OCCURRENCE OF THE FUNGUS <u>COELOMOMYCES</u> IN LARVAL POPULATIONS OF NORTH CAROLINA SALT MARSH MOSQUITOES

by

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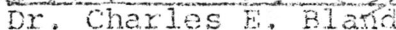
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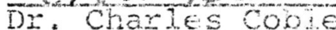
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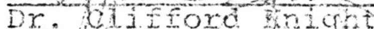
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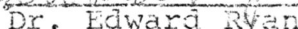
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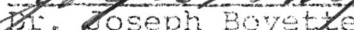
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ABSTRACT

Dale Ross Kiser. OCCURRENCE OF THE FUNGUS COELOMOMYCES IN LARVAL POPULATIONS OF NORTH CAROLINA SALT MARSH MOSQUITOES. (Under the direction of Dr. Charles E. Bland) Department of Biology, August, 1976

The aquatic larval stages of salt marsh mosquitoes were collected from natural breeding pools in designated marsh areas. Collections were made from March, 1974 until October, 1974. Pertinent environmental data was recorded for each collection. Each larva collected was identified to species and microscopically screened for signs of fungal infection. The overall level of infection for all species of salt marsh mosquito by the fungus *Coelomomyces* was calculated at 1.25%. Only *Aedes taeniorhynchus* was found to be infected. No single environmental parameter that was measured appears to have a direct influence on infection levels, although possible relationships are noticed. Germination experiments reveal that high salinity has a generally inhibitory effect on sporulation.

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OCCURRENCE OF THE FUNGUS *COELOMOMYCES* IN LARVAL POPULATIONS OF NORTH CAROLINA SALT MARSH MOSQUITOES

A Thesis

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Dale Ross Kiser

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INTRODUCTION AND REVIEW OF LITERATURE

Fungi of the genus *Coelomomyces* are obligate, internal parasites of mosquitoes. To date over thirty species of this genus have been described (Couch and Umphlett, 1963; Madelin, 1966; Pillai and Woo, 1973) with at least eleven occurring in the United States.

Although *Coelomomyces* has been collected previously from North Carolina, it has never been reported from North Carolina salt marsh mosquitoes. It was thus the objective of this study to collect *Coelomomyces* from North Carolina salt marsh areas and to monitor and analyze any environmental variables which may have influenced this infection among larval populations.

The ovoid, resting sporangia of this fungus were originally described by Keilin (1921) from a single specimen of *Stegomyia scutellaris* collected in Malaya. Iyengar (1935) described two new species of *Coelomomyces* from mosquito larvae of the genus *Anopheles* collected in India. In his observations, Iyengar noted the tubular, dichotomously branched hyphae as well as the thick-walled, resting sporangia of this fungus. Based on these characteristics, Iyengar tentatively placed the newly discovered organism among the Chytridiales. However, Couch (1945), noting similarities between the wall structure of the

resting sporangium of Coelomomyces and that of certain species of Allomyces as well as the rather extensive mycelial development of Coelomomyces, placed the organism in the order Blastocladiales and in a new family, the Coelomomycetaceae. This classification has been accepted by most taxonomists.

Although Coelomomyces has been reported from a species of the genus Notonecta (Backswimmer) collected in Russia (Bogoyavlensky, 1922), and from Simulium metallicum (Black Fly) (Garnham and Lewis, 1959), a Dipteran native to British Honduras, the principle host organism is the mosquito. Usually confined to the aquatic larval stages, several authors have also observed mycelial masses and resting sporangia in the hemocoele (Manalang, 1930) and in the ovaries of adult females (Walker, 1938; Van Thiel, 1953; Lum, 1963). The latter could provide a primary means of distribution of the fungus with sporangia possibly passing out of the ovaries and into new habitats as the eggs are laid. Past observations have also indicated a rather high degree of host specificity for the various species of Coelomomyces. At least thirteen species of Coelomomyces are known to parasitize only one species of host mosquito (Couch and Umphlett, 1963). In addition, the species of Coelomomyces which attack more than one species of mosquito host usually occur as different varieties.

Development of Coelomomyces occurs entirely within the hemocoele of the larval host. Although no definite

rhizoids have been observed, the hyphae appear to be attached to the fat bodies or other internal tissues from which the parasite draws nourishment. Subsequent destruction of these structures usually results in death of the larva before pupation. The appearance of spherical or irregularly shaped cell bodies called "hyphagens" in the hemolymph of the host is the first indication of infection. These structures are recognized by the slightly darkened cytoplasm and clusters of lipid droplets surrounded by what appears to be a single plasma membrane. Martin (1969) first described these bodies in second instar larvae of *Anopheles quadrimaculatus* and observed that they appeared to serve as the growth centers for hyphae. The hyphagens of *Coelomomyces punctatus* ranged in size from 5.4 to 20.3 microns in diameter. They were observed either floating freely within the hemolymph or adhering to host tissues. Slightly larger hyphagen growth centers were observed in third instar larvae, some of which could proliferate as many as eighteen individual hyphae. Branching of these coenocytic hyphae in subsequent development appeared to be irregular with the cytoplasm becoming dense and granular (Martin, 1969). As mycelial development proceeds, some of the hyphal tips undergo a slight swelling, eventually forming spherical, bulbous structures which contain a hyaline cytoplasm (Umphlett, 1964). The hypha then pinches inward at the base of the young sporangium, which has now become ovoid and developed a thickened cell wall. The

pinching process continues until the last remaining threads of the hyphal membrane are severed and the sporangium is free within the host hemocoele. Further development in most species of Coelomomyces results in the formation of a thick outer sporangial wall which is usually pitted or ridged. Sporangial maturation is essentially complete with the appearance of a germination slit or line of dehiscence (Couch and Dodge, 1947) in the outer wall.

The precise mechanism of host infection by Coelomomyces remains largely a mystery. For many years following the discovery of this fungus, the prevailing theory was that the posteriorly uniflagellate swimming zoospore was the infective unit. The exact mechanism of spore entry into the host hemolymph remained an unanswered question. But many experts believed that resting sporangia derived from the bodies of larvae of previous generations killed by the parasitic action of the fungus were ingested by actively feeding larvae and ultimately led to infection.

Recent studies have confirmed the existence of an alternate host in the life cycle of at least one species of Coelomomyces. Whisler, Zebold, and Shemanchuck (1974) have obtained high levels of laboratory infection of Culiseta inornata with Coelomomyces psorophorae by the addition of copepods of the species Cyclops vernalis to pans containing water and uninfected mosquito larvae. These workers observed what appeared to be an unwalled plasmodium and gametes within the copepod body cavity. It

was proposed that this represents the sexually reproductive phase in the life history of *Coelomomyces*, with meiosis presumably occurring within the resting sporangia. The posteriorly uniflagellate zoospores from these sporangia would therefore be meiospores and would enter the body cavity of the copepod host to produce a haploid plasmodium. The plasmodia later differentiate haploid, isogametes which fuse either inside the copepod body cavity or in the external aquatic environment to form diploid, biflagellate zoospores which in turn infect the mosquito larvae.

Since its discovery by Keilin in 1921, researchers have viewed *Coelomomyces* with interest regarding its possible application as a biological control agent for populations of mosquito pest species. Laboratory studies have consistently demonstrated the ability of certain species of this genus to attack and attain high levels of infection among cage populations of various species of mosquito larvae. One of the primary factors which may influence these infection levels is sporangial germination. Experiments have indicated that spore discharge can be hastened by both high temperature and low oxygen concentration (Couch, 1967; Couch and Umphlett, 1963). Other workers, notably Pillai and O'Loughlin (1972), have studied effects of salinity and temperature on spore discharge and motility. Results indicate that the optimum temperature for sporulation is about 23°C for *Coelomomyces psorophorae* and *C. punctatus* (Couch, 1967), but that sporulation will

occur within a range of 10° - 35°C. Germination of resting sporangia of Coelomomyces opifexi (Pillai, 1971) has been obtained at temperatures as low as 5°C and at salinities as high as 17o/oo. Although inconclusive, these tests indicate that none of the above environmental factors is absolutely critical for the release of zoospores, but that a combination or interaction of one or more of the above may facilitate sporulation.

Preliminary success at artificial infection was obtained by Walker (1938) using a larvae of Anopheles gambiae reared in an outdoor concrete tank and infected with Coelomomyces africanus. Muspratt (1946) also obtained infection of Anopheles gambiae with an unknown species of Coelomomyces. He observed an infection level of 15% in one experimental rearing. Laird (1956) recorded the first successful laboratory infection between two different species of mosquitoes. In this experiment, larvae of Aedes aegypti were infected with C. stegomyiae derived from the bodies of Aedes albopictus. The A. aegypti larvae were reared in pans of distilled water buffered to pH 6.6 and containing dried sporangia of C. stegomyiae as well as sediment from the original container which held parasitized A. albopictus larvae. Infection of previously uninfected A. aegypti larvae was observed. Studies using larvae of Anopheles quadrimaculatus infected with Coelomomyces punctatus by Couch (1967) have also produced high levels of laboratory infection. Couch's method of placing germinating

and "go" stage sporangia in a rearing pan with hatching eggs of A. quadrimaculatus yielded infection levels of up to 100%. Rearing pans contained soil from the original collection site. Infection was also observed in pans which contained no soil from the collection site as well as in pans containing rain water, lake water, and aerated tap water. Madelin (1968) also reported success with laboratory infection of Anopheles gambiae larvae by Coelomomyces indicus in both circular polyethylene basins and larger, rectangular, porcelain pans. The results indicated several conditions that appeared to facilitate infection. These factors included the presence of mopane clay from the infection area, daily illumination of the infection pans, a water temperature of 28-32°C, and a pH of 7.8 to 8.0.

Another successful cross-infection experiment was performed by Pillai (1969), using inoculum of Coelomomyces opifexi derived from Opifex fuscus. These results are especially interesting because both species of mosquito larvae share the same natural habitat. Thus the possibility arises that Aedes australis may serve as an additional host to Opifex fuscus in natural conditions. Continuous infections of Aedes australis larvae with Coelomomyces opifexi have been maintained by Pillai since 1969. Possible correlations between the infection levels in cage populations of A. australis and such factors as salinity and temperature have been indicated (Pillai and Woo, 1973). The most extensive work with laboratory

infection has been undertaken by Couch (1972), using Coelomomyces punctatus to infect Anopheles quadrimaculatus. Infection was observed in lake water, rain water, and snow water within a temperature range of 18-35°C and in a variety of artificial containers. Good success was obtained in pans without soil from the collection area, and results indicated that the presence of certain species of unicellular green algae is valuable in increasing infection levels.

