Effects of the Fungus *Lagenidium giganteum* Couch on Mosquitoes.

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ABSTRACT

Culex quinquefasciatus Say was used as a control organism in a study to determine if *Lagenidium giganteum* Couch was pathogenic to second-third instar larval stages of the mosquitoes *Psorophora columbiae* (Dyar & Knab) and *Anopheles quadrimaculatus* Say. This was done by homogenizing varying sections of sporulating agar plate cultures of *Lagenidium* in one liter of distilled water and spraying these one liter mixtures into pans containing second-third instar larvae of the three mosquito species. This study also attempted to find the concentrations of *Lagenidium* needed to give high larval mortality in the laboratory for all three mosquito species. Mortality induced by *Lagenidium* on all three species was erratic, and the concentrations needed to give high mortality for the mosquitoes were not determined. *Psorophora* and *Anopheles* were found to be susceptible to *Lagenidium* infections, and *Anopheles* was able to propagate the parasite. A preference test conducted with *Lagenidium* zoospores indicated that the zoospores are attracted to mosquito larval food.

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DEDICATION

This paper is dedicated to my parents, Dr. Telford H. Work and Dr. Martine Jozan Work whose assistance made my college education possible.

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INTRODUCTION

Forty years ago in Chapel Hill, North Carolina, John Couch (1935) isolated an aquatic fungus from Daphne and copepods and identified it as Lagenidium giganteum Couch. This fungus was also found to be a facultative parasite of mosquito larvae; however, experiments done by Couch (1935) on Lagenidium's effects on mosquitoes were inconclusive. After Couch's work, interest in this parasite became dormant until the early 1970's, and since then, there has been an increasing amount of research on L. giganteum's potential as a biological mosquito control agent.

McCray et al. (1973) found L. giganteum to be an infective parasite of the larval stages of the mosquitoes Culex tarsalis Coquillett and Aedes nigromaculis (Ludlow) and described the asexual and sexual cycle of L. giganteum. In the asexual cycle, motile reproductive stages of the fungus called zoospores are either ingested by the larva or attach to the larva's cuticle. Hyphae then grow from the spores into the larva, and these hyphae eventually become partitioned into round sporangia that encroach the larva's coelom. Larvae usually die at this stage of infection. One to two exit tubes then grow from each sporangia, and the contents of the sporangia flow out of these tubes and differentiate into zoospores. In the sexual cycle, male and female hyphal segments conjugate to form a zygote that

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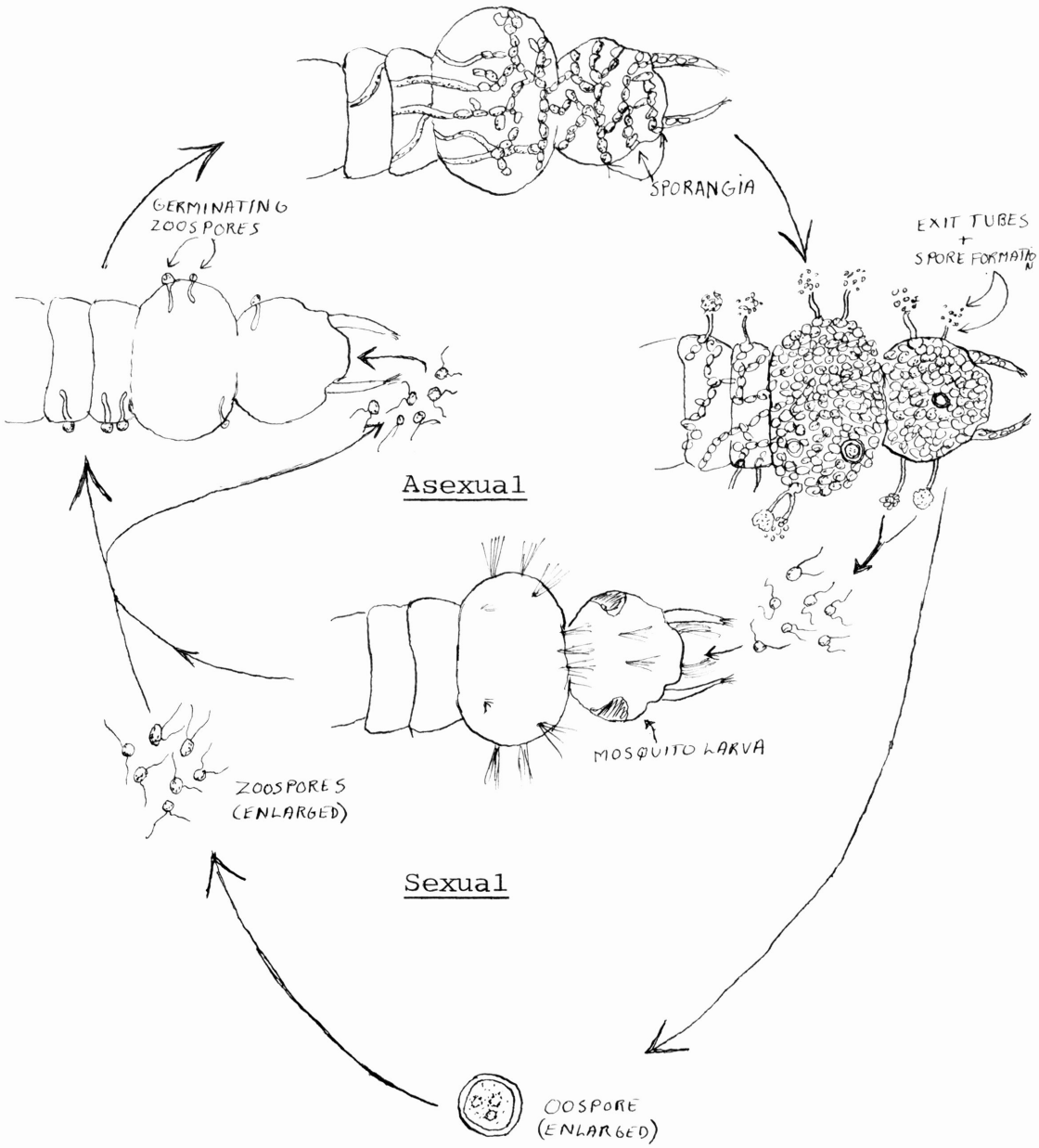
differentiates into an oospore. Oospores can remain dormant in dry conditions until stimulated by flooding to germinate and produce zoospores. These two life cycles are illustrated in Figure 1.

