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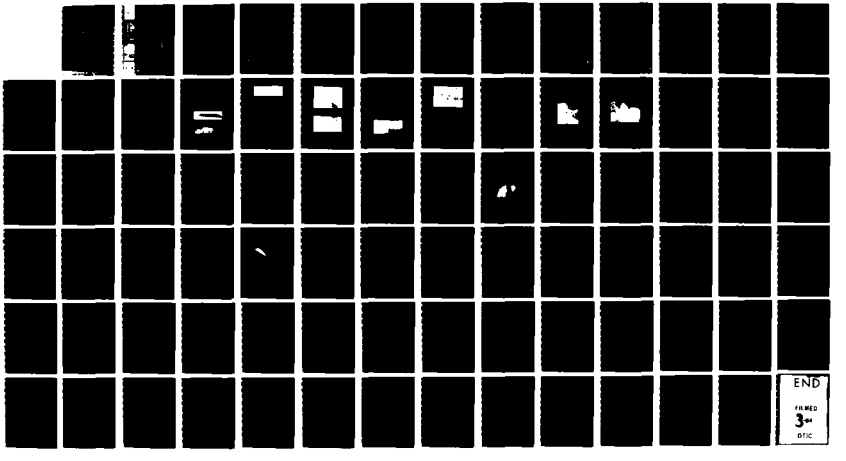

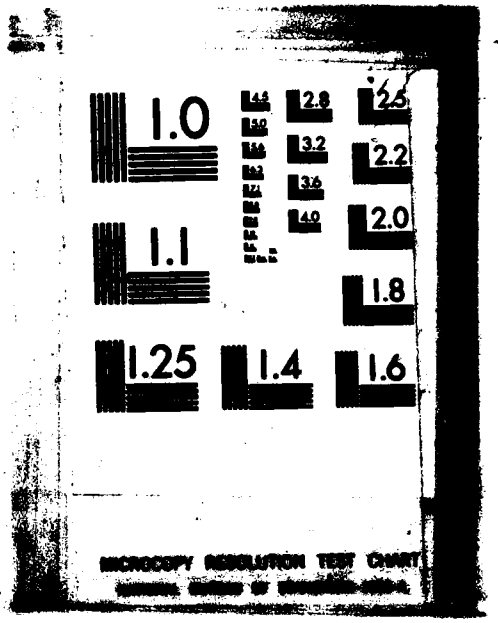
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AQUATIC PLANT CONTROL
RESEARCH PROGRAM

MISCELLANEOUS PAPER A-83-9

U.S. DEPARTMENT OF AGRICULTURE/ CORPS OF ENGINEERS COOPERATIVE AQUATIC PLANT CONTROL RESEARCH— ANNUAL REPORT FOR FY 1981

BIOLOGICAL AND CHEMICAL CONTROL TECHNOLOGIES

by

Aquatic Plant Management Laboratory
U. S. Department of Agriculture
3205 College Avenue
Fort Lauderdale, Fla. 33314

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October 1983
Final Report

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Prepared for Office, Chief of Engineers, U. S. Army
Washington, D. C. 20314

Monitored by Environmental Laboratory
U. S. Army Engineer Waterways Experiment Station
P. O. Box 631, Vicksburg, Miss. 39180

Under Agreement Nos.
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Chapter 1

Classical biological control, i.e., the importation and establishment of a natural enemy (usually an insect) from the home range of the target pest, is a proven technique for controlling some terrestrial weeds. To date all the insects released to control aquatic plants in the United States have been imported from South America to control alligatorweed and waterhyacinth, both natives of South America.

Currently, the United States has no scientists overseas working on biological control of aquatic plants. This is especially unfortunate in view of the long time periods necessary to discover, evaluate, import, and establish a new biological control agent. As a result of the lack of foreign exploration for natural enemies, there are now no exotic insects awaiting release for the other aquatic weeds in the United States. This includes the noxious plant hydrilla.

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20. ABSTRACT (Continued).

In June 1980, the U.S. Department of Agriculture entered into a specific cooperative agreement with the University of Florida entitled "Foreign Search for Biological Agents to Control Aquatic Weeds." The initial searches focused on tropical Asia, which is considered by most experts to be the area of origin for hydrilla and which is an area where the insects associated with hydrilla are poorly known. During the visit to Asia, hydrilla was observed in all countries visited and firsthand knowledge was gained of requirements and difficulties for collecting and testing in each country.

Progress was made in the overall project goal of locating possible biological control agents for hydrilla. Several species of a small aquatic weevil belonging to the genus *Bagous* were collected feeding on hydrilla in south-central India that appeared to be very promising natural enemies.

Potential biological control agents for other aquatic plants were also noted during this trip. Much research has been done in Thailand with the moth *Episammia pectinicornis*, which is very destructive to waterlettuce, *Pistia stratiotes*, and which appears to be very specific to this floating aquaphyte.

This trip was noteworthy not only because of these accomplishments, but also because there were several general indications that natural control agents exist in Asia: (a) hydrilla usually becomes a problem only in recently formed (within the last 20 years) reservoirs; and (b) on the relatively few occasions hydrilla was known to have been established for a long time, it was usually not the dominant macrophyte and was being outcompeted by native vegetation such as coontail, or by more recently introduced plants such as waterhyacinth and *Salvinia molesta*. Thus, while hydrilla may have been abundant in some, usually newly formed, aquatic systems, where populations of its natural enemies may not have yet become established, in general, it appeared to be less abundant and less competitive than in Florida. In view of the tremendous expenditures currently required for partial, temporary control, it would appear highly advisable to more thoroughly investigate the natural enemies of hydrilla in these areas, in case some of them may prove useful in controlling this nuisance in the United States.

Chapter 2

Sameodes albiguttalis, a pyralid moth species, was released in Florida for the biological control of waterhyacinth in 1977. Several populations became well established, most of which were in south Florida, and, by January 1979, the range of these populations began to expand. Within 18 months, *S. albiguttalis* could be found throughout the peninsular portion of the state. The most dramatic dispersal period occurred during mid-summer 1979 when a range extension which averaged ca. 4 km/day occurred. After the dispersal phase, population intensities varied seasonally and geographically and were somewhat dependent upon the type of waterhyacinth plant present. When the data were analyzed in such a way as to remove the effects of plant type, the populations seemed to be higher in the south during the spring and summer than during the winter and fall. The reverse was true in the north and little seasonal variation occurred in the central part of the state. Once populations became established, they persisted throughout all areas of the state in spite of a very cold winter in the northern regions.

Chapter 3

This chapter presents the results for FY 81 of an ongoing program to evaluate chemical formulations to determine their potential as aquatic plant control herbicides.

The objective of this project was to expand evaluation research on the use of chemicals for aquatic weed management in an attempt to discover safer and more effective herbicides and growth regulators.

Recently, several techniques of formulating effective chemicals within various polymer or matrix structures have been developed to provide controlled release over time, allowing a prolonged exposure of target plants to a sustained low concentration of a given herbicide. The effective use of controlled release herbicide formulations (CRHF) appears to hold great potential for long-term management of nuisance aquatic plant growth with much less herbicide required for the same period of activity.

During FY 81, our principal activity was to implement the protocol for evaluating CRHF's of MOE 2,4-D/GMA, Poly GMA 2,4-D, 2,4-D Kraft Lignin, and various formulations of diquat and dichlobenil. Progress on the implementation of the protocol as well as the results of the conventional herbicide evaluation program will be discussed in this chapter.

The polymer GMA 2,4-D was shown to be efficacious in constancy of 2,4-D release in static tests. After an initial "wash-out" during the first few days posttreatment, release rates stabilized at approximately 2.6 mg 2,4-D/g polymer/day in reconstituted water. Complete control of watermilfoil (*Myriophyllum spicatum* L.) was obtained in flowing-water bioassays with poly GMA 2,4-D at treatment rates calculated to maintain constant levels of 0.05 and 0.10 mg/ℓ 2,4-D in the flowing water.

The experimental herbicides DPX-4189 and DPX-5648 provided complete control of waterhyacinth and several other floating and emergent plants at treatment rates of 0.010 to 0.020 kg/ha after 8 weeks posttreatment. The chemicals were taken up readily by both the foliage and roots of waterhyacinth. Also, severe growth retardation was observed at treatment rates of 0.02 to 0.05 kg/ha, suggesting their possible use in combination with a biological control agent in an integrated management program for hyacinth.

DPX-4189 applied up to 20 mg/ℓ did not inhibit hydrilla tuber germination. However, growth and development of the newly germinating sprouts were severely retarded by treatments of 0.01 mg/ℓ or higher. Procedures have been developed for inducing tuber formation by hydrilla under controlled growth conditions in the laboratory. Preliminary evaluations indicated that the herbicides fluridone and DPX-4189 inhibited hydrilla tuber formation under experimental conditions, at treatment rates of 0.05 mg/ℓ and 0.10 mg/ℓ, respectively.

The susceptibility of *Hygrophila polysperma* and *Cabomba caroliniana* var. *multipartita* to aquatic herbicides now available or under development was determined.

PREFACE

The work reported herein was performed under Agreement Nos. 12-14-7001-995 (Biological Control) and 12-14-7001-992 (Herbicide Evaluation) between the U. S. Department of Agriculture (USDA) and the U. S. Army Engineer Waterways Experiment Station (WES). Corps of Engineers funds for the work were provided by the Office, Chief of Engineers (OCE), under Department of the Army Appropriation No. 96X3122, Construction General, 902740, through the Aquatic Plant Control Research Program (APCRP) at WES. USDA funds were provided through the USDA Organic Act of 1862 (5USC511) and the Research and Marketing Act of 1946 as amended (7USC427, 1621). Mr. Dwight Quarles was OCE Technical Monitor.

Principal Investigators at the USDA Aquatic Plant Management Laboratory, Fort Lauderdale, Fla., were: Dr. Joseph K. Balciunas (Chapter 1), Dr. Ted D. Center (Chapter 2), and Dr. Thai K. Van and Dr. Kerry K. Steward (Chapter 3). Dr. L. W. Larson was Associate Area Director, and Mr. Dean F. Davis was Area Director, USDA Florida-Antilles Area.

At WES, the biological control studies were monitored by Mr. Edwin A. Theriot and the herbicide evaluations were monitored by Dr. Howard E. Westerdahl. During preparation of this report, Mr. J. Lewis Decell was Manager, APCRP. Dr. John Harrison was Chief, Environmental Laboratory, WES.

Commander and Director of WES during preparation of this report was COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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INTRODUCTION

For several years, the Corps of Engineers (CE), through the Aquatic Plant Control Research Program (APCRP), assigned to the U. S. Army Engineer Waterways Experiment Station (WES), has conducted a cooperative program of research and evaluation with the United States Department of Agriculture, Science and Education Administration, Southern Region (USDA-SEA-SR). This program consists of two major technical areas: the search for and quarantine evaluation of host-specific insects to control problem aquatic plants, and the laboratory and small-scale testing of herbicides for potential use in the aquatic environment. These efforts are conducted by scientists at USDA-SEA-SR facilities in Gainesville and Fort Lauderdale, Florida.

Until the present publication, each separate research effort was reported and published as a separate APCRP technical publication. In an effort to reduce publication costs, it was decided that all research efforts conducted under the CE/USDA cooperative agreement would be published in an annual report. This publication is the first of the annual reports.

In addition to the cost savings, this effort should relieve the USDA cooperating scientists of the necessity to document their efforts in two formats; one for the CE publications and another that meets their in-house publications requirements. This annual APCRP publication will contain papers submitted in the format serving the USDA requirements. The existence of this report will also serve to document research conducted for funding provided by the Corps of Engineers APCRP.

Chapter 1

OVERSEAS SEARCH FOR INSECTS FOR CONTROL OF AQUATIC PLANTS

by

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OVERSEAS SEARCH FOR INSECTS FOR CONTROL OF AQUATIC PLANTS

INTRODUCTION

Many exotic aquatic plant species have become established in aquatic ecosystems of the United States. Some, such as alligatorweed (*Alternanthera philoxeroides*), waterhyacinth (*Eichhornia crassipes*), Eurasian watermilfoil (*Myriophyllum spicatum*), and hydrilla (*Hydrilla verticillata*), have become serious weed problems, while some very recent introductions, such as hygrophila (*Hygrophila* prob. *polysperma*), are just beginning to show their pest potential.

Currently, the management of these and other aquatic plants relies primarily on herbicides. However, the use of these chemicals is being increasingly restricted for environmental and other reasons, and their cost is becoming prohibitive for wide-scale application. Mechanical methods, while usually less risky environmentally, are so expensive that their use is usually limited to portions of high-priority waters. Accordingly, the use of natural enemies to control aquatic vegetation is receiving increased attention.

Classical biological control, i.e., the importation and establishment of a natural enemy (usually an insect) from the home range of the target pest, is a proven technique for controlling some terrestial weeds with the control of kalamath weed in western United States and of prickly-pear in Australia being the most notable in a long list of successes. Imported insects have also controlled aquatic plants with the control of alligatorweed by the beetle *Agasicles hydrophila* being the best example. Waterhyacinth is now also being partially controlled, at least in Louisiana, by imported weevils, *Neochetina* spp. The most recently released species, the moth *Sameodes albiguttalis*, is now also beginning to show control of this floating nuisance at some of the first release sites in Florida.

To date all the insects released to control aquatic plants in the United States have been imported from South America to control alligatorweed and waterhyacinth, both natives of South America. These successful introductions were primarily the result of work performed at the United States Department of Agriculture's (USDA's) laboratory at Hurlingham, Argentina. Currently, the United States has no scientists overseas working on biological control of aquatic plants. This is especially unfortunate in view of the long time periods necessary to discover, evaluate, import, and establish a new biological control agent. The three insect species imported for the control of waterhyacinth averaged approximately 10 years each from initial discovery to release in the United States.

As a result of the lack of foreign exploration for natural enemies, there are now no exotic insects awaiting release for the other aquatic weeds in the United States. This includes the noxious plant hydrilla, which, since its introduction 20 years

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ago, has become established in almost all of the southern states and which is difficult and expensive to control by herbicides. While some overseas searches for possible biocontrol agents for hydrilla have been conducted, primarily PL 480 projects in India and Pakistan, no agents suitable for importation and release have been found. The natural enemies of hydrilla in its native range remain largely unknown.

In June 1980, USDA entered into a specific cooperative agreement with the University of Florida entitled "Foreign Search for Biological Agents to Control Aquatic Weeds." Most of the funds for this project would be provided by U. S. Army Engineer Waterways Experiment Station, Aquatic Plant Control Research Program. Initial emphasis would be on locating possible biological control agents for hydrilla. Initial searches would be focused in tropical Asia, which is considered by most experts to be the area of origin for hydrilla and which is an area where the insects associated with hydrilla are poorly known.

Replies to questionnaires sent to possible cooperators in Asia, along with advice from colleagues here in the United States, allowed plans to be made for an approximately 4-month trip, beginning in February 1981. The primary objectives of this first trip were to:

a. Learn hydrilla's Asian distribution, recommended collecting areas, etc., during a 3- to 4-day visit with Dr. C. D. K. Cook in Zurich, Switzerland.

b. Visit India, Burma, Thailand, Malaysia, and northern Australia for 1 to 2 weeks each, and meet with possible cooperators in each country who might be willing to assist in later, long-term surveys.

c. Determine costs, travel and collecting restrictions, probable areas for collecting, and other information necessary for realistic planning of an extended survey in each country.

d. Attempt to locate additional sources of funds and technical assistance for extended surveys in each country.

e. If possible, collect hydrilla and its associated insects in each country.

f. Learn of any biological agents in any of these countries which may be useful in controlling aquatic plants other than hydrilla.

g. Spend approximately 2 months on various islands of Indonesia. Repeat objectives *b-f*.

h. If a probable biological control candidate is found in Indonesia, do sufficient host specificity and life history testing to allow organism to be imported into U. S. quarantine facilities for more complete testing.

i. Bring back insects collected on hydrilla as well as voucher herbarium specimens.

j. Identify organisms collected.

k. Consult with experts concerning the feasibility of any of the species collected serving as a biological control agent.

l. Use all information gained in making decision for probable destinations for subsequent trips.

RESULTS

An unexpected delay in obtaining an Indoneasian visa caused a sudden postponement of the trip for 3-1/2 months, with final departure occurring on 21 June 1981. A map of the main routes of the Asiatic portion of the trip is shown in Figure 1. Table 1 presents a list of the collection sites in Asia. This list does not include the many sites inspected, but which were not infested by hydrilla.

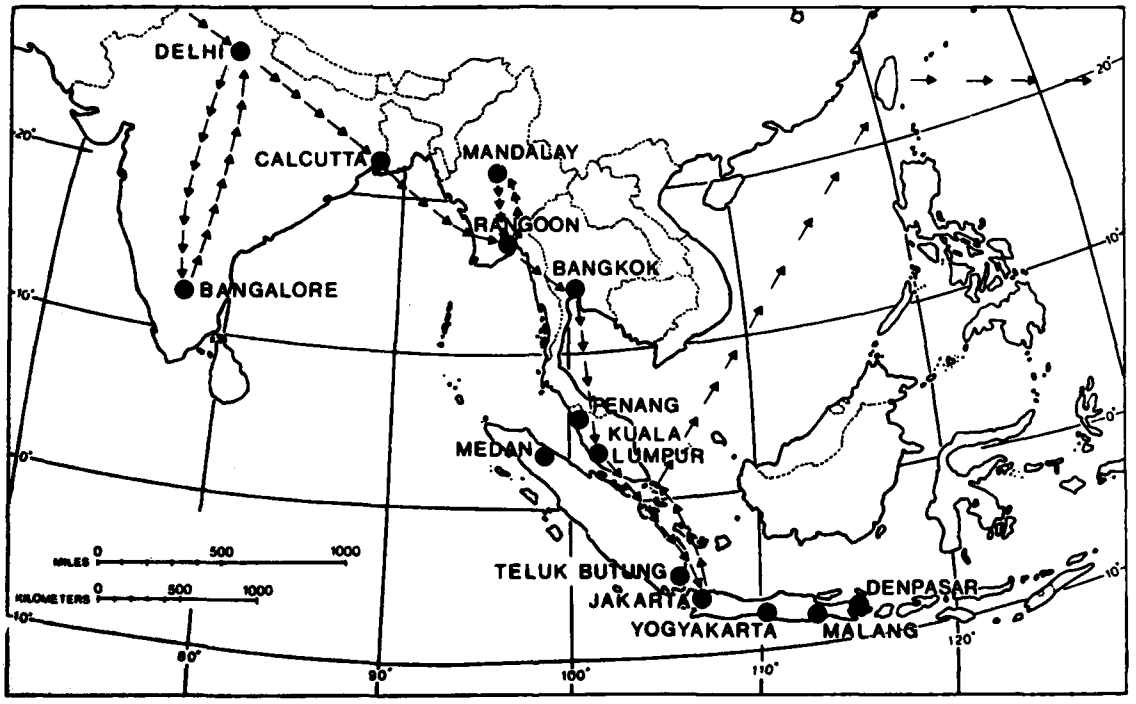

Figure 1. Map illustrating routes of major travels during initial trip to Asia in search of natural enemies of aquatic plants (21 June - 23 October 1981)

A preliminary list of the insects and other organisms collected in Asia is presented in Table 2. This list is extremely preliminary since the identifications are based on knowledge of the U. S. fauna. The more important groups, i.e., probable and possible natural enemies of hydrilla, are being identified by experts. The scanty and scattered taxonomic literature is also being assembled for aquatic insects of tropical Asia. A more detailed list of insect species will then be prepared.

The more important insects found, as well as other pertinent observations, appear in the trip highlights which follow.

Table 1

Collection Sites in Asia

Collection No.	*Host Plant*	*Date*	*Site*	*Location*	*State or City*	*Country*
81KAR201	*Hydrilla verticillata*	01 JUL 81	Arkavarti Stream	12 km N Bangalore	Karnataka	India
81KAR202		01 JUL 81	Kulunanahalli Pond	30 km S of Tumkur		
81KAR203		01 JUL 81	Baragenahalli Pond	Baragenahalli Village		
81KAR204		03 JUL 81	Ummulugodu Pond	Ummulugodu Village		
81KAR205		03 JUL 81	Seshagirihalli Pond	Seshagirihalli Village		
81KAR206		03 JUL 81	Dasappadoddi Pond	Dasappadoddi Village		
81BUR201		10 JUL 81	Inya Lake	Washington Park	Rangoon	Burma
81BUR801	Alligatorweed	10 JUL 81	Inya Lake	Washington Park	Rangoon	Burma
81BUR202	*Hydrilla verticillata*	14 JUL 81	Mandalay Moat	Near SW corner of pond	Mandalay	Burma
81PEN201		31 JUL 81	Irrigation Ditch	Near Balik Pulau Village	Penang	Malaysia
81JAV201		11 AUG 81	Cibinong Pond	300 M Cibinong Village	West Java	Java
81JAV202		19 AUG 81	Curug Reservoir	North end of Reservoir	West Java	Java
81JAV203		27 AUG 81	Rawa Pening Reservoir	Near Village of Tuntang	Central Java	Java
81JAV204		28 AUG 81	Jombor Lake	10 km S of Klaten	Central Java	Java
81JAV205		01 SEP 81	Senggreng Lake	25 km S of Malang	East Java	Java
81JAV206		01 SEP 81	Kediri Canal	3 km N of Kediri	East Java	Java
81SUM201		07 SEP 81	Canal BBGK1	1 km from Lake Sappan	Lumpong Prov.	Sumatra
81SUM501	*Myriophyllum spicatum*	11 SEP 81	Lake Toba	E shore of Samosir Island	N Sumatra	Sumatra
81SUM202	*Hydrilla verticillata*	11 SEP 81	Lake Toba	E shore of Samosir Island		
81SUM801	*Potomageton* sp.	12 SEP 81	Lake Toba	NE shore of Samosir Island		
81SUM502	*Myriophyllum spicatum*	12 SEP 81	Lake Toba	E shore of Samosir Island		
81SUM802	*Potomageton* sp.	14 SEP 81	Lake Toba	SE end of Samosir Island		
81SUM203	*Hydrilla verticillata*	14 SEP 81	Lake Toba	SE shore of Samosir Island		
81SUM204	*Hydrilla verticillata*	15 SEP 81	Kutabaru Canal	Kutabaru Village		
81SUM205	*Hydrilla verticillata*	16 SEP 81	Tanjung Kililing Pond	50 km SW of Medan		

Table 2

Preliminary List of Insects and Other Macroinvertebrates Collected on Hydrilla in Asia (21 June-23 October 1981)

Name	*Country*	*No. of Specimens*	*Collection Numbers*
Suborder Anisoptera (unid.)	Sumatra	1	Sum81205
	Java	1	Jav81201
Gomphidae	Java	1	Jav81205
Libellulidae	Java	3	Jav81201, Jav81202, Jav81204
	Sumatra	2	Sum81204
	India	2	Kar81202, Kar81206
	Malaysia	1	Pen81201
		11	
Suborder Zygoptera (unid.)	Sumatra	1	Sum81204
Coenagriidae (unid.)	Java	3	Jav81201, Jav81204
Pseudagrion rubriceps	Java	7	Jav81201, Jav81203, Jav81205
	Sumatra	15	Sum81204
		26	
Order Ephemeroptera	India	2	Kar81205, Kar81206
Neoephemeridae	Malaysia	1	Pen81201
Caenidae	Java	2	Jav81203
		5	
Order Hemiptera			
Nepidae			
Ranatra sp.	Java	2	Jav81204
Nepa sp.	Sumatra	1	Sum81204
		3	
Order Trichoptera (unid.)	Java	1	Jav81201
Brachycentridae	Sumatra	3	Sum81205
	India	1	Kar81202
		5	
Order Lepidoptera			
Pyralidae			
Paraponyx sp.	India	17	Kar81201, Kar81204, Kar81205
	Sumatra	3	Sum81201, Sum81204, Sum81205
	Java	28	Jav81201, Jav81203.H, Jav81204, Jav81205,
		5	Jav81206
Order Lepidoptera			
Pyralidae	India	5	Kar81201, Kar81203, Kar81205
		53	
Order Coleoptera			
Chrysomelidae	Burma	10	Bur81601
Hydrophilidae	India	3	Kar81202, Kar81206
Curculionidae	India	6	Kar81205, Kar81206
Elmidae	Sumatra	3	Sum81205
Dytiscidae	Java	1	Jav81201
		23	

(Continued)

Table 2 (Concluded)

Name	*Country*	*No. of Specimens*	*Collection Numbers*
Order Diptera			
Stratiomyidae	Java	1	Jav81204
Chironomidae	India	3	Kar81203
	Sumatra	9	Sum81201, Sum81801
	Java	163	Jav81201, Jav81204, Jav81205
		176	
Order Decapoda (unid.)	Java	1	Jav81202
Shrimp			
Palaemonidae	India	1	Kar81203
	Sumatra	13	Sum81201, Sum81204
	Java	3	Jav81202, Jav81203
		18	
Order Delecypoda			
Clams	Sumatra	10	Sum81201, Sum81202, Sum81205
Order Gastropoda			
Hydrobiidae	Burma	1	Bur81201
	Java	12	Jav81201, Jav81203
	India	20	Kar81201, Kar81203, Kar81204, Kar81205, Kar81206
	Sumatra	30	Sum81201, Sum81202, Sum81203, Sum81204, Sum81205, Sum81502
		63	
Order Gastropoda			
Planorbiidae	Java	91	Jav81201, Jav81204, Jav81206
	India	21	Kar81201, Kar81202, Kar81205
	Sumatra	1	Sum81204
	Burma	35	Bur81201, Bur81202
		148	
Physidae	India	4	Kar81201, Kar81205
	Java	1	Jav81201
	Sumatra	3	Sum81205
		8	
Lymnaeidae	India	1	Kar81203
	Sumatra	1	Sum81204
	Java	17	Jav81202, Jav81203.H, Jav81205
		19	
Order Tricladida			
Planariidae	Sumatra	4	Sum81501

HIGHLIGHTS OF ASIATIC COLLECTING TRIP (21 JUNE to 23 OCTOBER 1981)

Zurich (22-25 June)

Dr. C. D. K. Cook, Director, Zurich Botanical Gardens, showed me the botanical gardens, the collection of aquatic plants, and other facilities. We discussed the distribution of hydrilla in Asia and elsewhere and how to distinguish hydrilla from aquatic plants similar in appearance. He provided me with locations of hydrilla from herbaria specimens cited in his forthcoming monograph on *Hydrilla*. Hydrilla is very common in India, especially the southern portion, and Cook believes this would be a good area to search for natural enemies although he does not recall ever seeing any "moth-eaten" hydrilla. He showed me slides of hydrilla and other aquatic plants in Kerala State (on the southwest coast of India). He also provided a list of former students now in Asia who might be able to offer assistance.