A significant discovery resulting from these studies was that infection levels of nearly 100% with high rates of mortality could be obtained by adding germinating sporangia to the infection pans at intervals coinciding with larval ecdysis. Although the above laboratory studies have indicated possible relationships between the levels of infection and pH, salinity, temperature, presence of aquatic biota, and other environmental variables, Coelomomyces appears to have a wide range of tolerance to most of the factors that have been tested.

To further evaluate the biological control potential of Coelomomyces there have been attempts to introduce certain species of Coelomomyces into natural, parasite-free populations of mosquitoes. Success has been limited, but Laird and Colless (1962) managed to introduce C. stegomyiae into a healthy population of Aedes polynesiensis on an atoll in the Toukelau Islands, and observed some population reduction. Also, Coelomomyces indicus was successfully

transmitted to an uninfected population of *Anopheles gambiae* in Zambia by Muspratt (1963). Couch (1972) obtained limited success with field tests using *Anopheles quadrimaculatus* and *C. punctatus*, although the mosquito population in this case was not a naturally breeding one.

Although these laboratory studies are useful in providing a means to observe the morphology, cytology, and biology of host infection of *Coelomomyces*, they provide no insight as to the actual occurrence and distribution of the fungus among naturally breeding mosquito populations. The artificial infection of both cage and wild larval populations has, however, given an indication of the actual and potential host ranges for particular species of *Coelomomyces*. There has also been evidence as to the possible ecological variables that might prove limiting to both the incidence of the organism among natural populations of mosquitoes and the levels of infection that may be reached by various species of *Coelomomyces* in a given environmental situation. More information on the ecology of infection is necessary, however, before the results of laboratory studies can be efficiently applied to field situations. Understanding the subtleties involved in the parasites' behavior among natural populations of mosquitoes will be useful in the manipulation of the fungus in regard to the pressure that it places on the target population of insect pests. This knowledge will aid in the evaluation of the overall potential of *Coelomomyces* as an effective biological control agent.

Coelomomyces has been reported from every major continent and sub-continent except the sub-artic American regions (Laird, 1959). It was first reported in the United States by Couch (1945) from specimens of *Psorophora ciliata* collected by the Georgia State Malaria Control Authority in 1942. The new fungus was described by Couch as *Coelomomyces psorophorae*. From material sent subsequently to him by H. R. Dodge, Couch described four additional species of *Coelomomyces*. In a second publication, Couch and Dodge (1947) included the description of six more previously unknown species of *Coelomomyces*, as well as two new varieties. The fungus has since been reported from Canada in the larval host *Culiseta inornata* (Shemanchuk, 1959), from Louisiana in the hosts *Culex restuans*, *Culex salinarius*, *Aedes sollicitans* (Chapman and Woodard, 1966) and *Toxorhynchites rutilus* (Nolan *et al.*, 1973), from Mississippi in *Psorophora ciliata*, from Minnesota in *Aedes vexans*, *Anopheles walkerei*, and *Anopheles earlei* (Laird, 1961), and from Florida in *Psorophora howardii* and *Aedes taeniorhynchus* (Lum, 1963). Collections by Shemanchuk (1959) of larvae of *Culiseta inornata* from irrigation ditches in southern Alberta showed 12% of the larvae to be infected with *Coelomomyces psorophorae*. Although field collections by Lum (1963) turned up both infected adults and larvae, no specific environmental parameters were measured. An infection level of 5% was indicated for natural larval populations of *Aedes*

taeniorhynchus from these data. Chapman and Woodard (1966) tabulated data on infection levels in wild populations of eight different species of mosquito larvae from Louisiana. These levels ranged from less than 1% in *Aedes vexans*, *Aedes taeniorhynchus*, and *Aedes sollicitans* to as high as 50% in certain wild populations of *Culiseta inornata*. Data from field observations also indicated the conspicuous absence of infection in many companion species which inhabited the same breeding area with infected species. Many of these co-inhabitants were known to harbor *Coelomomyces* in other locations. A study conducted in Egypt by Gad, Sadek, and Fateen (1967) revealed an infection level of 27.4% of the fourth instar larvae of *Culex antennatus* with *Coelomomyces indicus*, compared with a figure of 4.7% infection of the larvae of *Anopheles pharoensis*.

In addition to studies concerned purely with occurrence and distribution, there have been several investigations concerning the level of infection by *Coelomomyces* among natural mosquito populations and the ecological factors which may regulate these infections. Muspratt (1963) observed a mortality rate of nearly 100% in larval populations of *Anopheles gambiae* infected with a species of *Coelomomyces* collected from the Livingstone province of Northern Rhodesia. Temperature and pH readings were taken but no differences between infected and uninfected pools were noted with respect to these factors.

Umphlett (1968, 1969) studied the environmental factors affecting the incidence of infection of *Anopheles quadrimaculatus* with *Coelomomyces punctatus*. Beginning in 1966, samples of larvae were taken at weekly intervals from two different coves (designated as A and B) in a small lake near Chapel Hill, North Carolina. During this study no infected larvae were taken from cove B, which was designated as a control area. Data on dissolved oxygen, pH, air temperature, and relative humidity were recorded, as well as notes on aquatic vegetation. No precise correlations between the levels of infection and any of the above variables were found, but possible relationships between pH, dissolved O_2, and temperature ranges, and the occurrence of *Coelomomyces* were indicated. Umphlett also noticed a steadily decreasing level of infection during the three year period. Although differences in temperature ranges, pH, and dissolved oxygen between the two coves were apparent in the 1966 survey, these factors seemed less likely to be biologically limiting according to measurements taken in 1967.

Chapman and Glenn (1972), in a four year study of field observations beginning in 1966, recorded incidence of infection of two separate larval populations of *Anopheles crucians*; one by *Coelomomyces punctatus* and the other by *C. dodgei*. The infection pattern observed for *C. punctatus* in this study was similar to that observed by Umphlett for the same species. However, a two year study of *C. dodgei*

in a separate population of *Anopheles crucians* revealed an increase in the level of infection. Finally, Pillai (1971), in a three year study beginning in 1967, recorded temperature, hydrogen ion concentration, and salinity of supralittoral pools on rocky outcrops of the southeastern coast of New Zealand. These pools contained larvae of *Aedes australis* and *Opifex fuscus* which in 21 of the 400 pools surveyed were parasitized by *Coelomomyces opifexi*. Data also indicated possible relationships between the presence and levels of fungal infection and salinity ranges.

Although numerous studies have been made concerning *Coelomomyces* occurring on fresh water mosquito species, data on *Coelomomyces* infections of salt marsh mosquito species is limited. *Coelomomyces* has been reported in *Aedes taeniorhynchus* collected from Florida (Lum, 1963) and in *Aedes sollicitans* from Louisiana salt marshes (Chapman and Woodard, 1966). However, no ecological data was recorded during these investigations. Thus, little is known about the ecology of *Coelomomyces* infections in eastern United States salt marsh areas. The purpose of the present study was to determine presence and to what extent *Coelomomyces* occurred among extant larval populations of mosquitoes in North Carolina salt marshes. The data presented constitutes the first report of a naturally occurring infection in a North Carolina salt marsh mosquito species.

MATERIALS AND METHODS

The general study area for this project was the Atlantic coastal region of eastern Carteret County, North Carolina. Mosquito larvae were collected from temporary pools found in the extensive *Juncus* marshes which cover these areas. The two most abundant species of mosquito present in these areas are *Aedes sollicitans* and *Aedes taeniorhynchus*, both of which are known to serve as hosts for certain species of *Coelomomyces*.

Sampling Procedure:

Collection of larvae was begun on March 21, 1974 and continued until October 24, 1974, after which no larvae were found. Collections were made in six marsh areas (Fig. 1), each of which contained from one to five pools which were sampled continually. Each pool was marked and numbered with a short stake for identification. Most pools averaged less than 3.05 meters in diameter and 0.6 meters in depth. Although not subject to regular tidal flooding, some were occasionally inundated by irregular high tides and salt spray which influenced the salinity of many pools. The amount of salt spray received was dependent on the proximity of the pools to the sound water. Pools were normally flooded with rain water which made collection of larvae dependent upon rainfall patterns. During extensive droughts, all pools became dry.

When larvae were present in the pools, samples were taken with a 0.49 litre aluminum dipper using fifteen dips per pool. The number of dips was often decreased to five or ten according to the number of larvae present and water level fluctuations. Sampling was carried on weekly between March 21 and May 25. Subsequent sampling was conducted on a daily basis. Collected larvae were transferred to glass jars labeled to correspond with the pool from which each sample was taken. Specimens were then transported to the laboratory to be microscopically screened for signs of fungal infection. The procedure for screening involved placing rows of larvae on a glass slide and observing each specimen under 100X magnification. Fourth instar larvae were screened within a day following collection. Earlier instars were placed in holding pans for one to three days so that development of the fungus could continue to the point that infection could be observed. Species identification was made of all third and fourth instar larvae, and of earlier instars if possible. All larvae which could not be positively identified were excluded from the data.

Air and water temperature measurements were taken from each location at the time of collection. Air temperature measurements were taken in partially shaded conditions. Salinity readings were also taken at the time of collection with an American Optical model 10402 Goldberg T/C refractometer, and converted into parts per

thousand. Water samples from each pool were analyzed for pH with a Fisher Accumet model 220 pH meter in the laboratory. Collections of specimens were carried out at approximately the same time of day, usually between 9:00 A.M. and 12:00 noon.

Infected larvae were separated from the samples and stored on a double sheet of Whatman (#1) filter paper. The paper was moistened and placed in a petri dish, covered, and refrigerated at 10°C. The filter paper was periodically remoistened to prevent complete dessication of the resting sporangia contained within larval bodies.

Germination Experiments:

Germination of resting sporangia was accomplished using the damp chamber method devised by Couch (1967) in which infected larvae are dissected in a drop of water on a glass slide. The slide is then placed on a glass block which rests atop a sheet of moist filter paper enclosed in a petri dish. This apparatus was maintained at a temperature range of 18° to 20°C for five to eight days during which time the slides were periodically removed and inspected. When approximately 50% or more of the sporangia were observed to exhibit the characteristic lateral bulge commonly called the "go" stage (Couch, 1967), a cover slip was placed over the preparation to stimulate sporulation. After eight days in the damp chamber, cover slips were added regardless of the number of "go" stage sporangia present.

