63-4-2

AD No. 408401

DDC FILE COPY

BIOLOGICAL CONTROL OF INSECTS OF MEDICAL IMPORTANCE

...ICAL FIELD
...LABORATORY
CAMP LEJEUNE, N.C.

... 28 '61

408 401

Published by
American Institute of Biological Sciences
2000 P Street, NW
Washington 6, D. C.

DDC
JUL 16 1963
TISIA D

④ $11.00 ⑤ 48 400

⑥ Conference on

BIOLOGICAL CONTROL OF INSECTS OF MEDICAL IMPORTANCE

Sponsored by:

The American Institute of Biological Sciences

The Armed Forces Pest Control Board

The Office of Naval Research

The Army Chemical Corps

Moderator

E. A. Steinhaus

Technical Editor

D. W. Jenkins

Convened at ~~Dart Auditorium,~~

Armed Forces Institute of Patholgy

Washington, D. C.

February 3-4, 1960

Technical Report

November 1960

C O N T E N T S

WEDNESDAY MORNING SESSION

February 3, 1960

A conference on Biological Control of Insects of Medical Importance, sponsored by the Office of Naval Research, the American Institute of Biological Sciences, the Armed Forces Pest Control Board, and the Army Chemical Corps convened at the Armed Forces Institute of Pathology, Walter Reed Army Medical Center, Washington, D. C.

WELCOME

Hiden T. Cox, Executive Secretary
American Institute of Biological Sciences

It is my great privilege and pleasure to welcome you to this conference on Biological Control of Insects of Medical Importance on behalf of the American Institute of Biological Sciences. You will note that we are one of four co-sponsors. I assure you that the Institute welcomes this opportunity to co-sponsor a symposium of importance and significance in biology. During the last five years the AIBS has sponsored or co-sponsored well over 125 conferences of this type. It is one of the major activities of the Institute.

One of the purposes of the Institute is to foster the exchange of information between biologists, between biologists and other scientists, and between biologists and the general public.

As might be suspected, the AIBS has a major role in publications. At the present time, we are publishing about eleven journals; these range from the Quarterly Review of Biology through the AIBS Bulletin.

If there is anything I or members of my staff can do to help you to make the meetings more meaningful to you, you have but to call upon us.

Now I would like to introduce Colonel Bunn, who has had much to do with arranging this conference, and is particularly well qualified to sketch the major objectives of this meeting.

OBJECTIVES OF CONFERENCE ON BIOLOGICAL CONTROL OF INSECTS OF MEDICAL IMPORTANCE

Ralph W. Bunn, Executive Secretary
Armed Forces Pest Control Board
Washington, D. C.

Oliver Wendell Holmes is credited with saying "... no man can be truly called an entomologist, sir; the subject is too vast for any single human intelligence to grasp." I could never be more fully in agreement with Dr. Holmes than at a time like this when confronted with a program involving not only entomology but the interrelationships of entomology with bacteriology, mycology, protozoology, virology, parasitology and nematology. However, as our more simple research problems are solved and we move on to the more complex ones, it will be increasingly necessary to focus the combined knowledge of two or more professions upon those problems in order to arrive at a solution. It is for this reason that this conference has been convened.

The problem we are faced with is what to do to control those insects which have developed the ability to tolerate nearly all the toxicants the entomologists and chemists have been able to devise. This resistance phenomenon is constantly becoming more widespread and is so generally known that it need not be elaborated upon at this time. However, it is such a serious problem, particularly with insects of medical significance, that no avenue of approach to the problem should be left unexplored.

With this thought in mind, Dr. Dale Jenkins began a comprehensive review of the literature relating to the biological control of insects of medical importance about two or three years ago. You will hear from him on this subject shortly.

Subsequently, preliminary plans were made to hold a biological control conference in the spring of 1959 with the Office of Naval Research, the American Institute of Biological Sciences, the Army Chemical Corps and the Armed Forces Pest Control Board as co-sponsors. However, as certain of our key participants could not attend at that time, the conference was postponed until later in the year. About the time plans for the conference were again well organized, it was learned that the broad field of biological control had been selected as the central theme of the joint meeting of the Entomological Society of America and the Entomological Societies of Canada and of Ontario, to be held in Detroit in November-December 1959. Reluctantly, therefore, it was agreed that once again our conference would be postponed -- this time until after the Detroit meetings, in the belief that it would be well first to hear a broad discussion of biological control and then to narrow the field down to considerations of its application to insects of medical importance.

Our postponements have finally ended and it is a pleasure for me to welcome you on behalf of the Armed Forces Pest Control Board and to thank you for your participation.

We hope that this conference will review and critically evaluate available information on the various aspects of biological control as they relate to insects of medical importance; that if these lines of investigation appear to offer promise, that this group will indicate the more promising areas of investigation and the most urgently needed information; and that suggestions will be made concerning ways and means of stimulating research, if this seems to be warranted.

To achieve these ends we hope that everyone here will participate freely in discussions. We have purposely limited the size of the conference as there seems to be an inverse relationship between the number of people present and the extent of participation in discussion - at least on the part of the more reticent (and frequently the better informed) individual. Please, therefore, do not feel that discussion should be limited to those sitting around these tables. All are invited to contribute their ideas and we hope this will be an interesting, stimulating and fruitful conference.

We will now turn the meeting over to Dr. E. A. Steinhaus, as Moderator. He is one of those we thought we could not have the conference without.

INTRODUCTION

Edward A. Steinhaus, Director
Laboratory of Insect Pathology
Department of Biological Control
University of California, Berkeley

I am flattered and honored to be asked to moderate this conference, although I have many misgivings as to being able to do it in a manner to please everyone. Those of you who know me know that I am not very much of a master of ceremonies; so I am simply going to reply upon each of you to extend to me your cooperation throughout the meeting and to participate, as Colonel Bunn requested, fully and actively. I am sure, if you will help me in this regard, we will have a fine meeting.

My instructions from Dr. Olive were to follow the agenda any way I saw fit. This, of course, gives me leeway to the point of being dangerous. However, I can assure you that we have such an excellent program that I shall not be taking advantage of this liberty.

I was assured that this conference would be strictly informal and one of the problems of a moderator is how to initiate, how to engender, this informality. I shall do my best to do this even to the point of not giving long introductions of the speakers, as I am sure that all of you here know them, if not personally, certainly by reputation.

Originally, I was asked to introduce the subject of "Potentialities of Biological Methods", and to discuss this matter briefly. However, since two of the speakers on our program for tomorrow are scheduled to speak on that subject, I ask to be relieved of this responsibility, because I am sure those two speakers will cover the subject adequately.

We should keep in mind just what we are considering when we speak of "biological control" or "biological methods of control." I am not going to be so presumptuous as to attempt to define these terms for the speakers, but I hope that each of the speakers will make it clear what he means when he gets into those areas in which there is some doubt as to just what is biological control and what is not. We all recognize that the use of predators (vertebrate or invertebrate), the use of parasites, and the use of pathogens, have long represented classical types of biological control. Thus we have such definitions as "biological control is the action of parasites, predators, or pathogens on a host or prey population which produces a lower general equilibrium position that would prevail in the absence of these agents." (Stern, et al.). Some authorities prefer to restrict biological control to man's manipulation of these agencies. But perhaps some of you will want to extend this into other areas, such as the use of hormones, the use of secretions from insects or other types of life, the use of naturally repellent substances, the use of the sterile-male technique and there are other examples.

Also, I should like to ask that we keep in mind, during the discussions, that we are speaking primarily about insects of <u>medical</u> importance. I have found difficulty here in my own thinking, in that I lapse over into examples in the area of insects of agricultural importance. Of course, I am not inferring that we should not draw upon the lessons and examples we have in other groups; but our main objective at this conference is with insects of medical importance.

I am confident that the conference will be an interesting and important one. I think if we all will participate actively, this will be guaranteed, because biological methods of control are just beginning to be exploited. Certainly, biology itself appears to have a great new day dawning. Dr. Vannevar Bush recently said, when asked what area of work he would go into if he had it all to do over again, that if he could begin again in science, he would go into biology, because he thought the most stimulating frontiers, the great new horizons, were really in the fields of biology. When we read such recent books as those by Jean Rostand, or Warren Weaver's latest Rockefeller Foundation Report, we can begin to appreciate the tremendous possibilities before us, remembering what George Wall has called "the deep truths of biology." So that, in its own way, "the potentialities of biological control," are truly great. That is, "biological power," if I may use such a term, is truly as great, in its way, as atomic power is in its way.

But who is to say when or where or how this reservoir of biological power is to be realized? Perhaps we can gain some idea as to this aspect of biological control in a conference such as this. I do not know how the rest of you feel, but sometimes the thought of manipulating this potential biological power is quite overwhelming. When you consider, for example, that there are 30,000 or more species of protozoa, 20,000 nematodes, 25,000 crustaceans, 90,000 mollusks, 20,000 fishes, a million insects, and so on -- the potentials involved in manipulating this mass of life is truly cause for thought.

While I have the floor I should like to make just one more comment. It may not be necessary, but for the record I think we should make it clear that in our consideration of biological control we are fully cognizant of the necessary role which chemicals play and will always play in controlling pest populations.

As far as insects of medical importance are concerned, I am sure it will help us to gain some idea of the potentialities of biological control as we listen to our next speaker, Dr. Dale Jenkins give us a report of an extensive literature survey. He will cover at least three categories (the three P's) Pathogens, Parasites, and Predators of medically important insects.

PATHOGENS, PARASITES AND PREDATORS OF MEDICALLY IMPORTANT INSECTS

Dale W. Jenkins
Chief, Entomology Division
Fort Detrick, Frederick, Md.

Biological control of insects by disease pathogens, parasites, and predators is now being studied more intensively by entomologists with a hopeful but critical attitude. There was formerly much interest in the subject and the literature contains many observations of predation and parasitism on injurious or harmful species of insects. These observations were frequently accompanied by comments that these parasites and predators would have great promise in controlling pest insects. Attempts to grow the parasites or predators and to control pest insects in the field often (with several notable exceptions) gave disappointing or equivocable results and interest declined. These tests pointed out the very complex ecological interacting factors involved and the necessity for understanding the ecology of the parasite or predator as well as the insect to be controlled.

The introduction of effective residual insecticides such as DDT resulted in a loss of interest in natural control. The careful and comprehensive natural control studies by Steinhaus in California, the forest insect control program in Canada, studies by Weiser in Czechoslovakia, and a few others are exceptions to this generalization. Widespread and irresponsible large scale use of residual and other insecticides has resulted in many changes of the natural fauna, including decreasing the number of parasitic and predaceous forms that normally maintained natural balances.

Use of chemical control, when accompanied by neglect of sanitation practices in control of houseflies, and draining and eliminating mosquito breeding places, decreases natural parasites and predators and puts one on a treadmill of chemical control. The discontinuation of chemical applications, or the development of resistance by the insects to insecticides spells trouble. Use of greater quantities of insecticides and of other more toxic chemicals or combinations of chemicals are required. The WHO Expert Committee on Insecticides stated in 1958 "resistance is at present the most important single problem facing vector control programmes". Over 1000 scientific papers have been published to date on the subject of insect resistance to insecticides. A large number of medically important insects have developed resistance to insecticides including about 36 species of mosquitoes, plus various species of fleas, body lice, flies, ticks, cockroaches, bedbugs and other insects.

PATHOGENS, PARASITES AND PREDATORS OF MEDICALLY IMPORTANT INSECTS

Dale W. Jenkins
Chief, Entomology Division
Fort Detrick, Frederick, Md.

Biological control of insects by disease pathogens, parasites, and predators is now being studied more intensively by entomologists with a hopeful but critical attitude. There was formerly much interest in the subject and the literature contains many observations of predation and parasitism on injurious or harmful species of insects. These observations were frequently accompanied by comments that these parasites and predators would have great promise in controlling pest insects. Attempts to grow the parasites or predators and to control pest insects in the field often (with several notable exceptions) gave disappointing or equivocable results and interest declined. These tests pointed out the very complex ecological interacting factors involved and the necessity for understanding the ecology of the parasite or predator as well as the insect to be controlled.

The introduction of effective residual insecticides such as DDT resulted in a loss of interest in natural control. The careful and comprehensive natural control studies by Steinhaus in California, the forest insect control program in Canada, studies by Weiser in Czechoslovakia, and a few others are exceptions to this generalization. Widespread and irresponsible large scale use of residual and other insecticides has resulted in many changes of the natural fauna, including decreasing the number of parasitic and predaceous forms that normally maintained natural balances.

Use of chemical control, when accompanied by neglect of sanitation practices in control of houseflies, and draining and eliminating mosquito breeding places, decreases natural parasites and predators and puts one on a treadmill of chemical control. The discontinuation of chemical applications, or the development of resistance by the insects to insecticides spells trouble. Use of greater quantities of insecticides and of other more toxic chemicals or combinations of chemicals are required. The WHO Expert Committee on Insecticides stated in 1958 "resistance is at present the most important single problem facing vector control programmes". Over 1000 scientific papers have been published to date on the subject of insect resistance to insecticides. A large number of medically important insects have developed resistance to insecticides including about 36 species of mosquitoes, plus various species of fleas, body lice, flies, ticks, cockroaches, bedbugs and other insects.

really in the fields of biology. When we read such recent books as those by Jean Rostand, or Warren Weaver's latest Rockefeller Foundation Report, we can begin to appreciate the tremendous possibilities before us, remembering what George Wall has called "the deep truths of biology." So that, in its own way, "the potentialities of biological control," are truly great. That is, "biological power," if I may use such a term, is truly as great, in its way, as atomic power is in its way.

But who is to say when or where or how this reservoir of biological power is to be realized? Perhaps we can gain some idea as to this aspect of biological control in a conference such as this. I do not know how the rest of you feel, but sometimes the thought of manipulating this potential biological power is quite overwhelming. When you consider, for example, that there are 30,000 or more species of protozoa, 20,000 nematodes, 25,000 crustaceans, 90,000 mollusks, 20,000 fishes, a million insects, and so on -- the potentials involved in manipulating this mass of life is truly cause for thought.

While I have the floor I should like to make just one more comment. It may not be necessary, but for the record I think we should make it clear that in our consideration of biological control we are fully cognizant of the necessary role which chemicals play and will always play in controlling pest populations.

As far as insects of medical importance are concerned, I am sure it will help us to gain some idea of the potentialities of biological control as we listen to our next speaker, Dr. Dale Jenkins give us a report of an extensive literature survey. He will cover at least three categories (the three P's) Pathogens, Parasites, and Predators of medically important insects.

The development of insecticide resistance and the failure of insecticides to effectively control certain injurious and harmful insects has necessarily created interest in other means of control. Practical and effective solutions to the insecticide resistance problem may be found by critically investigating environmental and biological control methods, particularly in resistant populations.

Biological control of certain injurious and harmful insects offers great promise if approached from a realistic viewpoint. This requires comprehensive knowledge of the ecology of the parasites and predators, and their ecological relationships, as well as a full knowledge of the ecology of the harmful insects. In general, natural control methods are restricted to use of specific parasites or predators against specific insects, frequently limited to certain geographical areas or to certain times of the year. Even with these actual or possible limiting parameters, natural control of certain insects is considered to have a very great potential.

During recent years when many reports indicated the ineffectiveness of insecticide control of various medically important insects, natural control has been discussed. Due to the lack of sufficient definitive information and the record of some past failures in biological control attempts, few firm research or control programs have been initiated.

The present report attempts to summarize a rather extensive survey of the literature on the pathogens, parasites and predators of medically important insects. An attempt is made to make a preliminary critical evaluation of the potential of natural control of medically important insects. The pathogens, parasites, and predators of each group of insects have been compiled based on data from abstracting over 1,200 references. This includes the references in the Review of Applied Entomology and all available literature. The type of damage, mortality rates, and quantitative data available have been compiled and evaluated with all known field test data on attempts to control medically important insects. After evaluation and comparison, certain parasites and a few predators have been tentatively selected as being the most promising candidates for more intensive study and test.

Detailed literature studies of the life history, ecology and other available information have been made on the most promising parasites and predators. The literature on the pathogens, parasites and predators is voluminous but scattered and fragmentary. Several limited reviews have been published and have been of great value, including reviews on mosquitoes by Howard et al. (1912), Speer (1927), Hinman (1934 a & b), Gerberich (1946), Christophers (1952); on houseflies by West (1951); on blackflies by Grenier (1943); on tsetse flies by Buxton (1955) and general reviews by Steinhaus (1946 and 1949). A recent review by Laird (1959) discusses the insecticide and resistance problems and the part natural control could play with medically important insects. Laird states "There is a real need for an annotated bibliography of the large and widely scattered literature on parasites and predators of all arthropods and molluscs of medical interest. Such a synopsis is prerequisite to a general evaluation of the biological control potentialities of these organisms."

As a result of the present intensive literature study and some field observations, the following general preliminary statements can be made with regard to biological control of medically important insects. Various pathogens and parasites including rickettsiae, bacteria, protozoa, fungi, nematodes, and insects (Table 1) are considered to be of potential importance in controlling these insects. Certain fungi, protozoa, bacteria, nematodes, and parasitic insects appear to offer real promise. Although there are very large numbers of predators (Table 2) from a preliminary study of available data they do not appear to be of great value in decreasing excessive insect populations in an area (except for the use of fish or predacious mosquito larvae). They are not thought to have as great a potential value for biological control of medically important insects as the pathogens and parasites.

This conference is fortunate in having present outstanding specialists on the various groups of pathogens, parasites and predators. It is highly gratifying that they will discuss the various organisms in their areas of specialization and consider the technical aspects of the potentials of these organisms for natural control of medically important insects. It is hoped that it may be possible to then select the most promising organisms on a priority basis for recommending a well considered program which can be justified and supported by various agencies and research organizations.

This literature review has been organized according to the taxonomic groups of medically important insects. Under each group, the pathogens, parasites, and predators have been listed in taxonomic sequence with the specific name of the organism followed by the host insects affected or killed, and with annotations, and the authority and date of publication. The groups of insects considered include mosquitoes, black flies, houseflies, tabanids, *Stomoxys*, *Glossina*, ceratopogonids and heleids, ticks and mites, fleas, triatomids, bed bugs, cockroaches, lice, spiders, and urticating *Lepidoptera*. Only a small part of the review can be reported in summary form.

1. Biological Control of Mosquitoes

Use of natural enemies of mosquito larvae and adults for biological control has been considered by many scientists, but except for use of fish, few serious attempts have been made. Effective

Table 1. <u>Recorded Parasites of Medically Important Insects</u>

	Rickettsiae	Bacteria	Fungi	Protozoa	Nematodes	Arachnida	Diptera	Hymenoptera	Total Parasites
Mosquitoes	3	23	50	75	20	12			183
Black flies		1	4	16	4	4	1	2	32
Houseflies		9	6	5	5	14		27	66
Tabanids				3	4	3	7	20	37
Tsetse flies		1	1	6	2		11	24	45
Ceratopogonids		4	3	11	1	4		1	24
Ticks		5	3	6			1	6	21
Fleas	1	6		18	3	2		1	31
Cockroaches		6	3	2	3	2		12	28
Lice	10	2		1					13
Stable flies		2	1	2	1	5		8	19
Totals	14	59	71	145	43	46	20	101	499

Table 2. Recorded Predators of Medically Important Insects

	Crustacea	Arachnida	Odonata	Hemiptera	Trichoptera	Diptera	Coleoptera	Hymenoptera	Fish	Amphibia	Birds	Mammals	Total Predators
Mosquito larvae	18	3	18	42	3	69	45		164	18	14		394
Mosquito adults		15	27	7		21	1	8		6	4	1	90
Black flies		2	3	4	7	25	1	4	10	1	9		66
Houseflies		9				8	7	18					42
Stable flies		1	2			2	1	4			1		11
Tsetse flies		2	4			2	4	6			3	3	24
Ceratopogonids						6			1				7
Ticks		4		1			8	4			5	2	24
Fleas							5						5
Cockroaches		10		1				5		3	2	1	22
Lice								3					3
Totals	18	46	54	55	10	133	72	52	175	28	38	7	688

control of mosquitoes requires use of all available methods such as filling, draining, and sanitation, use of chemicals, and use of natural parasites and predators. The development of resistance to insecticides by many species of mosquitoes is a further recommendation for developing the use of biological control.

Mosquitoes in all stages have many natural parasitic and predatory enemies that are extremely important in maintaining a balance in nature. Mosquito larvae and/or adults are killed or incapacitated by many species of bacteria, rickettsiae, fungi, protozoa, coelenterates, flat and round worms, molluscs, crustacea, acarina, insects, fish, amphibia, reptiles, birds and mammals.

No viruses are known at present to have a deleterious effect on mosquitoes. Of the large number of arbor viruses transmitted to man, no effect on longevity has been shown in infected mosquitoes. While three species of rickettsiae are known to affect mosquitoes and to produce some pathological effects, present information does not indicate them to have outstanding promise in natural control. However, more critical work is required.

Over 20 species of bacteria in 15 genera have been found to be injurious or to kill mosquitoes. Bacteria have been found to kill large numbers of mosquito larvae. A promising species of bacteria appears to be *Leptothrix buccalis*. A number of species cause death when they are abundant in the larval rearing medium.

Fungi in 16 genera have been shown to be pathogenic for mosquitoes. Some of these need to be investigated to determine their potential use for biological control. The most promising appears to be certain species of the genus *Coelomomyces*. Twenty-six species have been described which are fairly specific to certain host species of mosquitoes. Field studies by Muspratt in Africa and by Laird and Colless in the Pacific Islands (being reported at this conference) show members of this genus to be very promising. Certain members of the genus *Entomophthora* should not be overlooked, but no critical data are available to evaluate them.

There are many genera and species of *Protozoa* which attack mosquitoes in all stages. In the *Mastigophora*, species of various genera commonly attack mosquitoes but the resulting mortalities are low. The *Sporozoa* contains many injurious species, particularly members of the *Microsporidia* such as the genera *Nosema* and *Thelohania*. The protozoan *Thelohania legeri* appears to be promising for special study, and the other 10 described species should be considered. The potential value of the genera *Stempellia* and *Plistophora* could not be determined. The effect of the epibiotic *Ciliophora* protozoans on mosquito larvae is still controversial. Observations of the effect of the vorticella-like protozoans range from 100% mortality rates to no effect, with the majority of authors agreeing that they are injurious to the mosquito larvae. Over 20 species of Ciliophora in 12 genera have been observed on mosquito larvae.

Mermithid-type nematodes cause great mortality in mosquito larvae and adults in certain geographic localities. How generally abundant and important these worms are in natural control is not known, although they have been found in 41 species of mosquitoes throughout the world. The taxonomy of these nematodes is insufficiently known, but five genera of mermithids affecting mosquitoes have been described. Mermithids appear to have real promise for biological control of mosquitoes and deserve special study. Various other nematodes and trematodes have been discovered in mosquitoes but their value is unknown.

Parasitic Hydracarina mites (especially Arrenurus spp.) attack mosquitoes and cause some mortality, but the effectiveness has not been evaluated to date.

Mosquito predators are extremely numerous and effective in maintaining a population balance. They are rarely observed to eliminate mosquito populations except under unusual circumstances. Of the predators of mosquito larvae, fish (especially the top minnow Gambusia) are known to be effective under certain conditions. More critical studies on fish predators are needed. The larvae of Toxorhynchites mosquitoes are very effective predators on pest mosquito larvae and deserve additional study. The field tests in Hawaii, Fiji and other Pacific Islands indicate limited success in control. The potential of using various insect predators, although they are very effective in devouring mosquito larvae, is unknown. It is possible that certain dragon fly larvae, hemiptera, beetles, and particularly Chaoborinae may have value under specific conditions.

The value of predators for practical control of adult mosquitoes cannot be ascertained. Bats, birds (especially swallows), dragon flies, and robber flies are the most effective predators of adults.

2. Biological Control of Houseflies

Use of parasites against the housefly appears very promising and deserves special studies.

No viruses or rickettsiae appear to have promise in control of houseflies. Although a very large number of species of bacteria have been found associated with the housefly, only 9 species of 6 genera are known to be truly pathogenic and have a potential for biological control. Several of these species should be studied more extensively for determining their practical use, especially Bacillus lutzae and Staphylococcus muscae.

The fungus Empusa muscae appears to offer the greatest potential for housefly control. Several field tests have been carried out with varying degrees of success. This fungus deserves intensive study. The value of other known parasitic fungi and the few species of protozoans found in flies is undetermined. The value of certain Platyhelminthes and Nemathelminthes cannot be determined at present, but they do not appear promising. Various mites and pseudoscorpions are found on flies, but they are not reported to be of any real importance in natural control.

The hymenopterous parasites of fly pupae appear to be of real value for biological control of houseflies, particularly species of the genus Spalangia which have been used in field tests in Pacific Islands and Puerto Rico with some success. Other Chalcidoidae and Cynipidae offer potential candidates for natural control.

Housefly predators are very effective in reducing populations. They are observed to cut down populations during every stage of the life history. Ants are extremely important but do not appear to hold promise for artificial introduction to obtain high mortalities. The wasp Polistes hebraeus when introduced in Fiji reduced the fly population to some extent. The beetles Hister chinensis and Copris incertus prociduus were introduced into Fiji but were not effective in fly control. No known predators presently appear to deserve special study for housefly control.

3. Biological Control of Tabanids

The viruses, rickettsiae, bacteria, protozoans, and fungi do not appear to be of importance as parasites of tabanids. However, hymenopterous egg parasites appear to be of real importance, especially Phanurus emersoni. Several field tests have indicated its value in control, especially in Texas where 50% control of a Tabanus was obtained. Other hymenopterous parasites appear to offer promise, especially the genus Telenomus. The importance of parasitic mites on horse fly adults is unknown, as is the effect of certain Nemathelminthes parasites.

The predators of importance on adult tabanids include the Odonata, robber flies, Bembicidae, and birds. There are a few larval predators such as tipulid larvae. Cannibalism among species of tabanid larvae is said to be of local importance in control. None of these appear to deserve special study as potential means of biological control.

4. Biological Control of Black Flies

Protozoans and mermithid worms appear to be the most important parasites of black flies. The protozoans Thelohania spp. are very important in natural control and deserve special study. The importance of other protozoans should not be overlooked, but cannot be evaluated at present. Mermithid nematodes have been observed to parasitize 15 species of black flies and to produce significant mortality. Infestation usually results in death, and parasitization rates are fairly high in some localities. They appear to have promise for biological control of black flies.

Various insect predators, especially Trichoptera larvae, feed on black fly larvae and adults but little quantitative data are available. Certain stream inhabiting fish, especially suckers, appear to deserve investigation.

No particularly promising parasites or predators were found for potential control of Phlebotomus, Culicoides or Ceratopogon, although several parasites and predators are known. Empusa papatassi may have promise for control of Phlebotomus but no data are available.

5. Biological Control of Stable Flies

Bacterium delendae-muscae appears to have promise in control of stable flies, but critical data are lacking. The hymenopterous parasite Spalangia appears most promising and should be studied in more detail.

6. Biological Control of Tsetse Flies

Bacterium mathisi, a phycomycete fungus, a mermithid nematode, and several protozoans attack Glossina spp. but do not appear to have much promise for biological control. The hymenopterous parasites appear most promising and over 20 species are known to parasitize tsetse flies. Released eulophid parasites Syntomosphyrum glossinae caused a 50% increase in parasitization of Glossina morsitans in a field test, but other tests were not effective due to ecological factors such as type of soil.

A variety of predators of Glossina are known including spiders, dragonflies, robber flies, various birds and mammals. Ants and beetles eat numbers of pupae but their use for biological control appears to be limited.

7. Biological Control of Fleas

Certain rickettsiae, protozoans, nematodes, and trematodes affect fleas and cause damage or mortality, but no data are available to indicate potential for natural control of fleas. The plague bacterium Pasteurella pestis causes earlier death of fleas but would be unsuitable for natural control, so that no known parasites appear to offer promise. The only predators of note are staphylinid and histerid beetles in animal burrows where the fleas develop, but these do not appear to have a potential for biological control.

8. Biological Control of Hemiptera, Lice, and Cockroaches

Certain triatomids are parasitized in the egg stage by the hymenopteran *Telenomus fariai* which may have promise in natural control. The bed bug appears to be relatively free of important parasites and predators according to the available literature.

Body lice are affected by a number of species of bacteria and by certain rickettsiae, none of which appear to have practical biological control potential.

Cockroaches are susceptible to several species of bacteria which might be considered for biological control. Hymenopteran parasites offer a most promising potential, particularly against the egg stage of the roaches.

9. Biological Control of Ticks

While ticks are known to be killed by certain species of bacteria, protozoa, and fungi the potential for biological control appears limited in comparison with the value of the enchytrid parasite *Hunterellus hookeri* found to parasitize 26 species of ticks throughout the world. Other hymenopterous tick parasites include *Hunterellus theileri*, *Ixodiphagus hirtus*, *I. mysorensis*, and *I. texanus*. All of these tick parasites deserve more investigation although several field tests gave inconclusive results, indicating that more complete ecological data are required before use.

There are a fairly large number of tick predators known of which the tick bird *Buphagus* is probably most important, but it has relatively limited distribution.

Many generalizations have been made regarding the potential of various pathogens, parasites, and predators. It should be remembered that these are tentative comments based on data available at present and are subject to change with more information. The objective of stating these general summary comments is to provide a starting basis for discussing the relative merits of each type of parasite or predator for potential control. The possibilities of using selected pathogens, parasites, and predators for control of medically important insects is exciting and I am most anxious to hear the papers and comments by the specialists convened here.

Literature Cited

Buxton, P. A. 1955. The Natural History of Tsetse Flies. Lond. Sch. Trop. Med., Mem. 10. H.K. Lewis & Co., Ltd., London, 816 pp.

Christophers, S. R. 1952. The Recorded Parasites of Mosquitoes. Riv. di Parasit. 13(1):21-28.

Gerberich, J. B. 1946. An Annotated Bibliography of Papers Relating to the Control of Mosquitoes by the Use of Fish. Amer. Midl. Nat. 36:87-131.

Grenier, P. 1943. Observations sur quelques stations de simulies. Parasites et predateurs des larves et nymphes. Bull. Soc. Path. Exot. 36:105-110.

Hinman, E. H. 1934a. Predators of the Culicidae. I. The Predators of Larvae and Pupae Exclusive of Fish. J. Trop. Med. Hyg. 37: 129-134.

Hinman, E. H. 1934b. Predators of the Culicidae Mosquitoes. II. Predators of Adult Mosquitoes. J. Trop. Med. Hyg. 37:145-150.

Howard, L. O., Dyar, H. G., and Knab, F. 1912. The Mosquitoes of North and Central America and the West Indies. Carnegie Inst. Publ. Vol. 1. pp. 156-179.

Laird, M. 1959. Biological Solutions to Problems Arising from the Use of Modern Insecticides in the Field of Public Health. Acta Tropica 16(4):331-355.

Speer, A. J. 1927. Compendium of the Parasites of Mosquitoes (Culicidae). Treasury Dept., USPHS, Hyg. Lab. Bull. 146:1-36.