New Delhi, India (26-29 June)

Dr. Stanley Stone, Director FERRO, USDA, advised me on medical and other precautions necessary for health and safety while in India. Dr. Stone also expressed a great interest in biological control of aquatic plants and said that he would be glad to see a substantial amount of PL 480 monies (approx. $200,000) devoted to a project in this area. To acquaint me with the problems and procedures in initiating PL 480 projects, he showed me the files of current and previous PL 480 projects in India. It appeared that the Indian government is reluctant to approve new cooperative projects since this entails large monetary appropriations on their part. Also, ongoing projects are frequently delayed or suspended when one of the Indian principal investigators leaves the project to take a position elsewhere.

Bangalore (30 June - 5 July)

Dr. T. Sankaran, head of Indian Commonwealth Institute of Biological Control (CIBC) Station, and I discussed my current project and previous related work in this area, in which he is quite knowledgeable. He stated that Mr. W. Rex Ingram has been selected to head up the new CIBC Laboratory in Kenya. The USDA has a contract with CIBC for a survey of natural enemies of hydrilla in Africa, but research is not expected to begin there until 1982. Dr. Sankaran also provided me with reports of past PL 480 projects concerning aquatic plants in India. He did not believe that a new PL 480 project on aquatic weeds had a chance of approval by the Indian government. He was turned down by them recently on a proposal for biological control of waterhyacinth. However, he believes that embassy PL 480 monies might be used to assist in my survey. He and his assistants could collect insects and other data; do life history studies, feeding tests, etc.; ship the specimens and data; and send the bill to the U. S. Embassy in New Delhi who would pay CIBC with PL 480 monies. While the costs per shipment would be relatively high since

CIBC must charge for all costs (hourly rate for technicians, vehicle costs, overhead, etc.), we would obtain a lot of information with no additional expense to our research budget. He is currently doing the same sort of thing with natural enemies of scales. A similar arrangement might be possible to pay for my use of a CIBC vehicle on a future extended collecting trip.

I spent a couple of days collecting insects on hydrilla at six different locations within 120 km of Bangalore. Although the monsoon usually begins in May in the Bangalore area, it appeared to have "failed" this year. While many ponds that may have had hydrilla are now completely dry, many of the more permanent bodies of water were infested and the hydrilla was easy to observe and collect. Although only a short time (approx. 1 hr) was spent searching through the hydrilla at each site, many insects, including some causing damage, were found. Moth larvae (Figure 2) similar to *Parapoynx diminutalis* were present at most sites. It was very common in the pools of a dried up stream where more than 50 percent of the stems had larval (or pupal) cases. A few larvae of another nymphuline species were also collected.

Small weevils (Figure 3), comprising at least two species of *Bagous*, were found on hydrilla and appeared to be feeding on it. Weevil larvae (Figure 4), probably also

Figure 2. Caterpillar (*Parapoynx* sp.) found feeding on hydrilla growing in a pool of a stream near Bangalore, in south-central India. A similar caterpillar was also found feeding on hydrilla growing in Sumatra and Java. This caterpillar closely resembles the hydrilla-damaging *Parapoynx diminutalis*, an Asiatic species recently established in Florida

Figure 3. Adult of *Bagous* species A. Several species of these small aquatic weevils were collected and appeared to be feeding on hydrilla growing in ponds in Karnataka State in south-central India. Since the New World *Bagous* are usually very host specific and have short developmental times (about 2 weeks from egg to adult), these weevils will be studied in greater detail during a future trip to India, to allow importation to U. S. quarantine facilities for testing required before release in the field

Figure 4. Weevil larvae, prob. a *Bagous* sp., found burrowing in hydrilla stems growing in a pond in south-central India

Bagous spp., were found boring in hydrilla stems. Since *Bagous* spp. are usually very host specific and since they significantly damage hydrilla, they should be investigated further for importation to the United States as possible biological control agents on hydrilla. Dr. Sankaran agreed to ship the plant and insect specimens back to the United States.

Rangoon, Burma (10-13 July)

In Rangoon I met with Mr. Eugene Dorris, Science Officer, and Mr. Saw Laik, Agricultural Advisor, at the U. S. Embassy. Mr. Dorris explained that Burmese government officials must have permission from the Burmese Foreign Minister before meeting with foreigners. It usually takes 2 months to obtain this permission. All Burmese scientists working at Universities, the Agriculture Department, and elsewhere are government employees and therefore cannot have "official" contact with me. Mr. Saw Laik took me to meet "unofficially" with several professors from Rangoon University who are personal friends of his.

I visited aquatic sites in Rangoon vicinity with Mr. Saw Laik and Mr. Hla Mint Phu. Guides are essential here since many areas are considered "sensitive," e.g., a government official lives in the vicinity, and any unauthorized person faces severe interrogation. Hydrilla was present at only one site, Lake Inya, and it showed very little damage from insects or other organisms. Alligatorweed, however, was severely attacked by a chrysomelid beetle (Figure 5), with both the adults and larvae (Figure 6) feeding on it.

While not presently involved with aquatic plants, Mr. Terry Crowe and Mr. Hugh Rendell are interested in biocontrol of pest species, especially waterhyacinth. They agreed to collect aquatic plants and associated insects, especially if we send them information so they can tell what kind of plants they are collecting. In a meeting with Mr. U. Percy Mao, Chief Engineer of Water and Sewer Division, I learned that he is concerned with "underwater waterhyacinth," actually hydrilla. Unfortunately we cannot help him with that. Floating waterhyacinth, *E. crassipes*, ". . . is no problem" since they remove it manually.

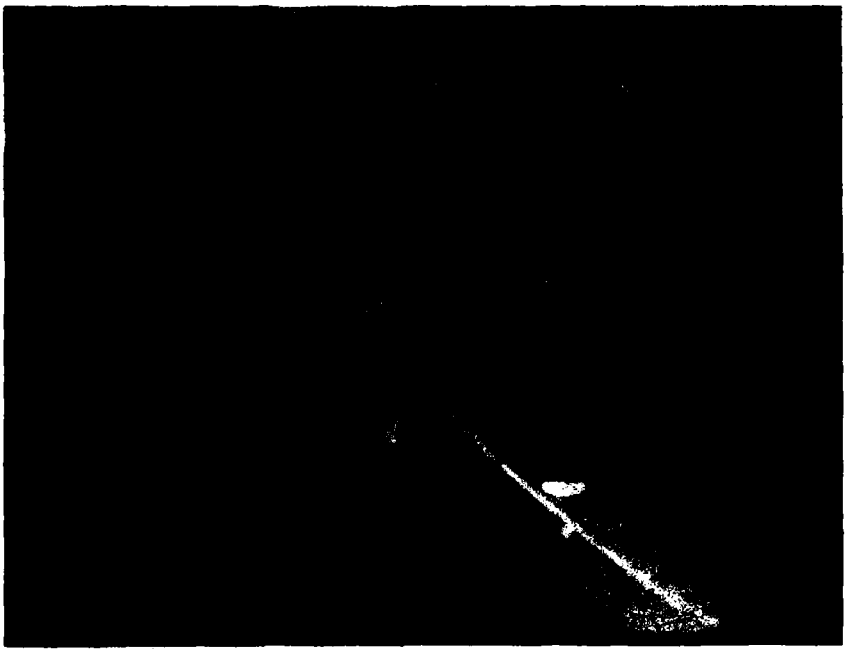

Figure 5. Leaves of alligatorweed, *Alternanthera philoxeroides*, from Lake Inya, Rangoon, Burma, showing feeding damage by a tortoise beetle (Coleoptera:Chrysomelidae). Three of these metallic, golden-green beetles are on the center of lowermost leaf in the photograph

Figure 6. Close-up of the larva of tortoise beetle (Coleoptera:Chrysomelidae) found feeding on alligatorweed growing at Lake Inya, Rangoon, Burma. Both the adults and larvae feed voraciously on alligatorweed

Mandalay, Burma (14-15 July)

In the Mandalay-Sagaing area I searched for hydrilla and associated insects with Mr. Mya Maung. There is a very old (1826) herbarium record of hydrilla from here, but due to heavy monsoon rains and heavy flooding, hydrilla is not present in flooded areas. There are patches of hydrilla in the moat surrounding Mandalay Fort. This hydrilla also shows little evidence of feeding damage, but appears to be possibly infected by a pathogen since the leaves and stems are very brittle and fracture with very slight pressure. The most common aquatic plant in Mandalay Moat is coontail, *Ceratophyllum* sp.

Bangkok, Thailand (16-22 July)

In Bangkok I discussed project goals and related research with Dr. Banpot Napompeth, Director, National Biological Control Research Center (NBCRC).

No hydrilla was found while inspecting aquatic nuisances at lakes 200 km north and 150 km east of Bangkok. The main problem plants were *Mimosa pigra*, *Eichhornia crassipes*, and *Ceratophyllum*. *Mimosa pigra* (Figures 7 and 8) was introduced into Thailand for erosion control, but it has now become a serious problem in aquatic and semiaquatic areas.

On 20 July I inspected the NBCRC facilities and met the personnel. Mr. Wiwat showed me slides of a moth, *Episammia pectinicornis*, specific to waterlettuce, *Pistia stratiotes*, and as effective as herbicide in removing *Pistia*. This moth should receive serious consideration for importation to the United States and Panama as a biological control agent for *Pistia*. In the afternoon, I met Ms. Saewanee Thamsara, head of Thailand Department of Irrigation, who is quite

Figure 7. ***Mimosa pigra*** **infestation in a shallow lake near Saraburi in central Thailand. This shrub is a recent introduction in Thailand and has now become the number one aquatic nuisance in that country. This plant has not yet been introduced into the United States**

Figure 8. Flowers and fruit of *Mimosa pigra*

knowledgeable about aquatic plants. She knows many locations in Thailand where hydrilla is present, but most are in northern Thailand. She made an appointment for one of her assistants to take me to a hydrilla infestation 200 km south of Bangkok. She has many species of aquatic plants growing in concrete pools behind her office. In one pool, we found a moth larvae, similar to *P. diminutalis*, on hydrilla although it appeared to be more abundant on the *Potomageton* sp. also growing in the same pool.

Bumbong Lima, Malaysia (29-31 July)

Mr. Baki, Dr. Supaad Mohd (head-of-station), and other MARDI staff members informed me that several people in the biology department of Malaysia University of Science (USM) are working on biological control of aquatic weeds and I should meet with them. Mr. Baki said that, by the far, the most important aquatic weed in Malaysia is waterhyacinth. Coontail is the most common submersed plant. Hydrilla appears sporadically at some locations, mostly in northern Malaysia but none nearby.

On 30 July I met with Dr. Ivor G. Caunter, Dr. Raj, Dr. Tan, and other faculty at the USM Biology Department. Dr. Ivor has a grant to study the utilization and control of waterhyacinth. As a plant pathologist, he has primarily been looking at pathogens of waterhyacinth, but has also enlisted the help of an entomologist colleague, Dr. Raj, to study the insects. He is very interested in extending his study to include hydrilla and he agreed to hire a technician who will collect hydrilla insects and send them to me in the United States.

A hydrilla infestation on Penang Island showed little damage. No insects which might feed on it were found.

Central Malaysia (2-4 August)

On 2-4 August I drove a rental car from Penang Island on the west coast, south through central Malaysia, then to Kuantan on the east coast, then back to Kuala Lumpur (see Figure 9). Various aquatic habitats were inspected, but no hydrilla was found. Because of the Hari Raya holidays, most hotels were booked and almost a quarter of the total of 1400 km was driven at night.

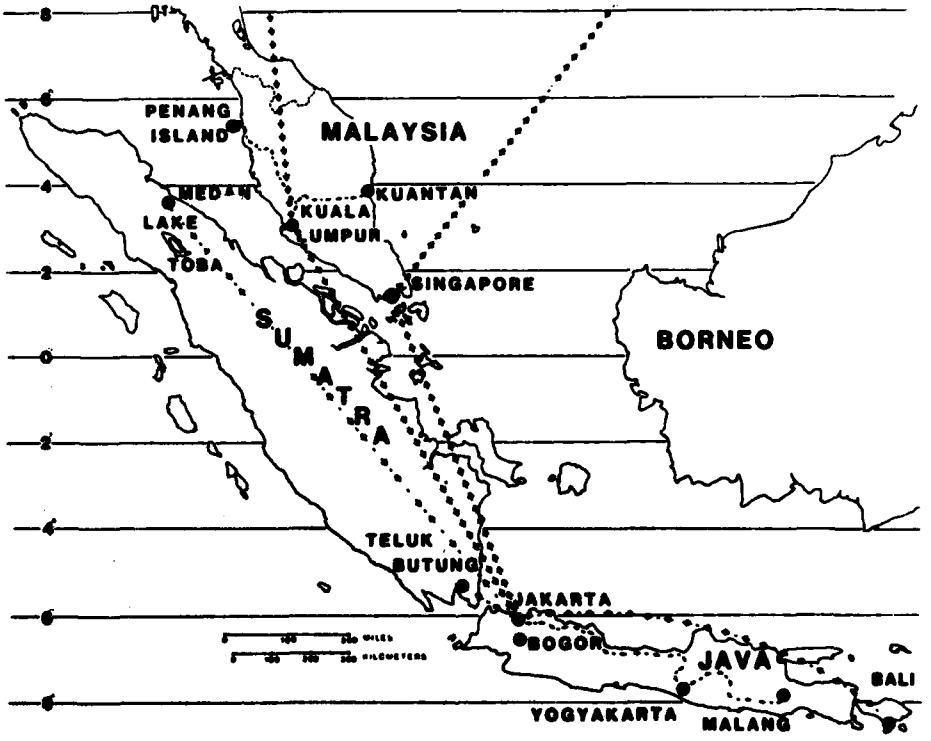

Figure 9. Map depicting major travels in peninsular Malaysia and the Indonesian islands of Java, Sumatra, and Bali in search of natural enemies of aquatic plants

Jakarta, Indonesia (6-8 August)

In Jakarta I met with Allan Trick, Dan Conable, and other staff members at the Agricultural Office of the U. S. Embassy. Dr. Karnandi, Assistant Director of SEAMEO Regional Center for Tropical Biology (Biotrop), drove me to the Biotrop headquarters near Bogor, about 60 km from Jakarta, where I met with Dr. Ishemat Soerianegara, Director of Biotrop, and other administrators. Here I was introduced to Mr. Kasno, Biotrop scientist, who has some experience with biological control of aquatic plants with whom I will be working.

While collecting with Biotrop staff near Bogor and other West Java sites, a moth larvae similar to *Parapoynx diminutalis* was found to be damaging hydrilla at most sites. Caddisfly (Trichoptera) larvae were abundant at one site but did not appear to be damaging hydrilla.

An extended collecting trip (24 August - 4 September) was taken with Mr. Kasno and Biotrop driver to central and east Java (see Figure 9). Hydrilla infestations were found causing problems in new reservoirs and irrigation systems. *Parapoynx diminutalis* was damaging hydrilla at all sites. Midge larvae (Chironomidae) were found associated with hydrilla at approximately half of the collecting sites, but did not appear to be causing damage. Eggs of a waterscorpion (Hermiptera:Nepidae) were found inserted into stems of hydrilla (see Figure 10) at a central Java site. While this caused hydrilla to fragment more easily, it did not otherwise seem to damage the plant.

Figure 10. Egg of a waterscorpion (Hemiptera:Nepidae) inserted into hydrilla stem growing in central Java. While this oviposition site may cause easier fragmentation of the hydrilla stem or serve as an entrance point for a pathogen, the hydrilla is otherwise unharmed by waterscorpions since they are predators on a wide variety of aquatic fauna

Sumatra (7-18 September)

In Sumatra (see Figure 9) almost 1 week was spent collecting with Mr. Aderis, Biotrop technician, at Lake Toba, the largest lake in Southeast Asia. A deep (500 m), volcanic lake at an elevation of over 900 m, Lake Toba had a variety of

submersed plants, mostly confined to narrow bands along the shoreline. Eurasian watermilfoil, *Myriophyllum spicatum*, and a pondweed, *Potomageton* sp., were the most noticeable submersed plants. Waterhyacinth, *Eichhornia crassipes*, was the dominant floating plant, while *Mimosa pigra*, introduced less than 10 years ago, was well established along the shoreline and in shallow water. While both the milfoil and pondweed reached the surface even from depths exceeding 5 m, hydrilla was usually prostrate on the hydrosoil and could usually be located only by diving. This unusual habit of the hydrilla appeared to be due to severe grazing of the apical portions of the plant (probably by a fish) resulting in a stunted, sprawling plant (see Figure 11). Insects at Lake Toba were extremely rare in all samples of the three submersed plant species examined.

Figure 11. Hydrilla, *Hydrilla verticillata* (right), Eurasian watermilfoil, *Myriophyllum spicatum* (center), and a pondweed, *Potomageton* sp. (left), collected from the same vicinity of Lake Toba, North Sumatra. Note the short, stunted appearance of the hydrilla. This specimen was among the largest found. All specimens of hydrilla exhibited severe grazing of the apical portions (probably by a fish) resulting in short, prostrate plants that usually could only be located by diving

Parapoynx diminutalis **larvae (or a similar species) were damaging hydrilla at all other Sumatra infestations examined. Some sites also had Chironomid and Tricoptera larvae associated with the hydrilla. Again, there was no persuasive evidence that either of these were damaging the hydrilla.**

Denpasar, Bali (1-3 October)

In October I examined aquatic habitats in Denpasar vicinity for hydrilla. No hydrilla was located. Since it was just before the beginning of the monsoon season, many aquatic systems were dry. The use of ducks seemed to be quite effective in keeping aquatic plants controlled in smaller irrigation systems.

DIFFICULTIES IN CONDUCTING RESEARCH IN TROPICAL ASIA

Preliminary preparations before initiation of actual travel were time-consuming. Long delays in communications with potential cooperators in the countries to be visited were common. Visa requirements for each country varied considerably and numerous itineraries, letters of recommendation, photographs, etc., had to be submitted. A research visa for Indonesia required a *minimum* of 6 months to process and the procedure resembles a job application; a resume, career and research goals, list of publications, letters of recommendation, and a local scientific sponsor are among the requirements. The University paperwork necessary to obtain permission to travel and travel advances entailed over a month of preparation and several months of processing.

Maintenance of personal health became a primary concern when traveling in Asia. Malaria is prevalent in almost all areas visited and a rigid malarial prophylaxis program had to be adhered to. This provides protection against nonresistant strains of the most common malaria, but does not prevent infection by any of the other three species of malaria. Other parasitic diseases are common as well as those such as cholera and typhoid usually related to poor hygenic conditions. However, these major diseases were, after arriving in Asia, usually relegated to the back recesses of consciousness since day-to-day survival becomes dependent on preventing gastrointestinal infections. My diet had to be restricted to prepared food, preferably still hot, and to beverages bottled by "approved" companies or boiled or treated water. Better, more expensive "tourist" hotels were generally considered to have far safer accommodations and food than those utilized by locals.

While diet and other hygienic considerations were a primary concern, the amount of research, especially fieldwork, was severely limited by several other factors. Communication, especially by telephone, was slow and unreliable, as was transportation. A trip to a collecting site 160 km away would frequently involve 2 to 3 days of travel. Permission to collect at a given site was usually considered essential, and several days would be spent making appointments and in personal meetings with reservoir managers and other officials.

Philosophically and practically, time is not considered to be very valuable or important in the Orient. In order to reconfirm an airplane reservation, you must go to the airport 1 or 2 days prior to the flight and have your name written on the flight manifest. Then you have to report to the ticket counter 2 hours before the scheduled

flight (3 hours before international flights) and wait for a departure which will invariably be late, sometimes 4 to 6 hours late.

Other, more minor difficulties, were encountered due to a lack of facility in the many languages encountered (India has 40 major languages while Indonesia has some 250). Also the large amount of equipment, about 50 kg, frequently posed a problem, especially when passing through customs.

SUMMARY

This initial trip to Asia was very successful. Since the potential area to be explored for natural enemies was enormous, and since I had never been in Asia and did not know what difficulties to expect, some of the primary aims of this trip were to learn the distribution of hydrilla in various parts of Asia, the difficulties in collecting and testing insects associated with hydrilla, and become acquainted with scientists living in these countries who might be able to offer assistance on future trips. All these goals were achieved. Hydrilla was observed in all countries visited and firsthand knowledge was gained of requirements and difficulties for collecting and testing in the country. Scientists who would be willing to lend assistance were located in each country. In Burma, however, the amount of assistance that can be provided by Burmese scientists is severely restricted by the Burmese government. In Malaysia, a cooperative project to search for hydrilla insects was initiated with Dr. Ivor Caunter at Malaysia's University of Science.

Progress was also made in the overall project goal of locating possible biological control agents for hydrilla. Several species of a small aquatic weevil belonging to the genus *Bagous* were collected feeding on hydrilla in south-central India that appeared to be very promising natural enemies. Dr. Charlie Obrien of Florida A&M University, who is the world authority on this genus, says that most *Bagous* are extremely host specific, have short generation times (around 2 weeks), are attracted to black-lights, and all immature life stage are usually confined on or in the plant host. These are ideal characteristics for a potential biocontrol agent. During my next trip to Asia, I plan to test the *Bagous* weevils in India to see if they are, in fact, host specific. If they are sufficiently host specific, I will ship living specimens to quarantine facilities in Gainesville, Florida, for more complete testing. Since weevils are frequently very destructive to their plant hosts and with the weevil *Neochetina* spp. now demonstrating control of waterhyacinth at some locations in the United States, we are encouraged by the discovery of these natural enemies of hydrilla.