Sporangial germination success was tested in distilled water, natural pool water at salinities of 5.5 and 20.5 parts per thousand, and in saline solutions of 10, 15, 20, 25, 30, and 35 o/oo. Each solution was tested using three damp chambers, and an estimate of the percentage of "go" stage sporangia which germinated was recorded.

RESULTS

Table I indicates the various species of mosquito larvae that were collected during the seven month study period, as well as the total number of each species of mosquito, the pools from which they were collected, and the percentage of the cumulative total that each species represents. From these data *Aedes sollicitans* and *Aedes taeniorhynchus* are shown to be the two most prevalent and widely distributed species among the larval populations sampled. Collectively, they accounted for 89.2% of all larvae collected. These species were followed in order of prevalence by *Culex salinarius* and *Anopheles bradleyi*.

Description of Collection Area:

The Ward Creek Area (Fig. 2) contained five pools (101-105) from which larvae were routinely collected. High levels of breeding were noted in pools 102, 103, and 105 throughout nearly the entire study period, with 102 and 103 accounting for 60.3% of all larvae collected. Dominant plant species in this area were *Juncus roemerianus*, *Spartina patens*, and *Myrica cerifera*. *Baccharis sp.* was occasionally observed in the area. Although pools 101 to 104 were all in close proximity to the sound, only 104 was ever observed to be inundated at high tide. Pools 102 and 103 were contiguous with a marsh area which lay between

them during periods of high water. They were treated as separate pools because the water connection was dry during most of the period when larvae were collected. Pool 105 was a large brackish water pool of approximately 0.2 hectare and with an average water depth of 0.15 meters. This pool was arbitrarily designated as a control because of the apparent absence of *Coelomomyces* and the more consistent breeding of salt marsh mosquitoes that occurred within it.

Collection area two (Fig. 1) was located at the edge of a ditched *Juncus* marsh. Breeding was low in the three pools sampled; broods of mosquitoes were produced only sporadically during the study.

A third area from which larvae were routinely collected was the King Point marsh. This was a large, natural marsh dominated by *J. roemerianus* and *S. patens*, with large quantities of *Distichlus spicata* and *Limonium carolinianum*. Of five pools sampled, breeding was observed in two.

The fourth collection site contained three pools and was located near a trash dump at the edge of a ditched *Juncus* marsh near Stacy, North Carolina (Fig. 1). Two pools from this area contained large numbers of larvae but breeding was sporadic.

Area five was an abandoned construction site near East Carteret High School (Fig. 1). The area was overgrown by *Juncus* and *Myrica cerifera*, and although three pools

at this site were checked regularly, larvae were found only once.

The final experimental area was found at Sea Level, North Carolina, in a small section of *Juncus* marsh near a stand of *M. cerifera*. One breeding pool was located here and large numbers of larvae were collected with breeding persisting throughout most of the study period.

Levels of Mosquito Breeding:

Additional marsh areas were explored and new breeding pools were located as time permitted. Specimens collected from these other areas are included in the data presented in Table I, and are noted separately in Table III. Also shown in Table III are the numbers of larvae collected from each pool and the percentage of the total that each pool contributed. These data indicate that pools 102 and 103 were the most heavily used breeding areas, followed by pools 104 and 105. Moderate amounts of breeding took place in pools 601, 401, 402, and 301.

Levels of Coelomomyces Infection:

Infection by Coelomomyces was only found in the two pools with greatest breeding activity. Levels of infection in pools 102 and 103 were monitored from the time of discovery of the first infected specimen until the final infected larva was collected on September 24. Table II shows the relative number of infected larvae taken from these pools. These data indicate that *Coelomomyces* does

not infect any species of larvae inhabiting the breeding pools except *Aedes taeniorhynchus*. This is interesting in view of the fact that larvae of *Aedes sollicitans* were present in both infected pools in large numbers, and are nearly identical in morphology and life history to those of *A. taeniorhynchus*. Infected larvae collected from both infected pools amounts to 2.07% of the total number of larvae collected. As a percentage of the total number of larvae collected from all breeding areas, this figure drops to 1.25%.

According to Table II there is a considerable difference in the overall level of infection between pools 102 and 103. Figure 3 compares the level of infection between the pools in time. Infection of larvae in both pools was sporadic prior to August 10 through August 17, when infected larvae were collected from pool 103 for eight consecutive days. Although *Coelomomyces* appeared earlier in pool 102 than in 103, the number of days infected larvae were collected were fewer in 102. Daily levels of infection were generally higher than those for pool 102. Data in Figure 3 was undoubtedly influenced by prolonged periods of drying which persisted in both pools during much of the study. Periods of drought are indicated in Table IV as time intervals where no salinity or water temperature ranges are shown. Data also indicate similarities in ranges of salinity and water temperature during the intervals in which the two pools are comparable.

More accurate comparisons of daily trends in salinity, water temperature, and pH values between the two infection pools and the control pool are shown graphically in Figs. 4 to 9. The difference in initial dates between plots 102 and 103 was because no larvae were observed in pool 103 prior to May 28, 1974. Salinity values show perhaps the most dramatic fluctuations and the widest degree of variation between the three pools than any other environmental parameter. Salinity readings were relatively high in both infection pools during periods in May, June, and July when Coelomomyces was found. The peak salinity readings were recorded August 16, reaching 28 o/oo in pool 102. Differences in water temperature in the three pools are much less obvious (Figs. 6 and 7). These results are not surprising since readings for all three pools were taken at approximately the same time each day. The graphs for pools 102 and 103 show moderately high temperatures on the dates corresponding with infection, with water temperatures during the eight day infection period in August oscillating between 23 and 26°C. Comparisons of pH trends within pools 102, 103, and 105 are shown in Figs. 8 and 9. Trends for both infection pools appear to be similar with pH values at times of infection ranging between 6.3 and 7.5. Readings of salinity, pH, and water temperature were not taken in pool 105 on days subsequent to August 9 because the site was dry. These dry conditions persisted throughout the rest of the

summer even though precipitation was sufficient to fill pools 102 and 103 on several occasions.

Description of Species:

Hyphae of *Coelomomyces* from *A. taeniorhynchus* were not observed in sufficient detail to provide an accurate description at this time. Resting sporangia were observed occasionally in third instar and mostly in fourth instar larvae of *Aedes taeniorhynchus*. Such sporangia were ovoid in shape, rarely spherical, and often slightly flattened laterally. Of forty sporangia measured, the size ranges were 26.6-42.6 X 41.2-87.8 microns, with an average of 34.6 X 62.5 microns. The wall of mature sporangia varied from green to light brown in color. The outer layer of the sporangial wall contained numerous, minute pits (Fig. 19). A longitudinal germination slit was also present. The inner layer of the wall was smooth and approximately the same thickness as the outer layer. The cytoplasm of mature sporangia appeared hyaline when observed within freshly collected larvae. Plasmolysis occurred in many sporangia when stored on filter paper for over a few days.

Germination Experiments:

Sporulation followed exposure of healthy sporangia to water after dissection of the larval body. After five to seven days, a slight swelling of the sporangium in the general area of the preformed germination slit became

apparent. This swelling resulted in a lateral bulge in the sporangial wall (Fig. 10). The next recognizable stage in the germination sequence was observed when the cytoplasm assumed a somewhat granular appearance (Fig. 11). Application of a cover slip to the preparation caused extrusion of a thin, translucent layer of refractive material through the germination slit (Fig. 12.) Within a few minutes incompletely cleaved spore plasm moved out of the sporangium and underneath this boundary material. The spore plasm moved out of the sporangium for two to five minutes as the translucent layer expanded. Within another two to three minutes the translucent layer enclosed a slowly moving mass of cleaved spores (Figs. 14, 15, 16). This separation was followed within a few seconds by rupture of the translucent layer and subsequent escape of the posteriorly uniflagellate spores (Figs. 15, 16, 17).

Results of germination experiments revealed differences in relative amounts of sporulation of sporangia under controlled laboratory conditions. Sporulation of freshly collected material was observed in distilled water and in water from the collection site with a salinity of 5.5 o/oo. An estimated 50% to 75% of these sporangia were observed to sporulate. Germination success was also tested in pool water with a salinity of 20.5 o/oo. Actual sporulation was not observed after a five day exposure period but significant numbers of sporangia exhibited the characteristic lateral bulge. This stage of sporangial

development was termed the "go" stage by Couch (1967) to indicate the condition of the sporangium immediately prior to sporulation. Germination of stored sporangia was observed in distilled water as long as eight months after collection, with approximately 50% of the go stage sporangia releasing motile spores. Germination of stored sporangia was observed in tap water with approximately 25% of the go stage sporangia releasing spores. In saline solution of 10 o/oo and 15 o/oo a 25% to 50% rate of successful sporulation was observed, but in solutions of 30 o/oo and 35 o/oo less than 25% of the go stage sporangia underwent sporulation. Go stages were also observed after exposure to saline solution of 20 o/oo and 25 o/oo, but no germination occurred.

Considerable numbers of refrigerated sporangia were plasmolyzed after the larval bodies were dissected in the test solution. Following storage at 10°C, 30% to 75% of the sporangia from a given larva were plasmolyzed and non-viable. The percentage of sporangia so affected depended on storage time.

DISCUSSION

The fact that *Aedes sollicitans* and *A. taeniorhynchus* were found to be more abundant than any other species of mosquito collected is not surprising since most experts have known that these were the two most important salt marsh breeders. However, the presence of *Coelomomyces* in only a single marsh area and in a single species of mosquito is unusual when compared with other field studies. In addition, the overall level of infection which was calculated at 1.25% for this study appears rather low when compared with results of recent studies (Umphlett, 1968, 1969; Chapman and Glenn, 1972) which show much higher levels of infection over a longer period of time.

There are no ecological facts presented in these data which indicated that *Coelomomyces* would not be found in other salt marsh mosquito breeding areas. Most breeding pools sampled underwent nearly the same pH, water temperature and salinity fluctuations encountered in pools 102 and 103. The next two most densely populated pools, 105 and 601, presented less stressful habitat conditions for germination of *Coelomomyces* sporangia and subsequent infection and growth. An ecological variable not studied was the presence (or absence) of copepods in sampled pools. Recent investigations have found that these organisms greatly

enhance infection of mosquito larvae in the laboratory and may be a necessary component for completion of the life cycle of *Coelomomyces* in natural habitats (Whisler, Zebold, and Schemanchuk, 1974, 1975). Another ecological factor not directly studied was the possibility that this species of *Coelomomyces* requires more stressful environmental conditions encountered in infection pools as opposed to the control pool. This could eliminate those pools in which wider ranges of pH, salinity, and water temperature were not observed as a likely habitat for *Coelomomyces*. It does not explain the absence of the fungus from many small breeding pools such as 104, 301, and 402 where conditions were very similar to those of the infection pools. A more likely explanation for the limited distribution of *Coelomomyces* was the low level of infection. The infection level of 2.18% calculated for *A. taeniorhyncus* indicates less than one infected larva in forty-five under natural conditions. Fairly large daily collections of larvae would be necessary to observe infection in the field. The heavy breeding of *A. taeniorhynchus* present in pools 102 and 103 allow larger collections of this species than in the other pools which were studied. Nearly 79% of all *A. taeniorhynchus* collected were found in these two pools. This may account in part for the limited distribution of *Coelomomyces*.