The ability of L. giganteum to remain dormant in the field was demonstrated by Fetter-Lasko (1977) who recovered the fungus in a ricefield one year after initial inoculation. In other test sites associated with ricefields, the fungus was found to be infective on mosquito larvae four years after initial inoculation (Fetter-Lasko 1977). This information is important since this aspect of L. giganteum could be used advantageously to control ricefield-breeding mosquitoes such as Psorophora columbiae (Dyar & Knab) and Anopheles quadrimaculatus Say, the mosquitoes included in portions of this study.

Ps. columbiae is a ricefield breeding mosquito that poses problems to human beings and animals in these areas because of its large populations and the aggressive feeding behavior of the female. According to Bishopp (1933), swarms of blood seeking Ps. columbiae caused the death of livestock in Florida and forced people in the area to wear heavy clothing as protection against the mosquitoes. A less severe case was reported by Steelman et al. (1973), who found that heavy densities of Ps. columbiae feeding on cattle could cause weight reductions of 0.3lb/head/day. In addition to the physical nuisance presented by this mosquito, it has been implicated by Davey et al. (1978) as a potential vector

FIGURE 1[a]

Life Cycles of Lagenidium giganteum



a.) Dr. Jim Kerwin (personal communication)

of Venezuelan Equine Encephalitis in horses and humans and Anaplasmosis (bovine anemia) in cattle.

An. quadrimaculatus like Ps. columbiae also breeds in ricefields, and it presents a danger to human and animal populations in the Southern and Southeastern portions of America since it is an established vector of malaria in those areas (Carpenter and LaCasse 1955). Lewandowski, Jr. et al. (1980) found that An. quadrimaculatus is also an excellent host for the dog heartworm as well as the most important potential vector of the parasite.

Controlling these ricefield breeding mosquitoes with aerial insecticide application might seem to be a solution to the problems that they harbor; however, as Olson[1] points out, ricefield farmers hesitate to do this, since they fear that the insecticides may react with herbicides applied to the fields, and this might result in the destruction of rice plants. Therefore, a possible solution to this problem would be to control An. quadrimaculatus and Ps. columbiae in their ricefield habitats by supplementing already existing biological control agents with L. giganteum. Such a solution would also appear to be ecologically sound since McCray et al. (1973) found that L. giganteum seems to be specific for mosquito larvae and does not affect other aquatic organisms. At the present time, there is no information regarding the infectivity of L. giganteum on Ps. columbiae and

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1.Personal communication

An. quadrimaculatus nor as to the fungus concentrations needed to give high mortality for these two species and Cx. quinquefasciatus in the laboratory. This study attempted to find this information.

Cx. quinquefasciatus was used in the study described herein for the following reasons. Since Cx. quinquefasciatus is known to be susceptible to infection by L. giganteum, this mosquito was used as a control organism in all of the experiments to ensure that the fungus used in any of the tests was indeed infective. The promise of L. giganteum as a control organism for Cx. quinquefasciatus in the field is slight however, since this mosquito species commonly breeds in polluted water, and Jaronski and Axtell (1982) found that L. giganteum operates poorly in such habitats.