Potential biological control agents for other aquatic plants were also noted during this trip. Much research has been done in Thailand with the moth *Episammia pectinicornis*, which is very destructive to waterlettuce, *Pistia stratiotes*, and which appears to be very specific to this floating aquaphyte. Since waterlettuce is a major problem in the Panama Canal, and is ranked as the third most noxious aquatic weed in Florida, and since it is a major aquatic pest in other

southern states, we believe that this insect should be seriously considered for importation and release in the United States. We have begun to assemble information to support the application to import this insect into quarantine for further testing. Once received into quarantine, this insect may receive clearance for release relatively rapidly (1 to 2 years) in view of the extensive prior testing conducted in Thailand.

This trip was noteworthy not only because of these accomplishments, but also because there were several general indications that natural control agents exist in Asia: (a) hydrilla usually becomes a problem only in recently formed (within the last 20 years) reservoirs; and (b) on the relatively few occasions hydrilla was known to have been established for a long time, it was usually not the dominant macrophyte and was being outcompeted by native vegetation such as coontail, or by more recently introduced plants such as waterhyacinth and *Salvinia molesta*. Thus, while hydrilla may have been abundant in some, usually newly formed, aquatic systems, where populations of its natural enemies may not have yet become established, in general, it appeared to be less abundant and competitive than in Florida. In view of the tremendous expenditures currently required for partial, temporary control, it would appear highly advisable to more thoroughly investigate the natural enemies of hydrilla in these areas, in case some of them may prove useful in controlling this nuisance in the United States.

Chapter 2

DISPERSAL OF *SAMEODES* WITHIN THE PENINSULAR PORTION OF FLORIDA AND THE EFFECTS OF SEASON, LATITUDE, AND WATERHYACINTH MORPHOTYPE UPON INFESTATION INTENSITIES

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DISPERSAL OF *SAMEODES* WITHIN THE PENINSULAR PORTION OF FLORIDA AND THE EFFECTS OF SEASON, LATITUDE, AND WATERHYACINTH MORPHOTYPE UPON INFESTATION INTENSITIES

INTRODUCTION

Holm et al. (1977) rank waterhyacinth as eighth among the world's most serious weeds. Because it is distributed primarily in the tropics, underdeveloped countries face the greatest problems from this weed, which include interference with virtually every conceivable use of water resources (Holm 1969). Although effective herbicidal controls are available (Little 1968), these are often impractical because of the vast acreages frequently involved (Little 1965). Environmental concerns over the use of potentially toxic substances in potable water make the registration of new herbicides for use in aquatic systems difficult. Rising world oil prices have resulted in higher costs for presently available herbicidal products as well as increased application expenses. It is, therefore, desirable to reduce the present dependence upon the chemical control of aquatic weeds and, in response to this, the U.S. Department of Agriculture (USDA), in cooperation with the U.S. Army Corps of Engineers Aquatic Plant Control Research Program (APCRP) and the Florida Department of Natural Resources (FDNR), have undertaken the task of developing a program of biological control.

The early work on the biological control of aquatic weeds has been reviewed by Blackburn, Sutton, and Taylor (1969) and Andres and Bennett (1975). Surveys in South America identified several insects potentially useful for the biological control of waterhyacinth (Bennett and Zwolfer 1968; Perkins 1974) and life history studies and host specificity tests were conducted for a few of these species (Silveira-Guido and Perkins 1975; DeLoach 1976; DeLoach and Cordo 1976a,b, 1978; Deloach et al. 1980; Cordo and DeLoach 1978; Perkins and Maddox 1976). These studies have thus far led to the release of three insect species in the United States. The first two were the weevils *Neochetina eichhorniae* Warner and *N. bruchi* Hustache, released in 1972 and 1974, respectively (Perkins and Maddox 1976; Perkins 1973). The third was the pyralid *Sameodes albiguttalis* (Warren), first released in 1977 and reported as well established by 1979 (Center and Durden 1981).

Following the initial establishment of the two weevil species, a great deal of emphasis was placed upon operational aspects of the dissemination of these insects in Florida (Zeiger 1979) and Louisiana (Manning 1979). These were extremely labor-intensive exercises by aquatic plant management agencies in which weevils were field collected or reared in greenhouses and thousands were

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released at hundreds of sites in these two states. These two species appear to be extremely slow to disperse and this effort was probably necessary to ensure a maximum dispersion of the insects in a minimum amount of time. In one 33-ha lake, for example, almost 2 years was required for *N. eichhorniae* to become relatively evenly distributed throughout the lake after the release (unpublished data).

In contrast to the *Neochetina* spp., *S. albiguttalis* disperses rapidly. Population numbers increase quickly because of their relatively short generation time and high fecundity. They also seem to be strong fliers and rapid dispersal away from release sites has been noted (Center and Durden 1981). From this early information, it appeared that an extensive operational collection and release program would not be required for the wide dissemination of this insect. The survey described in this paper was designed to determine if this speculation was accurate. Within 18 months after we first noted extensive dispersal beginning, *S. albiguttalis* had spread throughout peninsular Florida.

METHODS AND MATERIALS

Waterhyacinth plants within the original release sites were closely examined at frequent intervals to determine the state of development of the founder populations. When pupae were first noted, adjacent sites were examined for signs of larval activity. Larvae are more likely to feed on certain forms of the plant (Center and Durden 1981), and, by concentrating on examining the proper plant morphotype, the time required to ascertain the insects presence or absence could be reduced to a manageable level.

The original 20 release sites were located in three general areas. These three areas were considered loci from which dispersal could take place. After it had been determined that some local movement of the populations had begun, zones of interception were established and waterhyacinth populations in these zones were intensively monitored for signs of the presence of *S. albiguttalis*. The oldest site was in central Florida on the Pinellas Peninsula, which is nearly surrounded by Tampa Bay and the Gulf of Mexico. The rivers which empty into the bay include the Manatee, Little Manatee, Alafia, and Hillsborough and are across the bay from the release site. Only the Manatee and Hillsborough Rivers had large aggregations of waterhyacinth. Hence, these two rivers to the east and Lake Tarpon to the north were used as monitoring areas to determine when the insects had begun to disperse away from the Pinellas Peninsula.

Most of the release sites were located in south Florida and this constituted the second of the three population loci. Populations were well established in this area and all were located south of U.S. Route 84 primarily in the extensive canal systems of the Everglades Conservation Areas. Extensive monitoring was conducted in the area north of this highway in order to determine when northward dispersal had begun.

The third population locus was in Gainesville in north-central Florida at Lake Alice on the University of Florida campus. Additional waterhyacinth populations occurred primarily to the south and east of this site. Monitoring sites included Biven's Arm, Orange, Newnans, and Lochloosa Lakes as well as Payne's Prairie and the interconnecting canal systems.

After the presence of *S. albiguttalis* was established in the interception areas, sites well in advance of the advancing population fronts were examined. The search area was thus repeatedly expanded until areas were included in which *S. albiguttalis* could not be found and which were peripheral to the known populations. When that point was determined, sites were examined from there back towards the known populations until the limits of the range of the insect were determined. Then, areas progressively farther away were reexamined more intensively to ensure that *S. albiguttalis* could not be found. Although it was not possible to test the accuracy of this system, it appeared to be very efficient and repeatable and provided a good estimate of the distributional limits of the populations.

Other sites, which were not necessarily near to known infestations of *S. albiguttalis*, were examined in a less systematic manner. These examinations were somewhat opportunistic in nature and, as time and resources allowed, as many sites as possible were examined as often as possible. This was to determine whether the insect was scattering in random pattern away from known populations rather than in a regular radial expansion.

Each site was accurately plotted on a 1:250,000-scale map of Florida and coded to the three possible results of the examinations. At any specific site, waterhyacinth may not have been present, waterhyacinth may have been present but no evidence of *S. albiguttalis* found, or both waterhyacinth and *S. albiguttalis* may have been found. After these data were plotted, a grid was superimposed on the map. The grid corresponded with the U.S. Geological Survey 7.5-min quadrangles and was identified by a coordinate system. Each quadrangle was then coded as to whether or not *S. albiguttalis* was present at any site within it and the date that its presence was first verified. At the end of the study, the coordinates and the time the insect had been present within each quadrangle were plotted as a contour map on an outline of the state using the SYMAP system (Dougenik and Sheehan 1979). The data for the north, central, and south areas of the state were mapped separately using a third-order polynominal smoothing routine. This produced isolines that estimated the limits of distribution on various dates. These contours were then transferred to the large-scale map and used to estimate dispersal rates.

In March 1980, a second survey was begun in an effort to derive quantitative information on the distribution of *S. albiguttalis* along a north-south transect that extended the entire length of the state. Ten sites were selected within 1/2° latitudinal intervals (Figure 1) and waterhyacinth plants were sampled on a quarterly basis in these areas. All sites were sampled within a contiguous 4- to 5-week period every 3 months over a period of 15 months. Occasionally, upon

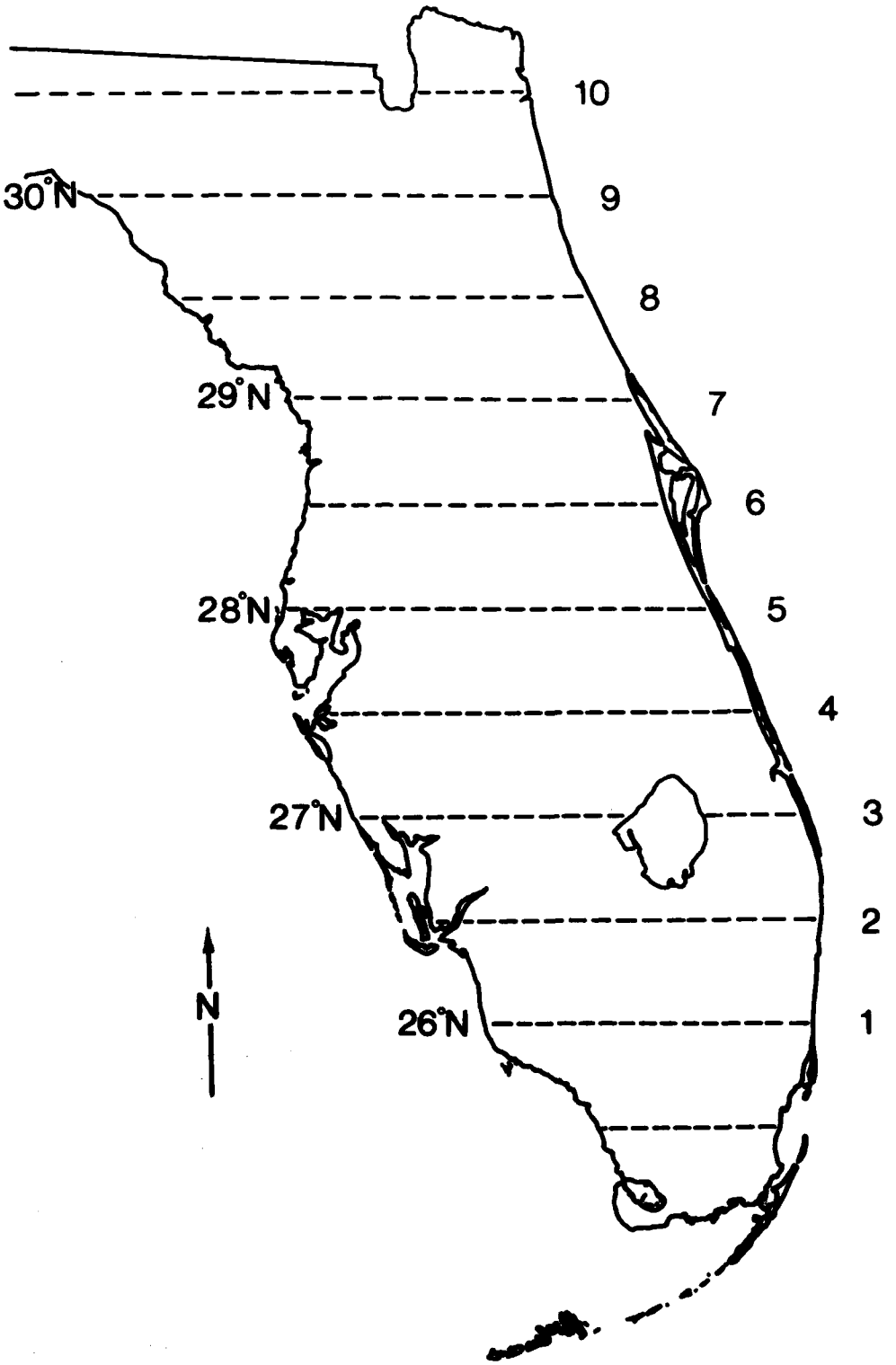

Figure 1. Map of Florida showing the location of each latitudinal zone at which quarterly quantitative estimates of the *S. albiguttalis* population intensities were derived. Sites were located predominantly along the east coast and as close to the 1/2° latitude interval as possible

returning to a site, the waterhyacinths would not be present. In these instances, alternative sites would be selected within the same latitudinal zone.

Samples were collected at each site by slowly piloting an airboat along the edge of the mat and manually grabbing clumps of plants at ca. 20-m intervals. The first 20 plants withdrawn from each clump were examined closely for signs of *S. albiguttalis* damage and 10 such samples were collected. Hence, at each site, an estimate of the percentage of the plants damaged based upon a total of 200 plants was derived. Based upon findings from a previous study (Center and Durden 1981), sampling was confined to areas where the plants appeared to be suitable for *S. albiguttalis* so that the data would reflect differences due to the abundances of the insects rather than the form of plant. Root length, leaf length, lamina length, lamina width, petiole length, and petiole width (Figure 2) were measured on ten plants, one randomly selected from each sample, to confirm that the plants represented similar morphotypes. The leaf measurements were always of the third youngest leaf. In addition to the original variables, the ratio of the lamina length to width, lamina length to total leaf length, root to leaf length, and petiole length to diameter were considered.

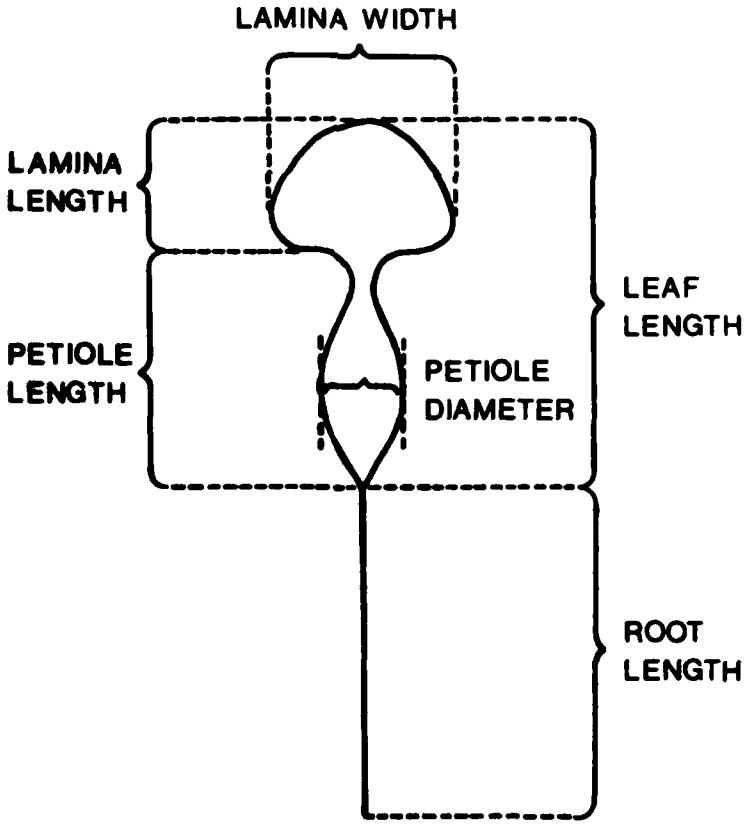

Figure 2. Schematic diagram of a waterhyacinth plant showing the leaf and root measurements used to define plant type. Measurements were of the third leaf as counted from the center of the rosette

Data were analyzed using the SAS (Barr et al. 1979) and BMDP (Dixon and Brown 1977) computer program libraries. Plant measurements were first analyzed using a multivariate analysis of variance (MANOVA) in order to obtain a simultaneous test of significance of differences among areas by season observations. Both area and season were considered as class variables. Univariate analyses of variance were also performed to compare each variable.

It became apparent during the 15-month study that it would be virtually impossible to sample uniform plant types, and the variation encountered in plant type could account for variation in damage estimates. If plants were sampled uniformly or if the majority of the variation was within a site (hereafter the term "site" will refer to a single location and date) rather than among sites, then it would not be possible to identify a site from the plant measurements. To determine if this discrimination was possible, a stepwise multivariate discriminant analysis was performed in which sites were designated as the group variable and plant measurements converted to log 10 were the classifying variables. As a prelude to this, a test of homogeneity of the within groups' covariance matrix was performed and the assumption of homogeneity of variances was confirmed.

Since the original purpose of this study was to examine seasonal and latitudinal variation in the proportion of "susceptible" plants damaged by *S. albiguttalis*, it was desirable to remove the effects of plant type from the data and thereby examine the "pure" effects of the season and latitude. Hence, the overall term "plant type" was considered a covariate and the data were analyzed as an analysis of covariance. Since six original variables and four transformed variables were necessary to define plant type, and because several of these variables were correlated, it was desirable to reduce the number of variables yet retain the intercorrelations among them. Principal axis factor analysis with varimax rotation was used to reduce the variables to a few orthogonal factors. These factors represented linear combinations of the original variables loaded in such a way as to weight a set of correlated variables within each factor. This procedure reduced the observed variables to a few nonobserved variables (comprised of subsets of the original variables), which should be manifestations of underlying factors (Sinha 1977).

Factor scores for each site were then used as indices of plant type and analyzed as covariates in a multiple linear regression procedure with latitudinal area, quarter, and a quarter by area interactions as the main effects entered as continuous variables. Also, partial correlation analysis was performed to examine the correlations between percentage damage and latitude controlling for the linear effects of quarter and plant type and between percentage damages and quarter controlling for the linear effects of latitude and plant type.

RESULTS

Although the oldest established population of *S. albiguttalis* existed in west-central Florida on the Pinellas Peninsula, the size of the populations was

persistently small. The insects were released in this area in September and October 1977, but no evidence of their dispersal to other sites was obtained until the spring of 1979. During April and May, populations began to appear in the rivers to the east of Tampa Bay and in Tarpon Lake to the north of the peninsula. During this period of time, these west-central populations were distinctly separate from the southern or northern populations. The slow dispersal of *S. albiguttalis* away from this site was probably a result of the almost islandlike character of this area.

Because of the abundance of the suitable forms of waterhyacinth in the canal systems in the Everglades Conservation Areas, the southern populations increased rapidly. (The variation in forms of waterhyacinth is shown in Figure 3.) *Sameodes albiguttalis* was well established throughout this area and by February 1979 the range of these populations had begun to expand northward. During the spring, the movement of these populations continued northward primarily through the North New River Canal and by May they could be found at the southern end of Lake Okeechobee. By June, *S. albiguttalis* was ubiquitous throughout Lake Okeechobee. Populations had spread northward through the Kissimmee River and were present in Lake Istokpoga by July.

Because the dispersal of the southern populations was through a continuous system of canals, lakes, and rivers, the continuity of the populations was evident and their movement was relatively easily monitored. The southern and west-central populations remained disjunct up until July 1979. During the following few months, however, numerous populations began to appear throughout central Florida and by August the central and southern population fronts could no longer be distinguished and continuous populations existed throughout the southern half of the state.

Sameodes albiguttalis first appeared in the headwaters of the St. Johns River at Blue Cypress Lake in early July 1979. Since the St. Johns flows northward, the populations dispersed very rapidly once they reached this system, so much so that it was difficult to trace their movement accurately. By late November 1979, continuous populations occurred throughout the river from Blue Cypress Lake to Lake George and a few populations were found even farther north. One small population was found as far north as Green Cove Springs at the mouth of Black Creek, ca. 25 km south of Jacksonville, as early as 10 October. This was not surprising since waterhyacinth is a floating plant and often drifts with river currents, which would tend to accelerate the dispersal rates of the insects in a downstream direction. Several severe frosts during the winter seemed to extirpate the more northerly populations, however, and, by January 1980, the insects could not be found farther north than the southern end of Lake George. This apparent extirpation was probably due more to a loss of the plants than to the direct lethal effects of the low temperatures on the insects. By March, populations were again dispersing northward and could be found abundantly near Palatka, at Crescent Lake, and in the Ocklawaha River. In the spring of 1980, the range of populations expanded dramatically, and by July populations were present as far west as Lake City and as far north as the Nassau River near the Florida-Georgia border.

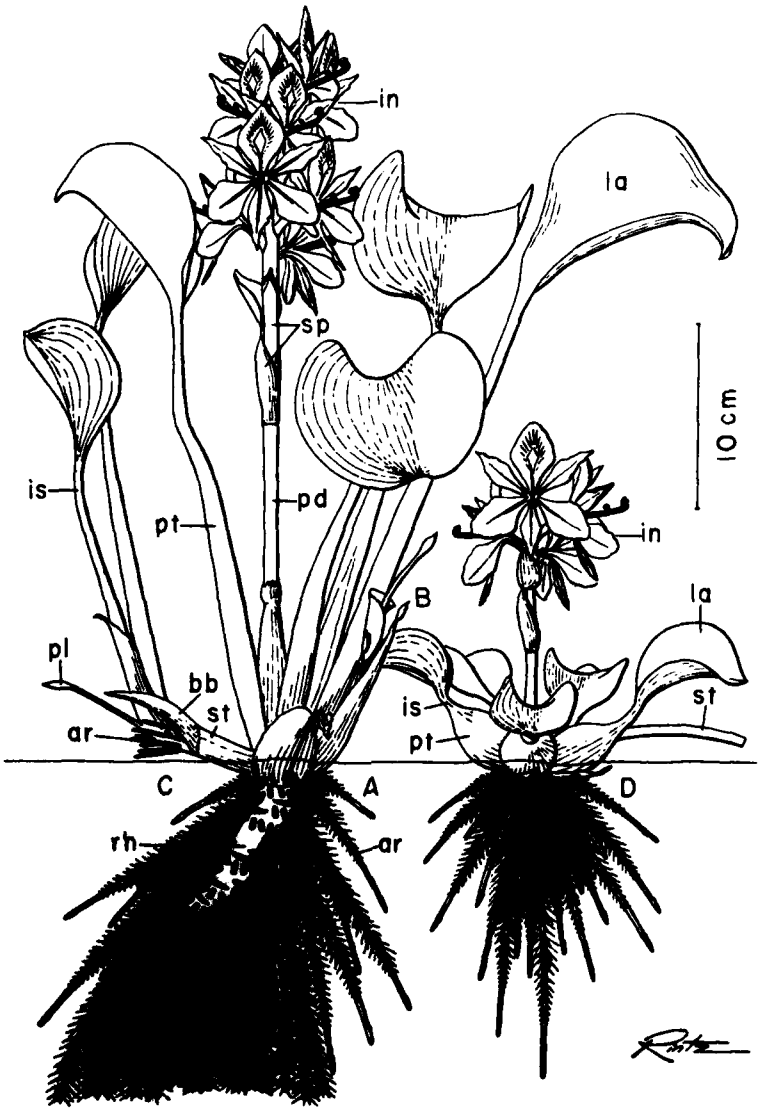

Figure 3. A generalized sketch of waterhyacinth plants showing the variation in form often encountered. Plants in dense stands tend to produce elongate petioles (A) whereas those along the fringe or in open areas tend to have inflated, bulbous petioles (D). Offsets (C) are produced from axillary buds (B). The major morphological features are: (ar) adventitious roots; (bb) bud bract or prophyllum; (in) inflorescence; (is) leaf isthmus; (la) leaf lamina; (pd) peduncle; (pl) primary leaf; (pt) leaf petiole; (rh) rhizome or stem; (sp) spathe; and (st) stolon

The northernmost release site at Lake Alice in Gainesville did not result in the establishment of widespread populations. Although the insect did persist there, the population was a very marginal one. Following the last release at this site in March 1979, nearby sites were repeatedly examined with negative results. In May 1980, however, *S. albiguttalis* was found in Orange and Newnan's Lakes and in June it

was finally found in Biven's Arm Lake, the site nearest the original release. By this time, however, populations were present throughout the northern part of the state as a result of the dispersal of the more southerly populations. It seemed likely that the insects reached these lakes by dispersing from the extensive populations in the Ocklawaha River through Orange Creek to Orange Lake and then through the connecting canals and streams to these other sites. Hence, the releases at Lake Alice probably played a minor role as a source of insects for the colonization of other sites. This was probably due to the land-locked nature of the site, the marginal suitability of the preponderence of the plants, and the resultant low insect population numbers. Hence, the site never developed a large population of insects and dispersal to other sites by those few was probably difficult.