Although the failure of this species of *Coelomomyces* to infect other species of mosquito larvae that were

co-inhabitants of the infection pools with A. taeniorhynchus appears unusual, the results are not particularly surprising when compared with other field studies dealing with infection of salt marsh species. Naturally occurring infection of A. taeniorhynchus larvae was first reported by Lum (1963) from collections in Florida. Although infection levels for this variety of Coelomomyces psorophorae in A. taeniorhynchus ranged as high as 5%, there was a conspicuous lack of infection in closely associated species such as Aedes sollicitans, A. atlanticus, A. infirmatus, and Culex nigripalpus, all of which shared the breeding areas. A single infected larva of A. taeniorhynchus was collected from a Louisiana salt marsh by Chapman and Woodard (1966). The specimen was found among a collection of several hundred thousand larvae, none of which showed signs of infection. Several infected specimens of A. sollicitans were collected also from the same general area, but from different infection pools. The infection level was below 1%, with no infection recorded in associated species in the locations from which infected A. sollicitans larvae were taken. These sympatric species included A. taeniorhynchus, Culex salinarius, and Anopheles bradleyi.

The species of Coelomomyces described here appeared somewhat similar to that described by Lum (1963) from collections in Florida. No explanation for the absence of the fungus in associated species of salt marsh mosquitoes

can be offered based on this study, other than the possibility that this species of *Coelomomyces* may be host specific for *A. taeniorhynchus* under these particular ecological conditions. Certainly this is an area where additional research is needed. The 2.18% infection level for *A. taeniorhynchus* is similar to the infection levels reported for other studies in salt marsh areas. The overall infection level of 1.25% compares with an average figure of 1.5% calculated from data of other field studies on *Coelomomyces* (Couch and Umphlett, 1963; Gad, Sadek, and Fateen, 1967). Figures presented in this study would seem to be reasonable estimates of the occurrence of *Coelomomyces* among larval populations of salt marsh mosquitoes, even when compared with the more lengthy studies of Umphlett (1968, 1969) and Chapman and Glenn (1972). Although these authors reported higher infection levels, habitat areas and ecological conditions were different from the salt marsh breeding areas described here. In addition, the species of mosquitoes and of *Coelomomyces* were different than those with which this study was concerned, which may also account for the higher infection levels observed in other studies. More extensive collections may, however, turn up additional infection areas as well as additional species of *Coelomomyces* among native North Carolina salt marsh species.

Differences observed between the infection levels for pools 102 and 103 are difficult to explain, but there

are several factors which may resolve these differences. One principle difference between the two pools was the number of days in which dry conditions persisted. Data in Table IV indicates that pool 102 was dry for 12 more days than 103. Two of these dry days for pool 102 fell in mid-June, and three more in mid-August. Both were periods in which a number of infected larvae were collected from pool 103. Thus, the probability that the lower incidence of Coelomomyces observed in pool 102 was influenced by these critical dry periods is high. The fact that pool 105 remained dry for even longer periods than either pool 102 or 103 is difficult to explain since both of the smaller pools were subject to conditions which would presumably cause a more rapid rate of evaporation. One possible explanation is that though 105 was larger and more shaded than either 102 or 103, its thick mud bottom and lush vegetation quickly absorbed or utilized moderate amounts of rainfall that were received in early August. By contrast, the hard packed mud bottoms and more sparse vegetative growth characteristic of the infection pools enabled them to hold the water received from small amounts of precipitation.

Differences observed in salinity, pH, and water temperature between pools 102 and 103 are generally of a small magnitude during periods in which Coelomomyces was present. Although conclusions concerning the effects these small differences may have had on infection cannot

be drawn, possibly one or a combination of the above variables may have influenced infection levels. This can be seen more clearly in Figures 4 to 9. The general oscillations for the three variables shown for pools 102 and 103 are similar over the time interval in which these values are plotted. The ranges between the maximum and minimum values during the August infection period are slightly greater for pool 102 (Figs. 4 to 9). It is possible that the lower fluctuations measured in pool 103 over this same time interval would cause less environmental stress on hatching and growing larvae and the fungal parasite. This would seem to account for observed differences in infection levels between pool 102 and 103 better than any single physical factor that was measured. If salinity values are considered, graphs in Figure 3 indicate higher readings in pool 103 on and prior to June 20. Salinity readings in pool 102 showed a more rapid rise from August 10 to 17. *Coelomomyces* was collected from pool 103 during both periods, yet salinity fluctuations appear to have no direct correlation with its presence. This is unexpected when results of germination experiments indicate a lower level of sporangial germination with increasing salinity. Field studies by Pillai (1971) reported lower incidences of *Coelomomyces* in pools of higher average salinities. These data indicate differences in salinity between pools 102 and 103 during periods of infection are very small, usually less than 2 to 4 o/oo.

Differences in infection levels due to salinity may be attributed to the amount of fluctuation. The only difference is a slightly lower range between maximum and minimum values recorded for pool 103 during the latter portion of the study when infection was most consistent. Salinity readings for pool 105 were lower than for pool 102 and pool 103 due in part to the fact that surrounding vegetation probably gave more shelter from the effects of salt spray.

Differences in overall fluctuations in pH and water temperature (Figs. 6 to 9) were less variable, with differences in pH rarely exceeding 0.3 units and differences in water temperature never exceeding 2°C. Slightly lower water temperature readings for pool 105 can probably be accounted for by the increased amount of shading of the larger control pool, in contrast to the smaller water volumes of pools 102 and 103 which were exposed to direct sunlight.

On the basis of the data presented here, it appears that a combination of these physical factors seems to be the most logical explanation for discrepancies in the infection levels of the two pools in which *Coelomomyces* was found.

TABLE I

SPECIES OF MOSQUITO COLLECTED, TOTAL NUMBER OF EACH SPECIES, PERCENT CUMULATIVE TOTAL OF EACH SPECIES, AND NUMBER OF POOL FROM WHICH EACH WAS COLLECTED

Species	Number Collected	Percent Total	Pool Number
Aedes taeniorhynchus	7244	57.16	101 - 105, 201, 202, 301, 302 401, 402, 601
Aedes sollicitans	4059	32.04	101 - 105, 201-203, 301, 302 401, 402, 601
Aedes atlanticus	1	0.01	102
Culex salinarius	1039	8.20	102, 105, 401, 601
Culex pipiens	54	0.42	401, 601
Anopheles bradleyi	210	1.66	101 - 103, 105, 201 - 203, 301, 401, 601
Psorophora ciliata	8	0.06	105
Psorophora confinnus	60	0.45	103, 105, 401, 601
TOTAL	12,675	100	

TABLE II

TOTAL NUMBER OF LARVAE OF DOMINANT SPECIES COLLECTED FROM POOLS 102 AND 103, NUMBER OF INFECTED LARVAE, AND PERCENT OF LARVAE INFECTED

Species	Number Collected	Number Infected	Percent Infected
Pool 102			
Aedes taeniorhynchus	2238	12	0.41
Aedes sollicitans	529	0	0
Other	129	0	0
Totals	2896	12	0.41
Pool 103			
Aedes taeniorhynchus	3465	146	3.07
Aedes sollicitans	1241	0	0
Other	46	0	0
Totals	4752	146	3.07
TOTALS	7648	158	2.07

TABLE III

NUMBER OF LARVAE COLLECTED FROM EACH STUDY POOL AND PERCENTAGE OF TOTAL THAT EACH POOL CONTRIBUTED

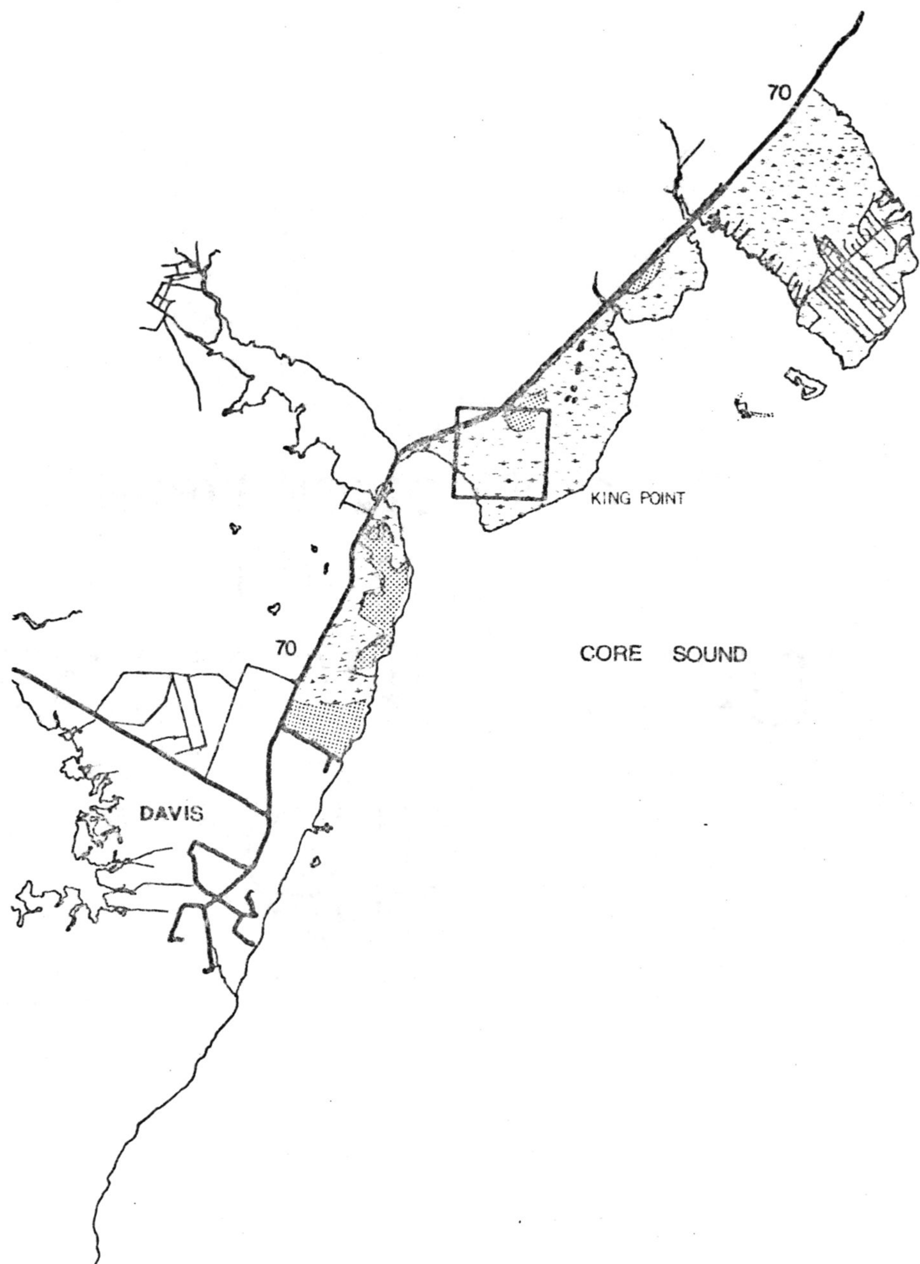
Pool Number	Number Larvae Collected	Percent Total
101	7	0.06
102	2896	22.85
103	4752	37.49
104	2259	17.82
105	956	7.54
201	39	0.30
202	48	0.38
203	42	0.33
301	148	1.17
302	7	0.06
303	0	0
304	0	0
305	0	0
401	360	2.84
402	117	0.92
403	0	0
501	7	0.06
502	0	0
503	0	0
601	502	3.96
Other	535	4.22
TOTAL	12,675	100.00

