versus the 3-week-old plants used here). Additional studies conducted in our laboratory indicate young milfoil plants (7 day-old apical cuttings) were much more sensitive to BSM than plants allowed to grow for 21 days pretreatment (data not presented). Other studies have also reported increased efficacy with BSM on younger plants. Haller *et al.* (1992) observed that in the field, hydrilla sprouting from tubers and turions was more susceptible to BSM than mature plants.

Under our experimental conditions, an exposure period of 21 days to concentrations of 25 to 50 µg/l was necessary to maintain acceptable growth suppression for the duration of the experiment (5 weeks following treatment). Although plants of these treatments had not yet formed a canopy, regrowth was apparent and that given more time (1 to 2 weeks), new growth would have probably reached the water surface on these treatments.

Study 2. Initial response of milfoil to all BSM treatments was evident 1 week after application and symptoms included reddening of shoot tips, downward bending of upper leaves, and bunched or compacted leaves at shoot apices. Effects were more pronounced with increasing chemical concentration.

At 2 weeks posttreatment, untreated plants had formed a dense canopy at the water surface and new growth was visible on plants that had been subjected to a 7-day exposure of 230, 1150, and 1730 µg/l of BSM. New growth emerged from rootcrowns, injured shoot tips, and along lateral stem nodes, and was green but not robust. Leaves were visually smaller than those of untreated plants and new stem growth was spindly. Very little growth was evident on plants treated with a 7-day exposure to concentrations of 2300 and 4600 µg/l. In fact, injury symptoms were still prevalent and necrosis was visible causing some stem breakage. New growth that was present showed signs of chemical injury (as previously described). Plants exposed for 14 days to 1150 and 2300 µg/l were unhealthy, with severe stem and leaf necrosis, stem breakage, and no sign of new shoot development.

Visual observations recorded at 4 weeks posttreatment revealed that all treatments showed signs of recovery; new growth emerged from rootcrowns, injured shoot tips, floating plant segments (detached from decaying stems), and lateral nodes along stems. Similar to the first study regrowth occurred 1 to 2 weeks following removal of the chemically treated water.

At the conclusion of the experiment (6 weeks posttreatment), final biomass data showed that all treatments significantly reduced shoot and root growth (Figure 2). Compared to untreated plants, biomass reductions ranged from 39 to 86% for shoots and 43 to 73% for roots, with the most effective treatment being a 14-day exposure to 2300 µg/l of BSM. Root biomass decreased below pretreatment levels with several treatments, indicating root tissues were decaying. It should be noted that the degree of chemical activity or effectiveness was not proportional to increasing BSM concentrations, as most treatments were not significantly different from each other (*e.g.*, 7-day exposure to 1150 µg/l versus 4600 µg/l). However, plants treated with the same chemical concentration (2300 µg/l) but exposed for different lengths of time (7 and 14 days) were significantly different. Thus, a longer exposure period was more efficacious than increasing BSM concentration. A flat response to increasing sulfonylurea concentration, similar to that observed in this study, has been observed by Brewster and Appleby (1983).

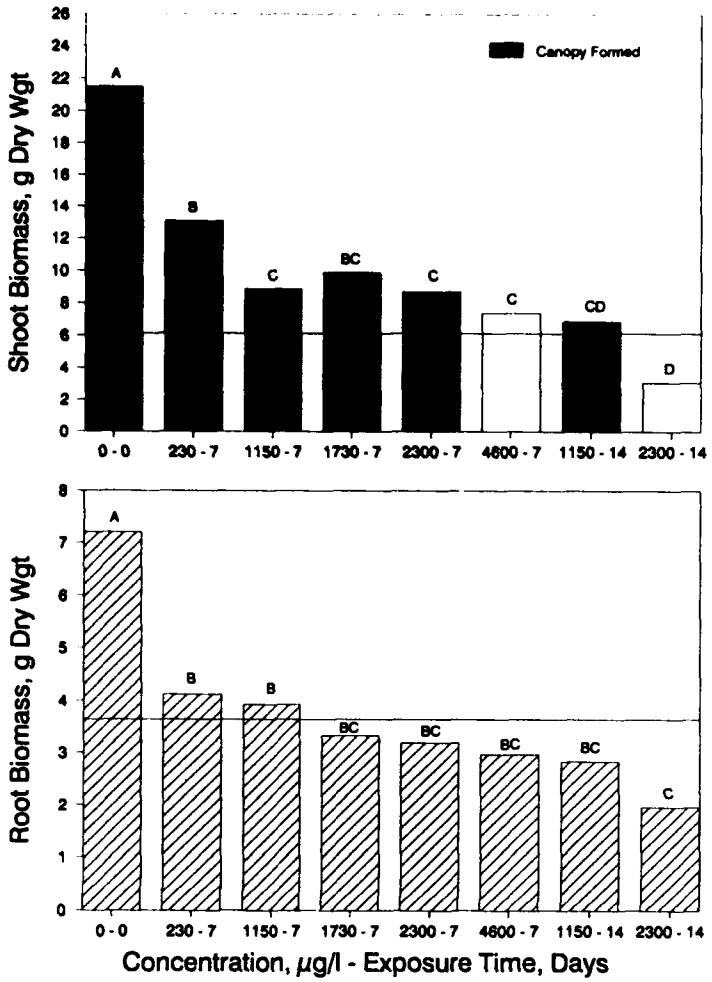

Figure 2. Effects of bensulfuron methyl on shoot and root biomass of Eurasian watermilfoil harvested at 6 weeks posttreatment. Horizontal lines represent pretreatment biomass; shoots = 6.1 ± 1.8 g DW, roots = 3.7 ± 1.3 g DW. Different letters among treatments indicate significant differences at the 5% level according to Waller-Duncan *k*-ratio *t* Test. For shoot biomass, unfilled bars represent those treatments in which a vegetative canopy did not form.

Despite significant differences in biomass production, plants had grown to the water surface (canopied) in all but two treatments (4600 µg/l at 7 days and 2300 µg/l at 14 days) by the end of the study. Extensive regrowth of milfoil to the surface is neither desirable nor acceptable in field situations. Moreover, total plant control was not achieved even though the application rates ranged as high as 46 times the recommended label rate of 100 µg/l. The ability of plants to recover from such high concentrations further suggests that milfoil may be capable of metabolizing BSM.

Results of Studies 1 and 2 show that BSM acts similarly to another aquatic herbicide, fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1*H*)-pyridinone), in that both require long exposure or contact times to achieve efficacy. Hall *et al.* (1984) and Van and Conant (1988) found that a long exposure (several days) to low and high fluridone concentrations was necessary for control of hydrilla and milfoil. Field treatments designed to maintain low doses of fluridone over long periods of time have also shown excellent control of hydrilla and milfoil in river and lake systems in Florida and Washington (Getsinger *et al.* 1992a). Van and Conant (1988) further state that systemic or translocated herbicides, such as fluridone, have much slower uptake rates than contact herbicides and thus require longer exposure times to be effective. Since BSM is also a systemic herbicide, the long contact times required for growth suppression in these studies was not surprising.

Study 3. Observations recorded 7 days after chemical treatment indicated that milfoil growth had slowed and plant injury (similar to that observed in Studies 1 and 2) was apparent. There was no visual difference in the degree of injury between plants treated with 50 µg/l and 150 µg/l. One week later, the number of stems with necrotic lesions and lateral buds had increased.

The first sign of recovery from BSM treatment was noted 21 days posttreatment on plants exposed for 7 and 14 days to all chemical concentrations. Lateral shoots developed and new growth appeared normal. One week later, regrowth from lateral buds was so extensive in these treatments, that they could not be visually distinguished from untreated plants. Recovery of other treatments occurred as in previous studies; 1 to 2 weeks following completion of the exposure period and removal of chemically treated water from the system. Even plants exposed to BSM concentrations of 150 µg/l for 42 days supported new growth by 56 days posttreatment.

Data collected at the conclusion of this study revealed that changes in shoot biomass were highly correlated with exposure time (Figure 3). Statistical comparison of regression coefficients (t-test) indicated that linear relationships between biomass and exposure time were the same at all concentrations. Compared to untreated plants, shoot biomass production decreased by an average of 10 to 86% as exposure time to BSM concentrations increased from 7 to 42 days. Results agree with data from Studies 1 and 2 and add support to the finding that longer BSM contact times are critical to maintaining suppression of milfoil growth. Root growth showed no linear relationship to exposure time or chemical concentration (data not presented).

Results of these studies showed that BSM was effective at reducing the growth of milfoil; however, complete plant control (total plant death) was not achieved at the rates and exposure times tested. Increasing exposure time was more efficacious than increasing BSM concentration. Contact time

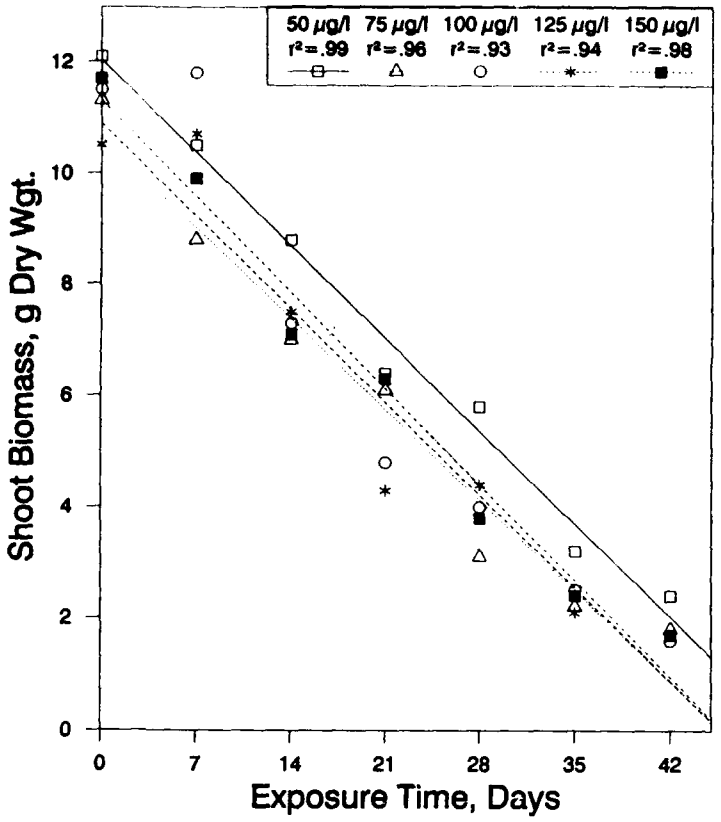

Figure 3. Effects of bensulfuron methyl on shoot biomass of Eurasian watermilfoil harvested at 8 weeks posttreatment. Pretreatment shoot biomass = 4.4 ± 0.5 g DW. Equations for regression lines are as follows: 50 µg/l, y = 12.2 - 0.238x; 75 µg/l, y = 10.93 - 0.239x; 100 µg/l, y = 11.96 - 0.271x; 125 µg/l, y = 11.0 - 0.239x; 150 µg/l, y = 11.4 - 0.251x.

was also critical for maintaining growth suppression, as plants showed strong regrowth 1 to 2 weeks after exposure to BSM was terminated.

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Changes in Nontarget Wetland Vegetation Following a Large-Scale Fluridone Application

STEPHEN M. FARONE AND T. M. MCNABB[1]

ABSTRACT

Surface areas of floating-leaved, emergent, and scrub-shrub wetland plant communities were measured using an aerial imaging methodology before and 1 yr after fluridone (1-methyl-3-phenyl-5-[-3 (triflurомethyl) phenyl]-4 (1*H*)-pyridinone) was applied in a 134-ha lake and two hydrologically connected 4-ha ponds in southwestern Washington to control Eurasian watermilfoil. No significant losses in surface area of emergent wetland or scrub-shrub wetland plant communities occurred, while loss of floating-leaved aquatic plant communities was 28% in the lake and 100% in the ponds. Large-scale fluridone application for Eurasian watermilfoil control can be accomplished in a manner sensitive to nontarget emergent and scrub-shrub wetland protection, although some losses of floating-leaved aquatic plants may be unavoidable. In addition, aerial survey utilizing video imaging technology can expedite wetland plant community measurements.

Key words: fluridone, SONAR™, wetlands, aerial imaging, hyperspectral, aerial photography.

INTRODUCTION

Fluridone is an aquatic herbicide that inhibits carotenoid synthesis in plants, thus exposing their chloroplasts to photodegradation (Bartels and Watson 1978). While the susceptibility of many aquatic plant species to fluridone has been studied (McCowen *et al.* 1979; Arnold 1979, Netherland *et al.* 1993), effects on heterogeneous wetland plant communities adjacent to lakes or ponds are not well documented.

Due to concerns over possible impacts of a proposed fluridone treatment on nontarget wetland species within and adjacent to a lake and wetland system, the Washington State Department of Ecology required a wetland monitoring and loss mitigation program to be in place before permitting the herbicide application. Under the monitoring program, surveys were to be completed immediately before and 1 yr after the herbicide treatment to assess impacts and need for mitigation. The aerial survey described herein was a part of that program.

Interpretation of aerial photographs and digital imagery gathered by satellite-borne sensors has often provided data for wetlands mapping and assessments (Carter 1976, Carter 1978, Tiner 1984). The use of airborne video remote sensing, however, is a relatively recent development (Meisner 1986), as is the development of hyperspectral imaging technology (Rinker 1990).

Our objective was to assess the impact of the fluridone application on nontarget wetlands by measuring the change in horizontal surface area of floating-leaved (predominantly waterlilies—*Nymphaea odorata* Ait.), emergent (predominantly cattails—*Typha latifolia* L.), and scrub-shrub (dominated by willows—*Salix* spp.) wetland plant communities within and adjacent to the treated lake and downstream wetland areas.

MATERIALS AND METHODS

The study was conducted on the Long Lake system in Thurston County, WA (47°01'N, 122°47'W). The system is comprised of Long Lake, 134 ha in area, Long's Pond, 4 ha and hydrologically connected to Long Lake by subsurface flow, and Lois Lake, 4 ha, which receives surface flow from Long Lake.

The applicator, Resource Management of Tumwater, WA, followed a "block treatment" plan developed in consultation with the U.S. Army Engineer Waterways Experiment Station (WES), Washington State Department of Ecology, and Thurston County staff. This program was based, in part, on previous fluridone concentration/exposure time evaluations (Netherland *et al.* 1993) and called for uniform dispersion of the herbicide while maintaining a fluridone concentration near 30 ppb for 6 to 8 weeks. Since the use of rhodamine WT dye to track water movement and fluridone dispersion, as specified in the

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program protocol, was not permitted by county officials, fluridone residues determined after each application were used by WES personnel to adjust the treatment plan as it proceeded (Getsinger 1992, personal communication).

Long Lake was treated with fluridone on July 2, July 17, July 31, and August 14, 1991, in 0.2- to 4.2-ha treatment block areas dispersed throughout the littoral zone. No two adjacent blocks were treated on the same date (Figure 1). One application was made in Long's Pond on July 17, while the hydrologically connected Lois Lake received no direct herbicide application at any time. In all areas, the herbicide was applied as an aqueous suspension just beneath the water's surface at a rate of 9.3 L/ha. On July 2 several floating-leaved, emergent, and scrub-shrub wetland plant communities in and adjacent to each waterbody were observed at close range to provide ground-based data for use in the aerial survey.

In the aerial survey methodology, color still photography was used first to locate and identify plant communities previously ground-truthed. On June 14, 1991, Kodak Echtachrome 100Hc 35-mm film was used to collect oblique color photographs of all nearshore and wetland areas within the project. Before takeoff, the camera and lens package was mounted in a Cessna 172 airplane for oblique imaging at a 45-deg angle to the lake surface. Each frame provided a view of the lakeshore and adjacent nearshore and wetland areas. West banks were photographed from 11:00 am to 12:00 pm DST and east banks from 2:00 pm to 3:00 pm DST to minimize shadow interference. Overlap between image frames permitted a continuous view to aid in referencing locations seen. On July 17, 1992, post-treatment oblique photography was collected in the same manner described above, except that images in both color and Echtachrome 2236 false-color infrared (CIR) 35-mm slide film were collected at all locations.

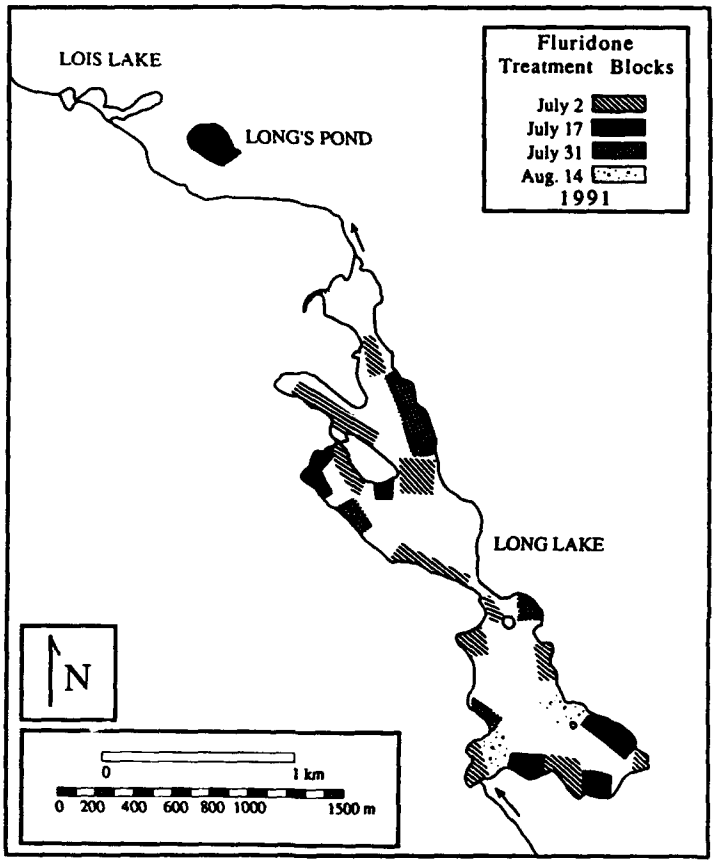

Figure 1. Locations and approximate sizes of treatment block areas receiving fluridone applications in 1991. The areas indicated as treated on July 2 cumulatively covered 33.4 ha, those treated on July 17 cumulatively covered 16.7 ha, those treated on July 31 cumulatively covered 8.3 ha, and areas treated on August 14 cumulatively covered 8.3 ha.

After film processing, the 1991 color images and 1992 color and CIR images were referenced to 7.5 Minute USGS Topographic maps. The set of visible and infrared images were then loaded into an image magnifier, and images were viewed at 20X and 80X magnification. Notes were made regarding vegetation conditions, and each color image was compared with the corresponding CIR image. Each floating-leaved, emergent, and scrub-shrub wetland community viewed on the 1991 imagery was roughly mapped for reference during the video analysis phase.