MATERIALS AND METHODS

Source of Materials:

The L. giganteum cultures were grown on PYG agar (containing 1.25g/L peptone, 1/25g/L yeast extract, 3g/L glucose, and 1.2% agar supplemented with 25ppm cholesterol, 50ppm lecithin, and 1g/L corn oil) and sent in 3.5 inch diameter petri dishes from the University of California at Davis (U.C. Davis) Department of Entomology. Upon their arrival at Texas A&M University (T.A.M.U), these dishes were stored at 27^{o}C in a covered box containing a moist cellucotton pad. The Ps. columbiae larvae were raised from eggs laid by females captured in a field near South Bend (Chamber's County), Tx. The Cx. quinquefasciatus and An. quadrimaculatus larvae were raised from eggs obtained from the mosquito colonies housed in the T.A.M.U Mosquito Research Laboratory. This laboratory also provided other necessary material such as small (11.5 x 7 x 2 inch) and large (14 x 9 x 2 inch) enamel coated rearing pans, a pump up pressure sprayer, stand up incubators, a walk in incubator, a blender, and deionized water which was the only type of water used throughout this study.

Maintenance of Mosquitoes:

The wild caught Ps. columbiae adults were kept in 1 x 1 x 7 inch plexiglass cages that were open and covered with screen mesh on two long sides. Each cage held 10-15 adults,

and under each cage was a strip of cheesecloth on which the adults oviposited (Figure 2a). Five to six cages were placed side by side and screen side down in a large rearing pan that contained a cellucotton pad wrapped in cheesecloth and soaked in water. The pan was tilted on its long side, and enough water was placed in the pan so that a small pool of water formed on the bottom (Figure 2b). This provided a moisture gradient on the cellucotton pad so that the adults could find the most favorable site on the cheesecloth strip for oviposition. A small cellucotton pad soaked in 5% sucrose solution was placed at the top of each cage to provide food for the adults. The pans and cages were stored at 27°C in a stand up incubator.

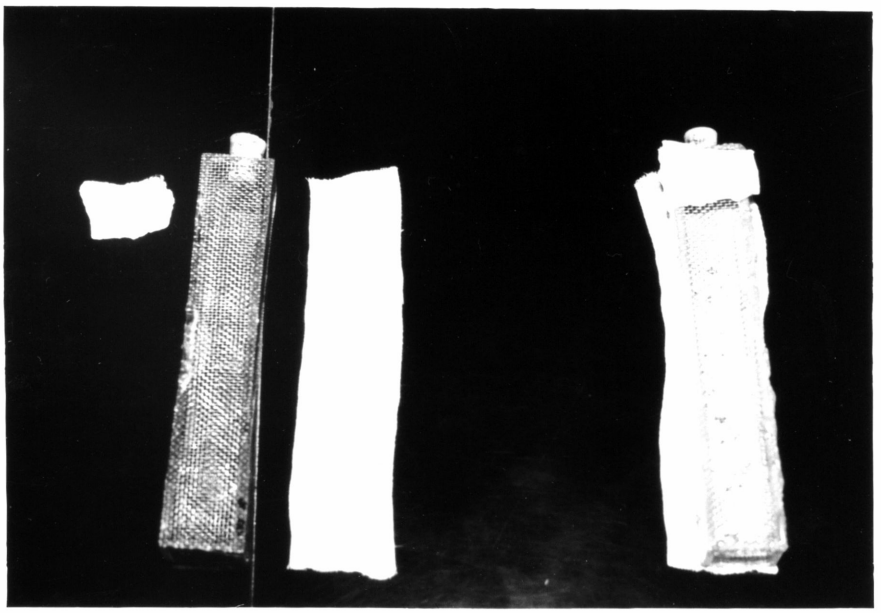
An. quadrimaculatus and Cx. quinquefasciatus adults were kept in 18 inch^3 cages made of a wooden floor, three plexiglass sides, a wooden door, and a screen roof. A wet cellucotton pad at the bottom of each cage maintained a humid environment, and each cage was kept in a walk in incubator (Figure 3) at 27°C. A baby food jar containing a 5% sucrose solution with a cellucotton wick provided food for the adults of both species.

Collection of Eggs:

The Ps. columbiae were fed on a human subject by wrapping 7 to 10 1 x 1 x 7 inch cages around the leg with tape so that the adults could feed through the screen. Two days after feeding, the females oviposited on the cheesecloth

FIGURE 2

a) Feeding pad, Psorophora cage, and cheesecloth strip. separate (left) and assembled (right)



b) Psorophora cages aligned on moisture gradient pad.