TABLE IV

COMPARISON OF SALINITY AND TEMPERATURE RANGES BETWEEN
POOLS 102 AND 103 AT TWO WEEK INTERVALS
NO FIGURES INDICATE DRY PERIOD

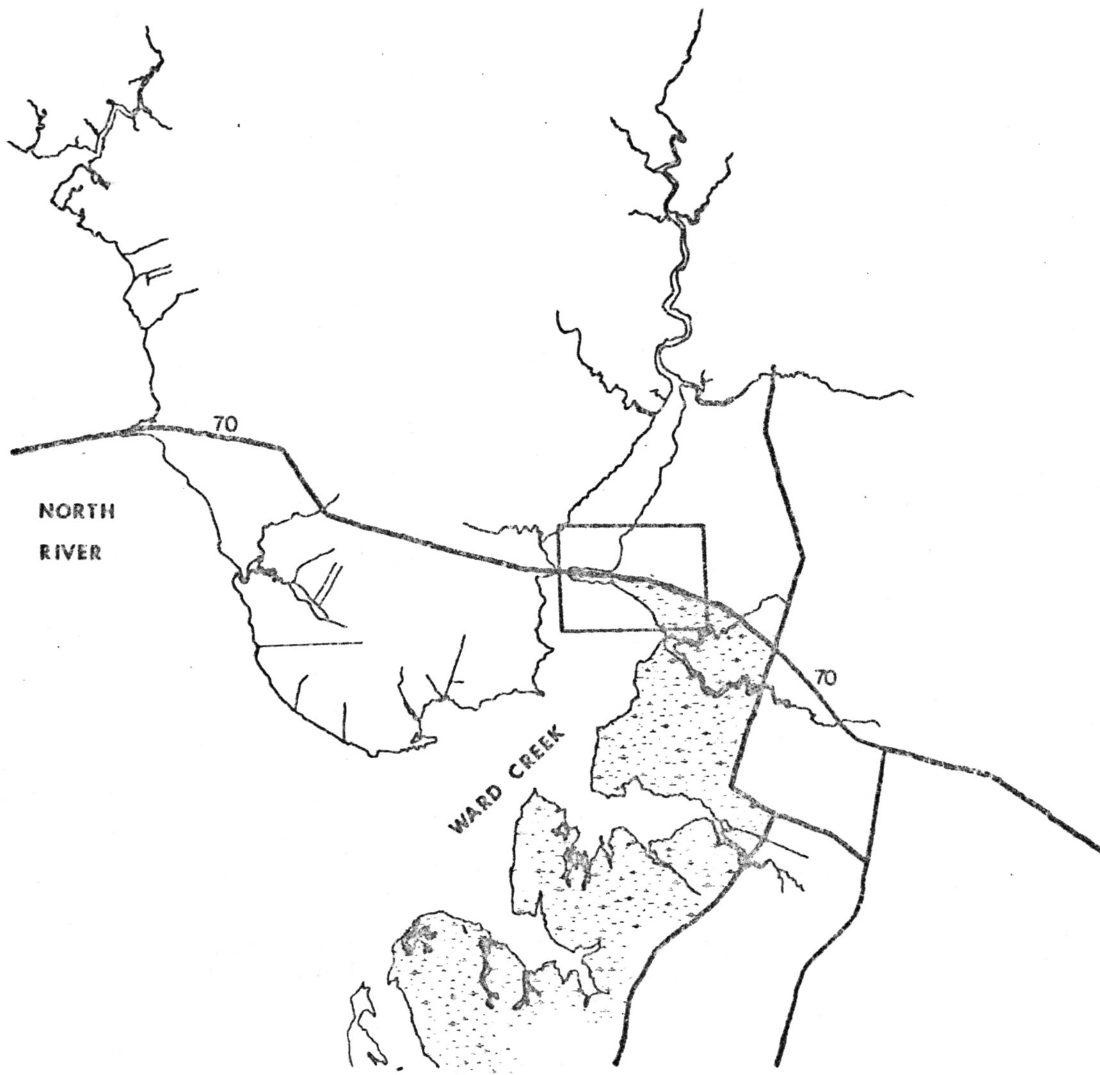
		Pool 102		Pool 103	
		Salinity (o/oo)	Water Temperature °C	Salinity (o/oo)	Water Temperature °C
May	14-31	13.4-24.4	21-27.5	---	---
June	1-14	---	---	5.0-19.5	21.5-27.0
	15-30	3.4-18.2	22-26	5.5-18.8	23.0-29.0
July	1-14	6.5-18.8	29-33	8.2-20.5	29.0-31.0
	15-31	---	---	---	---
Aug.	1-14	1.0-16.5	23-24.5	2.0-17.8	24.0-25.5
	15-30	16.5-28.8	24-25.5	18.8-25.0	25.0-26.5
Sept.	1-14	---	---	---	---
	15-30	---	---	20.5-25.0	24.0-26.0

Figure 1: Map showing location of six regular collection areas.



70
KING POINT
70
CORE SOUND
DAVIS

Figure 2: Map showing location of Ward Creek collection area.



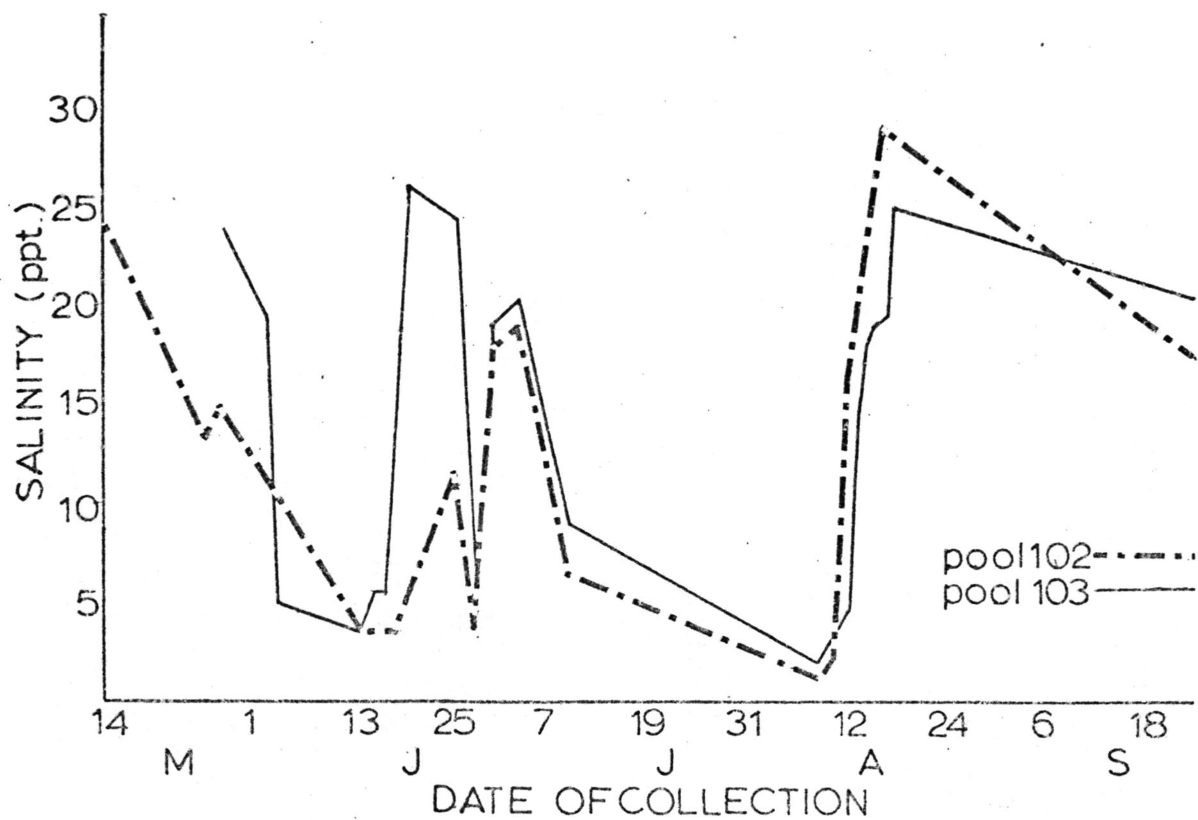
70
NORTH
RIVER
70
WARD CREEK

Figure 3: Histogram indicating daily percent of larval infection by *Coelomomyces*.

20
18
16
14
12
10
8
6
4
2
% LARVAE INFECTED
pool 102
pool 103
14
M
20
J
30 2
J
10
11
12
13
14
15
16
17
A
24
S
DATE OF COLLECTION

Figure 4: Plot of salinity readings taken from pools 102 and 103 from May 14, 1974 until September 24, 1974.