On June 14, 1991, and July 20, 1992, aerial hyperspectral video imagery was collected. During each flight session, imagery was collected from 12:00 pm to 2:00 pm DST to minimize shadows from the vertical perspective. An industrial grade RCA black and white VHS video camera specially equipped with a modified Ultracon tube was installed vertically in the floor of a Cessna-172 airplane and connected to a power supply, professional grade portable VHS recorder, and monitor. The camera was filtered to receive only those wavelengths of light useful for detecting variation in chlorophyll-*a* absorbance in green plants.[2] Transects were flown over all project areas at an altitude of 400 m MSL (approximately 350 m above Long Lake's surface). This provided a ground footpath of 250 m with footpaths of adjacent transects overlapping approximately 20%.[3]

To analyze the hyperspectral imagery for plant community detection and measurement, an IBM-based airborne video analysis system (Water Watch™) was used. Selected

[2]Spectral band selection for chlorophyll-*a* absorbance is discussed by Dr. Forest Dierberg in Remote Sensing for Water Quality Monitoring in the Tennessee Valley: Field Test of Two Systems. Tennessee Valley Authority, Water Resources Division. August 1992. TVA/WR-92/17. 123 pp.

[3]For a discussion of scale and image dimension considerations peculiar to video format, see the previously cited article by Meisner 1986.

video frames were digitized by the system's 512 x 512 8-bit image capture board yielding picture elements (pixels). The resulting image resolution equaled the resolution of the original VHS image. The image capture board distinguished 256 shades of gray and assigned each pixel a value (1 to 256) based on recorded light intensity. Reflectance characteristics of various plant community types were determined by sampling the gray-scale values of selected ground-truthed plant communities. A spectral classification and false-coloring scheme (Figure 2) was then developed to delineate and visually enhance all plant communities previously identified on color and CIR imagery (Figure 3).

Figure 2. One wetland community area as seen on hyperspectral video imagery. On the left is the black and white hyperspectral image showing variation in chlorophyll-*a* absorbance, on the right is a black and white reproduction of the color-enhanced classified image produced to delineate plant community boundaries.

To quantify the impacts of fluridone on aquatic plants and wetlands, the digitized video imagery was scaled for measurements by the computer system. Scaling targets were measured on the ground and on the video monitor, and these measurements were used by the system's software to calculate the ground area represented by each pixel, approximately 0.24 m^2. All delineated plant communities were then measured in hectares on both the pre-treatment 1991 imagery, and the post-treatment 1992 imagery. Similar wetland plant communities seen within any video frame were measured together as one "wetland community area." Comparisons of pre and post-treatment areas of wetland communities were then made.

RESULTS AND DISCUSSION

Change in area of emergent wetlands (Table 1) was -3% in Long Lake -4% in Long's Pond, and-+3% in Lois Lake. Change in area of scrub-shrub wetlands was +4% in Long Lake, and +7% in Long's Pond. The scrub-shrub component of the Lois Lake wetland community was included with the emergent community area for that area because scrub-shrub species were mixed with emergent wetland plants and were not easily discernible on imagery. The changes in areas of emergent and scrub-shrub wetlands do not appear significant in terms of indicating fluridone impacts because of interference in the area comparison caused by two factors. First, there was a chlorotic appearance in some of the surviving wetland vegetation after the fluridone treatment. Chlorosis in plants surviving in fluridone-treated waters has been observed by researchers including Bartels and Watson (1978) and Van and Steward (1985), and is an expected symptom of fluridone uptake. Chlorosis in portions of surviving plants seen in 1992 imagery made the delineation of these wetland communities by spectral classification more difficult, which may have resulted in a loss of accuracy in area measurements. Second, 1991-92 was an unusually dry winter and drier-than-normal wetland soil conditions in 1992 may have allowed expansion of emergent and scrub-shrub wetlands into lower areas,

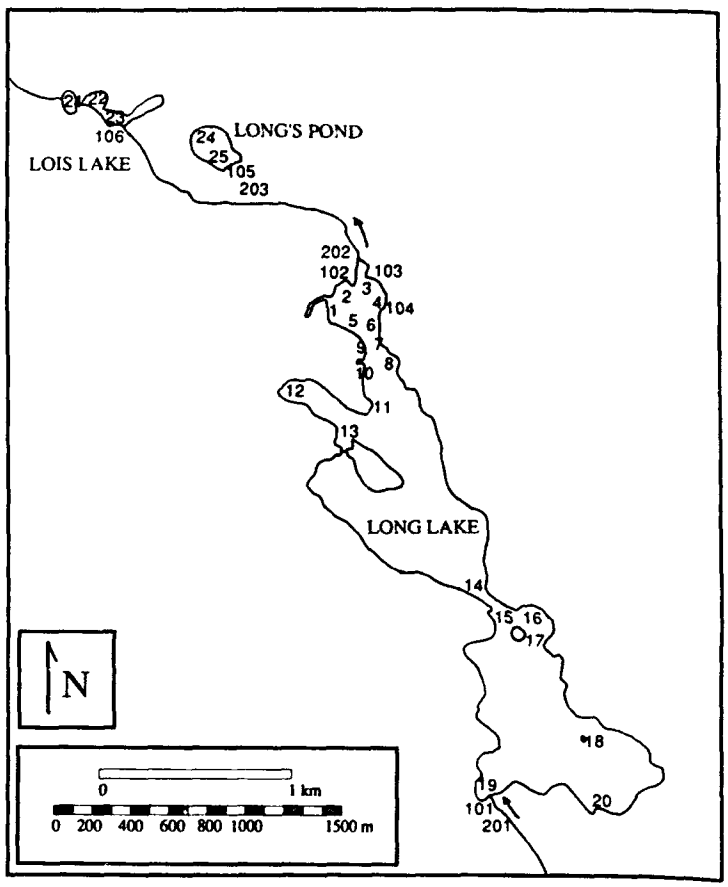

Figure 3. Locations of wetland community areas measured. Numbering refers to the Image Numbers listed in Table 1.

TABLE 1. MEASUREMENTS OF WETLAND COMMUNITY AREAS DELINEATED ON THE LONG LAKE SYSTEM IN THURSTON COUNTY, WA, USING THE WATER WATCH™ HYPERSPECTRAL VIDEO IMAGE PROCESSING SYSTEM.

Image No.	1991 (ha)	1992 (ha)	% change
Emergent wetland[1]			
Long Lake			
101	0.32	0.31	-4
102	0.08	0.09	10
103	0.15	0.14	-8
104	0.12	0.11	-3
Total	0.67	0.65	-3
Long's Pond			
105	0.51	0.49	-4
Lois Lake			
106	0.61	0.63	3
Scrub-shrub wetland[2]			
Long Lake			
201	0.21	0.22	4
202	0.08	0.08	5
Total	0.29	0.30	4
Long's Pond			
203	0.25	0.26	7
Lois Lake scrub/shrub community is included in emergent class.			
Floating-leaved aquatic plants[3]			
Long Lake			
1	0.58	0.47	-19
2	0.47	0.19	-60
3	0.94	0.54	-43
4	0.93	0.62	-34
5	0.69	0.44	-36
6	0.32	0.27	-15
7	0.53	0.55	3
8	0.25	0.20	-20
9	0.20	0.18	-10
10	0.28	0.29	4
11	0.26	0.23	-12
12	0.29	0.28	-3
13	0.20	0.17	-12
14	0.68	0.40	-41
15	0.70	0.63	-11
16	0.47	0.33	-30
17	0.36	0.32	-12
18	0.27	0.24	-9
19	0.46	0.14	-70
20	0.64	0.33	-49
Total	9.52	6.81	-28
Long's Pond			
24	0.14	0.00	-100
25	0.50	0.00	-100
Total	0.64	0.00	-100
Lois Lake			
21	0.19	0.00	-100
22	0.11	0.00	-100
23	0.19	0.00	-100
Total	0.49	0.00	-100

[1]Dominated by *Typha latifolia.*
[2]Dominated by *Salix* spp.
[3]Dominated by *Nymphaea odorata.*

possibly obscuring losses. As the measured areas showed changes of only 3 to 7%, it is likely these factors could have resulted in part or all of the detected changes in vegetation.

Chlorosis in leaves did not hamper the delineation of floating-leaved aquatic plant communities by spectral classification as they were floristically simple and their boundaries distinct. Detected loss in surface area of all floating-leaved aquatic plant communities in Long Lake was 28%, and change in area varied from +4% to -70% for different community areas. Those communities suffering the greatest losses were near the inlet stream (Figure 3, #19), near the outlet stream (#2 and #3), just below the narrowest portion of the lake (#14), and one community in the southeast portion of the lake (#20), all of which lost over 40% of their surface areas. Meanwhile, Long's Pond and Lois Lake showed 100% loss of floating-leaved aquatic plant communities.

Causes of the considerable variation in losses to floating-leaved aquatic plant communities are not clear from our data. All of the plant communities in Long Lake which lost over 40% of their surface area were located within fluridone-treatment block areas, except for the two near Long Lake's outlet stream. However, the location of a community within a treatment block area would not appear to account for greater control since residue sampling performed by Thurston County personnel throughout the treatment period indicated that the treatment block areas of Long Lake contained similar fluridone residues (mean 33.6 ppb, range 10 to 60, standard deviation 13.2, n = 43) as other parts of the lake (mean 33.7 ppb, range 19 to 42, standard deviation 8.5, n = 4), while the mean of the concentrations recorded in Lois Lake was 24.7 ppb (range 19 to 30, standard deviation 1.53, n = 9) and concentration in Long's Pond on August 15 was 30 ppb. Also, residue uniformity increased throughout the lake over the treatment period as shown by the decrease in the standard deviation of residue measurements from 11.7 for those taken on July 11 to 3.6 for those taken on August 15 (Thurston County Public Works 1991).

Although post-treatment chlorosis and drought conditions may have reduced the accuracy of area measurements in the emergent and scrub-shrub communities, results of this aerial survey suggest that large-scale fluridone application targeting Eurasian watermilfoil can be accomplished without significant losses of nontarget emergent or scrub-shrub wetlands. Waterlily decline resulted from this treatment program; however, the program's July/August time frame may have exacerbated this decline. The original WES recommendation was for treatments during spring. Regrowth of the waterlilies will be monitored along with other communities in future phases of the project. The aerial survey methodology utilizing hyperspectral video imaging proved valuable in

expediting the wetland plant community measurements and has provided imagery which will be useful as baseline data in future phases of the monitoring program.

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J. Aquat. Plant Manage. 31: 189-194

Fluridone Concentration and Exposure Time Requirements for Control of Eurasian Watermilfoil and Hydrilla

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ABSTRACT

Fluridone concentration and exposure time requirements were evaluated for Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* (L.f.) Royle) under controlled-environment conditions. Results indicated that fluridone effectively inhibited growth and reduced biomass at rates of 12, 24 and 48 µg/l. Shoot and root biomass and total chlorophyll were reduced from 70 to 98% following 30-, 60- and 90-days exposures to fluridone. However, removal of fluridone at 30 and 60 days resulted in extensive regrowth following a 30-day recovery period. One exception was milfoil exposed to 48 µg/l for 60 days which was reduced by approximately 98% with no evidence of regrowth. The 48-µg/l treatment often resulted in greater biomass reduction in both species than the other rates; however, no significant differences were noted between the treatment rates of 12 and 24 µg/l. Results indicate that maintaining fluridone concentrations for >60 days at rates as low as 12 µg/l is critical for successful fluridone treatments.

Key words: aquatic weeds, herbicide, *Myriophyllum spicatum*, *Hydrilla verticillata*.

INTRODUCTION

Previous laboratory studies have shown that the efficacy of fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone} against Eurasian watermilfoil and hydrilla is dependent upon the length of time these plants remain exposed to given concentrations of the herbicide (Hall *et al.* 1984, Van and Conant 1988, Netherland 1992). Studies with several other aquatic herbicides indicated concentration/exposure time (CET) requirements for these plants ranged from 6 hr to 4 days (Green and Westerdahl 1990,

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Netherland *et al.* 1991, Netherland and Getsinger 1992), whereas initial fluridone CET evaluations demonstrated that much longer exposures (21 to 84 days) were required to provide comparable reductions in biomass (Hall *et al.* 1984, Netherland 1992). In addition, these investigations showed that by maintaining low levels of fluridone (10 to 25 µg/l) in the water column over long periods of time plant control similar to that provided by much higher fluridone treatment rates could be achieved. In an effort to verify preliminary laboratory-based fluridone CET relationships, sequential applications of fluridone have been made to flowing-water systems in which low concentrations of the herbicide were maintained over long periods of time (Getsinger *et al.* 1992). These low-dose, long-exposure treatments have provided excellent plant control for at least one growing season.

Although successful plant control in previous laboratory and field treatments has been linked to maintaining low concentrations of fluridone for an extended period of time, these evaluations have only broadly quantified the relationship between concentration, exposure time, and efficacy. Therefore, this study was designed to more precisely determine fluridone CET relationships for controlling Eurasian watermilfoil and hydrilla. Further quantification of fluridone CET relationships will provide guidance for improving the management of Eurasian watermilfoil and hydrilla, particularly in flowing and other high water exchange environments.

MATERIALS AND METHODS

This study was conducted in a controlled-environment growth chamber with a photosynthetic photon flux density of 520 ± 50 µmoles/m/sec at the water surface, a 14L:10D photoperiod, and a water temperature of 24 ± 2C. Lighting was provided by 400-W high pressure sodium and GE multivapor lamps. Sediment was collected from Brown's Lake, Vicksburg, MS, and amended with fertilizer (Rapid-Gro® 20:15:15 (3 g/l) and slow-release Osmocote® 15:15:15 (5 g/l)). Glass beakers (300 ml) were filled with sediment and four 10- to 15-cm apical shoots were planted in each beaker. A thin layer (0.5 cm) of silica sand was added to the sediment surface of each beaker to prevent suspension of sediment during water exchange periods. Ten beakers containing four shoots of a single target species were placed in each 55-L aquarium (0.9 m tall by 0.09 m^2). Aquaria were independently supplied with a water culture solution (Smart and Barko 1984) via peristaltic pumps that were calibrated to provide a complete water volume exchange every 24 hr. Air was bubbled through each aquarium to provide a source of CO_2 and thorough mixing of the water column.

Eurasian watermilfoil (hereafter called milfoil) and hydrilla, collected from the Suwannee River, FL, were grown separately in 55-L aquaria. Milfoil was grown for 3 weeks prior to fluridone treatment while hydrilla was allowed to grow for 4 weeks. These periods allowed the actively growing plants to reach the water surface and encouraged the development of a healthy root mass. Immediately prior to treatment, one randomly selected beaker was removed from each aquarium. Mean shoot and root dry weights (DW ± SD) were measured and these values, multiplied by the number of beakers remaining in each aquarium, provided an estimate of pretreatment biomass. Pretreatment shoot weights (105 g DW/m^2 for milfoil and 90 g DW/m^2 for hydrilla) approximated spring to early summer field biomass reported for milfoil and hydrilla (Grace and Wetzel 1978, Bowes *et al.* 1979, Harlan *et al.* 1985). Following the initial growth period, plants were treated with fluridone at concentrations of 12 and 24 µg/l for a period of 30, 60, and 90 days, and 48 µg/l for 30 and 60 days. Each treatment (including untreated controls) was replicated three times and randomly assigned to a test aquarium.

Fluridone stock solutions were prepared from the commercial formulation Sonar® AS (4 lb active ingredient per gallon). All treatment concentrations are reported as µg/l (ppb) of the active ingredient fluridone. At the time of treatment, the flow-through water system was deactivated and fluridone was added to the aquaria. Following a 30-day exposure, all aquaria (including controls) were thoroughly drained. Rhodamine WT dye was added (10 µg/l) to each aquarium prior to draining and measured using a Turner Design® fluorometer. It was assumed that once dye concentrations reached zero, herbicide removal from the water column was complete. Residue analyses from previous studies conducted in this system showed that only 5 to 12% of the fluridone degraded over a 42-day period (data not shown). Minimal degradation in the chamber is attributed to the exclusion (due to 0.6-cm glass cover plates) of the ultraviolet light component (297 to 325 nm) primarily responsible for photolysis of fluridone (Mossler *et al.* 1989), and the fact that plant uptake accounts for a very small fraction of the fluridone removed from the water column over time (Marquis *et al.* 1981, Van and Steward 1986, Van and Conant 1988). Although degradation was not a major concern, due to the length of this study, aquaria were drained and re-treated at 30-day intervals to allow for an exchange of fresh water. Treatments designated as 60- and 90-day exposures were re-treated immediately following the drain procedure.

Plant response to fluridone treatment was monitored for a 90-day period, which allowed for potential plant recovery following the 30- and 60-day exposure periods. Visual assessments were used to characterize initial plant response to fluridone, progression of injury symptoms, and initiation of regrowth from shoots or rootcrowns. Two shoot apices (4 to 6 cm) per aquarium were sampled at 6, 30, 60, and 90 days

and analyzed for total chlorophyll (a and b) using a dimethyl sulfoxide (DMSO) extraction method (Hiscox and Israelstam 1979). Three beakers were removed from each aquarium at 30, 60 and 90 days, and shoots and roots were separated and oven-dried (70C for 48 hr) to a constant weight. Biomass and chlorophyll data were subjected to analysis of variance (ANOVA). Effects of the fluridone treatments on shoot biomass and chlorophyll content were examined by regression analysis to test for a linear response of each parameter over sampling time and between treatments at each sampling time.

RESULTS AND DISCUSSION

Growth of untreated milfoil and hydrilla was characterized by the formation of dense surface canopies that persisted throughout the study. Although biomass per harvest increased over time (Figures 1 and 2), the total biomass per aquarium remained fairly constant from 30 through 90 days. By removing beakers over time, individual plants were able to increase biomass through utilization of open space.

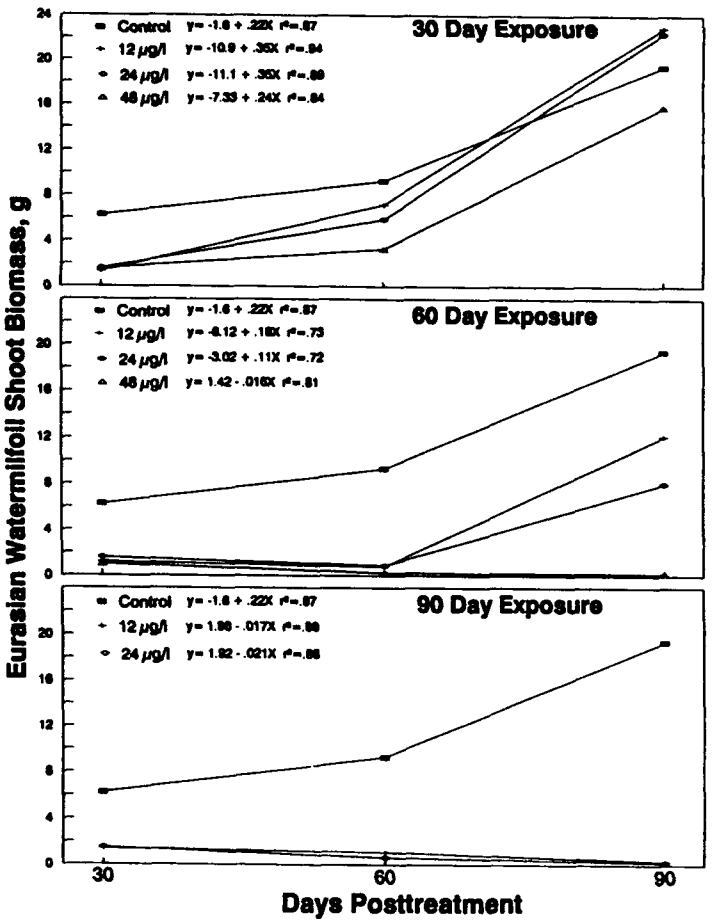

Figure 1. Effects of fluridone on shoot biomass of Eurasian watermilfoil harvested at 30, 60, and 90 days. Data points represent actual values. Regression equations (y = shoot biomass, x = days posttreatment) were calculated to determine if biomass showed a linear response to treatment over time.