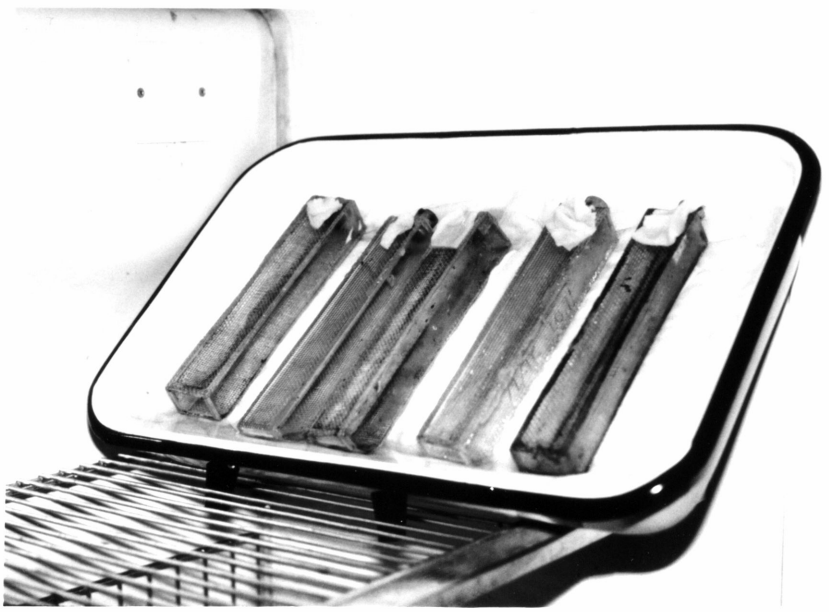
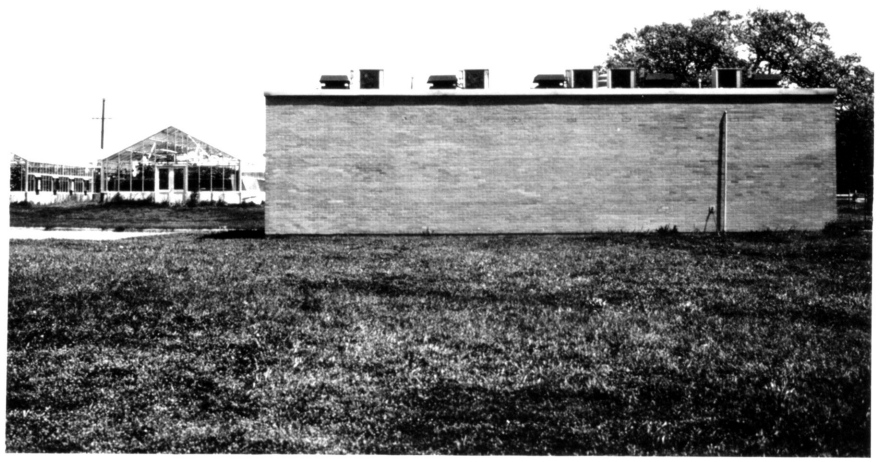


FIGURE 3

Walk in Incubator



strips. The eggs were rinsed off of the strips, filtered with a #120 sieve, and placed on one inch squares of filter paper in such a way that none of the eggs touched each other. Extraneous material was picked away from around the eggs with forceps to discourage fungus growth, and the eggs and filter paper were placed in covered petri dishes that held a moist cellucotton pad. The dishes were stored in a covered box containing a wet sponge to maintain a humid environment, and the box was stored in a stand up incubator at 27°C.

The An. quadrimaculatus were bloodfed by placing a guinea pig wrapped in cheesecloth in the mosquitoes' cage overnight. The same was done with Cx. quinquefasciatus except that the animal used was a chicken. Beakers of water were placed in the cages after the mosquitoes had fed, and both species oviposited in these beakers.

Hatching of Eggs:

Ps. columbiae were hatched by immersing a square of filter paper plus eggs in hatching solution which contained water mixed with a compound consisting of 3g/L bactobeef extract and 5g/L bactopeptone. Hatching continued for about 30 minutes, and the larvae were taken out as they hatched. The larvae were transferred into a solution of larval mosquito food which consisted of water mixed with a powder containing one part each of lactalbumin, lab chow, and brewer's yeast. The larvae were kept in the larval food solution for 8 hours and then were transferred into rearing pans

containing water. They were fed using an eye-dropper by placing spots of a slurry of larval food on the bottom of each pan.

Anopheles and Culex eggs were hatched by putting them in a rearing pan with water. The Cx. quinquefasciatus were fed in the same manner as Ps. columbiae, and the An. quadrimaculatus were fed a powder, consisting of 1 part brewer's yeast to 3 parts lab chow, that was sprinkled on the surface of the water. All pans containing mosquito larvae were covered with glass lids to prevent stray mosquitoes from laying eggs in the water.

Experimental Procedures:

The basic procedure for innoculating mosquito larvae with L. giganteum came from the U.C. Davis entomology department. They suggested taking 1/2 of a 3.5 inch diameter fungus pad and homogenizing it in one liter of water. By spraying this mixture over a one square meter (m^2) area containing Culex larvae, high mortality could be obtained. In order to place mosquito larvae in a 1 m^2 area, a "set" was used which consisted of 4 large (14 x 9 x 2 inch) rearing pans each containing 20 second and third instar mosquito larvae of the same species and 900 ml of water. These 4 pans were equally spaced in a 1 m^2 area and evenly sprayed using a pump up garden sprayer containing varying concentrations (1/4 pad, 1/2 pad, etc.) of the fungus in one liter of water (Figure 4). All innoculations were done

FIGURE 4

A set being sprayed with Lagenidium.

outside. The control sets (4 pans each) were taken outside, spaced in a 1 m^2 area with the lids removed, and left there for the amount of time needed to spray 1 liter of fungus solution; however, they were in fact not sprayed with anything. After the test, all pans containing Culex and Anopheles were kept in the walk in incubator at 27°C, and the pans with Psorophora were kept in the stand up incubator at 27°C. All larvae were fed once each day as described above throughout the post-treatment period. Different eye-droppers were used to feed the control and innoculated Psorophora and Culex in order to prevent cross contamination of the controls with the fungus. This was not necessary for Anopheles since the feeding apparatus for this species never came into contact with infected water.