Steinhaus, E. A. 1946. Insect Microbiology: An Account of the Microbes Associated with Insects and Ticks with Special Reference to the Biological Relationships Involved. Comstock Pub. Co., Ithaca, N.Y., 763 pp.

Steinhaus, E. A. 1949. Principles of Insect Pathology. McGraw Hill, New York. 757 pp.

West, L. S. 1951. The House Fly. Comstock Pub. Co., Ithaca, N.Y., 584 pp.

World Health Organization 1958. Expert Committee on Insecticides. Eighth Report. Wld. Health Org. Tech. Ser. 153.

DR. STEINHAUS: Thank you, Dr. Jenkins.

I am sure we are all impressed by the multitude of organisms associated with insects of medical importance, and the terrific job Dr. Jenkins has done in compiling this information. He is quite truthful when he says this is only a small part of what he has assembled, because I have seen his manuscript; it is a tremendous accomplishment. I am happy to know that Dr. Jenkins' paper is to be published, and will be available *in extenso*.

I am sure there are a good many here who have comments or expressions of thought on the subjects dealt with by Dr. Jenkins.

DR. DUTKY: Most of our work has been done with agricultural insects, but we have done quite a bit with the *Diptera*, including flies and mosquitoes. Among the fungi, the entomophthorales and fungi imperfecti attack medically important insects. Some of the fungi imperfecti are more readily cultured.

A single species of nematode, which has not been described yet, will attack a wide variety of these forms. It causes high mortality of larvae in Pupipara, houseflies, and *Sarcophaga*; also, it will attack many of the other groups. With cockroaches, it seems to be exclusive, that is, it attacks with tremendous rapidity the lobster cockroach, but most other cockroaches are considerably more resistant. The nematodes could be used, I think, to attack many of the insects which are in contact with the soil.

DR. WILLIAMS: The speaker stated that perhaps the greatest potentiality for predators lay with control of mosquitoes through *Gambusia* fish.

I remember in the early days in the United States, *Gambusia* came in for a great deal of advertising. I helped construct perhaps 25 or 30 hatcheries for *Gambusia*. The observation had been that they ate mosquito larvae; but the corollary observation was that they could not reach the mosquito larvae in the presence of plants. That led to the initiation of clearing the shoreline of impounded waters, so that the *Gambusia* could reach the larvae. We had the experience of clearing shore lines in a number of new ponds which did not have any *Gambusia*; when the flotage was removed so that the *Gambusia* could get to the larvae, you did not need the *Gambusia*. I know of no cases, over a period of some 40 years, where *Gambusia* put into natural waters have controlled production of malaria mosquitoes.

COLONEL TRAUB: I would like to express my sentiments in fully agreeing with the Chairman as to the enormous amount of work Dr. Jenkins has put into the presentation, and how valuable it is. I would also like to comment on two points. One is that it is undoubtedly true that there is not much hope that predators will prove to be of practical value in the control of medically important insects on a large scale, but I can think of one example which does not seem to be in the literature and which perhaps may indicate a field which should receive further consideration. I am referring to pseudoscorpions, which are quite common in rodent nests in various parts of the world. I have repeatedly seen pseudoscorpions running around with a flea larva in each claw. Since dozens, or even hundreds, of pseudoscorpions may be present in one nest, they may exert a detrimental effect on the numbers of fleas present. However it is possible that the observed behaviour was atypical and occurred only when nests were artificially distrubed and exposed to light.

In utilizing predators for control of fleas, it is quite obvious that one problem would be that of distribution of the predator, such as the pseudoscorpion, from nest to nest. Here we are in a somewhat fortunate situation, in that pseudoscorpions are often found hitchhiking on rodents which live in the burrows. I have taken as many as 40 or 50 pseudoscorpions from the backs of rats in Malaya and other parts of the Orient, and have seen the same thing in Mexico. Pseudoscorpions of various sizes, of both sexes, will clutch the fur very tightly. I do not know how far they go but it is quite a common phenomenon.

The other point leads me to say, with due respect to Dr. Knipling and Dr. Lindquist, that the sterile-male technique is not new because it has been used for eons in the case of fleas. The Russians, particularly, have noted that it is very common to find fleas which have been castrated by nematodes. This same phenomenon occurs in this country. Several fleas have been described which turned out to be synonyms, because their genitalia, used as the basis for considering them undescribed species, had actually been deformed by such nematodes. I do not know how many fleas survive or how many are killed by this process, but I have seen quite a few with genitalia which were so deformed that the fleas were certainly sterile.

DR. LAIRD: In his remarks on Gambusia, Dr. Williams put his finger on one of the big problems that is going to face us in applying precise biological control techniques in public health entomology. The early work with Gambusia in many ways parallels the postwar work with insecticides. People said: "Here we have a fish which destroys mosquitoes, so let us go ahead and put it in places where mosquitoes breed." They proceeded to stock a wide variety of surface waters with it, without regard to the optimum habitat requirements of Gambusia.

In the South Pacific, I have seen top minnows placed in taro irrigation ditches which run dry every few weeks, and this sort of lack of prior ecological study and planning undoubtedly accounts for many of the control failures with Gambusia. DDT has been used with similar recklessness in recent years, and there has been tremendous destruction of aquatic organisms which, in the normal course of events, exercise a considerable measure of mosquito control.

What we must do now is to learn more about population-regulating factors in nature, with a view to manipulating their effectiveness to our advantage.

I support Dr. Jenkins' choice of Coelomomyces and mermithids as two very promising mosquito biological control agents ready to hand for immediate research purposes. But as Dr. Jenkins has made obvious, the extensive literature on the parasites of mosquitoes and other medically important arthropods is very largely an incidental one. A high percentage of these organisms, and predators too, have been described by entomologists engaged primarily on taxonomic studies.

It is likely that for every such organism which has been described, several others have been poured down the sink or thrown away, because there is nothing that upsets a systematic entomologist more than a battered and imperfect mosquito. A distorted larva, too, makes a very indifferent slide.

One of our targets for the immediate future is the arousing of interest, among entomologists generally, in the possibility of turning to effective advantage some of the parasites revealed from time to time in the course of their studies. There is need here for some sort of central establishment to which organisms turned up by investigators, not parasitologists in their own right, might be referred for identification and evaluation.

Shortly before I left Singapore three years ago, some anopheline eggs harbouring an unknown organism were received from the Philippines. Dr. Colless and I identified the poorly preserved larvae they contained as dipterous or hymenopterous, probably the latter. No parasites of either group have been described from mosquitoes. Local difficulties unfortunately prevented the collection of further material for rearing purposes.

This instance serves to suggest that interest aroused among field entomologists by well organized experiments commenced now with such mosquito parasites as are to hand, might well lead to the discovery of new biological control agents of wider use than those known to us at present.

MAJOR BARNETT: I would like to say a few words also on the subject of Gambusia, not to disagree at all with Dr. Williams, but to point out that in recent years there has been a developing interest in culicine control, particularly in the genus Culex, as a result of its association with the various viral encephalitides.

This past year, in Iran in an area along the Caspian Sea where rice is grown, we found *Culex tritaeniorhynchus*, the vector of Japanese encephalitis. However, its distribution appeared to be limited to those rice paddies in which top minnows did not occur. Whenever we found top minnows, we could not find this mosquito. Of course, this is purely an association, and does not prove cause and effect. I think the earlier workers probably recognized that *Gambusia* was somewhat more effective for culicines than for *Anopheles*; I believe Dr. Williams' remarks were really directed at *Anopheles*.

DR. WILLIAMS: That is right.

MAJOR BARNETT: I would like to ask two questions of Dr. Jenkins.

First, I think there was no reference to viruses in the slides you showed.

Second, with respect to the rickettsial organisms you referred to, has it been definitely proven that these organisms are, in effect, pathogenic for the insects indicated; or are they possibly intracellular symbiotes?

DR. JENKINS: With regard to viruses, I know of none which cause mortality or significant pathogenicity in medically important insects, other than in urticating *Lepidoptera*. It has been shown that eastern equine encephalitis caused visible pathogenicity in the fat body of the mosquito; but this did not kill or shorten the life of the mosquitoes. Many viruses occur and multiply in insects but they are not known to cause any mortality or detrimental pathogenicity in medically important insects.

Rickettsiae, however, do cause considerable pathology and mortality in certain insects. In mosquitoes, *Rickettsia culicis* is destructive to stomach epithelial cells, but *Wolbachia pipientis* is rarely pathogenic but may cause degeneration of certain host cells. In fleas some rickettsiae cause the gut cells to burst but do not cause significant mortality. Rickettsiae are now known to be pathogenic to the bed bug. The body lice are highly susceptible to rickettsiae. The most pathogenic species causing high mortality in lice are *Rickettsia prowazeki*, *R. mooseri*, *R. rickettsii*, *R. tsutsugamushi*, and *R. conori* (Marseilles fever strain). The pathogenicity to lice may vary with the strain in some species. No rickettsiae are known which presently appear to offer a good means of control of any medically important insects.

BACTERIAL AND VIRAL DISEASES OF INSECTS OF MEDICAL IMPORTANCE

Edward A. Steinhaus
Laboratory of Insect Pathology
Department of Biological Control
University of California, Berkeley

Abstract

Of the few bacteria reported as causing disease in insects of medical importance in nature, virtually none has been shown to be a consistent or reliable pathogen. None of these bacteria appears to have strong potentialities as an effective microbial control agent, but ~~adequate testing has not been done. Massive doses may~~ give more promising results than those usually obtained in ordinary experimental procedures. Some medically important insects, such as the housefly, are susceptible to _Bacillus thuringiensis_ Berliner, and under certain conditions may be effectively controlled by it.

As yet, no virus diseases have been found in insects of outstanding medical importance. Polyhedroses and granuloses have been found in urticating Lepidoptera, and unidentified virus infections have been reported in venomous insects such as the honey bee. The recent findings of virus infections in phytophagous mites may indicate that such agents will eventually be discovered in mites of medical importance.

If we are to know more about the role of disease among insects of medical importance, it is first necessary to discover more of the diseases concerned. One way of doing this is to submit to insect pathology laboratories specimens of diseased insects medical entomologists encounter during the course of their laboratory and field work. Diagnostic services for the identification of the causative agents involved are available and should be used.

* * *

I certainly am grateful to the Program Committee for assigning to me what is undoubtedly the simplest and easiest subject of the conference; namely, the bacteria and viruses associated with or causing disease in medically important insects.

Perhaps I could most easily discharge my responsibility by saying that, as far as viruses are concerned, there is virtually none known in insects of medical importance. As far as bacteria are concerned, with two or three exceptions, they do not appear to be very significant on the basis of what we now know. With that, I could conclude my talk. However, perhaps a few comments are in order in this general subject area.

I know of no virus infections in insects of outstanding medical importance. This, of course, echoes the statement which Dr. Jenkins made just before our recess. However, I am sure that most of you realize that insects of some groups do suffer from virus infections, particularly certain Lepidoptera, fewer species of Hymenoptera and Diptera, and, as recently reported, Neuroptera. As far as these virus infections are concerned, they have not been found in any insect that may be considered of medical importance, unless we include the urticating Lepidoptera. Certainly, when a physician has an urticating Lepidoptera problem on his hands, he would consider it of medical importance.

There are two general types of virus infections which are commonly found in Lepidoptera: the polyhedroses and the granuloses. Examples of both of these do occur in urticating Lepidoptera. There are at least 10 such examples. Undoubtedly, urticating Lepidoptera other than those so far reported also suffer from virus disease but have not been observed to do so as yet. In all, I might say, between two and three hundred species of insects are known to suffer from virus infection of one type or another.

Just offhand, I should say that as far as the polyhedroses and the granuloses are concerned, there is nothing to indicate that we can expect to find these in most insects of medical importance. These types of virus infection appear to be so characteristic for the hosts in which they are known to occur, that we should not expect to find them, let us say, in fleas or lice. When it comes to the Diptera the possibility increases, because in one or two instances viruses appear to have been found in certain dipterous insects.

The honey bee, being a venomous insect, can be considered of medical importance too, at least under certain circumstances. This insect has been reported to suffer from two noninclusion virus diseases. Neither one of these virus infections has been very well described; the etiological agent has not been isolated with certainty in either case.

Although no virus infections have as yet been found in mites or ticks of medical importance, recently two species of phytophagous mites have been found to suffer from attack by noninclusion viruses.

This might indicate, if one is thinking of the problem on a phylogenetic basis, that possibly we may eventually find similar viruses in mites of medical importance. As yet, however, none has been reported.

As far as the bacterial diseases are concerned, Dr. Jenkins covered the situation very well. Dr. Briggs, who is next on the program, will consider certain other aspects of bacterial infections in medically important insects. So the only remarks I care to make at this time have to do with emphasizing the fact that most of these bacterial infections -- I believe Dr. Jenkins listed nine for houseflies -- have been found under rather artificial circumstances. To be sure, there are species like Bacillus lutzae and Staphylococcus muscae, as Dr. Jenkins mentioned. But as yet these have not shown the degree of pathogenicity which one would ordinarily expect in a pathogen which could be used for practical control purposes.

Everything I am saying is predicated on an assumption that with more knowledge and more work, we may be able to adapt pathogens to our purposes. I have been speaking of the situation as it appears to be at the moment.

Dr. Briggs will discuss with you the use of the sporeformer Bacillus thuringiensis, which is now being marketed as a microbial insecticide, and which has shown some promise against such medically important insects as the housefly. But here we have a rather artificial situation. In other words, it underlines my statement that, as far as natural infections are concerned, the bacterial diseases are not well known in insects of medical importance.

However, I wish to qualify that statement a bit. I believe there may be such infections in nature. I am saying only that we do not know much about them, that the really true or frank type of infection, or the vigorous type of pathogenic action we would hope to find in bacterial pathogens, has not yet been evidenced in the instances so far observed. For example, in mosquito larvae, quite a number of "bacterial infections" have been reported in the literature, usually without any identification as to the species of bacterium concerned. There is evidence that under some circumstances mosquito larvae do suffer a rather high incidence of bacterial infection. The bacteria involved are usually gram-negative small rods. But, again, the situation seems to be a fortuitous one. So it is very difficult in our present state of knowledge, or lack of knowledge, to say anything very concrete or knowledgeable with regard to bacterial pathogens in medically important insects.

I should expect that, when we do find more natural infections in these insects and are able to generalize a little bit better, we shall find that they are the same general type of bacterial infections as occur in other insects. In other words, they will probably manifest themselves largely as septicemias or sometimes as toxemias. As you probably know, whereas most of the fungi invade their insect hosts through the integument, most of the bacteria, protozoa, and viruses are ingested by the insect, and invade the animal through the intestinal wall. Very probably, that is the type of bacterial or virus infection we might expect to find in insects of medical importance.

We must remember also that the chance of exposure to bacteria is limited in many ways in the case of insects of medical importance. The flea or the louse or the tick or the mite certainly does not take into the digestive tract the type of microbiota which one would expect with many of the phytophagous insects, for example. I remember, however, an exception to this which was rather dramatic to us some years ago when I was with the Rocky Mountain Laboratory in Hamilton, Montana. We began to find that ticks (Dermacentor andersoni) which we were feeding on guinea pigs had a high mortality rate, and obviously were dying of bacterial infection. It was found very quickly that the bacterium concerned was a Salmonella, which had been causing infection ~~in the guinea pigs. What happened was that the tick picked up from~~ the bloodstream of the guinea pig this bacterium which, for reasons we did not determine, was pathogenic for the tick as well. Just how frequently this occurs in nature I cannot even guess, but I imagine there may be more of it than we suspect.

I should like to emphasize that the identification of many of the bacteria which have been reported as being associated with and which have been recorded as being pathogenic for insects of medical importance is very poor. The taconomy -- the systematics -- concerned with these species of bacteria is certainly inadequate; most of the species have been reported once, barely described, than never recorded again. In some cases, it is clear that although the bacteria were given names (I presume simply because to name a bacterium is frequently easier than to identify it), they are obviously synonyms of other well known species. So we have a rather confused situation with regard to the bacteria associated not only with medically important insects but with insects in general.

I mentioned cases of laboratory infections in mosquitoes which have been held in aquaria. In other such instances, certain bacteria such as Serratia marcescens, with which I am sure most of you are familiar, cause disease in insects when the latter are held under similar conditions, such as in cages, but have not been reported from the same insects in nature. This, I think, might typify many of the situations reported in the literature as constituting "bacterial infection."

I will not labor the subject further. Dr. Jenkins said he wanted me to comment with regard to potentialities of Bacillus lutzae and Staphylococcus muscae. I think it would be presumptuous of me to attempt to prognosticate on this point, except that from what we know of insect pathogens in general, I would not hold out much hope for either of these species as being really effective pathogens from the control standpoint. On the other hand, one never knows but that when a bacterium is available in mass quantities and used in large numbers, the effectiveness may be completely different from that apparent in natural epizootics. I think there is already evidence that this can be the case. Again, I shall not go further along this line, because I believe Dr. Briggs will touch on this point.

I should like to take the liberty of speaking just a moment more on the subject of diagnosis.

To learn more about the diseases of insects of medical importance, it will be necessary to find more diseases, and more pathogens, and to study them thoroughly. One way of doing this is to be more attentive to the diagnostic work relating to diseases in this group of insects. I feel that one of the wisest things we could do -- one of the things I would recommend -- would be to increase our search for more pathogens of insects of medical importance.

Dr. Laird mentioned a while ago that frequently the entomologist sees the diseased insect and the quicker he can get rid of it the better; or he tosses it aside or waits too long before investigating it. We probably lose quite a large number of interesting pathogens in this manner. In an effort to overcome this neglect, with regard to insects in general, our laboratory at the University of California has, since 1944, been receiving specimens of diseased and dead insects for the purpose of diagnosis. In other words, we have established a diagnostic service.

As of January 1, just past, this service has been reorganized and enlarged, so that it is our intention to be able to provide anyone with a service that will attempt to identify the diseases of insects submitted to us, whenever this is possible. Our findings, of course, are reported back to the person submitting the specimens.

This service is not entirely altruism on our part. Certainly we do not have sufficient staff or field men who could go out and collect diseased insects for us. (Incidentally, it is not quite so easy to find diseased insects as it is to find certain parasites and predators). Therefore, we are increasingly depending upon other specialists, other people working with the insects, to send us specimens they come upon which are diseased, and which they may not wish to study themselves. Of course, we are not asking anyone to send us material they may wish to incorporate and study in detail in their own research projects, although we are always happy to help out in any way we can in such projects.

We prefer, when possible, to work with living diseased material, but this is not always possible or practical to obtain or to submit. For one thing, there are the quarantine restrictions, both state and federal, that restrict the sending of living insects about the country, between states in many instances, and certainly into the country from abroad. In the case of California, it is required that a special state permit be obtained for living insects to be sent into the state. However, not being able to study living insects does not always bother us too much because, as those of you who work with diseased insects know, many of the diseases or their agents can still be identified from dead insects. I would generalize this point by saying that we should like for you to send us dead or dying insects, when you can, (as soon after death or as near death as possible), and to send them to us in the most rapid way available to you. When you do have insects that have been dead for quite some time, do not despair. Some pathogens can be detected after months, or even longer, in a dead insect. So don't feel it would be foolhardy to send in old material. If the material cannot be worked up, we shall inform you accordingly. Material of any kind, in any condition, is acceptable. If we do need living material, we shall communicate with you, and help arrange for the necessary quarantine permit if it is an insect that can be brought into our state. As far as medically important insects are concerned, this is frequently not difficult. No one is going to object too much to sending a cat flea into California, because after all, we have our own supply. Nevertheless, the law requires that a permit be obtained.

Dead specimens may be sent through the mails, but please do not place them in an envelope. Everyone month or so, we receive two or three such shipments. Of course, the material is almost always smashed and unuseable by the time it reaches us. Simply place the diseased insects in small vials, pill boxes, or some other convenient container and ship them to us, preferably by airmail. We used to say "airmail or air express." For some reason, "express" to many people means "express collect." Moreover, we have to drive over to the San Francisco airport to pick up air express packages, and also pay certain terminal charges. We prefer to receive most specimens by mail.

One other very important point: Do not place the specimens in a chemical preservative of any kind. I realize that sometimes entomologists, particularly systematists, do not detect diseased material until after it has been preserved. In such cases, we are still willing to receive it and do what we can. But when a preservative is added, we lose the opportunity to culture and otherwise examine the material properly.

Other points could be mentioned, but my time is coming to a close. I should just like to say that if any of you are interested in sending us diseased material, we shall be glad to send you a pamphlet which describes all the procedures involved. Anything we find out about your material is always reported back to you so that you can use it in your own reports or in your own papers, and publish it as you wish. The terms of this arrangement are explained in the pamphlet.

It is a real pleasure to introduce the next speaker. Dr. Briggs is a modest chap; he may not tell you that he heads what I believe is the first, or one of the first, true laboratory of insect pathology in an industrial organization. I hope he will enlarge upon this aspect of his work. Some of you know him also for showing experimentally that the principles of immunity in certain insects may be quite different from those we know in vertebrates.

BACTERIAL CONTROL TECHNIQUES

John D. Briggs
Bioferm Corporation
Wasco, California

In biological control, we are experiencing an industrial revolution. The tools for biological control are beginning to be made available through the scaling-up of methods from the laboratory bench to the production line. Today, we witness the arrival of a microbial control product, a spore forming bacteria, _Bacillus thuringiensis_, produced on artificial media by the fermentation industry. Tomorrow, this same industry may produce the artificial diets for the maintainence of parasite and predator populations in the field, or for the mass production of these beneficial insects over and above the quantities heretofore considered possible because of the limitation of natural host material.

When we speak of the industrialization of a process or technique, we immediately think of mass production, and mass production means the availability of the commodity in large amounts. A spore forming bacteria, pathogenic for insects, grown in 12,000 gallon fermentation tanks, means many hundreds of pounds, tons of this biological control material. This availability permits us, as researchers, to enter into many new activities, as well as expanding existing programs, to date limited because of lack of material.

At this point, a discussion in answer to several questions is appropriate. What is the nature of the microbial control material now available? What other types of bacterial insecticides can we expect? How may these biological control materials be used? i.e., What are the possible _materials_ and _methods_ for bacterial control? For discussion, let's imagine that we have a bacteria which will grow in a liquid nutrient medium. This may be a sporeformer or a nonsporeforming insect pathogen. Introduced into the nutrient medium, it is brought through a logarithmic phase of growth to maturity. In the case of a spore former, this means that vegetative growth has stopped and spore formation is nearly complete. We now have a tank full of bacteria and "spent" liquid media referred to as the final whole culture. What do we do with it? For presently available bacterial spore preparations, spores are harvested from the final whole culture by centrifugation or filtration, and the resulting spore concentrate is dried and formulated as a wettable powder, dust or granular preparation. These formulations are used in the same manner as similar chemical formulations.

For other bacteria without a convenient resistant resting stage, these procedures may not be possible. Consider the alternative measures. We may use this final whole culture as is. For dry application, we could adsorb it onto a dry carrier such as finely divided clay, or clay

Figure 1

Small fermentation tanks for developmental studies of microbial insecticides and production of small quantities of experimental materials for research purposes.

Figure 2

Fermentation facilities for large scale production of microbial insecticides.

granules. For ease in handling, we might take this final whole culture and concentrate it by elimination of water. As a concentrate, it could be frozen similar to frozen orange juice, or freeze-dried, in either form to be reconstituted. As a frozen concentrate, there would be a necessity for refrigeration, whereas in a freeze-dried condition, it could be maintained in unrefrigerated containers. A cell concentration might be mixed with a bacteriostat, which not only would prevent further growth of the microorganism in which we are primarily interested, but would discourage contaminants. Upon dilution of the concentrate, the bacteriostat would not interfere with the activity of the pathogen. In all of the foregoing examples, we have considered the subject bacteria to be alive. If viability were not paramount, the final whole culture could be sterilized and packaged aseptically for storage or shipment.

For obvious reasons, it might be to our advantage to reduce the bulk of our material and concentrate the bacterial cells from the final whole culture. Not only would this reduce the volume of the active material, but it would certainly reduce the possibility of materials present in the culture medium interfering with the activity of the bacteria, or nutrient materials providing substrates for contaminating organisms. The cell concentrate could be treated in the same manner as the final whole culture concentrate previously mentioned. It could be frozen as a wet concentrate, or freeze-dried. It could be packaged with a bacteriostat, or the cell concentrate could be inactivated by heat or other means. To this point, we have considered the bacteria as the active principal, or we have at least assumed this role for the bacteria. Two other possibilities for insect active bacterial products offer fascinating possibilities. The first are the intracellular insect active bacterial products. Extraction of this intracellular produced principal would be necessary from a concentration of bacterial cells. Secondly, serious consideration should be given the production in a fermentation process of extra-cellular materials which have activity against insects. Such a material would be not unlike an antibiotic, in reality, it would be an antibiotic. In the work with intracellular and extra-cellular active principals, industrial groups should excel. Certainly, industrial experience with vitamins and antibiotics will contribute greatly to the discovery, development, and eventual availability of biological control agents produced by bacteria or other microorganisms.

The materials we have considered represent both insoluble and soluble products. Bacterial cells, spores, or whole culture, being particulate in nature, could obviously be formulated as wettable powders or dust, or perhaps colloidal suspensions. Extracts of bacterial cells or by-products soluble in the culture medium would be readily formulated for use in any form.

In addition to spraying and dusting as a means for applying insecticidal preparations of bacteria, the toxic specificity for certain insects of presently available bacterial preparations offer the possibility of other methods for application. For example, studies in Illinois on the incorporation of Bacillus thuringiensis spore powder into the daily ration of laying hens demonstrated a reduction in the number of house flies emerging from the feces of hens on the treated diet (Briggs, 1960). Preliminary studies in California indicate that this same method can be applied to control of house flies in cattle droppings (Dunn, 1960). To date, the control measures are limited to situations where the animals in question are under a supervised feeding program. In many cases, it is under these conditions that our greatest fly problems exist, particularly in close proximity to urban areas.

The existence of soluble principals produced by insect pathogens of bacterial nature, offers us the opportunity to utilize materials that may be entirely noninjurious to mammals as systemic insecticides in order to combat insects living within or preying upon vertebrate hosts. For example, the feeding or injection of livestock with biologically produced materials for controlling dipterous parasites. Similar treatment of pets for the control of fleas, or of human beings for fleas or ticks may be possible.

Techniques for control measures with bacteria are not different from those which you may consider with a nonbiological insecticide. However, the safety to vertebrates of these biological materials produced to date allow us great latitude in their handling and a challenge to our imagination for new applications.

Literature Cited

Briggs, John D., 1960. Feeding layers Bacillus thuringiensis Berliner to control Musca domestica L. developing in chicken feces. First Joint Meeting Ent. Soc. Am., Ent. Soc. Can., Ent. Soc. Ont., Detroit, Mich. Sec. C, sub-sec. a. (Submitted for publication to the J. Insect Pathol.)

Dunn, Paul H., 1960. Control of house flies in bovine feces by a feed additive containing Bacillus thuringiensis var. thuringiensis Berliner. J. Insect Pathol. 2 (1): 13-16.

DR. STEINHAUS: Discussion is open on Dr. Briggs' paper.

QUESTION: What happened to egg production when spore powder was added to the diet of chickens?

DR. BRIGGS: There was no effect on egg production. We followed this for 20 months on one series of hens. There was no detection of the bacillus within the egg. We had no mortality in our flocks which could be attributed to it. All dead hens were submitted to the Laboratory of Veterinary Medicine, University of Illinois. We finally consumed the remaining test animals ourselves.

DR. STEINHAUS: I wonder if you care to say anything as to mosquito larvae. I know you referred to it in your remarks; but perhaps you have further comments to make.

DR. BRIGGS: Our tests with mosquito larvae grew out of a program we had in cooperation with the State Bureau of Vector Control, Fresno, California. The laboratory tests showed a high mosquito mortality with Bacillus thuringiensis. As you might expect, there was considerable interest shown in this immediately. However, in field applications, alongside chemical plots, the material was very erratic in its performance.

Proper formulation of these materials is perhaps one of the greatest problems we have in industrial development of microbial insecticides. This is an area in which we are now spending a great deal of time in finding what the proper formulation might be for a particular use. In forest work, one type of formulation is needed; in agricultural, it might be another. Certainly, for work on mosquitoes and perhaps on flies, special formulations will have to be developed.

DR. STEINHAUS: Do you consider the use of this microbial insecticide against the house fly as fairly well established now?

DR. BRIGGS: No. However, since the study reported here, the pathogenicity of this spore preparation has been increased considerably; tests are still in progress on the use of this material in feeding programs.

DR. STEINHAUS: Dr. Kramer, do you have any comments you would care to make on your work on house fly diseases?

DR. KRAMER: Our work is restricted to studying what happens to the fly populations in the field, and what microorganisms might be responsible for the mortality we observed. We have not run across any bacteria which are described and readily identified in the literature. For example, the microorganism which was mentioned earlier by Dr.

Jenkins as Micrococcus melitensis is not even listed in the latest edition of Bergey's Manual of Determinative Bacteriology. So as far as the bacteria are concerned, we have nothing to report.

DR. PRATT: Have you tried Bacillus thuringiensis on cockroaches and other household pests, using a massive, overwhelming number of spores in a given cubage of water or air space?

DR. BRIGGS: I have not tested this against cockroaches.

DR. DUTKY: We have been doing work with house flies and contemplate using the house fly larva as a screening organism. Some of the pathogens, as picked up and tested by injection, are capable of killing larvae. The usual time of death is within 17 hours. It is very possible that the house fly larva is much more susceptible than any other stage. This would not limit the use of the organism for this purpose. I think we can rather quickly scan quite a lot of organisms by this technique.

DR. STEINHAUS: Speaking for myself, I am sure that anything I may know about Coelomomyces fungi I have learned from Dr. Couch's writings; not only his formal publications, but from letters. He has been most indulgent with us as far as identifying species is concerned. I do not know whether he will appreciate my pointing this out. Maybe you would not want to be flooded with work of this kind, Dr. Couch. But all of us in insect pathology owe you a real debt for the monumental work you have done in this field. So it is with particular pleasure that I introduce Dr. Couch to speak on the entomogenous fungi.

SOME FUNGAL PARASITES OF MOSQUITOES

J. N. Couch
University of North Carolina

A survey of the more important literature on *Coelomomyces*, a genus of aquatic fungi in the order *Blastocladiales*, is given. The members of this genus are obligate parasites on mosquitoes usually in the larval stage but sometimes in adults. The infected insect is recognized by the presence of large, oval, yellowish to yellow-brown, thick-walled sporangia in the body cavity of the insect including the thorax and head. If the infection is heavy, the body cavity may be rather closely packed with these sporangia and the larva fails to change into an adult and dies. Light infections do not prevent metamorphosis, and infection of adults has been reported in the Philippines and Africa. The mycelium is without cell walls and is unlike that of any known fungus. The development of the fungus is incompletely known but it has been reported by two workers that the fat body surrounding the intestine is destroyed and that as a rule the other body organs are left intact. Light infections in adult females may be confined to the ovaries, in which cases the eggs fail to develop. In some species both thin and thick-walled sporangia have been reported but in most only the latter have been observed. Muspratt reported on the germination of both thin and thick-walled sporangia and his observations have been confirmed on the thick-walled ones. The agents of infection are unknown but it is assumed that the zoospores are responsible. Some information is presented on the geographical distribution and seasonal occurrence of *Coelomomyces*. Some suggestions for future work are proposed.