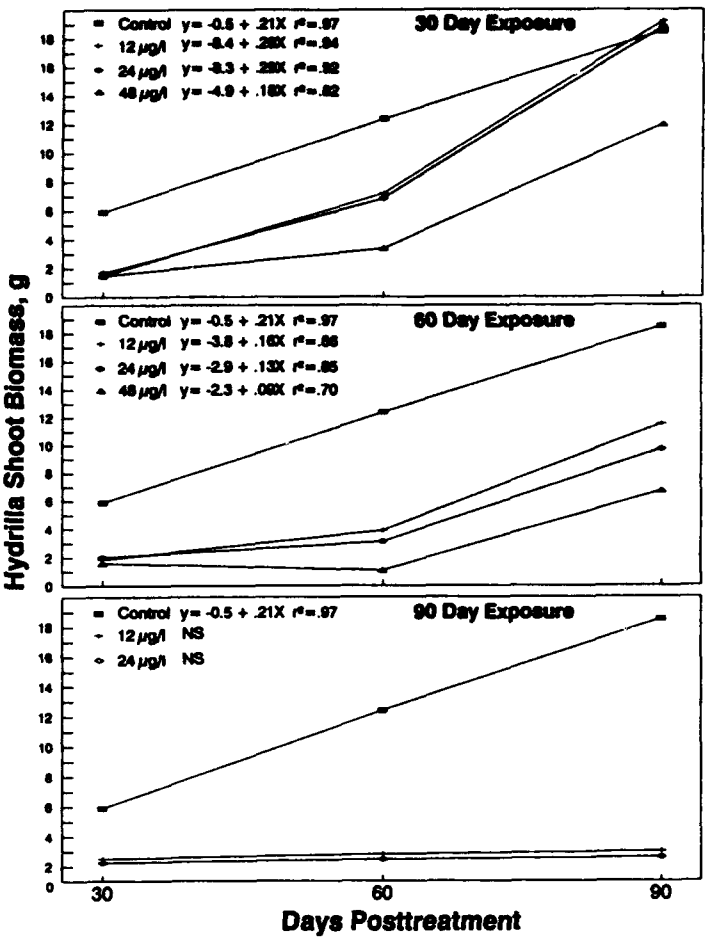

Figure 2. Effects of fluridone on shoot biomass of hydrilla harvested at 30, 60, and 90 days. Data points represent actual values. Regression equations (y = shoot biomass, x = days posttreatment) were calculated to determine if biomass showed a linear response to treatment over time. If the ANOVA procedure indicated that no significant differences existed between treatments, regression equations were labeled NS.

Milfoil began to manifest fluridone symptoms by 6 days after treatment (DAT), as indicated by the 47 to 74% reduction of total chlorophyll in shoot tips (Table 1). Although actively growing apical shoots became albescent (bleached), elongation and growth of this tissue continued to occur for approximately 7 days. Growth had ceased by 10 DAT and albescent tissue became necrotic and detached from the stem. By 21 DAT all new growth from apical shoots had decayed (a canopy no longer existed) and lateral buds began to emerge from existing stem tissue or rootcrowns. All new shoot growth showed characteristic fluridone symptoms, yet stem tissue below the active growing points maintained a healthy green appearance. Results of the 30-day harvest indicated

that fluridone reduced milfoil biomass by 75% (Figure 1). Although treatment rate resulted in no significant differences in shoot biomass ($p = 0.97$), data indicated that the 24- and 48-µg/l treatments resulted in a 50 to 77% greater reduction of chlorophyll than the 12-µg/l treatment (Table 1).

TABLE 1. CHLOROPHYLL CONTENT OF EURASIAN WATER-MILFOIL APICAL SHOOTS SAMPLED AT 6, 30, 60, AND 90 DAYS AFTER INITIAL FLURIDONE TREATMENT.

Treatment (µg/l/day)	Chlorophyll content (mg/g fresh weight) 6 DAT	30 DAT	60 DAT	90 DAT	Linear response[1]
Control	1.19	1.21	1.05	1.25	NS
12/30	0.64	0.45	0.86	1.31	0.05
24/30	0.43	0.23	0.85	1.20	0.05
48/30	0.31	0.16	0.72	1.13	0.05
12/60	0.62	0.42	0.39	0.96	NS
24/60	0.41	0.21	0.21	0.97	NS
48/60	0.28	0.13	0.04	0.02	0.05
12/90	0.59	0.47	0.28	0.09	0.05
24/90	0.37	0.19	0.11	0.02	0.05

[1]Test for linear response of chlorophyll content over sampling time within each treatment. NS = not significant at the 0.05 level.

Immediately following the 30-day treatment period, milfoil no longer exposed to fluridone began to recover. Regrowth from lateral buds and rootcrowns in the 12- and 24-µg/l treatments was rapid and plants reformed a canopy within 12 days. No residual response to fluridone was noted during the recovery period. Milfoil regrowth from the 48-µg/l treatments was delayed, and some of the early regrowth showed symptoms of residual fluridone. Following a 30-day recovery period (60-day harvest), it was difficult to discern fluridone-treated plants from untreated plants. Milfoil treated at 48 µg/l lagged behind the other treatments in biomass recovery (Figure 1); however, these plants were actively growing and forming a canopy. Shoot biomass recovery decreased linearly in response to increasing treatment concentrations (biomass = 8.44- 0.11*conc. $r^2 = 0.97$) following 30 days of recovery. Following 60 days of recovery the 12- and 24-µg/l treatments exceeded reference aquaria in biomass (Figure 1). The biomass and chlorophyll of the 48-µg/l treatment remained reduced, but the trend toward an increase in biomass over time indicated a complete recovery was likely following 30 days of fluridone exposure. In contrast, milfoil that remained exposed to fluridone (60- and 90-day exposures) continued to decline and new growth was limited to a few albescent shoots from lateral buds or rootcrowns. Stems were further defoliated and less vigorous, but, overall, the plants remained dormant. This was verified by the fact that shoot and root biomass levels of treated plants changed very little from 30 to 60 days. Shoot biomass at 60 days showed an 87% reduction in all treatments compared to the reference aquaria (Figure 1). Although no significant differences in shoot ($p = 0.68$) or root ($p = 0.51$) biomass existed between fluridone treatments, the 48-µg/l treatment was much less vigorous as stems were completely defoliated and brittle at harvest.

Milfoil recovery was slower following the 60-day fluridone exposure period. Although the 12-µg/l treatment began to recover immediately following the removal of fluridone, the 24- and 48-µg/l treatments remained inactive for 5 days following fluridone removal. By 14 days following removal of the fluridone, both the 12- and 24-µg/l treatments were actively growing and recovering. At the 90-day harvest, shoot biomass of these treatments, though 36 to 57% less than untreated plants, had recovered dramatically (8- to 12-fold increase in biomass) over the 30-day recovery period (Figure 1). The 30-day recovery, following the 60-day exposure, also showed that shoot biomass decreased linearly as treatment rates increased (biomass = 17.8 - 0.38*conc. $r^2 = 0.96$). The 48-µg/l treatments produced few new shoots from lateral buds; furthermore, these new tips were brittle and somewhat albescent. Biomass and chlorophyll continued to decrease over the 30-day recovery period (Figure 1), indicating the inability of milfoil to recover following this treatment.

Milfoil biomass and chlorophyll content continued to decrease during the 90-day exposure period. Following 90 days of exposure to fluridone, biomass and chlorophyll were reduced by approximately 93 to 99% compared to untreated controls (Figure 1, Table 1). The defoliated stems lacked shoot tips and were flaccid at harvest; however, some root tissue remained attached to rootcrowns. The fragile condition and extremely reduced biomass of the milfoil following the 90-day exposure indicated that recovery was unlikely, even in the optimal regrowth conditions experienced in the growth chamber.

Results indicated that fluridone exposure time was critical for the long-term control of milfoil. Growth ceased and biomass declined in the presence of all fluridone treatments tested. However, the 48-µg/l treatment was the only rate that prevented rapid regrowth following 60 days of exposure. Immediate regrowth following removal of fluridone from the water column indicates that the herbicide was not sequestered in plant tissue at phytotoxic levels. The minimum level of fluridone that must be maintained to produce phytotoxic symptoms has not been determined, and is likely dependent on the species and growth stage of the plant (Van and Conant 1988, Spencer and Ksander 1989, Spencer *et al.* 1989, Netherland 1992). However, Netherland (1992) reported that milfoil

exposed to 5 µg/l for 70 days was reduced by 40% compared to untreated plants, but treated plants continued to produce chlorophyll and significantly increased biomass over pretreatment levels.

Exposure of hydrilla to fluridone led to a 85 to 92% reduction in total chlorophyll by 6 DAT (Table 2). Albescent tissue continued to elongate and maintained its integrity during the 30-day exposure. This was in contrast to milfoil which ceased elongating at 7 days, as bleached tissue became necrotic and detached from the stem. Following the 30-day exposure period at concentrations of 12, 24 and 48 µg/l, hydrilla shoot mass was reduced 70% compared to untreated controls (Figure 2). Total chlorophyll at 30 DAT remained reduced by 85 to 92%, whereas root biomass was reduced by only 5 to 18% compared to untreated controls.

TABLE 2. CHLOROPHYLL CONTENT OF HYDRILLA APICAL SHOOTS SAMPLED AT 6, 30, 60, AND 90 DAYS AFTER INITIAL FLURIDONE TREATMENT.

Treatment (µg/l/day)	Chlorophyll content (mg/g fresh weight)				Linear response[1]
	6 DAT	30 DAT	60 DAT	90 DAT	
Control	1.05	1.01	1.12	1.04	NS
12/30	0.15	0.16	0.97	1.22	0.05
24/30	0.13	0.09	1.18	1.06	0.05
48/30	0.09	0.09	1.02	0.98	0.05
12/60	0.10	0.08	0.19	0.81	0.05
24/60	0.09	0.14	0.19	0.92	0.05
48/60	0.08	0.10	0.21	1.01	0.05
12/90	0.12	0.12	0.14	0.08	—[2]
24/90	0.07	0.08	0.06	0.04	—[2]

[1]Test for linear response of chlorophyll content over sampling time within each treatment. NS = not significant at the 0.05 level.
[2]ANOVA indicated no significant difference between treatments at the 0.05 level.

Removal of fluridone-treated water at 30 days resulted in an initial rapid growth of green shoot tips for a 4-day period, followed by a return of fluridone symptoms at 7 days. The reappearance of fluridone symptoms 1 week following the drain procedure indicates that fluridone was either not adequately removed from the system, or the compound remained sequestered within the plant tissue. This recurrence of symptoms indicates that the level of fluridone activity may be well below 12 µg/l. By 15 days recovery, hydrilla again produced healthy green shoots from stems and rootcrowns. The 60-day harvest (30 days of recovery) resulted in a 46% shoot biomass reduction in both the 12- and 24-µg/l treatments, and a 70% reduction in the 48-µg/l treatment (Figure 2). Although shoot biomass remained significantly decreased, total chlorophyll recovered to approach untreated control levels (Table 2), indicating active regrowth. This lag between the resumption of hydrilla regrowth and chlorophyll recovery following fluridone treatment also was noted by Spencer and Ksander (1989). Results of the 30-day exposure period indicated that biomass recovery was linear over time (30 and 60 days of recovery) and was reduced (35%) only by the 48-µg/l treatment (Figure 2).

Hydrilla exposed to fluridone for 60 days continued to produce albescent shoots from rootcrowns. Stems remained foliated and buoyant but were not actively growing. Shoot biomass was reduced from 70 to 85% by 60 DAT (Figure 2), whereas root biomass was only reduced 35 to 50%. Immediately following the 60-day drain procedure, all treated plants began to recover (no residual fluridone symptoms were apparent). During this 30-day recovery period hydrilla biomass nearly tripled (Figure 2). Although biomass was reduced by 35 to 60%, chlorophyll values and canopy formation by actively growing shoots indicated that recovery from all treatments was likely to occur.

Hydrilla exposed to fluridone for 90 days remained reduced by 88% at all harvest intervals (30, 60, and 90 days) compared to untreated controls (Figure 2). Although stems were flaccid and defoliated and chlorophyll was greatly reduced, no significant linear response in biomass reduction was noted over time. This was in contrast to milfoil biomass and chlorophyll which continued to decline over time.

Results indicated that fluridone exposure time was critical for the sustained control of hydrilla. Although significantly reduced following a 60-day exposure, hydrilla was able to recover from all fluridone rates tested. The ability of the plant to recover from 90-day exposures remained unclear.

Previous laboratory research has been conducted on fluridone CET effects on hydrilla (Van and Steward 1986, Van and Conant 1988, Spencer and Ksander 1989). These studies showed that increasing fluridone rates from 50 to 1,000 µg/l (150 µg/l is the maximum labeled rate) could reduce contact time requirements; however, a 10-fold increase in fluridone concentration often led to only marginal increases in efficacy. Hall *et al.* (1984) treated hydrilla and milfoil with fluridone at rates of 10 to 90 µg/l for a 12-week period and achieved a 75 to 90% reduction in shoot biomass; yet increasing the rate of fluridone did not result in a significant difference in shoot mass. These studies showed that over a long exposure period, low fluridone rates (~10 µg/l) were effective at inhibiting growth of submersed plants. Our results indicated that the shorter exposure periods (30 and 60 days) effectively reduced shoot mass, but were ineffective at preventing regrowth following removal of fluridone. One exception was milfoil treated at 48 µg/l for 60 days, which

significantly reduced biomass (98%) and prevented regrowth. Since the laboratory offers optimal conditions for plant regrowth following herbicide treatment (*e.g.* readily available light, stable water quality and temperature, low mechanical stress, etc.), perhaps an underestimation of efficacy can occur.

Based on information from the laboratory and the field, it is likely that the key to a successful fluridone treatment is in maintaining herbicidally active concentrations for periods exceeding 60 days. Moreover, recent success of sequential applications of fluridone to lotic systems can be explained by the ability to maintain low concentrations (<40 µg/l) over long periods of time (8 to 16 weeks).

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Effects of Fluridone on Hydrilla Growth and Reproduction[1]

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ABSTRACT

The effects of fluridone on hydrilla growth and reproduction were evaluated over time. Newly established (young) and 8-month-old (mature) hydrilla plants were exposed to 0.0, 0.05, 0.5, 5.0, or 50 ppb fluridone and maintained outdoors under ambient short-day conditions. An untreated long-day control was also included. Fresh and dry weight, number of flowers, and axillary and subterranean turions were determined 0, 2, 4, 6, 8, and 12 weeks after fluridone treatment. Short-day conditions promoted flower and axillary and subterranean turion production in mature plants. Low concentrations of fluridone (0.05 and 0.5 ppb) caused transient increases in the number of both subterranean and axillary turions by mature hydrilla, but higher concentrations (5 and 50 ppb) inhibited development of these tissues. Growth (shoot dry weight) of young plants treated with low concentrations of fluridone (0.05 and 0.5 ppb) was not affected. The 5.0 ppb-fluridone treatment did not affect the growth of young plants until after 6 weeks of exposure. The 50 ppb fluridone treatment prevented any significant change in young plant shoot dry weight over the 12-week study. There was no significant change in shoot dry weight of mature plants regardless of the treatment.

Key words: subterranean turion, axillary turion, herbicide, tubers, turion, photoperiod, abscisic acid.

INTRODUCTION

Hydrilla (*Hydrilla verticillata* (L.f.) Royle) is an exotic submersed aquatic macrophyte that infests fresh water ecosystems throughout the world (12,26). Hydrilla was introduced to Florida from Asia in the late 1950s by the aquarium industry (5,7) and is currently Florida's most serious aquatic weed problem (7). Hydrilla possesses unique photosynthetic characteristics such as C_4 metabolism and a low light compensation point that allow this species to out-compete other aquatic macrophytes (1,6,19,22). In addition, hydrilla can reproduce through several vegetative mechanisms including fragmentation (11), and specialized structures [axillary turions (turions) and subterranean turions (tubers)](7,24). Tubers are formed at the end of positively geotropic rhizomes, while turions are found in leaf axials or occasionally at the end of shoots (21).

Fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1H)-pyridinone) was registered for aquatic use in 1986 and usually provides good control, but results are often unpredictable in terms of area treated and area controlled (14,20). Fluridone was the first herbicide to provide long-term hydrilla control; however, it was difficult to produce consistent results. In addition, methods to best utilize several unique characteristics of fluridone to improve control have not been completely developed.

Fluridone, applied at normal use rates, decreases carotenoid levels sufficiently to cause chlorophyll decomposition and plant death (4,17). Because abscisic acid is a carotenoid metabolite, fluridone is also used in physiological studies to regulate abscisic acid (ABA) levels (16,18) . Abscisic acid has been associated with turion formation (initiation) in green milfoil (*Myriophyllum verticillatum* L.) (25) and giant duckweed (*Spirodela polyrrhiza* L.) (13). Therefore, fluridone could potentially prevent the production of turions in hydrilla.

Carotenoids protect chlorophyll from decomposition and are essential to the survival of all plants. The ability to regulate the reproduction of hydrilla without killing the plant would be dependent on a critical concentration of fluridone. A growth-regulating concentration of fluridone could potentially lower carotenoids (but not enough to cause a critical decrease in chlorophylls) enough to reduce ABA below a critical concentration thus preventing vegetative reproduction. These studies were conducted to evaluate the relationship between fluridone concentration and regulation of reproduction and growth in hydrilla.

MATERIALS AND METHODS

On February 22, 1991, four 10-cm apical stem segments of hydrilla were planted in 10-cm square pots. These pots were filled with potting media (Metro-mix 200 amended with fertilizer) and a 1.3-cm deep sand cap was added to prevent

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floating of the media. These plants were grown in a greenhouse for 8 months under the following environmental conditions: 16 hr light/8 hr dark photoperiod, day temperature of 30 ± 5C, night temperature of 25 ± 5C with a mean light intensity at noon of 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (PPFD). Under these conditions the hydrilla quickly reached the surface of the water and formed a dense mat.

On October 10, 1991, plants (mature) were transferred outdoors to 900-L concrete vaults. In addition, two 10-cm hydrilla apical stem segments per pot were established in the vaults in the same manner described previously. Segments were taken from the mature plants. All plants were treated on October 16, 1991, with fluridone at the following rates: 0.0, 0.05, 0.5, 5.0, and 50 ppb and maintained outdoors under ambient short-day conditions (<12-hr daylength) in Gainesville, FL. In addition, control groups representing both age groups of plants were maintained outdoors but supplied with floodlights timed to extend the photoperiod to 16 hr. Representative plants (n = 4) were harvested from both age groups at this time to establish the status of the plants at the time of treatment.