Figure 5: Plot of salinity readings taken from pool 105 from May 14, 1974 until August 12, 1974. Pool 105 was dry from August 12 until September 24, 1974.


30
25
20
15
10
5
SALINITY (ppt.)
pool102
pool 103
14
1
13
25
7
19
31
12
24
6
18
M
J
J
A
S
DATE OF COLLECTION

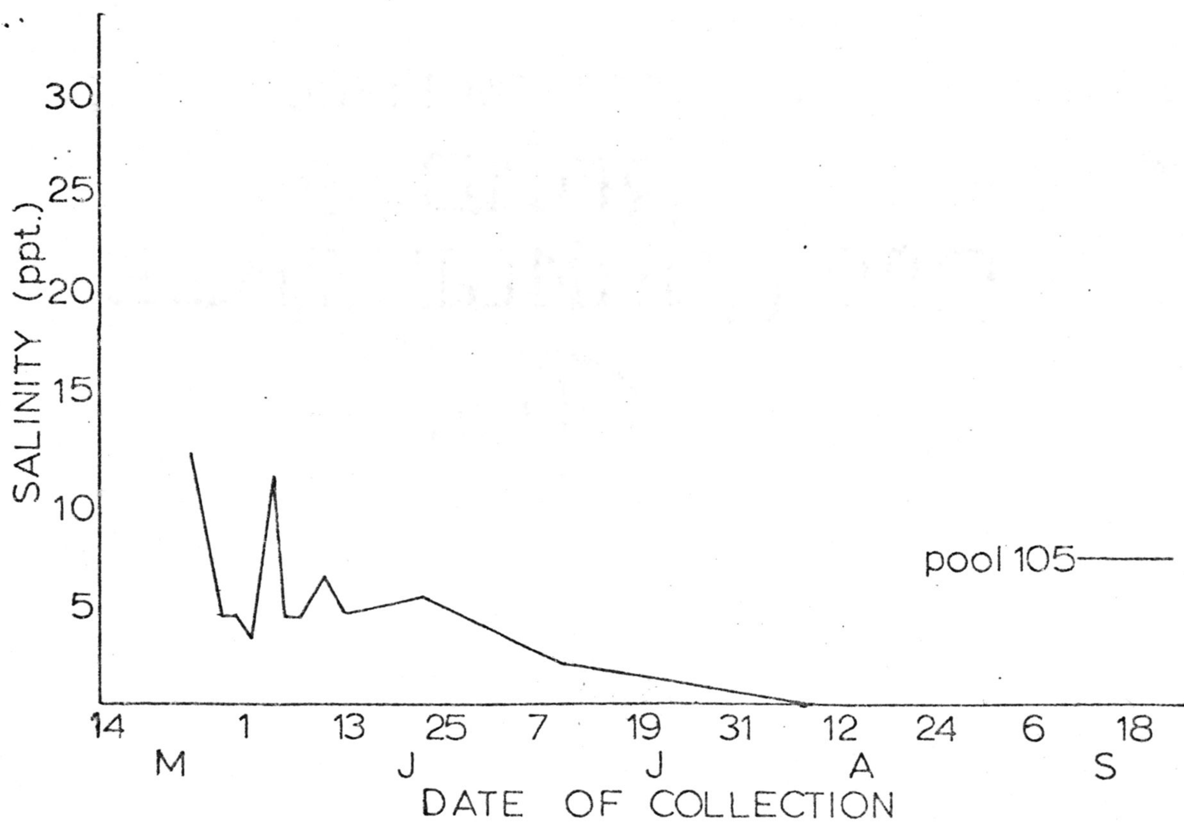
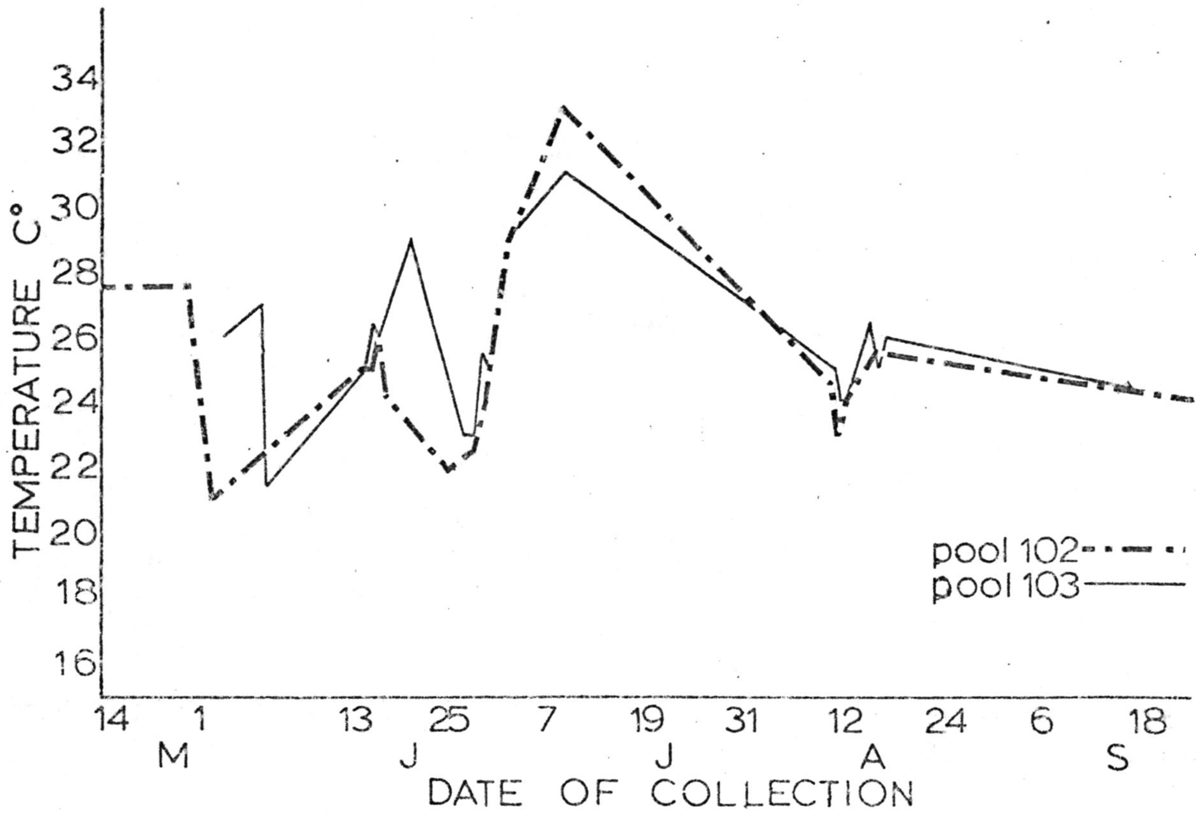

30
25
20
15
10
5
SALINITY (ppt.)
pool 105
14
1
13
25
7
19
31
12
24
6
18
M
J
J
A
S
DATE OF COLLECTION

Figure 6: Plot of water temperature readings taken from pools 102 and 103 from May k4, 1974 until September 24, 1974.

Figure 7: Plot of water temperature readings taken from pool 105 from May 14, 1974 until August 12, 1974. Pool 105 was dry from August 12 until September 24, 1974.


TEMPERATURE C°
34
32
30
28
26
24
22
20
18
16
14
1
13
25
7
19
31
12
24
6
18
M
J
J
A
S
DATE OF COLLECTION
pool 102
pool 103

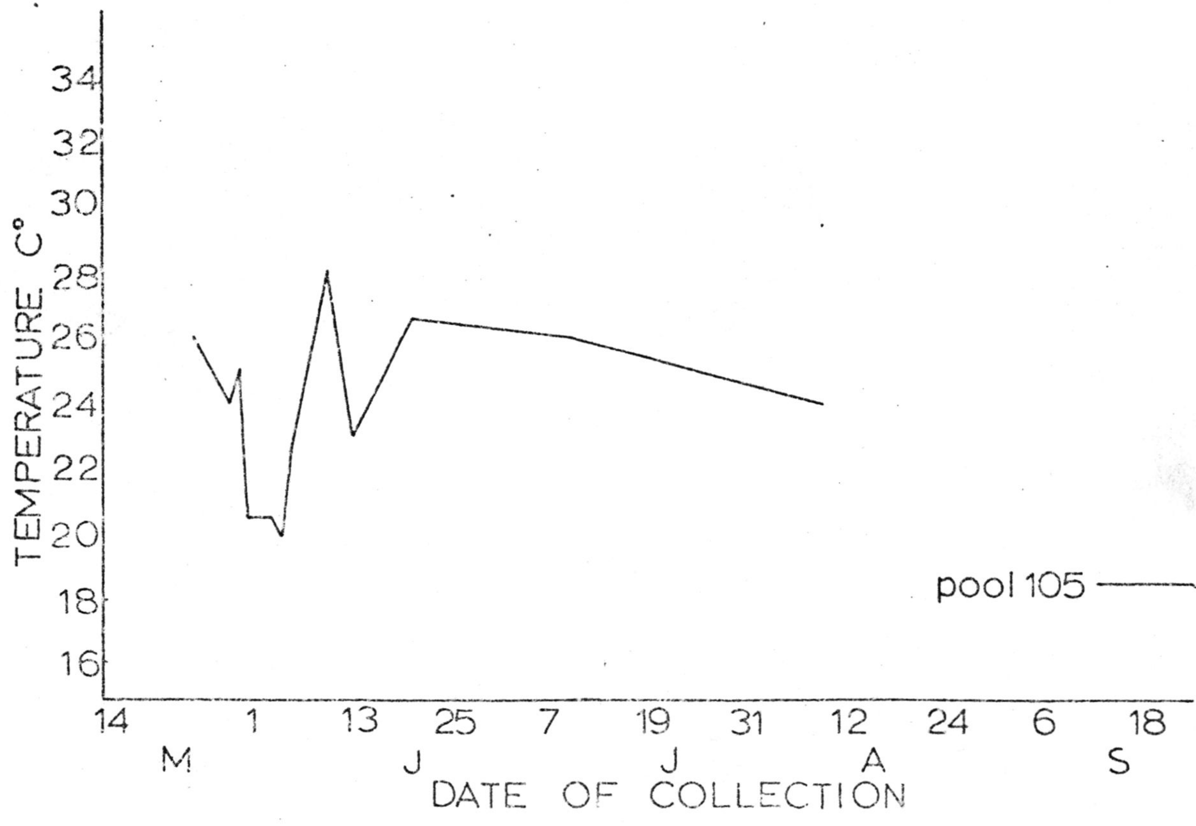
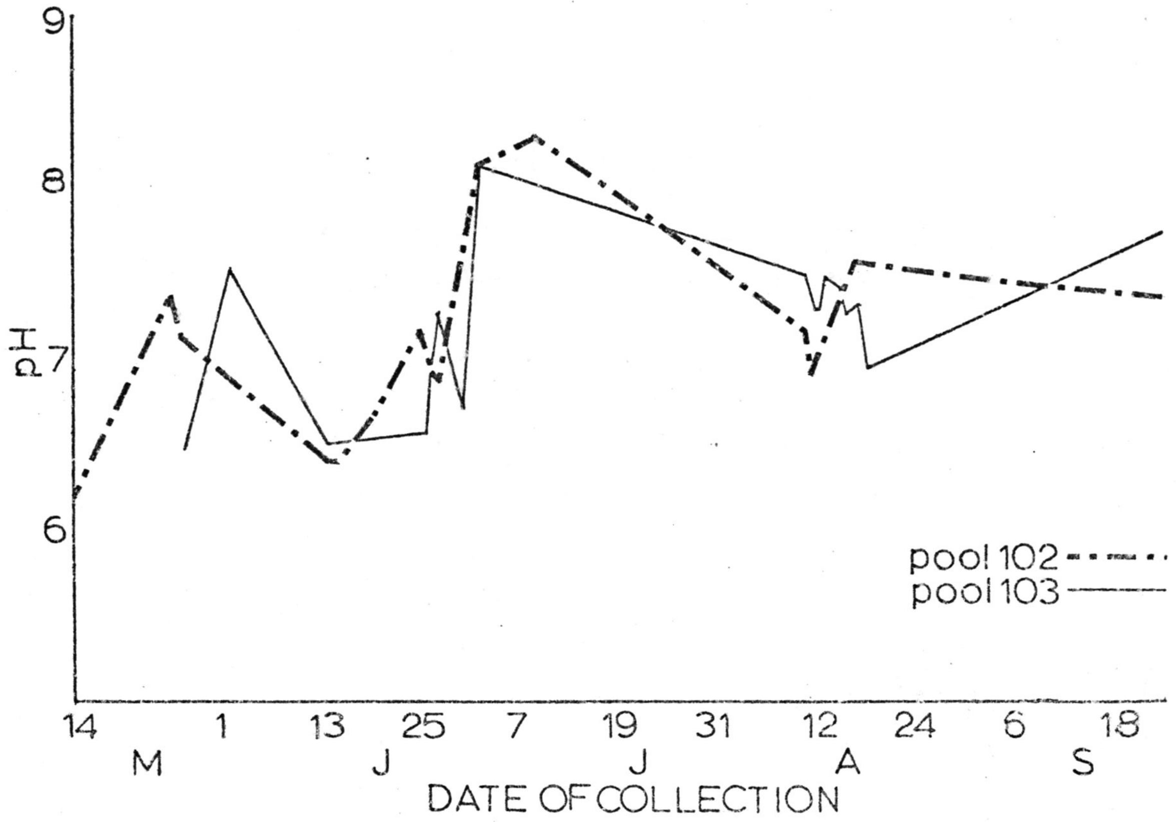