After innoculating the sets, 2 days were allowed to pass in order for the fungus to establish itself, and on the second day, larval mortality data was initiated. Subsequently, mortality data was taken once each day for each set until all the larvae had either pupated or died. All larval cadavers were inspected microscopically for indications of fungal infection (presence of sporangia). At the end of each test, percent test mortality was calculated for each innoculated set by dividing the total number of dead larvae and pupae for a particular set by the total number of larvae originally present in that set. A percent control mortality was similarly calculated for the control set. Using the

percent test mortality (%T) and the percent control mortality (%C), a <u>percent corrected mortality</u> (%CM) was calculated for each innoculated set using Abbot's formula:

$$\%CM = \frac{\%T - \%C}{100 - \%C} \times 100$$

The percent corrected mortality was equal to 100, times the percent test mortality minus the percent control mortality, all divided by 100 minus the percent control mortality.

RESULTS

Pilot Test:

In the Pilot Test, Ps. columbiae, An. quadrimaculatus, and Cx. quinquefasciatus were all used, and each species was assigned 3 sets, one set for each fungus concentration and one control set (0 pad). Concentrations of 1/2 pad of fungus culture (in a 3.5 inch diameter petri dish), the concentration recommended by U.C. Davis, and 1 pad each mixed with 1 liter of water and sprayed over a 1 m^2 area were used in the test. The results of this test are shown in Table 1. For Psorophora and Culex, a majority of the larvae died within the first 2 days, and during that time, sporangia were found in the larval cadavers; however, as the days went on, the number of cadavers with sporangia gradually decreased, and all Psorophora and Culex larvae had either pupated or were dead by day 5. Anopheles larval mortality was more gradual, and all larvae were dead or had pupated by day 10. The later pupation for Anopheles was expected since this species develops more slowly than Psorophora or Culex. The frequency of sporangia in Anopheles cadavers also decreased gradually as the days progressed, and no sporangia or hyphae were noticed in control larvae of all three species tested. A problem that occured in this test and that had to be solved in later tests was to get a more accurate count of larvae in each pan. After this test, 7 tests labelled Test I - VII

TABLE 1.--Mortality inflicted by varying concentrations of *Lagenidium* cultures on *Cx. quinquefasciatus*, *Ps. columbiae*, and *An. quadrimaculatus*.

Mosquito species		Amount of fungus culture/liter of water/m^2		
		Set 1 (1/2pad[1])	Set 2 (1pad)	Control (0pad)
Cx. quinquefasciatus	%TM[2]	50	100	17
	%CM[3]	40	100	
Ps. columbiae	%TM	95	70	38
	%CM	92	52	
An. quadrimaculatus	%TM	78	80	54
	%CM	52	56	

1. pad = amount of culture held in one 3.5 inch diameter petri dish.
2. %TM = percent test mortality.
3. %CM = percent corrected mortality.

were conducted. The results of these tests are described below.

Main Tests:

Test I: The number of mosquito species included in this test was reduced to 2 species (Ps. columbiae and Cx. quinquefasciatus) in order to more accurately conduct the experiment. Also, methods were developed to provide a more accurate count of larvae in each pan. Again, 1 pad and 1/2 pad-fungus concentrations were used, and the results of this test are in Table 2. The larvae of the 2 species did not die as rapidly in this test as they did in the Pilot Test, and for both Psorophora and Culex, all larvae died or pupated by the fifth day. The larval cadavers of both species from innoculated pans had sporangia, and the control larvae of both species that died had no signs of Lagenidium infections. Since the supply of Psorophora eggs was limited, and because Anopheles took a long time to raise to second or third instar, it was decided to use only Cx. quinquefasciatus for the next few tests until the fungus concentration that would give the highest mortality for this species was found. This concentration would then be used on Ps. columbiae and An. quadrimaculatus and modified as needed for each species until high mortality was found for both species. Because higher mortality occured with the lower Lagenidium concentration for Culex in Test I, it was decided to further reduce the concentration in Test II.

TABLE 2.--Mortality inflicted by varying concentrations of Lagenidium cultures on Cx. quinquefasciatus and Ps. columbiae.

Mosquito species		Amount of fungus culture/liter of water/m^2		
		Set 1 (1/2pad[1])	Set 2 (1pad)	Control (0pad)
Cx. quinquefasciatus	%TM[2]	99	10	3
	%CM[3]	98	7.2	
Ps. columbiae	%TM	19	49	6
	%CM	14	46	

1. pad = amount of fungus culture held in one 3.5 inch diameter petri dish.
2. %TM = percent test mortality.
3. %CM = percent corrected mortality.