Plants were harvested at intervals of 2, 4, 6, 8, and 12 weeks after treatment beginning October 31, 1991. Three groups of plants from each age group were harvested from each vault at each sampling period. Parameters evaluated were fresh weight (g) and the numbers of flowers, turions, and tubers. After measurements were taken, plant material was dried at 60C for 72 hr and dry weights were determined on a whole plant basis.

Data are the average of three replicates for both mature (4 plants/pot) and young (2 plants/pot) plants. Data were initially analyzed by analysis of variance to test for photoperiod and rate effects and interactions. Time was considered a repeated measure. There was a significant ($P < 0.05$) time by rate interaction for all parameters, therefore data are presented for individual harvest dates. Dunnett's "t" test ($\alpha = 0.05$) was used to separate the effect of fluridone rate on tubers and turions. Regression analysis was used to determine the relationship between dry weight and time as a function of fluridone concentration in the young plants. Means are presented with standard errors.

RESULTS

Tuber production in mature hydrilla occurred only under short-day conditions (Table 1). Fluridone at concentrations of 5.0 and 50 ppb reduced tuber formation by hydrilla grown under short days. Fluridone at 0.05 and 0.5 ppb caused an initial stimulation (2 weeks after treatment) in tuber production of 49 and 61% compared to the untreated short-day plants. Flowers were produced under short-day conditions but variability precluded any definitive conclusions to be drawn (data not shown).

TABLE 1. THE INFLUENCE OF FLURIDONE AND PHOTOPERIOD ON TUBER PRODUCTION (number of tubers/plant) BY MATURE HYDRILLA.

Photoperiod[1]	Fluridone (ppb)	Time after treatment (weeks) 2	4	8	12
LD	0	0	0	0	
SD	0	2.6 ± 0.6*[2]	2.7 ± 1.0*	3.2 ± 0.5*	3.2 ± 0.5*
SD	0.05	3.8 ± 0.8*	4.3 ± 0.6*	3.7 ± 1.4*	3.4 ± 0.8*
SD	0.5	4.1 ± 0.8*	2.2 ± 0.3	2.7 ± 0.2*	3.6 ± 0.7*
SD	5.0	0.1 ± 0.1	0.2 ± 0.2	0.3 ± 0.3	0.2 ± 0.2
SD	50	0.5 ± 0.5	0	0	0

[1]LD = long day (16 hr light/8 hr dark).
SD = ambient short day (<12 hr light).
[2]Means are followed by standard errors. Values followed by * are significantly different from the untreated long-day control within each week (Dunnett's "t" test at the 0.05 level).

Mature hydrilla produced fewer turions than tubers in response to short-day conditions with the exception of plants exposed to 0.5-ppb fluridone for 12 weeks (Table 2). Long photoperiod and fluridone at 5.0 and 50 ppb inhibited turion production. The lower rates of fluridone (0.05 and 0.5 ppb) caused an increase in the number of turions with the 0.5-ppb rate causing a five-fold increase after 12 weeks of treatment.

Tuber and turion production by young plants was too low and variable to draw any conclusions (data not shown). In addition, no flowers were produced by the young plants regardless of treatment.

Fluridone did not cause any change ($P > 0.05$) in the dry weight of mature plant (data not shown). Young untreated

TABLE 2. THE INFLUENCE OF FLURIDONE AND PHOTOPERIOD ON TURION PRODUCTION (number of turions/plant) BY MATURE HYDRILLA.

Photoperiod[1]	Fluridone (ppb)	Time after treatment (weeks) 8	12
LD	0	0	0
SD	0	1.6 ± 0.1	1.4 ± 0.7
SD	0.05	2.4 ± 0.8	3.0 ± 0.8
SD	0.5	4.5 ± 2.2*[2]	8.8 ± 1.6*
SD	5.0	0	0
SD	50	0	0

[1]LD = long day (16 hr light/8 hr dark).
SD = ambient short day (<12 hr light).
[2]Means are followed by standard errors. Values followed by * are significantly different from the untreated long-day control within each week (Dunnett's "t" test at the 0.05 level).

plants and plants treated with fluridone at 0.05 and 0.5 ppb grew linearly over the study period (Figure 1). Young plants exposed to 5.0-ppb fluridone also grew linearly ($y = 0.02x + 0.06$; $R^2 = 0.91$) for 6 weeks, but subsequently stopped growing. Plants treated with 50-ppb fluridone did not produce any significant increase in biomass.

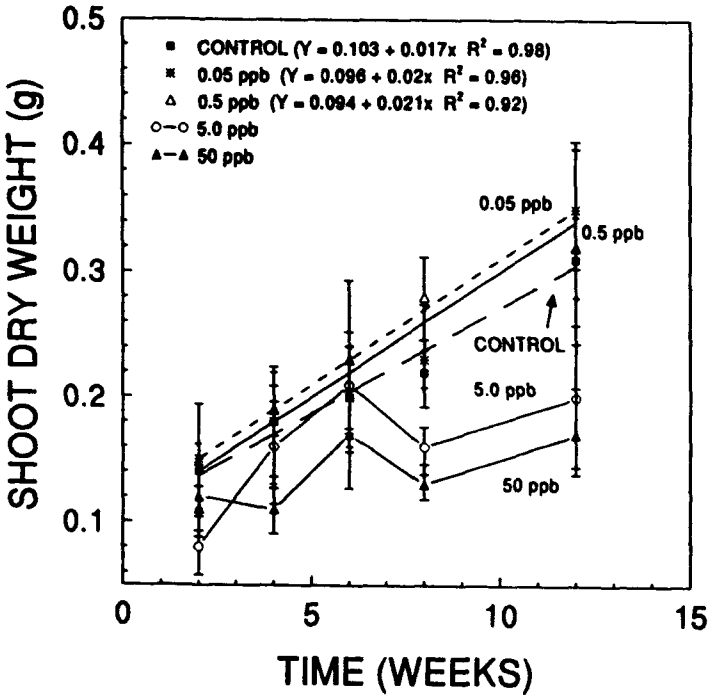

Figure 1. The effect of time and fluridone on the shoot dry weight of young hydrilla plants over 12 weeks.

DISCUSSION

Plants that have been treated with a lethal dose of fluridone generally become bleached white or pink, due to a loss of carotenoids and chlorophyll, and a cessation of growth occurs (2,4). This effect is exacerbated in young plants due to the critical dependency of newly emerging photosynthetic tissue to provide carbohydrate (photosynthate) for the plant and the lack of pre-existing carotenoids at the time of treatment. This could explain the lack of a growth response in mature hydrilla to fluridone over the 12-week period of the experiment. The lethal concentration of fluridone on hydrilla under field conditions is believed to be between 5 and 10 ppb (8). Significant growth inhibition by 5-ppb fluridone took 6 weeks in young plants, but was immediate at 50 ppb. This latter response has been reported previously, but the importance of contact time and concentration have not been fully characterized.

Tuber and turion production in hydrilla has been reported to be a photoperiodic response (23,24), regulated via phytochrome (10). Research has shown that exogenously applied ABA caused tuber and turion formation under long days (24) which indicated that high levels of ABA were involved (either directly or indirectly) in the formation of these reproductive propagules under short days. During this study, fluridone at 5 and 50 ppb inhibited growth and reproduction (tuber and turion formation). Growth inhibition occurred due to the lack of carotenoids but was probably not the cause of inhibiting reproduction. There was no significant tuber initiation at 5- and 50-ppb fluridone, even though vegetative growth occurred for 6 weeks at 5 ppb. Reproduction can occur in the absence of photosynthesis *in vitro*, when sucrose is supplied exogenously (Kane, unpublished data), and sugars required for tuber formation should not have been lacking in the mature plants. Fluridone is often utilized to study the effect of ABA, because of its ability to block ABA biosynthesis (through an inhibition of carotenoid biosynthesis) (15,16). Therefore, it is possible that fluridone at 5.0 ppb was inhibiting tuber and turion formation by lowering ABA to levels below that required for tuber and turion initiation.

At concentrations of 0.05- and 0.5-ppb fluridone, a transient stimulation was observed for young but not mature plants in tuber production. Many plants respond to stress by an elevation of ABA levels (3,9,27) and an increase in or sudden shift toward reproduction. This was probably the case for the 0.05- and 0.5-ppb treated hydrilla plants, where tuber and turion production was initially high due to an increase in the level of stress induced by the herbicide.

In conclusion, regulation of tuber and turion formation in hydrilla may be possible with sublethal rates of fluridone due to the separate mechanisms by which fluridone inhibits growth and reproduction. The critical concentration of fluridone required to potentially cause this effect remains to be determined but appears to be between 0.5 and 5.0 ppb. Due to the mechanism-of-action of fluridone, contact time, light intensity, and carotenoid levels would be very critical to obtaining a growth-regulating versus growth-inhibiting effect.

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Factors Influencing the Efficacy of Glyphosate on Torpedograss (*Panicum repens* L.)[1]

B. E. SMITH, D. G. SHILLING, W. T. HALLER, AND G. E. MACDONALD[2]

ABSTRACT

The effects of burning, disking, and stage of development at the time of glyphosate[3] [(N-phosphonomethyl)glycine] application and a combination of these were evaluated on a natural population of torpedograss (*Panicum repens* L.) during a drawdown of East Lake Tohopekaliga. Disking alone caused an initial reduction of greater than 75% torpedograss in rhizome biomass, and rhizome biomass remained reduced after 1 year by 26% and 44% in 1990 and 1991, respectively. Glyphosate application rates of 1.13, 2.26, and 4.52 kg/ha provided the same level of control regardless of when it was applied or whether the chemical treatment followed disking. Following burning and disking, shoot biomass increased continuously over the 750-day study period. After an initial decrease in rhizome biomass following burning and disking (greater reduction in disked rhizomes), a slight recovery occurred to 20% of the original biomass and then remained constant. The amount of nonstructural carbohydrate in rhizomes increased in the fall suggesting that later herbicide applications might have increased efficacy. Based on these data a single application of glyphosate would provide the best control if applied in the late-fall prior to cold temperatures.

Key words: Disking, burning, mechanical control, stage of development, herbicide, nonstructural carbohydrate.

INTRODUCTION

Torpedograss is a major aquatic and terrestrial weed in the Southeastern United States and other subtropical and tropical regions of the world (3,7,17). Torpedograss has an aggressive growth habit that is supported by an extensive rhizome system. The principal means of dissemination for torpedograss is by rhizomes (4,7,10,15) which are vigorous, competitive, and persistent (7). Torpedograss is especially problematic along shoreline areas of many Florida lakes. Lake margins provide excellent habitat for torpedograss and it forms dense monotypic stands that displace desirable vegetation (16).

The control of torpedograss growing in standing water has proven difficult partially due to the limited amount of herbicide contact (1,14). However, lake levels fluctuate naturally or are controlled artificially. Low water levels provide an exceptional opportunity for intensive torpedograss management. Low water periods expose all the torpedograss foliage allowing better herbicide coverage. Glyphosate is a systemic foliar applied herbicide used for torpedograss control. Generally, glyphosate provides excellent control of many perennial species (9); however, the complete control of torpedograss with a single application rarely occurs (10).

To control a rhizomatous species for a relatively long period, the rhizomes must be destroyed. The herbicide must be absorbed and translocated to the rhizomes in quantities that are phytotoxic (8,18). Glyphosate readily translocates and follows the movement of photosynthates to areas of high metabolic activity (6,12,13). To best control torpedograss with glyphosate, the rhizomes must be the predominant carbohydrate sink at the time of application. A key to improving the control of torpedograss with glyphosate is to better understand the source-sink relationship.

Disturbing rhizomes and allowing regrowth prior to the application of glyphosate has been shown to improve the control of other perennial species (2). Tillage would reduce the stand of torpedograss through direct physical injury, and exposure of the rhizomes to the effects of desiccation, pathogens, and deep burial. Peng and Twu in 1979 (11) reported that disking alone reduced torpedograss biomass by 74%. Furthermore, disking will induce previously inactive axillary buds on the rhizome to produce shoots (3). Additional shoots provide increased sites-of-entry and cause nodes to become active sinks. In addition, by cutting rhizomes into smaller sections, the shoot-to-rhizome ratio will increase. Thus, there will be an increase in the amount of herbicide absorption relative to the mass of rhizome to control.

The objectives of this study were to 1) determine if disking can enhance the activity of glyphosate on torpedograss, 2) determine if time of application influences the

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efficacy of glyphosate, 3) monitor the regrowth of torpedograss following burning and burning plus disking, and 4) assay the seasonal allocation of nonstructural carbohydrates in torpedograss.

METHODS AND MATERIALS

Field studies were conducted during drawdowns of East Lake Tohopekaliga, located near St. Cloud, FL, in 1990 and 1992 on natural stands of torpedograss growing in the littoral zone. In May 1990, a 23-ha field of torpedograss was burned and subsequently one half of this area was disked to a depth of 20 cm. Glyphosate was applied at 1.13, 2.26, and 4.52 kg ae/ha (with 0.5% v/v nonionic surfactant) using a CO_2 backpack sprayer calibrated to deliver 152 L/ha. Untreated plots were included as a control in both the burned and tilled areas. Torpedograss was treated in early July, late July, and August (prebloom, bloom, and late bloom stages of development). Plot size was 15 m^2.

In 1991, burning was not possible, so mowing was substituted for burning. A 1-ha field of torpedograss was mowed and subsequently half this area was disked. Glyphosate was applied at 0.565, 1.13, 2.26, and 4.52 kg ae/ha in the same manner described previously. Due to an unexpected rise in water level only the prebloom treatment was applied.

Shoot and rhizome biomass was collected 1 year after the final treatment in each study. Shoot and rhizome samples were collected from a 1-m^2 quadrat and a 2,000-cm^3 soil core, respectively, from each plot. The soil core sampled to a soil depth of 20 cm. The plant tissue was dried for 72 hr at 70C so that dry weights could be determined.

Time of application and rate were factorially arranged within each of the mechanical practices. The experimental design was a completely randomized block with three replications. The study was conducted twice. Because of logistics, the disking treatment was not randomized and data were analyzed and are presented accordingly. Data were analyzed by analysis of variance to test for main factor effects (rate, stage of development, and year) and interactions. The effects of glyphosate rate were separated using Dunnett's two-tailed "t" test at the 0.05-level of significance. Data are presented as percent (%) inhibition as compared to the untreated control. Mean values are presented with one standard deviation.

Torpedograss regrowth, in areas not treated with a herbicide, was monitored from the burned alone and burned plus disked area from July 1990 through May 1992. Shoot and rhizome samples were collected from a 0.25-m^2 quadrat and a 2,000-cm^3 soil core, respectively. Data are presented as the mean of six replications with one standard deviation.

The rhizome tissue from the soil cores was heat shocked for 1 hr at 100C, then dried at 70C for 72 hr and stored neat for later carbohydrate analysis. Growth parameters measured were shoot number, flower number, shoot dry weight, and rhizome dry weight. Representative shoot and rhizome samples were also analyzed for total nonstructural carbohydrate content utilizing the procedure published by Christiansen (5).

RESULTS

There was no interaction between stage of development or year and herbicide application rate ($P > 0.05$); therefore, rate data are presented averaged across the other two variables. Two months after treatment (based on visual observations), all rates of glyphosate, regardless of other treatments, provided 100% control of treated foliage (data not shown). Regrown shoot and rhizome biomass 1 yr after treatment was inhibited equally by 1.13, 2.26, and 4.52 kg/ha of glyphosate regardless of mechanical practice (Table 1). With the exception of regrown shoots in the disked area, 0.57 kg/ha of glyphosate caused less inhibition of torpedograss than the higher three rates. Rhizome growth was less sensitive to glyphosate than shoot growth in either mechanical practice.

TABLE 1. THE EFFECT OF GLYPHOSATE AND CULTURAL PRACTICES ON TORPEDOGRASS CONTROL IN EAST LAKE TOHOPEKALIGA. THE DATA ARE % INHIBITION BASED ON THE DIFFERENCE BETWEEN SHOOT AND RHIZOME BIOMASS IN CONTROL AND TREATED PLOTS 1 YR POST TREATMENT.[1]

	Disked		Nondisked[2]	
Rate kg/ha	Shoot	Rhizome	Shoot	Rhizome
1.6[3]	89	31	55	56
	(1)	(2)	(5)	(12)
1.1	78	66	88	74
	(5)	(12)	(5)	(4)
2.3	79	55	94	74
	(4)	(15)	(3)	(9)
4.5	82	66	92	68
	(4)	(6)	(2)	(8)

[1]Means represent average of 2 yr, physiological stages of development, and 12 replications each followed by standard deviation. All values are significantly different from respective controls according to Dunnett's "t" test (0.05).
[2]Nondisked area was burned in 1990 or mowed in 1991.
[3]Applied only in 1991.

Burning effectively removed all torpedograss foliage and thatch (initial weight and height of 4725 g/m^2 and 40 cm, respectively). Shoot regrowth was similar in both the burned and disked areas. Regrowth began immediately and continued to increase during the 2-yr study. The final biomass in both areas was approximately 925 g/m^2 which was only 20% (Figure 1). Rhizome biomass was reduced by 66% and 93%

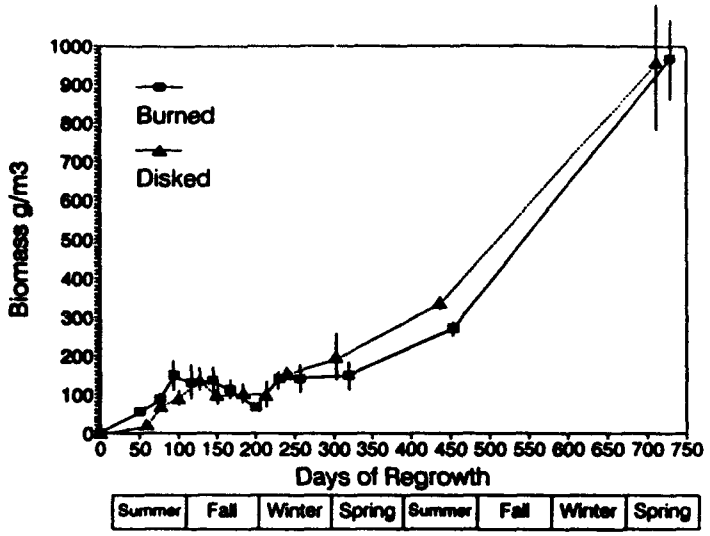

Figure 1. The influence of burning and disking on the regrowth of torpedograss shoots. Data points represent means of six replicates with one standard deviation. At the time of burning, shoot biomass was 4725 g/m^3.