TEMPERATURE C°
34
32
30
28
26
24
22
20
18
16
14
1
13
25
7
19
31
12
24
6
18
M
J
J
A
S
DATE OF COLLECTION
pool 105

Figure 8: Plot of pH readings taken from pools 102 and 103 from May 14, 1974 until September 24, 1974.

Figure 9: Plot of pH readings taken from pool 105 from May 14, 1974 until September 24, 1974. Pool 105 was dry from August 12 until September 24, 1974.


9
8
pH 7
6
pool 102
pool 103
14
1
13
25
7
19
31
12
24
6
18
M
J
J
A
S
DATE OF COLLECTION

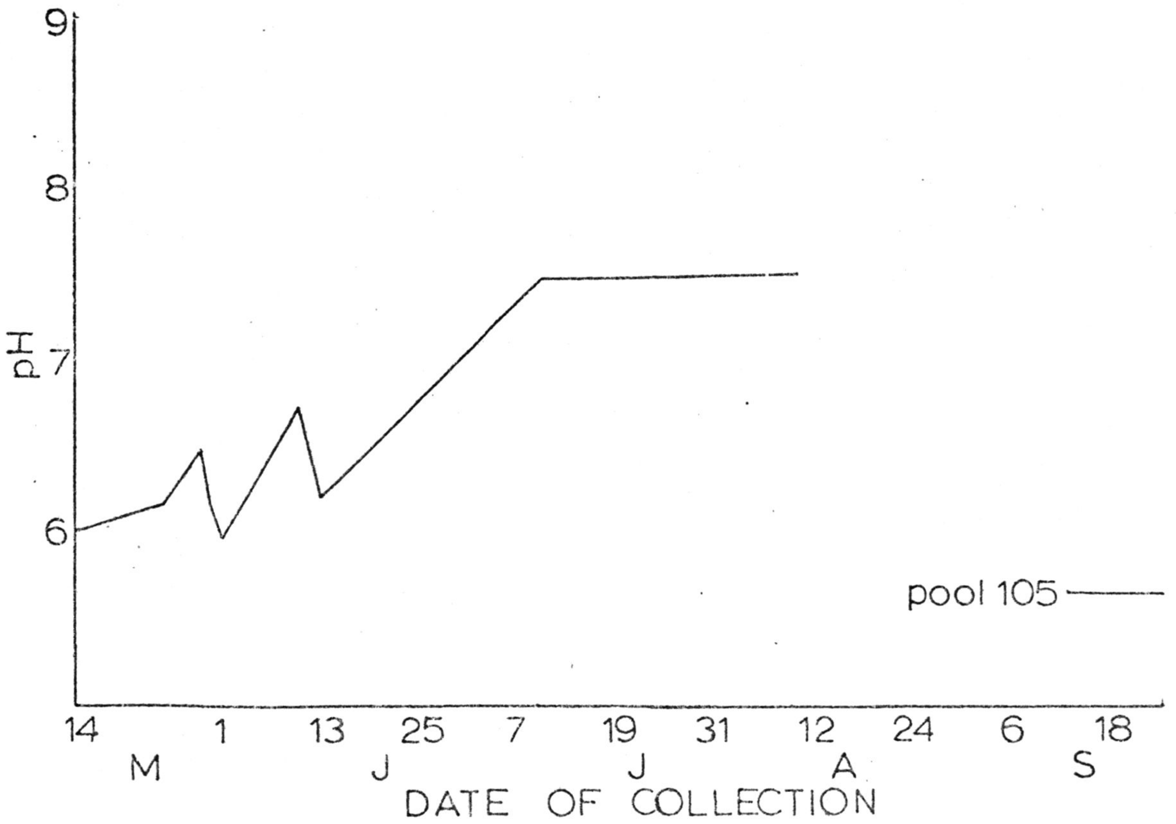

9
8
pH 7
6
pool 105
14
1
13
25
7
19
31
12
24
6
18
M
J
J
A
S
DATE OF COLLECTION

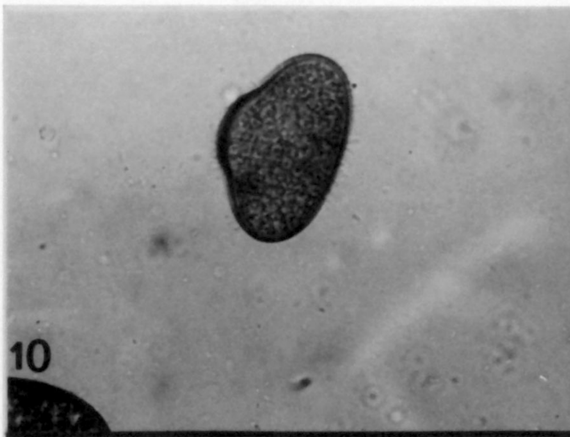
Figure 10: Sporangium of *Coelomomyces* showing lateral bulge which is present prior to germination. (X450)

Figure 11: Sporangium showing granular appearance of cytoplasm prior to sporulation. (X625)

Figure 12: Initial stage of sporulation with extrusion of inner wall layer through germination slit. (X625)

Figure 13: Cytoplasmic discharge with incompletely cleaved sporeplasm flowing outward behind translucent layer. (X625)