Test II: Cx. quinquefasciatus was the only mosquito species used in Test II, and the Lagenidium concentrations tested were 1/8 pad, 1/4 pad, 1/2 pad, 1 pad, and a control set (0 pad). Results of this test are shown in Table 3. All of the cadavers found in the 1/8 pad set were in between the larval and pupal stage, yet no sporangia or hyphae were found in those cadavers. The control cadavers were all in the larval stage of development, and had no signs of fungus infection.

Test III: Cx. quinquefasciatus was again the only mosquito used in Test III, and the Lagenidium concentrations were further reduced since higher mortality was encountered with the lower fungus concentration in Test II. The fungus concentrations used were 1/32 pad, 1/16 pad, 1/8 pad, 1/4 pad, and 0 pad (control). The results of Test III are shown in Table 4. All of the larval cadavers occuring in treated pans were in the same state as those found in the 1/8 pad concentration in Test II, and none of them had sporangia. During the interim period between Tests III and IV, a means for recognizing the fungal zoospores was learned, and it was partly as a result of this that some modifications to the remaining tests were made. Since before this time, it was not certain that the fungus pads that were being used were in fact sporulating, this situation was rectified by testing each agar fungus pad for sporulation before using it. This was done by scraping some Lagenidium from the top of a pad

TABLE 3.--Effects of varying concentrations of the fungus L. giganteum on the mosquito Cx. quinquefasciatus; Test II.

	Amount of fungus culture/liter of water/m^2				
	Set 1(1/8pad[1])	Set 2(1/4pad)	Set 3(1/2 pad)	Set 4(1pad)	Control (0pad)
%TM[2]	10	0	0	0	1.3
%CM[3]	9	0	0	0	

1. pad = amount of fungus culture in one 3.5 inch petri dish.
2. %TM = percent test mortality.
3. %CM = percent corrected mortality.

TABLE 4.--Mortality inflicted by varying concentrations of Lagenidium cultures on Cx. quinquefasciatus; Test III.

	Amount of fungus culture/liter of water/m^2				
	Set 1(1/32pad[1])	Set 2(1/16pad)	Set 3(1/8pad)	Set 4(1/4pad)	Control (0pad)
%TM[2]	36	1.25	1.25	0	0
%CM[3]	36	1.25	1.25	0	

1. pad = amount of fungus culture held in one 3.5 inch petri dish.
2. %TM = percent test mortality.
3. %CM = percent corrected mortality.

and immersing this sample in a petri dish of water. Eight hours later, the sample was checked microscopically for spore formation, and if spores were present, the agar pad was used. The other modifications made to the remaining tests was that the controls were handled before any contact was made with Lagenidium. Also, treatment of the larvae with fungus was performed on one side of a building, and the control larvae were set out on the other side of the building. These last two modifications were made to minimize the chances of cross contamination. It was during this interim period that the Ps. columbiae eggs became dessicated, thus this mosquito had to be dropped from further tests.

Test IV: In this test, Cx. quinquefasciatus larvae were used, and the fungus concentrations tested were the same as those of Test III. The results of Test IV are shown in Table 5. Like Test III, the highest mortality was with the lowest Lagenidium concentrations, and all larval cadavers from treated pans were identical to those in Test III. All of the control cadavers were in the larval stage, and none of the cadavers from either control or innoculated pans had sporangia. All of the cadavers were filled with lipid like droplets, and these were put in vials of water in the hope that these droplets were immature oospores. However, no zoospores were ever formed, and the droplets eventually disappeared.

Test V: Cx. quinquefasciatus was again the species used in this test; however, since Test IV results still indicated

TABLE 5.--Effects of varying concentrations of Lagenidium cultures on the mosquito Cx. quinquefasciatus; Test IV.

	Amount of fungus culture/liter of water/m^2				
	Set 1(1/32pad[1])	Set 2(1/16pad)	Set 3(1/8pad)	Set 4(1/4pad)	Control (0pad)
%TM[2]	15	0	0	0	1.3
%CM[3]	14	0	0	0	

1. pad = amount of fungus culture held in one 3.5 inch petri dish.
2. %TM = percent test mortality.
3. %CM = percent corrected mortality.

that higher mortalities were occuring with the lower Lagenidium concentrations, these were reduced further to 1/256 pad, 1/128 pad, 1/64 pad, 1/32 pad, and a control set (0 pad). The results of this test are shown in Table 6. Again, all cadavers from treated pans were in between the larval and pupal stage, and none had sporangia. The control cadavers were in the larval stage of development, and they also had no sporangia.

Test VI: This test was an exact repeat of Test V except that in Test VI, there was 0% mortality for all fungus concentrations used.