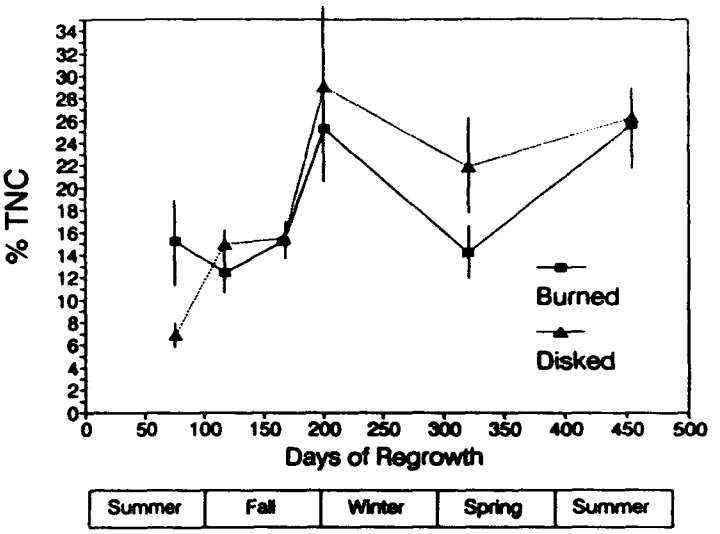

Figure 3. Total nonstructural carbohydrate (TNC) content in torpedograss rhizomes over time in burned and disked areas. Data points represent means of six replicates with one standard deviation.

100 days after burning or disking, respectively (Figure 2). Rhizome biomass recovered to approximately 20% of the original biomass (40 kg/m^3) after 250 days and remained constant through 750 days in both the burned and disked areas.

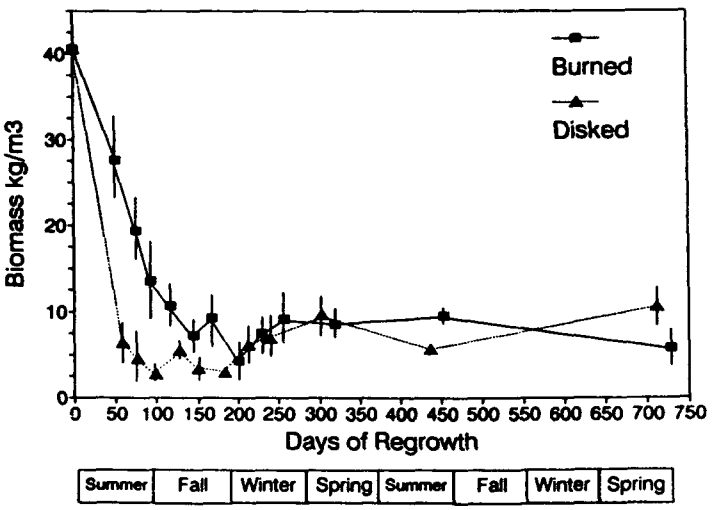

Figure 2. The influence of burning and disking on the regrowth of torpedograss rhizomes. Data points represent means of six replicates with one standard deviation.

Total nonstructural carbohydrate (TNC) contents in the rhizomes were 15% and 7% in the burned and disked areas, respectively, after 76 days of regrowth (DOR) (Figure 3). After 167 DOR (*i.e.* midfall), the TNC content in the rhizomes of the disked area had recovered to 15%, similar to that in the burned area. From the midfall through the beginning of winter (167 to 201 DOR), TNC content in the rhizomes increased sharply in the burned and disked areas. TNC content decreased in both mechanical practices through midspring (320 DOR). TNC concentration increased to 26% from midspring through the end of summer in rhizomes regardless of mechanical practice.

DISCUSSION

Torpedograss was well established in the experimental area and had produced extensive thatch over years of growth. Torpedograss was burned or mowed for four reasons: 1) to provide a uniform population of torpedograss for the evaluation of stage of development as a factor affecting the activity of glyphosate, 2) as a possible mechanical control method, 3) to determine if these mechanical control methods interactively influence the control of torpedograss with glyphosate and 4) to allow for disking to be more effective.

Although all rates of glyphosate provided excellent initial control of torpedograss, long-term control of regrowth was not achieved. In order to obtain long-term control of any perennial plant, the response of rhizomes must be considered. However, data based on rhizome biomass could be misleading as this tissue was present in a low oxygen environment which would reduce the rate of decomposition of dead tissue. This lack of decay would tend to lower control estimates based on rhizome biomass.

The three mechanical practices (burning, mowing, and disking) and stage of development did not enhance the activity of glyphosate on torpedograss. These factors have been previously shown to improve the activity of glyphosate on other

perennial weeds (2). Disking was hypothesized to have worked additively with glyphosate, because disking reduces the amount of viable rhizomes to be controlled by glyphosate. The lack of an additive or synergistic effect was probably due to not applying glyphosate late enough in the year. Percent TNC in the rhizomes markedly increased from midfall through early winter. However, the last application of glyphosate (1990) was made in late summer due to rising water levels. Therefore, had glyphosate been applied during this time of basipetal (*i.e.* maximum percent TNC in rhizomes) translocation, better control might have been achieved. In addition, the higher percent TNC in disked rhizomes indicated a greater translocation potential which would have been exploited if the herbicide application was properly timed.

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Control of *Microcystis aeruginosa* by Decomposing Barley Straw

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ABSTRACT

Growth of the blue-green alga *Microcystis aeruginosa* is inhibited by the presence of decomposing barley straw in laboratory culture to levels of 6% of that achieved in control experiments. The effect appears to be algistatic rather than algicidal. Final biomass in regrowth experiments is independent of previous treatment. Values for regrowth from control treatments ($2.96.10^6$ cells cm^{-3}) were not significantly different from values for regrowth of cells from the most inhibitory treatment ($2.67.10^6$ cells cm^{-3}). Cells inhibited by exposure to straw recovered, achieving the same growth rate as untreated cells when reinoculated into straw-free media. Growth inhibition of 95% can be achieved with 2.57 g straw (dry weight) m^{-3} water. These results are compared to the results of a survey in Great Britain and Ireland on the use of straw to control algae. Decomposing barley straw inhibits the growth of both filamentous and blue-green algal species in all types of water bodies so far assessed. Possible causes of the inhibitory effect are discussed.

Key words: algae, blue-green, growth inhibition.

INTRODUCTION

Problems associated with the development of large blooms of potentially toxin-producing cyanophyte algae have recently become a matter of public concern in the United Kingdom. The generally low rainfall in the U.K. during the past 4 yr has exacerbated algal problems, and the adoption of environmentally sound solutions to the growing number of algal problems in all areas of the water industry is now becoming more important.

Restrictions on the use of herbicides in potable water supplies and some environmentally sensitive areas have encouraged the use of alternative algal control strategies. The presence of decomposing barley straw in water can reduce the growth of a range of algal species under field (Welch *et al.* 1990) and laboratory conditions (Gibson *et al.* 1990). The mechanism by which growth inhibition is achieved is still largely unknown. However, the conditions necessary for the production of the inhibitory effect are now well established as a result of direct experimentation and many field observations. They are, primarily, the maintenance of aerobic conditions in the straw mass and development of a diverse microbial community leading to decomposition of the straw.

Previous observations, in a series of unreplicated field trials, have shown that decomposing barley straw can prevent the growth of *Microcystis aeruginosa* and other blue-green unicellular and green filamentous algal species (Barrett, pers. obs.). The work reported here demonstrates that the growth of *M. aeruginosa* in controlled laboratory conditions can be inhibited by the presence of decomposing barley straw or straw liquor (water in which straw was rotted) (see also Foundation for Water Research 1992).

MATERIALS AND METHODS

Barley (*Hordeum vulgare* var. Atem) straw was added to aged (2 weeks) dechlorinated tap water in fiberglass tanks in a glasshouse on 22 May 1991 at a rate of 1 kg straw m^{-3} water. The tanks were maintained at 20 ± 3C with natural daylight irradiance and continuous aeration provided by an aquarium pump. Samples were taken from the tanks between 78 and 92 days after the start of the aquatic decomposition process. Previous experiments have indicated that this is sufficient time for a significant algistatic effect to be produced (Gibson *et al.* 1990).

A culture of *Microcystis aeruginosa* Kützing emend Elenkin 1924 strain CCAP 1450/6 was obtained from the Cambridge Collection of Algae and Protozoa at the Institute of Freshwater Ecology in Ambleside, U.K. Jaworski's culture medium (JM) (Thompson *et al.* 1988) was prepared with sterile filtered straw liquor, and autoclaved if required. The inhibitory effect was different in autoclaved straw liquor JM. The control medium of JM in dechlorinated aged tap water was always autoclaved. An inoculum culture was prepared 3 days before the experiment to produce cells just before the onset of log-phase growth. This minimized the duration of the experiment, permitted the use of low inoculum cell densities, and made inhibitory effects easier to detect.

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To determine the effect of straw and straw liquor on the growth of *M. aeruginosa*, 5 cm (0.08 g wet weight) of barley straw was added to flasks containing 50 cm^3 sterile filtered or autoclaved straw liquor JM or tap water JM. The same weight of plastic straws was added to control experiments to provide a surface for colonization. Plastic straws do not affect the growth of *M. aeruginosa* (unpublished results). Flasks were inoculated with 7.10^4 cells cm^{-3} of an early log-phase culture, and incubated on an orbital shaker at 75 rpm at 20C and 150 µmol photons $m^{-2}s^{-1}$ for 72 hr. Cells were counted with a haemocytometer in triplicate samples from each flask and growth was expressed as a percentage of growth in control cultures.

To determine cell viability after exposure to straw or straw liquor, aliquots containing an equal number of cells were removed from each treatment. The samples were centrifuged for 2 min and the pellet was resuspended in 10 cm^3 distilled water JM, and the suspension re-centrifuged. The pellet was resuspended in 3 cm^3 distilled water JM, and 1 cm^3 was inoculated into each of three lots of 50 cm^3 in 250 cm^3 conical flasks. The cultures were grown for 72 hr as described above and the number of cells in each flask was counted.

To determine if the inhibitory effect caused by decomposing barley straw exhibited dose response characteristics, straw, which had been rotting for 85 days, was washed five times in distilled water and cut into 1-mm and 1-cm pieces. Straw pieces (not autoclaved) were put into 50 cm^3 autoclaved straw liquor culture medium in conical flasks. There were three replicates of each of the following application rates; 1, 2, 3, 4 and 5 mm and 1, 2, 3, 4, 5, 10 and 25 cm. Control flasks did not have any added straw. The flasks were inoculated with a culture of *M. aeruginosa* growing logarithmically, and cells were counted in all flasks after 72 hr. Growth was expressed as a percentage of control values. A dose response effect was not observed when straw liquor JM was diluted with distilled water in the absence of straw pieces.

To assess the effects of decomposing straw on the growth of blue-green algae under field conditions, a survey was carried out among people who had contacted the authors for advice on the application of straw. Blue-green algae occurred in 47% of the sites surveyed. An arbitrary score was assigned for algal control based on the personal opinions of the site managers or, in some cases, on the basis of cell counts. Data on factors such as area of water body, geographical location, nutrient loading, use, extent of weed problem, straw application rate, duration of control and any associated benefits or problems were collected.

RESULTS AND DISCUSSION

Decomposing barley straw in combination with all the types of culture medium tested inhibited the growth of *M. aeruginosa* (Table 1). The inhibitory effect was enhanced when straw liquor and barley straw were used in combination. Straw liquor and straw in tap water culture medium both produced an effect but to a lesser extent. The inhibitory effect was lost when straw liquor was autoclaved. Loss of inhibition was also noted by Gibson *et al.* (1990) when straw was autoclaved. Stimulation of growth by 93% was observed in treatments of autoclaved straw liquor with plastic straws. This effect has been observed in other unpublished experiments. The compounds released by decomposing straw are a complex mixture of stimulatory and inhibitory factors, and it may be that autoclaving selectively destroys the inhibitory component(s) of the liquor. There was less inhibition in filter-sterilized straw liquor without straw, which may suggest the inhibitory substance is continually produced by the decomposing straw or that it does not have a long persistence time in aqueous solution. This hypothesis is supported by comparing the data for autoclaved straw liquor/barley straw with those for autoclaved straw liquor/plastic straws (Table 1). There is an inhibitory effect with barley straw which is not evident with plastic straws.

TABLE 1. GROWTH OF *M. aeruginosa* IN JAWORSKI'S MEDIUM CONTAINING BARLEY STRAW OR PLASTIC STRAWS EXPRESSED AS PERCENT OF CONTROL VALUES. NUMBERS IN PARENTHESES ARE STANDARD ERRORS OF THE MEAN OF THREE REPLICATES.

Treatment	Growth	
Barley straw/Tap water	30.9	(3.2)
Barley straw/Autoclaved straw liquor	6.0	(1.3)
Barley straw/Sterile filtered straw liquor	11.6	(4.7)
Plastic straw/Autoclaved straw liquor	193.0	(12.6)
Plastic straw/Sterile filtered straw liquor	67.3	(4.7)
Plastic straw/Tap water	100.0	(12.7)

When cells were removed from the treatments and reinoculated into fresh culture media at the same inoculation density, the biomass achieved at the end of 72 hr was the same for each sample (Table 2). This indicates that cells remain viable after exposure to straw in the conditions used in these experiments, and that the effect of decomposing straw is algistatic rather than algicidal.

TABLE 2. GROWTH OF *M. aeruginosa* AFTER EXPOSURE TO DECOMPOSING BARLEY STRAW OR STRAW LIQUOR AND TRANSFER TO FRESH MEDIUM. GROWTH MEASURED AFTER 72 HR AND EXPRESSED AS CELL NUMBERS (10^6 cm^{-3}). NUMBERS IN PARENTHESES ARE STANDARD ERRORS OF THE MEAN OF THREE REPLICATES.

Treatment	Growth	
Barley straw/Autoclaved straw liquor	2.67	(0.30)
Barley straw/Sterile filtered straw liquor	2.31	(0.12)
Plastic straw/Autoclaved straw liquor	1.96	(0.44)
Plastic straw/Sterile filtered straw liquor	2.10	(0.12)
Plastic straw/Tap water (control)	2.96	(0.75)

The degree of growth inhibition of *M. aeruginosa* was dependent on the amount of straw in the flask (Figure 1). At the lowest dose tested, growth was only 5% of that in control flasks. This dose of 2.57 g m^{-3} corresponds well with the minimum effective application rates used in field conditions of three 20-kg bales per hectare.

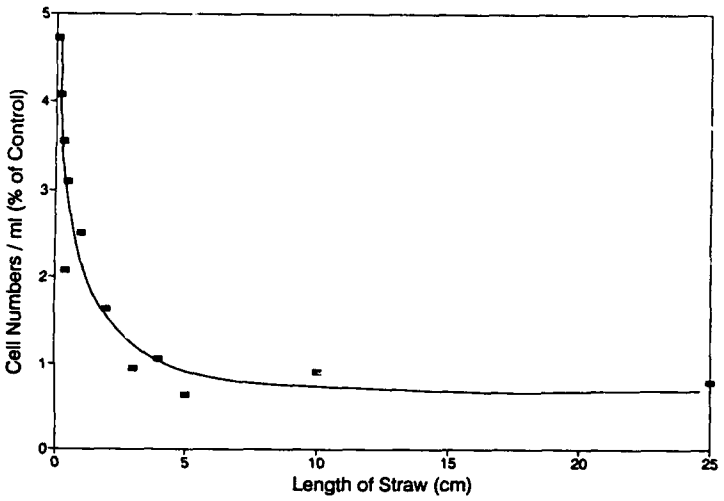

Figure 1. Relationship between length of 92-day-old decomposing barley straw in sterile filtered straw liquor and growth of *M. aeruginosa* expressed as a percentage of growth in control experiments. n = 1. The curve fitted by regression in Lotus Freelance has the equation $Y = 1.97 X^{-0.39}$, $r^2 = 0.87$.

Results from a survey of field sites in which straw has been applied to control blue-green and green filamentous algae, or a mixture of both, are given in Table 3. The survey included a range of types of water body in all areas of the U.K. and Eire. These sites were all static waters and consisted of a mixture of ponds, lakes and reservoirs. Blue-green algae were the main cause of the algal problem in 47% of the sites surveyed. The data were supplied by the site owners or managers, and were based on various assessment techniques of the amount of algal growth before and after the application of straw. Some assessments were made visually and others were based on cell counts. These results are from unreplicated field trials and, as such, information derived from them can only be used to indicate trends. However, the information gained shows that algal control was achieved to some extent in all types of water body but was better in the smaller ponds. This may be an artifact that results from the difficulties of applying straw to large water bodies where inadequate distribution of the algistatic factors could have occurred.

TABLE 3. RESULTS OF A SURVEY ON THE USE OF BARLEY STRAW TO CONTROL ALGAL GROWTH IN GREAT BRITAIN AND IRELAND. VALUES GIVEN ARE THE MEANS OF AN ASSESSMENT OF ALGAL GROWTH CARRIED OUT BY THE OWNER/MANAGER BASED ON A SCALE OF 0 TO 9 (0 = no control, 9 = no algal growth). THERE HAS BEEN NO INDEPENDENT ASSESSMENT OF THE SCORES GIVEN IN THIS TABLE. NUMBER OF SITES ASSESSED IN PARENTHESES.

Type of water body	Mean score	(SE)
Pond (8)	6.75	(0.88)
Drainage ditch (6)	6.17	(0.87)
Lake (15)	6.00	(0.83)
Canal (3)	5.67	(2.85)
Reservoir(4)	3.75	(1.89)
Saltwater pond (1)	9	

The results of laboratory experiments reported here, and by Gibson *et al.* (1990), strongly suggest that the inhibitory effect of straw on algal growth is caused by the release of a chemical during aerobic microbial decomposition of the straw. This chemical, or mixture of chemicals, so far unidentified, is algistatic rather than algicidal. This has implications for control of *M. aeruginosa* and other algae in the field. To achieve long-term control, the straw should remain in the water continuously during the period when algal growth might occur, and should be replaced before it has totally decomposed to keep sufficient concentrations of the algistatic factors in the water.

The response of blue-green algae to straw appears to have different characteristics than those of green filamentous algae. Although the effect demonstrated here is algistatic, blue-green algae appear to have a much shorter survival time than filamentous green algae when exposed to the algistatic factor. When straw was added to a sewage works settlement pond in Australia, containing a bloom of *Anabaena sp.*, the algal population decreased to near zero within a week (M. Hindmarsh, pers. comm.). Similar observations have been made by the authors in a 6-ha lake containing a bloom of *Oscillatoria agardhii*. Before the addition of straw the number of filaments was 10,000 cm^{-3}; 3 weeks after the addition

of straw as anchored bales to the lake, *O. agardhii* was undetectable. In contrast, at least 2 months decomposition was required before control of the filamentous green alga *Cladophora glomerata* became significant (Welch *et al.* 1990).

There may be several reasons for the inhibition of algal growth caused by decomposing straw, which include the production of antibiotics by the fungal flora and the release of straw cell wall components modified during microbial decomposition. The production of antibiotics by soil microorganisms in association with wheat straw residues (McCalla and Norstadt 1974, Wright 1956) has not been examined in aquatic situations with barley straw. However, a dense population of a very wide range of microorganisms (bacteria, fungi, actinomycetes) is associated with decomposing barley straw (Pillinger, pers. comm.) and the possibility of *in situ* antibiotic production cannot be ruled out.