Figure 4 shows the results of the dispersal survey with the approximate distributional limits of *S. albiguttalis* on various dates during the term of the study.

Figure 5 shows the results of the quarterly survey in terms of the proportion of the presumably susceptible plants that showed symptoms of damage by *S. albiguttalis* within each latitudinal zone. The first sampling period was during the spring of 1980 and a distinct latitudinal gradient seemed to occur at that time. Most of the plants in the southernmost areas were damaged while none in the northern areas were. Curiously, no damage was detected in the area between 29.5° and 30°N latitude. The area sampled was near Welaka on the St. Johns River and *S. albiguttalis* was known to occur in that area at that time (see Figure 4). Apparently, it was not sufficiently abundant to be found in a random sampling of plants. By the summer quarter, the relative frequency of damage had begun to decrease in the south and increase in the north. Although the numbers were low at the two northernmost sites, the insects were detectable. By the fall quarter, the latitudinal gradient had disappeared and, in fact, damage was most evident at the northernmost site. Although the presence of the insect could be detected throughout the state during each subsequent quarter, latitudinal trends were generally not evident and damage frequencies were erratic. In the spring of 1981, damage frequencies were very low in the northern areas. Several hard freezes occurred in February and had a devestating effect upon the waterhyacinth populations at these northern sites. As a result, both the plant and the insect populations were low. A massive resurgence of the plants manifested as a flush of growth occurred in the spring, which further diluted the populations. Hence, the low measure of relative damage resulted from an increase in the plant density, which caused an apparent reduction of the insects. This was generally true throughout the state but to a lesser degree in the south. Although the numbers were low in the north during the spring of 1981, the presence of *S. albiguttalis* was detectable. This contrasted with the data for the spring of 1980 when no damage was apparent in the samples north of ca. 29.5°N latitude.

Because of the lack of apparent trends in Figure 5, these data were analyzed to determine if either latitudinal and/or seasonal trends were obscured by changes in the acceptability of the plants. Although an attempt was made to sample plants that appeared to be suitable to *S. albiguttalis*, an "ideal" type could not be defined.

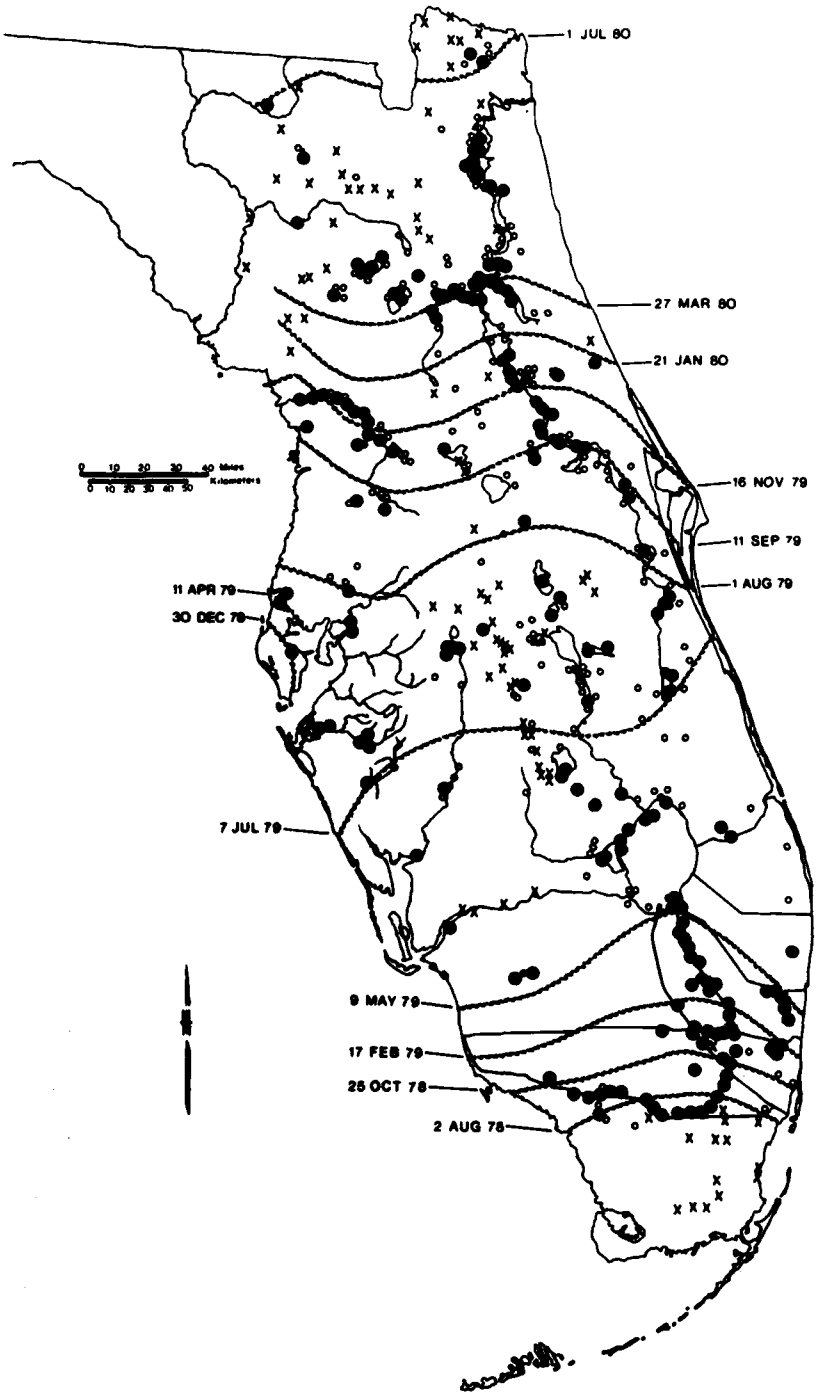

Figure 4. Map of peninsular Florida showing the distribution of *S. albiguttalis*. The closed circles represent localities where *S. albiguttalis* was found. The open circles represent localities where waterhyacinth was present but where the presence of *S. albiguttalis* was not confirmed. The X's represent sites examined in which no waterhyacinth were found. The contour lines represent the distributional limits of the insect population for various dates

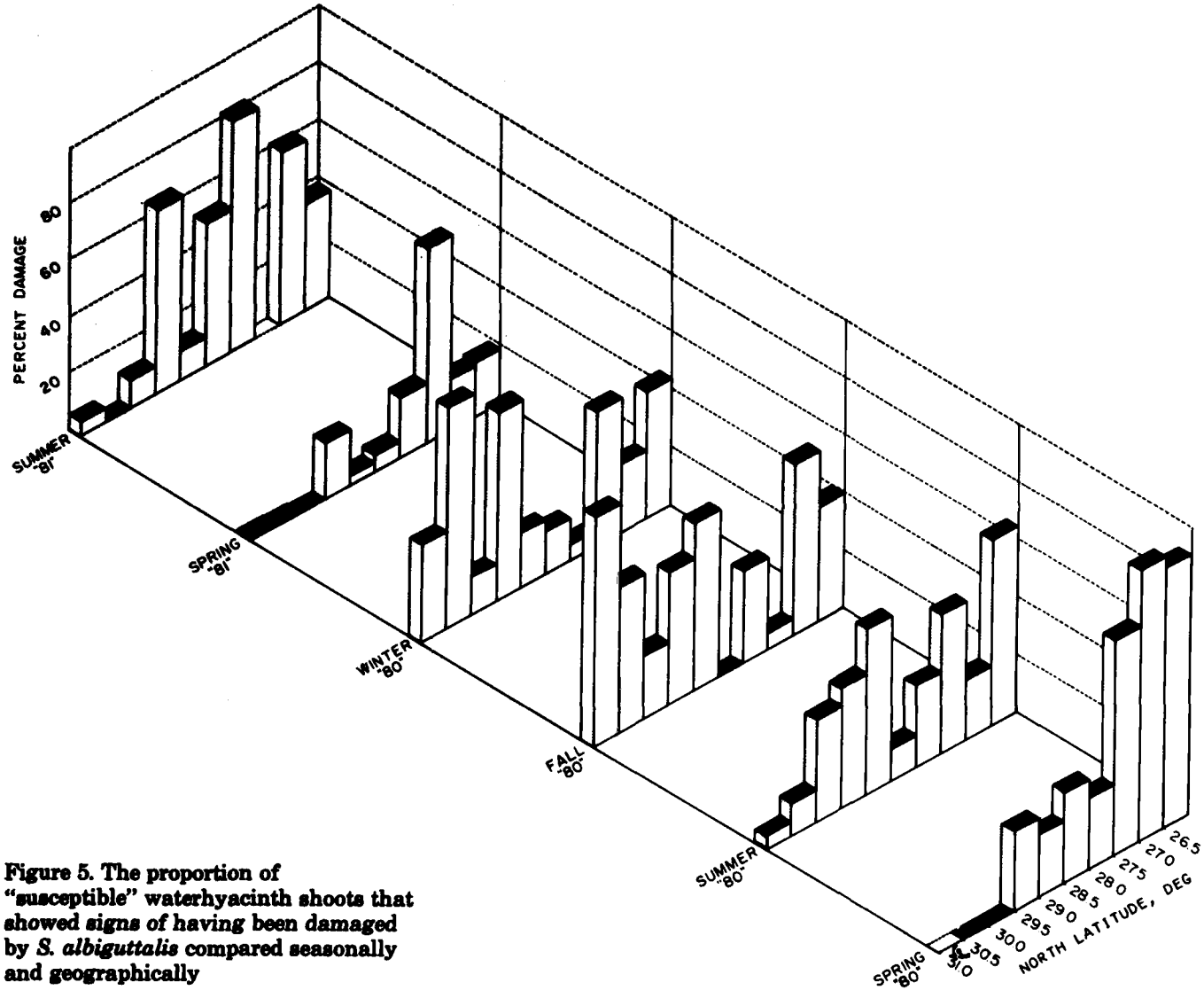

Figure 5. The proportion of "susceptible" waterhyacinth shoots that showed *signs of* having been damaged by *S. albiguttalis* compared seasonally and geographically

The type of plant sampled, therefore, varied considerably (see Figure 6) and consisted of the plants considered most likely to be infested among those available at each site. These usually were plants growing along the fringes of a stand. A multivariate analysis of variance was performed on the plant measurements to determine if the plant type varied significantly. Wilks' criterion showed a significant area by quarter interaction in a simultaneous test of significance over all plant measurements (see Table 1). Since the multivariate interaction was significant, the results of univariate analyses were inconsequential and plant type was not uniform.

A discriminant analysis was performed to analyze the pattern of dispersion of the various plant types among the sites. The results of this analysis are summarized in Table 2 and Figure 7. Site discrimination required only five of the

Table 1

Results of Univariate and Multivariate Analysis of Variance. The Numbers are F-Values and all are Significant at P>0.01 Unless Otherwise Indicated

	Effects			
Variable	*Area*	*Quarter*	*Area × Quarter*	*Overall*
Root length	7.34	21.27	5.76	7.37
Leaf length	8.57	60.97	5.75	11.04
Petiole length	8.64	49.22	5.12	9.54
Petiole width	9.02	40.58	4.32	8.24
Lamina length	8.40	86.08	6.04	13.44
Lamina width	10.97	35.58	6.26	9.48
Petiole ratio	3.16	51.32	5.46	9.12
Leaf ratio	3.37	29.03	2.53	4.99
Lamina ratio	3.03	79.26	2.70	9.46
Root to leaf	8.11	6.51	4.03	4.89
Multiple	1.30*	4.53	3.36	-

*Prob. >F = 0.06.

Table 2

Standardized Coefficients of the Canonical Variables Formed in the Discriminant Analysis in which Plant Measurements (Converted to log 10) Were Used as Criteria for Discrimination Among Sites

Variable	*Standard Deviation*	*CV I*	*CV II*	*CV III*	*CV IV*	*CV V*
Root length	0.18	0.29	0.25	0.18	0.93	-0.10
Leaf length	0.12	0.06	-0.66	-1.71	0.48	-0.43
Petiole width	0.09	-0.49	0.55	-0.32	-0.04	-0.73
Lamina length	0.10	0.65	1.09	1.19	-0.97	1.07
Lamina L:W	0.06	0.22	-0.48	0.15	0.08	-1.15
Cumulative proportion of total dispersion	-	0.52	0.72	0.85	0.94	1.00

NOTE: Coefficients were standardized by multiplication with the standard deviations of the original variables.

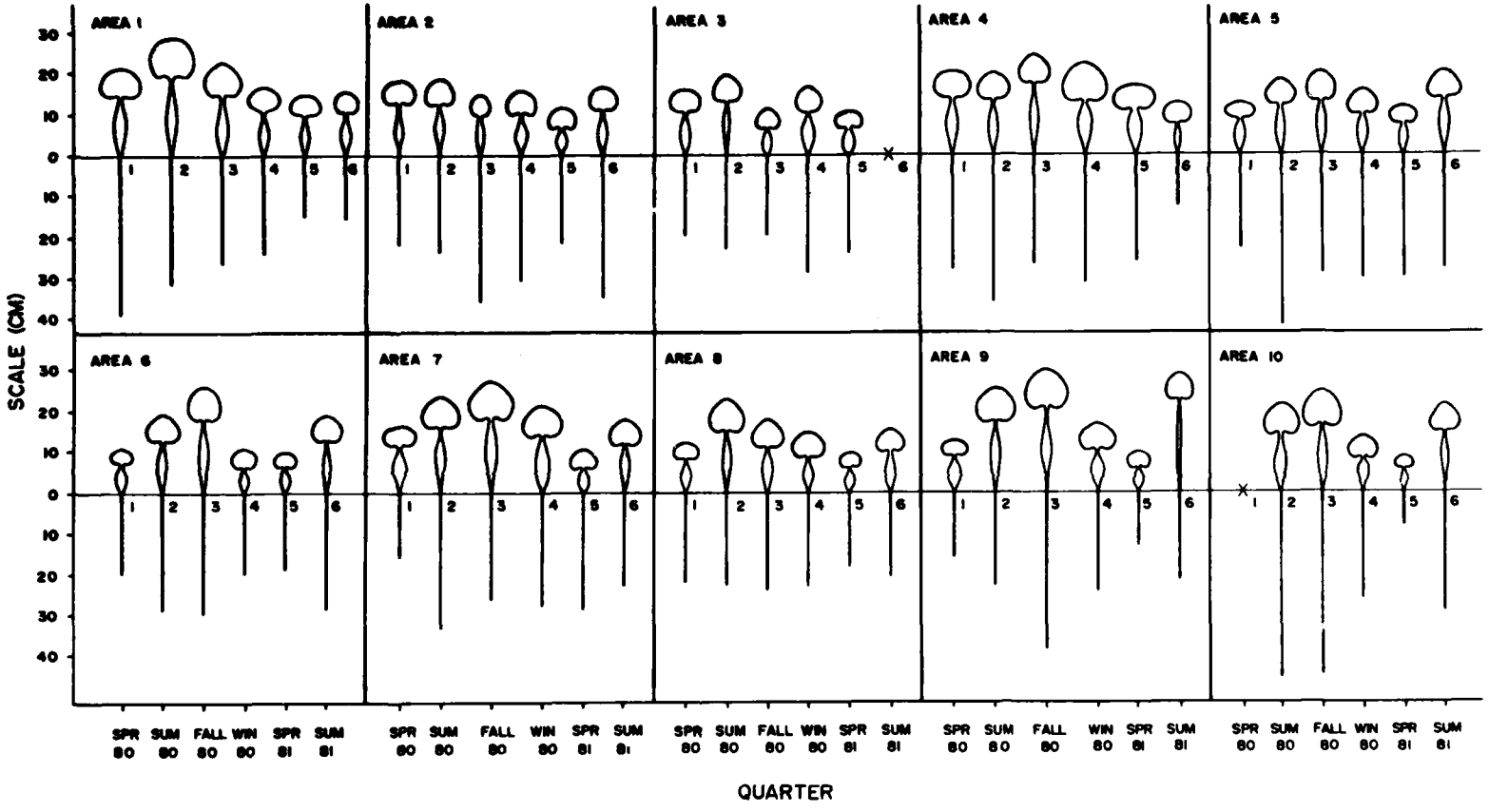

Figure 6. Schematic representation of the various forms of waterhyacinth "types" sampled seasonally and geographically. Areas represent the latitudinal zones as indicated in Figure 1. Numbers 1 through 6 within an area represent the sampling quarters, spring 80 through summer 81, respectively. For an explanation of the measurements these diagrams are based upon, see Figure 2. All are drawn to scale

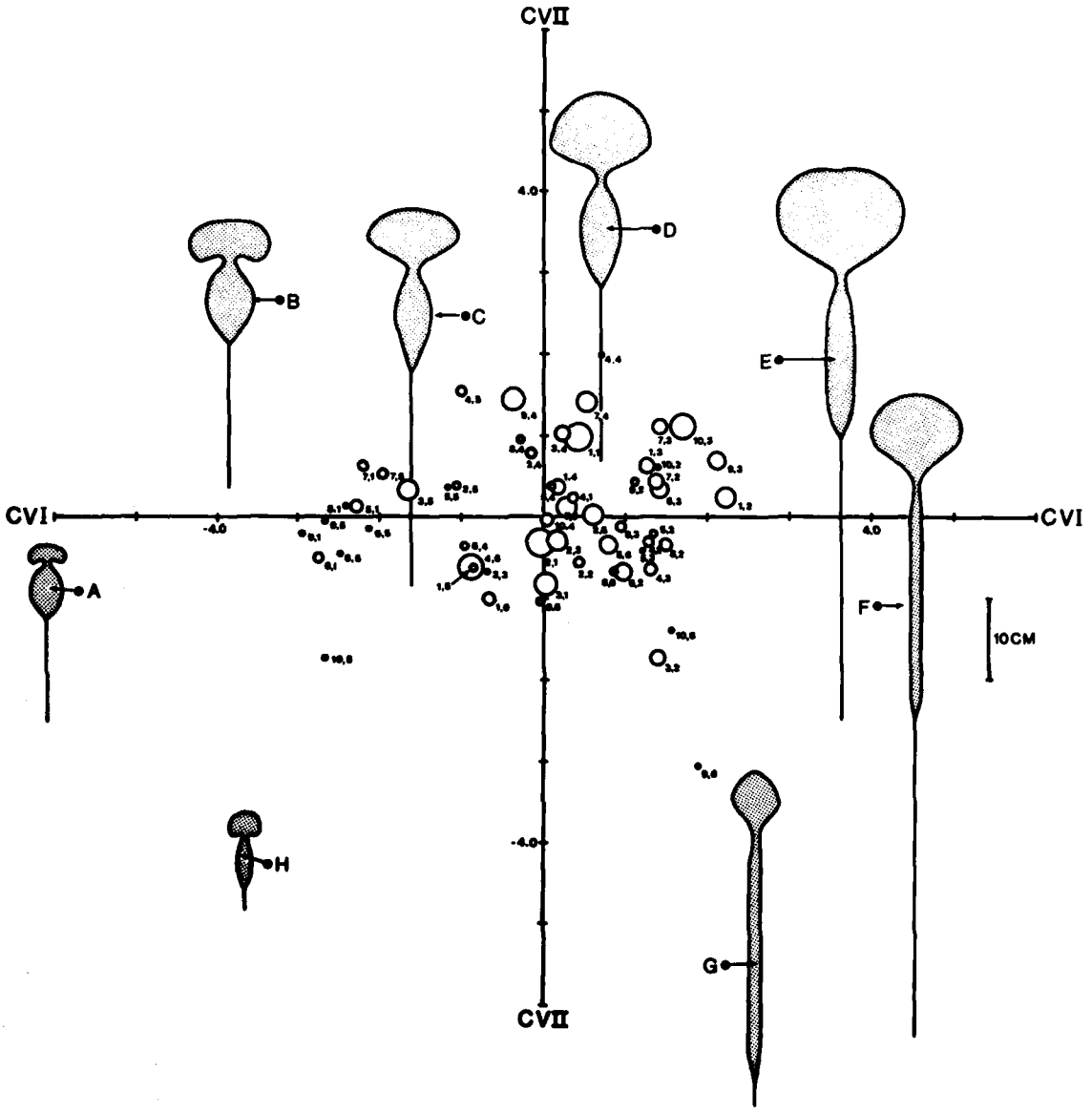

Figure 7. Results of the discriminant analyses in which plant measurements were used to classify the plants as to site. The circles are the centroids representing the means for each site and are proportional in size to the population intensity of *S. albiguttalis* for each site. The numbers by each circle represent the area and quarter for the site (e.g., 5,6 indicates Area 5, Quarter 6 or ca. 28°N, summer 1981). The diagrams indicated by points A-H represent examples of plants at various points on this ordination

original variables and the first two canonical variables (CV's) accounted for 72 percent of the total dispersion (Table 2). The mean values of the first two canonical variates for each site are plotted in Figure 7. As the schematic illustrations of the plants show, those with large laminae are positioned towards the positive side of the scale on CVI, whereas those with wide petioles are positioned towards the negative in this ordination. Those plants with wide petioles and large laminae are

at the positive end of the scale on CV II, whereas those with long leaves and petioles and a lanceolate leaf shape (a high lamina length to width ratio) are towards the negative end. Sites at which the plants were heavily infested by *S. albiguttalis* tend towards the origin and the upper right quadrant in the ordination.

The results of these first analyses indicated that the plant type sampled was not uniform, that sites could be discrimated based upon the plant types present, and a possible relationship between plant type and *S. albiguttalis* infestation levels existed.

Factor analysis was conducted as a means of combining correlated plant measurements into sets of fewer variables and thereby reducing the dimensionality of the "plant type" characterization. This effectively reduced the original 10 variables to 4 factors that accounted for 97.8 percent of the total variance (Table 3). The first factor was comprised of high loading coefficients for lamina width, lamina length, leaf length, petiole length, the ratio of lamina length to width, root length, and the ratio of lamina length to width, in this respective order. All of these coefficents were positive; hence, high values for any of these variables would tend to increase the score for Factor 1 in a positive direction. The factor may be interpreted as an index of plant size since all of the included variables would have large values for large plants and small values for small plants. The plants in Area 1 during Quarter 2 had the highest positive value for Factor 1 while those at Area 10 during Quarter 5 had the highest negative values. A comparison of the schematics of those plants in Figure 6 helps in the interpretation of the meaning of Factor 1.

The only two variables that loaded heavily on Factor 2 were root length and the ratio of root length to leaf length. Again, the coefficients were both positive. This factor can be interpreted as indicative of the extent of development of the root system, especially relative to the shoot. Plants with long roots and short leaves

Table 3

Rotated Factor Pattern Showing the Loading Of Each Original Variable on Each Factor Score. Any Value of 0.4 or Less Was Considered Zero Loading

Variable	*Factor 1*	*Factor 2*	*Factor 3*	*Factor 4*
Root length	0.627	0.749	0.071	0.100
Leaf length	0.881	-0.046	0.208	0.348
Petiole length	0.823	-0.061	0.216	0.473
Petiole width	0.006	0.035	-0.984	-0.137
Lamina length	0.945	0.001	0.161	0.031
Lamina width	0.963	0.027	-0.153	0.051
Petiole ratio	0.643	-0.074	0.594	0.427
Leaf ratio	-0.166	0.154	-0.153	-0.962
Lamina ratio	0.575	-0.042	0.490	0.013
Root to shoot	-0.232	0.928	-0.101	-0.250
Cumulative % variation	58.8	77.3	89.6	97.8

would tend to have high positive values, whereas those with short roots and long leaves would tend towards negative values. Site 5,2 (Area 5, Quarter 2) had the highest positive score and site 10,5 again had the highest negative score. Compare these in Figure 6.

Factor 3 was primarily indicative of leaf shape. Plants with spindly petioles and lanceolate laminae had high positive scores whereas those with robust, thick petioles and more uniform laminae had high negative scores. Compare site 3,2 and site 7,1 for the extremes on Factor 3.