* * *

In our laboratory, we have done considerable work on fungi parasitic on various animals such as scale insects, flies, caterpillars, liverflukes, crabs, nematodes, and mosquitoes. However, because of our primary interest at this conference in fungi which attack insects of medical importance, I shall limit my talk to *Coelomomyces*, a genus of fungi parasitic mostly on mosquitoes.

An excellent discussion of the earlier work on this genus is given in Steinhaus (1949). Since a good many of the original papers have been published in journals which are not readily available, a summary of the more important work on *Coelomomyces* is given here.

The genus was described by Keilin (1921) from material found in one infected larva of Aëdes albopictus sent to him in England from Malaya by Dr. Lamborn. With this larva were five others of the same species which contained a ciliate described by Keilin as Lambornella stegomyiae n.g., n. sp. Keilin calls attention to the similarity of the cysts of the ciliate to the thick-walled sporangia of the Coelomomyces. From sections he found that the larva though heavily infected by the fungus had apparently healthy internal organs but that the fat body had completely disappeared. The mycelium was well developed around the viscera and beneath the hypoderm but there was little in the body cavity. It was branched, multi-nucleate and without cross-walls. The sporangia arose as terminal swellings which became separated from the mycelium and completed their maturation floating within the body cavity. He described the mature sporangia as of two kinds, thick and thin-walled. On the thick-walled sporangia a fine line was evident from pole to pole. He correctly predicted that the escape of the spores results from the rupture of the sporangium along this line. Keilin suggested that the fungus belonged to the Phycomycetes, showing some resemblance to the Chytridineae. He pointed out that the mycelium of Coelomomyces was better developed than in any known member of the Chytridineae and that the structure of the sporangia of Coelomomyces was different from that of the chytrids. He finally concluded that the systematic position of Coelomomyces cannot be finally established until more abundant and living material is available for a detailed study of its structure. It is of interest to note that Keilin had available for consultation one of the leading mycologists, the late F. T. Brooks.

Bogoyavlensky, in 1922, unfamiliar with Keilin's work, described a second species as a new genus and species, Zografia notonectae. Keilin, in 1927, indicated that this was a species of Coelomomyces. It is the only species of the genus so far described which was not found on mosquitoes. He described the vegetative stages as consisting of a plasmodium of irregular, much branched and anastomosing threads without independent motion and covered with a scarcely noticeable pellicle. The parasite destroyed the fat body, though not penetrating the cells, leaving the rest of the insect intact, apparently causing no real harm and not preventing the laying of eggs. He described and illustrated the sporangia but considered them as spores. He suggested a relationship to the Myxomycetes, but finally concluded that it was closer to Sporomyxa but should be an independent genus. It should be noted that neither Keilin nor Bogoyavlensky mention a cell wall around the mycelium.

Manalang (1930) described two varieties from the Philippines of what he considered to be coccidia. One to two percent of all adult mosquitoes dissected were infected. This is of interest since Iyengar in India found that only rarely did an infected larva pupate and the adult emerge. Walker (1938) was the first to point out that Manalang's coccidial infections were perhaps Coelomomyces.

Iyengar, in 1935, described in some detail two new species which parasitized *Anopheles* larvae in India. He described the mycelium as attached to the fat body by very thin hyphae, confirming for the most part the observations of Keilin and Bogoyavlensky. He describes the wall as very thin and membranous. One of the species of *Coelomomyces* described by Iyengar parasitized eight different species of *Anopheles* larvae and the other, four species. Both were widely distributed over India.

Walker in 1938, working in West Africa, recorded four forms of *Coelomomyces* in the larval and adult stages of *Anopheles costalis* and *A. funestus*. Infected larvae were much more abundant than were infected adults. He tried to culture the fungi, using sporangia and mycelium on various artificial media, but was unsuccessful. Most interesting of Walker's results was his success in producing artificial infection of laboratory-bred mosquito larvae.

DeMeillon and Muspratt, in 1943, first obtained germination of the thick-walled sporangia in a species of *Coelomomyces* but were unable to classify the fungus correctly from this very important observation. In 1946, Muspratt reported interesting results of observations carried on during the rainy seasons of 1941 to 1945 in Northern Rhodesia. During this four-year period, Muspratt estimated the mortality of the larvae in these pools about 95 per cent caused by the *Coelomomyces* infection. He states that the larvae may become infected in any stage of their growth. In a later paper the same year (1946) he recorded the experimental infection of laboratory-hatched larvae of *Anopheles gambiae* by putting them in a concrete trough filled with rain water and containing mopane clay and several hundred dead larvae of *A. gambiae*, whose bodies were packed with the thick-walled resting-sporangia of Coelomomyces. The mopane clay and the infected larvae (stored in jars in soil from the breeding place) had been dried for eight months before the experiment was started. The water was allowed to evaporate to dryness every two or three weeks and the trough to remain dry for three or four days before it was refilled and another batch of newly hatched larvae put in. No infected ones were seen in the first lot, but about fifteen out of 100 larvae of the second batch became heavily infected, and a few in later batches. Musspratt thinks the zoospores are responsible for infection.

Laird (1959a) reported from Singapore successful laboratory infection experiments with the larvae of *Aëdes aegypti* when reared in pans of distilled water buffered to an acidity of pH 6.6 into which were put dried sporangia derived from parasitized *Aedes albopictus*, and sediment from the container of the latter. In other papers (1956a, 1956b, 1959b) he has added a new species (a total of five), and extended the host range and geographical distribution of several of the old species. In a recent paper (1959b) he presents a key to the 21 species so far described.

Since this conference is interested primarily in the possible use of parasites, predators, etc. in the biological control of insects of medical importance, I will first give an example of a fungal parasite of mosquitoes which I think offers only minor opportunities as an agent of biological control.

This fungus belongs to the genus *Lagenidium*, the other species of which are parasites on algae and certain lowly, fresh and salt water animals. It was first found (Couch, 1935) on Daphne and Copepods in the lake at Mountain Lake, Virginia and a few weeks later was found on mosquito larvae near Chapel Hill, North Carolina. The penetration of the fungus into the insect larvae was not observed. It forms a segmented, rather coarse mycelium which branches and extends throughout the larva, eventually killing and destroying the internal organs. When mature, each segment may form a sporangium. The sporangia develop long tubes, one from each sporangium, which emerge through the cuticle and are 6 - 10 microns thick and 50 - 300 microns long. These tubes form a white fringe around the larva. The zoospores are formed in large quantities and are doubtless the agents of infection. Sexual reproduction was not observed.

The fungus was isolated in pure culture from spores and cultured on a variety of media for several years. A few experiments were carried out to see if it would attack healthy mosquito larvae. The experiments, though partly successful, were done on too small a scale to warrant conclusions of any value. This fungus seems unsuitable for biological control of mosquitoes since it is a weak parasite and has too wide a host range. However, if we could find it again, we would test its parasitic possibilities more throughly.

I am going to list what I consider desirable qualities in an organism to be used for biological control.

First, it should be a virulent parasite capable of killing the host.

Second, it should be limited in host range and harmless to desirable organisms.

Third, it should be culturable in large quantities either in pure culture or on laboratory-reared hosts, or capable of being introduced into a new area on a new and susceptible host (as done by Laird in the South Pacific).

Fourth, it should be easily and cheaply dispersed over the host range. To do this it must have some sort of reproductive spores or zygotes which survive prolonged dessication and can be mixed with a proper carrier.

I think you will see how well *Coelomomyces* fits these requirements.

I first became acquainted with Coelomomyces during the second World War. A slide showing two beautifully preserved larvae of Anopheles quadrimaculatus whose bodies were chock-full from head to tail with oval-shaped brown bodies was sent to me by H. R. Dodge from Georgia. In size, shape and structure these suggested the resting bodies of Allomyces but since there seemed to be no mycelium present, I suspected that the bodies might be cysts of protozoa or worm eggs and had them examined by our protozoologist, who suggested that the brown bodies did not belong to any animal with which he was familiar. Meanwhile in fresh living material from Mr. Dodge, I had found the brown bodies germinating to form zoospores somewhat similar to those in the Blastocladiales. Fortunately at this time I was working on two species of Catenaria, one parasitic on nematodes and liverfluke eggs and the other parasitic on all the species of Allomyces and some species of Blastocladiella. As soon as germination of the resting bodies in the mosquito parasite had been seen, the striking similarity of the resting bodies of Coelomomyces and those of some of the Blastocladiales was obvious. One of the most striking features in common is the preformed line extending more or less lengthwise on the wall of the resting sporangia of many Blastocladiaceous fungi. When the spores are discharged, the sporangia crack and the wall spreads open at this fissure because of internal pressure, thus permitting the escape of the zoospores. These thick-walled, ovoid, brown bodies are thus homologous with the resting sporangia of Allomyces and the other Blastocladiales.

In Coelomomyces the thick-walled sporangia are formed from hyphal bodies which are separated from the hyphae and float freely in the insect's haemocoel. In all the rest of the Blastocladiales the sporangia complete their development while attached to the parent hypha. Indeed this peculiar method of sporangial formation seems to occur only in Coelomomyces.

Our knowledge of Coelomomyces is very limited. For purposes of classification all we have to go on at present is the structure of the mycelium and the sporangia.

I will discuss the mycelium first. When the body of the larva becomes filled with sporangia it is difficult or impossible to find any mycelium since it has been used up largely in the formation ofsporangia. Without some sporangia, it is hard to recognize an infected insect for the hyphae are easily mistaken for some of the shredded structures on the insect's body. To find the mycelium it is best to select a larva in which a few young sporangia can be recognized and then to dissect the body in saline or in 7% formalin. If the living body of the insect is torn apart in water, the mycelium rapidly swells and finally is dissolved and disappears completely. If, however, the live larvae have been fixed in formalin before dissecting, the mycelium does not swell and disappear but persists. This dissolving of the mycelium in water indicates the absence of a cell wall and confirms the observations of Bogoyavlensky (1922). Coelomomyces is the only fungus known which has a mycelium lacking cell walls. This is one of the reasons it deserves family rank in the Blastocladiales.

Even though our knowledge of the mycelium is very scant, its structure seems to be of some value in separating groups of species. The more important features of the mycelium are the width of the individual threads, the method of branching and the way in which the hyphal bodies which develop into sporangia are separated from themycelium. The aid which mycelial characters may give in the recognition of species is well shown in Coelomomyces quadrangulatus and C. pentangulatus. In the two species the resting bodies are about the same size and are much alike in the structure of the wall. The main difference in the resting bodies is that in the first they are four angled in end view while in C. pentangulatus they are five angled in end view. This difference alone might not justify the establishment of the two as separate species but in the two the mycelium is distinct. In C. quadrangulatus the mycelium is the most vigorous of any seen so far, averaging over twice the thickness as that of C. pentangulatus. Also in the latter the hyphae break up into pieces with many short branches. These two appear to be distinct species but since the two occur on different hosts they may be physiologic races of the same species and the differences in mycelium and resting sporangia may be the result of different hosts. The only way in which this and similar problems can be solved is by cross inoculation experiments.

The resting bodies or thick-walled sporangia in Coelomomyces are separated from the hyphae and formed in a way that appears to be unique in the fungi. On this, however, observations are needed with living material since all studies on sporangial development so far reported are from preserved specimens. In all species the sporangia appear to develop from naked hyphal bodies which are pinched off or otherwise separated from the mycelium without the formation of a cross wall. In C. pentangulatus large pieces of branched mycelium are severed from the mycelium and each branch is then pinched off and develops into a sporangium. The hyphal bodies when first pinched off may be rounded or irregular in shape but they soon assume a more or less oval shape and float in the hemocoele. As they mature into sporangia they become smaller and take on a more regular oval or rounded shape. The thick-walled resting sporangia are usually surrounded by the thin plasma membrane and the wall proper consists of two layers, a thinner, hyaline, internal part and a thicker, yellowish to brownish, sculptured, or rarely smooth external layer. A very distinctive structural feature of the sporangia is a fine line in the wall extending longitudinally. This performed line or fissure marks the place where cleavage of the sporangium occurs when the spores are mature and ready to emerge.

The size, shape and structure of the wall of the resting sporangia are of prime importance in separating species. Indeed in several species this is all the morphology we have to go on at present.

The resting sporangia in the different species vary greatly in size and considerably in shape. The smallest so far described are those of C. africanus Walker occurring in Anopheles costalis. These are 12-18 x 20-35 microns. The largest resting sporangia are those of C. psorophorae on Theobaldia inornata, which are up to 58 x 119 microns. In most species the resting sporangia are oval with a slightly flattened side, in some they are slightly allantoid and in one species somewhat kidney shaped. The most unusual shape of a resting sporangium so far reported is that of C. anophelesica Iyengar which is discoid in face view but with one edge flat.

The color of the wall, its thickness and its ornamentation seem to be of great value in determining species. The wall is composed of two distinct layers in all but two or three species in which the thinner inner layer if present could not be made out. The pigment if present is located in the outer layer and varies from a pale yellow to a deep brown. In a few species, C. notonectae, C. walkeri, C. solomonis, the wall is unpitted and unornamented. In C. stegomyiae the wall is smooth in outline but with minute pits, this is true for C. psorophorae, C. tasmaniensis and C. keilini. In C. lativittatus, C. dodgei and perhaps in C. ascariformis (as illustrated in Manalang fig. 1, 1930) the outer wall is composed of several bands which extend longitudinally or are irregularly arranged on the sporangial wall. C. punctatus which is related to C. dodgei has narrow bands so closely and irregularly arranged as to form rounded or elongated pits. These bands whether wide or narrow are closely arranged and low and thus readily enable one to recognize the species in this group. Another very distinctive group is the one to which C. bisymmetricus, C. sculptosporus, C. cribrosus and C. africanus belong. In C. bisymmetricus the bands are of two sizes- low narrow and broad high ones, which alternate and encircle the sporangium in such fashion as to give the sporangium a bilateral symmetry. In this species most of the sporangia show this symmetry but in some the symmetry is lacking. The other three species C. sculptosporus, C. cribrosus and C. africanus appear to be related to C. bisymmetricus but can easily be separated by the ornaments on the wall. The two species, C. indiana and C. anophelesica, described from India by Iyengar stand out in comparison with all previously described species by having very prominent ridges spaced widely apart and extending longitudinally or anastomosing to form a net or arranged sometimes in C. anophelesica in concentric circles. Another distinct group of species which are closely related is C. cairnsensis, C. macleayae, and C. finlayae all described from Queensland by Laird. The distinctive feature of this group is the sharp ridges which usually anastomose to form more or less regular polygonal areas. In section these ridges appear in two of the species as equilateral triangles. Sometimes the ridges may extend almost straight from one end of the sporangium to the other; again the ridges may appear wavy. C. uranotaeniae may be related to this group as suggested by Laird (1959). It has the sharp ridges but these are fewer and they extend longitudinally on both sides of the resting sporangia and never anastomose to form the polygonal areas so distinct in C. cairnsensis and related species. A final and very distinct group is the C. quadrangulatus, C. pentangulatus series. These have small resting bodies which show their distinctive features best in cross section or end view. In the former the resting sporangium is four angled, while in the latter it is five angled in cross section view.

Keilin (1921) and others have recorded the presence of thin walled sporangia in C. stegomyiae and other species. The only report of the germination of thin-walled sporangia was by Muspratt (1945a) in his types a and c. The thin-walled sporangia germinate if the larval remains containing the sporangia are left in the water from the breeding-place, the zoospores usually emerging within three to six days. In most of the species thin-walled sporangia have not been observed. In related genera as Allomyces and Blastocladiella several types of life histories have been described. In all species of these two genera except one, thin-walled sporangia have been recorded. These always appear in advance of the thick-walled ones, the thin-walled sporangia releasing their zoospores in large quantities into the water and thus serving to disseminate and increase the fungus. Since Coelomomyces is completely enclosed within the body cavity of the insects, thin-walled sporangia discharging zoospores in the body fluid would be of no survival value to the fungus sunce the zoospores would be unable to escape and bring about the dissemination of the fungus. The thick-walled sporangia are probably able to withstand prolonged dessication and are very likely the condition in which the fungus is distributed in wind-blown dust and by other agents. In some cases of light infection the insects may reach the adult winged stages with resting sporangia in the coelom (Manalang, 1930; Walker, 1938; Haddow, 1942) and thus be responsible for desseminating the parasite.

The germination of the thick-walled resting sporangia was first observed by deMeillon and Muspratt (1943). In a later paper Muspratt (1946a) describes the technics used to get the thick-walled sporangia to germinate. The larval remains containing the sporangia of type "c" were dried on a slide two or three days after the death of the larvae, when slight decomposition had begun. These were then put in an incubator at 28° C for two to three months before being placed in water again. In another experiment the dead larvae were kept in water at room-temperature (Johannesburg) for three months; then dried and incubated dry at 28° C for three weeks before being wetted again. Both methods led to successful germination. Muspratt states: "....failures have been experienced subsequently when trying to repeat the above experiments; so it is evident that all the factors involved in the germination process are not fully known." It was found by Muspratt that light-stimulus plays a part in the liberation of the zoospores. Germination up to the splitting of the thick-wall may proceed in darkness but the light of the microscope lamp seems to hasten the extrusion of the sporangial contents and the liberation of the zoospores, which may start after about 15 minutes of light-stimulation.

The germination of the thick-walled sporangia was also observed by Couch (1945) and was reported in some detail by Couch and Dodge in 1947. Germination was seen in three closely related species C. dodgei, C. punctatus, and C. lativittatus. The most complete observations were on the latter species. Material collected January 23, 1945 in Georgia was received January 26. The infected larvae were ~~dead but their bodies had not disintegrated.~~ One heavily infected larva was cut into three parts and each part put into a petri dish of charcoal treated water. After six days some of the sporangia had started to germinate. It is of interest to note that these sporangia germinated without being previously dried. Since the process of germination has been described in detail it need not be repeated here.

The zoospores are rather small for the Blastocladiales. When quiescent and more or less rounded they are 4-5 microns thick and when active they are elongated, 2.6-3.8 x 5.2-6.3 microns. There is a single posterior flagellum, a distinct nuclear cap and a clump of lipoid bodies close to the nucleus, all characteristics of the Blastocladiales. In a medium sized sporangium of C. lativittatus I would extimate there are from one to several hundred zoospores. These are most likely the agents of infection. It is not known, however, what are the agents of infection or when infection occurs.

Coelomomyces has now been reported from all the continents except South America. It doubtless occurs there but has not been looked for. There do seem to be some areas in which Coelomomyces is much more abundant than in others but before any sound conclusions about its distribution and abundance can be drawn much more work should be done. Manalang (1930) reported coccidial (Coelomomyces) infections in about one to two per cent of each adult species dissected in the Philippines. Muspratt (1946) worked at Livingstone in Northern Rhodesia during the rainy seasons of 1941 to 1945 on the Coelomomyces infections of Anopheles gambiae larvae. He estimated the mortality to be as high as 95 per cent of the larvae which hatch out during the rainy season. Laird (1959) from personal observations and studies by Colless reports that C. stegomyiae is established in from three to five per cent of larval habitats of Aëdes albopictus on Singapore Island. Shemanchuk (1959) reports from Alberta, Canada that Coelomomyces psorophorae in larvae of Culiseta inornata

(Williston) is widely distributed and well established in Southern Alberta. Weekly samples were taken in 1957 in all irrigated districts from July 8 to September 3 and twelve per cent of all larvae of C. inornata examined were infected. Laird (1959) in his studies of isolated Pacific atolls found that even intensive sampling has so far failed to reveal any infections. I have collected larvae in considerable numbers in North Carolina from the coast to over 4000 feet but have so far failed to find Coelomomyces. One larva of Anopheles crucians heavily infected with C. quadrangulatus was found in a large number examined in the collections stored in the State Board of Health Laboratory at Raleigh, North Carolina. It was in a collection from Elizabeth City, North Carolina.

The collections made so far would seem to indicate that Coelomomyces is more abundant on the species of Anopheles and Aëdes than other genera. At first glance this would certainly seem to be the case in the collections from Georgia since of the eleven species described from that state eight are on species of Anopheles. It must be kept in mind, however, that the collectors were working for the malaria control authorities in military areas and were looking for the breeding places and abundance of the mosquitoes which transmitted malaria. A general survey of the occurrence of Coelomomyces in all kinds of mosquito larvae would doubtless present a different picture.

Muspratt (1946), in the studies referred to above in Northern Rhodesia, found larvae infected with Coelomomyces only during three or four months of the year, and these are the last months of the rainy season which begins in October and lasts until the beginning of May. There are no pools and no larvae during most of the dry season. In Georgia where the rainfall is about evenly distributed throughout the year infected larvae have been collected throughout the year. They were most abundant during the month of May, followed by June, April, March, July, September, November, December, October, January, and February in decreasing order of parasitized larvae. The number for May was almost twice that for June and about four times that for April. The lowest records were for January and February which were about the same, but for each of these months the numbers of parasitized larvae dropped to about one ninetieth of the number collected in May.

In closing, I would like to suggest some of the unanswered questions concerning Coelomomyces. How wide spread and how common is Coelomomyces and what are the factors that determine its distribution? What are the agents of infection and at what stage in the insect's life cycle does infection occur? What parts of the insect's body are attacked? Will one species of Coelomomyces parasitize more than one kind of mosquito larva, i.e. can controlled cross inoculations be carried out? Is there an alternation of generations as in certain other related fungi? Is there an alternation of hosts? Can

Coelomomyces be cultivated on artificial media? Can it be grown in quantity on mosquito larvae artificially reared in the laboratory? Can any of the species of Coelomomyces be used for biological control of mosquitoes?

Literature Cited

Bogoyavlensky, N. 1922. Zografia notonectae, n.g., n. sp. Arch. Soc. Russe Protist. 1: 113-119

Couch, J. N. 1935. A new saprophytic species of Lagenidium, with notes on other forms. Mycologia 27: 376-387.

Couch, J. N. 1945. Revision of the genus Coelomomyces, parasitic in insect larvae. J. Elisha Mitch. Sci. Soc. 61: 124-136.

Couch, J. N. and H. R. Dodge. 1947. Further observations on Coelomomyces, parasitic on mosquito larvae. J. Elisha Mitch. Sci. Soc. 63: 69-79.

DeMeillion, B. and J. Muspratt. 1943. Germination of the sporangia of Coelomomyces Keilin. Nature 152: 507-509.

Haddow, A. J. 1942. The mosquito fauna and climate of native huts at Kisumu, Kenya. Bull. Ent. Res. 33: 91-142.

Iyenger, M. O. T. 1935. Two new fungi of the genus Coelomomyces parasitic in larvae of Anopheles. Parasitology 27: 440-449.

Keilin, D. 1921. On a new type of fungus: Coelomomyces stegomyiae n. g., n. sp., parasitic in the body cavity of the larva of Stegomyia scutellaris Walker. Parasitology 13: 225-234.

Keilin, D. 1927. On Coelomomyces stegomyiae and Zografia notonectae, fungi parasitic in insects. Parasitology 19: 365-367.

Laird, Marshall. 1956a. Studies of mosquitoes and freshwater ecology in the South Pacific. Roy. Soc. of N. Zealand Bull. No. 6: 1-213.

Laird, Marshall. 1956b. A new species of Coelomomyces (Fungi) from Tasmanian mosquito larvae. J. Parasitol. 42: 53-55.

Laird, Marshall. 1959a. Parasites of Singapore mosquitoes, with particular reference to the significance of larval epibionts as an index of habitat pollution. Ecology 40: 206-221.

Laird, Marshall. 1959b. Fungal parasites of mosquito larvae from the oriental and Australian regions with a key to the genus Coelomomyces (Blastocladiales: Coelomomycetaceae) Can. J. Zoology 37: 781-791.

Manalang, C. 1930. Coccidiosis in Anopheles mosquitoes. Philippine J. Sci. 42: 279-281.

Muspratt, J. 1946a. On Coelomomyces fungi causing high mortality of Anopheles gambiae larvae in Rhodesia. Ann. Trop. Med. & Parasitol. 40: 10-17.

Muspratt, J. 1946b. Experimental infection of the larvae of Anopheles gambiae (Dipt., Culicidae) with a Coelomomyces fungus. Nature 158: 202-203.

Shemanchuck, J. A. 1959. Note on Coelomomyces psorophorae Couch, a fungus parasitic on mosquito larvae. Can. Ent. 91: 743-744.

Steinhaus, E. J. 1949. Principles of Insect Pathology, McGraw-Hill Book Co. New York.

van Thiel, P. H. 1954. Trematode, gregarine and fungus parasites of Anopoeles mosquitoes. J. Parasitol. 40: 271-279.

Walker, A. J. 1938. Fungal infections of mosquitoes especially of Anopheles costalis. Ann. Trop. Med. Parasitol. 32: 321-244.

DR. STEINHAUS: Thank you, Dr. Couch. Is there discussion?

DR. LAIRD: I understand that botanists still insist upon a Latin summary of the characters of species, if these can be regarded as valid. If this is so, I am afraid that Coelomomyces does not actually exist, because Keilin, when he described the species in Latin, described nothing. I would like some botanical clarification of this point.

DR. COUCH: The rule requiring a Latin description dates from January 1, 1935. The Mycological Society of America voted to accept this rule about 1940. All species of fungi adequately described before 1935, and this of course includes Keilin's, are valid. Species described since then if published without a Latin diagnosis will be accepted provided they have been adequately described. Unfortunately as pointed out by Laird not all of the species that have been given names have been completely described in any language.

But for the zoologists, who do not require Latin descriptions, we very likely would know nothing about Coelomomyces.

DR. STEINHAUS: Of course this also applies to some of the things which have been described since that rule went into effect.

DR. JENKINS: I would like to ask Dr. Couch what he thinks of the potential of the Entomophthoraceae for use against mosquitoes or houseflies.

DR. COUCH: Some of the Entomophthoraceae as Conidiobolus are weak parasites; many others are obligate parasites on certain insects in nature and some of these strong parasites could probably be used in biological control of harmful insects. I doubt, however, if any of the Entomophthoras would be effective against mosquitoes since these fungi are spread by airborne conidia, but Empusa muscae has certainly been effective at times in killing house flies.

WEDNESDAY AFTERNOON SESSION

February 3, 1960

The meeting reconvened at one-thirty o'clock, Dr. Steinhaus presiding.

DR. STEINHAUS: Certainly, when it comes to the subject of protozoa, particularly the Microsporidia, associated with insects, everyone knows the name and work of our next speaker. It is a real pleasure for me to introduce Dr. Kudo.

PROTOZOAN PARASITES IN CERTAIN INSECTS OF MEDICAL IMPORTANCE

R. R. Kudo
Department of Zoology
Southern Illinois University
Carbondale, Illinois

Abstract

The occurrence of microsporidian parasites in the mosquitoes, the midges and the black flies, and their geographical distribution are reviewed. The incidence of infections in anopheline and culicine larvae, and the effect of infection upon the host larvae are examined and discussed. The possibility of utilizing microsporidian parasites for control of insects of medical importance is suggested.

* * *

A number of protozoan parasites are known to occur in insects of medical importance. Coelozoic parasites do not, as a rule, hinder the normal activity of the host insect. Some gregarines invade the host cells during early development, but appear not to bring about serious damage on the host body. Cytozoic protozoa, on the other hand, attack the cells of various tissues and organs and grow and multiply at the expense of the cell substances. Consequently the host insects when heavily infected are visibly affected and not infrequently succumb to death.

Such appears to be the case with a group of protozoa known as Microsporidia, which is characterized by the production of spores that contain a sporoplasm and a coiled polar filament. Up to date more than 300 species of Microsporidia are known. Of these some 48 species have been reported from mosquitoes, midges, and black flies. This number includes some forms that were noticed in a small number of host insects as well as others that were mentioned incompletely by workers engaged in another phase of research and, therefore, their microsporidian nature has not been established definitely.