Figure 14: Cleavage of spores. (X625)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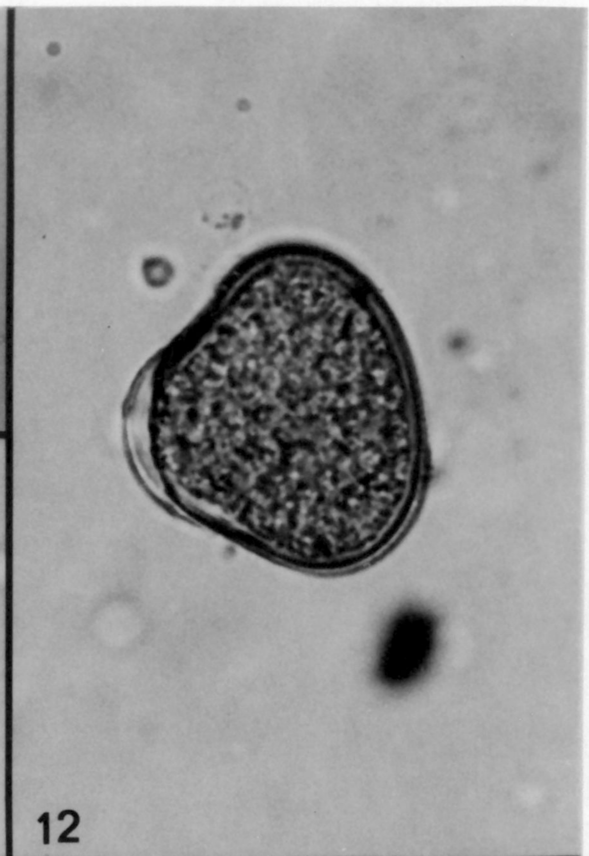
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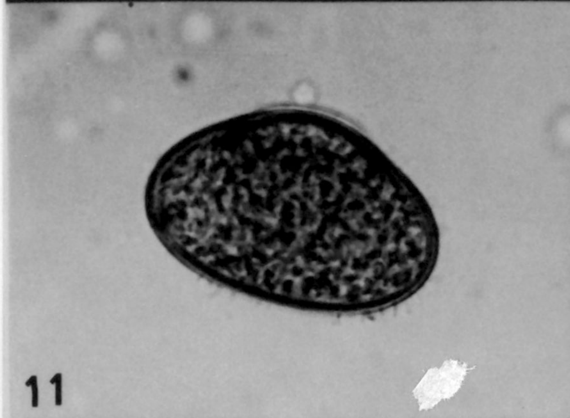
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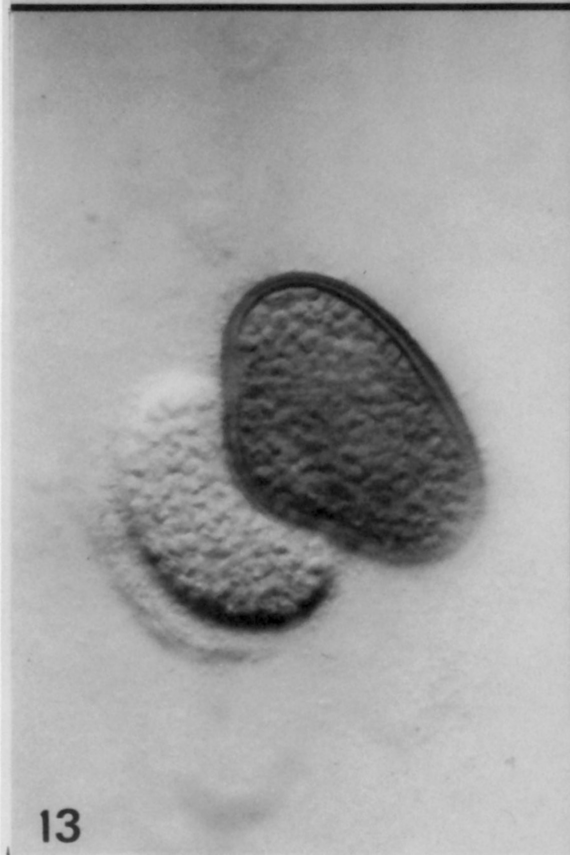
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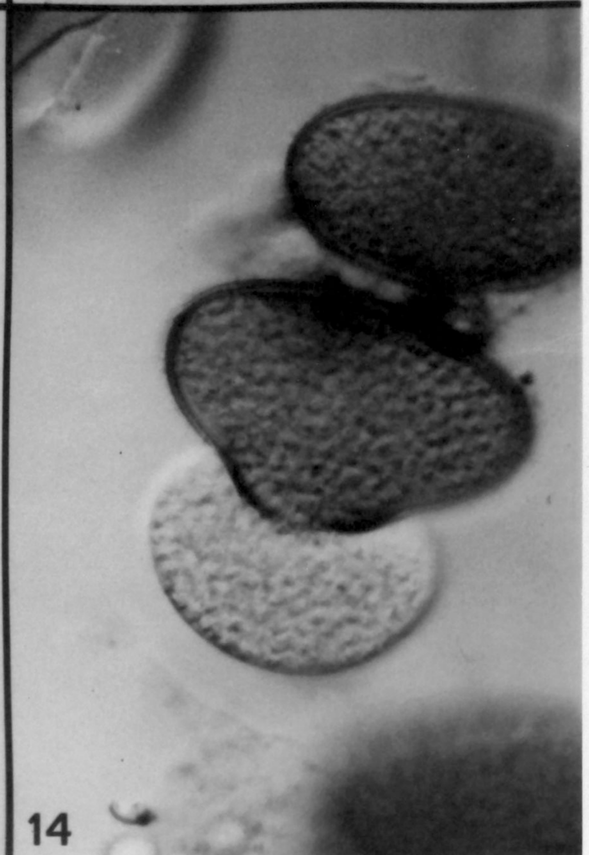

14

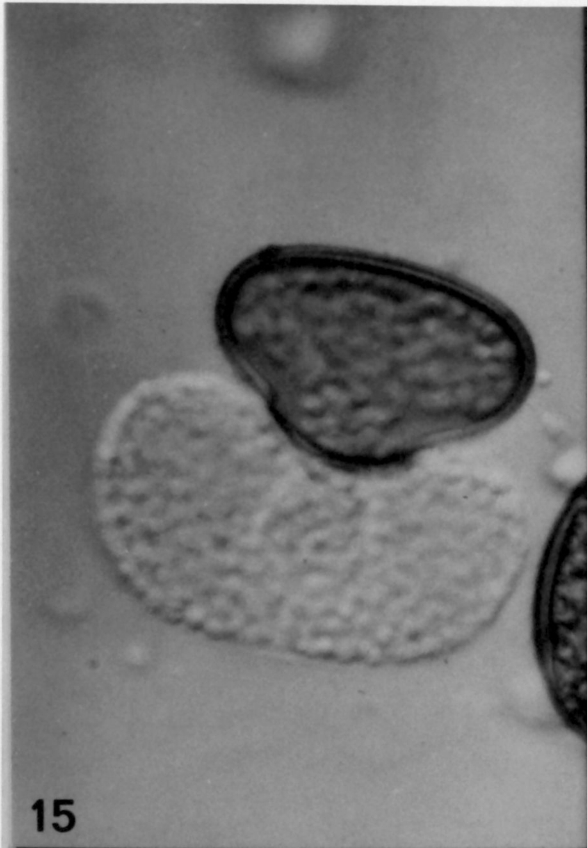
Figure 15: Large mass of spores immediately prior to spore release. (X625)

Figure 16: Same sporangium as in Fig. 15 showing rupture of membrane and escape of spores. (X625)

Figure 17: Same sporangium as in Fig. 15 showing release of spores. (X625)

Figure 18: Same sporangium as in Fig. 15 nearly empty of its content of spores. (X450)

Figure 19: Empty sporangium of *Coelomomyces* showing pitted structure of outer wall. (Phase contrast, X2,250 Initial)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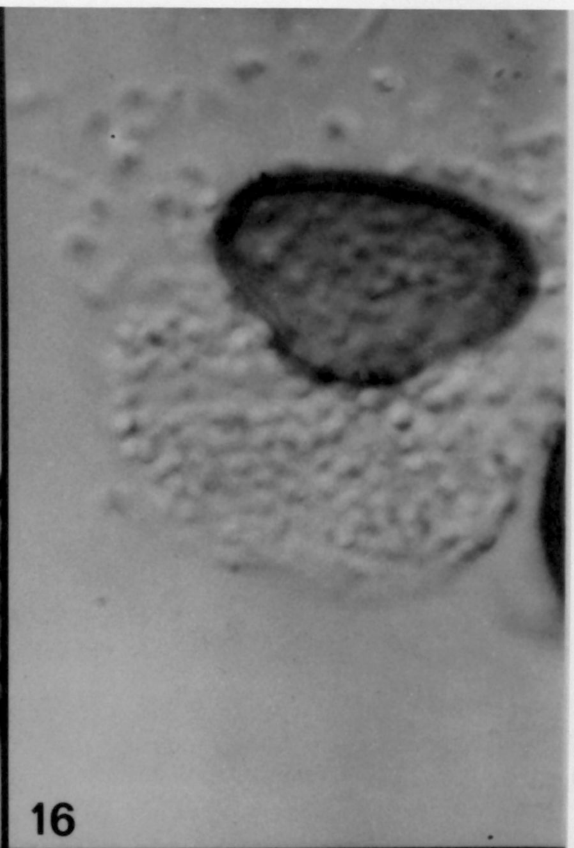
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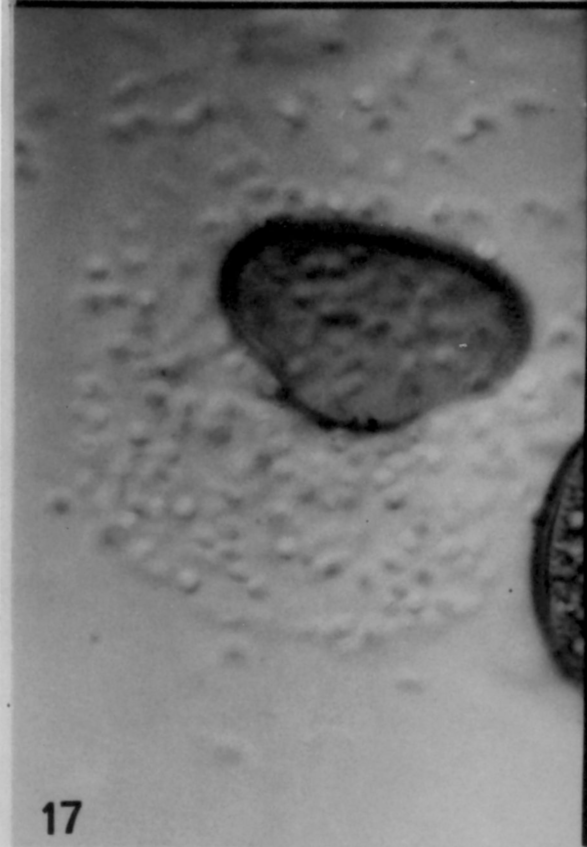
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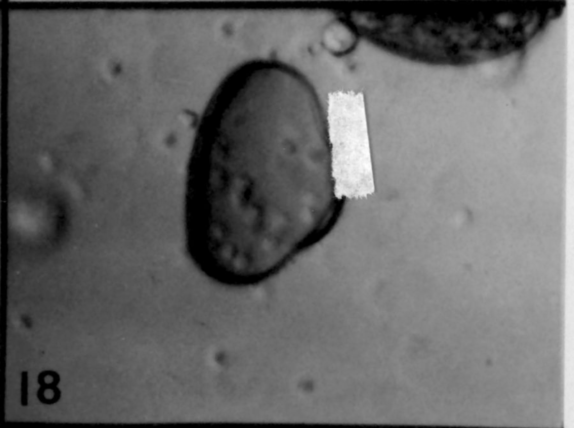
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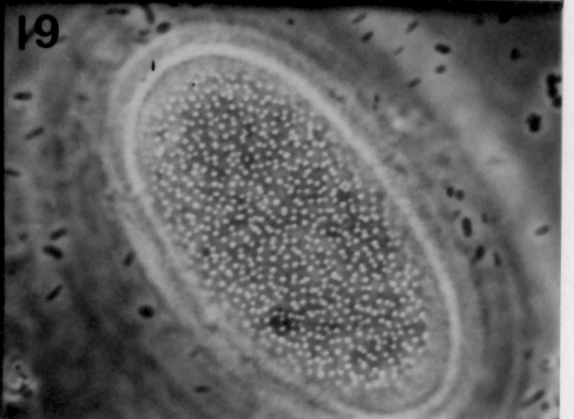
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