Factor 4 again contrasted long, thin, spindly petioles (high positive) with short, fat, robust ones (high negative) but additionally considered the length of the lamina relative to the total leaf length. Hence, plants with short leaves but proportionally long laminae would also tend towards the negative end of the scale. Thus, the factor probably is indicative of leaf size since only very short leaves would have high lamina length to petiole length ratios. Also, only large leaves would have high petiole length to diameter ratios. The extremes on this factor were site 9,6 (positive) and site 8,4 (negative).

Factor analysis proved to be an excellent approach for defining plant type in a quantitative manner. The factors created were, by definition, orthogonal and, by using these as variables for plant type variables, intercorrelations were eliminated. Partial correlation analyses were then conducted to determine if either area or quarter were correlated with percentage infestation after the linear effects of plant type were removed. Area was considered a continuous variable with values of one to ten and the partial correlation of area with percentage infestation was only -0.316. Quarter was considered a continuous variable with values from one to six, and the partial correlation with percentage infestation was only -0.084. Hence, either the area at which and the date during which the samples were taken had no bearing upon the percentage of the plants infested or these relationships were not linear. Since the latter was suspected, the data were further analyzed using a multiple regression procedure.

Samples were collected during the course of this study over more than a year. If the insect populations underwent an annual cycle, then one would expect that the percentage infestation would vary over the time period in a curvilinear fashion. Also, since the climates of north Florida and south Florida were radically different, one would expect that the pattern of variation in this annual cycle would be different from north to south. Hence, a time by latitude interaction should be anticipated. Assuming that at least a second-order polynomial would be needed to describe variation patterns, second-order terms were used in the analysis for both area and quarter, as well as the various interaction terms. Factor scores for each site were included as covariates to allow for the effect of plant type. Hence, the model tested was $PCI = A + AQ + A^2 + Q^2 + A^2Q + AQ^2 + A^2Q^2 +$ Factor 1 + Factor 2 + Factor 3 + Factor 4, where A = latitudinal area, Q = time as quarters, and PCI = percentage infestation. Factor 1 to Factor 4 were the factor scores for plant type for each site. When all possible regressions were tested, the model which explained the greatest variance with the fewest variables included A, Q, AQ, Q^2,

AQ^2, Factor 1, and Factor 4 and accounted for 52 percent of the total variation. A further analysis of this model is presented in Table 4.

Although this model was significant, 48 percent of the variance was not explained. There are, of course, many other models that may be more efficient. These may include plant type by area or by quarter interactions or higher order polynomial equations. Much of the variation may simply be random and not accountable with the parameters examined.

By setting the values for plant type equal to the overall averages in the regression equation, one may examine the effects of area and quarter by calculating predicted values for each site. Figure 8 shows the predicted output of the regression equation adjusted to equal plant types. The resultant pattern shows definite seasonal

Table 4

Regression Analysis Explaining the Variation in the Percentage of Waterhyacinth Plants Damaged by *S. albiguttalis* as a Function of the Geographic Location (Area), the Date (Quarter), and the Type of Plants Present

ANOVA:

Source	*DF*	*Sum of Squares*	*Mean Square*	*F-Value*	*Pr>F*	R^2	*C.V.*
Model	7	21452	3064	7.74	0.0001	0.52	59%
Error	50	19809	396				
Corr. Total	57	41260					

STD. DEV.	*MEAN*
19.9	33.5

Source	*DF*	*Type I SS*	*F-Value*	*Pr>F*	*Type IV SS*	*F-Value*	*Pr>F*
Area (A)	1	4524	11.42	0.0014	7559	19.08	0.0001
Quarter (Q)	1	743	1.88	0.1769	8958	22.61	0.0001
A*Q	1	504	1.27	0.2647	6475	16.35	0.0002
Q^2	1	63	0.16	0.6925	8618	21.75	0.0001
$A*Q^2$	1	9519	24.03	0.0001	6212	15.68	0.0002
Factor 1	1	6061	15.35	0.0003	6028	15.22	0.0003
Factor 4	1	17	0.04	0.8354	17	0.04	0.8354

REGRESSION:

Parameter	*Estimate (B)*	*T for Ho: Parameter = 0*	*Pr>T*
Intercept	162.3	6.32	0.0001
Area	-30.5	4.37	0.0001
Quarter	-76.9	4.76	0.0001
A*Q	12.0	4.04	0.0002
Q^2	10.8	4.66	0.0001
$A*Q^2$	-1.6	3.96	0.0002
Factor 1	11.6	3.90	0.0003
Factor 4	-0.8	0.21	0.8354

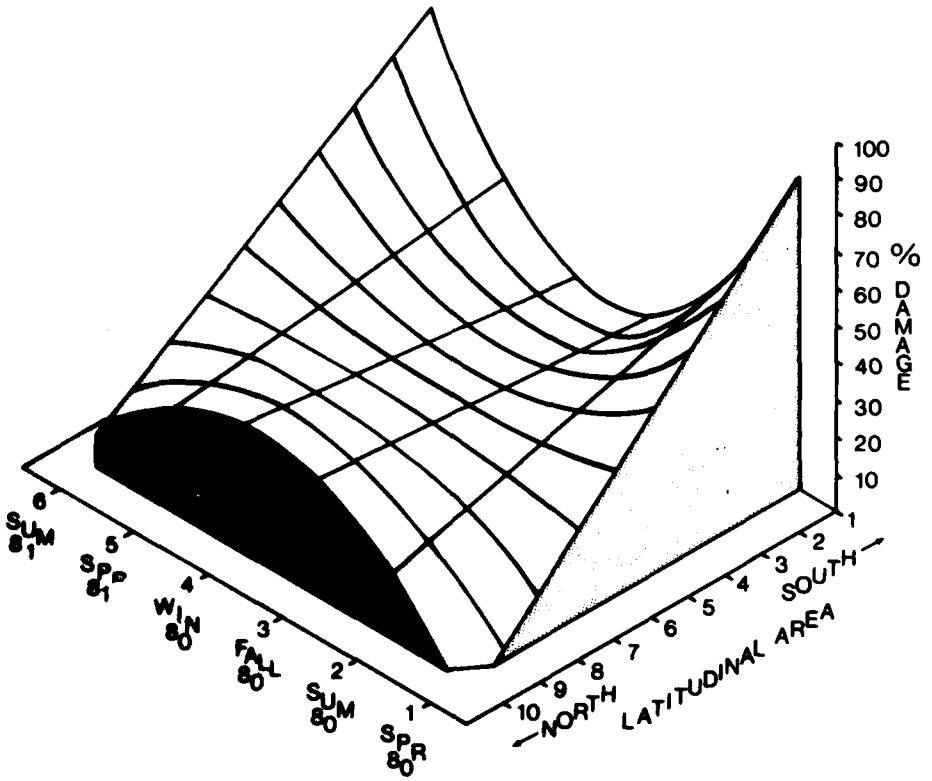

Figure 8. A three-dimensional illustration of a regression model showing the pattern of *S. albiguttalis* population intensities as influenced by latitude and season

patterns in both north and south Florida, but a lack of seasonality in the central areas. Interestingly, the pattern seems to reverse from north to south with high values in the spring and summer and low values in the fall and winter in the south. In the north, the high values tend to be in fall and winter and the low values in the spring and summer.

DISCUSSION

This study was designed to determine if an operational release program would be necessary to disseminate *S. albiguttalis* throughout the range of waterhyacinth after the initial establishment of field populations was accomplished. If the original releases resulted in very localized population centers that were concentrated at the release sites, and if dispersal away from these areas was slow, then an operational program would probably be necessary. This would also be true if extirpation of the populations were frequent and recolonizations failed to occur. An operational program for the purpose of establishing populations of insects in new areas or recolonizing areas at which populations were lost would be difficult and expensive. Massive rearing or collecting efforts would be required in order to obtain adequate numbers of insects for this purpose.

The results of the dispersal surveys described herein clearly show that *S. albiguttalis* dispersed readily and rapidly after several populations were established. In fact, in the 550 days following 1 Jan 1979, the range of this insect expanded northward at least 528 km, an average of slightly less than 1 km/day. This expansion did not occur at a constant rate, of course, but rather tended to exponentiate during the first 8 months of 1979 then decrease during the fall and winter, and increase rapidly again during the spring and summer of 1980. The most rapid dispersal rates occurred during July and August of 1979 when the range increased northward by ca. 105 km within 1 month at a rate of ca. 4 km/day. It is apparent, then, that it is not necessary to invest the required time and effort in an extensive operational release program to disseminate populations of *S. albiguttalis* over a large geographical range. Instead, it is more appropriate to concentrate the release of the insects in a fairly restricted region to ensure that viable populations become established. This is to say that the evidence indicates that the insects themselves can saturate their resource and distribute themselves throughout the range of the waterhyacinth without human assistance. This is not a generalization, however, in that the same cannot be said for all species of insects that one might work with but it does seem to be so in the case of *S. albiguttalis*.

The quantitative aspects of this project have provided a great deal of insight into the patterns of resource utilization by *S. albiguttalis*. It is apparent that population intensities vary tremendously both spatially and temporally. It is also clear that waterhyacinth may be considered a coarse-grained resource with regard to the pattern of utilization by *S. albiguttalis*. The insect does not appear to perceive all morphotypes or growth forms of the plant as alike, but rather discriminates among them in some manner and utilizes them differently. Hence, no amount of effort will establish populations of this insect on plants that the insect perceives as unsuitable. Our problem is to define the dimensions upon which this perception is based. This discrimination for plant type was evident by a tendency towards higher infestation intensities on the more robust plants even amongst plants which all appeared to be suitable to *S. albiguttalis*.

When the effects of plant type were removed in order to ascertain more clearly seasonal and latitudinal variations in infestation intensities, it was determined that seasonal variation was curvilinear whereas latitudinal variation was linear and latitude affected the characteristics of the seasonal curve. In the south, populations were high during the hottest part of the year and lower during the cooler months. The reverse was true in the north. This pattern is difficult to explain without a great deal of speculation and should be the subject of further research. The specific purpose of this study was not so much to determine seasonal patterns of insect abundance as to determine if populations persisted at different latitudes throughout the year. With this in mind, it has been concluded that the *S. albiguttalis* populations can and do survive throughout all areas of Florida over the entire year. This is even true for those populations in the north which are sometimes exposed to extremely cold winter conditions. Although the populations appear to be low in the north during the spring, it is felt that this is due to the

resurgence of the waterhyacinth populations and a rapid increase in the number of plants. The insect populations do not numerically respond with equal rapidity, so a temporary dilution of the relative intensity results. Because these populations do persist, it should be concluded that there is no need for annual restocking of these areas. In fact, it appears that there is no need for further releases of *S. albiguttalis* in Florida.

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Chapter 3

EVALUATION OF CHEMICALS FOR AQUATIC PLANT CONTROL

by

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INTRODUCTION

It is generally agreed that aquatic weed problems are becoming more prevalent and more serious. Rapid eutrophication of water resources caused by increased urbanization, more intensive use of fertilizers, and greater use of available water is commonly considered to be the major cause of the upward trend in severity of aquatic weed infestations. The introduction of several species of exceedingly noxious aquatic weeds from other areas of the world has added greatly to the problems and the need for research to develop control measures.

The impact of aquatic weeds on utilization of water resources is well documented. Nearly every conceivable water use can be prevented or at least curtailed by unmanaged growth of these weeds. Aquatic weeds cause severe problems to navigation in streams and inland waterways. They interfere with flow and utilization of water for irrigated agriculture, prevent fishing and recreation, depress real estate value, and present health hazards.

Management of aquatic plants is primarily accomplished with herbicides. Since 1968, however, the number of chemicals registered nationally for aquatic use and available to the water manager has decreased approximately 63 percent, from 38 to 14.

The reduction in the number of available chemicals is due to the loss of registration of older chemicals, usually because of adverse environment impact, and to the reduction in number of new chemicals being developed by industry.

With the assistance of government, industry, and university laboratories, the search for new chemicals and new technology should be expanded.

Safer and more effective herbicides and growth regulators need to be developed for selective removal and for regulation of growth of noxious aquatic species at a lower cost. Techniques of formulating chemicals used in water which will reduce environmental impact, as well as increase efficacy, also need to be investigated.

Recently, several techniques of formulating effective chemicals within various polymer or matrix structures have been developed to provide controlled release over time, allowing a prolonged exposure of target plants to a sustained low concentration of a given herbicide. The effective use of controlled release herbicide formulations (CRHF) appears to hold great potential for long-term management of nuisance aquatic plant growth with much less herbicide required for the same period of activity.

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A protocol for evaluating this potential has been developed and involves determinations of: (a) chemical release rates; (b) stability of the released chemicals (degradation rate); (c) constancy and chemical release from the formulation (reliability); and (d) efficacy of the formulation in managing or eliminating aquatic plant problems. All four of the above evaluation phases are initially conducted in the laboratory. Confirmation of findings from the last two phases is attempted in outdoor studies conducted in large aquaria under environmental conditions more closely approximating those in the field.

Aquatic herbicides, such as phenoxy esters and dichlobenil, have been incorporated into various polymer solid matrices, including polyvinyl chloride (Steward and Nelson 1972) and polyethylene (Harris, Noris, and Post 1973) to provide long-term control of Eurasian watermilfoil (*Myriophyllum spicatum* L.). Other CRHF's of buthoxyethanol ester 2,4-D which have been tested successfully against watermilfoil included rubber-based compounds as sinking pellets slowly releasing the herbicide (Cardarelli 1976). Although these formulations have considerable potential, a drawback to their production and use has been the large amount of inert polymer carrier (70 to 90 percent w/w) that must be employed.

Another experimental approach is the synthesis of hydrophilic copolymers that contain a high percentage of the phenoxy herbicides as pendent side chains (Harris 1977). The herbicides are slowly released from these systems by the hydrolysis of the herbicide-polymer bonds at a nearly constant rate, making them excellent candidates as CRHF's for aquatic weed control. Further, the herbicide release rates from the copolymer can be altered by varying the degree of hydrophilicity around the herbicide ester bonds so that release rates from several weeks to several years may be obtained (Harris 1977). One example of these controlled release (CR) systems is the copolymer 2-methacryloyloxyethyl 2,4-dichlorophenoxyacetate with glycerylmethacrylate (MOE 2,4-D/GMA).

The major emphasis of this project has been to implement the protocol for evaluating various CRHF's provided by different cooperating formulators. Progress on the implementation of the protocol as well as the results of the conventional herbicide evaluation program will be discussed herein.

Aquatic weeds treated in FY 1981 are listed below:

Alligatorweed	***Alternanthera philoxeroides*** **(Mart.) Griseb.**
Cabomba	***Cabomba caroliniana*** **Gray**
Chara	***Chara*** **spp.**
Duckweed	***Lemna*** **spp.**
Hydrilla	***Hydrilla verticillata*** **Royle**
Hygrophila	***Hygrophila polysperma*** **(Roxb.) Anderson**
Sago pondweed	***Potamogeton pectinatus*** **L.**
Torpedograss	***Panicum repens*** **L.**
Waterhyacinth	***Eichhornia crassipes*** **(Mart.) Solms**
Waterlettuce	***Pistia stratiotes*** **L.**
Watermilfoil	***Myriophyllum spicatum*** **L.**

The names and sources of chemical compounds evaluated in 1981 are listed in Table 1.

Table 1
Names and Sources of Chemicals Evaluated in Fiscal Year 1981

Common Name	*Chemical Name*	*Source*
Acrolein	2-propenal	Union Carbide Corporation Tarrytown, New York 10591
Copper EDA	Copper-Ethylenediamine Complex	Sandoz, Inc., Crop Protection Komeen 480 Camino Del Rio South, San Diego, California 92108
Dicamba	3,6-dichloro-o-anisic acid	Velsicol Chemical Corporation, 341 East Ohio Street, Chicago, Illinois 60611
Dichlobenil	2,6-dichlorobenzo-nitrite	Thompson Hayward Chemical Co., P.O. Box 2383, Kansas City, Kansas 66110
Dichlobenil/Urea Formaldehyde resins	Controlled release formulations	Dr. Etiene Schact, Laboratory of Organic Chemistry, Gent, Belgium
Diquat	6,7-dihydrodipyrido (1,2-a:2',1'c) pyrazinediium dibromide	Chevron Chemical Company, Ortho Division, 940 Hensley Street, Richmond, California 93710
Diquat sinking pellets, Diquat floating pellets	Controlled release formulations	Creative Biology Laboratory, Inc., 3070 Cleveland-Massillon Rd., Barbeton, Ohio 44203
Diquat grains + 1.5% Kelzan Diquat grains + 3.5% PVA Diquat grains + 3% PVP	Controlled release formulations	Chevron Chemical Company, Ortho
Diuron	3-(3,4-dichlorophenyl)-1,1-dimethylurea	E.I. duPont de Nemours & Co., Biochemicals Department, Wilmington, Delaware 19898
DPX-4189	2-chloro-N- [(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl] -benzene-sulfonamide	E.I. duPont de Nemours & Co.
DPX-5648	Methyl 2- [(4,6-dimethyl-2-pyrimidinyl) amino] -carbonyl] amino] sulfonyl] benzoate	E.I. duPont deNemours & Co.
Endothall	Salts of 7-oxabicyclo (2.2.1)heptane-2,3-dicarboxylic acid	Pennwalt Corporation, Agricultural Chemical Division, 1630 East Shaw Avenue, Fresno, California 93710
Fenac	Salts of 2,3,6-trichloro-phenylacetic acid	Union Carbide, Agricultural Products Co., Inc., 300 Brookside Ave., Ambler, Pennsylvania 19002
Fluridone	1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1H)-pyridinone	Lilly Research Laboratories, Division of Eli Lilly and Co., P.O. Box 708, Greenfield, Indiana 46140
Glyphosate	N-(phosphonomethyl)-glycine	Monsanto Co., Agricultural Products, St. Louis, Missouri 63166
Simazine	2-chloro-4,6,bis(ethyl-amino)-s-triazine	Ciba-Geigy Corporation, Agricultural Division, P.O. Box 11422, Greensboro, NC 27409
Terbutryn	2-(tert-butylamino)-4-ethyl-amino)-6-(methylthio)-s-triazine(2-methylthio)-4-ethyl-amino-6-tert-butylamino-s-triazine	Ciba-Geigy Corporation
2,4-D DMA	Dimethylamine salt of 2,4-dichlorophenoxy acetic acid	Union Carbide
MOE 2,4-D/GMA Poly GMA 2,4-D	2-methacrylolyloxyethyl 2,4-dichlorophenoxyacetate/ glycerylmethacrylate	Dr. Frank Harris, Wright State University, Dayton, Ohio 44231
2,4-D/Kraft lignin	Controlled release formulation	Westvaco Corporation, P.O. Box 5207, N. Charleston, South Carolina 29406

MATERIALS AND METHODS

Evaluation of Controlled Release Formulations

Controlled release formulations. On 19 September 1980, approximately 2 kg of Emathite clay pellet formulation with MOE 2,4-D/GMA copolymer (10% a.i.) was received from Allen Edgar, Mid-Florida Mining Company, Lowell, Fla. The pellets were formulated with a water binder and stored in a moist condition. Release rate was specified as 1.2 mg 2,4-D/g copolymer/day.

On 27 March 1981, approximately 2 kg of a clay pellet formulation containing 18 percent by weight Poly GMA 2,4-D was received from Mid-Florida Mining Company. The copolymer in the new clay formulation contained 58 percent by weight 2,4-D acid, plus about 5 percent 2,4-D unreacted with the copolymer backbone.

In February 1981, approximately 1 kg of Westvaco's 2,4-D/lignin formulation was received from the U. S. Army Engineer Waterways Experiment Station (WES). The lignin formulation contained 50 percent by weight of 2,4-D acid, with a specified release rate of 1 to 2 mg 2,4-D/g pelletized formulation/day.

Also, on 11 September 1980, four urea-formaldehyde resin formulations of dichlobenil were received from Dr. Etienne Schact, Laboratory of Organic Chemistry, Gent, Belgium.

Determination of release rate in static water. Release rates of the CRHF were determined first in static water tests under controlled laboratory conditions at 28 ± 2°C.

Treatments were made to 3.7 ℓ water with amounts of CRHF calculated to produce a concentration of 0.10 mg/ℓ herbicide every 24 hours, based on estimated release rates specified by the cooperating formulators. Treatments were replicated four times.

Natural water from a dug pond on the Agricultural Research Center grounds was used. Water quality was monitored monthly in March, June, and September (Table 2).

For interlaboratory comparisons, release rate data were also determined in reconstituted distilled water pH 8.0, containing 192 mg $NaHCO_3$, 120 mg $CaSO_4 \cdot 2H_2$, 120 mg $MgSO_4$, and 8 mg KCl per litre.

Water samples were taken from each container at various times throughout the experiment. Measurements of the herbicide concentrations were determined by high-pressure liquid chromatography and by gas chromatography for 2,4-D and dichlobenil, respectively.

Determination of release rate in flowing natural water. Treatments of 2,4-D CRHF to maintain various herbicide concentrations were made to 19 ℓ of flowing

Table 2
Water Quality Control Analysis

Date	Oxygen ppm	Conductivity µmhos	pH	Alkalinity mg/ℓ $CaCO_3$	Hardness mg/ℓ $CaCO_3$	Air Temp, °C
March 81	8.19	410	7.59	147.4	176.7	22.8
June 81	6.43	331	7.96	149.9	174.8	26.4
Sept 81	5.86	318	7.41	144.4	179.9	24.9

Date	Water Temp, °C	Phosphate mg/ℓ	Nitrate mg/ℓ	Ammonia mg/ℓ	Total Solids mg/ℓ	Suspended Solids, mg/ℓ
March 81	25.0	0.19	2.8	0.10	263	3.0
June 81	28.2	0.25	0.4	0.17	282	1.0
Sept 81	27.4	0.02	—	—	224	6.0

natural water in glass, flow-through culture vessels, with and without plants and soil. Natural pond water was used and treatments were replicated four times.

Regulated flowing water provided by a multichannel tubing pump (Eldex Laboratories Inc., 3551 Heaven Ave., Menlo Park, Calif.) was delivered to the bottom of individual culture vessels at a rate to provide one volume change in 24 hours. Water flow was checked at least once a week and adjusted when necessary.

Wastewater flowed out through side arms near the top of the vessels and was carried outside. Residual 2,4-D in solution was removed by passage of the wastewater through a series of three connected 19-ℓ containers filled with activated charcoal.

Fifty-millilitre water samples were taken from each vessel at 1, 2, 3, 4, 7, 14, 21, 42, and 56 days after treatment. The samples were concentrated in SEP PAC® C_{18} cartridges and analyzed for herbicide residues.

Evaluation of efficacy against watermilfoil and sago pondweed in flowing water. Mature plants of watermilfoil were obtained from Lake Seminole, Georgia. The plants were maintained as a stock culture in an outdoor pool until use. Germinated tubers of sago pondweed were obtained from Wildlife Nurseries, Inc., Oshkosh, Wis.

The plants were established in standard soil mix (70 percent sand and 30 percent organic peat) in 250-ml glass beakers. Three beakers each of watermilfoil and sago pondweed were placed in the culture vessels and allowed to establish for 4 weeks before chemical treatment was applied. Culture vessels were subjected to 14-hr days of 150 µE/m²/sec from a combination of fluorescent and incandescent lamps. Temperature was maintained at 28 ± 2°C.

Treatments were applied to vessels containing watermilfoil and to vessels without plants in order to determine the effect of plants and soil on herbicide concentrations. Culture vessels with and without plants to which treatments were not applied served as plant and water controls.

Response of watermilfoil plants to chemical treatments under flowing water conditions was evaluated closely throughout the experiment. The plants were harvested 8 weeks after treatment and evaluated for percent survival. Stem lengths and plant weights were measured.

Residue analyses. Complete details of the analytical procedures used for determining 2,4-D and dichlobenil residues have been discussed in a previous publication (Steward 1981). Briefly, 2,4-D was analyzed by high pressure liquid chromatography with a Perkin-Elmer series 3B HPLC, a Perkin-Elmer LC 75 detector (285 nm), and a Perkin-Elmer Sigma 10 integrator. The chromatographic column was HCODS SIL X (reversed phase). The mobile phase was acetonitrile:1 percent acetic acid (35:65), and solvent flow rate 1.5 ml/min. The detection limit was determined to be 50 ng 2,4-D.