The following list indicates the species of Microsporidia, their hosts, and developmental stages and infected organs or tissues of the hosts:

Microsporidia	Hosts	Infected*	
		Stage	Organ, tissue
Nosema aedis Kudo, 1930	Aedes aegypti	L	Fat body
N. anophelis Kudo, 1924	Anopheles quadrimaculatus	L A	Gut Gut, fat body
N. culicis Bresslau, 1919	Culex pipiens	L	-
N. lutzi Kudo, 1929 (N. stegomyiae L.& Sp., 1908)	Aedes calopus	A	Gut
N. stricklandi Jirovec, 1943	Simulium sp.	L	Fat body
N. zavreli Weiser, 1944	Chironomus thumi	L	Mid-gut
N. sp. Martini, 1920	Aedes sp.	L	-
N. sp. Noller, 1920	A. nemorosus; A. cantans	L	-
Thelohania bracteata (Strick., 1913) Deb.& Gast., 1919	Simulium bracteatum; S. hirtipes; S. maculata; S. ochraceum; S. venustum; S. sp.; Eusimulium latipes	L	Fat body
T. breindli Weiser, 1946	Chironomus thumi	L	Mid-gut
T. chironomi Jirovec, 1940	C. plumosus; C. thumi; Trichocladius sp.	L	Fat body
T. fibrata (Strickland, 1913) Debaisieux & Gastaldi, 1919	Simulium bracteatum; S. hirtipes; S. maculata; S. ochraceum; S. ornatum; S. venustum; S. sp.	L	Fat body
T. grassi Missiroli, 1929	Anopheles maculipennis	A	Egg, fat body
T. indica Kudo, 1929	A. hyrcanus	L	Fat body
T. legeri Hesse, 1904 (T. illinoisensis Kudo, 1921)	A. barbirostris; A. bifurcatus; A. crucians; A. fuliginosus; A. funestus; A. gambiae, A. hyrcanus; A. maculipennis; A. punctipennis; A. quadrimaculatus; A. ramsayi; A. subpictus	L,A	Fat body

Microsporidia	Hosts	Infected*	
		Stage	Organ, Tissue
T. minuta Kudo, 1924	*Culex leprincei*	L,P	Fat body muscle, ganglion
T. obesa Kudo, 1924	*Anopheles quadrimaculatus*	L	Fat body
T. obscura Kudo, 1929	*A. funestus*	L	-
T. opacita Kudo, 1922	*Culex* [illegible]; *C. territans*; *Aedes nemorosus*	L	Fat body
T. pinguis Hesse, 1903	*Tanypus varius*	L	Fat body
T. pyriformis Kudo, 1924	*Anopheles sp.*	L	Fat body
T. rotunda Kudo, 1924	*Culex leprincei*	L	Fat body
T. varians (Leger, 1897) Debaisieux, 1919	*Simulium ornatum*; *S. reptans*; *S. sp.*	L	Fat body
T. sp. Bresslau, 1919	*Culiseta annulata*	L	-
T. sp. Iturbe & Gonzalez, 1921	*Culex pipiens*	L	-
T. sp. Noller, 1920	*Aedes nemorosus*	L	Fat body
T. sp. Ross, 1906	*A. sp.*; *Culex fatigans*	A	Nerve chord
T. sp. Wenyon, 1926	*Aedes nemorosus*	L	Fat body
Stempellia magna Kudo, 1924 (*Thelohania magna* Kudo, 1921)	*Culex pipiens*; *C. territans*	L	Fat body
Plistophora chironomi Debaisieux, 1931	*Chironomus sp.* *Camptochironomus tentans*	L	Fat body
P. collessi Laird, 1959	*Culex tritaeniorhynchus*; *C. gelidus*	A	Egg follicles
P. culicis Weiser, 1947 (*P. kudoi* Weiser, 1946)	*Culex pipiens*; *Anopheles gambiae*; *A. stephensi*	L A	Malp. tubules Mal. tub., fat body
P. debaisieuxi Jirovec, 1943	*Simulium maculata*; *S. sp.*	L	Fat body
P. jiroveci Weiser, 1942	*Chironomus thumi*; *Prochironomus anomalus*; *Glyptotendipes sp.*	L	Fat body

Microsporidia	Hosts	Infected*	
		Stage	Organ, Tissue
P. simulii (L.& Sp., 1904) Deb.& Gast, 1919 (Thelohania multispora (Str. 1913))	Simulium bracteatum S. maculata; S. morsitans; S. ochraceum; S. venustum; S. vittatum; S. sp.	L	Fat body
P. stegomyiae (Marchoux, Salimbeni & Simond, 1903) Chatton, 1911	Aedes aegypti Anopheles gambiae; A. melas	L,A A	Various organs Various organs
P. thienemanni Weiser, 1943 (Thelohania chironomi Deb. 1919	Chironomus sp.	L	Gut
Coccospora micrococcus (Leger & Hesse, 1921) Kudo, 1925 Cocconema micrococcys L.&H.)	Tanypus setiger	L	Fat body
C. octospora (L. & H., 1921)	Tanytarsus sp.	L	Gut
C. polyspora (L. & H., 1921)	Tanypus sp.	L	Fat body
Mrazekia brevicauda (L. & H., 1916)	Chironomus plumosus; C. thumi; C. anthracinus	L	Fat body
Octosporea chironomi Weiser, 1943)	Camptochironomus tentans	L	Fat body
O. simulii Debaisieux, 1929	Simulium sp.	L	Gut
Bacillidium bacilliforme (L. & H., 1922) Jirovec, 1936	Orthocladius sp.; Chironomus sp., Endochironomus juncicola	L	Fat body
Toxoglugea chironomi Debaisieux, 1931	Chironomus sp.	L	Fat body
T. vibrio Leger and Hesse, 1922	Ceratopogon sp.	L	Fat body
Spiroglugea octospora Leger and Hesse, 1922	Ceratopogon sp.	L	Fat Body
Caudosporo simulii Weiser, 1946	Simulium latipes; S. Sp.,	L	Fat Body
*Stage: L - larva, A - adult P - pupa			

As noted above, the great majority of microsporidian parasites of the insects under consideration are capable of parasitizing species belonging to one genus; namely, they seem to be host genus specific. For example, *Thelohania legeri* is found to parasitize the larvae and less frequently adults of no less than 12 species of *Anopheles* in various regions of the world, but has not been found in the mosquitoes of other genera. This genus specificity is noticed even when *Anopheles* larvae, some of which were infected with microsporidian parasites, lived mingled with *Culex* larvae with their own parasites in the same body of water, there being no cross infection (Kudo, 1925). All *Culex* microsporidians seem to manifest a similar specificity except *Thelohania opacita*, which was originally found in two species of Culex in the United States, was reported later to occur also in a species of *Aedes* in Czechoslovakia (Weiser, 1946).

Recently Garnham (1946) and Canning (1957) reported the occurrence of a microsporidian which parasitized the epithelium of Malpighian tubules and adjacent fat bodies of 50 to 100 per cent of laboratory bred adult *Anopheles gamibiae* and *A. stephensi* in London. Canning maintains that it is probably the same as *Plistophora culicis* (Weiser, 1947) which parasitizes the cells of Malpighian tubules of the larvae of *Culex pipiens* (Weiser, 1946). Fox and Weiser (1959) found a microsporidian in various organs of 50% or more of laboratory bred adult *Anopheles gambiae* and *A. melas* in Liberia and considered it identical with *Plistophora stegomyiae* (Marchoux, Salimbeni & Simond) Chatton, which had been reported to parasitize the larvae and adults of *Aedes aegypti*. If these identifications are correct, it would indicate that certain microsporidian parasites are capable of infecting mosquitoes belonging to different genera.

The following list shows the host species, their microsporidian parasites and localities where infected hosts were found:

HOST INSECTS	MICROSPORIDIA	LOCALITIES
Mosquitoes:		
Aedes aegypti	Plistophoro stegomyiae Nosema aedis	Brazil Puerto Rico
A. calopus	Nosema lutzi	Brazil
A. cantans	N. sp. Noller	Germany
~~A. nemorosus~~	~~N. sp. Noller~~ Thelohania opacita T. sp. Noller	~~Germany~~ Czechoslovakia Germany
A. sp.	T. sp. (?) Ross	India
Culex fatigans	T. sp. (?) Ross	India
C. gelidus	Plistophora collessi	Malaya
C. leprincei	Thelohania minuta T. rotunda	U. S. A. U. S. A.
C. pipiens	Nosema culicis T. sp. Iturbe and Gonzalez Stempellia magna Plistophora culicis	Germany Venezuela U. S. A. Czechoslovakia
C. territans	Thelohania opacita Stempellia magna	U. S. A. U. S. A.
C. testaceus	Thelohania opacita	U. S. A.
C. tritaeniorhynchus	Plistophora collessi	Malaya
Culiseta annulatus	Thelohania sp. Bresslau	Germany
Anopheles barbirostris A. bifurcatus A. crucians	T. legeri T. legeri T. legeri	India France U. S. A.
A. fuliginosus	T. legeri	India

Mosquitoes: (Cont'd)		
A. funestus (A. varuna)	T. legeri	India
	T. obscura	India
A. gambiae	T. legeri	Zululand (Fantham et al)
	Plistophora culicis	England
	P. stegomyiae	Liberia
A. hyrcanus	Thelohania legeri	India
	T. indica	India
A. maculipennis	T. legeri	France
		Czechoslovakia
	T. grassi	Italy
A. melas	Plistophora stegomyiae	Liberia
A. punctipennis	Thelohania legeri	U. S. A., Canada
A. quadrimaculatus	T. legeri	U. S. A.
	T. obesa	U. S. A.
A. ramsayi	T. legeri	India
A. stephensi	Plistophora culicis	England
A. subpictus (A. rossi)	Thelohania legeri	India
A. sp.	T. pyriformis	U. S. A.
Midges:		
Chironomus anthracinus	Mrazekia brevicauda	Czechoslovakia
C. plumosus	Thelohania chironomi	Czechoslovakia
	Plistophora jiroveci	Czechoslovakia
	Mrazekia brevicauda	France, Germany
C. thumi	Nosema zavreli	Czechoslovakia
	Thelohania breindli	Czechoslovakia
	T. chironomi	Czechoslovakia
	Mrazekia brevicauda	Czechoslovakia
C. sp.	Plistophora thienemanni	Belgium
	P. chironomi	Belgium
	Toxoglugea chironomi	Belgium
	Bacillidium bacilliforme	France

Midges: (Cont'd)

Camptochironomus tentans	Plistophora chironomi Octosporea chironomi	Czechoslovakia Germany
Endochironomus juncicola	Bacillidium bacilliforme	Czechoslovakia
Prochironomus anomalus	Plistophora jiroveci	Czechoslovakia
Ceratopogon sp.	Toxoglugea vibrio Spiroglugea octospora	France
Glyptotendipes sp.	Plistophora jiroveci	Czechoslovakia
Tanypus setiger	Coccospora micrococcus	France
T. varius	Thelohania pinguis	France Czechoslovakia
T. sp.	Coccospora polyspora	France
Tanytarsus sp.	C. octospora	France
Trichocladius sp.	Thelohania chironomi	Czechoslovakia

Blackflies:

Simulium bracteatum	Thelohania bracteata T. fibrata Plistophora simulii	U. S. A. Canada U. S. A. U. S. A.
S. hirtipes	Thelohania bracteata T. fibrata	U. S. A. U. S. A.
S. latipes	Caudospora simulii	Czechoslovakia
S. maculata	Thelohania bracteata T. fibrata Plistophora debaisieuxi P. simulii	Belgium Belgium Belgium Belgium
S. morsitans	P. simulii	Czechoslovakia
S. ochraceum	Thelohania bracteata T. fibrata Plistophora simulii	Brazil Brazil Brazil
S. ornatum	Thelohania varians T. fibrata	France, Belgium Czechoslovakia

Blackflies: (Cont'd)		
S. reptans	*T. varians*	France, Belgium
S. venustum	*T. bracteata*	Brazil, Canada
	T. fibrata	Brazil, Canada
	Plistophora simulii	Brazil
S. vittatum	*P. simulii*	U. S. A.
S. sp.	*Nosema stricklandi*	Czechoslovakia
	Thelohania bracteata	Belgian Congo (Henrard)
	T. fibrata	Belgian Congo
	T. varians	Czechslovakia
	Plistophora debaisieuxi	Czechoslovakia
	P. simulii	Belgian Congo
	Octosporea simulii	Belgium
	Caudospora simulii	Czechslovakia
Eusimulium latipes	*Thelohania bracteata*	Czechoslovakia

Incidence of Microsporidian Infection

The incidence of infection in a natural population of the host insects is unknown in many species. The following data appear in published papers:

Microsporidia and Hosts	Number Examined	Infected		Observer
		Number	%	
Thelohania grassi				
in Anopheles maculipennis (adults)	216	3	1.4	Missiroli (1929)
Thelohania legeri				
in Anopheles maculipennis (larvae)	40	2	5	Hesse (1904)
A. punctipennis (larvae)	34	2	6	Kudo, 1921
A. punctipennis (larvae)	70	2	2.5	Fantham et all 1941
A. quadrimaculatus (larvae)	54	3	5.5	Kudo, 1922
A. quadrimaculatus (adults)	235	5	2.1	Kudo, 1925
A. quad., A. punct., A. crucian (larvae)	1450	54	3.7	Kudo, 1925
Thelohania opacita				
in Culex tetaceus (larvae)	51	3	5.9	Kudo, 1922
Stempellia magna				
in C. pipiens (larvae)	60	7	12	Kudo, 1921
C. territans (larvae)	290	43	15	Kudo, 1925
Plistophora collessi				
in C. tritaeniorhynchus (adults)	1000	11	1	Laird, 1959
Plistophora culicis				
in C. pipiens (larvae)	several hundreds	2		Weiser, 1946
A. gambiae (adults)			50-100	Canning, 1957
Plistophora stegomyiae				
in Aedes aegypti (adults)	300	40	13	Marchoux, et al, 1903
A. aegypti (adults)	200	3	1.5	
A. aegypti (larvae)	"less frequent"			
Anopheles gambiae (adults)	171	77	45	Fox & Weiser 1959
Thelohania bracteata				
in Simulium bracteatum and S. hirtipes (larvae)			Ca. 10	Strickland,1913
Thelohania fibrata				
in Simulium bracteatum and S. hirtipes (larvae)			Ca. 5	Strickland, 1913
Thelohania pinguis				
in Tanypus varius (larvae)	1000	2		Hesse, 1903

These data, though meager, reveal that the infection rate of microsporidian parasites among the insects under consideration is low. This is not difficult to understand, as the spores freed in water by disintegration of infected larvae, would disperse through disturbances in water caused by current or wind and soon sink to the bottom. Therefore, the host larvae will have little chance of ingesting them. In addition, there appears to be a close microsporidia host relationship for many species so that the chance of ingestion by host larvae of the spores of a microsporidian to which they are susceptible, will be small.

It seems probable that the size of the body of water where host larvae breed has a bearing on the incidence of infection. For example, 5.9% of *Culex* larvae collected from a slow flowing creek were infected by *Thelohania opacita*, while 12% of *Culex* larvae inhabiting a small pool and 15% of *Culex* larvae found in a small unused boat were infected by *Stempellia magna*. Thus the incidence of infection is higher in a dense population occupying a small area than in a sparse population.

The low incidence (2.5 to 5.5%) of *Thelohania legeri* infection among *Anopheles* larvae may be due in part to their surface feeding habit, since the fragment of dead larvae with the microspordian will sink to the bottom and will be unavailable to the larvae, unless there happens some obstruction such as a thick growth of filamentous algae which would hold up the infectious material to be ingested by healthy larvae. The two instances of extremely heavy microspordian infection with infection rates of 45% and 50 to 100%, among laboratory bred adult *Anopheles* are difficult to comprehend on the basis of information we have regarding Microsporidia.

The Effect of Microsporidian Infection Upon the Host

The fat body is the most common site of microsporidian infection. In case of a heavy localized or general infection, the infected fat bodies are completely filled with various developmental stages of the parasite and the host cells become distended and their nuclei undergo an extreme hypertrophy, a characteristic feature resulting from a microsporidian infection.

Heavily infected larvae are opaque white or yellowish in color by which they can be easily detected among the healthy or lightly infected larvae. They show also deformities of body of all sorts. They may diminish in size or become distended due to the pressure of enlarged fat bodies.

The activity of host larvae decrease visibly as a result of heavy infection. Even when the muscles are not infected, they appear not to be able to function normally. When uninfected and heavily infected larvae that are kept in a glass jar, go down as the surface water is disturbed, the infected larvae are usually slower in reaching the bottom than the uninfected ones, and when they come up to the surface sluggishly, they seem to have difficulty in orienting themselves. Several rearing experiments indicate that heavily parasitized host larvae cannot pupate and perish sooner or later.

In the case of light infection, the larvae appear to complete metamorphosis and emerge as infected adults, which seemingly disseminate the infectious spores in breeding places.

How the Microsporidian Infections Begin

It is generally believed that the infection begins with the entrance of mature spores into the digestive tract of a specific host animal. This has been experimentally established in a number of Microsporidia including Thelohania legeri and Stempellia magna. In addition in certain species such as Nosema bombycis, infected eggs give arise to young infected larvae. The great majority of microsporidian infections among the insects under consideration also take place through the entrance of spores into proper host insects. Three species are known to parasitize ovaries of mosquitoes. Marchoux and coworkers (1903) found that the eggs of Aëdes aegypti infected by a microsporidian, which they considered would produce infected larvae upon hatching. However, in the case of Plistophora collessi which parasitizes exclusively the ovaries of two species of Malayan Culex, Laird (1959) found that the infection resulted in the destruction of eggs and mature eggs were free from the parasite. He considered that "the spores are deposited in the larval habitat when infected females attempt to oviposit". A similar situation was noted in the laboratory bred Anopheles by Fox and Weiser (1959) who found no infected larvae, but as high as 45% infection among adults. The microsporidian attacks and completely destroys the eggs so that mature eggs are free from the microsporidian. The two observers supposed that the infection takes place in adult mosquitoes through contaminated water kept in the insectary. Thus germinal infection which would give rise to young infected larvae is not definitely known among these insects.

Conclusion

That the host larvae whose fat body is heavily infected by microsporidian parasites are unable to pupate and perish, seems to be fairly well established. Laird's and Fox and Weiser's observations point to ~~the reduction in number of eggs laid by mosquitoes, due to destruction~~ of eggs infected by microsporidian parasites. Unfortunately the information on microsporidia in the insects under consideration is still fragmentary and incomplete. In nature the incidence of infection seems to be low, which led some to remark that Microsporidia and host now exist in a state of static equilibrium. Why not then break up this state by mass production of microsporidian parasites thereby increasing the infection rate? But before practical application of these organisms is undertaken, experimental laboratory work and field observation and experimentation must be conducted in order to find more about host-parasite relationship, means of mass production of useful microsporidians, and ecological factors in relation to practical application.

PAPERS CITED

Bresslau, E., and Buschkiel, M. 1919. Parasiten der Stechmückenlarven. Biol. Zentralbl., 39:325-336.

Canning, E. U. 1957. On the occurrence of Plistophora culicis Weiser in Anopheles gambiae. Riv. Malariol., 36:39-50.

Chatton, E. 1911. Microsporidies considérées comme causes d'erreurs dans l'étude de cycle évolutif des trypanosomides chez les insectes. Bull. soc. path. exot., 4:662-664.

Debaisieux, P. 1919. Microsporidies parasites des larves de Simulium: Thelohania varians. La Cellule, 30:47-79.

1929. Etudes cytologiques sur quelques microsporidies. La Cellule, 38:387-450.

1931. Deux microsporidies nouvelles de larves de Chironomus. C. R. soc. biol., Paris, 107:913-915.

Debaisieux, P., and Gastaldi, L. 1919. Les microsporidies parasites des larves de Simulium. II. La Cellule, 30:187-213.

Fanthan, H. B., Porter, A., and Richardson, L. R. 1941. Some Microsporidia found in certain fishes and insects in eastern Canada. Parasitology, 33:186-208.

Fox, R. M., and Weiser, J. 1959. A microsporidian parasite of Anopheles gambiae in Liberia. J. Parasit., 45:21-30.

Garnham, P. C. C. 1956. Microsporidia in laboratory colonies of Anopheles. Bull. World Health Org., 15:845-847.

Henrard, C. 1930. Quelques protozoaires parasites des larves de Simulium congolais. Rev. Zool. Bot. Afr., 19:226-231.

Hesse, E. 1903. Sur la présence de microsporidie du genre Thelohania chez les insectes. C. R. acad. sci., Paris, 137:418-419.

1904. Thelohania legeri n. sp., microsporidie nouvelle, parasite des larves d'Anopheles maculipennis. C. R. soc. Biol., 57:570-571.

1904a. Sur le developpement de Thelohania legeri Hesse. C. R. soc. biol., 57:571-572.

Iturbe, J., and Gonzalez, E. 1921. Contribucion del Laboratorio Iturbe en el 3er Congress Venezolano de Medicine. 35 pp.

Jírovec, O. 1936. Studien ueber Mikrosporidien. Mem. Soc. Zool. Czech., 4:1-75.

1940. Zur Kenntnis der in Chironomidenlarven parasitierenden Microsporidien. Zool. Anz., 130:123-127.

1943. Revision der in Simulium-Larven parasitierenden Mikrosporidien. Zool. Anz., 142:173-179.

Kudo, R. R. 1921. Studies on Microsporidia with special reference to those parasitic in mosquitoes. J. Morph., 35:153-193.

1922. Studies on Microsporidia parasitic in mosquitoes. II. On the effect of the parasites upon the host body. J. Parasit., 8:70-77.

1924. A biologic and taxonomic study of the Microsporidia. Illinois Biol. Monogr., 9:76-344.

1924a. Studies on Microsporidia parasitic in mosquitoes. III. On Thelohania legeri Hesse (Th. illinoisensis Kudo). Arch. Protistenk., 49:147-162.

1924b. VI. On the development of Thelohania opacita, a culicine parasite. J. Parasit., 11:84-89.

1925. IV. Observations upon the Microsporidia found in the mosquitoes of Georgia, U. S. A. Centralbl. Bakt. I. Orig., 96:428-440.

1925a. V. Further observations upon Stempellia (Thelohania) magna Kudo, parasitic in Culex pipiens and C. territans. Biol. Bull., 98:112-127.

1925b. Microsporidia. Science 61:366.

1929. VII. Notes on Microsporidia of some Indian mosquitoes. Arch. Protistenk., 67:1-10.

1930. VIII. On a microsporidian, Nosema aedis nov. spec., parasitic in a larva of Aedes aegypti of Porto Rico. Arch. Protistenk., 48:23-38.

Laird, M. 1959. Malayan Protozoa. I. Plistophora collessi n. sp. (sporozoa: Microsporidia), an ovarian parasite of Singapore mosquitoes. J. Protozool., 6:37-45.

Léger, L. 1897. sur une nouvelle myxosporidié de la famille des Glugéidées. C. R. acad. sci., 125:260-262.

Léger, L., and Hesse, E. 1916. Mrazekia, genre nouveau de microsporidies à spores tubuleuses. C. R. soc. biol., 79:345-348.

Léger, L. and Hesse, E. 1921. Microsporidies à spores sphériques. C. R. acad. sci., 173-1419-1421.

1922. Microsporidies bactériformis et essai de systematique du groupe. C. R. acad. sci., 174:327-330.

Lutz, A., and Splendore, A. 1904. Ueber Pebrine und verwandte Mikrosporidien. Nachtrag zur ersten Mitteilung. Centralbl. Bakt. I. Orig., 36:645-650.

1908. Ueber Pebrine und verwandte Mikrosporidien. Zweite Mitteilung. Centralbl. Bakt. I. Orig., 46:311-315.

Marchoux, E., Salimbeni, A., and Simond, P. L. 1903. La fièvre jaune. Ann. Inst. Pasteur, 17:665-731.

Martini, E. 1920. Ueber Stechmücken besonders deren europäische Arten und ihre Bekämpfung. Beihefte Arch. Schiffs-Trop. Hyg., 24:1-267.

Missiroli A. 1929. Sui microsporidi parassiti dell 'Anopheles maculipennis. Riv. Malar., 8:393-400.

Nöller W. 1920. Kleine Beobachtungen an parasitischem Protozoen. Arch. Protistenk., 41:169-189.

Ross, R. 1906. Notes on the parasites of mosquitoes found in India between 1895 and 1899. J. Hyg., 6:101-108.

Strickland, E. H. 1913. Further observations on the parasites of Simulium larvae. J. Morph., 24:23-102.

Weiser, J. 1942. Zur Kenntnis der Mikrosporidien aus Chironomiden-Larven. Zool. Anz., 140:126-128.

1943. Zur Kenntnis der Mikrosporidien aus Chironomus-Larven. II Zool. Anz., 141:255-264.

1944. Zur Kenntnis der Mikrosporidien aus Chironomus-Larven. III. Zool. Ans., 145.

1946. The Mikrosporidia of insect larvae. Véstnik. Csl. Zool. Spol., 10:245-272.

1946a. The microsporidia from Chironomid larvae. Ibid., 10:273-292.

1947. Klič k určování mikrosporidii. Práce Morav. Přír. Spol., 18:1-64.

Wenyon, C. M. 1926. Protozoology. Vol. 1.

DR. STEINHAUS: Thank you, Dr. Kudo.

We have the usual problem of having a full schedule and trying to get it all accomplished in the time allotted. I know there is a great deal of interest in the subject Dr. Kudo discussed. I do want to bring it back for discussion. If not today, then tomorrow when we have our general discussion.

With Dr. Kudo's permission, we shall proceed with the next speaker, who will tell us about an increasingly interesting subject with regard to insect pathology; that is the nematodes pathogenic for insects.

Dr. Welch.

POTENTIALITIES OF NEMATODES IN THE BIOLOGICAL CONTROL OF INSECTS OF MEDICAL IMPORTANCE

H. E. Welch
Entomology Research Institute for Biological Control
Canada Department of Agriculture
Bellville, Ontario, Canada

Most of the 150 records of nematodes parasitizing insects of medical importance are mermithids in mosquitoes and black flies. These nematodes have much the same type of life history, reduce host activity during parasitism and kill the host upon emergence, and have, as yet, an undefined range of host specificity below the family level. Rates of parasitism vary widely, and black flies appear more generally attacked than mosquitoes. Most authorities consider nematodes of less importance than protozoa in the regulation of mosquito and black fly populations. Dutky's nematode, DD136, was tested in the laboratory against larvae of Aedes aegypti L., and found to kill the larvae in 36-48 hours at room temperature. Potentialities for the utilization of mermithids consist of introductions into new environments in much the same manner as insect parasites and predators, and in the dispersal of DD136, and closely related species of Neoaplectana in much the same manner as microbes are disseminated against insects.

* * *

Medical entomologists are well acquainted with the more noxious nematodes and the diseases that they cause but may not be aware of the existence of beneficial nematodes. These species, mainly primary parasites of insects, occur in nine families of the phylum and are associated in a wide variety of relationships with insects of most orders. Of the nine families, three, the Steinernematidae, Allantonematidae, and Mermithidae, are of chief concern as possible biological agents for their species usually kill the host, or at least reduce its reproductive potential.

The majority of the 150 host records of nematodes associated with insects of medical importance are species of the family Mermithidae. The taxonomy of this poorly known family has long been in a state of confusion. I have just completed the first draft of a taxonomic review of the group. and hope that this may solve some of the problems. Much confusion arises from the fact that the commonly encountered parasitic stages are immature and lack useful taxonomic characters. The existing taxonomy of the family is based on adult characters, and unless one is prepared to rear the larval mermithids until they mature it is impossible to provide specific, and in many cases, generic identification. Stiles (1903) sought to circumvent this by erecting an artificial group, Agamomermis, as a repository for species based on immature specimens. While it does serve a useful purpose, the group should be used with knowledge and caution.

Mermithid Parasites of Mosquitoes

Stiles description of an immature mermithid in Aedes sollicitans (Walk.) in New Jersey in 1903 is the earliest of some fifty host records in the literature. Mermithid parasites are recorded in species of Culex, Aedes, and Anopheles, and several minor genera. The records are world-wide with 13 from the Nearctic region, 5 from the Paleoarctic, 11 from the Ethiopian, 12 from the Oriental, and 4 from the Australian.

Only three of these records identify and name the mermithid. Some species were, and most could be, assigned to Agamomermis, and are probably immature stages of Hydromermis and Limnomermis. Welch (1960b) examined the specimens recorded from the Nearctic region and stated on the basis of his studies of Hydromermis churchillensis Welch, (1960a) that three species were involved. Laird (1956) reviewed the world literature and pointed out that at least four species were involved.

The life histories described by Iyengar (1928) and Welch (1960a) are similar and probably indicate the pattern for most other species. Freshly-hatched larvae, armed with spears, pierce the cuticle and enter the haemocoele of first and second instar larval hosts in single or multiple infections. The nematodes grow and eventually so fill the host body cavity that they coil around and through the organs. The nematodes usually emerge from the larval host just prior to the time when pupation would normally occur. Parasitism of and emergence from adult mosquitoes also occurs. Following emergence the larval nematodes become free-living in the bottom of the pool. They molt, become adults, mate, then the males die and the females oviposit.

All authors agree that host larvae die following the emergence of the worms. Death may result from bleeding or from damage to the organs from the extensive parasite movement during emergence. Welch (1960a) showed that parasitized larvae are less active than healthy ones, and Stabler (1952) established that the presence of the parasite inhibited histoblast formation and prevented pupation. Shakhov (1927) noted that infected adults were sterile, while Iyengar (1928) described their death following parasite emergence.

Evidence concerning host specificity is contradictory. Some data suggests specificity at the species and others at the generic level. Specificity may arise less from adaptation and immunological processes than from the concurrence of the hosts and parasites at a time when mermithids are seeking hosts. In several instances mermithids were found in culicids but not in other insects in the same environment so that these mermithids may be specific to mosquitoes. The problem awaits more adequate taxonomy and cross-infection experiments.

Iyengar's (1928) observation that "in some ponds mosquito larvae were parasitized to a large extent while in others the larvae were free" summarizes the findings of most investigators. This was true at Churchill, Manitoba, where parasites occurred in the larvae of one pool but not in those of a neighbouring pool, and pools infested in one year were not infested in the next. Comparison of temperature records, pH determinations, chemical analyses of the bottom, and water levels showed no significant differences between infested and uninfested pool populations.

Available data show that less than a third and usually near a tenth of the pools contain parasitized larvae. Therefore, no matter how high the percentage parasitism may be in a particualr pool, its effect is reduced by the fact that this pool is only one of the many from which the mosquitoes of the area emerge. Redistribution of parasites into previously unoccupied pools would be more beneficial than measures to increase the parasite's abundance in a particular pool.

The level of parasitism in pools where the parasite is present usually ranges from 30 to 50 percent, but may reach 80 percent. Percentage parasitism data should be accepted with caution, for infected larvae develop slower, and do not pupate. Thus their numbers tend to increase in proportion to the numbers of healthy larvae. This was true at Churchill where the apparent percentage parasitism rose from 25 to 60 percent in samples from one pool during the period of pupation.

Little opinion is given in the literature on the value of nematodes in the regulation of mosquito numbers. This is not surprising for there is little data on the value of any biological factor, the main interest and consequently importance having been attached to physical factors. Bates' view (1949) that predators are more important than parasites is supported by most authorities, though all admit that the quantitative basis for such statements is inadequate.

Among the parasites the protozoa appear more abundant and generally distributed than the mermithids. This was apparent at Churchill, where peritrichs were found in all larval populations, microsporidians in 75 percent, and nematodes in 40 percent of the pools in four study areas. The average infestation per pool of microsporidians was little more than that of nematodes so that the level of parasitism of microsporidians in each of the four study areas was not much greater than that of the nematodes.

Mermithid Parasites of the Simuliidae

At least 55 host records of mermithid parasites of simuliids occur in the world literature. The hosts include species of the genera Simulium, Prosimulium, Gymnopais, and Wilhelmina. The records are mainly from the northern hemisphere with 42 from North America, 10 from Europe, and 3 from the U.S.S.R. but one record from Sudan and another from Brazil suggest mermithid parasitism also occurs in tropical regions.

Two of the parasites were named but are inadequately described and must be considered species indeterminata. Most of the mermithids are probably, as in the case of the mosquito parasites, immature forms of species of the two aquatic genera Limnomermis and Hydromermis.

The life history of these mermithids is much the same as that described for mermithid parasites of mosquitoes. A freshly hatched larval mermithid penetrates the cuticle and enters the haemocoele of the host. A few authors suggested that the black fly becomes infected by consuming mermithid eggs. This is unlikely as this means of infection exists only in one terrestrial and rather abberant mermithid genus. The parasite develops and eventually emerges to begin a free-living existence in the bottom of the stream. Adults develop, mate, and oviposit. Parasitism occurs usually in all host stages, but may be restricted to only larvae.

When the mermithids emerge from their aquatic hosts they are carried a short distance dwonstream by the current. One would expect that after many generations the mermithid population would be moved beyond the black fly breeding region of the stream, but this does not happen. It seems probable that the parasitism of adult black flies by a certain proportion of the mermithid population provides the mechanism whereby the nematodes maintain themselves in the stream. Infected black flies are often found and probably transport the mermithids upstream. Grunin's observation (1949) of nematodes emerging from females in ovipositing flight illustrates how the cycle might be completed. This hypothesis may have some basis, as Dr. B. V. Peterson, Entomology Laboratory, Guelph, Canada, informs me in litt. that he finds higher incidence of mermithids in black flies that breed on dams or waterfalls, and fly upstream to oviposit than in those that breed away from dams or waterfalls.

Emergence of the mermithid causes the death of the larval and pupal host. Strickland (1911, 1913) observed that the presence of the parasite often prevents histoblast formation and host pupation. In black flies the mermithids destroy the gonads and leave cavities in the abdomen after emerging. Nocking and Pickering (1954) were the first to associate these cavities with parasite activity.

In surveying the literature one gains the impression that mermithids are found more often in black fly than in mosquito populations. This arises chiefly from the fact that most references are of general occurrences in an area rather than a specific occurrence in a place. This is true at Churchill where mermithid parasites occurred in all of the localities.