The release of phenolic compounds such as ferulic acid and *p*-coumaric acid from decomposition of straw cell walls, and other aromatic compounds from the incomplete decomposition of lignin may also contribute to the effect. However, assuming that 1% of the carbon in the straw is released as *p*-coumaric acid, the concentration achieved at the dose rates used here would be 2.3 ng ml^{-1}. This is lower than the concentrations reported to reduce algal growth in laboratory conditions (Dedonder and Van Sumere 1971).

The complexity of the mixture released suggests that the cause of the inhibition is not due to any single chemical and that the observed effect may be produced by synergistic interaction of all inhibitory components of the system. This is supported by Rice (1984) and although allelopathy is not a strictly correct description of this situation, some of the chemicals possibly responsible for the inhibition of growth are produced by algae as allelochemicals, notably fatty acids and phenolic acids.

The use of decomposing barley straw to inhibit the growth of algae is increasing as the technique becomes more widely known. The presence of decomposing straw in water can help to prevent the development of blue-green algal blooms in most situations by preventing the rapid increase in population numbers. The survey data reported here and other current laboratory and field experiments provide evidence for the control of both blue-green and filamentous green algae with barley straw under natural environmental conditions.

There do not appear to be any limits imposed by the type of water body on the use of straw to reduce algal problems, and the technique could have a wide application. The conditions which encourage the development of algal blooms, such as high temperatures, also encourage the decomposition of the straw, and a close relationship between production of an anti-algal effect and development of blooms can be envisaged. Work is continuing into the identification of the active principles involved in the inhibition, what aspect of algal metabolism is specifically inhibited, and development and optimization of the method of application to different types of water body.

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Leaf Protein Concentrate from Water Hyacinth

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ABSTRACT

Leaf protein was extracted from water hyacinth and from 16 other aquatic weeds in Thailand. The water hyacinth leaves showed 22.6%, those of morning glory had 29.4% and water chestnut contained only 4.3% protein. Aqueous extraction of the leaves at pH 8.5 was judged suitable for water hyacinth. The water hyacinth protein was further processed into Leaf Protein Concentrate (LPC) using acid and thermal precipitation at pH 4.0 and 82C. The precipitated LPC was rinsed and dried at 60C. Chemical analysis of the water hyacinth LPC indicated 55.4% protein, 3.1% fatty acids, 1.0% fiber, 5.0% ash, and 35.5% carbohydrate. The protein fraction contained most essential amino acids and was particularly rich in leucine (5.1%) and phenylalanine (3.4%).

Key words: *Eichhornia crassipes*, aquatic macrophyte, green protein.

INTRODUCTION

Leaf Protein Concentrate (LPC) contains proteins prepared from disrupted plant cells. The proteins in leaf juice were heat coagulated into green chloroplastic LPC or white cytoplasmic LPC (Telek and Graham 1983).

LPC may be used in vegetarian dishes or as supplementary food. It is likely to be nutritious because of the high protein content, and the content of unsaturated fats, carotenes, xanthophyll, starch, and minerals such as iron, calcium and phosphorus. LPC may also be used in animal feeds for swine, calves, chickens and fish (Telek and Graham 1983).

In a tropical region like Thailand where aquatic weeds are abundant, it would be of interest to determine the possibility of using these plants for the production of LPC. This investigation describes the use of water hyacinth for the preparation of LPC and determines its value as a food or feed.

MATERIAL AND METHODS

Seventeen aquatic weeds commonly found in Thailand including *Wolffia globosa*, *Pistia stratiotes*, *Potamogeton malaianus*, *Typha angustifolia*, *Nelumbo nucifera*, *Eichhornia crassipes*, *Ipomoea aquatica*, *Alternanthera philoxeroides*, *Mimosa pigra*, *Coix aquatica*, *Sesbania javanica*, *Ceratophyllum demersum*, *Chara zeylanica*, *Hydrilla verticillata*, *Eleocharis dulcis*, *Polygonum tomentosum* and *Brachiaria mutica* were collected from a swampy area in a suburb of Bangkok. The leaves were dried at 80C for 48 hr and analyzed for protein content using the Kjeldahl method. *Leucaena leucocephala*, a nonaquatic forage commonly used as feed in Southeast Asia for its high protein value was used for comparison. Water hyacinth was further processed for the preparation of LPC. The leaves, carefully selected from the apex down to the fifth leaf, were washed with water, cut into pieces, and crushed, with water at pH 8.5, having a solid-to-liquid ratio of 1:3, in a blender for 3 min. The slurry was filtered through cheesecloth and the juice separated. The residue was repeatedly ground and all filtered juice combined. The green juice fraction was obtained by centrifugation of the filtered juice at 1465 g for 3 min. It was acidified with 1.0 N HCl to pH 4.0 and heated at 82C for 5 min. LPC precipitated by the heat treatment was separated by centrifugation at 23,500 g for 3 min and washed with 95% ethanol or acetone or water. It was dried at 60C and ground into LPC powder.

Chemical constituents of the LPC were analyzed for protein, fat, ash, fiber, carbohydrate and amino acids.

Data, collected in 1992, on the nutrient concentrations of water in the collection area were obtained from the Royal Irrigation Department, Nonthaburi, Thailand.

Data on LPC were statistically analyzed by the Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

Data on the leaf protein content of 17 aquatic weeds and *L. leucocephala* are summarized in Table 1. Morning glory (*Ipomoea aquatica*) showed the highest leaf protein content (29.4%) followed by the nonaquatic *L. leucocephala* (26.8%). Water hyacinth (*Eichhornia crassipes*) had a somewhat lower leaf protein content of 22.6%, which is similar to data reported earlier (24.9%; Meksongsee 1984). It is generally recommended that a leaf suitable for the preparation of LPC should contain at least 20% protein (Nagy *et al.* 1978). Many of the other aquatic weeds tested here did not pass this initial criterion. However, the ranking of leaf protein content could well change under different growth conditions, where the nutrient concentrations in the water were different.

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TABLE 1. LEAF PROTEIN CONTENT (% dry wt.) OF AQUATIC WEEDS COMMONLY FOUND IN THAILAND[1] IN CONTRAST TO A NONAQUATIC *L. leucocephala*.

Plant types	Protein content
Ipomoea aquatica Forsk.	29.4 ± 0.4
Nelumbo nucifera Gaertn	25.3 ± 0.1
Sesbania javanica Miq.	25.3 ± 1.8
Pistia stratiotes Linn.	25.2 ± 1.6
Alternanthera philoxeroides (Mart.) Griseb.	24.4 ± 2.8
Eichhornia crassipes (Mart.) Solms	22.6 ± 1.4
Hydrilla verticillata Presl	22.3 ± 1.8
Mimosa pigra Linn.	21.7 ± 2.1
Wolffia globosa Hartog & Plas	20.1 ± 0.4
Polygonum tomentosum Wild	19.8 ± 3.0
Chara zealanica Kl. ex Wild	15.5 ± 0.7
Ceratophyllum demersum Linn.	14.2 ± 1.1
Brachiaria mutica Stapf	14.1 ± 2.5
Potamogeton malaianus Miquel	13.7 ± 0.6
Coix aquatica Roxb.	11.7 ± 1.6
Typha angustifolia Linn.	8.3 ± 0.9
Eleocharis dulcis (Burm.f.) Henschel	4.3 ± 0.3
Leucaena leucocephala de Wit	26.8 ± 2.6

[1]Nutrient concentrations of water in the collection area, reported in ppm, are 0.31 (ammonia nitrogen), 0.80 (organic nitrogen), 0.24 (nitrite), 0.38 (nitrate), 0.67 (phosphate), 0.01 (dissolved iron), 0.012 (copper) and 0.099 (manganese).

The preparation of LPC from water hyacinth by aqueous extraction of the leaves at pH 8.5, acidification (pH 4.0) and thermal precipitation at 82C were found to be satisfactory. Final rinsing with an organic solvent, such as 95% ethanol or acetone, gave higher protein yields than rinsing with water (Table 2). The higher protein yields (obtained using acetone or ethanol) were, respectively, 4.3 and 4.6 100 g^{-1} dry leaves. The LPC produced with either ethanol or acetone treatment contained 57% protein, as opposed to the 50.7% protein by

TABLE 2. PROTEIN YIELDS FOR THE LPC PREPARATION FROM WATER HYACINTH. DATA SHOW THE YIELD FOR VARIOUS RINSING AGENTS.

Rinsing agent	LPC yield (g)	Protein (% dry wt LPC)	Protein yield (g/100 g fresh leaves)	Protein yield (g/100 g dry leaves)
Water	1.1[a]	50.7[b]	0.56[b]	3.98[b]
Acetone	1.1[a]	57.3[b]	0.61[a]	4.34[a]
95% Ethyl alc.	1.1[a]	57.0[b]	0.65[a]	4.64[a]

[a,b]Data statistically analyzed by the SAS and are significantly different between a and b.

rinsing with water. It is likely that the use of an organic solvent decreases some of the contaminants such as fat from the LPC as indicated by the chemical analysis of the LPC products (Table 3). The water hyacinth LPC was found to contain not only protein but also fat, ash, fiber and carbohydrates. The fat content can be made lower with the ethanol rinsing or made fat-free with the soxhlet extraction. The reduction in fat content helped reduce the rancidity of the products.

TABLE 3. CHEMICAL ANALYSIS (% dry wt) OF THE WATER HYACINTH LPC TREATED WITH WATER, 95% ETHYL ALCOHOL OR SOXHLET EXTRACTION.

Treatment	Protein	Fat	Ash	Fiber	Carbohydrate
Water rinsing	49.52[a]	10.21[a]	5.63[a]	1.15[a]	33.49[a]
95% ethyl alc. rinsing	55.39[b]	3.08[b]	5.02[a]	0.97[a]	35.54[a]
Soxhlet extraction	61.04[c]	—	4.98[a]	1.02[a]	32.96[a]

[a,b,c]Data statistically analyzed by the SAS and are significantly different between a, b and c.

The protein fraction of LPC was found to contain most essential amino acids (Table 4). It was particularly rich in

TABLE 4. AMINO ACID CONTENT (g/100 g^{-1}) OF THE WATER HYACINTH LPC COMPARED TO THE FAO (1965) PROVISIONAL RECOMMENDATION.

Amino acid	LPC	FAO (1965) provisional recommendation
Ala	3.40	NA
Arg	3.56	NA
Asp	5.05	NA
Cys	0.42	NA
Cys	0.84	NA
Glu	5.90	NA
Gly	3.02	NA
His	1.10	NA
Ile	2.31	4.2
Leu	5.06	4.8
Lys	2.69	4.2
Met	1.27	2.2
Phe	3.39	2.8
Pro	2.72	NA
Ser	2.56	NA
Thr	2.63	2.8
Trp	NA	1.4
Tyr	2.16	2.8
Val	2.79	4.2

leucine (5.1%) and phenylalanine (3.4%), which exceeded the FAO (1965) provisional recommendation (Pirie 1971).

In conclusion, we have found that water hyacinth can be successfully used for the preparation of the LPC. The product appeared to have nutritional values for it contained protein, fat, carbohydrate, fiber and ash. The fat content of the LPC may be reduced after the ethanol or soxhlet treatments. The protein fraction contained most essential amino acids at appreciable concentrations. Therefore, the water hyacinth LPC has a potential of being a good source of protein in food or feed in regions where this aquatic weed is widespread. Further research concerning the animal feeding experiments are recommended in order to determine if antinutritional factors are present.

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PROGRAM EVALUATION

Benefits of the British Columbia Aquatic Plant Management Program

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ABSTRACT

Following about 20 yr of management of aquatic plants in the Province of British Columbia, the rationale, objectives, methods, results and costs of the Okanagan Valley part of that program have been assessed. Eurasian watermilfoil control projects in 16 British Columbia lakes are being implemented by the Water Quality Branch and five local agencies. The Province provides most of the control equipment, gives technical advice on control methods and approaches, provides 75% of the funding, and monitors performance. Local authorities administer control, decide on treatment priorities, hire staff to operate equipment and provide the remaining operating funds. A consultant was selected to review the socioeconomic benefits of management in one of the cost-shared projects, implemented by the Okanagan Basin Water Board in eight Okanagan Valley lakes. The study reviewed available statistical data on the control project and the resources affected by Eurasian watermilfoil. Surveys of over 470 persons measured project effectiveness and benefits. Although treatments are made in only 15% of littoral areas affected in these lakes, analysis showed that control has promoted economic development, that most residents and tourism operators are satisfied, and that control is cost-effective. The analysis projected that termination of the control program (1990 cost $350,000) would lead to about $85 million decline of regional tourism revenues, and affect about 1700 tourism industry jobs and $360 million of real estate values. Recommendations include support for cost-sharing, encouragement for more derooting as a control method and more research on other longer term control technologies, and greater efforts to advise the public on project results.

Key words: Eurasian watermilfoil, Myriophyllum spicatum, economic analysis, impacts, control.

INTRODUCTION

A wide range of technical and scientific approaches have been applied during the evolution of aquatic plant management. Many fields of science and engineering have been used to help managers control nuisance aquatic plants with biological, mechanical and chemical approaches. However, application of economic analyses, especially with studies of social needs and attitudes, is rarely described in context with aquatic plant management (Thunberg 1991, Henderson 1991). Fishery managers historically have used economic assessments to help support their planning, and in some cases aquatic plants are addressed in their analyses (Milon *et al.* 1986).

Ongoing competition for resources now makes it essential that cost-benefit analysis be included with other technical analyses to support recommendations for action to limit adverse impacts of aquatic plants. This paper describes a recent use of economic and social data in analysis of management of Eurasian watermilfoil (*Myriophyllum spicatum* L.; EWM) in British Columbia (B.C.), Canada.

Details of the history and procedures of this program, managed by the Water Quality Branch, B. C. Ministry of Environment, Lands and Parks, were presented by Newroth (1986, 1988, 1990). Major program objectives have included:

a. Aquatic plant documentation and mapping.
b. Reducing spread of noxious exotic species, such as EWM.
c. Developing and evaluating control measures for nuisance aquatic plants.
d. Planning and monitoring cost-shared control programs.

There has been a consistent effort to document demonstration projects, and to learn from successes and failures. Measures of success of preventive approaches to reduce spread of EWM include the degree of public support and satisfaction and the rate of expansion of EWM infestations. While EWM is distributed in a variety of habitats in southern B. C., it is now known only in 80 water bodies of about 2000 that have been checked. The rate of spread apparently has

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been slowed since only 20 new infestations have been found in the past 10 yr. Also, support for intensive management has been demonstrated by local authorities, which contribute 25% of control costs.

In respect to overall cost-effectiveness, the unit cost of treatments has declined from about $4000/ha in 1981 to about $3000/ha in 1990 (excluding equipment purchases and administrative costs).

Five agreements (75% Provincial, 25% local costs) for treatments of EWM in 15 lakes throughout B. C. are now being implemented. In 1991, the Okanagan Basin Water Board received a Provincial contribution of $265,000 to treat 8 Okanagan Valley lakes. In common with other cost-sharing arrangements, this local agency is responsible for administering control, prioritizing treatments, hiring staff and providing the remaining 25% of funds.

Although there was strong political support for continued control (ongoing for a 10-yr period) and good cost-effectiveness was apparent, in 1991 the B. C. Treasury Board requested a socioeconomic study, with a budget ceiling of $20,000.

PROCEDURES FOR ECONOMIC ANALYSIS

Ference Weicker & Company of Vancouver, B. C., was selected to review available statistical data on the control project and to identify the resources affected by EWM. They performed their review in two phases (Anon. 1991). A review of program details and initial meetings with Provincial and local representatives preceded development of a final evaluation plan, used in the second phase, which included:

a. Reviews of available statistical information published by B.C. Ministry of Tourism and local and Federal government agencies on the value of the resources and activities (tourism, water-based recreation, real estate) potentially affected by EWM.

b. In-depth personal and telephone surveys of 13 program representatives (local and Provincial agencies) to identify program impacts, effects, levels of satisfaction and alternatives.

c. Telephone surveys of 60 of the 244 accommodation/boat rental and marina operators located immediately adjacent to affected Okanagan Valley lakes to determine their awareness and satisfaction with the program, the impact that control program termination would have on business, willingness of operators to pay to expand control and their recommendations for program improvement.

d. Telephone surveys of 50 residents selected randomly in major Okanagan cities (Vernon, Kelowna, Penticton, Summerland and Osoyoos) to determine their levels of participation and expenditures in water-based recreation, satisfaction with control, willingness to pay to expand the program, impacts that program termination would have on their visitors, and recommendations for improvement.

e. Interviews of 75 beach users (local and visitors) to determine reactions as for the group above and to define characteristics of their beach use and the attractive and unattractive features of Okanagan beaches. Visitors also were surveyed to determine the impact of water-based recreation on their decision to visit the area, the length of stay and alternative destinations.

f. Analysis of 270 questionnaires (of 1400 distributed to 70 tourism operators) completed by B. C. and out-of-Province visitors to determine the importance of beach recreation in attracting visitors and the proportion of visitors that would have shortened their stay if recreation was restricted by EWM.

g. Interviews of five real estate professionals to estimate values of lakeshore property, based on length of shoreline and market and tax-assessed values of bare and developed waterfront properties.

In addition to consultation with Provincial and local personnel associated with the control program, considerable baseline statistical information already was available to the consultants for the B. C. tourism industry and especially for this recreationally important Okanagan region.

RESULTS AND DISCUSSION

The Ference Weicker report (Anon. 1991) addressed specific evaluation issues; the main results within each area are outlined below.

1. *To what extent are the rationale and intended impacts of the Okanagan Valley control program still relevant?*

EWM can have major impacts on recreation. Areas affected by this plant have increased over the past 15 yr and require continuous control. Despite annual control of 140 to 180 ha of high-use recreational areas since 1982, substantial area remains untreated (about 1000 ha in eight lakes); 37% of tourism operators said EWM impacted their business and 52% of residents indicated that aquatic plants impacted water-based recreation. Generally, both groups surveyed supported the program and believed that a severe impact would result from its termination.

In the Okanagan, population increased 33% in 15 yr, visitors increased 23% in 10 yr and beach-days increased from 3.9 million to about 8.5 million since 1970. Expec-

tations are increasing for better "product quality" and there now is increased competition from other travel destinations. These factors indicate that the resources affected by EWM (*e.g.*, clean, safe beaches) are of increasing value.

2. *What social and economic value is associated with the Okanagan Valley water-based recreational resources affected by EWM?*

Findings in this area included tourism incomes, participation in water-based recreation and beach use, and real estate values. Based on the annual visits of 3 million visitors (who were estimated to have used beaches for over 4 million days), averaging over 3 nights stay and multiplying by the average expenditure of $30/day (including accommodation, transportation and meals), the annual Okanagan tourism revenue totals $320 million. $185 million of this total is estimated to be contributed by B. C. residents and the remainder from outside the Province. Restricted only to visitors in summer months, this tourism revenue is estimated at $182 million.

Interviews of 75 beach users at 8 public beaches showed increasing use of beaches by residents as compared to 1980 surveys and the emergence of parking, aquatic weeds and crowding as unattractive features of beaches. Beach use in 1991 by Okanagan residents was estimated at 4.7 million beach-days, plus over 4 million beach-days by visitors.