Dichlobenil was partitioned into hexane from 9-ml water samples and analyzed with a Perkin-Elmer Model 3920 gas chromatograph equipped with a ^{63}Ni electron-capture detector. The column was 1.5% OV 17/1.95% QF-1 on 80/100 mesh Chromosorb Q. Chromatographic conditions: Column (140-195C at 32 degrees/min), detector (275C), injection port (230C). The detection limit was determined to be 0.05 ng.

Evaluation of Conventional Formulations

Laboratory evaluation techniques for submersed aquatic plants. Apical sections of submersed plants were planted in the aforementioned sand-soil mix in small plastic pots and placed in 3.8- or 19-ℓ jars filled with pond water. Plants were then allowed to become established for approximately 1 week under controlled conditions of temperature (25°C) and light (25 to 40 $\mu E/m^2/sec$), from Gro-lux fluorescent tubes, 14-hr photoperiods. The plants were treated by injecting treatment solutions into the water with a hypodermic syringe. The treatments were then evaluated biweekly for phytotoxicity.

Laboratory evaluations of chemicals for growth inhibition of hydrilla propagules. Vegetative propagules (tubers) of hydrilla were planted in four 5-cm pots (five tubers per pot). These pots were placed in a 3.8-ℓ jar filled with water. Chemical treatments were applied at the time of planting. Effects on germination were recorded along with phytotoxic response of sprouted plants. These tests were conducted in a growth lab under conditions of controlled light and temperature as described above.

Greenhouse evaluation techniques for emergent and floating aquatic plants. Plants to be treated were grown in polyethylene-lined, 12-ℓ capacity plastic containers, and allowed to become established in the greenhouse for a period of

approximately one to four weeks prior to treatment. Each replicated treatment was applied by placing the containers in a 929-cm^2 enclosure with an open top. The plants were then uniformly sprayed with a small atomizer. The total spray volume is equivalent to 935 ℓ/ha. Following application of the chemicals, the plants were moved to a greenhouse where treatments were periodically evaluated for phytotoxicity.

Evaluation techniques in outside aquaria. Evaluations were conducted in aquaria of two sizes and types. One type consisted of circular, vinyl-lined containers manufactured for use as swimming or wading pools. The dimensions were 3.05 m in diameter (7.3×10^{-4} ha) with a maximum depth of 74 cm. The maximum volume was 5400 ℓ. The pools were filled to a 53-cm depth, which resulted in a volume of 3870 ℓ.

The second type of aquarium consisted of rectangular-shaped concrete boxes. The interior of each box was covered with two coats of white epoxy paint. The dimensions were 77 cm wide by 219 cm long (1.7×10^{-4} ha) with depth varying from 48 to 65 cm. The maximum capacity of these containers ranged from 815 to 1095 ℓ and the normal volume after adding soil was 500 to 825 ℓ.

When these aquaria were used to evaluate herbicide efficacy of submersed plants, apical cuttings of individual species were established by planting cuttings 15 cm in length into holes on 5.1-cm centers (428 stems/sq m). The holes were punched into a 15-cm layer of sand-organic soil mix on the bottom of each aquarium. Water levels were then slowly raised in the aquaria and the plants were subjected to a continuous water flow until treatments were applied. For evaluation of herbicide efficacy on floating plant species, field-collected plants were established in the aquaria and allowed to completely cover the water surface before treatment.

All chemical treatment rates were replicated a minimum of three times and were applied on an area (kilograms per hectare) or volume (milligrams per litre) basis. Phytotoxicity ratings, determined at various times after treatment, were made on a scale of 0 to 100 percent injury: 0 percent = no injury, and 100 percent = complete elimination of live plant tissue.

RESULTS AND DISCUSSION

Evaluation of Controlled Released Formulations

Release of 2,4-D from clay pellets MOE 2,4-D/GMA in static water. Figure 1 illustrates the cumulative release of 2,4-D from clay pellets MOE 2,4-D/GMA in reconstituted water over a period of 70 days. The increasing levels of herbicide with time indicated that release from the formulation had occurred. Regression analysis of the release data revealed a significant relationship between release and time as would be expected.

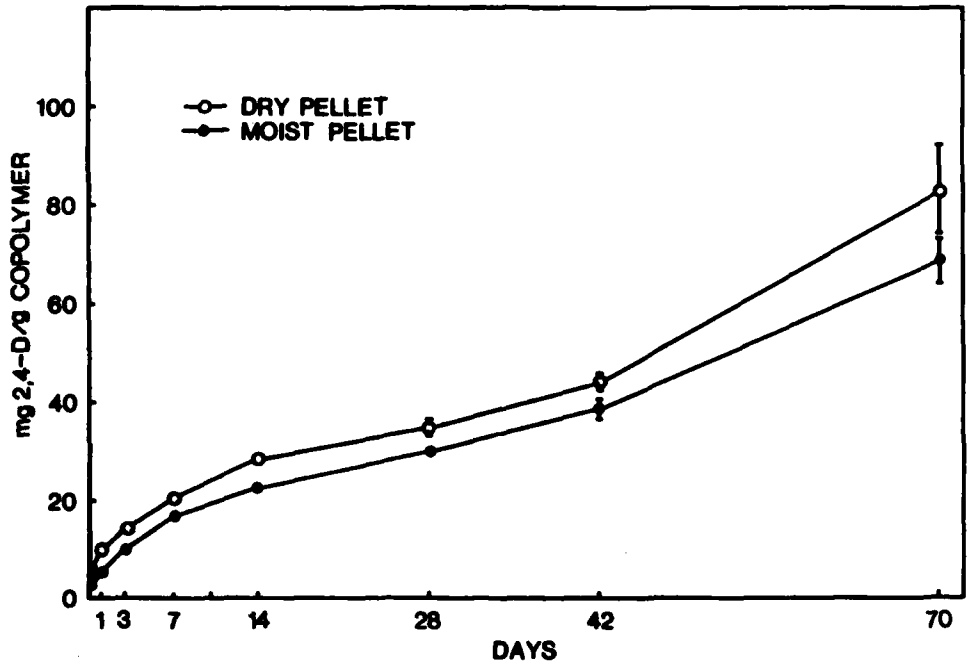

Figure 1. Release of 2,4-D from moist and dried clay pellets MOE 2,4-D/GMA in static natural water. Each point is the mean of four replicates ± S.E.

It was determined that an average of 5.5 mg 2,4-D/g copolymer was released within the first 24 hours after treatment (Table 3). However, the release rate appeared to be stabilized at approximately 0.8 mg 2,4-D/g copolymer/day throughout the remaining time of the experiment. The regression equation of release rates from day 3 to day 70 was:

$$Y = 9.5 + 0.8X, \; R = 0.99$$

Formulation of the copolymer MOE 2,4-D/GMA in clay pellets apparently significantly slowed down the release of 2,4-D from the copolymer, as previously observed (Steward 1981).

The test was repeated with pellets of MOE 2,4-D/GMA which had been air dried for 72 hours in the laboratory (Figure 1). Initial release of 2,4-D from the dried pellets was about twice the amount released from moist pellets, with approximately 9.8 mg 2,4-D released per gram copolymer per day during the first 24 hour posttreatment. The dried pellets were observed to lose integrity immediately after application. Release then stabilized at about the same rates as those observed with moist pellets. Regression equation of release rates from dried pellets was

$$Y = 11.7 + 0.9X, \; R = 0.98$$

Table 3
2,4-D Release from MOE 2,4-D/GMA Copolymer in Static Water

Treatment	Days after Treatment										
	1/12	1/6	1/4	1	3	3-1/4	7	14	28	42	133
Copolymer in reconstituted water				1.7*	7.0		15.8		72.3	92.7	408.0
				1.7**	2.3		2.3		2.6	2.2	3.1
Copolymer in natural water				1.8*	6.1		14.8		63.0	184.1	256.7
				1.8**	2.0		2.1		2.2	4.4	1.9
Wet clay pellet in reconstituted water	3.03	3.58	4.03	5.5*	10.0		16.8	22.4	30.2	38.5	
				5.5**	3.3		2.4	1.6	1.1	0.9	
Dry clay pellet in reconstituted water	6.9	7.6	7.9	9.8*		14.4	20.4	28.4	35.7	43.9	
				9.8**		4.4	2.9	2.0	1.3	1.0	

* mg/g copolymer.
** mg/g copolymer/day.

Release of 2,4-D from clay pellets MOE 2,4-D/GMA in natural flowing water and efficacy against watermilfoil. Treatments of clay pellets MOE 2,4-D/GMA to maintain 0.01 and 0.05 mg/ℓ 2,4-D were made to 19ℓ of flowing natural water with and without plants. The constancy of release and efficacy of the formulations in controlling watermilfoil were evaluated.

Figures 2 and 3 illustrate the concentrations of 2,4-D observed over an 8-week period for treatments with and without plants at rates of 0.01 and 0.05 mg/ℓ, respectively. A fast initial release of 2,4-D from the formulation was observed, with an average release rate of approximately 3.4 mg 2,4-D/g copolymer during the first 24 hours in both treatment rates (Table 4).

After 1 day, the measured concentrations of 2,4-D in the flowing water were 0.02-0.03 and 0.13-0.14 mg/ℓ for the treatment rates calculated to maintain 0.01 and 0.05 mg/ℓ, respectively. The herbicide concentrations then decreased sharply during the next day in all treatments. However, 2,4-D concentrations appeared to stabilize around the expected levels of 0.01 mg/ℓ (Figure 2) and 0.05 mg/ℓ (Figure 3) for a period of about 14 days, after which a decline was observed.

Concentrations of 2,4-D in treatments with plants were significantly lower than those in treatments without plants, possibly due to adsorption/absorption by plants and soil.

Table 4

Measured Release of 2,4-D from Emathite Clay Formulation with MOE 2,4-D/GMA Copolymer in Flowing Natural Water Conditions as Influenced by the Presence of Plants and Soil

2,4-D Treatment mg/ℓ	*mg/ℓ Concentration of 2,4-D, Days after Treatment*							
	1	*2*	*4*	*7*	*14*	*30*	*42*	*56*
Plant control								
A	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0
Average	0	0	0	0	0	0	0	0
0.01 no plants								
A	0.026	0.014	0.010	0.008	0.016	0.002	0.005	0.004
B	0.035	0.016	0.016	0.014	0.014	0.011	0.006	0.005
C	0.024	0.011	0.011	0.014	0.012	0.009	0.007	0.004
D	0.028	0.012	0.016	0.023	0.012	0.011	0.010	0.004
Average	0.028	0.013	0.013	0.015	0.012	0.008	0.007	0.004
0.01 with plants								
A	0.019	0.012	0.009	0.009	0.007	0.005	0.003	0.002
B	0.037	0.011	0.012	—	0.008	0.003	0.001	0.003
C	0.025	0.011	0.012	0.005	0.007	0.002	0.000	0.000
D	0.010	—	0.013	0.009	0.006	0.003	0.002	0.002
Average	0.023	0.011	0.012	0.008	0.007	0.004	0.002	0.002
0.05 no plants								
A	0.112	0.057	0.052	0.046	0.048	0.034	0.020	0.014
B	0.146	0.063	0.065	0.047	0.049	0.038	0.025	0.021
C	0.132	0.062	0.068	0.044	0.043	0.024	0.017	0.017
D	0.146	0.066	0.054	0.047	0.047	0.031	0.024	0.020
Average	0.134	0.062	0.060	0.046	0.047	0.032	0.022	0.018
0.05 with plants								
A	0.121	0.090	0.057	0.045	0.042	0.004	0.008	0.000
B	0.157	0.076	0.054	0.045	0.032	0.017	0.015	0.006
C	0.151	0.055	0.045	0.047	0.038	0.021	0.019	0.010
D	0.126	0.047	0.038	0.048	0.028	0.007	0.006	0.000
Average	0.139	0.067	0.048	0.046	0.035	0.012	0.013	0.004
mg 2,4-D released per gram copolymer (Average of 4 replicates)								
Treatment #2	3.36	1.68	1.56	1.8	1.56	0.96	0.84	0.48
Treatment #4	3.32	1.49	1.45	1.11	1.16	0.77	0.53	0.43

The watermilfoil plants were harvested 8 weeks after treatment and evaluated for percent survival. Responses of watermilfoil plants to treatments are presented in Table 5. No significant differences in several growth parameters were observed with plants treated at the 0.01-mg/ℓ level. However, percent survival as well as growth in stem length were significantly lower in plants treated at the 0.05-mg/ℓ level.

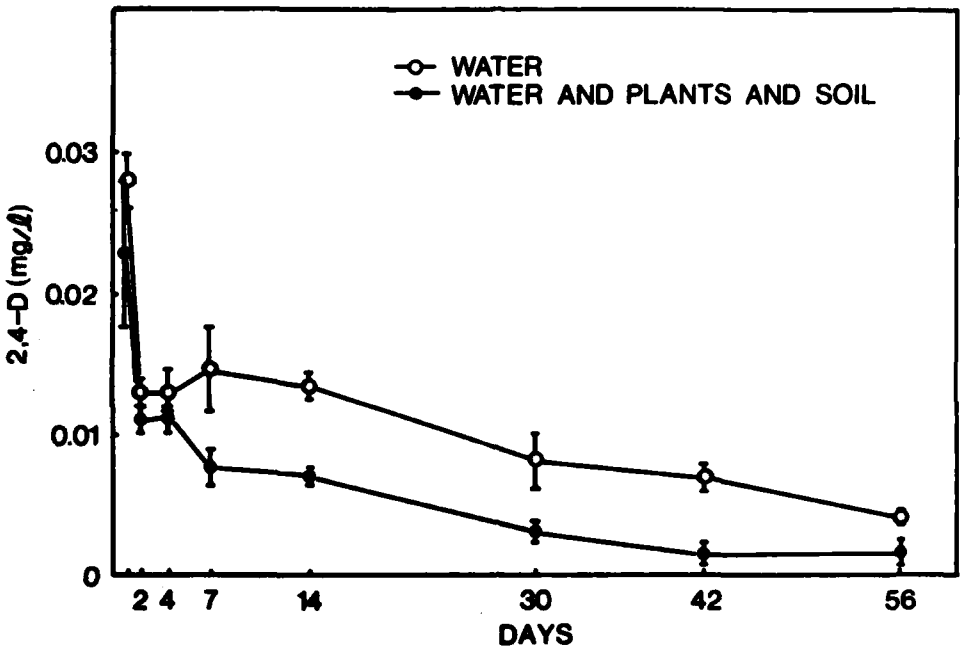

Figure 2. Release of 2,4-D from clay pellets MOE 2,4-D/GMA in natural flowing water. Treatments were made to maintain 0.01 mg/l 2,4-D. Each point is the mean of four replicates ± S.E.

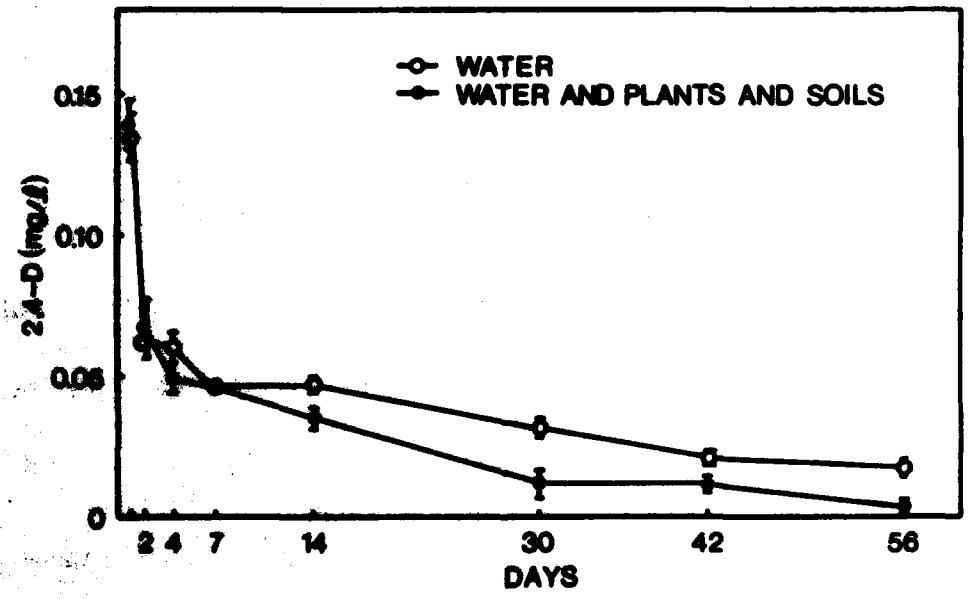

Figure 3. Release of 2,4-D from clay pellets MOE 2,4-D/GMA in natural flowing water. Treatments were made to maintain 0.05 mg/l 2,4-D. Each point is the mean of four replicates ± S.E.

Table 5
Effect of 2,4-D Release from Clay Formulation of MOE 2,4-D/GMA Copolymer on Watermilfoil Growth after 8 Weeks in Flowing Water*

Treatments	*% Survival*	*Stem Length cm*	*Fresh Wt/Plant g*
Control	60[a]	43.4[a]	1.94[a]
0.01 mg/ℓ	52[a]	35.9[a]	1.98[a]
0.05 mg/ℓ	38[b]	20.7[b]	1.66[a]

* Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of four replications.

Release rates of 2,4-D from clay pellets Poly GMA 2,4-D in static water. Release of 2,4-D from clay pellets Poly GMA 2,4-D was determined first in static water tests under controlled laboratory conditions at 28 ± 2°C. Treatments of the clay pellets were made to 3.7ℓ water with amounts calculated to produce a concentration of 0.1 mg/ℓ 2,4-D every 24 hours.

Results of 2,4-D measurements are presented in Figure 4 for treatments in reconstituted water. It was determined that an average of 64.2 mg/g polymer was released within the first 24 hours after treatment. This initial "wash-out" was probably due to the portion of 2,4-D acid unreacted with the polymer in the formulation (Harris, personal communication).

The release rates remained high during the next 3 days, with about 17 percent of the total 2,4-D applied being released by the end of this period. However, the release rate appeared to be stabilized at around 2.6 mg/g polymer/day throughout the remaining time of the experiment.

The regression equation of release rates from day 7 to day 84 was

$$Y = 96.25 + 2.61X, \quad R = 0.99$$

Based on this release rate, we estimated that the polymer formulation would be depleted of all 2,4-D in approximately 185 days.

Similar results were found with treatments of Poly GMA 2,4-D in natural water (Figure 5). After 1 week, the release rates stabilized at 2.3 mg/g polymer/day. The regression equation of release rates in natural water from day 7 to day 84 was

$$Y = 97.50 + 2.29X, \quad R = 0.98$$

Figure 6 compares cumulative release of the herbicide from Poly GMA 2,4-D into reconstituted and natural water over a period of 24 weeks.

Similar release rates of 2,4-D were observed in treatments with reconstituted water and natural water during the first 8 weeks of the experiment. Release rates into reconstituted water remained unchanged in later sampling periods, indicating the constancy and reliability of release.

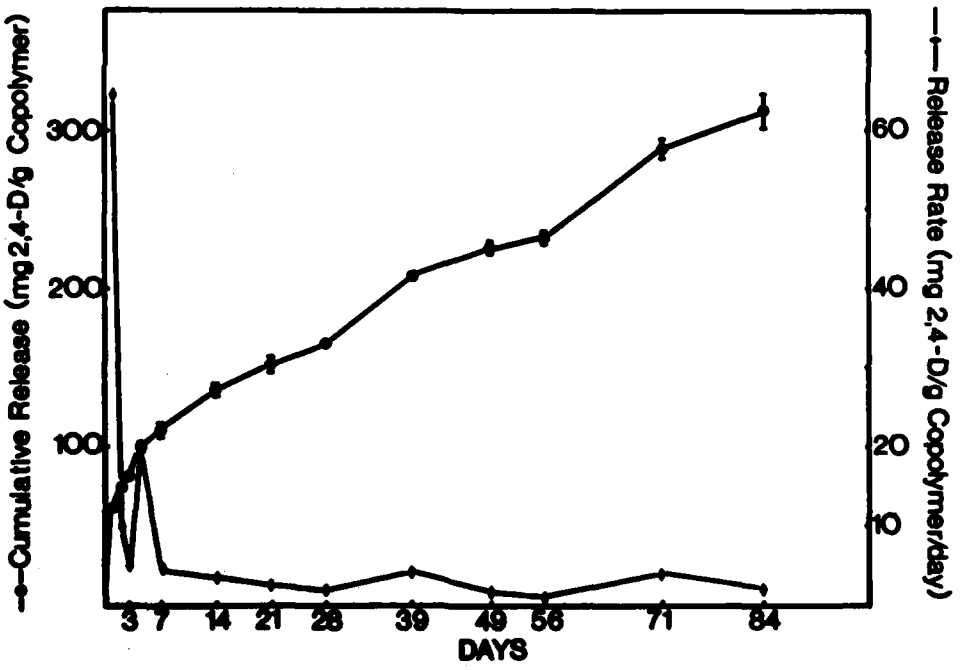

Figure 4. Release of 2,4-D from clay pellets Poly GMA 2,4-D in static reconstituted water. Each point is the mean of four replicates ± S.E.

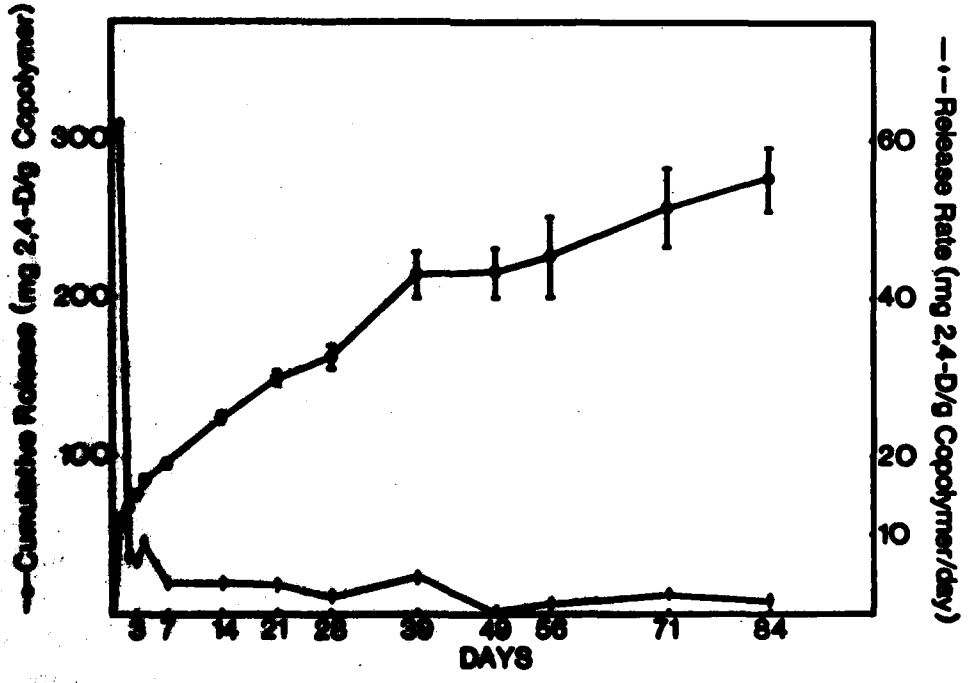

Figure 5. Release of 2,4-D from clay pellets Poly GMA 2,4-D in static natural water. Each point is the mean of four replicates ± S.E.