Percentage parasitism of larval hosts usually ranges from five to 30 percent, but may reach 80 percent. Adult host parasitism usually ranges from five to 20 percent with a few occurrences at 50 percent. Examination of black flies should include tallies of adults with large abdominal cavities. At Churchill such numbers revealed that parasitism was about 20 rather than 2 percent as shown by the acutal presence of worms.

Opinion varies on the value of biological agents in the natural control of balck fly populations. Cameron (1922) considered them minor compared to such physical factors as temperature and water level, whereas Rubtsov (1950) suggested that massive outbreaks of black flies occurred in those regions of Russian rivers where the topography of the river caused the absence of parasites.

More unanimity exists on the role of nematodes relative to other biological agents. Davies (1957) felt that nematodes ranked second to aquatic hydracarina, and Cameron (1922) suggested that invertebrate predators were more important. Strickland (1911), Sommerman et al. (1955), and Rubtsov (1950) placed mermithids second to protozoa in occurrence. This was true of S. venustum Say at Churchill, where microsporidians occurred more frequently than nematodes in larvae but less frequently in pupae or adults. If the parasitism percentages of all stages of the black fly population are totalled, the nematodes are of equal importance to the microsporidians. Furthermore it must be remembered that a single nematode infection is fatal to a black fly whereas multiple infection is necessary by the protozoa.

Nematodes and Bacteria in Insects

In 1955 Dutky and Hough reported the discovery of a nematode and an associated bacteria in the coddling moth. Their discovery stimulated considerable interest by entomologists in entomophilic nematodes, and refocussed attention on a rather unusual group of nematodes.

Dutky's nematode, named DD136, gains entrance to the haemocole of the host, and actively or passively releases the bacteria. The bacteria multiplies rapidly and soon kills the host. The nematodes feed in the cadaver, develop, mate, and reproduce several generations. Larval nematodes eventually leave the host carrying with them the bacteria.

Dutky's nematode is closely related to the eight species of the genus Neoaplectana. Some evidence suggests that most of these nematodes also have bacterial associates, and that theoretically it might be possible to chose a nematode and bacteria that were particularly suited for a given host.

Dutky developed techniques of rearing and handling the nematode, DD136, and succeeded in infecting many kinds of insects. In 1957 Dutky gave a culture of the nematode to the Belleville laboratory and it was tested against several Canadian pests.

In preliminary experiments the nematode was tested against larvae of Aedes aegypti L. in small dishes in the laboratory. When infected, the mosquitoes died within 24-36 hours at room temperature, while the nematodes take three to four more days to develop and to emerge from the host cadaver. Several experiments were conducted to determine what dosages of the nematode were necessary to cause both mosquito mortality, and to infect a proportion of the host with one male and one female per host so as to obtain reproduction. The LD_{50} for mosquito larvae at equivalent field densities of 800 to 5000 per square metre was found to range proportionately from 500,000 to 150,000 nematodes per square metre. At this dosage approximately 10 percent of the mosquitoes were infected with mating pairs that reproduced in the host cadaver. As a gravid female reproduces 300 to 400 larvae in a mosquito, these dosages would be sufficient to ensure establishment of the nematode. This work was conducted in smooth-bottomed containers and no provision made for rough surfaces such as leaf-strewn forest depressions, where the unevenness of the surface would decrease the chances of the grazing larvae encountering nematodes. Further laboratory studies and field trials will be conducted in 1960.

Oldham (1933) was the first to formally suggest the use of nematodes in biological control work. Since then other authors discussed the possibilities of their use, the most recent discussions being those of Theodorides (1950) and Welch (1958). Attempts to use nematodes were made only with Neoaplectana. Girth, McCoy and Glaser (1940) tested N. glaseri Steiner, 1929, against Japanese beetle in the New England states and obtained establishment of the parasite. Attempts to establish two species of Neoaplectana in New Zealand have not been successful (Dumbleton, 1945). In field experiments at Belleville with DD136 limited parasitism of the Colorado potato beetle and an economically significant reduction of the population of the cabbage root maggot were obtained. These experiments have shown that high moisture content in the environment, moderate temperatures, and high host populations are necessary for introduction and establishment.

Nematodes would seem to have high potentialities for use against mosquitoes and black flies, for ideal conditions seem to occur in the habitats of these insects. Much more knowledge should be obtained concerning their taxonomy, distribution, life history and behaviour, before extensive trials are made.

There are sufficient data to indicate along what lines the potentialities of nematodes may develop. The first family, the Mermithidae, resemble insect parasites. Infections usually involve a small number of parasites; there is no known method to mass-produce them efficiently and economically; they kill or sterilize the host more by their activity than by their number; each generation takes a relatively long period of time. Mermithids will probably be transferred from one country to another for biological control work in much the same manner as insect parasites. Control of the host will require considerable time.

The Neoaplectana on the other hand are more akin to microbes. They infect the host in large numbers; they can be mass-produced efficiently; they kill their host quickly by their numbers and interference in the physiological state of the host; they reproduce very quickly. They probably will be used in much the same manner as microbes are utilized at present. Control of the host will follow closely after release.

References Cited

Bates, M. 1949. The natural history of mosquitoes. The Macmillan Co., N. Y., pp. 1-379.

Cameron, A. E. 1922. The morphology and biology of a Canadian cattle-infesting black fly. Canada Dept. Agric. Tech. Bull. 5, n. s. p. 21.

Davies, D. M. 1957. Some parasites of Canandian Black flies (Diptera, Simuliidae). XVth Inter. Cong. of Zoology, Sect. VIII, Paper 3.

Dumbleton, L. J. 1945. Bacterial and nematode parasites of soil insects. New Zealand J. Sci. Technol. 27A: 76-81

Dutky, S. R., and W. S. Hough. 1955. Note on a parasitic nematode from the codling moth larvae, Carpocapsa pomonella (Lepidoptera, Olethreutidae). Proc. Ent. Soc. Wash. 57(5): 244.

Girth, H. B., E. E. McCoy, and R. W. Glaser. 1940. Field experiments with a nematode parasite of the Japanese beetle. New Jersey Dept. Agr. Circ. 317: 3-21.

Grunin, K. Y. 1949. (A mistake in instinct resulting from parasitic castration in Prosimulium hirtipes Fries (Diptera, Simuliidae)). Doklady Akad. Nauk S.S.S.R. (N.S.) 66(2): 305-307.

Hocking, B., and L. R. Pickering. 1954. Observations on the bionomics of some northern species of Simuliidae (Diptera). Canadian J. Zool. 32(2): 99-119.

Iyengar, M.O.T. 1928. Parasitic nematodes of Anopheles in Bengal. Far East. Assoc. of Trop. Med. Trans. 7th Congr. (Brit. India Calcutta) 3: 128-135.

Laird, M. 1956. Studies of mosquitoes and freshwater ecology in the South Pacific. Roy. Soc. of New Zealand Bull. 6: 213 pp.

Oldham, J. N. 1933. Helminths in the biological control of insect pests. Imp. Bur. Agr. Parasitol. Notes and Memoranda 9: 6 p.

Rubtsov, I. A. 1950. (Massive propagation of the black fly and its probable explanation.) Priroda Populiarnyi Estestuennoistoricheskii Zhurnal Izdau. Akad. Nauk S.S.S.R. (2): 16-22.

Shakhov, S. D. 1927. Über das Parasitieren von Agamomermis Stiles bei den Mücken Aëdes dorsalis Mg. und Aëdes cantans Mg. in der Umgegend der Stadt Charkov (Russian Test - German Summary). Russk. Entom. Obozr. 21(1-2): 27-32.

Stabler, R. M. 1952. Parasitism of mosquito larvae by mermithids (Nemotoda). J. Parasitol. 38(2): 130-132.

Stiles, C. W. 1903. A parasitic roundworm (Agamomermis culicis n.g., n.sp.) in American mosquitoes (Culex sollicitans). U.S. Pub. Health and Mar. Hosp. Serv. Hyg. Lab. Bull. 13: 15-17.

Strickland, E. H. 1911. Some parasites of Simulium larvae and their effects on the development of the host. Biol. Bull. 21: 302-338.

Strickland, E. H. 1913. Some parasites of Simulium larvae and their possible economic value. Canadian Ent. 45: 405-413.

Sommerman, K. M., R. I. Sailer, and C. O. Esselbaugh. 1955. Biology of Alaskan black flies (Simuliidae, Diptera). Ecol. Monogr. 25(4): 345-385.

Theodorides, J. 1950. Les nematodes dans la lutte biologique contre les insecte nuisibles. Bull. Analytique du C.N.R.S. Complement au tome 9 (1948) Fascicule II, 73-82.

Welch, H. E. 1958. A review of recent work on nematodes associated with insects with regard to their utilization as biological control agents. Proc. X Int. Congr. Ent. 4: 863-868.

Welch, H. E. 1960a. Hydromermis churchillensis n.sp. (Nematoda: Mermithidae) a parasite of Aedes communis (DeG.) from Churchill, Manitoba, with observations of its incidence and bionomics. Canadian J. Zool. In press.

Welch, H. E. 1960b. Notes on the identities of mermithid parasites of North American mosquitoes, and a redescription of Agamomermis culicis Stiles, 1903. Proc. Helm. Soc. Wash. In press.

DR. STEINHAUS: Thank you, Dr. Welch. There are so many things which could be discussed on the matters raised. However, I think we would be remiss if we did not use our remaining minute to hear Dr. Dutky's ideas on this general subject, in light of all the work he has done in this field.

DR. DUTKY: There are many nematodes, many of this some group. The organisms which are associated are different for each major group of nematodes. The DD136 nematode is capable of attacking in good numbers more than 130 different species of insects, occupying almost all the orders we have tested. It is particularly desirable, I think, to try it for some of these medically important insects. The tabanids, for example, spend almost a full year in the soil, and would be excellent targets for attack.

The control of mosquitoes, as Dr. Welch has pointed out, is a challenge to the nematode, because it is heavy and sinks to the bottom, so that it is then not in contact with the host.

We contemplate using one of the Sarcophaga as a host. It will yield about 60,000 nematodes per insect, or about a third of the yield we get from our present insect host. We have tremendous yields of a million and a half nematodes from a gram of insect tissue. In artificial culture we can achieve about one-fifth of this yield in the better cultures. Dr. Welch pointed out that the attack is a very rapid one. Some of the insect species are invaded by the nematode under certain conditions of population density inside a half-hour; death ensues within 16 or 20 hours. Some of the insects which are not killed for 24 hours will be prevented from feeding almost immediately; that is, they cease feeding within a few minutes after attack.

DR. STEINHAUS: Thank you very much.

We proceed now to what some people consider the more classical area of biological control; that is, the area having to do with insect parasites and predators. I am pleased to introduce Dr. Sailer for this discussion.

INSECT PARASITES AND PREDATORS OF MEDICALLY IMPORTANT ARTHROPODS

R. I. Sailer
Entomology Research Division
Agricultural Research Service, USDA

All insect pests, including those of medical importance, are attacked in one or more of their stages by one or more species of insect parasites or predators. Despite the very considerable amount of work on the classification and biology of entomophagous arthropods that has been accomplished, we have only a fraction of the information needed to make intelligent use of these organisms as counterpests. Each pest species must be studied with regard to potential ecological niches that it affords parasites or predators, and the latter organisms must be studied and evaluated in terms of their ability to occupy vacant or inefficiently utilized inches. In this way maximum biotic pressure can be brought to bear on pest species and their abundance reduced to the lowest possible levels.

As an example of an unoccupied niche, reference is made to the temporary pools in the arctic and subarctic that produce swarms of Aedes mosquitoes. It is possible that a careful inventory of the predaceous culicids belonging to the subfamily Chaoborinae would reveal species suited to live in this niche, for at present only 15 of the 75 known species have been found in North America. Toxorhynchites is cited as another group of culicids that are predaceous on mosquito wigglers and may be useful in temperate and tropical regions. At least 20 families of insects belonging to 7 orders include species that are known to prey on mosquito larvae, pupae or adults. The actual potential of this array of mosquito enemies for purposes of biological control cannot be fully assessed until the identity of all species is known and the individual habits and ecological requirements of each is defined.

*   *   *

Entomologists in search of lethal agents to use against medically important arthropods can scarcely hope to find a more numerous and versatile array than those to be found among the insects. It can be said with complete confidence that no arthropod vector of disease or pest species escapes attack by another arthropod in one or more of its life stages. In fact the great number of counterpests and their diversity of habits has been an obstacle to their rational use in pest control.

Little more is known about most of the species than the fact that they were found at a certain locality and observed to parasitize or prey on a pest species.

The entire complex of natural enemies of no pest has been thoroughly studied. Extensive exploration has successfully located surprisingly large numbers of parasitic and predaceous insects of potential use against crop pests. Nine years work by U.S.D.A. personnel in Japan and Korea on the oriental fruit moth (*Grapholitha molesta* Busck) yielded 50 species of parasites, of which at least half were primary in habit. Studies on the European corn borer (*Pyrausta nubilalis* (Hbn.)) in Europe, Japan, Korea, and Manchuria provided similar results with 24 primary species being discovered. In the case of the black scale (*Saessetia oleae* Bern) each instar, as well as the egg and adult stages, was found to serve as the primary host for one or more of 35 species of parasitic Hymenoptera. Of the 84 odd species associated with these three pests, 22 have actually been established in the United States - six on the corn borer, one on the oriental fruit moth, and 15 on the black scale. Of the established species only two have been outstandingly successful in reducing the abundance of their host. This is not a good record, and we can properly inquire why greater benefits have not been achieved.

In view of the effort expended to find parasites of the three above mentioned pests surprisingly little was learned about the biology and behavior of the beneficial species. In fact, it is only a fraction of what we should know if we are to make intelligent use of the impressive array of counterpests. In the case of some of the black scale parasites, there is evidence that strains originating from different localities have different host preferences and different tolerance to weather conditions. These intraspecific differences are highly significant for they suggest that by crossbreeding and selection, superiorly adapted strains could be developed that would be more effective than the wild parental stock.

It is also a recognized rule that few parasites are able to occupy the full range of their host. Where several species are competing for the same host they normally stake out a particular stage of the host or a geographic area and will be the dominant factor in controlling that segment of the host population.

If maximum benefits are to be obtained from a biological control program the entomologist must have as much information as possible about each member of a host-parasite complex. Without such information, his efforts can only be of the trial and error kind in which he reaches blindfolded into a bag and attempts to use whatever comes to hand. It is scarcely cause for surprise that a majority of the beneficial insects that have been introduced into the United States in the past 75 years have failed to become established and still fewer have succeeded in providing worthwhile control of their hosts.

Despite these handicaps there have been several highly successful introductions. Notable examples in which the host was controlled are the cottony-cushion scale (Icerya purchasi Mask.) by the vedalia Rodolia cardinalis (Muls.) imported from Australia; the citrus black fly (Aleurocanthus woglumi Ashby) in Cuba by Eretmocerus serius Silv. from Malaya and in Mexico by Prospallella clypealis Silv., P. opulenta Silv., and Amitus hesperidum Silv. all imported from Western India and Pakistan; the sugarcane planthopper (Perkinsiella saccharicida Kirkaldy) in Hawaii by Tytthus mundulus (Breddin) from Fiji; the Comstock mealybug (Pseudococcus comstocki Kuw.) in eastern U. S. by Allotropa burelli Mues., Pseudaphycus malinus Gahan and Clausenia purpurea Ishii from Japan; the satin moth (Stilpnotia salicis (L.)) in New England and Washington by Apanteles solitarius (Ratz.), Compsilura concinnata (Meig.) and Eupteromalus nidulans (Thoms.)

Many more examples, from many parts of the world could be named, and taken as a whole, undoubtedly place biological control far over on the credit side of the ledger. Strangely enough biocontrol has ~~not capitalized on this credit. No doubt the spectacular success of~~ DDT and later insecticides is largely responsible. However, it is also in part attributable to the nature of a successful biological control program. People soon forget that the problem ever existed, while with chemicals they may see a threatened loss and a spectacular control with every crop season.

It is also true that biological control projects have seldom been carried out in a way that permitted proper evaluation of results. Preliminary studies of host population dynamics needed to evaluate the impact of new natural enemies are largely lacking and follow-up studies are few. In fact, there is an inverse ratio between the success of a biological control project and the amount of effort likely to be expended in evaluating the results. Once a problem has been solved there is generally a feeling that evaluation studies should have lower priority than new studies needed to solve the pressing problems that seem to be queued up waiting their turn.

Biological control of medically important insects has been confronted by all these difficulties and others in addition. One problem stems from the fact that many, if not most, species having medical importance are indigenous species; whereas many of the most important crop pests are introduced and presumably live in a new home largely free from natural enemies that regulated their abundance in their old home ranges.

This does not mean that parasites or predators cannot be manipulated and effectively used against medically important insects. However, it may mean that biological control programs will require more detailed knowledge of both pest and counterpest than is needed for introduced crop pests and will demand more sustained effort to hold ground that is gained.

Biological control of medically important insects suffers another handicap that is less serious in the case of most crop pests. Many arthropod vectors of diseases are normally represented in endemic areas by populations so low that they will not sustain an introduced counterpest. Furthermore, because of emergencies arising from war or other disaster immediate control is often required. At the present time, this cannot be achieved through use of any insect parasite or predator. A long-term approach, perhaps coordinated with other forms of environment modification designed to reduce breeding sites, or adult shelter is necessary.

My contact with medical entomology has mostly been confined to the problem presented by Aedes in the arctic and subarctic regions. Here I have observed that predators such as chaoborines, dytiscids, corixids, and Odonata effectively exclude mosquitoes from some semi-permanent and most permanent pools (Sailer and Lienk 1954). Unquestionably these predators prevent serious troubles from mosquitoes in years when the temporary pools fail to persist long enough to produce the early developing species.

In Alaska the importance of these predators is further emphasized by a change that occurs, as one moves north, in the type of pool found to produce the early-season species of Aedes. Few of the predators normally attacking mosquito larvae are found in numbers beyond the tree line. In treeless tundra such temporary snow-melt pool species as Aedes communis (DeGeer) and A. punctor (Kirby) thrive in semi-permanent and permanent pools. Hence to attack the mosquito problem in Alaska by biological methods, it will be necessary to find predaceous species capable of living in these unoccupied niches. No doubt the same situation prevails in irrigated lands and the coastal areas of eastern United States.

It has been common knowledge since 1903 (Underwood 1903; Howard, Dyar, and Knab 1912) that many species of the culicid subfamily Chaoborinae are predaceous on mosquito wigglers. Yet prior to 1956, we had no clear idea of how many species occurred in North America or how they could be identified. Cook (1956), in his revision of the Nearctic species of this subfamily, summarized the available information of 15 species belonging to four genera. From his account it is evident that within the subfamily, individual species have different habitat requirements and that there is a great diversity of habitats represented when the entire group is considered. Furthermore, as Cook mentions in his introduction, additional species found in the rest of the world increases the number of known genera to seven and known species to 75. Little is known regarding the habitat requirements of these species, but enough is known to assure us that almost certainly there are species or adapted strains capable of living in some of the habitats in Alaska where tremendous numbers of arctic and subarctic Aedes are produced. For 57 years entomologists have known that some species of chaoborines destroy mosquitoes; yet this knowledge has not yet been put to any practical use.

In another group of predaceous culicids, the culicine genus Toxorhynchites, two species have been introduced into Samoa, Guam, and Fiji (Hoyt 1957) for control of Aedes polynesiensis, and into the Hawaiian Islands (Bonnet and Hu 1951, Weber 1955) for control of A. albopictus (Skuse). Here again there is evidence of great diversity of habitats, for there are 57 known species of Toxorhynchites and their distribution ranges from the tropics to Amur, Siberia (Stone, Knight, and Starke 1959).

Certainly the entomologist looking for means to kill mosquitoes will have no trouble finding them among the insects. At least 20 families belonging to seven orders include species predaceous on mosquito larvae, pupae, or adults (Hinman 1934). The problem is to obtain and organize the information about the potentially useful species. In medical entomology excellent progress has been made in making known our biological liabilities; now it is necessary to inventory our biological assets. Therefore, any concerted effort toward greater utilization of biological methods must place first emphasis on studies that will find and identify the potentially useful species and provide information about their ecological requirements. Until this information is available, control efforts through utilization of insect parasites or predators will not only be inefficient but will contribute little to our basic understanding of the factors that regulate the abundance of the pests and their counterpests.

Well-planned work, adequately supported and energetically executed, can be expected to solve or at least alleviate many problems, but no miracles should be expected. Anyone holding the view that biological agents provide a poor man's method of pest control is almost certain to be disappointed. The acquisition, storage, retrieval, and utilization of information essential for their successful use in controlling medically important insects will be costly, though perhaps no more so than the development of new insecticides to solve such problems as increasing resistance and residue hazards.

Biological control is a natural phenomenon constantly functioning around us, and there is no organism that would not soon become a problem if it were able to reproduce without the restraint of natural enemies. To depreciate the usefulness of natural enemies because they are not 100 percent effective, is tantamount to depreciating the value of law-enforcement agencies because of the lawless acts of a few hoodlums. Certainly insect parasites and predators play an important role in control of insects that transmit diseases and otherwise annoy man. Every effort should be made to use them more effectively.

Literature Cited

Bonnet, D.D., and Hu, S. M. K. 1951. The introduction of *Toxorhynchites brevipalpis* Theobold into the Territory of Hawaii. Hawaii Ent. Soc. Proc. 14(2): 237-242.

Cook, E.F. 1956. The Nearctic Chaoborinae (Diptera: Culicidae). Univ. Minnesota Agr. Expt. Station Tech. Bull. 218, 102 pp.

Hinman, E. H. 1934. Predators of the Culicidae. J. Trop. Med. and Hygiene. 37(9): 129-134; 37(10):145-150.

Howard, L.O., Dyar, H.G., and Knab, K. 1912. The mosquitoes of North and Central America and the West Indies. Carnegie Institution of Washington, Publ. 159, Vol. I pp 1-520.

Hoyt, C.P. 1957. Parasites and predators introduced into the Pacific Islands for the biological control of insects and other pests. South Pacific Commission Tech. Paper 101, Noumea, New Caledonia, 38 pp.

Sailer, R. I., and Lienk, S. 1954. Insect predators of mosquito larvae and pupae in Alaska. Mosquito News 14(1):14-16.

Stone, A., Knight, K.L., and Starke, H. 1959. A synoptic catalogue of the Mosquitoes of the World. (Diptera: Culicidae). Thomas Say Foundation, Vol. 6, 358 pp.

Weber, P.W. 1955. Recent liberations of beneficial insects in Hawaii - 4. Hawaii Ent. Soc. Proc. 15(3): 635-638.

Underwood, W.L. 1903. A new mosquito. Science, Vol. 18 n.s. 182-184.

DR. STEINHAUS: Thank you, Dr. Sailer. We shall proceed to the next talk, which will be concerned primarily with the Coelomomyces, by Dr. Marshall Laird.

COELOMOMYCES, AND THE BIOLOGICAL CONTROL OF MOSQUITOES [1]

Marshall Laird [2]

Much fundamental work remains to be done before we become able to manipulate pathogens and other biological control agents to wide advantage in the field of public health. Each individual ecological situation offers its own distinct problems, whether prejudicial or favourable to the successful establishment of introduced predators and parasites, and must therefore be well understood before such introductions are attempted. The present paper concerns mosquito parasites of the fungal genus *Coelomomyces*, and the planning of a pilot project in the South Pacific to test their usefulness in this form of control.

Coelomomyces

Eleven of the 21 recognizable species of *Coelomomyces* were described from material collected in Georgia during World War II (Couch, 1945; Couch and Dodge, 1947). Astonishingly little attention has been paid to them elsewhere in the U.S.A., and only one of them has been recorded from Canada. Six more were discovered in Australasia where two others first described from India and Malaya are also known to occur (Laird, 1956, 1959). The remaining two have only been reported from the type localities, in Russia and India. Two of the American species have been recognized in the Oriental Region, and there are several generic records from Africa and a solitary one from South America. Although at first sight it would thus seem that some extensive areas are conspicuously free from *Coelomomyces*, closer examination of the data discloses that they reveal to us not the geographical distribution of this genus, but merely that of the relatively few people who happen to have interested themselves in it.

---

[1]Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

[2]Associate Professor of Parasitology, McGill University. Member of the Expert Advisory Panel on Insecticides, World Health Organization.

Nevertheless, although a great deal remains to be learnt about these fungi, their virtual restriction to mosquito hosts is already well established, as is the fact that they cause the death of most infected larva before pupation. The single Russian and South American records concern a back swimmer (Hemiptera: Notonectidae) and a black fly (Diptera: Simuliidae) respectively, but with these exceptions Coelomomyces is known from the Culicidae alone. While some species parasitize a wide range of mosquitoes, others appear to have a high degree of host specificity. One of the latter is the genotype, Coelomomyces stegomyiae Keilin, the subject of the present project. There is a recent record of this species from Singapore Armigeres larvae sharing a tree hole habitat with infected Aedes (Stegomyia) albopictus Skuse (Laird, 1959), all other findings concerning members of the subgenus Stegomyia alone.

Pacific Islands as Sites for Biological Control Research

One of these findings was made on Rennel Island, British Solomon Islands Protectorate, which my wife and I visited during an anopheline survey conducted for the Royal New Zealand Air Force and the N. Z. Department of Scientific and Industrial Research in 1952-54. The primary subject of our assignment was the distribution of malaria and its vectors in the South Pacific, with particular reference to the investigation of ecological factors that might help to explain the absence of Anopheles from the islands east of the New Hebrides and south of Aneityum in that group. Rapidly expanding aviation in that part of the world had made it desirable to establish whether conditions are in fact favourable to anophelines in the extensive zone as yet free from these insects, as a background to disinsectization and airport insect control developments. Our studies, which took the form of a detailed investigation of larval habitat ecology in many islands on sides of the malaria perimeter, revealed no natural barriers whatsoever to the establishment of anophelines should these ever be accidentally transported beyond "Buxton's Line". They also highlighted some basic trends in the dispersal of mosquitoes in general, and other aquatic organisms, in Oceania.

Prominent among these trends is the progressive simplification in insular freshwater faunae and floras with increasing distance eastward from Indo-Australian sources. An insect order including numerous predators on mosquito larvae, the Hemiptera, furnishes a good illustration of this. Its families Naucoridae and Nepidae do not occur east of Queensland, Rhagovelia (Veliidae) drops out in the Solomons, and the Belostomatidae do not range beyond New Caledonia. The Hydrometridae and Corixidae reach their easterly limits in the Loyalties, the Pleidae and Mesoveliidae attaining theirs in the New

Hebrides and Samoa respectively. Of the three families ranging further afield, the Gerridae and Notonectidae are strong fliers, while minute veliids are subject to accidental transportation by man (Laird, 1956).

Faunal and floral impoverishment on islands reflects not only the presence of natural barriers to dispersal, but also habitat restriction. Isolated Pacific atolls, lacking streams, large natural ponds, and various other types of freshwater bodies, exemplify this.

Nukunono, one of the three atolls of the Tokelau group, some 300 miles north of Samoa, has no fresh water beyond that which accumulates in natural and artificial containers. The former include tree holes and coconut shells, the latter the palm-bole reservoirs (Fig. 1) termed "tungu" by the islanders, and metal drums. In these, the only mosquito of the atoll, Aëdes (Stegomyia) polynesiensis Marks, was found breeding in enormous numbers during a visit in June, 1953. It was observed that the larvae were neither subject to any form of arthropod predation, nor infected by harmful parasites or even epibionts common elsewhere.

Some 42 miles southeast of Nukunono and 53 miles northwest of it lie the other two islands of the Tokelaus, Fakaofo and Atafu. Approximate land areas are 1,350, 700 and 600 acres, each atoll consisting of many small islets strung out on a roughly circular reef. A. polynesiensis was the sole mosquito known from Atafu and Fakaofo too, and, as elsewhere in Polynesia, this insect is the vector of Wuchereria bancrofti throughout the group, the total population of which was approximately 1,600. Here, then, was an island group with a small-scale vector control problem awaiting a solution that, once found, might prove referable to numerous similar situations throughout Oceania. At that time, the insecticide resistance problem, and control difficulties arising from the non-selective nature of modern larvicides, were beginning to cause uneasiness among medical entomologists. It was accordingly suggested that advantage be taken of the remarkable freedom of Nukunono mosquitoes from parasites and selective predators, and of the group's isolation and limitation of fauna, flora, habitats and area, to plan field experiments with biological control agents. Coelomomyces stegomyiae was recommended as the subject of the pilot project.

The Pilot Project in the Tokelau Islands

There the matter rested until November, 1957, when the Expert Committee on Insecticides of the World Health Organization, at its eighth session in Geneva, recommended that "attention should be directed towards determining those factors in nature which are important in limiting the population of vector species" and declared that "although naturalistic control measures cannot be expected to give immediate widespread benefits, the continued development of resistance demands that this field should not be overlooked" (WHO, 1958). It had recently been announced that the New Zealand authorities were planning a solar eclipse expedition to the Tokelau Islands in September/October, 1958, in connexion with the International Geophysical Year, and it was accordingly requested that consideration to be given to integrating a small WHO expedition with this one in an attempt to establish Coelomomyces stegomyiae on Nukunono. In the meantime it had been possible to do a little work with this fungus at Singapore, while on the staff of the Department of Parasitology, University of Malaya, following the conclusion of the South Pacific assignment already discussed. My former colleague at Singapore, Dr. D.H. Colless, undertook to collect quantities of infective material, he and his field assistants devoting considerable time to this, and the necessary joint arrangements having been agreed to by the World Health Organization and the New Zealand Government we flew to Suva, Fiji, to accompany the IGY party to the Tokelaus at the beginning of September, 1958.

Our plan was to introduce Coelomomyces stegomyiae sporangia into the more permanent larval habitats of Aëdes polynesiensis at Nukunono, and to undertake a parallel experiment with an insecticide at Atafu, where dieldrin-cement briquettes (as developed by Dr. L. J. Bruce-Chwatt) would be placed in as many larval habitats as possible. Fakaofo would be left untreated as an experimental control, preliminary estimates of larval and adult abundance of Aëdes polynesiensis being made on all three atolls, the group thus being utilized as a great outdoor laboratory.

The fungal inoculum itself was recognized from the start as the weakest factor in the project as well as the key to its success. Although some laboratory infections had been achieved at Singapore, the mechanisms concerned were inadequately understood and culture techniques had not been developed. Nevertheless, faced with the prospect of a field experiment that would be both timely and comparatively inexpensive because of the special circumstances already detailed, it was felt that the risk of failure should be accepted. To cover as wide a range of possibilities as practicable, five batches of inoculum were prepared. Two of them consisted of bottom debris from laboratory containers in which the sporangia-rich remains of parasitized Aëdes albopictus larvae, brought in from the field, had been allowed to accumulate. Sediment from a tree hole from which infected larvae had been taken for several months (Fig. 2) formed batch no. 3, all this material being intermittently flooded and dried before use in order to stimulate the hatching of any insect eggs present. The remaining two batches were made up of the bodies of parasitized larvae individually dried on to pieces of filter paper, and living larvae exhibiting sporangia. Most of the latter batch died

Fig. 1

A tungu, or reservoir hollowed into the lower part of the bole of a coconut palm, at Nukunono. Note rainwater ducts.