Property value for the 1.3 million feet of Okanagan lakefront was estimated by local real estate professionals to exceed $2 billion, or about 23% of the 1992 assessed values.

3. *What local and Provincial economic and social benefits are generated by the control program?*

Ference Weicker estimated that summer tourism revenue was about $130 million, calculated by multiplying the number of visitor beach-days (over 4 million) by their daily expenditure ($32 per person). Based on combined surveys of tourism operators (46 respondents) and a visitor survey (270 respondents), it was estimated that about 47% of party-nights would be lost through failure to control EWM. This would result in loss of about $84 million annually and translate into loss of 1700 employment positions of a total of 4700 tourism jobs in the Okanagan Valley.

About 60% of beach users felt the control program cost of about $5/household in the Okanagan (there were about 89,000 households in this area in 1991) was appropriate. A willingness to pay up to $185,000 extra annually for this program also was determined. Based on realtors' estimates that uncontrolled EWM infestation could cause a 10% devaluation of a "weedy" waterfront property and reduce the overall value of lakefront real estate values by 2%, an impact of $360 million was estimated on property values.

Provincial benefits were estimated by calculating the number of out-of-Province visitors who would not have visited another B. C. region if EWM were uncontrolled; application of this statistic was translated into lost revenue totaling $40 million annually. In turn this would lead to a loss of Provincial revenue (sales, income and room taxes) totaling $3 million.

4. *What is the relative distribution of program benefits to affected groups?*

Ference Weicker found that the control program benefits of about $450 million, including both annual and longer term periods, were split among the tourism operators, the general public and the Provincial government. Tourism operators (transportation, restaurants, accommodation and shopping elements) were estimated to gain about $85 million annually, with about 1700 Okanagan Valley jobs directly related to this economic activity. The general public was estimated to derive nearly $230 million and lakeshore residents about $130 million of the program benefits.

Ference Weicker calculated that impacts to the Provincial government totaling over $40 million annually could be expected to result should EWM control not be continued. This included losses of visitors from outside the Province and the associated local employment, and tax revenues. About 22% of out-of-Province visitors polled in the Ference Weicker surveys indicated that they definitely would not have visited another B. C. region. Also 72% of visitors indicated a probability ranging from 25% to 75% that they might have visited another B. C. region.

Annual Provincial tax revenue losses totaling about $3 million were calculated for general and liquor tax ($2.5 million), room tax revenues (8% rate amounting to $132,000) and corporate taxes ($360,000).

5. *Did the program give the effects intended?*

Control activities have been prioritized in public use areas and Ference Weicker concluded that water-based recreation has been facilitated, furthering tourism and generating increased government revenues. Surveys of 50 residents, 32 resident beach users and 60 tourism operators indicated most were moderately familiar with the program. Tourism operators, who have the greatest personal investment in use of the water and were more

familiar with the program than either other group surveyed, showed the greatest percentage of respondents who were not satisfied. However, 86%, 88% and 80% of residents, resident beach users and tourism operators, respectively, indicated in the surveys that they were moderately to extremely satisfied with the EWM control program.

Respondents expressing dissatisfaction often were unhappy with the length of time between treatments in their area. Also, the polls indicated that dissatisfied respondents had expectations that EWM could be eradicated and did not understand that this was impossible.

Generally the program has been cost-effective based on a number of subjective factors, perceptions by residents and tourism operators and as indicated by the increased public use of many treated areas. Also, Ference Weicker compared the cost of the harvesting element of the Okanagan program with the Metropolitan Seattle harvesting program from 1985 to 1990. They concluded that the Okanagan project cost about half as much as the Seattle project (averaging about $890 for work in Canada, compared to $1604 for work in Washington, both expressed in $U.S. per hectare).

6. *Are there more effective ways to achieve the intended results?*

Ference Weicker recommended expansion of the derooting component of the program to improve the overall level of control. Based on their surveys, they also recommended continuation of Provincial biocontrol research efforts to find long-term solutions to the EWM problem. They noted that survey respondents held misconceptions about the rates and capabilities of control methods and the natural fragmentation of EWM that might influence levels of public support and cooperation. Ference Weicker concluded that this could be resolved by increasing public information.

The present cost-sharing approach was supported and a user fee structure was considered difficult to implement. The need for more continuity in funding levels from year to year was recognized, saving time and permitting multi-year contracts. It was not recommended that private sector contracting be used for major program components. Ference Weicker felt that dependence on contractors might increase risk of delivery of a high quality program and probably would increase costs because of profit margins.

CONCLUSIONS

Provincial revenues attributed to the control program by Ference Weicker ($3 million annually) greatly exceed the direct provincial cost-share contribution ($265,000 in 1991), yielding a Benefit:Cost ratio of 11.3:1. The benefits to local businesses and the public are much greater, and the local Benefit:Cost ratio must be very high, considering that the 25% local contribution to control in 1991 was about $90,000. The overall degree of satisfaction expressed by local residents, visitors and tourism operators constitutes strong endorsement of the Okanagan EWM control program.

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Research Needs for Aquatic Plant Management in Developing Countries

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ABSTRACT

Excessive growth of aquatic weeds limits the sustained performance of many tropical irrigation and drainage systems, with consequent reductions in food and fiber production. A priority theme for the International Program for Technology Research in Irrigation and Drainage (IPTRID) is to improve technologies for channel maintenance which includes aquatic plant management. Field research, technology transfer and training initiatives with emphasis upon the needs of developing countries are required to achieve effective and affordable weed control in irrigation systems. New techniques and adaptations of cost-effective technologies are considered necessary to sustain effective operation and maintenance. Practical solutions for use by developing country irrigation operators are likely to be enhanced by interdisciplinary networks and closer collaboration between scientists, engineers and managers, a primary role of IPTRID.

Key words: irrigation, drainage, maintenance, control, channels.

INTRODUCTION

Population growth and changing food habits pose a major challenge for agricultural production in the 21st Century. Annual yields in irrigated agriculture will have to increase by 3% to meet future demands for food and fiber (World Bank/UNDP 1990). At present a significant proportion of the total global output of wheat and rice is from irrigated agriculture. In 1986, 73% of the gross irrigated area of the world, 253 million ha, was in developing countries. Future yield growth will depend largely on the improvement in productivity of existing irrigation systems since there are limits to expansion of land for irrigation in many parts of the developing world. Moreover, competition from nonagricultural uses is increasingly limiting the quantity of water available for irrigation.

Concern about the poor performance of developing country irrigation systems has been growing for some time with calls for new initiatives and action on policy, management and technology to secure the promised benefits of irrigation (Sagardoy 1982, Le Moigne *et al.* 1989, 1992, ICID 1989). To give new focus to technology, the International Program for Technology Research in Irrigation and Drainage (IPTRID), cosponsored by the United Nations Development Program (UNDP), the World Bank and the International Commission on Irrigation and Drainage (ICID), was created in 1991. IPTRID is a cooperative venture of development agencies and professional bodies in developed and developing countries aimed at promoting and strengthening adaptive technology research in the developing world. While IPTRID does not undertake or finance research, it does assist organizations and developing countries in identifying research priorities and stimulating collaborative research and technology transfer by means of networking and human resource development. Attention is focused on three priority themes. modernizing irrigation and drainage systems, ensuring sustainable land and water use, and improving technologies for maintenance.

IMPACT OF AQUATIC PLANTS ON IRRIGATION

Worldwide, irrigation development has created several million kilometers of delivery canals and main drains (excluding farm channels). Unlined channels provide an excellent environment for aquatic plant growth, with stable water levels and flows, moderate depths (1 m to 3 m), clear water (usually), and a good supply of nutrients. Even in some lined systems it is not unusual to find aquatic plants growing through cracks in the lining or in sediment deposits. Aquatic plants can impede flow and reduce the capacity of the channels to convey water to or from an area. Vegetation growth can lead to water being lost from the reservoirs or channels through increased evapotranspiration and exacerbated seepage. Aquatic plants also pose problems to human health by providing habitats for the vectors of schistosomiasis and malaria.

Many irrigation designs have not taken adequate account of the potential for aquatic weed growth with the result that operational targets cannot be achieved and weed control becomes an excessive burden on already limited maintenance budgets. The control of excessive vegetation growth is now one of the major maintenance tasks facing irrigation and drainage engineers. In Egypt, 78% of the maintenance expenditure

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in 1983/84 was for the removal and control of aquatic plants. Expenditure in that year on excavation, which includes weed cutting and removal, and chemical treatments was equivalent to U.S.$1100/km/year for a combined length of 39,400 km of canals and drains. Data from the Egyptian Ministry of Public Works and Water Resources indicate that the total maintenance budget has increased by 20% per year since 1987/88. Expenditure on contracts for sediment and weed control has grown by 50% per year over the same period.

Maintenance budgets in developing countries still appear to be inadequate for aquatic plant control. For example, in Pakistan, McLoughlin (1988) calculates that the operation and maintenance (O&M) expenditure for irrigation in 1985 probably needed to be four times the amount actually spent to achieve effective control. In Thailand, Plusquellec and Wickham (1985) found that equipment, manpower and budget were all insufficient to carry out maintenance work to the required standard. In many countries inefficient and inappropriate technologies, such as heavy construction equipment and the misapplication of herbicides (Brabben 1988), continue to be used resulting in the need for remedial work which in turn jeopardizes the performance of the irrigation systems and reduces crop yields.

There is little economic analysis and no effective literature about operations and maintenance (McLoughlin 1988). The economic, environmental and social impact of the lack of maintenance or ill-timed, inadequate weed control on irrigation efficiency, equity of water distribution and reliability of supply is not clearly quantified. Quantification of the benefits of maintenance and the costs when it is inadequate is an important step toward planning and managing maintenance activities. Maintenance activities can be more effective if technological improvements with better managerial and institutional arrangements are researched and applied (ICID 1989).

RESEARCH NEEDS

Irrigation and drainage maintenance often receives attention only when construction is complete. Without some operational experience of the system in question there is a tendency to be too optimistic about future system performance. More attention to the establishment and revision of the maintenance plan over the first few years of operation is required. In this way siltation and weed growth can be recognized and their impact gauged with timely identification of the need for further investigation of cost-effective control solutions.

A key factor in implementation of an irrigation maintenance plan is to know how the irrigation or drainage system is functioning. Technical information is needed to determine the hydraulic performance of the system, for which simple and reliable diagnostic methods are required. Background knowledge of how channel systems deteriorate is also relevant so that the correct or optimum level of resources for maintenance can be planned and allocated before deterioration increases beyond repair. For aquatic plants, the aim is to anticipate problems by implementing regular control programs. To be effective irrigation agencies require information or guidance on the effectiveness of particular control actions.

However, in most irrigation communities managers do not always have the information and guidance to make decisions based upon the latest practical technological research. Up-to-date information on machines and herbicides has to be more fully disseminated. The research community has to explain clearly the impacts of different weed control activities and provide policy makers with practical, widely applicable, longer term, biological methods.

Maintenance can involve specialized tasks but may not be carried out properly due to a lack of skilled personnel in existing operational organizations. Large irrigation and drainage undertakings should be able to afford technologically advanced hardware and invest in staff training. However the growing desire to pass the operation and maintenance to farmer groups or water users associations means that it is unlikely that these groups can make such operational investments. Sophisticated maintenance requirements will be beyond the reach of most farmer groups or individual farmers unless high technology solutions can be made manageable.

With adequate training and equipment, the use of herbicides can be safe and effective in most irrigation and drainage channels. However, in many developing countries, these conditions cannot be assumed. Suitable alternative methods integrating a variety of approaches, but offering a flexible response, are probably best for such situations. Egypt, due to environmental concerns over the use of herbicides by unskilled labor, banned herbicides for use in, on or around water channels in 1991. To deal with the aquatic plant problems in the future, existing mechanical and biological methods have to be either extended by the Egyptian engineers or other techniques have to replace what was previously done with herbicides. In the Sudan Gezira, aquatic plants constitute a major constraint to the irrigation system. Sudan's policy has been to emphasize chemical control. However, there is no concrete evidence in Sudan to show that chemical control is superior to any other method either theoretically, economically or environmentally. Other techniques which are more sustainable need to be reviewed for use in Sudan (Ahmed and Abdulla 1992).

Considerable high quality research on the botany of particular aquatic plant species and on ways to control their

spread has been undertaken worldwide. There is still a need, though, to develop, adapt and integrate these research results into techniques that can be applied and sustained on developing country irrigation and drainage systems given the constraints commonly experienced in these locations, limited access to foreign exchange, inadequate supervision and poorly functioning equipment.

If plant control is to be sustained, one approach could be to make canals and drains less suitable environments for aquatic vegetation. A principle of seeking to mimic nature may be appropriate like discouraging the growth of plants in sensitive areas by constructing deeper channels and providing shade (Brookes 1988 and Gardiner 1991). These "soft engineering" techniques, now being used by some river engineers in the USA and northern Europe, can potentially minimize weed control requirements. The construction and management of "environmentally acceptable channels" can provide hydraulically acceptable channels with reduced maintenance costs and a positive environmental impact (HR Wallingford 1988 and Fisher 1992). To be successful these methods will require a better understanding of the plant's growing cycle. "Professionals should recognize the characteristics of physical and biological systems which enhance sustainability" (Gardiner, in press). Guidance on the various techniques and their costs will have to be researched and developed if they are to be adopted by maintenance engineers in developing countries.

The partial cutting of aquatic plants from boats is now practiced as routine in some north European countries (Pitlo 1986). This has the advantage of speed, achieves an adequate conveyance, maintains a diverse aquatic environment and gives savings in cost by reducing staff, machine hours and material handling. Cutting vegetation from the bed of the channel gives the same hydraulic performance as a complete cut of the whole channel and does not involve as much material handling. The maintenance engineer needs guidance to decide on how much plant material to remove from a channel cross-section to achieve desired hydraulic objectives at minimal cost.

Cutting or herbicide applications to pre-empt the growth in the next season may also offer the potential for reducing costs (Westlake and Dawson 1986, 1988). Savings of between 30% and 50% in the annual cost of aquatic plant cutting may be possible. Adaptation of such techniques on irrigation and drainage channels offers the potential to make more effective use of manpower and machines. The research input of aquatic plant specialists in collaboration with irrigation engineers is needed to make this part of a routine maintenance plan.

Research is also needed to identify the most appropriate machinery for aquatic plant cutting and adapt it for use by farmers/farmer groups (IPTRID 1991a, 1991b). Pilot studies in Mexico and Egypt could be envisaged that make practical use of aquatic plant managers from developed countries in twinning arrangements to provide guidelines on how to choose and adapt off-the-shelf machines and herbicide techniques.

Biological control of submerged aquatic vegetation using grass carp (*Ctenopharyngodon idella* Val.) and the use of insects (*Neochtina bruchi* Hustache, *N. eichhorniae* Warner, etc.) to control floating plants have been shown to be effective in many locations (Pieterse and Murphy 1990). However, these methods do not give instantaneous results, unlike cutting or dredging, and engineers and farmers are reluctant to use them for this reason. Under favorable conditions 90% of water hyacinth plants (*Eichhornia crassipes* (Mart.) Solms.) can be controlled by insects within 3 yr (Harley 1990). Clear demonstrations of the benefits, in financial and environmental cost terms, are still required to convince skeptical users. How such methods can be more widely used and incorporated into maintenance activities will require an interdisciplinary approach.

With the increased interest in environmental management the use of West Indian Manatees (*Trichechus manatus* Linnaeus) to control vegetation has been proposed in Surinam where they occur naturally. Reports from Guyana (Haigh 1991) indicate success in keeping channels clear; in general though, little information appears to be available on how to keep and use manatees without endangering them. However in Florida, Etheridge *et al.* (1985) found that manatees were inefficient as control agents. More pilot studies, building on the experience from Guyana and Florida will be needed to develop management guidelines for concurrent plant control and manatee conservation.

Aquatic plants can be harvested and used for fiber and building materials or as food for farm animals or directly for human consumption (National Academy of Sciences 1976). In particular, the removal of water hyacinth plants has yielded source material for compost, fodder, fiber and biogas. In some pilot studies sale of plant material products has helped allay the costs of control and removal, with varying results. Techniques to ease handling and drying require continued research and application if maintenance costs are to be recovered in part.

Information about the susceptibility of particular plants to variations in environment, light, velocity of water, temperature of water and competition from nearby species is not well known by irrigation and drainage engineers (Dawson and Brabben 1991). Application of this knowledge for different approaches to aquatic plant control is worth considering in some circumstances. There is scope for existing networks, such as the Aquatic Plant Management Society, and the

European Weed Research Society to involve more engineers and increase the dissemination of relevant research to the developing world perhaps by working more closely with the ICID and other non-governmental organizations. The establishment and strengthening of networks bringing practicing maintenance professionals together with researchers from the hydraulic, biological and engineering professions, to provide the necessary transfer of knowledge and experience, is vital if irrigation and drainage systems in developing countries are to be sustained.

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J. Aquat. Plant Manage. 31: 218-220

WORKSHOP SUMMARIES

Evaluation of Invasions and Declines of Submersed Aquatic Macrophytes[1]

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ABSTRACT

During the past 60 yr, sightings of aquatic macrophyte species in geographic regions where they had previously not been found have occurred with increasing frequency, apparently due to both greater dispersal of the plants as a result of human activities as well as better documentation of plant distribution. Intercontinental invasions, such as *Myriophyllum spicatum* and *Hydrilla* into North America, *Elodea canadensis* into Europe and *Elodea nuttallii, Egeria densa* and *Cabomba caroliniana* into Japan, have generally been well documented. However, the spread of an exotic species across a continent after its initial introduction (*e.g.*, *Potamogeton crispus* in North America) or the expansion of a species native to a continent into hitherto unexploited territory (*e.g.*, the expansion of the North American native *Myriophyllum heterophyllum* into New England) have received little attention. Natural declines in aquatic macrophyte communities have also received little scientific study although there are many accounts of macrophyte declines. The best-documented example comes from the marine literature where extensive declines of eelgrass (*Zostera*) occurred in the 1930s along the Atlantic coast due to a pathogenic marine slime mold ("wasting disease").

The aim of this workshop was to identify examples of invasions or natural declines of aquatic macrophyte species throughout the world and assess the importance of environmental factors in their control. Forty-five scientists and aquatic plant managers from ten countries participated in the workshop. Eleven of the participants contributed written evaluations of species invasions and declines in their geographic region. These were distributed to registered participants prior to the meeting and served as the starting-point of workshop discussions. To address the topics raised in the working papers, the participants divided into four working groups to evaluate:

1. Environmental controls of species invasions.
2. Biotic controls of species declines.
3. Abiotic controls of species declines.
4. Impact of management practices on macrophyte invasions or declines.

Each working group was asked to identify existing evidence, the need for additional evidence and management implications of their topics and then requested to discuss their findings with the entire workshop at the conclusion of discussions.