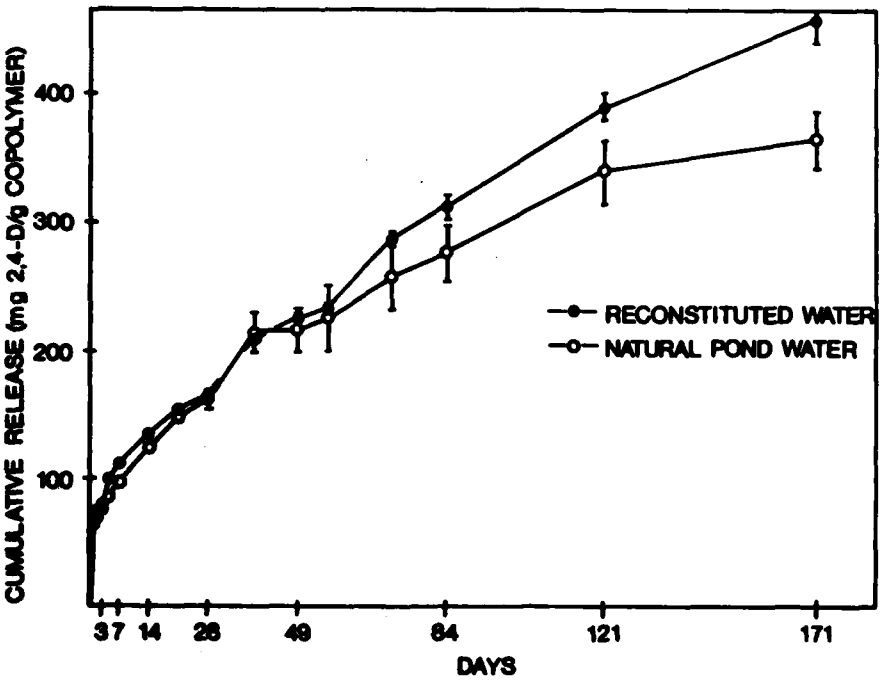

Figure 6. Cumulative release of 2,4-D from Poly GMA 2,4-D in reconstituted water and natural water. Each point is the mean of four replicates ± S.E.

In treatments with natural water, however, apparent release rates appeared to slow down significantly during the later part of the experiment. Higher microbial activity and algal growth were observed in treatments with natural pond water. These factors may have been partly responsible for faster disappearance of the released chemical in natural water.

Release rates of 2,4-D from Poly GMA 2,4-D into flowing natural water. Treatments of the pelletized Poly GMA formulation to maintain 0, 0.01, 0.02, 0.05, and 0.10 mg/*l* 2,4-D concentrations were made to 19*l* of flowing water in glass, flow-through culture vessels with Eurasian watermilfoil and sago pondweed.

Results of the analyses (Table 6) indicated again an initial "wash-out" of 2,4-D from the formulation, with an average release rate varying from 24.5 to 25.7 mg 2,4-D/g polymer for all treatment rates during the first 24 hours. These release rates were only about half of those observed in earlier static tests (Figure 5), presumably because of dilution by the flowing water, and absorption and/or adsorption by plants and soil in the culture jars. Furthermore, the release rates appeared independent from the four treatment rates applied (Table 6).

After 1 day, the average measured concentrations of 2,4-D in the flowing water were 0.22, 0.45, 1.03, and 2.16 mg/*l* for treatment rates calculated to maintain 0.01, 0.02, 0.05, and 0.10 mg/*l*, respectively. These 2,4-D concentrations gradually

Table 6
Measured Release of 2,4-D from Clay Pellets Poly GMA 2,4-D in Flowing Natural Water Conditions as Influenced by the Presence of Plants and Soil

2,4-D Treatment	*mg/l Concentration of 2,4-D, Days after Treatment*								
	1	*2*	*3*	*4*	*7*	*14*	*28*	*42*	*56*
0.01 mg/l									
A	0.17	0.08	0.08	0.05	0.03	0.04	0.00	0.00	0.00
B	0.26	0.14	0.08	0.06	0.05	0.01	0.00	0.00	0.00
C	0.20	0.09	0.07	0.05	0.03	0.04	0.00	0.00	0.00
D	0.31	0.14	0.08	0.06	0.03	0.01	0.00	0.00	0.00
Average	0.23	0.11	0.08	0.06	0.04	0.03	0.00	0.00	0.00
0.02 mg/l									
A	0.42	0.26	0.16	0.10	0.08	0.10	0.00	0.00	0.00
B	0.37	0.27	0.16	0.11	0.07	0.01	0.00	0.00	0.00
C	0.37	0.25	0.17	0.12	0.04	0.01	0.00	0.00	0.00
D	0.64	0.30	0.17	0.11	0.07	0.05	0.01	0.01	0.00
Average	0.45	0.27	0.17	0.11	0.07	0.04	0.00	—	0.00
0.05 mg/l									
A	0.95	0.55	0.39	—	0.19	0.00	0.00	0.00	0.00
B	1.09	0.61	0.37	0.27	0.20	0.08	0.02	BDL	BDL
C	1.13	0.52	0.41	0.31	0.20	0.00	0.00	0.00	0.00
D	0.95	0.56	—	0.32	0.11	0.10	0.13	BDL	0.00
Average	1.03	0.57	0.39	0.30	0.18	0.05	0.04	—	—
0.10 mg/l									
A	2.16	1.48	0.81	0.65	0.35	0.02	0.04	0.00	0.00
B	1.58	0.88	—	0.60	0.24	0.11	—	0.00	BDL
C	2.18	1.19	0.73	0.56	0.36	—	0.02	BDL	0.00
D	2.69	1.36	0.75	0.60	0.37	0.14	0.06	0.02	0.01
Average	2.16	1.23	0.76	0.60	0.33	0.09	0.04	—	—
mg 2,4-D released per gram copolymer (Means of 4 replicates)									
Treatment #1	25.2	12.6	8.5	6.0	3.6	3.3	0.0	0.0	0.0
Treatment #2	26.0	15.0	9.2	5.8	3.4	2.2	0.0	0.0	0.0
Treatment #3	24.5	13.2	9.3	7.2	4.2	1.1	1.0	—	—
Treatment #4	25.7	14.5	9.1	7.2	4.0	1.1	0.5	—	—

declined during the first week after treatment; however, they still remained at levels severalfold higher than those expected from the specified release rate of the formulation.

The initial high levels of 2,4-D in the flowing water may have been responsible for the rapid injury response by watermilfoil plants. Heavy plant decay and algal growth were observed in the culture vessels 4 weeks after treatment at all treatment rates. These factors may act as sinks in taking up chemical, resulting in the disappearance of 2,4-D from the flowing water at later sampling periods (Table 6).

Figure 7 illustrates the influence of plants and soil on the measured herbicide levels in the flowing water. Treatments of Poly GMA 2,4-D were made with amounts calculated to maintain a constant 2,4-D concentration of 0.10 mg/ℓ in the flowing water, based on the release rate of 2.3 mg 2,4-D/g polymer/day observed in static natural water (Figure 5).

The actual 2,4-D concentrations measured after 24 hours were 1.6 and 1.8 mg/ℓ, reflecting the initial "wash-out" of the formulation as observed earlier. Concentrations of the herbicide then decreased rapidly in treatments with plants and soil, and disappeared from the flowing water during the last 4 weeks of the experiment.

In the absence of plants and soil, however, 2,4-D concentrations appeared to maintain around the expected level of 0.10 mg/ℓ for a period of 4 weeks, after which a decline was observed.

The influence of various components of the experimental system that may act as sinks in taking up the released chemical was further investigated (Figure 8). The presence of soil in the culture vessels appeared to decrease the herbicide levels in water by approximately 40 percent when compared to concentration in vessels without soil. Adding plants to the culture vessels further decreased the levels of 2,4-D in water and complete loss of the herbicide in the flowing water was again observed by 6 weeks posttreatment.

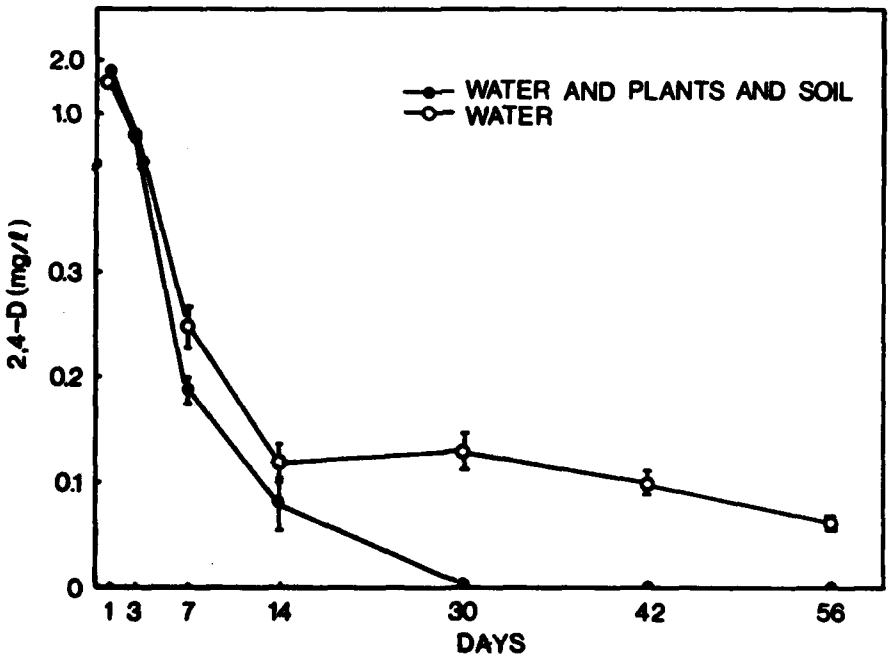

Figure 7. Release of 2,4-D from clay pellets Poly GMA 2,4-D in flowing natural water. Each point is the mean of four replicates ± S.E.

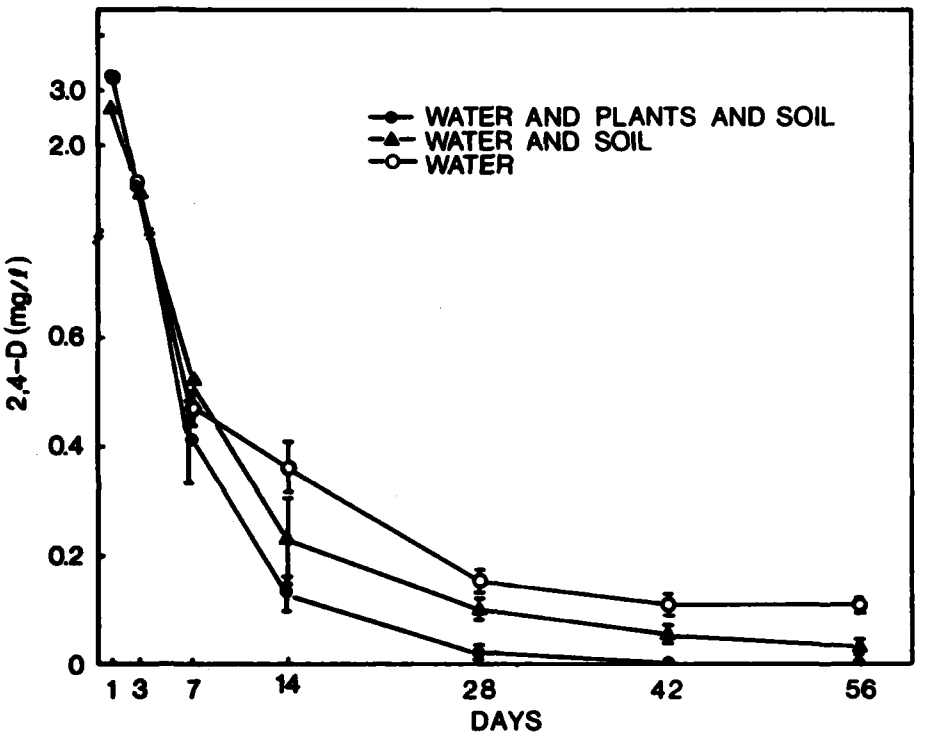

Figure 8. Influence of soil and plants on 2,4-D concentration in flowing natural water as released from clay pellets Poly GMA 2,4-D. Each point is the mean of four replicates ± S.E.

These results suggested that higher release rates of chemical may be necessary, so that the formulation will be effectively delivering enough herbicide not only to control plant growth, but also to compensate for any other components in the aquatic system acting as sinks in taking up the chemical.

Efficacy of Poly GMA 2,4-D against watermilfoil and sago pondweed. Response of watermilfoil and sago pondweed to the 2,4-D formulation under flowing water conditions was evaluated closely during the first 2 weeks of the experiment. Elongation of main stems was observed in watermilfoil plants after 2 days in all treatment levels. However, further growth of the plants appeared completely suspended after this initial elongation phase. At treatment levels of 0.05 and 0.10 mg/l, a slight epinastic response of leaves at or near stem apices was observed 3 days after treatment, and was very pronounced after 1 week. At the end of 2 weeks, the upper portions of the main stems appeared darkened as though undergoing necrosis. Necrosis then spread down to near the base of the plants, and complete kill was obvious by 3 to 4 weeks after treatment at all four 2,4-D treatment levels. All control plants appeared healthy, erect, and of good color.

After 5 weeks, a slight regrowth occurred in treatment levels in 0.01 and 0.02 mg/l in the form of a single branch arising from the nodes of damaged stems.

However, the new branches eventually showed some injury response and none survived at the end of the experiment.

Sago pondweed appeared more tolerant to the treated levels of 2,4-D. All plants survived through the end of the experiment. No visual injury was observed on treated plants at 0.01 and 0.02 mg/ℓ; however, several necrotic leaves and stems were apparent in higher treatment rates. The average injury after 8 weeks was 15 and 32 percent in plants treated at rates of 0.05 and 0.10 mg/ℓ, respectively (Table 7). All plants were harvested 8 weeks after treatment, and stem lengths and plant weights were measured. The dry weight as well as growth in stem length was significantly lower in plants treated at the 0.05- and 0.10-mg/ℓ levels.

Table 7
Effect of 2,4-D Release from Clay Formulation of Poly GMA 2,4-D on Watermilfoil and Sago Pondweed after 8 Weeks in Flowing Water*

	Watermilfoil			*Sago Pondweed*		
Treatment	*% Injury*	*Stem Length cm*	*Dry Wt/Plant g*	*% Injury*	*Stem Length cm*	*Dry Wt/Plant g*
Control	0	42	1.57	0	62[a]	2.19[a]
0.01 mg/ℓ	100	0	0	0	67[a]	1.79[a]
0.02 mg/ℓ	100	0	0	0	63[a]	1.88[a]
Control	0	42	1.28	0	69[a]	1.18[a]
0.05 mg/ℓ	100	0	0	15	58[ab]	0.87[b]
0.10 mg/ℓ	100	0	0	32	48[b]	0.69[b]

*Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of three replications.

Release rates of 2,4-D from Westvaco lignin formulation in static reconstituted and natural water. Release rates of 2,4-D from Westvaco lignin formulation in static reconstituted and natural water are presented in Figure 9. Linear regression analyses of the data indicated that release rates from day 1 to day 21 were approximately tenfold higher than the theoretical (designed) rate, with averages being 13.8 and 14.5 mg/g pellet for treatments in reconstituted and natural water, respectively.

From day 21 to day 71, release rates slowed down significantly, being 2.1 mg/g pellet in both reconstituted and natural water. By day 71, approximately 86 percent of the total 2,4-D applied had been released. No further release was apparent from day 71 to day 84.

Release rates of dichlobenil from formulations of urea-formaldehyde resins in static reconstituted water. Treatments of the different formulations were made to reconstituted water to release a total of 12.1 mg dichlobenil. The four different formulations evaluated are as follows:

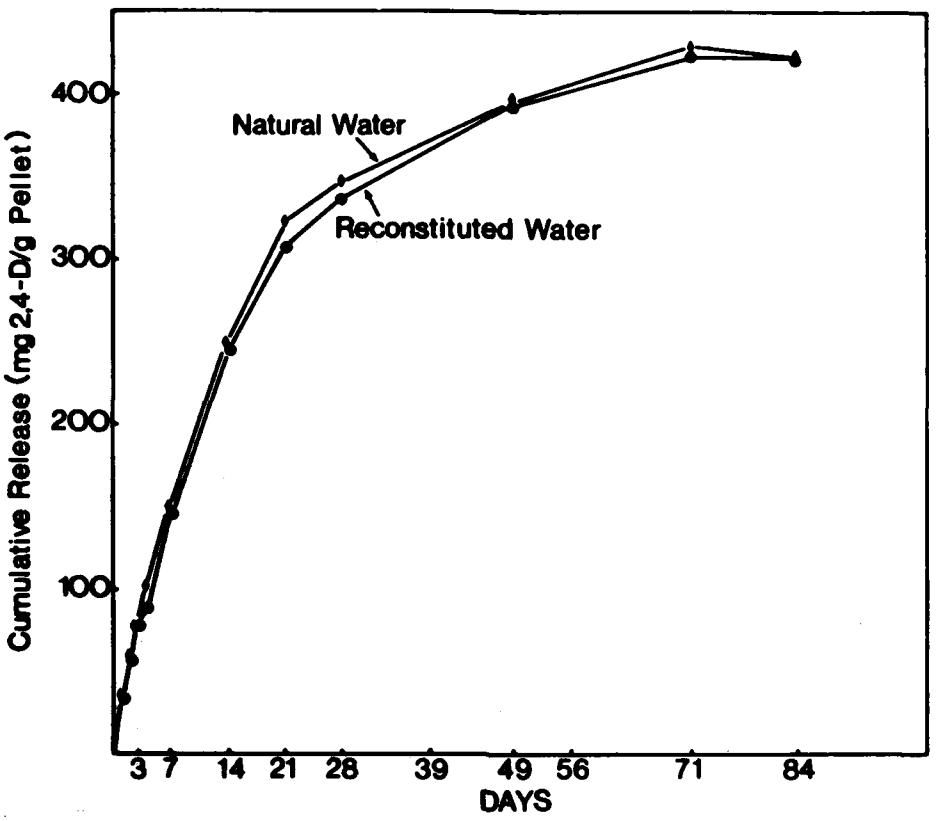

Figure 9. Cumulative release of 2,4-D from Westvaco 2,4-D/Kraft lignin in static reconstituted water and natural water

P UF_1	Ureaform and chlorthiamid. 52 percent chlorthiamid covalently bound onto the resin, 24 percent a.i.
P UF_2	Ureaform and chlorthiamid. 100 percent chlorthiamid convalently bound into the resin, 23.3 percent a.i.
C UF	Physical combination of dichlobenil and ureaform, 18 percent a.i.
MP UF	Chemical combinations of N-methylochlorthiamid and ureaform, 24 percent a.i.

Results of dichlobenil analyses are presented in Figure 10. The rate of dichlobenil released from the physical mixture, C UF, was the fastest. The percent chlorthiamid bound to the resin appeared to affect the release rate of dichlobenil inversely: P UF_1 released significantly faster than P UF_2.

The release rate of dichlobenil from all formulations was not constant, with rapid initial release followed by slow release for the next 3 weeks, and an increasing release rate up to 6 weeks (Table 8). The limited amount of material available precluded extensive investigation of these formulations.

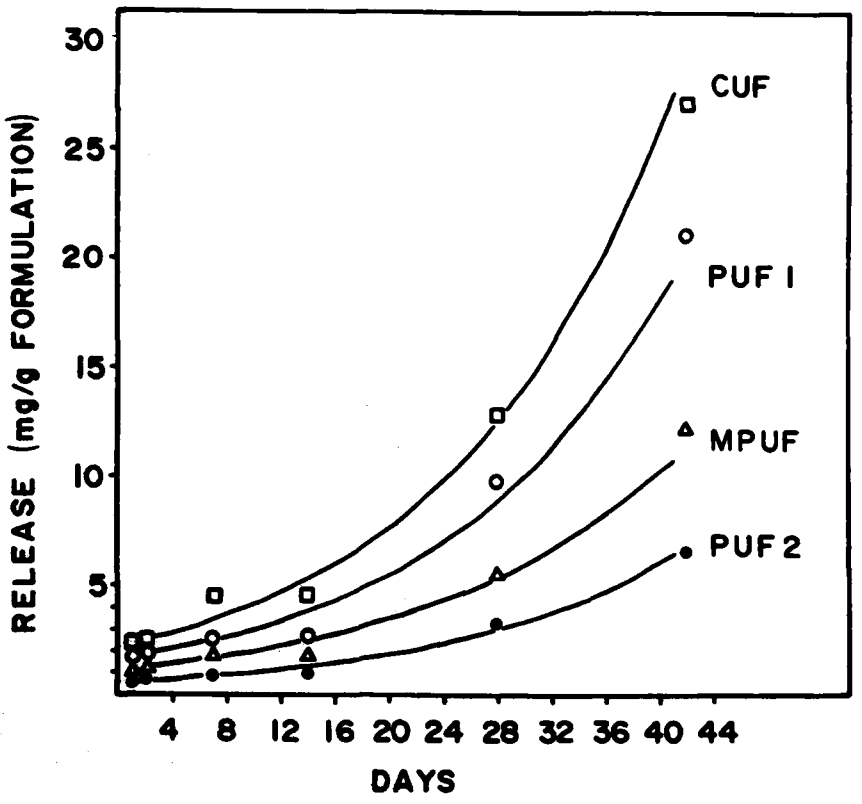

Figure 10. Release of dichlobenil from urea-formaldehyde resin formulations into static reconstituted water

Table 8
Dichlobenil Release from Four Formulations of Urea-Formaldehyde Resins in Reconstituted Water

	Days After Treatment					
	1	*2*	*7*	*14*	*28*	*42*
	mg/g formulation (average of 3 replicates)					
P UF_1	1.79	2.16	2.47	2.60	9.70	20.94
P UF_2	0.53	0.73	0.85	0.98	3.21	6.64
C UF	1.95	2.26	4.39	4.49	13.93	27.01
MP UF	1.30	1.37	1.71	1.73	5.47	12.07

Evaluation of Conventional Formulations

Hydrilla tuber germination, tuber formation. DPX-4189 was evaluated in the laboratory for efficacy in preventing germination of hydrilla tubers (Table 9). The chemical was also bioassayed for toxicity toward the new sprouts emerging from germinated tubers planted in soil (Table 10).

The results indicated that DPX-4189 applied at a rate of up to 20 mg/ℓ did not inhibit hydrilla tuber germination. However, growth and development of the newly germinating sprouts were severely retarded by treatments of 0.01 mg/ℓ or higher.

The study was repeated with dichlobenil, fenac, and fluridone, as well as DPX-4189 (Table 11). None of these chemicals were found to be effective in preventing hydrilla tuber germination. However, fenac and dichlobenil at 0.25 mg/ℓ provided more than 90-percent control of regrowth. Retardation of hydrilla regrowth was also observed in fluridone treatments at 0.025 mg/ℓ or higher.

Procedures have been developed for inducing tuber formation by hydrilla under controlled growth conditions in the laboratory. Preliminary evaluations indicated that the herbicides fluridone and DPX-4189 inhibited hydrilla tuber formation under experimental conditions, at treatment rates of 0.05 mg/ℓ and 0.10 mg/ℓ, respectively (Table 12).

The potential use of these chemicals for management of hydrilla regrowth is being further investigated at this laboratory.

Table 9
Effect of DPX-4189 on Hydrilla Tuber Germination*

Concentration mg/ℓ	*Percent Germination Days Posttreatment*			
	5	*7*	*10*	*17*
0	13	47	85	92[a]
0.1	8	48	87	88[a]
0.5	20	58	82	88[a]
1	2	38	83	88[a]
5	3	40	80	83[a]
10	5	28	83	88[a]
20	10	40	83	87[a]

* Sixty tubers per treatment. No significant differences between means followed by the same letter at P=0.05 as determined by Duncan's Multiple Range Test.

Table 10
Effect of DPX-4189 on Growth of Hydrilla Tubers after 28 Days*

Concentration mg/ℓ	*Germination %*	*Shoot Length mm*
0	63[a]	201[c]
0.01	50[a]	88[b]
0.02	52[a]	63[b]
0.05	62[a]	39[a]
0.10	60[a]	39[a]
0.25	60[a]	36[a]
0.50	47[a]	30[a]

* Sixty tubers per treatment. No significant difference between means followed by the same letter at P=0.05 as determined by Duncan's Multiple Range Test.

Table 11
Effect of Various Herbicides on Hydrilla Tuber Germination and Growth after Six Weeks*

Chemical Treatment Rate mg/l	*Germination %*	*Shoot Length mm*	*Injury %*
FLURIDONE			
0.010	54	370	70
0.025	44	230	73
0.050	44	110	88
0.100	40	110	57
DPX-4189			
0.010	40	150	70
0.025	50	80	73
0.050	54	70	77
0.100	44	40	60
FENAC			
0.10	46	270	10
0.25	76	120	90
0.50	56	100	97
1.0	86	120	96
DICHLOBENIL			
0.25	66	0	100
0.50	56	0	100
1.0	44	0	100
Control	44	310	3

* Sixty tubers per treatment.