Fig. 2

Collecting sediment from one of the natural sources of Coelomomyces stegomyiae at Singapore, prior to the Tokelau introduction prior to arrival at Nukunono, but their bodies yielded a rich concentration of what was hoped would prove infective material. Inoculum No. 5 did in fact produce infections in second and third instar Aëdes polynesiensis larvae kept under observation at the field laboratory. Although these died prematurely due to unsatisfactory rearing conditions, evidence was thus obtained of the susceptibility of the species to Coelomomyces stegomyiae -- the only such evidence procured during the three weeks spent at Nukunono. (Laird and Colless, 1959).

In these three weeks, all 45 islets of the atoll were systematically searched for larval habitats. Periodic microscopic examination of the inoculum proved that Coelomomyces sporangia remained plentiful until the end, by which time 761 larval habitats (tungu, other tree holes, old and well dried coconuts and bracts) had been seeded. None of the Aëdes polynesiensis larvae sampled throughout the operation proved to harbour any parasites other than the cosmopolitan and supposedly harmless protozoon, Lankesteria culicis (Ross), of high incidence in Stegomyia populations whereever it is searched for.

Population baseline estimates were made here as planned, also at Fakaofo during five days spent there and at Atafu in the course of a three-week stay. By the time we reached the latter island, at the end of September, a sufficient stock of dieldrin-cement briquettes had been prepared for us from Dr. Bruce-Chwatt's instructions by members of the eclipse party under the supervision of the district Officer, Mr. H. L. Webber. It was desired to obtain as high a kill as possible, and a briquette was placed in each larval habitat discovered, regardless of its permanence. In all, 6,500 breeding places (chiefly rat-gnawed coconuts and tree holes) were treated, in the hope that a perceptible reduction in the incidence of Aëdes polynesiensis might be obtained for comparison with any such reduction achieved by the fungus. It was confirmed that Aëdes polynesiensis is the sole mosquito present at Atafu also, but it should be mentioned that a second species widespread in the South Pacific but new to the group, Aëdes (Aëdimorphus) vexans nocturnus (Theobald), was found breeding in the pools and ditches of a taro patch on one of the islets of Fakaofo.

A return visit to the Tokelaus, for assessment purposes, is to take place within the next few months. However, thanks to New Zealand cooperation, an interim report has already been obtained. On November 10-11 last, Wing Commander J.W.G. McDougall, Deputy Director of Medical Services, Royal New Zealand Air Force, and Mr. G.W. Gibbs of Victoria University of Wellington, briefly visited all three atolls. Thirty-one water drums containing briquettes were located on the village islet of Atafu. None of these held larvae, in contrast to 25 of 49 similar containers without briquettes. A number of briquettes were taken away and forwarded to Dr. Colless at Singapore, who tested them against Aëdes albopictus larvae and was able to confirm that they were still 100% effective despite the fact that they had spent 13 months submerged in water. Also, an hour's work on an islet at Nukunono disclosed 11 larval habitats, specimens from two of which exhibited numerous sporangia of Coelomomyces stegomyiae -- an encouraging result in the light of the fact that the preliminary collecting at Singapore revealed parasitized Aëdes albopictus larvae in only 48 (2.0%) of 2,454 containers.

It is hoped that by the end of this project we will have learnt something of the handling of these fungi for biological control purposes, and that it may eventually be possible to use Coelomomyces where required to cause mortalities comparable with those reported from nature by Muspratt (1946) who observed that an undescribed species of the genus caused the death of 95 percent of Anopheles gambiae larvae hatching in a complex of pools in Rhodesia over a four-year period. The absence from the Tokelaus of selective predators on mosquitoes would, it is submitted, justify extending the scope of these studies to investigate still further "those factors in nature which are important in limiting the population of vector species". Candidate predators include a Brazilian crane fly, Sigmatomera shannoniana Alexander, the voracious larvae of which devour large numbers of larval mosquitoes in tree holes, and an emesine bug, Bagauda gilletti Miller, which haunts tree trunks and destroys Stegomyia adults as they leave their breeding places in Uganda (Laird and Colless, 1959).

Data derived from such field experiments under carefully controlled conditions, may prove not only of basic value but also of real benefit to all small and underdeveloped islands where filariasis poses the main mosquito control problem, the rate of this disease, the severity of individual cases and the occurrence of elephantiasis all being intimately linked with vector abundance.

Summary

Knowledge of these fungi, almost all members of which parasitize mosquitoes alone, is still very incomplete. The genus merits serious study by medical entomologists, its mosquito control potential having become evident almost twenty years ago when an undescribed species proved responsible for a sustained larval mortality rate of 95 percent in a Rhodesian Anopheles gambiae population. Sound fundamental understanding of the particular problem concerned is, of course, prerequisite to the profitable employment of Coelomomyces or any other biological control agent. Such understanding is likely to be more quickly obtained under insular conditions than continental ones. In the Pacific, progressive simplification of island freshwater faunae and floras accompanies increasing distance eastward from the Indo-Australian area. This trend reflects both

the occurrence of successive natural barriers to dispersal, and habitat restriction, the latter reaching its ultimate on Nukunono (one of the three atolls of the Tokelau Group) which is without fresh water beyond that accumulating in natural and artificial containers. These are utilized as breeding places by but one mosquito, the Wuchereria vector Aëdes polynesiensis. A visit in 1953 indicated that larvae of this insect throve there in freedom from pathogens or arthropod predators, and suggested that local conditions would favour experiments in the biological control of mosquitoes. The Tokelau Islands were thus chosen as the site of the World Health Organization's pilot project, the rationale and inception of which are outlined in this paper.

References Cited

Couch, J.N. 1945. Revision of the genus *Coelomomyces*, parasitic in insect larvae. J. Elisha Mitchell Sci. Soc. 61: 124-136.

Couch, J.N. & Dodge, H.R. 1947. Further observations on *Coelomomyces*, parasitic on mosquito larvae. J. Elisha Mitchell Sci. Soc. 63: 69-79.

Laird, M. 1956. Studies of mosquitoes and freshwater ecology in the South Pacific. Royal Soc. New Zealand Bull. No. 6: 213 pp.

Laird, M. 1959. Fungal parasites of mosquito larvae from the Oriental and Australian Regions, with a key to the genus *Coelomomyces* (Blastocladiales: Coelomomycetaceae). Can. J. Zool. 37: 781-791.

Laird, M. & Colless, D.H. 1959. Experiments towards the biological control of mosquitoes in the Tokelau Islands. First Report, August-October, 1958. World Health Organization. Mimeogr., 39 pp. (Restricted).

Muspratte, J. 1946. On *Coelomomyces* Fungi causing high mortality of *Anopheles* gambiae larvae in Rhodesia. Ann. Trop. Med. Parasitol. 40: 10-17.

WHO 1958. Insect resistance and vector control. (Eighth Report of the Expert Committee on Insecticides). Wld. Hlth Org. techn. Rep. Ser. No. 153, 67 pp.

DR. STEINHAUS: Thank you, Dr. Laird. I feel badly that we are not having time for discussion; let us hope that we shall have sufficient time for this tomorrow. I am pleased to present to you Dr. B. P. Beirne, speaking on "Biological-Control Research in Canada."

BIOLOGICAL CONTROL - RESEARCH IN CANADA

B. P. Beirne
Director, Entomology Research Institute for Biological Control
Research Branch, Canada Department of Agriculture, Belleville, Ontario

Abstract

In Canada biting flies are important because of their direct attacks, and not as disease carriers. Their natural enemies are being identified and their importance evaluated as a preliminary to the possible introduction of additional species from abroad. Mosquito larvae are attacked by many predators. Parasites are of some importance in control of tabanids, but natural enemies appear to be of lesser importance in control of black flies. Effects of sounds and atmospheric electricity on activity of adult mosquitoes, and of ultrasonics on the larvae, are under investigation. Competition between biting and non-biting species of mosquitoes should be investigated as a possible control method.

* * *

The insects of greatest medical importance in Canada are biting flies, and they are important because they bite and not because they carry human diseases. They are thus primarily a major nuisance or irritation, at times to an extreme degree in most parts of the country. To stop them from being a major irritation in a locality requires the virtual elimination there of the biting fly population. Biological control does not normally virtually eliminate a population: when successful, it reduces the population from one level of abundance to a lower level. With biting flies that are not disease carriers this does not solve the situation in so far as human well-being is concerned, though it may ameliorate it to some degree. Probably for this reason relatively little work is in progress in Canada on biological control of these insects.

The centre of research on biting flies in Canada is the Entomology Research Institute, Ottawa, of the Research Branch of the Canada Department of Agriculture. There ecology and behaviour are studied by J. A. Downes and his associates, and taxonomy by G. E. Shewell and others. Biting flies are investigated at several of the universities, notably by Professor B. Hocking at the University of Alberta, Professor D. M. Davies at McMaster University, and at certain Regional Research Stations or Laboratories, of the Research Branch, notably at Guelph, Ontario, Lethbridge, Alberta, and Saskatoon, Saskatchewan.

However, there appears to be only one full-time investigation in progress on the biological control of biting flies; by H. G. James at the Entomology Research Institute for Biological Control, Belleville. This is also an Institute of the Research Branch, Canada Department of Agriculture. In earlier work at Belleville, Baldwin, James, and Welch demonstrated, by radio-active tracer techniques, that predators can be important in causing decreases in populations of larval mosquitoes, and Welch worked out methods, to be published shortly, of estimating population sizes.

James is working out the identities and relative importance of the native natural enemies of biting flies. This work was started at Churchill, Manitoba, on various species but in recent years has been done in the Belleville district where it is concentrated on mosquitoes. The ultimate objective is to see if there are any situations or any stages in the lifecycle where these insects are relatively free from attack and where new natural enemies introduced from abroad might be effective supplements to the native species.

A considerable number of predators on the immature stages of mosquitoes were identified and studied and their importance evaluated. Twenty-seven species of predators were found in the Churchill area and 16 to date in the Belleville district. All the important species, however, apparently require food additional to immature mosquitoes, and, with one exception, the predacious larval stages of the more important predacious water-beetles do not coincide adequately with the presence of the aquatic stages of the mosquitoes. However, predators, notably the water-beetles, are important in the spring when they kill up to 40 percent of the immature stages of the mosquitoes in certain pools. They give greater control in permanent than in temporary pools.

In view of the ultimate objective of this work, we plan to initiate similar studies in Europe this summer, through the Commonwealth Institute of Biological Control.

Studies by James indicate that arthropod natural enemies are not significant control factors for black flies in the Churchill area. However, parasitic insects, especially egg-parasites, appear to be of some significance in the natural control of tabanids in both the Churchill and Belleville districts.

The primary research function of the Belleville Institute is not to investigate the natural enemies of particular pest species but to engage in basic research on principles of biological control. In research on principles we sometimes use biting flies as tools, and some results of this indicate lines of work related to the subject of this Conference that might be profitable to pursue further.

In work on effects of sounds on insects G. Wishart and D. F. Riordan analyzed the sounds that attract male mosquitoes to females, and identified and reproduced artificially the components that are significant in this respect. The fundamental is the only tone that produces good response, and it produces its effects even in the presence of considerable amounts of background noise. The males of *Aedes aegypti* respond to sine sounds of 300 to 800 cycles per second, with 500 to 550 the most effective; and they respond to favourable sounds of 34 to 119 decibels, with 68 decibels producing the greatest effect. By inserting an electrode in the Johnston's organ, the hearing organ of the mosquito, Wishart, VanSickle, and Riordan found that this organ acts as a true microphone that is only receptive in the range that causes response. Both sexes of the mosquito "hear" the same sounds, but only the male responds to them.

Mosquito larvae in water could be killed by ultrasonics of 40,000 cycles per second. Death results from rupture of the tracheae caused by sudden expansion of the enclosed air. Mature larvae and pupae are more resistant than young larvae. The ultrasonics also killed other aquatic organisms that contained free air within their bodies, but organisms that did not were relatively unaffected. The amount of power required and the short life of the transducers used appeared to rule out any practical application of ultrasonics for mosquito control with the equipment that we had available.

In investigations at Belleville on effects of atmospheric electricity on insects M. G. Maw found that electrical potentials of about 40 volts caused evasive flight patterns in both sexes of *Aedes aegypti*, but that potentials of not less that 150 volts were necessary to cause resting mosquitoes to fly. In the field electrical potentials differ at different times and between different kinds of plants. We might speculate on the possibility that, because of this, certain kinds of plants at most times and most kinds at certain times may either attract or repel mosquitoes seeking resting-sites. Information on this could have some practical application.

Of the work at Belleville that I have discussed, that by James on arthropod natural enemies as well as that by Welch on nematodes, will continue, but that with sounds and with static electricity will not necessarily continue with biting flies as tools.

In my opinion effective biological control attempts by manipulating natural enemies of biting flies usually will be both more difficult and less feasible than such attempts against agricultural and forestry pests. This is because, in general, biological factors may be of relatively much less importance than physical factors in the control of biting flies, and consequently more difficult to manipulate. The immature stages of these insects, and their natural enemies, usually inhabit water or water-logged situations and are thus subject to frequent and severe harmful changes that do not affect insects surrounded by air: the disappearance of their environment when it dries up; the appearance of harmful materials in suspension or solution; the reduction of useful materials in suspension or solution.

This is not an opinion that biological control of biting flies might be so difficult and ineffective as to be a waste of time. It is an opinion that, because effective control is likely to be difficult to achieve, broadened and intensified investigation is essential, not only in the more classic areas of biological control -- the use of natural enemies -- but from new viewpoints.

I have indicated possibilities in the use of sounds and ultrasonics and of static electricity, and in conclusion I will suggest that more attention be given to: the use of competition between species of biting flies whose immature stages have virtually identical habits and ecological requirements. Can we eliminate a species that bites man from a locality by introducing from another part of the world a species that does not bite man, that has virtually the same habits and ecological requirements and that, because of some advantageous attribute, successfully competes with and eliminates the biting species when the immature stages of the two occur together? The result would not be to reduce the total fly population of the locality but to change it from a pest, or biting population to a non-pest, or non-biting, population and thereby to eliminate it as a medical insect problem.

In conclusion I will reiterate what has been indicated by other speakers: we have only begun to explore possible ways of controlling insects of medical importance by biological means, and attention has centered on identification rather than evaluation. Much further exploration is necessary before we can say that we can have biological control measures that appear both practical and satisfactorily effective.

ACTIVITIES OF THE COMMONWEALTH INSTITUTE OF BIOLOGICAL CONTROL

F. J. Simmonds, Director,
Commonwealth Institute of Biological Control.
(Presented by Dr. Beirne)

Although the title of this paper would encompass the work of the Commonwealth Institute in the field of biological control in general, I think that in this instance it might be more to the point to confine my remarks to ways in which our Institute might be of value in connection with insects of medical importance.

There have been, in the past, relatively few attempts at biological control of insects of medical importance - for example the use of the fishes Gambusia and Lebistes against mosquito larvae, that of the predacious mosquito Megarhinus against mosquitoes in Fiji and elsewhere, and in the same islands the successful introduction of Pachylister sinensis against house flies that breed in animal droppings in the field. In addition there has been some tentative work done on the use of insect pathogens against mosquitoes. The natural enemies of a number of medical insects have been investigated in different areas - mosquitoes, Glossina spp., house flies, Simuliids, etc. However, it may certainly be claimed that the field of biological control in this regard has been comparatively neglected.

In general, in the last decade or more the control of a number of medically important insects, and of other insect pests, by means of chemical insecticides has been very spectacular, and the benefits derived from this mothod of control have quite obviously been enormous and far-reaching. However in the medical, as in the agricultural, field there is increasing realization that the use of these modern highly effective insecticides has created a number of additional problems - the most important in the present connection being perhaps the development of resistant strains of the noxious species, with a consequent increased difficulty in controlling them. It is quite obvious that the chemists, well aware of this situation, are developing and will continue to develop, insecticides of different types aimed at counteracting this problem of resistance.

However, this development, as well as a widespread concern as to the manner in which these modern insecticides may affect the general ecology of a number of insects, and of other species living in habitats treated with insecticides, has given rise to a very definite trend of thought that we may perhaps be over-emphasizing the value of chemical control, and that we should in fact look a little more deeply into the possibilities of natural control, or of manipulating the environment. This would include the introduction of additional natural enemies of the pest species in such a way as to favour all controlling factors and thus diminish the numbers of the noxious species - utilizing chemical methods only where absolutely necessary as an emergency measure and not as a routine procedure.

Such a trend of thought naturally raises the question as to whether biological control might not possible be utilized more effectively against some of these medical, as well as agricultural and forest, pests. Even if the successful introduction of predators, parasites and diseases of individual pests into certain areas might not of themselves give spectacular control, they might contribute something towards the control of the pest in conjunction with other factors.

For these reasons I think that it might be useful at this time to consider and in some detail, on a world-wide basis, to what extent biological control methods might be more fully explored in relation to insects of medical importance, even though what little ~~work has been done in the past in this field would indicate that~~ possibilities may be rather limited.

Our organization, the Commonwealth Institute of Biological Control, has carried out investigations in this general field with regard to agricultural and forest pests for many years - with several spectacular successes to its credit. We have not, however, been active in the field of medical entomology because we have not been requested to carry out such investigations by any of our contributing Governments.

In view of the success achieved by Pachylister against Musca in Fiji, I did try to colonize this predator in several West Indian islands, British Guiana, and Mauritius, but without success. Last year I was asked by Dr. Marshall Laird whether it would be possible to obtain material of a predacious Reduviid from Uganda and a predacious Tipulid from Brazil for trial against tree-hole mosquitoes in the Tokela Islands in the Pacific. We could, I think, have arranged to get this material, but the project must have collapsed since we heard nothing further about it.

I have recently been on tour through Pakistan, India, Ceylon, Malaya, New Guinea, etc., and was very impressed with the varying abundance of Musca spp. in different areas, and the fact that in some places where apparently conditions appeared favourable for rapid multiplication of the flies there were, in fact, very few present. It seemed possible that this might be due at least in part to natural control, and it appeared to be worth while pursuing further - if funds could be found to develop this line of research.

Whilst in Hawaii I saw the work being done there on the biological control of water-snails, which it seems to me might be developed considerably in other areas in view of the importance of a number of species in this group as secondary hosts for organisms of medical and veterinary importance.

Having sketched very briefly the general type of possibilities for biological control in this field I may outline the organization and facilities afforded by the Commonwealth Institute of Biological Control, to give some idea of the opportunities which this Institute affords for research in this field - and in this instance, of course, in that of insects of medical importance.

The Commonwealth Institute of Biological Control has developed gradually, from small beginnings as the Farnham House Laboratory of the Commonwealth Institute of Entomology, which laboratory was established in England in 1927. Its present organization consists of headquarters in Ottawa, Canada (but this is little more than an office and library) and five Stations where active research is carried out. These are the European Station at Delemont, Switzerland, the West Indian Station in Trinidad, the Californian Station at Fontana, the Indian Station at Bangalore in South India and the Pakistan Station at Rawalpindi, West Pakistan. At each of these Stations there are entomologists and adequate laboratory facilities for carrying out research work into biological control problems - some 24 projects over a wide field in 1958 - and from these Stations members of the staff make exploratory and investigational trips to different areas to study various pests and their natural enemies. If results warrant, sub-stations of a temporary nature (which may be several years) are set up. After general studies, selected species of parasites and predators are collected in the field or bred in the laboratory for shipment to the country where the particular pest occurs and where biological control is required. There supervised releases of natural enemies are made in attempts to establish particular biological control agents in the new environment, followed as far as the project will allow by an assessment of the value of the parasite or predator introduction.

This work is financed in part by annual contributions made to the general Commonwealth Agricultural Bureaux organization by all British Commonwealth countries. The size of these contributions and the general work of the organization is reviewed at a Commonwealth Conference held every five years. These contributions meet the basic expenses of the organization and are sufficient to keep it in existence. When a definite biological control investigation is undertaken for any Commonwealth country additional funds have to be provided by that country to cover the expenses incurred specifically in connection with that project - travelling, equipment, labor etc. The possibility of utilizing the Institute has now been extended to countries outside the British Commonwealth, and also to international bodies such as F.A.O., and W.H.O., with the proviso that the projects undertaken do not interfere with work for the Commonwealth.

Since many or most of the insect problems of medical importance are of world-wide interest, or at any rate affect a number of different countries, it would seem to me that if it were considered worth while attempting the biological control of some of these species our Institute might prove most useful, with its strategically situated Stations in different countries. It would seem worth while carrying out investigations in as many areas as possible to gain the maximum knowledge of the possibilities of biological control, even where a priori these appear to be unpromising - house flies, a number of species of mosquitoes, Simuliids, Culicoids, certain Reduviids, tse-tse flies etc., with a beginning perhaps in the preparation of detailed memoranda on prevoius work, and present possibilities, in connection with each species.

I should be very glad to assist in any way possible in this regard if it is felt by this Conference that biological control of insects of medical importance warrants further investigation - by means of parasites, predators or micro-organisms.

I sincerely hope that some work will be done in this field, perhaps initiated or encouraged by this group, in a sustained effort to ascertain why it is that in some of these places there is such a low fly population. Sanitation must play a part, because during the breakdown of normal sanitary practices in World War II there were far more house flies, and a year or so after the War their population was low again.

DR. STEINHAUS: Thank you, gentlemen, for your remarks. The title of the next talk appears to me to be somewhat deceptive; and an example of modesty. Because of the very exciting work Dr. Lindquist and his associates have been doing, I feel that the title "New Aspects of Biological Control" is much to mundane for such highly exciting and important work. Dr. Lindquist!

NEW ASPECTS OF BIOLOGICAL CONTROL

A. W. Lindquist
Entomology Research Division, Agricultural Research Service, USDA,
Beltsville, Md.

Abstract

Increase of resistance to insecticides and growing concern over residues in meat, milk, and plants have stimulated entomologists to seek new methods of controlling arthropod enemies of man and animals. An exciting new approach is the sterile-male technique, by which reared males are rendered sexually sterile and released to mate with native females, thus destroying reproductive potential.

Recent successful use of this method in eradicating the screw-worm from the Southeast followed a research program directed to studies on the ecology, habits, and incidence of this pest and the development of artificial larval media for rearing them. Sterilization studies were begun in about 1950; these demonstrated the amount of radiation necessary and the optimum time for treatment. Research on extending the sterile-male technique to other insects, following the announcement in 1954 of the successful eradication of the screw-worm from Curacao, has been slow. However, studies have now been initiated with the Mediterranean and oriental fruit flies, white pine weevils, common malaria mosquitoes, boll weevils, codling moths, European corn borers, and tsetse flies to determine if the method is useful and practical.

Chemical sterilants are also being considered. In theory, these could be incorporated in a bait and distributed to render males and females sterile without the necessity of laboratory rearing in large numbers. Recent studies have shown that a single feeding of amethopterin prevents oviposition of house flies.

Other methods include release of inferior genetic material and use of chemicals to interfere with normal development.

* * *

The increase of insecticide resistance in insects and the growing concern over residues in meat, milk, and plants have stimulated entomologists to seek new methods of controlling arthropod enemies of man and animals. An exciting new approach to insect control is the sterile-male technique. This method of biological control consists in releasing sterile males, which mate with native females and destroy their ability to reproduce. The recent successful use of this method in eradication of the screw-worm from the Southeastern States marks one of the most important advances in man's struggle to combat insect pests.

This eradication project followed a research program, including a few small-scale field trials, that had been conducted over several years. It was in August 1957 that the U.S. Department of Agriculture joined with the State of Florida in providing funds for screw-worm eradication. The U.S.D.A. Animal Disease Eradication Division was charged with the administrative responsibility of the project, and the Florida Livestock Board carried out the State's obligations. Because of the complexity of the program, several entomologists from the Entomology Research Division were asked to assist with its technical direction.

An enormous rearing facility was completed in July 1958. Production was aimed at having 50 million screw-worm flies for release each week. The flies were released systematically in special cardboard containers over the entire State of Florida and parts of Georgia and Alabama. After about 7 months it was impossible to find any screw-worm cases or adult flies of the species in Florida, Georgia, and Alabama. Release of insects was stopped on November 14, 1959, and none have been found since that time. Therefore, it is concluded that the species is indeed eradicated. It is possible that it might be re-introduced into the Southeastern States, especially Florida, from infested areas in the Southwest, but a tight quarantine of livestock shipments is enforced along the Mississippi River.

Let us review briefly the research that made this eradication possible. The sterile-male method did not suddenly appear as a complete, ready-to-apply technique, as one might suppose from reading popular articles on the subject. Many people had worked on the biology and ecology of this pest since about 1915, but it was not until about 1938 that E. F. Knipling (Lindquist 1955) began considering the possibility of controlling it by releasing sexually sterile flies in a natural environment. In 1955 Dr. Knipling elaborated on research pertaining to the sterility method. The research information available at that time indicated that in parts of Texas the number of flies per square mile was in the order of a few hundred rather than thousands as had been supposed. These small numbers suggested to him that it might be practical to rear and release more flies than existed in nature, at least in selected areas. Other biological and ecological information also supported his theory that sterile males could be used to control or eradicate this pest.

Actual sterilization experiments were begun in about 1950. Bushland and Hopkins (1951, 1953) found the optimum time for sterilizing the screw-worm to be 5 to 6 days after pupation at 80°F., or 2 to 3 days before adult emergence. A dose of 2500 r caused sterility of males and 5000 r caused sterility of females without interfering with their normal behavior. At first X-rays were used, but later it was found that gamma rays produced by cobalt-60 provided a more practical means of sterilizing the numbers of insects needed. Gradually other information was obtained that had an important bearing on the problem. For rearing such obligatory parasites in large numbers an artificial medium had to be developed. An important early finding was that a mixture of ground lean beef, blood, and a small amount of formaldehyde provided a satisfactory medium, which was kept at approximately 100°F. (Melvin and Bushland 1940). This was modified in 1957 so as to be practical for large-scale operations. (Graham and Dudley 1959). It was also found that the screw-worm females mate only once, which seemed to favor use of the method. However, it should be pointed out that single matings are not prerequisite to the successful use of the sterile-male method, at least in laboratory experiments. Theoretically, it is possible for multiple-mating species to be used, since a population containing ten times as many sterile as normal males gives an advantage to the steriles.

A great deal of research conducted on the possible use of this technique against the Mediterranean and oriental fruit flies has led to the initiation of field experiments with these flies. The U.S.D.A. has announced that island tests will be conducted near Guam in cooperation with the Navy and the Trust Territories. It is planned to rear and sterilize about 3 million fruit flies each week for distribution by airplane over the island selected.

Jaynes and Godwin (1957) studied the sexual sterilization of multiple-mating white-pine weevils by exposing them to various levels of gamma radiation. They found that normal females mated with sterile males produced infertile eggs but subsequent matings of these females with normal males resulted in 81 percent egg fertility. The normal males could in this way nullify insemination by irradiated males. However, in this experiment the sexes were equal in number and a high preponderance of sterile to normal males might have produced more favorable results.

Laboratory and small-cage tests have shown that the common malaria mosquito responds to irradiation and mating in a manner similar to that of the screw-worm. The laboratory work was so promising that it was decided last fall to test the practicality of rearing and releasing sterile males over a small area. A 5-square-mile peninsula on the south side of Lake Okeechobee, in Florida, was selected as a test site. Releases were made at a rate of about 1500-

2000 males per square mile per week, which should exceed by several times the normal population. Checking the results of the releases has proved to be exceedingly difficult. One of the methods employed is the collection of females from resting stations and giving them an opportunity to deposit eggs in small vials containing a few milliliters of water. Failure of the eggs to hatch or the larva to develop was believed to indicate a mating with a released sterile male. The experiment has not yet been continued long enough to determine whether the method of control will work or is practical.

The paucity of information on the habits of this mosquito under field conditions has hampered work in the release experiment. An understanding of the normal situation in the field is of great importance and often is absolutely vital to designing tests and to determine if the method is practical. For example, how far do released male mosquitoes migrate in search of females and how effective are they in mating behavior? What time of day does mating usually occur and under what conditions? This could be critical in relation to time of day of releases. Does mating occur near emergence sites or in some special ecological type of situation? This again has a bearing on where release of sterile males will be most effective.

Preliminary research on the effects of gamma radiation and the probable use of the sterile-male method has been conducted on several other insects, such as the boll weevil, the codling moth, the European corn borer, and the ststse fly, but none of the work has advanced to a field-test stage. Furthermore, the method may never be practical against these and other insects for a variety of reasons.

It is strange that so little attention has been given to extending the sterile-male technique to other insects, since the successful eradication of the screw-worm from Curacao was announced in 1954. One would expect that research entomologists would have given immediate attention to exploratory investigations on many different insect pests. Among the reasons for the slow progress have been insufficient funds and incomplete understanding of the entire problem. With any insect one naturally appraises the situation in terms of requirements that must be met if the technique can be used with any degree of practicality. In most instances much imaginative planning and intensive research will be required to explore possible use of the method. The requirements to consider seem to be rather severe and have been mentioned many times in various publications but they will be reviewed again.

(1) An economical method of rearing large numbers of insects must be available, or at least the problem must be reasonably amenable to development.

(2) The insect must be of a type that can be readily dispersed by aircraft and other means, and the males must have the ability to

search effectively for the opposite sex and mate in competition with native males.

(3) The sterilizing procedure must not adversely affect mating behavior or injure the males appreciably.

(4) The species to be controlled must have a comparatively low population or be subject to reduction by insecticide or other means. Advantage may be taken of seasonal fluctuations, since most insects are less numerous at some seasons of the year than others.

(5) The area to be treated must be reasonably protected against re-infestation, preferably isolated by water, mountains, or other barriers.

(6) The males to be released must not be harmful to man, animals, or plants.

(7) Mechanical and low-cost means of separating the sexes before release must be available in cases where the female is harmful. For example, to release female mosquitoes in large numbers would create an intolerable condition for man and animals within the area. This problem could be exceedingly difficult, although progress has been made in mechanically separating mosquito pupae.

(8) A thorough knowledge of the habits and ecology of the insect is essential. This will include the number of annual generations, the length of the various stages under different conditions, the rate of emergence in natural habitats, the distribution of emerged broods, when and where mating occurs, the population per unit area and other factors. We know very little regarding the incidence of an insect per unit area. Most assessments of population density are on the basis of rates of incidence rather than total numbers. A great deal of careful research is needed and it may be found that total numbers, especially at the low level of annual incidence, are not nearly as great as supposed. For example, the Anopheles quadrimaculatus adult population may be about 200-400 per square mile of the land area in Florida. These figures are based on estimates of numbers of adults in resting stations and are to be considered guesses rather than an enlightened estimate.

A great deal of effort should be directed to radiation effects on different species of insects. One must know the best time to irradiate and the best conditions under which to expose different stages to radiation. For example, recent work on the screw-worm indicates that anoxia, or absence of oxygen, may influence the amount of radiation necessary to cause sterility. The influence of temperature during radiation may be highly important. Perhaps other gases, other chemicals, or physical conditions may affect somatic as well as genetic tissues. With the screw-worm we were fortunate that a lack of knowledge of radiation effects did not hamper our progress. It is now known that several plant-feeding insects have not responded favorably to irradiation. The boll weevil, for example, is injured greatly by an exposure that will cause sexual sterility. A knowledge of radiation effects, therefore, may help in devising ways of exposure without harmful effects other than sterility. In order to do this basic type of research much expensive equipment is required, such as a good gamma ray source.