Test VII: In this test, higher fungus concentrations (3/4 pad, 1/2 pad, 1/32 pad, and 0 pad) were used than had been the case in most of the previous tests. This was done since the Pilot Test indicated that perhaps higher fungus concentrations would give higher larval mortality for An. quadrimaculatus and Cx. quinquefasciatus, the mosquito species used in Test VII. The results of this test are in Table 7. The 1/32 pad concentration was used to insure that this concentration did not give high larval mortality. For Culex, all cadavers from treated pans were in the larval stage and had sporangia. For Anopheles, most of the cadavers were in the larval stage and had sporangia; however, some dead pupae with sporangia were also found. One Anopheles larva that was infected had a larger concentration of sporangia at the tail end that gradually decreased towards the head area. This was unusual since all infected larval

TABLE 6.--Effects of varying concentrations of the fungus *Lagenidium giganteum* on *Cx. quinquefasciatus*; Test V.

	Amount of fungus culture/liter of water/m^2				
	Set 1(1/256pad[1])	Set 2(1/128pad)	Set 3(1/64pad)	Set 4(1/32pad)	Con.[2](0pad)
%TM[3]	0	0	8	21	1.3
%CM[4]	0	0	7	20	

1. pad = amount of fungus culture held in one 3.5 inch petri dish.
2. Con. = Control
3. %TM = percent test mortality.
4. %CM = percent corrected mortality.

TABLE 7.--Mortality inflicted by varying concentrations of Lagenidium on An. quadrimaculatus and Cx. quinquefasciatus.

		Amount of fungus culture/liter of water/m[2]			
		Set 1 (1/32 pad[1])	Set 2 (1/2 pad)	Set 3 (3/4 pad)	Control (0 pad)
A[2]	%TM[4]	11	56	65	23
	%CM[5]	0	43	54	
B[3]	%TM	0	1.2	35	0
	%CM	0	1.2	35	

1. pad = amount of fungus culture in one 3.5 inch petri dish.
2. A = An. quadrimaculatus
3. B = Cx. quinquefasciatus
4. %TM = percent test mortality.
5. %CM = percent corrected mortality.

cadavers were either uniformly filled with sporangia or had heavier concentrations of sporangia at the head region that gradually decreased towards the tail region. The high larval mortality in the Anopheles control set was probably due to cross contamination since some of the cadavers in that set had sporangia.

Other Observations:

In addition to the above tests, a preference test with Lagenidium zoospores was performed to see if they were perhaps attracted to the larval mosquito food at the bottom of the Culex and Psorophora pans. To determine this, a small portion of fungal hyphae in water was allowed to sporulate in the well of a depression slide. A small block made of water and unflavored gelatin was placed on one end of the slide, and another block made of unflavored gelatin mixed with larval food was placed on the other end. The gelatin blocks were simultaneously connected to the center well with a small strip of water. This allowed the contents of the gelatin blocks (food or water) to slowly diffuse into the water strips. This also provided a path from the center well to either end of the slide for the motile zoospores. After 15 minutes, spores were counted on each side of the slide in the area of water between the gelatin block and the edge of the well (Figure 5). The results of this test are in Table 8. A total of 12 of these tests were conducted.

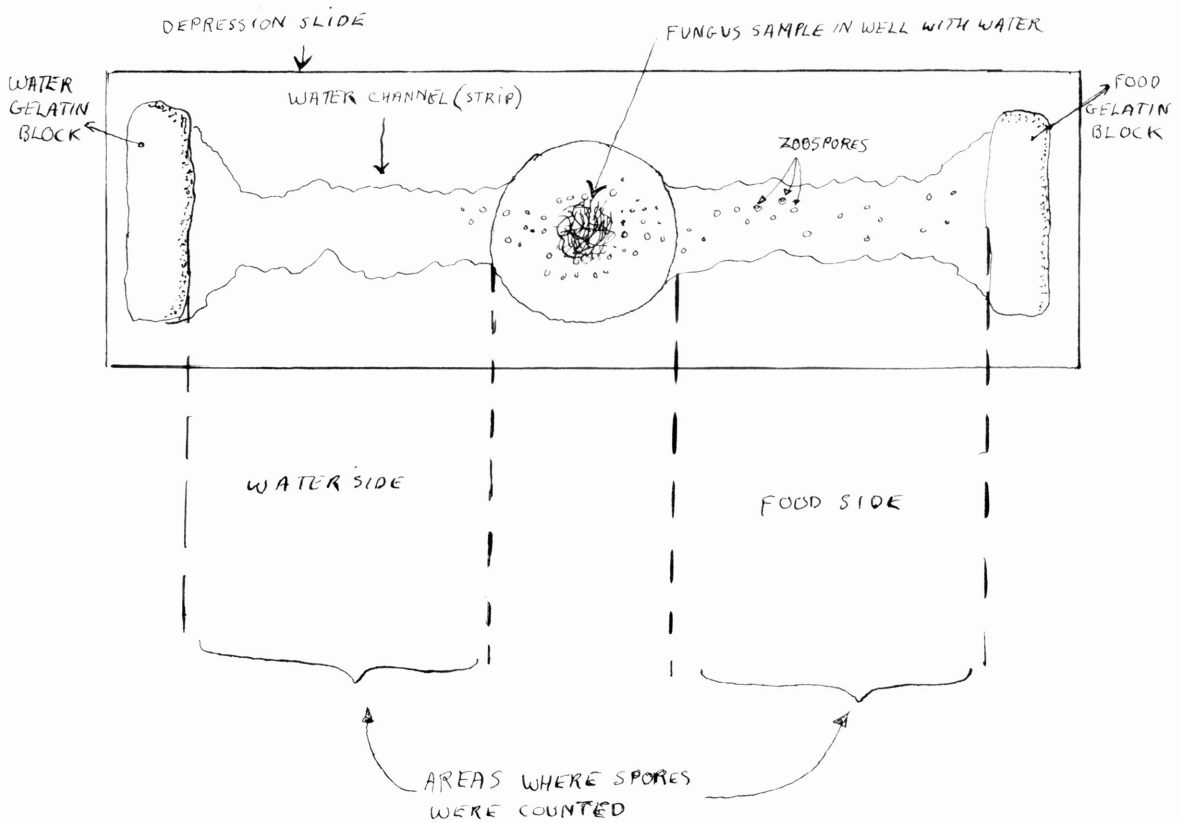
TABLE 8.--Preference indicated by Lagenidium zoospores when presented with the choice of larval mosquito food or distilled water.