Table 12
Effects of Various Herbicides on Vegetative Growth and Tuberization in Hydrilla under 10-hr Photoperiods*

Treatment	*Stems and Leaves*	*Roots*	*Rhizomes*	*Tubers*	*Tubers at 5 Weeks*
mg/l		*g dry wt.*			Number
Control	14.2	1.26	0.24	0.92	23
0.25 Fenac	9.0	0.73	0.06	0.03	6
0.25 Dichlobenil	11.9	0.37	0.11	0.08	2
0.10 DPX-4189	10.3	0.12	0.00	0.00	0
0.05 Fluridone	9.4	0.39	0.00	0.00	0

* Average of three replicates.

Submersed weeds. DPX-4189 and DPX-5648 were evaluated for efficacy in controlling hydrilla, watermilfoil, sago pondweed, and chara (Table 13), hygrophila, cabomba, bacopa, and coontail (Table 14). The two chemicals appeared to give a broad-spectrum control of the species tested. The herbicide action was slow, with approximately 8 to 10 weeks required for adequate control.

The macro alga chara showed no evidence of phytotoxicity at dose rates up to 5 mg/ℓ of DPX-4189 or DPX-5648. This selectivity would be desirable since chara is being used as a replacement species in the management of hydrilla in Florida. A treatment with these herbicides would selectively kill hydrilla, leaving chara behind which may serve as a physical barrier to prevent hydrilla regrowth.

The evaluation of glutaraldehyde against several submersed aquatic weed species was completed (Table 15). Over 90-percent control of chara was obtained after 4 weeks at treatment rates of 5 mg/ℓ or higher. The chemical was not effective against watermilfoil and cabomba, and only moderately effective against hydrilla at the 10-mg/ℓ rate. This rate would be environmentally and economically infeasible, however, based on priority information supplied by the manufacturer.

Table 13
Laboratory Evaluations of DPX-4189 and DPX-5648 for Phytotoxicity Toward Combined Hydrilla (HD), Watermilfoil (W), Sago pondweed (P), and Chara (CR)

Evaluation Date	Chemical Designation	Company or Source	Rate mg/ℓ	Posttreatment Control, percent											
				2 weeks				4 weeks				6 weeks			
				HD	W	P	CR	HD	W	P	CR	HD	W	P	CR
10/12/81	DPX-4189	duPont	0.5	0	3	0	2	13	7	3	3	28	27	13	3
			1.0	0	3	0	2	33	65	17	7	35	77	23	3
			2.0	0	0	3	0	23	10	27	5	43	63	28	3
			5.0	0	0	5	0	20	10	18	5	43	53	33	8
	DPX-5648	duPont	0.5	0	0	0	0	22	17	33	7	45	23	40	3
			1.0	0	0	8	3	12	10	22	7	37	27	30	8
			2.0	0	0	3	0	32	33	33	7	57	63	40	7
			5.0	0	0	0	2	17	37	35	7	40	90	43	18
	Control			0	0	2	0	0	2	3	2	2	2	7	3

				8 weeks				10 weeks			
				HD	W	P	CR	HD	W	P	CR
	DPX-4189	duPont	0.5	68	83	33	2	83	93	57	3
			1.0	73	97	33	5	93	97	60	5
			2.0	92	87	37	20	90	90	65	15
			5.0	90	90	40	22	90	100	65	17
	DPX-5648	duPont	0.5	87	93	53	13	85	95	85	10
			1.0	80	83	50	8	100	90	80	5
			2.0	93	97	73	30	100	100	80	23
			5.0	90	90	58	30	93	100	80	23
	Control			10	30	20	5	15	30	27	5

Table 14

Laboratory Evaluations of DPX-4189 and DPX-5648 for Phytotoxicity Toward Combined Hygrophila (HG), Cabomba (CB), Bacopa (B), and Coontail (CT)

Evaluation Date	Chemical Designation	Company or Source	Rate mg/l	Posttreatment Control, percent											
				2 weeks				4 weeks				6 weeks			
				HG	CB	B	CT	HG	CB	B	CT	HG	CB	B	CT
10/12/81	DPX-4189	duPont	0.5	0	0	0	7	0	5	7	67	0	5	27	80
			1.0	0	5	0	15	0	23	5	93	3	30	33	100
			2.0	0	0	0	8	10	18	13	70	30	40	53	80
			5.0	0	0	0	2	7	27	30	70	27	43	43	97
	DPX-5648	duPont	0.5	0	2	0	32	0	17	30	93	13	35	55	100
			1.0	3	0	0	7	17	7	13	90	20	27	30	100
			2.0	3	0	0	18	20	37	27	77	30	53	53	80
			5.0	0	0	0	7	13	8	3	90	27	53	37	100
	Control			0	0	0	0	0	0	0	0	0	0	0	0

				8 weeks				10 weeks			
				HG	CB	B	CT	HG	CB	B	CT
	DPX-4189	duPont	0.5	53	40	47	93	63	67	67	98
			1.0	90	73	80	100	93	97	97	100
			2.0	67	77	68	93	73	93	70	97
			5.0	67	90	93	100	88	97	97	100
	DPX-5648	duPont	0.5	67	63	68	100	73	90	75	100
			1.0	77	83	75	100	92	100	90	100
			2.0	67	73	60	93	78	93	67	100
			5.0	58	100	80	100	85	100	90	100
	Control			2	3	0	0	0	10	15	0

Table 15

Laboratory Evaluations of Glutaraldehyde for Phytotoxicity Toward Combined Hydrilla (H), Watermilfoil (W), Cabomba (CA), and Chara (CR)

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/l	Posttreatment Control, percent							
				2 Weeks				4 Weeks			
				H	W	CA	CR	H	W	CA	CR
06/13/81	Glutaraldehyde	Union Carbide	0.25	0	0	0	0	5	0	0	0
			0.5	2	0	13	0	7	0	27	2
			1.0	0	0	0	5	5	0	0	13
			2.0	3	0	7	10	3	0	13	17
			3.0	23	0	2	5	30	0	10	15
			5.0	37	0	2	30	75	0	3	90
			10.0	57	0	3	78	87	0	7	97
	Control			0	0	0	0	0	0	0	0

				6 Weeks				8 Weeks			
				H	W	CA	CR	H	W	CA	CR
	Glutaraldehyde	Union Carbide	0.25	5	0	45	5	3	5	55	5
			0.5	2	0	43	10	0	5	42	12
			1.0	0	0	12	15	0	10	33	18
			2.0	0	3	17	15	0	20	25	28
			3.0	30	0	32	22	23	10	37	38
			5.0	67	0	20	83	53	5	23	88
			10.0	83	0	18	100	80	15	23	100
	Control			2	0	3	2	0	5	25	2

Table 16
Laboratory Evaluations of Acrolein and Glutaraldehyde for Phytotoxicity Toward Combined Hydrilla (H), Watermilfoil (W), and Chara (CR)

Date of Evaluation	*Chemical Designation*	*Company or Source*	*Rate mg/l*	*Posttreatment Control, percent*					
				2 Weeks			*4 Weeks*		
				H	*W*	*CR*	*H*	*W*	*CR*
11/13/81	Acrolein	Union Carbide	0.5	0	3	7	2	7	3
			1.0	13	3	17	20	20	20
			2.0	30	73	100	60	70	97
			5.0	90	100	100	95	100	100
			10.0	98	97	100	100	100	100
	Glutaraldehyde	Union Carbide	0.5	0	0	0	0	0	3
			1.0	0	0	0	0	0	0
			2.0	3	0	10	3	0	13
			5.0	20	20	23	25	20	32
			10.0	53	27	63	70	22	100
				0	0	0	0	0	0
				6 Weeks			*8 Weeks*		
				H	*W*	*CR*	*H*	*W*	*CR*
	Acrolein	Union Carbide	0.5	7	3	10	3	13	43
			1.0	22	57	17	25	63	23
			2.0	53*	70*	93	30	32*	77*
			5.0	95	100	100	57	100	100
			10.0	100	100	100	100	100	100
	Glutaraldehyde	Union Carbide	0.5	7	10	2	0	0	23
			1.0	0	0	7	2	0	17
			2.0	0	0	8	2	0	13
			5.0	27	13	28	33	13	30
			10.0	53*	2	100	47*	0	98
	Control			0	0	0	0	0	0

*Regrowth was evident.

In comparative tests against hydrilla, watermilfoil, and chara (Table 16), coontail, cabomba, and lacuna (Table 17), acrolein appeared effective against all the tested species at dose rates of 2 mg/*l* or higher. In contrast, chara was the only species sensitive to glutaraldehyde treatments at rates up to 10 mg/*l*.

Regrowth was evident in several treatments of both acrolein and glutaraldehyde after 6 and 8 weeks, respectively, suggesting short-term control by these chemicals.

Diquat. Table 18 presents the results of laboratory evaluations of various formulations of diquat for phytotoxicity toward hydrilla. In these evaluations, the experimental diquat formulations were compared with the standard liquid formulation injected below the water surface.

There was little apparent difference in phytotoxicity produced by the new experimental formulations and by the reference liquid diquat. This pattern is indicative of a rapid release of herbicide from the formulations and a response to phytotoxic concentrations.

Table 17
Laboratory Evaluations of Acrolein and Glutaraldehyde for Phytotoxicity Toward Combined Coontail (CT), Cabomba (CA), and Bacopa (B)

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/l	Posttreatment Control, percent					
				2 Weeks			4 Weeks		
				CT	CA	B	CT	CA	B
11/13/81	Acrolein	Union Carbide	0.5	3	0	10	8	0	10
			1.0	7	0	3	37	7	5
			2.0	97	77	97	96	87	97
			5.0	97	90	97	100	96	100
			10.0	97	97	100	100	100	100
	Glutaraldehyde	Union Carbide	0.5	0	0	0	5	0	0
			1.0	0	0	0	0	0	0
			2.0	10	0	0	27	0	3
			5.0	37	5	0	53	7	10
			10.0	83	7	7	90	10	30
	Control			0	0	3	0	0	3
				6 Weeks			8 Weeks		
				CT	CA	B	CT	CA	B
	Acrolein	Union Carbide	0.5	5	17	10	2	37	20
			1.0	20	22	7	17	35	5
			2.0	92	83	96	60*	53*	87*
			5.0	96	96	100	100	83*	100
			10.0	100	100	100	100	100	100
	Glutaraldehyde	Union Carbide	0.5	2	23	12	3	67	10
			1.0	0	10	3	10	17	5
			2.0	23	30	8	35	63	10
			5.0	27*	10	10	30*	17	10
			10.0	77*	10	27	70*	33	17
	Control			0	0	0	0	0	0

*Regrowth was evident.

Table 18
Laboratory Evaluations of Various Formulations of Diquat for Phytotoxicity Toward Hydrilla

Date of Evaluation	Diquat Formulations	Company or Source	Rate mg/l	Percent Control Weeks Posttreatment				
				1	2	4	6	8
[illegible]	Gramo + 1.5% Kelzan	Chevron	0.25	40	63	83	97	90*
			0.50	37	70	97	100	100
			1.0	30	70	97	100	100
			2.0	22	83	100	100	100
	Gramo + 2.5% PVA	Chevron	0.25	33	43	80	95	77
			0.50	97	83	97	100	90*
			1.0	45	70	96	100	100
			2.0	27	83	100	100	100
	Gramo + 5% PVP	Chevron	0.25	35	45	70	73	83
	[illegible] Company		0.50	30	73	93	100	100
			1.0	43	73	93	100	100
			2.0	33	60	93	100	100
	Diquat liquid	Chevron	0.25	25	53	70	93	93
			0.50	33	70	97	100	100
			1.0	25	73	93	100	100
			2.0	33	80	93	100	100
	Control			0	0	0	0	10
[illegible]	Floating pellet		0.25		80	85	90	93
			0.50		97	100	100	100
			1.0		93	97	100	100
			2.0		93	100	100	100
	Sinking pellet		0.25		57	60	85	70
			0.50		80	93	100	100
			1.0		93	100	100	100
			2.0		97	100	100	100
	Diquat liquid	Chevron	0.25		93	93	93	100
			0.50		93	93	100	100
			1.0		93	100	100	100
			2.0		97	100	100	100
	Control				0	0	0	0
	[illegible]							

Hygrophila. Susceptibility of hygrophila (Table 19) and green cabomba (Table 20) to aquatic herbicides now available or under development was determined. Both hygrophila and cabomba were most sensitive to terbutryn. Complete control was obtained at all treatment rates from 0.63 to 5.0 mg/ℓ after 8 weeks.

The most rapid injury response was produced, however, by treatments with liquid amine formulation of endothall (Hydrothol 191®). This chemical gave 93-percent control at the 1.25-mg/ℓ treatment after 2 weeks and 4 weeks for hygrophila and cabomba, respectively.

Diuron produced complete control of both plant species at 5 mg/ℓ treatment after 8 weeks.

The 5-mg/ℓ treatment with benthiocarb gave 100-percent control of hygrophila after 6 weeks, but only 40 percent control of cabomba after 8 weeks.

Fluridone produced little damage to both tested species at dose rates up to 5 mg/ℓ in our experimental conditions. However, from the beginning of the test through termination, there was a sustained loss of chlorophyll in the young meristematic tissues. No other injury was evident.

Diquat was not effective against cabomba. However, more than 90-percent control was obtained for hygrophila after 6 weeks by diquat treatments of 0.63 mg/ℓ or higher.

Copper ethylenediamine complex (Komeen®) was found to be ineffective against both hygrophila and cabomba at dose rates up to 5 mg/ℓ.

Combinations of diquat and copper were compared with diquat and copper alone for efficacy against hygrophila (Table 21). No differences between treatments were apparent. Phytotoxicity to hygrophila appeared to be caused by diquat alone since additions of copper did not increase toxicity ratings.

Floating weeds. Efficacy tests for DPX-4189 and DPX-5648 were conducted against several floating and emergent weed species. Complete control of water-hyacinth was obtained with treatments of 0.010 kg/ha DPX-5648 or 0.020 kg/ha DPX-4189 over the foliage after 10 weeks (Table 22). Similar control was also obtained by treatments of 0.02 mg/ℓ DPX-4189 or DPX-5648 injected below the [illegible].

About 70 to 80-percent injury ratings were produced by dose rates of 1 to 5 g/ha [illegible] after 10 weeks. However, severe plant growth retardation was observed at these treatment rates.

[illegible] appeared weakened and became more susceptible to attacks by [illegible]. Efforts had to be made to spray the plants with a fungicide [illegible] once a week during the experiment to keep them from being severely [illegible] herbicide efficacy was being evaluated.

[illegible] were very sensitive to DPX-4189 with complete control obtained at [illegible] of 0.05 kg/ha or higher (Table 23).

Table 19

Laboratory Evaluations of Several Aquatic Herbicides for Efficacy Toward Hygrophila

Chemical Treatment Rate mg/l a.i.	Percent Injury Weeks Posttreatment* 1	2	4	6	8
BENTHIOCARB					
0.63	0	0	0	0	0
1.25	0	0	2	2	2
2.50	0	3	10	20	85
5.00	27	47	90	100	100
COPPER EDA**					
0.63	0	0	0	0	0
1.25	0	0	0	0	0
2.50	0	0	13	10	8
5.00	0	8	20	20	20
DICAMBA + 2,4D					
0.63	0	0	2	2	2
1.25	0	0	27	27	37
2.50	0	7	60	70	73
5.00	0	17	87	100	100
DICHLOBENIL					
0.63	0	10	32	52	63
1.25	0	10	17	27	52
2.50	0	10	13	22	53
5.00	10	10	47	63	78
DIQUAT					
0.63	10	70	77	90	90
1.25	20	70	87	90	100
2.50	33	77	100	100	100
5.00	60	87	100	100	100
DIURON					
0.63	0	0	0	0	3
1.25	0	0	0	5	32
2.50	0	0	0	50	90
5.00	0	0	8	95	100
DPX-4189					
0.63	0	0	0	10	58
1.25	0	0	10	32	93
2.50	0	0	20	20	67
5.00	0	0	20	27	67
ENDOTHALLAMINE (solid)					
0.63	5	5	7	5	5
1.25	15	22	28	28	28
2.50	72	77	90	92	92
5.00	80	85	100	100	100

Chemical Treatment Rate mg/l a.i.	Percent Injury Weeks Posttreatment* 1	2	4	6	8
ENDOTHALLAMINE (liquid)					
0.63	70	80	83	73	60†
1.25	80	93	97	92	88†
2.50	87	100	100	100	100
5.00	87	100	100	100	100
POTASSIUM ENDOTHALL					
0.63	0	0	2	3	5
1.25	0	0	2	7	2
2.50	13	25	15	15	15
5.00	42	72	77	77	80
FENAC					
0.63	0	0	0	0	0
1.25	0	0	0	0	33
2.50	0	0	0	3	47
5.00	0	0	15	30	93
FLURIDONE					
0.63	5	5	5	5	5
1.25	10	10	10	10	10
2.50	5	5	10	10	10
5.00	5	5	10	10	10
SIMAZINE					
0.63	0	0	0	0	0
1.25	0	0	5	5	5
2.50	0	0	0	5	5
5.00	0	0	0	0	5
TERBUTRYN					
0.63	0	0	63	92	100
1.25	0	0	93	98	100
2.50	0	23	93	100	100
5.00	17	60	100	100	100
2,4-D DMA††					
0.63	0	0	10	7	3
1.25	0	0	10	8	8
2.50	0	0	13	13	13
5.00	0	0	50	53	52
CONTROL	0	0	0	0	0

[redacted]

Table 20
Laboratory Evaluations of Several Aquatic Herbicides For Efficacy Toward Green Cabomba

Chemical Treatment Rate mg/l a.i.	Percent Injury Weeks Posttreatment* 1	2	4	6	8
BENTHIOCARB					
0.63	0	0	0	0	0
1.25	0	0	0	0	2
2.50	0	0	0	10	23
5.00	23	27	30	33	40
COPPER EDA**					
0.63	2	5	7	10	10
1.25	0	2	7	7	7
2.50	0	5	8	10	7
5.00	0	10	27	30	30
DICAMBA + 2,4D					
0.63	0	0	0	0	0
1.25	0	0	0	0	0
2.50	0	0	0	2	5
5.00	0	0	13	22	25
DICHLOBENIL					
0.63	0	0	0	0	0
1.25	0	0	0	0	2
2.50	0	0	2	2	2
5.00	3	12	13	27	30
DIQUAT					
0.63	0	0	17	22	18
1.25	3	13	20	20	15
2.50	8	–	33	50	53
5.00	30	57	67	77	80
DIURON					
0.63	0	0	0	5	7
1.25	0	0	0	10	13
2.50	0	0	3	5	8
5.00	0	0	33	100	100
DPZ-4189					
0.63	0	0	5	10	40
1.25	5	5	13	30	73
2.50	0	0	18	40	77
5.00	0	0	27	43	90
ENDOTHALLAMINE (solid)					
0.63	0	0	0	0	0
1.25	0	0	0	0	0
2.50	3	13	20	40	40
5.00	20	40	80	80	82

Chemical Treatment Rate mg/l a.i.	Percent Injury Weeks Posttreatment* 1	2	4	6	8
ENDOTHALLAMINE (liquid)					
0.63	50	60	80	78	83
1.25	67	83	93	90	90
2.50	60	83	93	100	100
5.00	70	80	100	100	100
POTASSIUM ENDOTHALL					
0.63	3	7	8	8	10
1.25	0	0	3	5	7
2.50	0	0	0	0	0
5.00	0	0	0	0	0
FENAC					
0.63	0	0	0	0	0
1.25	0	0	0	0	0
2.50	0	0	2	2	20
5.00	0	0	10	30	70
FLURIDONE					
0.63	0	5	5	5	5
1.25	5	5	10	10	10
2.50	10	10	10	10	10
5.00	10	10	10	17	18
SIMAZINE					
0.63	0	0	0	7	12
1.25	0	0	2	3	13
2.50	0	0	0	12	17
5.00	0	0	0	2	2
TERBUTRYN					
0.63	0	0	7	60	100
1.25	0	0	30	86	100
2.50	0	0	43	77	100
5.00	0	0	13	46	100
2,4-D DMA†					
0.63	0	0	10	10	10
1.25	0	0	0	0	0
2.50	0	0	0	0	0
5.00	0	0	0	0	0
CONTROL	0	0	0	0	0

* Average of three replicate treatments.
** Ethylenediamine complex.
† Dimethylamine salt.

Table 21
Evaluation of Copper-Ethylenediamine for Enhancing Efficacy of Diquat against Hygrophila

Date of Evaluation	Chemical Designation	Company or Source	Rate mg/l	Percent Injury Weeks Posttreatment 2	4	6	8
07/15/81	Diquat	Chevron	0.32	7	28	47	70
			0.63	35	65	78	91
	Cu		0.25	0	0	3	3
			0.50	0	3	2	3
	Diquat+Cu		0.32+0.25	7	30	33	43
			0.32+0.50	13	50	60	70
			0.63+0.25	27	60	73	88
			0.63+0.50	35	42	75	80
	Control			0	0	3	5

Table 22
Greenhouse Evaluation of DPX-4189 and DPX-5648 for Phytotoxicity Toward Waterhyacinth

Date of Evaluation	Chemical Designation	Company or Source	Rate kg/ha	Percent Injury Weeks Posttreatment 2	4	6	8	10
10/02/81 (Foliar Treatment)	DPX-4189	duPont	0.001	0	5	5	10	10
			0.002	0	5	7	10	20
			0.005	0	13	20	30	30
			0.010	0	35	43	47	73
			0.020	7	73	93	98	100
			0.030	7	87	95	100	100
	DPX-5648	duPont	0.005	0	27	33	50	50
			0.010	5	67	92	98	100
			0.020	10	70	97	100	100
			Rate mg/l	Percent Control Weeks Posttreatment 2	4	6	8	10
10/02/81 (Root Treatment)	DPX-4189	duPont	0.005	0	5	12	15	23
			0.010	0	5	10	23	37
			0.020	0	30	60	93	97
	DPX-5648	duPont	0.010	0	8	13	32	40
			0.020	0	20	47	80	87
	CONTROL			0	2	2	7	7

Table 23
Greenhouse Evaluation of DPX-4189 for Phytotoxicity Toward Floating Weeds

Date of Evaluation	Chemical Designation	Company or Source	Rate kg/ha	Percent Control Weeks Posttreatment 2	4	6	8	10
				WATER LETTUCE				
10/02/81	DPX-4189	duPont	0.005	10	90	93	93	95
			0.010	10	100	100	100	100
			0.020	20	98	96	96	100
			0.030	20	100	100	100	100
		Control	0	0	8	10	10	10
				DUCKWEED				
10/02/81	DPX-4189	duPont	0.005	0	0	13	23	30
			0.010	0	23	53	60	70
			0.020	0	43	73	88	83
			0.030	0	50	80	93	95
		Control	0	0	0	0	0	0

A phytotoxicity rating for duckweed of 95 percent was obtained with DPX-4189 treatment at 30 g/ha after 8 weeks.

Emergent weeds. DPX-4189, at 0.030 kg/ha, produced 95-percent control of alligatorweed after 6 weeks (Table 24). Continuation of the test through 10 weeks showed evidence of regrowth in treatment rates of 0.05 to 0.020 kg/ha.

Torpedograss and cattail showed no evidence of phytotoxicity by treatments of DPX-4189 up to 0.050 kg/ha.

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Table 24
Greenhouse Evaluation of DPX-4189 for Phytotoxicity Toward Emergent Weeds

Date of Evaluation	*Chemical Designation*	*Company or Source*	*Rate kg/ha*	*Percent Control Weeks Posttreatment*				
				2	*4*	*6*	*8*	*10*
					ALLIGATORWEED			
11/12/81	DPX-4189	duPont	0.005	5	28	35	37	0*
			0.010	25	73	68	65	20*
			0.020	47	82	82	94	80*
			0.030	60	92	92	92	95
		Control	0	0	0	0	0	0
					TORPEDOGRASS			
10/02/81	DPX-4189	duPont	0.005	0	3	3	2	2
			0.010	3	10	7	7	0
			0.020	0	8	3	2	0
			0.030	3	7	5	2	0
			0.050	2	8	5	3	2
		Control	0	0	0	0	0	0
					CATTAIL			
10/02/81	DPX-4189	duPont	0.010	0	0	0	0	0
			0.020	0	5	10	0	0
			0.030	0	10	10	2	0
		Control	0	0	0	0	0	0

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