We are certain that the gamma-induced sterility technique will not be applicable to all insects or in all situations. However, it may have wider application than we now believe. The method is spoken of as an eradication tool, but it may also have application as a temporary or possible annual type of control in some situations--for example where the mosquitoes causing trouble are short-range fliers and never move more than 2 or 3 miles from the source of breeding. A community having a disease problem might be protected by utilizing the sterile-male technique to either eradicate or bring the population down to nondangerous levels. If an area contains an estimated 1 million mosquitoes, half of which are females, perhaps only 5-10 million sterile males would need to be released per 7-14 days to depress the population. Of course, all of this is conjecture at this time, but certainly research should be directed to exploring the possibilities.

It has been known for many years that certain chemicals cause sexual sterility in insects, or at least prevent ovarian development. Recently, in view of the threats of resistance to insecticides and residues in meat, milk, and food crops, attention has been given to the finding of chemicals that have a sterilizing effect. In theory, chemical sterilants that could be incorporated into a bait -- for example, house fly bait -- and make both sexes sterile would provide an ideal method of control. The sterilized insects would act as carriers of a factor preventing the development of progeny from matings between normal field females and sterile males. This method of control has interesting possibilities, but of course safe and effective chemicals must be found.

Another approach is the use of chemicals that would interfere with normal development of immature stages of insects. The effects might include stunting of larvae, failure or delay in pupation, mortality of pupae, delayed adult emergence, and deformity of pupae and adults. Publicity has been given to the powerful effects of the so-called juvenile hormone of the Cecropia moth which prevents insects from moulting.

About a year ago our Orlando, Florida, laboratory started a screening program to find compounds having a sterilizing or growthinhibiting effect on the house fly. Our fruit fly laboratories in Mexico City and Hawaii are engaged in a similar screening program.

At Orlando approximately 300 compounds have been evaluated, and some of them have biological effects that are of interest. One of them, Methotrexate, prevents house flies from ovipositing but has no effect on the males. A single feeding of this compound at a concentration of 0.5 percent in sugar baits prevents oviposition. Oviposition by flies maintained in a room with a choice of treated and untreated food was only 2 percent as high as that by flies given only untreated food. However, males are not sterilized, and it seems that the full effectiveness of this method cannot be reached unless males are also sterile. Furthermore, the safety to man and animals or any compound used in this manner must be of a high order.

Another possible approach to the sterile male technique is that of rearing and releasing strains of insects having a high percentage of males. If a laboratory strain were available that would produce a high percentage of males and they would mate with normal field strains of females, the efficiency of rearing large numbers of males would be considerably improved. It has been reported that crosses between certain insect strains produce about 90-95 percent males.

If the characteristics of high male productivity is genetically controlled, one could expect the first generation, at least, resulting from crosses with normal females, to reduce the number of females within the wild population. If the characteristic is dominant, the effects on a normal population could be great, especially if the aberrant strain is released repeatedly in numbers exceeding the normal population.

Dr. Knipling (in press) has proposed the development of inferior strains for distribution in a natural population. The inferior strain might be easily reared under controlled conditions and, in the adult stage in nature, survive long enough to add its inferior genetic material to the native population. Some of the useful genetic deficiencies might include inability to diapause, inability to fly while retaining ability to walk or crawl, and deficiencies in immature stages such as deformed mouth parts. As far as is known, very little research has been done on the development of insect strains with one or more deficiencies that could be used in a practical way to destroy native populations. It might prove easier to select a strain for characteristics that would prove inferior to the species in its natural habitat, than to produce a strain with superior characteristics such as insecticide resistance. It would be especially easy to select the inferior strain if this inferiority were coupled with increased ease of laboratory culturing.

Literature Cited

Bushland, R.C., and Hopkins, D.E. 1951. Experiments with screw-worm flies sterilized by X-rays. J. Econ. Ent. 44:725-731.

Bushland, R.C., and Hopkins, D.E. 1953. Sterilization of screw-worm flies with X-rays and gamma rays. J. Econ. Ent. 46:648-656.

Graham, A.J., and Dudley, F.H. 1959. Culture methods for mass rearing of screw-worm larvae. J. Econ. Ent. 52:1006-1008.

Jaynes, H.A., and Godwin, P.A. 1957. Sterilization of the white-pine weevil with gamma radiation. J. Econ. Ent. 50:393-395.

Knipling, E.F. 1955. The possibilities of insect control or eradication through the use of sexually sterile males. J. Econ. Ent. 48:459-462.

Knipling, E.F. Use of insects for their own destruction. J. Econ. Ent. 53:415-420.

Lindquist, A.W. 1955. The use of gamma radiation for control or eradication of the screw-worm. J. Econ. Ent. 48:467-469.

Melvin, R. and Bushland, R.C. 1940. Nutritional requirements of screw-worm larvae. J. Econ. Ent. 33:850-852.

DR. STEINHAUS: Thank you very much, Dr. Lindquist.

DR. JENKINS: Are there any important parasites or predators of the screw-worm fly?

DR. LINDQUIST: As far as we have been able to determine--and there has been quite a bit of work on that--there are no parasites of the screw-worm. There are predators (ants, for example) which will take the larvae and pupae when they fall to the ground.

DR. WILLIAMS: What difference in the likelihood of success would there be in releasing sterile males among an insect population which copulates only once during the lifetime, versus one which copulates many times?

DR. LINDQUIST: We have been giving a lot of thought to this and have quite a bit of experimental work. The screw-worm female mates only once and it was thought at first that the sterile male technique would only work with species which mate but once. However, that is not necessarily true since, in the multiple mating species, if you release sterile males at a ratio of ten to one, you can reduce the population. There is something else which is very important. What happens if a normal male mates with a female and then, a little while later, a sterile male mates with that same female? Or vice versa, what happens if sterile mating occurs first, followed by mating with a normal male?

With fruitflies if the first mating is with a sterile male the female will lay eggs for life which are infertile. If the first mating is with a normal male and subsequent mating is with a sterile male, some infertile eggs result in spite of the fact that the first mating was with a normal male. This will differ with different insects and is a complicated problem.

QUESTION: We have been doing some work in the past few months on the effect of radiation on the susceptibility of mosquitoes to malaria. In this instance the radiation is of the larval stage and applies to both males and females. We find that there is a three-fold increase in susceptibility of Culex mosquitoes to Plasmodium in this instance, which is statistically significant; furthermore, this characteristic is passed on through at least four generations without additional exposure to radiation. There is, therefore, the possibility that other factors will have to be taken into consideration when we are attempting to sterilize insects with radiation.

DR. STEINHAUS: During the afternoon we have discussed protozoa, nematodes, insect parasites and predators, and fungi. Apropos of Dr. Kudo's talk he referred to Dr. Weiser, of Czechoslovakia, who is concerned with microsporidian diseases of insects. Dr. Weiser has a monograph on the Microsporidia, not only of insects but of other invertebrates, which was scheduled to be published in February or March, but I think it may be delayed a few months. But certainly, during the next year we can expect a very extensive and highly illustrated monograph on Microsporidia, published in the Czech language. He is also considering publication in German. In any case this important publication will be one we should translate into English.

DR. LINDQUIST: I would like to ask Dr. Laird, if any results of distribution of the fungi are yet known and what in your best judgment is going to be the result? Do you think you are going to depress or annihilate the population?

DR. LAIRD: My initial feeling, and that of other members of the 1957 Expert Committee on Insecticides, was that the chances of total failure were high, perhaps of the order of 90%. We went ahead with the operation because circumstances were such that the project could be carried out cheaply. Transportation was going to cost less than would normally be the case, and if the fungal introduction proved unsuccessful little would be lost.

However, we do have results to the extent of what the New Zealand party found in November of last year, 14 months after the introduction had taken place. It was found that two of 11 *Aedes polynesiensis* breeding places seeded in 1958, contained numerous infected larvae. In one case 20% of the larvae present were parasitized, almost twice the average Singapore figure.

We thus know that *Coelomomyces stegomyiae* is maintaining itself at Nukunono, although we must wait until this year's visit to assess the percentage of larval habitats infected. It is of course encouraging that two of 11 habitats yielded parasitized larvae during so short a visit as last November's, for only 2% of *Aedes albopictus* breeding places proved positive at Singapore.

At best, what it is hoped to achieve in the initial phase is a perceptible lowering of the mosquito population at Nukunono through the agency of the fungus. A material reduction in the vector population by *Coelomomyces*, perhaps supplemented by other biological control agents, should be reflected by a corresponding decrease in the *Wuchereria bancrofti* microfilarial index over an extended period, and a very substantial reduction might eliminate the possibility of elephantiasis. This kind of approach to vector control would suit the comparatively underdeveloped state of small, isolated Pacific islands the economic status of which is not such as to support the expense of a chemical control program.

DR. STEINHAUS: Dr. Welch, could you tell us whether you or anyone else is doing any work on the mass-rearing of nematodes?

DR. WELCH: Dr. Daugherty in California has developed techniques for rearing. A significant feature of nematodes is that few have been reared through their complete life history. There are different stages in rearing. Nematodes can be allowed to complete the life cycle in the laboratory; or go through one generation in the laboratory; or insectary-rearing where the host and the nematodes develop; or use of a chemical medium. Dr. Daugherty is the only one who has suggested chemical rearing, in vitro culture.

DR. STEINHAUS: Are you working on chemical rearing:

DR. WELCH: No, but we intend to try some techniques which have been developed by Dr. House at our laboratory. He has been trying to rear insect parasites in various media. The method might be amenable to this same technique.

DR. STEINHAUS: Thank you very much. The meeting is adjourned.

THURSDAY MORNING SESSION

February 4, 1960

DR. STEINHAUS: We shall begin the program this morning by hearing Dr. C. G. Thompson, of the Laboratory of Insect Pathology of the USDA, speak to us on "Potentialities of Biological Methods in the Control of Insects of Public Health Importance."
Dr. Thompson!

POTENTIALITIES OF BIOLOGICAL METHODS IN THE CONTROL OF INSECTS OF PUBLIC HEALTH IMPORTANCE

C. G. Thompson, Entomology Research Division
Agricultural Research Service, USDA
Beltsville, Maryland

The insects of public health importance which appears to offer the best prospects for biological control methods are those in the order Diptera. The lice and fleas, while not hopeless, do not appear to be immediate general prospects for biological control.

Biological control is commonly considered to be that control produced by parasitic and predacious insects. In recent years, the development of insect pathology has emphasized the importance of the microbial parasites of insects. The release of sterile males may also be considered a method of biological control.

Mosquitoes, particularly in the larval stages, would appear to present promising prospects for biological control through the use of pathogens. The results to date, however, have not been too encouraging in most cases. This probably reflects lack of intensive research in the field rather than a failure of the method.

A number of diseases of various types--bacterial, protozoan, fungus, and nematode diseases--have been reported from mosquitoes and a more active investigation would undoubtedly disclose more, perhaps including virus diseases. The aquatic environment of most mosquito larvae, while forming a barrier to some pathogens, forms an excellent means of dispersing others. The feeding habits of mosquito larvae should render them highly susceptible to infection by microbial agents that invade through the alimentary tract.

The artificial introduction of pathogens and insect parasites and predators is much less likely to upset natural control already in effect than is the application of an insecticide. Mosquito-eating fish such as Gambusia could be incorporated into biological control programs much more readily than in chemical control programs. Controlled water level fluctuations have been reported to be of value in aiding mosquito control in the TVA lakes.

Certain of the Entomophthorales fungi have been commonly observed killing adult mosquitoes. At times, oviposition and emergence sites are almost covered with fungus-killed mosquitoes. The artificial introduction of these fungi might be of considerable value in reducing adult populations.

Biological insecticides or toxins have been reported to have killed mosquito larvae under laboratory conditions. Water extracts of spores of Beauveria bassiana have demonstrated considerable insecticidal activity. The possibilities of materials of this type remain to be explored.

House flies and biting flies are subject to a variety of diseases. In addition, the sterile male release method is especially promising as an eradication method for certain flies. The success of screw-worm eradication in Curacao and in Florida has stimulated interest in this type of control. The possibility of eradicating the Tsetse fly is now under investigation in Africa. Since the success of sterile male release depends on relatively low wild populations, chemical suppression of populations may be necessary, in some cases, before sterile male release.

With discovery of a virus disease occurring in crane fly larvae, the possibilities of viruses attacking other Diptera appear brighter. Entomophthorales fungi are commonly found attacking adults of a number of species of flies and undoubtedly account for considerable reductions in adult populations. These fungi are generally difficult to manipulate artificially and applied biological control would appear to lend itself more readily to measures against the larvae.

Current investigations with Bacillus thuringiensis Berliner indicate a considerable value of this material in preventing emergence of adult house flies from the manure of treated animals. The possibilities of inducing epizootics of other diseases in breeding sites have not been adequately investigated but there would appear to be considerable potential in this field.

The pest and blood-sucking flies whose larvae develop in aquatic environments should offer possibilities similar to those previously discussed with the mosquitoes.

Potentially, the insects of public health importance offer the same possibilities for biological control as found with pests of agriculture and forest crops. As with these latter pests, some will undoubtedly be much more easily manipulated than others. These species which have a larval feeding stage not connected with a warm blooded animal host appear to be the most promising, although parasites and predators undoubtedly play an important role in natural control of many of those forms attacking mammalian hosts.

QUESTION: I was interested, Dr. Thompson, in your remark that the mosquitoes offered a real potentiality for biological control. It has been our experience in Canada that usually a mosquito population in the field is a tremendously complex association. I wonder if you have any comment to make on the fact that it is such a complex factor.

DR. THOMPSON: I think that is the first problem we run into in almost any biological control problem. It is a complex problem. We are not working with a simple problem such as putting DDT on a house fly, then seeing if it dies--but we are working with a very complex ecology, an animal relationship. Each case is going to be different. We may have a biological agent which is quite specific. In that case we may be somewhat restricting the organisms involved, but we also run into problems. For instance, if there are six mosquito species operating as pests, and we can only kill one of them, we are not doing very much.

DR. PRATT: Is your field work done on the basis of thorough known ecology of the host in relation to the biological control agent?

DR. THOMPSON: Not thoroughly. We go into the field before we fully understand all of the factors. That may present quite a hazard. For instance, anything which might tend to eradicate a species, at the same time eradicating all the biological enemies of that species, would pose a tremendous threat if the host were reintroduced into that environment. I regret to say we do not know when we should go into the field. We are trying to get results fast; maybe we are trying to get them too fast.

DR. PRATT: Do you think it is sometimes necessary to go to the field to learn what some of your environmental problems are, which you might not perceive or know about until you have gone to the field?

DR. THOMPSON: We learn as we go along. In trying to alleviate a pest situation we are doing some good, even though we do not know all we are doing. I think probably we are running less hazard in most cases with biological measures than we would be with a chemical-insecticide measure. People who are putting insecticides out do not fully understand what they may be doing to that environment either. I think they may be running more of a hazard than we are.

COLONEL BUNN: I believe there has been a little work with insecticide-pathogen combinations, apparently with some success; but I do not believe I heard much comment on that. Nor has there been comment on any work on the combination of pathogens.

DR. THOMPSON: Yes, there has been such work. Pathogens are quite compatible. We have at times tried combining several viruses and bacteria; there has also been some work done in Europe and Canada, combining chemical insecticides and pathogens, with the idea that the requirement of chemicals can be reduced considerably.

DR. BRIGGS: I visited recently with Dr. Haines in the Department of Entomology at Michigan University; he did quite a bit of work this past year on the combination of pathogens with various insecticides on fruit tree pests and has published a report. He had some surprising results; at least, I felt they were. There seemed to be some synergistic effect. There was an increased benefit from the combined treatment in a case using less insecticide than using either the insecticide alone, at the larger rate, or the pathogen alone. The insect pathogen used was a formulated commercial preparation, Thuricide. As far as my own research is concerned, I want to work with combinations of chemical and microbial ~~insecticides for use on house flies and mosquitoes. We have a good~~ situation in which to work in the central valley of California, where we have increasing resistance to chemical insecticides.

DR. STEINHAUS: There is work in Czechoslovakia where a synergistic effect between pathogens and chemical insecticides were noticed. A Nosema in Otiorrhynchus accelerated the effect of certain contact insecticides.

DR. LAIRD: It has been my impression, Dr. Thompson, that a lot of the trouble we have run into in the postwar period has resulted from our preoccupation with chemical control to the exclusion of naturalistic control practices developed in the pre-DDT era. I feel that we should not think in terms of any sort of dramatic replacement of chemical control by biological control; rather, we should strive for appropriate blendings of these two, so as to have the best of both worlds and slow down the development of resistance thereby.

DR. THOMPSON: I think we will have to live with chemical control insecticides. Of course, there will in the future be a large-scale replacement of chemical insecticides. I do hope that some problems can be solved by biological methods alone, others by a combination.

Some of our pathological work is close to chemical-insecticide work. Reports indicate that in some instances we almost have a chemical insecticide. Some of the fungus spores produce materials which behave as insecticides. I think when we evolve insect control to a higher status than it is now, we will find a complete gradation from pure chemical to pure biological control, with all steps in between.

DR. LAIRD: It would seem to be apparent, then, that both chemical and biological control will have their place in future programs.

DR. STEINHAUS: Thank you very much. I am going to ask Dr. Olive to recapitulate for us some of the ideas and thoughts which have been generating during the conference, and to add some of his own.

Summary

John R. Olive, American Institute of Biological Sciences

I find summarizing very difficult. There have been many things said in the sessions yesterday and this morning. So I think it is probably very inappropriate to call this a summary, or even a recapitulation. These are some thoughts I would like to express for your consideration, to possibly stimulate some thinking as you go into the session on recommendations.

It would seem to me that there has been one underlying theme which all of you have touched upon, the ecological relationships with man. Certainly, you are working with the entire world as your laboratory. About a year ago I suggested establishing an international ecological year. Such a program has some roots in the IGY. As I listen to the things which are going on in far-flung places, it occurred to me that this is an idea to which you could give some thought.

With regard to environment, as you have discussed it the last day and a half; that once you change any factor, one single factor within the environment, you have automatically changed them all.

A few concepts expressed by individuals I would like to bring to your attention again. Dr. Steinhaus yesterday morning started out with the idea of identifying and reporting true species. This seems to be a very vital thing. This brings the matter of communication into its proper focus, the problem of how to get the information to the people. This is something you can start thinking about as you go into your deliberations and recommendations today, the dissemination of the literature and translation services.

Our own organization, AIBS, is quite heavily involved in this area, as Dr. Cox told you yesterday. Perhaps an organization such as this can be helpful to you working in the field, possibly by centralization of literature. Then, as Dr. Laird mentioned, there is the matter of centralization of techniques. This is not the term he used; but, at least, he hinted at this. I hope I am not putting words in your mouth, Dr. Laird, when I say you feel that there should be more information going out to the field men as to how to get specimens and materials to the proper place at the proper time. This seems to be a very important feature.

Then I have taken the liberty of setting forth what I would like to call the Couch postulates. Dr. Couch set forth these ideas yesterday on the requirements of parasites: that the parasite must be virulent, it must be specific, it must be culturable, and it must be easily and cheaply dispersed. This is quite a charge to place before you, to find parasites or pathogens which would fulfill these requirements, because you are looking foward a parasite which actually kills its hosts. This is not a good parasite in the scheme of things, if we consider the over-all ecological picture.

Dr. Couch has (as have many of the following speakers) pointed out the need for basic research. I am very happy to see that there does not seem to be a conflict here between the so-called areas of basic and applied research.

On Dr. Kudo's very fine studies on the protozoans, here certainly is basic research at its best. There are many, many things which must be known taxonomically and morphologically before you can move into other areas of endeavor. All of this must come first or at least very closely allied with the other research.

Dr. Welch has done very fine work on the nematodes. Here, working in a rather difficult environment (I got the impression that much of his work is in the aquatic environment) there are many, many problems associated with the aquatic environment, which provides a continuously changing dynamic situation. One factor which I would like to mention is the possible role of antibiotics which may be released by algae. I do not believe this one has been mentioned.

Then I think Dr. Sailer's comments yesterday certainly struck at the heart of the problem, where he was talking about the inventory of assets and liabilities. In many instances here, we seem to be forcing the organism into an ecological niche which is nonexistent; or at least the relationships become very strained.

Dr. Laird's work in distributional problems seems to be on the frontiers of very important findings. Dr. Thompson mentioned the development of genetic strains. The term "lethal genes" I do not believe has crept in here; but, certainly, this is an area which might lend itself very well. I do not know how much work has been done.

Now let me turn just very briefly to a subject which has not appeared anywhere in the program. This is what you as scientists are doing in the area of education and recruitment. It seems that here is an area which might bear critical scrutiny; i.e., what is being done for the young graduate student, to bring him into a stimulating field such as you are in? Are there scholarships? Are there fellowships? What might you do to promote these? What is being done at your own university or your own institution?

DR. STEINHAUS: Thank you very much, Dr. Olive.

QUESTION: I think the impetus for this conference probably resulted from the development of insecticide resistance. I have heard no mention thus far of the potential for resistance or immunity developing in biological control. I feel that this is an area which has been omitted, and I should like to hear the chairman's comments.

DR. STEINHAUS: I shall be glad to respond, but I can express only my own personal viewpoint. There is no reason, theoretically, why resistance should not develop, at least to some insect pathogens. It may be a different story with the somewhat larger parasites. But, if for no other reason than through the processes of natural selection, we should expect some resistance to develop.

Of course, there are very puzzling instances which have been mentioned on occasion. There is for example a virus disease, a nuclear polyhedrosis, of the alfalfa caterpillar, which occurs every year in populations of this caterpillar. The disease usually breaks out too late really to protect the crop, although sometimes it does. This is the reason we are using the artificial distribution of the virus for control purposes. But it seemed only reasonable to me that surely this disease, which had been known to exist for fifty years or more in populations of this insect throughout California, should have left at least remnants of resistant populations. So I went up and down the state, sampling, expecting (naively, apparently) that, sooner or later, I would find some resistant individuals. I never did. When we artifically infected a group of larvae, there were usually some survivors. Upon reinfecting these survivors, however, they succumbed as readily as the others. Thus there was no evidence of immunity being produced in individuals.

It is true that resistance has not developed in most of the cases in which pathogens have been used against agricultural insects. There has been some recent work in England and in this country to indicate that perhaps a degree of resistance can develop or be forced. A slight degree of resistance has been detected in cases of some virus infections. Perhaps Dr. Thompson and others know of some examples from their own work. But no really major instance of resistance, on the level of that which develops with chemical insecticides, has so far appeared. I would not be the least surprised, however, if at any moment we hear that such resistance does develop, particularly with some of the commercially applied material.

There is another thing we must realize: So far, these insect pathogens have not been applied to the degree that chemical insecticides have been; so there is not that pressure on populations for resistance to develop. Of course, the microbial products Dr. Briggs talked about yesterday are beginning to be applied in greater and greater numbers. Possibly, when the pressures which are formed by these applications exert themselves, we shall see some evidence of resistance.

Dr. Thompson, do you have comments?

DR. THOMPSON: I agree with what you said; but if we get to working with something new, a new biological agent which has not previously encouraged us, we might run into a situation like the myxomatosis of the rabbits of Australia.

So far we have not had any indication of resistance. One of our major problems is that transmission often eliminates the progeny of survivors of the LD_{50} dose, so that we cannot reliably maintain a population which has survived virus exposure.

MR. MEAD: What real hope have we that a pathogenic control of insects will be any more successful than the one just mentioned, the myxomatosis of rabbits in Australia, or the fact that, in human infections, we have had some very severe epidemics, referring particularly to the black plague in England and on the Continent in the Middle Ages, which has taken a large toll in the populations of the susceptible hosts, but never has completely eradicated this host? Development of resistance to viruses is even less common than development of resistance to bacterial infections.

DR. STEINHAUS: How about ordinarly natural selection playing a role?

MR. MEAD: This goes back to my first question: How about natural selection in preventing biological control? I am speaking now of parasitic or pathogenic control of insects, because I wonder if you will not get a natural selection in protection, as in myxomatosis in rabbits. The breakdown of the balance of nature is important. A microorganism or a pathogen which is going to be useful in the control of pest insects must not be pathogenic for the beneficial ones.

DR. STEINHAUS: With reference to your last statement, I might volunteer a comment: Most insect pathogens (in fact, as far as we know; all true insect pathogens) are nonpathogenic for vertebrate animals. At least, this is true for all entomogenous microorganisms that have been tested. There have been no cases of spontaneous disease resulting in higher animals following application of microbial insecticides. Since they contain protein, sensitivities could develop, as can also happen with chemical insecticides. In fact, there are one or two known instances where Beauveria bassiana, one of the very common entomogenous fungi, has given a great deal of trouble to insect pathologists because, in working with it, they developed a sensitivity to it.

should insist that all industrial groups who manufacture insecticides get this sort of information.

As far as honey bees are concerned, a study was undertaken by the University of California at Davis; they found that there was absolutely no toxicity of Bacillus thuringiensis to worker bees. It was tried in almost every conceivable manner to see if there would be some toxicity when taken from flowers in the field back to the hive. These are some examples of the work which must be done in order to have confidence in these new biological materials.

The Food and Drug Administration is set up for chemicals; there are laws which certainly will cover microbial insecticides. But we are really growing together on this. Everybody in this room can help by making suggestions (1) to the companies which are involved, as far as information which is necessary is concerned; and (2) to the Food and Drug Administration or governmental authorities, to bring up some points about what we might consider. Many of these men are so busy with administrative duties, are not familiar with the biological areas, and are really appreciative of getting the points of view of people who have the perspective on this particular area.

DR. STEINHAUS: Dr. Briggs, your remarks applied primarily to bacteria, didn't they? In general, however, this is true across the board. But I should like to point out that perhaps there is more apprehension with the bacteria than with the viruses and the entomophilic protozoa; because the viruses, for example, although not necessarily species specific, are nevertheless highly specific. It is almost inconceivable that they would infect anything other than an insect; they are so restricted in their range of pathogenicity. Certainly, their wide-scale use in some areas already points out that there is virtually no danger from the use of these viruses. But, when you take a bacterial organism like the one Dr. Briggs is discussing, here the safeguards perhaps have to be emphasized all the more.

Also, I should like to add that I assume Dr. Briggs was speaking from the standpoint of the precautions taken by industrial concerns manufacturing these materials on a commercial scale. I think also that all of the laboratories of insect pathology (federal, state, and otherwise), both in this country and in Europe, are more and more taking these precautions, even on an experimental basis. In our own laboratory, for example, we are now careful to run at least certain basic or routine tests in experimental animals, just to be sure we do not have something unusual.

DR. SAILER: We are dealing with one of the biological facts of life when we are talking about the development of resistance. The very process of evolution is a response to processes of selection which reflect adaptation, two factors eliciting a resistance response.

In general, however, these entomogenous microorganisms are harmless to all types of life other than insects. Of course, the public and government have a perfect right (and our Food and Drug Administration has the duty) to see that microbial insecticides are safe, and that the proper tests are made, and that the proper precautions are taken.

I know that there are others here who could comment on this perhaps more fully; so it would be more appropriate for me to call on them. Dr. Briggs and his company ran most exhaustive tests in getting their permit for commercial production of Bacillus thuringiensis. Would you please comment about the safety of these materials, Dr. Briggs?

DR. BRIGGS: In Agriculture and Food Chemistry (October 1959), there is a paper entitled, "Toxicology of a Microbial Insecticide, Thuricide" by Fisher and Rosner. These tests were done in close cooperation with the Food and Drug Administration, and followed their suggestions in locating any possible toxic effect of the spores of Bacillus thuringiensis on vertebrate animals.

In getting clearance from the Food and Drug Administration for use of a microbial insecticide on a food crop--(this is a new line in some respects)--the materials must follow the Food, Drug, and Cosmetic Act regulations; tests and residue analyses have to be made on that basis.

But, more important, although there is a great deal of circumstantial evidence indicating that there is no toxicity of this particular material to beneficial insects and wildlife, we found it necessary to investigate this area quite extensively, particularly with insects or animals that we would come in contact with in the field.

For example, honey bees, trout, pheasants, earth worms and others have to be tested because, in every area where you are going to use this material, people are going to ask "Well, it does not hurt Gambusia; but could it hurt rainbow trout?"

We have had to test many of these things, and have been very fortunate in having close working relationships with the California Fish and Game Commission, for example, and with the laboratories for biological control in Canada and the Midwest, as far as the dipterous parasites are concerned.

These are the questions people ask; you have to try to anticipate them, or at least get as much information as possible. Obtaining this information is the job of an industrial group which is in a position to make quantities of the material in question. I think we

It is valuable to look at what is happening in the wheat-breeding problems. It has been recognized for a long while that there has been a race between the wheat breeders and the rust-disease organisms in this process of developing and responding to resistance; that is, the wheat breeders are trying to breed resistance into the wheat, while the rust organisms are constantly evolving new strains of rust which will attack the previously resistant strains of wheat.

What can be done about it? Apparently, we just continue doing what the wheat breeders are doing. They are trying to evolve new strains of wheat which will have broader resistance. I think we can make a flat statement that we will find resistance to any of these factors. The Canadians here can, I believe, speak to the development of resistance in larch sawflies to an insect parasite.

If we work at these long enough, expose them to sufficiently efficient parasites or predators, our pests will become resistant to them; we will have to take countermeasures.

COLONEL TRAUB: The question has been raised as to whether there is immunity or resistance to viruses. It has been suggested there is not. If we regard resistance to insecticides as analogous to immunity to disease, we may come up with some useful comparisons. For example, human viruses were mentioned, polio in particular.

If we consider Japanese encephalitis in Malaya where we worked, infection was very common. Virtually everybody (about 99 percent of the people) have neutralizing antibodies to the virus. Yet the disease is very, very rare clinically, both in the Asian and the non-Asian populations. We have an analogous situation with polio. We are all concerned about the numbers of polio cases; but we forget that, for everyone who gets sick, there are probably 10,000 who have been infected and did not realize they had anything but some mild disease. So there is some sort of immunity or resistance to viruses.

Then the question was raised as to whether we would aim at eradication with biological control. Often do not need eradication; it can only be hoped for in certain special instances, but adequate control may prove tantamount to eradication epidemiologically. Let us consider what happened to malaria in the United States. It is not so many years ago that this disease occurred as far north as Ithaca, New York, and the northern Midwest. It has been unknown in those areas for fifty years, except perhaps in returning soldiers. At least a few years ago, you could still find Anopheles quadrimaculatus in those areas where cases had once occurred; but the number of mosquitoes was so low that they could not maintain malarial infection of people. I am sure that in many other instances, if we can effect a suitable reduction of the number of vectors or insects of economic importance, we will have achieved a great deal.

I am glad the point was brought up that we must consider the public response when we start working with some of these agents, because undoubtedly the question will be raised as to whether some of these organisms are pathogenic to man.

DR. LAIRD: In connection with this matter of resistance, I would like to suggest that perhaps we are getting away from the really important basic difference between the use of chemical insecticides and that of biological control agents.