Trial no.	No. spores on food side[1]	No. spores on water side[2]
1	37	22
2	45	18
3	42	16
4	48	17
5	37	21
6	19	6
7	72	11
8	135	17
9	15	16
10	126	34
11	83	11
12	15	6

1. food side = end of microscope slide containing gelatin-food block.
2. water side = end of microscope slide containing gelatin-water block.

FIGURE 5

Components of the Preference Test



CONCLUSIONS

One of the major problems encountered with L. giganteum was that it behaves erratically in the laboratory. As can be noted by the results described herein, a particular concentration of fungus might result in high mortality in one test and little or no mortality in another. This trend continued in Tests IV - VII even after the fungus cultures that were going to be used were checked for sporulation. According to Dr. Jim Kerwin[3] at the U.C. Davis entomology department, such results have also been encountered with the U.C. Davis laboratory assays concerning this fungus and its infectivity towards mosquitoes. In the tests just described, the higher mortalities (80% - 90%) for all species seemed to occur with the higher fungus concentrations (1/2 pad and up). It was also only with these concentrations that sporangia were observed in larval cadavers. It was not determined why mortality occured at the 1/8 pad and 1/32 pad concentrations, nor as to why mosquito death at those concentrations occured between the larval and pupal stages. Since molting from a larva to a pupa is a stressful period for an insect, this stress may have been amplified by the presence of Lagenidium and caused larval death; however, this could not be verified since no signs of fungus infection were observed in these cadavers.

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3. Personal communication.

The majority of larval cadavers (all 3 species) with a heavier concentration of sporangia at the cephalic region implied that the route of infection for Lagenidium zoospores could have been via the ingestion of these spores by the larva or by the penetration of the cuticle in the head region; however, this information could not be determined. The one Anopheles larva (Test VII) with a higher concentration of sporangia in the tail region indicated that the zoospores probably started infecting the larva in that area; yet, this could not be determined. These results were similar to those of McCray et al.(1973) who infected Cx. tarsalis larvae with Lagenidium and found that a predominance of the larvae tested were infected via the head region; however, in 2% of the larvae, infection started at the tail region. It was not known if the dead Anopheles pupae with sporangia had been infected during the pupal stage or if the fungus infection carried over into the pupal stage when the larva molted.

In this study, it was determined that An. quadrimaculatus was susceptible to L. giganteum. The presence of exit tubes and the formation of zoospores out of these tubes also indicated that this mosquito species was able to propagate the parasite. These results are in contrast to those of Umphlett and Huang (1972) who infected An. quadrimaculatus with a species of Lagenidium yet were unable to determine if this fungus was L. giganteum. The

results described herein also contradict those of McCray et al. (1973) who found that L. giganteum did not infect An. quadrimaculatus. Ps. columbiae was also found to be susceptible to L. giganteum; however, it cannot be stated whether exit tubes were formed in this species since at the time Psorophora was being tested, the ability to recognize the tubes had not been developed.

The data from the preference experiments indicated that Lagenidium zoospores seemed to prefer the food side of the slide. This spore preference may have affected the results of the susceptibility tests involving Ps. columbiae and Cx. quinqefasciatus (who are both bottom feeders) by the fact that the majority of zoospores may have gone to the larval food at the bottom of the pans. This would have reduced the chances of contact between spores and mosquitoes on the surface of the water and increased the chances of contact between the spores and mosquitoes that fed at the bottom of the pans.

In summary, it appears that L. giganteum is a promising biocontrol agent for An. quadrimaculatus and Ps. columbiae; however, more work is needed to determine the doses of fungus necessary to give optimum mortality for these two species in the laboratory and in the field.

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