1. ENVIRONMENTAL CONTROLS OF SPECIES INVASIONS

While chance was acknowledged as a, if not "the," major factor determining species invasions, the environmental factors determining an invader's success were recognized to vary with scale such that different factors were more important on a continental or macroscale (*e.g.* Europe to North America) than on a regional or microscale (*e.g.* within a particular lake or river reach). On a macroscale, the potential for a species to invade hitherto unexploited territory depends upon opportunity, dispersal agents, and mode of dispersal/reproduction. Once introduced to a new area, its ability to establish and expand appears largely to depend upon climate (temperature and photoperiod). For example, Kunii noted that the northerly limit in Japan for the exotic *Egeria densa* is set by temperature while Chambers observed that the present-day distribution of *M. spicatum* in North America is generally limited to regions with mean annual dewpoint temperatures greater than 35C, suggesting that desiccation survival may limit aquatic plant dispersal in arid regions.

Once an exotic species is already present in a region, its introduction into any particular lake or river reach will be primarily determined by the level of human activity or, to a lesser extent, watershed barriers to dispersal (*e.g.* downstream

[1]This paper is a summary of a workshop held in conjunction with the International Symposium on the Biology and Management of Aquatic Plants, July 1992.

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flow). For example, Madsen noted that most exotics are introduced at sites of public access, particularly boat launches. Once introduced to a specific water body, an invader's ability to establish and expand will be determined by a variety of environmental factors including water and/or sediment chemistry, irradiance, stable water levels (particularly for plants in reservoirs) and water movement (flow or wave action). Disturbance or, conversely, community stability was also recognized as a factor that may contribute to species invasions in that disturbance creates a gap, thereby opening a community to invasion. Disturbance phenomena range in scale from geographic regions (*e.g.* hurricane activity) to entire watersheds or lakes (*e.g.* human development) to within communities (*e.g.* fish nests, turtle trails). Disturbance is not always a precursor to invasion but it may lead to opportunistic exploitation. For example, Nichols noted that in the Upper Great Lakes region, *P. crispus* and *M. spicatum* tended to invade lakes with histories of disturbance as a result of human activity.

To better identify environmental factors influencing species invasions, further research on species autecology and long-term monitoring to detect and track invasions were recommended. However, it is unlikely that intercontinental invasions will ever be predictable since they depend upon dispersal agents. Once an exotic species has become established in a region, it is almost a 100% certainty that it will invade other water bodies in that region. The development of models relating species survival and growth rates to environmental factors may assist in predicting the potential distribution of an exotic species throughout a region.

2. BIOTIC CONTROLS OF SPECIES DECLINES

Presently, there is limited quantitative evidence of biotic controls in species declines due to a lack of "before and after" data. Interspecific competition has often been cited as an important factor in the replacement of native species by exotics. For example, Nichols observed that *P. crispus* and *M. spicatum* had replaced native species in the Upper Great Lakes region, while Bates indicated that *Hydrilla* had displaced *Zostera* and *Najas* in the Mobile River delta, *C. demersum, Cabomba sp., M. spicatum* and *Potamogeton illinoensis* in Lake Seminole, and a variety of native species in Alabama and Georgia. Quantitative data to verify the role of interspecific competition in species declines are limited. However, Madsen reported that the expansion of *M. spicatum* throughout Lake George, New York, coincided with a significant decrease in species richness. The mechanism by which exotics appear to out-compete native species has yet to be elucidated. In addition, further research is required to determine if there are predictable replacement sequences (species A to species B to species C), environmental conditions controlling replacement sequences (*e.g.* disturbance, carrying capacity of the environment) and, in the case of native species, whether these changes in community dominance represent replacements or succession. There is no evidence to indicate the interspecific competition plays a role in natural declines of nuisance exotic species.

In addition to competition, herbivores and plant pathogens may also mediate species declines although little is known concerning these processes. Sheldon noted the decline in *M. heterophyllum* in a New Hampshire lake and *M. spicatum* in a Connecticut and several Vermont lakes was associated with the presence of high densities of aquatic herbivores (weevils and/or aquatic Lepidopteran). Likewise, the reduction in *M. spicatum* populations in some Ontario lakes has been attributed to weevil populations. The importance of plant pathogens in controlling macrophyte declines is even less well documented. The decline of *M. spicatum* in lakes near Madison, WI, has been attributed to Northeast disease, a possible viral pathogen. Shearer reported that research is presently underway to develop a fungal isolate as a biological control agent for commercial use. However, the management of nuisance aquatic macrophytes by herbivore or pathogenic biological control agents will likely be limited by quarantine regulations which restrict the introduction of non-native herbivores or pathogens and by the need for extensive testing to evaluate the action of herbivores or pathogens under conditions which mimic the natural situation with respect to the chemical and physical environment, and the vigor of the host population.

3. ABIOTIC CONTROLS OF SPECIES DECLINES

Few natural declines of aquatic macrophyte species have been studied quantitatively although personal accounts suggest that natural species declines may be common. In studying declines, it is important to identify the time interval (long term (*i.e.* >3 years) versus short term (≤ 1 year) declines) and the specificity (*i.e.*, one species versus all species). A variety of abiotic factors controlling declines have been identified including insufficient light caused by biogenic turbidity or suspended sediments, water movement (flow or wave action), temperature, substrate composition, and nutrient availability. Observations that changes in abiotic factors have brought about natural declines in aquatic macrophyte communities have led to management attempts aimed at manipulating one or more of these factors to reduce aquatic macrophyte growth. Chambers noted that reduced nutrient loading was related to decreased aquatic weed growth in a Canadian prairie river. While some attempts have been successful, difficulties arise because the impact of the factors and their interactions on

aquatic macrophyte growth differ between systems (*i.e.* lakes, rivers, reservoirs, tidal systems). With further research on interactions between abiotic factors and species autecology, life history strategies and resource allocation, manipulation of abiotic factors may become more useful as a management tool. In addition, studies of natural declines may assist in the development of conceptual models relating environmental variables to plant growth, the assessment of natural variability in aquatic plant communities and the development of realistic management goals.

4. IMPACT OF MANAGEMENT PRACTICES ON MACROPHYTE INVASIONS OR DECLINES

While most management practices aim at reducing aquatic plant abundance, it should be noted that efforts are underway in some regions to stock or preserve submerged vegetation, particularly native species. Management practices, be they positive or negative, are a disturbance to the system and can therefore affect susceptibility to invasion by opening a niche for invaders. However, as noted previously, disturbance does not necessarily lead to invasion since "pristine" areas have been invaded and not all disturbed areas have been invaded. Nichols noted that the invasion success of nuisance species appeared to increase after harvesting or herbicide treatment of native plants. However, management has also resulted in the replacement of some exotic species by native or less noxious plants. Bates noted that treatment of *Hydrilla* in Lake Seminole, Florida/Georgia, with fluridone led to the establishment of the native species *Potamogeton illinoensis* or *Chara*.

In addition to its effects on species invasions, management practices can also affect plant declines. Management may sustain exotic populations for a greater number of years than would occur without management intervention. This may relate to the failure of harvested beds to develop a herbivore community. For example, Sheldon noted that beds of *M. spicatum* in Lake Bomoseen that had been harvested for 8 years had significantly less weevils than "no-harvest" sites.

CONCLUSIONS

The environmental factors controlling aquatic macrophyte invasions differ between intercontinental invasions, where invasion success is largely determined by climate, and regional invasions, where the spread of an established exotic species throughout a region is largely a function of human activity.

At present, there is little quantitative information on the role of biotic factors (*e.g.* interspecific competition, pathogens, herbivores) in effecting species declines. In the future, biological control agents may be used to manage aquatic plant populations. However, their use will likely be limited by quarantine regulations which restrict the introduction of non-native herbivores or pathogens.

Abiotic factors have been documented as causing declines in aquatic macrophyte communities. While few attempts have been made to modify aquatic habitats in order to prevent or reduce aquatic macrophyte growth, manipulation of abiotic factors may become a widely used management tool in the future as the role of abiotic factors in the control of natural declines is better understood.

Management practices aimed at reducing aquatic plant abundance can affect the abundance and diversity of nontarget species by promoting the establishment of desirable or nuisance plant species. Management activities may also sustain exotic plant populations for a greater number of years than would occur without management intervention.

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Carbon Fixation and Concentrating Mechanisms[1]

TOM VINDBÆK MADSEN[2] AND GEORGE BOWES[3]

OVERVIEW

The objective of the workshop was to identify and discuss dissolved inorganic carbon (DIC) concentrating mechanisms as they affect inorganic carbon uptake characteristics and growth of submerged freshwater and marine macrophytes. However, it was not anticipated that the discussion would reach any consensus. Rather, it was hoped that the discussion would inspire the participants in their research and help them to identify important new problems to be investigated.

Each of the topics addressed was briefly introduced by a presenter and then discussed by the workshop participants for about 15 min. The topics included the following areas.

WHAT IS A CONCENTRATING MECHANISM?

A concentrating mechanism was defined as a mechanism which increases the CO_2 concentration around the rubisco (ribulose bisphosphate carboxylase-oxygenase) carboxylation site to levels above that in the bathing medium. The rationale for choosing CO_2 as the concentrating carbon species was that it is the species used by rubisco. Concentrating mechanisms generally involve an active process; passive uptake of CO_2 only leads to a higher DIC pool in the cells (providing the pH of the bulk medium is lower than pH of the cytosol), not a higher CO_2 concentration *per se*.

METHODS FOR MEASURING INTERNAL CO_2

The methods which have been used so far to measure internal CO_2 in submerged macrophytes were described, and their advantages and limitations were identified. The methods are based either on measurements of oxygen release into CO_2-free water or on ^{14}C labeling and subsequent extraction of the inorganic carbon from the plant tissue with strong acid. None of these methods measures internal CO_2 directly. It must be calculated from measured (or estimated) internal pH. The ^{14}C method was considered preferable, as it gives a direct measure of internal DIC and also allows for the use of inhibitors to probe the system. The drawbacks are that it is destructive and very time-consuming. For both methods, the uncertainties surrounding measurements of internal pH, especially in regard to specific pH values in the various intracellular compartments, adds a significant degree of uncertainty to the estimation of internal free CO_2 from the DIC actually measured.

PHYSIOLOGICAL ASPECTS: C_4-LIKE SYSTEMS. HOW DO THEY WORK AND WHERE DO THEY CONCENTRATE?

Submerged macrophytes do not have Kranz anatomy or other anatomical features which could separate the C_3 and C_4 carboxylation processes spatially. It was agreed that an intracellular separation between the two carboxylation events is required for the operation of a C_4-type system, and it was suggested that the separation of these processes between cytosolic and chloroplastic components may be the answer. The discussion then focused on mechanisms by which the plants could maintain high internal CO_2 and avoid or counterbalance backflux. Finally, the need for studies of carbon uptake kinetics on isolated chloroplasts and protoplasts, which could give valuable information on the site of concentrating, and techniques to isolate chloroplasts/protoplasts were debated.

PHYSIOLOGICAL ASPECTS: HCO_3^- / CO_2 UPTAKE. HOW DOES IT WORK?

The widely accepted models for HCO_3^- uptake based either on (a) acidification of the cell wall/boundary layer via an ATP-driven pump and subsequent passive uptake of CO_2 derived from conversion of HCO_3^- or (b) a H^+/ HCO_3^- symport mechanism were described. Bicarbonate pumps may not be confined to the plasmalemma, but may also be located at the chloroplast envelope. The possible role of facilitated diffusion of HCO_3^- mediated by anion exchange proteins was discussed. It was agreed that such a passive mechanism could concentrate CO_2 internally only if the pH in the compartment where CO_2 is concentrated is lower than the pH of the bathing medium.

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[1]This paper is a summary of a workshop held in conjunction with the International Symposium on the Biology and Management of Aquatic Plants, July 1992.

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COST EFFICIENCY OF DIC CONCENTRATING MECHANISMS

A theoretical balance sheet for inorganic carbon concentrating mechanisms was outlined. The balance sheet must include costs associated with synthesis, maintenance, and running of the carbon acquisition apparatus in plants with inorganic carbon concentrating systems relative to plants without such a system.

The cost efficiency will change within a canopy, and it is therefore important that the whole organism be taken into consideration when the cost efficiency of inorganic carbon mechanisms are evaluated.

ECOLOGICAL ROLE(S) OF INORGANIC CARBON CONCENTRATING MECHANISMS

The ecophysiological functions of inorganic carbon concentrating mechanisms were defined as the means to (a) reduce the $[O_2]/[CO_2]$ ratio at the site of fixation by rubisco, thus allowing it to operate closer to its maximum; (b) maintain the flux of inorganic carbon into the cell at low external DIC concentrations; and (c) minimize the loss of photorespiratory CO_2. Consequently, species possessing a concentrating system would be expected to have a competitive advantage in (a) habitats low in DIC; (b) habitats with substantial depletion of CO_2 and HCO_3^- during the day due to photosynthetic activity; and (c) dense mats where photosynthetic depletion of carbon is common. The carbon concentrating systems in submerged macrophytes are inducible systems which are activated/induced only under certain environmental conditions and are not always operative. The environmental parameters responsible are not fully elucidated, but the concentration of DIC may play a key triggering-role, as carbon concentrating systems can be induced under carbon limitation or stress. Finally, management implications of the invasion of species with efficient carbon concentrating systems, such as *Hydrilla verticillata* and *Myriophyllum spicatum*, were discussed. Although no consensus was reached, it was suggested that the degree to which this characteristic adds to the weed potential of certain aquatic plants needs to have more attention paid to it.

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New Frontiers in Biocontrol[1]

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ABSTRACT

This workshop was designed to give a perspective on new areas being examined in biological control of aquatic plants and to stimulate a discussion on how these new technologies could be integrated into current management practices. Five presenters provided brief discussions on the following subjects:

1. HISTORICAL PERSPECTIVE ON BIOCONTROL OF AQUATIC PLANTS WITH INSECTS

Dr. Ted Center presented a summary of aquatic weeds introduced into the United States and exotic insects used for classical biological control. These operations in many cases have been successful; however, additional control methods are still needed.

The need for greater interagency involvement/collaboration and the need for additional funding were discussed. Local, state, and Federal agencies need to participate in supporting research efforts, and increased communication between organizations is essential for an effective program. Dr. Center stressed the need to integrate control strategies, and emphasized that research on the use of current and new technologies in combination needs to be aggressively pursued. It was noted that about 20 million exotic plants are imported annually through Florida (Miami) with the risk that further undesirable aquatic plants will "escape" into Florida.

2. HISTORY OF BIOLOGICAL CONTROL OF AQUATIC PLANTS WITH PATHOGENS

Dr. Edwin Theriot reported that to date there has been no successful use of mycoherbicides for aquatic weeds and researchers need to expand the ways in which endemic pathogens can be utilized. Mycoherbicide development has been limited by technology development and funding and,

[1]This paper is a summary of a workshop held in conjunction with the International Symposium on the Biology and Management of Aquatic Plants, July 1992.

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further, the industrial or commercial sector is hesitant to market a product that is restricted in its effect (i.e., attacks only one aquatic weed). Companies feel this is not a cost-effective approach due in part to the costly registration requirements of mycoherbicides and the small size of the potential market.

3. ENDEMIC PATHOGENS FOR CONTROL OF AQUATIC PLANTS

Dr. Judy Shearer noted that the development of effective formulations encounters the same problems faced by manufacturers of aquatic herbicides (*i.e.*, contact time and retention rates needed to produce effects). There is a need to develop formulation technology of mycoherbicides specific for the aquatic environment rather than trying to adapt terrestrial technology to aquatic systems. Approval for field release of a mycoherbicide by the Environmental Protection Agency (EPA) and state agencies may not automatically ensure approval by the USDA-Animal, Plant and Health Inspection Service (APHIS). At present, APHIS does not have a standard set of rules which apply to all applicants, but rather each application is handled on a per case basis. Aquatic plant control achieved in laboratory experiments is not always reproducible in the field. Experimental results obtained from inoculation of aquatic plants raised in greenhouse conditions and grown in small confined units such as columns or aquaria may be unreliable indicators of field effectiveness of a biocontrol agent. Costs of producing experimental mycoherbicides put constraints on the size and number of field plots. The destructive nature of collecting biomass samples in the aquatic environment dictates the need for new technologies for assessing the effect of the mycoherbicide on target species.

4. PATHOGEN QUARANTINE FACILITY AT FREDERICK, MD

Dr. Bill Bruckart reported that over half of the major weeds in the United States are introduced species. The USDA-ARS research facility at Frederick, MD is the only quarantine facility in the continental United States which evaluates pathogens for weed control. The facility has 10,000 sq ft in containment: 7,500 sq ft under glass and the remainder includes laboratories, growth chambers and dew chambers. Although three clear advantages (low maintenance, long-term impact and cost effectiveness) characterize the use of classic biocontrol pathogens, there is a reluctance by regulatory authorities to introduce them. Authorities have to be convinced that the organism is safe, which requires extensive testing and evaluation, and legal and safety issues of introducing pathogens have to be addressed in a uniform manner.

5. THE RELEASE OF EXOTIC PLANT PATHOGENS IN AUSTRALIA

Dr. Wendy Forno noted that conflicts of interest regarding the control of a weed should be resolved before a program is started. In Australia, the "Biological Control Act of 1984" allows for a biological control authority to order a public inquiry if the issue of biological control of a weed is controversial. If the inquiry concludes that control should proceed, or if the authority concludes than an inquiry is unnecessary, the weed can be made a "declared target" which gives legal authority to proceed with the program. It should be standard practice to consider the use of pathogens as well as insects for classical biological control of weeds. It is cost-effective to contract a mycologist and an entomologist to work together in the initial surveys for agents attacking the target weed. It is desirable that these persons have a good botanical background in the family to which the weed belongs. It may be possible to employ a local mycologist to sample for pathogens and thus determine the seasonality of occurrence.

The importation of biological control agents into Australia, or to commence screening agents overseas, requires a carefully prepared document on the candidate insect/pathogen, and the proposed list of plants (80^{+}) to be tested. Approval must be granted by the Australian Quarantine Authority and the Australian National Park and Wildlife Service before the program can proceed. Australia is committed to using pathogens as classical biological control agents. Some have been introduced, others are under host specificity testing and still others are under preliminary investigation.

CONCLUSIONS/SUGGESTIONS

Regulatory authorities in the United States need to be convinced that the classical use of pathogens for biological control of weeds is a safe and effective method.

In new projects, a mycologist and an entomologist should be involved in the exploratory phase of the project.

In old projects, there is a need to revisit native ranges of aquatic weeds and look for pathogens which may be specific and damaging. This is because much of the exploratory work for biological control of aquatic weeds was carried out before pathogens had such a high profile in classical biological control.

Worldwide, $10 to $12 billion are spent annually on investment in irrigation. Aquatic weed problems threaten the

viability of this investment, particularly in developing countries where the use of herbicides is often undesirable and the cost is prohibitive. Successful technology for biological control of aquatic weeds is essential to ensuring a sustainable investment in water use.

There is a world need for control of aquatic weeds. There are projects where countries such as Australia and the United States can share research and development costs of control technology because they have the same weed problem. Results could be transferred with the technology to developing countries where aquatic weeds threaten agricultural production.

There is a need to evaluate the success of biological control of aquatic weeds as it relates to cost. An economist may be the best person to advise on the proper procedure for documenting the value of biological control.

Australia and the United States are committed to collaboration on biological control of floating aquatic weeds. Australia is currently involved in transferring the technology developed to countries in Southeast Asia, Africa and the Pacific basin.

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NOTES