Insecticides, even the residuals, have to be reapplied from time to time. After their effect has worn off, there is likely to be, at least temporarily, a greater abundance of the vector or pest species than there was before control commenced, because of the destruction of natural enemies.

On the other hand, once having injected a biological control agent of some kind into a population of insects previously free from that particular enemy, the biological balance finally achieved is established to the permanent disadvantage of the vector or pest species. Even if the resultant degree of control does not exceed five or ten per cent, the numbers of the insect concerned show lasting reduction.

I think that the fundamental issue in biological control in our field should be to devise combinations of pathogens and predators, suited to individual control problems, by which vector populations can be reduced to a point where, as Colonel Traub suggests, transmission of disease organisms ceases to be likely. Of course, even after attaining such an objective, the tangible and immediate benefits that chemical insecticides can confer will ensure their retaining an important place in control.

DR. STEINHAUS: I think before we go on to the formulation of recommendations we ought to talk a little bit about some of the points Dr. Olive raised, about organization, more liaison, and more cooperation.

Most of you know that in Europe they have organized quite formally their biological control work. The CILB (Commission Internationale de Lutte Biologique) in Western Europe is quite a large organization established for the express purpose of exchanging materials and information. They have a journal called Entomophaga which publishes papers relating to biological control. Other journals in Europe also carry such articles.

At the First International Conference for Insect Pathology and Biological Control held in Prague in 1958 some of us enjoyed very much meeting for the first time a number of the Russian insect pathologists. We were able to learn at first hand some of what is being done in this field in Russia. At this meeting, the Eastern countries organized their own group similar to the CILB, with the hope that eventually they could establish a strong liaison with the Western group.

In all of this discussion at Prague what was particularly interesting was realization that America, or the New World, had no such organization; indeed, many of the authorities in the United States have expressed themselves strongly that they want no such organization. So there was no attempt to establish formal liaison with the New World. Fortunately, however, the Commonwealth Bureau of Biological Control serves us in a very good capacity. When I say "us", I mean all of us, not just the Commonwealth, because they do have liaison throughout the world, which help many of us in areas where an organization of the type I am describing would be beneficial. Also, both the USDA and our own biological control group in California have by themselves established foreign contacts and mechanisms by which foreign sources can be made available.

Dr. Olive mentioned the matter of journals and other means of communication. Perhaps I may be permitted to mention that we now have a Journal of Insect Pathology. It has just finished its first year of publication. It is published by the Academic Press. The pages of this journal are available for papers pertaining to original research on diseases not only of insects but of all invertebrates. If your library does not get this journal, you might be interested in seeing that it does, because we are trying to put out a high-quality journal which will serve your interests in this area of biological science.

DR. BEIRNE: Most of us use the Review of Applied Entomology, published by the Bureau of Applied Entomology in England. The activities of this Institute are reviewed every five years. At one of the review periods this summer we can suggest changes and improvements in any of the activities which are of common interest. I wonder if anyone here has any suggestions as to how the Review of Applied Entomology might be improved or changed in any way to make it more effective.

COLONEL TRAUB: I think it would be appropriate if this group expressed its appreciation for the very useful Review of Applied Entomology. The interested parties who subsidize it are always glad to have people say that.

DR. STEINHAUS: I think we unanimously agree to that suggestion. Please relay these sentiments, Dr. Beirne.

Another matter in which I know Dr. Olive is interested is that of education, the training of people in this field; the training of specialists. By way of introduction, the University of California has one of the strongest programs, in the teaching of biological control subjects, with courses being given in both the predator-parasite aspects and in insect pathology. Dr. Sweetman at Massachusetts has also been teaching a course in biological control, and one or two others may be doing so. I know that one or two universities are considering initiating courses in insect pathology. So there is at least the genesis of this development taking place.

I do not know how other institutions are in this respect, but as far as our laboratory is concerned our primary need is space. It is getting to be that we can obtain adequate research money, but try to get room to do it in--that is a problem! This year alone we have had to turn away ten requests from visiting scientists who wanted to come to study with us. This hurts badly.

Does anybody else have comments on this subject of education or training?

COLONEL TRAUB: The problem you have mentioned is a very grave one. In the armed services, we are faced with an additional difficulty. Not only can we not get space; but we cannot get the personnel, because of congressional ceilings. I would like to point out that the Surgeon General of the Army is much interested in supporting new approaches in the field of insect control, particularly with respect to arthropods which are of interest to the military. We hope to get additional funds to support this type of work. The Army can now issue grants as well as contracts, contracts on an annual basis, grants for up to five years. If any of the men here, or their colleagues, want to submit projects, we would be very glad to receive them for consideration. They can be sent to the Army Medical Research and Development Command, Main Navy Building, Washington, D.C.

DR. LINDSEY: Is this support you have mentioned limited to United States institutions?

COLONEL TRAUB: No; we have contracts awarded to activities overseas.

DR. STEINHAUS: I would like to call on Dr. Wessel for discussion on the communications matter before we start our discussion on recommendations. Dr. Wessel.

DR. WESSEL: I would like to follow up the comment made by Dr. Olive on the science-communication part of this problem. In looking back over this meeting, the first paper was by Dr. Jenkins in which he reviewed the literature. This sort of set the stage for the whole conference.

This matter of scientific communication today is becoming more and more important, and is being recognized in all scientific fields. This has led to the formation of information centers. I speak now not as an entomologist or a mycologist. By training, I am a biochemist; but, by work, I am an information specialist. We run an information center on deterioration of materials due to natural climatic factors, such as mildew, some of the insects, chemical deterioration, and so on. The point I want to make is that this field of biological control could utilize the services of an information center. There are many such centers now in existence. I mentioned to Dr. Olive one which came to mind at Battelle. This is called the Defense-Metals Information Center; it has an annual budget of a million dollars. It is supported by the Department of Defense to provide information in the field of metals important to the defense efforts.

About two years ago the Air Force put out a list of information centers or organizations dealing in that sort of thing. This listed more than 100 organizations throughout the country which are handling information.

It would be a wonderful thing if you had such a service available to you in your own field, so that you would have better means of communication with each other and with the many, many other people in this field who are not here today, and in related fields which would help in this effort.

DR. STEINHAUS: Thank you, Dr. Wessel. I wonder how many of you noticed that one of the recent Science Newsletters included predictions for the next decade. One of them was to the effect that there would be great advances in biological control of pests. I do not know the basis of their prediction, but perhaps it is worth noting.

DR. JENKINS: I strongly corroborate Dr. Wessel's statement about the need for some sort of coordination center. As a result of quite a bit of effort in reviewing the literature, I doubt if we have brought together much more than half of the known information in the world. The data are scattered in obscure journals dealing with a wide variety of subjects. Also, new data are coming out very rapidly from many scattered sources.

DR. REID: Dr. Olive has suggested that we should have an international ecological year. I have been proposing for something over a year now an International Biological decade. I think it is time for the biologists to start thinking in broader terms. The geophysical year was a pretty good one; it ran eighteen months.

Biologists have a lot more to do and a much bigger problem. It seems as though something should be done along this line. I am delighted that this group has heard the proposal made by Dr. Olive; I hope they will give us at least their opinion or backing on going ahead with something of this nature.

DR. STEINHAUS: Can you encourage us on this wonderful idea by telling us if the AIBS is responding to your agitation for this? I think they would be one organization which would support it.

DR. REED: As I say, I have not talked to Dr. Olive about it, but I think they will respond with enthusiasm.

DR. STEINHAUS: On the matter of recommendations, the sponsors have requested that the conference make recommendations which would not only reflect what we have discussed here, but also light the way, for the future in this area of biological research.

Actually, a group met informally last night, discussed these matters and have developed some tentative ideas; but perhaps it would be better to have a plenary discussion of this matter before those subjects which were thought about last night are discussed. So I would like all of you to respond with any ideas you might have, both generally and with regard to your own specialty, as to what you think should be recommended.

COLONEL TRAUB: I am sure the group will want to support more research in this field. Rather than generalities, I hope we make some specific recommendations, at least along the lines of coordination and integration for collection of specimens. Some of the speakers pointed out that many of the disease organisms we work with are those which cropped up during rearing programs and by other rather artificial means. It is very difficult to get any sort of entomological specimens in from the field, and here we want specialized specimens--diseased insects. This means that we should educate potential sources as to what we want rather than continue working in a haphazard way. For instance, if we in the Army could get the brochure from the University of California and from others, we could request that the men in the field send in any specimens they see. We could get them either alive or frozen (a category that was presumably not mentioned), or dead. I think the Navy can do that, too.

The drug houses, in their search for new antibiotics, have a tremendous screening program, collecting soil from all over the world, and have thus found new medicines of tremendous value. While we have a lot of contacts overseas, we probably are overlooking a lot of disease organisms right in our own back yard. Instead of waiting for a man overseas to send something which looks like an antibiotic, we should consider establishing a definite program of research and consider local sources as well.

I know that the Agriculture Department has a definite program to send personnel to look for insect parasites overseas. Perhaps these representatives of the Department are already looking for diseases of insects. If this is being done, is the search restricted to agricultural pests or are collectors on the alert for parasites and predators of medical pests?

I have a strong suspicion that the agriculture entomologists already have more work than they can handle, and I would like to see some sort of program set up involving not only the Army, Navy, and Government, but universities, at home and abroad, which would be an integrated affair, utilizing a planned screening program, not merely blind screening where you test every microorganism, but one where you look for leads first, then study that organism and its relatives.

DR. SAILER: As many of you know, the Insect Identification and Parasite Introduction Research Branch of the Entomology Research Division does keep a certain number of people in foreign fields, searching for parasites or predators which would be used primarily against agricultural pests. In the last ten years, they have been alerted to examinations of insect diseases. As they have found these, they have occasionally sent in specimens to be diagnosed. This whole problem, I think, boils down to a question of proper support for taxonomy. We have talked around this problem; but there is nothing which is more discouraging to the field man who is collecting material and sending it in than to get absolutely no response from the people who are receiving it. They welcome the material for research collections but it does not receive prompt attention. Twenty or thirty years from now, somebody may be very grateful to the individual who collected it. We need a staff who can promptly classify the material which is sent in from field personnel. They should compile a biological inventory, or at least make a start at it. The inventory would not be complete until something was known about the value of the material.

At the present time, our own insect identification staff is completely swamped with requests for identification services; better than 90,000 lots were submitted last year to a staff of less than thirty people. There are many unfinished lots waiting for attention. Unfortunately, a number of those which have been reported have not been reported as completely as they should have been. They have been "Genus sp." or sometimes only a family identification. This does not encourage the field man.

With regard to recommendations, a group such as this cannot do better than to recommend that better support be given to the people who provide the means for communicating this information we are talking about. As Dr. Steinhaus mentioned, there are bacteria which have been named and mentioned once and no one has been able to recognize them again. With 800,000 insect species named, the probabilities are that there are a million and a half in existence. We therefore face a difficult problem in this matter of communication in talking about organisms discussing their biologies.

DR. LINDQUIST: Someone mentioned this morning the need for laboratory space to conduct research. That leads me to wonder about our assets as far as money is concerned. I wonder if anyone has ever figured out just how much goes for entomological research in federal, state, and private institutions, not counting industry. I have not, but just as a rough guess it may be on the order of 25 or 30 million. That is not very much in comparison with the sum total of insect damage. Then one might raise the question of how many of the available research dollars go for biological control? These things have a bearing on the question of how we can stimulate and increase activity in this field.

DR. THOMPSON: I hope to put one man on full time on handling diagnoses; seeing that they are performed and that the sendee is informed of the results. We have been very remiss in some of our work as we have been putting diagnosis aside.

DR. STEINHAUS: The diagnostic service which our laboratory offers, similar to that in other laboratories, is designed with the idea of trying to handle all types of disease in insects. From many of these diseased insects we isolate cultures of various types, particularly bacteria and fungi. We are also developing means of preserving some organisms which are not cultivable on artificial media, such as the protozoa and viruses. This has presented another problem. What type of culture collections can we or should we maintain? I am aware that the American Type Culture Collection in this country will receive cultures. But sometimes we could flood them with various strains or with similar cultures from different insect hosts.

We have a limited culture collection in our own laboratory. What we usually do, however, is to send most of our cultures for storage to the American Type Culture Collection. There is a collection of bacteria isolated from insects located in Prague, Czechoslovakia. It is being maintained by Dr. Lysenko of the Czechoslovakian Academy of Sciences. A similar collection is being maintained at the Sericulture Station in Ales, France. There are other general culture collections in other countries.

As far as the United States is concerned there are still some difficulties involved. It is largely that we need curators, space, and storage facilities. Many of our laboratories do not have these facilities. Finding these pathogens and studying them for a while does not finish the problem at all; we must properly maintain these cultures, and make them available to all workers.

DR. COUCH: May I take a minute to emphasize the need for maintaining support of the Bureau of Plant Industry collection of fungi at Beltsville; and, secondly, to support this American Type Culture Collection in Washington. We deposit all of our living material with this national type culture collection. There is another supported by the United States Department of Agriculture, the Northern Regional Research Laboratory at Peoria, where they have one of the finest collections of living fungi in the world.

This to me is a tremendously important thing, the depositing of type material of Coelomomyces, which I have not yet done, and of other fungal types at Beltsville, then sending living cultures either to Peoria or the American Type Culture Collection. I do not think we need any new organizations. We just need to support the things we already have and see to it that Congress gives enough money to support the type culture collections.

DR. STEINHAUS: Dr. Couch, what do you think of the idea of individual investigators (particularly those having organisms that are not easy to maintain in culture) preparing enough slides of their material for distribution to interested workers in the field, so that they can make comparisons. Not necessarily type slides, but slides which would give some idea as to the characteristics of the different species. Could we recommend that people working with noncultivable material do that; or would it be too much of a burden?

DR. COUCH: It is a burden; but I think it should be done.

DR. PRATT: Many of you may know that the Quartermaster of the Army maintains a culture collection of fungi. They have available in culture many organisms of this type and mail them all over the world.

The suggestion that this could be done for insect pathogens would be a very fine service. It might well be possible that they could add insect pathogens to the Quartermaster's culture collection.

DR. STEINHAUS: Dr. Kudo, what about microsporidian slide material? I refer to the general availability of such material to workers in this field.

DR. KUDO: I think the type specimen should be selected.

DR. STEINHAUS: How about slides of examples of various species of microsporidia?

DR. KUDO: Perhaps that could be also undertaken; but, as to the collection, as you know, no culture is possible.

DR. WILLIAMS: Mr. Chairman, wouldn't it be appropriate for this organization to strongly recommend that funds be provided for wider research on the ecology of insects and their predators and their infections? It seems to me that the lack of funds for students to make this kind of study, the fact that these studies are mostly made in conjunction with or incidental to some field projects aimed at something else has inhibited the search for both predators and infectious agents.

DR. STEINHAUS: I certainly agree with the point you make. Of course, as Dr. Traub pointed out, some of this is a matter of personnel. How do you think we should solve that? As far as our own situation is concerned, we are doing all we can, with limited space, to train people. Is there any way which occurs to you by which we may obtain more space and personnel to do what you suggested?

DR. WILLIAMS: I wish a real drive could develop aimed at getting a much larger scholarship fund from the United States Government.

DR. STEINHAUS: We should remember that some fellowships in biological sciences are available through the National Science Foundation and the U.S. Public Health Service.

DR. PRATT: I would like to second Dr. Williams' suggestion that one of our specific recommendations be that funds be provided from various governmental agencies and state agencies for this purpose. Those concerned with getting the work done can then worry about where to find the people and the space.

COLONEL TRAUB: Some of the support should be earmarked for undergraduates, too, because a lot of them are going into other fields where support is available. We can get support for the few who now are working along these lines; but we want to steer more into it. I think we should recommend undergraduate support as well.

DR. STEINHAUS: That is a very good point. For example, in entomology, the University of California has some 70 graduate students, but only two or three undergraduates.

DR. WESSEL: How many here are familiar with the program at the National Academy of Sciences on oceanography? This was a problem brought to the National Academy of Sciences by a large variety of people in oceanography. This resulted in the setting up of a committee to study the matter. As a result of the study and recommendations of this committee, it became a subject for Congressional study and action and a program is now in force called Oceanography 1960-1970. I think the magnitude of this is in the area of 650 million dollars for ten years. Perhaps, if this group wanted to follow a similar technique, you could attract the necessary support to carry through the kind of program you envisage.

COLONEL TRAUB: This is true; but, unfortunately, this is another instance where we are starting very late. We should take advantage of this approach, emphasize the strategic value, and point out the obvious value of knowing about diseases of insects. It may pay to stress how much other countries have done in this field.

DR. PRATT: You cannot support an undergraduate in insect pathology in most entomology departments in this country, because the professors are non-existent. Therefore, I recommend that we urge entomology departments of universities to establish professorships in insect pathology and biological control as a very urgent phase of entomology.

DR. BRIGGS: I would like to comment on the possible role of industrial groups in the development of this program. We have a research laboratory, a small one; but it is growing. Publications will be coming forth. I think one of the greatest services these industrial groups can render is to provide the facilities for making biological control materials available for the research scientist.

It should be kept in mind that there are industrial groups which have the personnel, organization, and facilities for handling this sort of job, whether it is special diet for a predator, or a fungus or bacterial preparation which has some promise in the eyes of an investigator.

As you saw from the pictures presented, Bioferm has a very flexible physical plant and an Insect Pathology facility made up of specialized laboratories.

DR. STEINHAUS: In the interest of impartiality, in addition to Bioferm, there are some other companies in this field, such as the Nutrilite Corporation and Rohm and Haas; and some of the biological houses are interested. There is an extending interest in this field among commercial concerns.

If there are no more suggestions or recommendations, I shall call upon Dr. Jenkins to review what transpired at the informal meeting last night.

DR. JENKINS: Apparently some of the specialists have not made comments now, because they made them last night at an informal meeting of Dr. Kudo, Dr. Couch, Dr. Welch, Dr. Laird, Dr. Lindsey, myself, and some others. The general conclusions and recommendations are as follows:

1. Pathogens, parasites, and some predators offer a real potential for natural control of medically important insects.

2. Tests and experiments to date have shown promise, and have also demonstrated the absolute requirement for basic knowledge of the ecology and life history of the pathogens, parasites, or predators, and insect hosts.

3. Direct basic research should be intensified on selected pathogens and parasites presently appearing to have a potential for natural control.

4. Emphasis should be placed on coordinated, intensive survey, collection, identification, and screening of pathogens, parasites, and predators.

5. Type-culture collections of promising pathogens and parasites should be established and maintained.

6. The need for accurate, quantitative field studies and field assessments is recognized.

7. Field-assessment sites should be carefully selected. Consideration should be given to selecting the sites where the parasites being tested do not occur.

8. Pathogen-free or specific pathogen-free colonies of mosquitoes and other medically important insects should be established and used in parasite and other studies.

9. Combinations of pathogens, and pathogens and insecticides should be considered.

10. Industrial groups should make available facilities and personnel for studies in this area.

11. Microorganism specimens should be distributed for identification and teaching.

12. Funds should be provided for study of pathogens, parasites, predators, and the ecology of medically important arthropods. This should include scholarships, teaching, and establishing programs in insect pathology and bi logical control at universities. More laboratory space is urgently needed.

COLONEL TRAUB: How are these recommendations to be implemented?

DR. JENKINS: Funds will be required. It is planned that the proceedings of the conference will be sent to granting groups, universities, and federal groups where research may be initiated and supported.

MR. HUTTON: I was struck by the broadness of the recommendations made by Dr. Reid, Dr. Wessel, and Dr. Olive concerning a rallying point, which not only the entomologist but the mycologists and many other scientists can rally around--an ecological year or biological decade, or a similar action. This is the type of suggestion which is extremely difficult to comprehend or act upon in such a meeting as this. Therefore, I suggest that the group consider establishing an informal working group comprised of the previously named gentlemen and Dr. Steinhaus and Colonel Bunn, to explore the possibility of bringing about such an effort, in which heavy emphasis would be placed upon biological control.

DR. STEINHAUS: Dr. Reid, what do you think of that idea?

DR. REID: It sounds good to me.

COLONEL BUNN: Perhaps part of this has been implied; but something specific which might follow this conference would be a recommendation to the effect that an information circular be prepared for distribution to field biologists, which would list available diagnostic services for biological control agents, and provide information on detection, collection, handling, and shipping of specimens. This would be subject to the will and the desires of the available diagnostic services; but I am sure that information on this point is lacking to a large extent among the field biologists.

DR. STEINHAUS: Is it the sense of your suggestion that included in such a recommendation would be a call for people who are interested in receiving material to notify some central group?

COLONEL BUNN: Yes. To accomplish this one would have to form an informal work group under the auspices of an organization such as AIBS to serve as the focal point for receiving information to be compiled.

DR. KERR: International organizations should not be forgotten; I am thinking particularly of the information circulars of the World Health Organization on malaria and insecticides--those are information sources--and these activities are international.

DR. JENKINS: I would recommend that a national organization be established in the United States under some agency such as the AIBS, NRC, a special group in California, the Armed Forces Pest Control Board, or other body. This would be very valuable to stimulate development of this field, and would also provide a national center to coordinate with international groups.

DR. LINDSEY: This may be perfectly obvious, Mr. Chairman, but I wonder whether anything of a very general nature might be included, something to the effect that we should, in any way we can, be cognizant of other information on insect control when we are considering biological control measures. In other words, we should perhaps try to incorporate or coordinate our biological control program with other types of control programs, including chemical.

DR. STEINHAUS: In some areas this is now being called integrated control.

DR. JENKINS: This was mentioned in one of the conclusions, but Dr. Lindquist's other biological methods could well be included.

DR. STEINHAUS: These suggestions are all being recorded.

DR. PRATT: Perhaps the international aspects could be taken care of if this body wished to recommend that the World Health Organization set up an expert committee on biological control.

DR. STEINHAUS: Certainly one can learn much from the Commonwealth Bureau and Canadian work.

Do you wish to proceed with the specific recommendations, Dr. Jenkins?

DR. JENKINS: We have specific recommendations for various groups of medically important insects. It would take too long to go through all of them; so specific recommendations for mosquitoes will be presented as an example.

1. No presently known viruses, rickettsiae, and perhaps bacteria appear to offer immediate potential for control of mosquitoes.

2. Fungi appear to offer the greatest potential for control. These include Beauvaria and Entomophthora, but Coelomomyces appears to have the greatest immediate potential and highest priority. Some of the major research problems--for Coelomomyces--include (a) research on taxonomy and specificity to hosts; (b) determine geographical and ecological distribution; (c) develop methods of culture; (d) determine infective stage and tissues of hosts attached; (e) learn full life history and methods of spore germination; (f) study of ecology with relation to other organisms; (g) establish and maintain type cultures; (h) carry out quantitative field assessments.

3. Of the many associated Protozoa, only the Microsporidia appear to cause high mortality. The most promising genera include Nosema, Thelohania, Stempellia, and Plistophora. The major research problems include (a) study of number of species in nature; (b) learn full life cycle and development; (c) develop rearing method; (d) determine host-parasite relationship and specificity; (e) determine factors affecting geographical and ecological distribution; (f) carry out quantitative field tests.

4. The Mermithid nematodes appear to have real promise, and deserve investigation. The major research problems are (a) conduct taxonomic studies; (b) develop rearing methods and carry out life-history studies; (c) conduct field studies on geographical and ecological distribution; (d) determine host specificity; (e) determine requirements for infection in the field, and methods of disseminating nematodes.

5. Studies on field effectiveness of larval and adult predators are needed. Additional studies, including quantitative field assessments on Toxorhynchites and larvivorous fish, are merited and should be carried out in carefully selected test sites to give valid and critical data.

We also have specific recommendations for houseflies, which include recommendations about fungi, especially Empusa muscae and the various parasitic wasps attacking the larvae and pupae, and certain promising predators. In the interest of time, we will not present specific recommendations for other groups.

In no way are we trying to tell the specialists what they should be doing, but from the combined standpoint of entomologists and specialist protozoologists, we are pointing out some of the major problems. Carrying out the program is up to the specialists. However, we thought it advisable to be quite specific in indicating some of the major problems with some of the most promising parasites and predators.

DR. STEINHAUS: Dr. Jenkins, you would be happy to receive, wouldn't you, any suggestion which participants might think of after they leave the group here?

DR. JENKINS: I would appreciate any comments now or by correspondence.

COLONEL TRAUB: Mr. Chairman, may we assume that there will be a committee to continue this type of integration and plan for a future meeting; or should we make such a recommendation?

DR. STEINHAUS: I know of no such plans; probably some of the sponsors do. Dr. Olive, does the AIBS have any?

DR. OLIVE: I know of no such plans; but I think this is certainly a must. We are talking rather broadly in terms of eventually getting together again, but I think we should definitely go on record with something to this effect.

DR. STEINHAUS: Colonel Traub, would you care to voice such a recommendation?

COLONEL TRAUB: I recommend that the organizing committee continue functioning after the meeting, not only to collect and disseminate the information which has been presented; but to implement such recommendations as have been made in the most practical manner, and to coordinate future activities and plan a future session.

DR. STEINHAUS: The Chair will entertain a motion approving the recommendations which Dr. Jenkins has read. I realize that they have been presented only tentatively; but I think it would lend strength to them if this group would go on record approving these recommendations.

Do I have a motion?

MR. HUTTON: I so move.

The motion was seconded by Dr. Thompson, put to a vote, and was passed unanimously.

DR. STEINHAUS: In bringing this meeting to a close, do any of the representatives of the sponsors have anything they wish to say?

DR. REID: Mr. Chairman, as a representative of one of the sponsors, I want to express our thanks to everyone who is here: the Chairman for conducting the meeting very efficiently, the speakers, and all participants. This has been a spendid meeting; I hope a great deal of good will come from it. I trust it will set the pattern for future meetings of this sort.

DR. STEINHAUS: Thank you. I should like to return the compliment for the group in expressing our thanks and appreciation to the sponsors for making all the arrangements, and certainly to the speakers. Rarely have I been surrounded by so many excellent and outstanding speakers and agile minds. It has been really inspiring to me; I thought, as I heard the various speakers, what an excellent selection the program committee had made. So I certainly wish to express our thanks to them.

I declare the meeting adjourned.

PARTICIPANTS

Dr. Lawrence M. Ames, Office of the Chief, Research and Development, Department of the Army, Washington, D.C.

Dr. R. W. Babione, Pan American Sanitary Bureau, Regional Office of World Health Organization, Washington, D.C.

Mr. John Bagby, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia

Maj. H. C. Barnett, Walter Reed Army Institute of Research, Washington, D.C.

Dr. Bryan P. Beirne, Entomology Research Institute for Biological Control, Canada Department of Agriculture, Belleville, Ontario

Dr. John D. Briggs, Bioferm Corporation, Wasco, California

Col. Ralph W. Bunn, Armed Forces Pest Control Board, Washington, D.C.

Dr. G. E. Cantwell, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

Capt. D. C. Cavanaugh, Walter Reed Army Institute of Research, Washington, D.C.

Dr. Gordon Clark, Patuxent Research Center, Laurel, Maryland

Dr. Floyd Clum, 2430 E Street, N.W., Washington, D.C.

Dr. John N. Couch, University of North Carolina, Chapel Hill, N.C.

Dr. Hiden T. Cox, American Institute of Biological Sciences, Washington, D.C.

Maj. David N. Dalton, U.S. Army Medical Research and Development Command, Washington, D.C.

Capt. J. D. DeCoursey, Bureau of Medicine and Surgery, Department of the Navy, Washington, D.C.

Mr. S. C. Dews, Corps of Engineers, Department of the Army, Washington, D.C.

Maj. Thomas B. Dunn, U.S. Army Medical Research and Development Command, Washington, D.C.

Dr. Samson R. Dutky, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

Mr. F. D. Enzie, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

Lt. Col. J. D. Galloway, RCAMC, Canadian Medical Liaison Officer, Department of the Army, Washington, D.C.

Dr. D. J. Gould, Walter Reed Army Institute of Research, Washington, D.C.

Dr. Carlton M. Herman, Patuxent Research Center, Laurel, Maryland

Dr. Robert Hurr, 2430 E Street, N.W., Washington, D.C.

Mr. George L. Hutton, Bureau of Yards and Docks, Department of the Navy, Washington, D.C.

Dr. Dale W. Jenkins, U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland

Dr. J. Austin Kerr, Pan American Sanitary Bureau, Regional Office of World Health Organization, Washington, D.C.

Dr. John P. Kramer, University of Illinois, Urbana, Illinois

Dr. R. R. Kudo, Southern Illinois University, Carbondale, Illinois

Dr. Marshall Laird, Institute of Parasitology, McGill University, Montreal, Canada

Dr. Joseph R. Leal, American Cyanamid Company, Washington, D.C.

Dr. A. W. Lindquist, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

Mr. Ian S. Lindsay, Defence Research Board, Ottawa, Ontario

Dr. Dale Lindsey, National Institutes of Health, Bethesda, Maryland

Mr. Bohdan Maksymiwk, Forest Insect Laboratory, U.S. Department of Agriculture, Beltsville, Maryland

Col. R. L. Marks, RAMC, British Joint Services Mission, Washington, D.C.

Mr. J. H. McLeod, Entomology Research Institute for Biological Control, Canada Department of Agriculture, Belleville, Ontario

Dr. John R. Olive, American Institute of Biological Sciences, Washington, D.C.

Mr. R. F. Philpitt, Olin Matheison Chemical Corporation, Baltimore, Maryland

Dr. J. J. Pratt, Jr., Quartermaster Research and Engineering Center, U.S. Army, Natick, Massachusetts

Mr. K. W. Quarterman, Technical Development Laboratories, U.S. Public Health Service, Savannah, Georgia

Mr. W. D. Reed, Corps of Engineers, Department of the Army, Washington, D.C.

Dr. Roger D. Reid, Naval Research Advisory Committee, Department of the Navy, Washington, D.C.

Dr. R. I. Sailer, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

Dr. Vincent Schultz, Atomic Energy Commission, Germantown, Maryland

Mr. George F. Shambaugh, Quartermaster Research and Engineering Center, U.S. Army, Natick, Massachusetts

Dr. F. J. Simmonds, Commonwealth Institute of Biological Control, Ottawa, Ontario

Mr. Paul F. Springer, Patuxent Research Center, Laurel, Maryland

Dr. Edward A. Steinhaus, Laboratory of Insect Pathology, University of California, Berkeley, California

Mr. B. Tannor, Office of the Quartermaster General, Department of the Army, Washington, D.C.

Dr. C. G. Thompson, Insect Pathology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland

Lt. Col. Robert Traub, U.S. Army Medical Research and Development Command, Washington, D.C.

Dr. R. A. Ward, Walter Reed Army Institute of Research, Washington, D.C.

Lt. Col. J. E. Webb, Jr., Office of the Surgeon General, Department of the Army, Washington, D.C.

Dr. H. E. Welch, Entomology Research Institute for Biological Control, Canada Department of Agriculture, Belleville, Ontario

Dr. C. G. Wessel, National Research Council, National Academy of Sciences, Washington, D.C.

Dr. Louis L. Williams, Pan American Sanitary Bureau, Regional Office of World Health Organization, Washington, D.C.

Dr. Gertraude Wittig, Insect Pathology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland