A study was made to determine biological relationships between the garden symphylan, *Scutigerella immaculata* (Newport), and an entomogenous fungus, *Entomophthora coronata* (Costantin), which attacks it under certain conditions. Until this fungus was found to infect this pest, no organism which seemed to offer promise of control had been discovered.

Symphylan populations were exposed to *E. coronata* and the pathology followed. When new symphylans were added to an infected culture as the diseased individuals died, an epizootic condition developed to a point at which symphylans were infected and killed in less than two days.

*E. coronata* survived in contaminated containers for as much
as five months without susceptible hosts as evidenced by infection of reintroduced symphylans. Wax moth larvae, Galleria, and European house crickets showed high mortality when injected with spores suspended in physiological saline solution. Wax moth larvae and mealworms, Tenebrio, were infected when they were dusted with spores and incubated at 15°C temperature in high humidity. Penetration of the cuticle by germ tubes from attached spores is the usual pathway of infection.

Temperature ranges for both organisms correspond closely. Both become active a few degrees above freezing and reach an optimum between 20° and 30°C. Lethal temperature for both is somewhat below 37°C.

Eight sulfonamides and an equal number of antibiotics were tested against the fungus. None showed inhibitory effects. Fungal inhibiting agents Mycostatin, methyl p-hydroxybenzoate, sorbic acid, and formalin were destructive at rates used in insect rearing media.

E. coronata grows well on a large number of high protein media. Mass-rearing for large experiments seems possible.
Biological and Ecological Relationships of the Fungus, *Entomophthora coronata* (Costantin) Kevorkian, and the Garden Symphylan, *Scutigerella immaculata* (Newport)

by

Robert Frank Koontz

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INTRODUCTION

The garden symphylan, *Scutigerella immaculata* (Newport), was reported to be causing damage to crops in Oregon over 40 years ago (Thompson, 1925). It has since become a major agricultural pest. Chemical control has never been as successful as could be desired and there is growing resistance to the use of insecticides in the soil. Recent reports show that the garden symphylan is becoming more widespread in fields, perhaps because irrigation is becoming more widespread. Morrison (1965) stated that while the most serious damage occurs in western Oregon, the pest is becoming increasingly important in other parts of the state. Beeler (1967) reported symphylan damage to corn in Indiana in the year 1966.

Except for limited predation by mites, centipedes and carabid beetles (Waterhouse, 1963), no indication of biological control had been reported until Getzin (1963) found *Entomophthora coronata* (Constantin) Kevorkian killing symphylans held in cans of soil for chemical testing. Getzin and Shanks (1964a) reported that cultures of the fungus had been introduced into soil both in cans and in field tests and that it had survived and infected symphylans for a month.
or two. This discovery seemed to offer an opportunity for further examination of the relationships between these two organisms in order to evaluate the possibility of using this pathogen to limit symphylan populations.

The species of the genus *Entomophthora* Fresenius, have been known as a destructive group of insect parasites since Cohn (1855) described the fly fungus, *Entomophthora muscae*, under the name *Empusa muscae*. They have probably been considered in terms of biological control as long as any group of insect pathogens because of the spectacular destruction they can inflict on insects such as grasshoppers. But in spite of the seemingly good opportunities for biological control offered by this group, attempts in this direction have been generally unsuccessful, apparently because of the inability of the experimenters to control the environment in such a way as to promote a high level of infection.

The fact that *E. coronata* can invade soil and kill symphylans seemed to offer an opportunity to use the more stable soil environment to explore the possibility of artificially inducing infections in dense populations of this pest and in this way limiting their numbers.

Symphylans would seem to be a promising species to try to control by biological means because their populations are quite stable and do not move rapidly from one area to another. Also a considerable population can be tolerated by many crops, thus making
the complete kill sought by chemical means not absolutely necessary.

For these reasons this investigation of the relationships between these two organisms was undertaken in the hope that some light might be thrown on the problem of a biological control of this pest.
TAXONOMY AND HISTORICAL REVIEW

Scutigerella immaculata (Newport)

Taxonomy

The biology of the garden symphylan, Scutigerella immaculata, (Figure 3, page 104) has been covered by Michelbacher (1938), Savos (1958), Edwards (1957), and Waterhouse (1963); the taxonomy by Michelbacher (1963), Edwards (1958), and to some extent by Waterhouse (1963); and the reproduction by Juberthie-Jupeau (1959, 1963, 1964). The present study was confined to the biological and pathological relationships of the fungus, Entomophthora coronata, to S. immaculata. Therefore, only those papers specifically concerned with this subject and those which relate to the taxonomic position of both organisms are reviewed.

The first species of the class Symphyla was described by Scopoli in 1763 (Michelbacher, 1938) under the name Scolopendra nivea. The generic name indicates that he thought he was dealing with a true centipede. In 1836 Gervais (1839) described a similar creature which he named Geophilus junior thinking it to be a larval stage. Later he changed the name to Scolopendrella notocantha (Gervais, 1840). Newport (1845) found a third species near London
and described it under the name *Scolopendrella immaculata*. He listed this genus under the Chilopoda.

Ryder (1880) raised this group to ordinal rank and called it Symphyla. The first American symphylan was reported by Packard (1873) who named it *Scolopendrella americana* Packard. Later he found *S. americana* to be synonymous with *S. immaculata* (Packard, 1881).

Ryder in 1882 divided the order into two genera, *Scolopendrella* Gervais and *Scutigerella* Ryder.

Pocock (1893) raised the Symphyla to the rank of class.

Hansen (1903) published a complete work on all the symphylan species known at the time and raised the number of known species from five to 24. He recognized Ryder's classification and considered the group to consist of a single family and two genera, *Scolopendrella* and *Scutigerella*, consisting of 12 species each.

Bagnall (1913) published a complete synopsis and key to the 35 species known at that time. He followed Ryder and Hansen in giving Symphyla the rank of order. Hansen's genera he raised to the rank of subfamilies, the Scutigerellinae under which he grouped eight genera and the Scolopendrellinae under which he placed three genera. All were placed under the single family Scolopendrellidae.

Michelbacher (1938) listed 54 species under nine genera. He published a key in 1942 to the eight species of the genus *Scutigerella*
which were known at that time. He followed Pocock (1893) in giving Symphyla the rank of class.

Edwards (1957) constructed a key to world genera of Symphyla in his thesis but this work seems not to have been published. Edwards (1959) published keys to British genera and species. In this work he gave family rank to Bagnall's subfamilies. These became Scutigerellidae and Scolopendrellidae.

Waterhouse (1963) listed 102 species arranged in 13 genera but did not separate them into families. He said that the only key to world species was for the genus Scutigerella published by Michelbacher in 1942, and that this needed revision.

Michelbacher (1938, p. 61) summarized the classification as follows:

**SYSTEMATIC POSITION**

**SYNONYMY**

- *Scolopendrella immaculata* Newport (1845)
- *Scolopendrella americana* Packard (1873)
- *Scutigerella immaculata* (Newport) Ryder (1882)
- *Scutigerella californica* Woodworth (1905)

**FULL CLASSIFICATION**

- Phylum: Arthropoda
- Class: Symphyla Pocock (1893)
- Order: Symphyla Ryder (1880)
- Family: Scutigerellidae Ribaut (1931)
- Subfamily: Scutigerellinae Bagnall (1913b)
- Genus: Scutigerella Ryder (1882)
- Species: immaculata Newport (1845)
Biological Control

As early as 1851 Menge noted that true centipedes were predacious on the "garden centipede". Since then Filinger (1928, 1931), Wymore (1931), Savos (1958), and Waterhouse (1963) reported similar observations on a total of seven species of centipedes.

Wymore (1931) reported a gasamid mite as attacking symphylans. Illingsworth (1927-1928) stated that two species of beetles attack symphylans. Savos (1958) reported that millipedes and Collembola fed on dead symphylans and their eggs.

Michelbacher (1938) remarks that the study of the natural enemies of the "garden centipede" has been neglected. Especially has this been true of parasites and diseases. Until Getzin (1963) and Getzin and Shanks (1964b) reported the isolation and identification of Entomophthora coronata and Metarrhizium anisopliae (Metchnikoff) Sorokin from symphylans held in soil cultures, there were only three references in the literature on disease organisms. Verhoeff (1933) found sporozoans in the digestive tract of S. immaculata but did not suggest that they were pathogenic. Remy (1942) found gregarines in their digestive tract but attached no importance to this discovery. Waterhouse (1963) found eggs and first instar symphylans were susceptible to attack by an undetermined species of Fungi Imperfecti.
Since the report of Getzin and Shanks (1964), Swenson (1966) was able to infect *S. immaculata* with the pathogenic nemitode DD-136 (Dutky, 1959). He also isolated *F. coronata* in symphylan cultures at Corvallis, Oregon (Swenson, 1967).

**Entomophthora coronata** (Costantin) Kevorkian

The order Entomophthorales of the class Phycomycetes consists of the single family Entomophthoraceae, which may be divided into six genera. They appear to be closely allied structurally, are chiefly parasitic on insects and related arthropods, but a few species are parasitic on plants and several are saprobic on animal matter such as the excreta of frogs and lizards. Some, like *F. coronata*, may be parasitic on arthropods or other fungi or weakly saprobic under certain conditions.

McLeod (1963) characterizes the order Entomophthorales, family Entomophthoraceae as follows:

Form chiefly parasitic and entomogenous, a few saprophytic, a few others parasitic on plants; mycelium not very extensive, at first coenocytic but sooner or later becoming septate and often falling apart into hyphal bodies; asexual reproduction almost always by conidia borne at the ends of specialized conidioshores and shot away (with violence) at maturity; sexual reproduction by the

---

1 Personal communication with Dr. K. G. Swenson, Professor of Entomology, Oregon State University, Corvallis, Oregon.
union of mycelial fragments (or of hyphal bodies) to form zygospores; zygospores frequently replaced by the parthenogenetic development of azygospores.

Key to Entomophthoraceae

Mycelium not entomogenous (i.e., not living on insects)

Parasitic in the gametophytes of ferns Completoria
Parasitic in the desmid genus Closterium Ancylistes
Saprophytic in the excrement of frogs and lizards Basidiobolus
Saprophytic or weakly parasitic on fungi Conidiobolus

Mycelium entomogenous

Conidia borne on conidiophores extruded through the body wall of the host, smooth-walled, discharged forcibly from the conidiophore Entomophthora
Conidia borne within the body of the host and spread by its disintegration, not shot away Massospora

(McLeod 1963, p. 190).

The genus Entomophthora was first described by Cohn (1853) under the name Empusa based on the fly fungus Empusa muscae which he designated as the type species.

Fresenius (1856) recognized that the name Empusa had been preempted by a genus of orchids and proposed the genus name Entomophthora.

Brefeld (1877) thought that there was enough difference between the known species to justify two genera, Empusa and Entomophthora.

Nowakowski (1884) subdivided the genus still further and created a new genus, Lamia.
According to this arrangement Empusa is characterized by the possession of unbranched conidiophores and the formation of azygospores and Entomophthora has branched conidiophores, forms rhizoids and cystidia, and produces zygospores. Lamia was intermediate, differing from Empusa chiefly in the possession of cystidia. These proposals subsequently led to some confusion in the taxonomy of this group of fungi (McLeod, 1963, p. 191).

Tarichium Cohn was described from a "resting spore of some Empusa" (Thaxter, 1888) and remains still unconnected with a vegetative organism.

Thaxter (1888) after examining the known species, decided that borderline species made Nowakowski's separation invalid. He, therefore, united all the species under the generic name Empusa, which he selected because of its priority and weight of authority.

But even after the appearance of Thaxter's publication, confusion still persisted as to the question of the number of genera involved, and which generic names should be used.

"In general, contemporary workers have treated this group of fungi as a single genus" (McLeod, 1963). But there was still disagreement as to whether Empusa or Entomophthora should be used as the generic name. In order to resolve this question McLeod (1963) discussed the problem with Dr. J. W. Groves, Head, Mycological Section, Plant Research Institute, Science Service Ottawa. His opinion was that a strict application of the International Rules of Botanical Nomenclature requires the adoption of Entomophthora Fresenius as the proper designation for the genus.
Entomophthora coronata (Costantin) Kevorkian 1937

Synonyms (according to Kevorkian 1937)

Boudierella coronata Costantin 1897

Delacroixia coronata Sacc. and Syd. (Saccardo 1899)

Conidiobolus villosus Martin 1925

Costantin (1897) discovered an unfamiliar Entomophthorales growing on the gills of a mushroom of the genus Psalliota or on an insect which had died between the lamellae. He was not sure which. This fungus showed up in the culture tubes in which he was trying to germinate mushroom spores. It ejected conidia measuring from 26 to 45 μ in diameter which germinated on the wall of the tubes to produce secondary conidia. He described it as Boudierella coronata Costantin.

Saccardo and Sydow (Saccardo, 1899) renamed it Delacroixia coronata (Costantin) Sac. and Syd. since the generic name Boudierella had been preempted.

Martin (1925) described an Entomophthorales fungus which he had isolated from decayed wood in Iowa and which he named Conidiobolus villosus Martin.

He was able to cultivate it on "ordinary nutrient media" both solid and liquid. The solid media is referred to as agar of an unspecified type and the liquid media as concoctions of prunes and
green beans.

He recorded the size of the papillate conidia as ranging from 12 to 46μ. He mentioned that the smaller conidia were probably of "secondary, tertiary, or a higher order". The primary conidia were thrown 25 to 30 mm from the conidiophore. Cultures a few days old produced another spore with short hairlike (villous) projections. These he called resting spores and have thus been referred to since (Figure 8, page 106).

Because he had isolated the fungus from a fungal complex on decayed wood and had cultured it on artificial medium, he put it in the saprophytic genus Conidiobolus Brefeld (Brefeld, 1884). Because of the peculiar "resting spores" he named the species villosus.

Kevorkian (1937) isolated a fungus from termites of the genus Nasutitermes in Cuba which produced "villous resting spores" and conidia measuring from 12 to 36μ with an average of 32μ. He considered it to be identical with a saprophytic species described by Martin as Conidiobolus villosus. On further observation he came to the conclusion that it was the same fungus as Delacroixia coronata described by Costantin. Since he found it evidently parasitic on insects he placed it in the entomogenous genus Entomophthora and proposed the new combination Entomophthora coronata (Costantin) Kevorkian, which is still in use.

Gallaud (1905) isolated this species in France from Orchid


seeds. Gilbert (1919) isolated *E. coronata* from fern prothalia. White (1937) found this fungus as a contaminant in plates used to germinate *Pezizia* ascospores. Couch (1939) found it growing as a weak endophytic parasite on stalks of *Nardia*. Schaefer (1941) isolated it from ants; Harris (1948) isolated it from an aphid in the United States. Hall and Dunn (1957) isolated *Entomophthora coronata* from spotted alfalfa aphids, *Thrioaphis* maculata (Buckton). Prosertphon (1963) infected larvae of *Galleria mellonella* with this fungus. Getzin and Shanks (1964) isolated it from the symphylan, *S. immaculata* and Rameseshian (1964) isolated it from a species of aphid infesting crucifers in India. Gustaffson (1965) isolated it from greenhouse soil in Sweden.

*Entomophthora coronata* has always been easily isolated on various organic media. It is this ability to grow on nonliving material that has caused much of the uncertainty as to whether it should be placed in the genus *Conidiobolus* or *Entomophthora*. But this tolerance makes it a suitable organism to experiment with. Wolf (1951) cultivated it in a chemically defined diet. Prosertphon (1963) tested the pathogenicity of five strains for the larvae of the greater wax moth, *Galleria mellonella*. Ali, Heitfuss and Fuchs (1965) showed that a strain of *E. coronata* isolated from an aphid was able to digest chiten and metabolize the N-acetylglucosamine. It could grow on a substrate of pure chiten. Gabriel (1965) made a
biochemical and histochemical study of the penetration of the insect cuticle by germinating E. coronata conidia.

Recently E. coronata has been reported to be pathogenic to mammals. Emmons and Bridges (1961) reported E. coronata to have caused serious mycosis in the nostrils and lips of horses in Texas. Bras et al. (1965) recovered E. coronata from a mycosis in the nose of a boy in Jamaica.

The taxonomic position of Entomophthora coronata has lately come under discussion again. The identity of this species has always been somewhat uncertain because of the lack of information about the zygospores. These sexually produced spores develop in characteristic structures on the hyphae and have thick resistant walls which display peculiarities of structure which are much used in identification. Couch (1939) said that the taxonomy of this species should be put off until zygospore formation had been observed. Emmons and Bridges (1961) reported finding smooth, thick-walled spores slightly smaller than the average size of primary conidia which they thought might be zygospores but they had not observed with certainty the manner in which they originated.

Srinivasan and Thirumalachar (1964) proposed the new combination Conidiobolus coronatus (Costantin) Srinivasan and Thirumalachar. They based their position on three arguments. The first is that potential pathogenicity is not enough to separate
the general Entomophthora and Conidiobolus because most of the Entomophthora species have been grown on artificial media. In the second place the conidiophores of coronata are of the type found in Conidiobolus. And in the third place the villous outgrowths on the so called "resting spores" represent "sterigmoid branches on which the microconidia have failed to develop." They gave a formal description of the new combination.

In the same year Batko (1964), publishing a paper given in 1962, proposed the combination Conidiobolus coronatus (Constantin) Batko. His reasons were similar to the above authors but emphasized morphological features more.

Because the present investigation is not taxonomic and the validity of the new name has not been tested by use, the old term of Entomophthora coronata as used by Bessey (1961), McLeod (1963) and Gustafsson (1965) will be retained in this paper for convenience.
MATERIALS AND METHODS

The symphyllans used were collected locally as needed. Extra specimens were retained for future use in plastic containers on ground hemlock bark.

The fungus was obtained from Dr. L. W. Getzin, Western Washington Experiment Station, Washington State University, Puyallup, Washington, in September, 1963. He had isolated the organism from symphyllans held in culture for insecticide screening tests at Southwestern Washington Experiment Station, Vancouver, Washington. In 1962 he had submitted cultures to E. A. Steinhouse at the University of California, Berkeley, California, who identified it as *Entomophthora coronata* (Costantin) Kevorkian. Prosertphon (1963) states that this strain was given the designation FO-40-1 and was the most virulent of four strains he tested against the greater wax moth, *Galleria mellonella*. Material from the present experiments was reidentified as *E. coronata* by Dr. W. C. Denison, Curator of the Mycological Herbarium, Department of Botany and Plant Pathology at Oregon State University in May, 1967.

The equipment used in maintenance and propagation of *Entomophthora coronata* consisted of the usual mycological tools: bunsen burners, transfer loops, Petri dishes, test tubes, bottles, and flasks. A pressure sterilizer was used to sterilize media and utensils. A Waring blender aided in preparing some of the media.
An incubator kept at 15°C and refrigerators located in other laboratories made temperatures below that of the laboratory possible. Several cabinets were available with temperatures higher than those in the laboratory.

The methods used were the customary mycological techniques—steam sterilization, flaming of loops and tubes and flask mouths. The fact that E. coronata conidia are shot away from the surface of the cultures made possible the use of jump plates and slants. These are made by bringing a piece of sporulating colony on agar medium or on an infected symphylan or insect, within a distance of one to five millimeters of the surface of a sterile plate or slant and allowing the spores to shower onto the fresh surface. Best results were obtained if the surface to be inoculated was inverted over the infected specimen. In this position condensation flowing from the specimen or debris falling from it could not contaminate the new culture. This made the isolation of relatively pure cultures rather easy in spite of the fact that this fungus, like other phycomycetes (Burges, 1958), seemed to show no antibiotic action against other microorganisms. Old cultures tended to fall prey to bacteria.

Aspirators, brushes and forceps were employed for the transfer of individual symphylans. Alcohol and Clorox were employed as disinfectants. Utensils that could stand heat were autoclaved at 15 pounds pressure for a suitable time.
The cages required for the symphylan colonies consisted of three kinds. Rectangular plastic refrigerator crisper chests 12" X 18" X 4" with tight lids, layered with three inches of bark chips, were used for storage of symphyllans to be used in later experiments. Small observation cages were made from 90 mm X 15 mm Petri dishes with a layer of various materials such as sand or plaster of Paris serving as a substrate (Figure 1, page 103). Enamel pans 16" X 10" X 2" containing a one-fourth to one-half inch layer of sand, with glass covers, served as large observation cages (Figure 2, page 103).

Aspirators, camel's hair brushes, a spatula, and forceps were employed in the transfer of specimens from soil or from one colony to another.

The methods used in caring for the symphyllans were patterned after Savos (1963) and Michelbacher (1938). The substrate was kept moist and vegetable food was added from time to time.

Exposure to _E. coronata_ was made by placing an infected symphylan or a piece of agar culture in the cage where the symphyllans would come in contact with it.
ECOLOGY AND EPIZOOLOGY OF SYMPHYLAN POPULATIONS UNDER CONTROLLED ENVIRONMENTS

The purpose of this experiment was to follow the interaction of symphylan populations and the disease organism over a prolonged period of time with particular attention to epizootic tendencies.

The method used was to confine symphylans to controlled environments where they could be observed with a minimum of disturbance and to expose some populations to the pathogen while others were kept free from contamination to act as controls.

Petri dishes with several types of substrate proved suitable for colonies of from 10 to 50 individuals while enamel pans covered with panes of glass were used for larger populations. The glass covers permitted observation with less disturbance to the organisms than would be possible in soil.

It was felt that the conditions in these culture dishes would approximate those in the soil as similar high humidities existed and many of the same microorganisms were present, being introduced with the symphyans and the food. The survival in these containers of symphyans, collembolans and the disease organism over the time period of the investigation suggests that such conditions were approximated. Colonies allowed to die from desiccation gave data as to whether or not the parasite could survive without the host
for any considerable length of time at low humidities.

The assumption was made that symphylans collected in the field or from cultures held in the laboratory, which showed no decrease in numbers, were free from infection. For this reason checks for each introduction were not maintained. The uninfected colonies which survived and reproduced over long periods served as checks on the over-all experiment.

Preliminary experiments in inoculating symphylan colonies were carried out soon after the fungus cultures were obtained from Dr. L. W. Getzin in 1963. On 9 December 1965 ten Petri dish colonies were selected for continuing observation. At this time four new colonies were started in $12'' \times 16'' \times 2''$ white enamel pans. The type of container and details of substrate will be described in the histories of various colonies.

Various foods were used including lettuce, beet leaf and slices of root, carrot slices and sunflower seeds. Carrot slices tend to cork over and remain alive for a long time in a moist atmosphere and resist attack by molds. The hull was removed from the sunflower seeds and the kernel broken in half. If left whole, so much energy was available to the sprout that it often pushed off the covers of Petri dishes. Because they were growing, these sprouting seeds were not often overgrown with molds and were available as food for the symphylans for two or three weeks. The combination of carrot
and sunflower seed made a food supply that did not need replacement as regularly as portions of leaves such as lettuce. With these long-lasting foods, and the ability of symphylans to eat certain fungi and decaying organic matter, the most crucial requirement was the replacement of moisture in the substrate. The only apparent cause of the death of colonies, aside from attack by Entomophthora coronata was desiccation or overwatering to the point where drowning took place.

As the biological details of symphylan development was not the aim of this investigation the symphylan cultures were examined, fed, and condition recorded if any change was noted, on an average of once every week or two.

Colony Number I

A colony was set up 28 October 1964 in a 100 mm X 20 mm Petri dish using an 8 mm layer of diatomaceous earth as substrate at the request of Dr. Andrew Duncan of the Department of Horticulture at Oregon State University to test the efficiency of diatomaceous earth as a pesticide. Far from being detrimental to the symphylans this material supported the most densely populated and most continuous colony in this experiment. The diatomaceous earth held large amounts of water, dried out slowly and offered crevices in which the symphylans could hide.
The objection to this type of substrate is that it is almost the same color as the symphylans and provides too many places to hide. Both of these characteristics make observation of symphylans difficult.

On 13 July 1965, eight and one-half months after introduction, an attempt to infect this colony with E. coronata from an agar culture was made. A small cube of the sporulating fungus was fastened to the top of a Petri dish cover and this was inverted over the colony so that ejected spores would be released into the environment and on to the symphylans. No drop in population and no dead symphylans were seen. Evidently the spores failed to infect. No more attempts were made to infect this culture.

Five months later, 17 December 1965, 50 symphylans were transferred to Colony Number XIV which was set up on diatomaceous earth in a 10" X 16" enamel pan.

The original colony remained healthy and again contained an estimated 50 symphylans on 1 April 1967 when the observations were terminated. The colony seemed to have stabilized as few immature individuals were seen. The light substrate was somewhat discolored by food and the excretion of the symphylans, but otherwise was quite clean of molds or other accumulation except for cast skins. No trace of dead symphylans could be found. If any had died, they were either eaten by other members of the colony or were broken down.
immediately by decay organisms in the environment. It is characteristic of symphylan habitats in the field that cast skins may be very abundant but dead symphylans are almost never found except for those injured by the movement of soil during the examination.

**Colony Number II**

This colony was set up on 25 June 1965 in a 100 mm X 20 mm Petri dish. An 8 mm layer of diatomaceous earth was used as a substrate. Ten symphylans were introduced. A cover with a sporulating piece of *E. coronata* culture medium fastened to it was inverted over the colony immediately after the symphylans were introduced.

Examination of 8 and 22 July showed no evidence of infection. Ten more symphylans were added on 8 July. On 22 July a dead symphylan from a colony infected with *E. coronata* was laid on the piece of lettuce offered for food. By 14 August all symphylans had died. The plate lay uninhabited until 7 September 1965 when 20 more symphylans from the Entomology Farm were added. By 11 October only one symphylan could be seen, but due to the difficulty of observing symphylans on the light-colored background a few more could have been present and not been observed. On 9 November 1965 20 more symphylans were added. One symphylan was found dead on 23 December. Ten more symphylans were added on this date.
On 1 January 1966 several healthy symphylans were seen but there were two dead ones. One of the dead ones was transferred to a jump-slant of milk agar which became infested with *E. coronata* by introduction of the dead symphylan.

From this time on to the end of the observation the history of this colony is one of introduction at varying intervals of time of five groups of symphylans, all of which eventually succumbed to *E. coronata* infection.

**Colony Number III**

This colony was maintained on a plaster of Paris-soil layer similar to Filinger's "muck plates" (Filinger, 1928) except that the plaster mixture was poured into a 100 mm X 15 mm Petri dish instead of a Stender dish. The surface was grooved and a microscopic slide provided as an object under which the symphylans could hide. This was an old substrate set up in late 1964. On 9 December 1965 the colony consisted of nine adults and four young symphylans. On 9 March 1966 there were 12 healthy adults. They were fed and watered at intervals and showed no incidence of disease until 12 October 1966 when the colony was found to be dead. The cause seemed to be desiccation with no disease symptoms evident. This type of substrate does not hold water as long as the diatomaceous earth or plates made of plaster of Paris and lampblack.
On 12 November 1966 ten more symphylans from the Entomology Farm were introduced with moisture and food. They did well producing five young by 18 January 1967.

On 17 March 1967 the plate was again noted to be desiccated but four symphylans were still alive. One of the survivors was dead on 18 March but no Entomophthora was isolated from it. The remaining three symphylans persisted until the end of the experiment.

This is a case of a plate which was uncontaminated with Entomophthora supporting disease-free symphylans for a period of three years. Although the colonies were subjected to occasional desiccation and limitation of food they developed nothing that could be recognized as an infection.

**Colony Number IV**

The substrate for this colony was composed of four parts plaster of Paris to one part lampblack, poured to a depth of 8 mm in a 100 mm × 15 mm Petri dish (Peterson, 1964). The surface was grooved and a glass slide laid over the grooves. The first symphylans were introduced 20 November 1964. On 13 April 1965 the colony was exposed to infection by sticking a cube of agar from a sporulating culture of *E. coronata* to the cover of the plate. When this plate was chosen for further study on 9 December 1965 there was one
surviving symphylan and a few white subterranean collembolans.

On 10 December 1965 ten more symphyllans were added. There seemed to be no disease problem. Reproduction was observed in March and April of 1966 and in January and February 1967.

This is a case of a colony once showing mortality from E. coronata but in which a lone survivor and individuals introduced later escaped infection. There is the possibility that the long period during which the plate was inhabited by collembola may have had something to do with the elimination of the disease. The collembolans which were transferred from the soil at various times have never been definitely shown to suffer from Entomophthora infection. They are scavengers and fungus eaters and it is possible that they destroyed the spores remaining in the plate before the new symphyllans were introduced. Collembola of another species were seen feeding on E. coronata spores which were deposited on a glass surface covering a sporulating colony of the fungus.

Colony Number V

The substrate for this plate was plaster of Paris and lampblack upon which a microscope slide was laid. This was an old plate from preliminary experiments in 1963 when it had been colonized with symphyllans and infected with Entomophthora from
the Getzin culture.

On 9 December 1965, when the present study was started, ten more symphylans were added. On 23 December three were still alive. Two of these were dead by 30 December. One of these showed typical sporulation. Ten new symphylans were added on this date. Nine days later, 1 January 1966, all were dead but two. Two dead symphylans which were still not decomposed were transferred to milk agar slants. One of these showed a typical \textit{E. coronata} colony. The other slant was overgrown by a filamentous fungus of undetermined species. Several species of fungus behaved as secondary invaders but were never identified as causing the death of symphylans. The saprophytes often overran the specimen making identification of \textit{E. coronata} difficult.

On 23 January 1966 one specimen was still alive. Ten more were added. On 2 February, four days later, only three were still alive. One dead one was transferred to a jump-slant. Three days later a mixed culture was obtained of what appeared to be \textit{E. coronata} and a reddish colony of bacteria. The color suggests \textit{Serratia marcescens}. This bacterium, usually a saprophyte, sometimes may be an invertebrate parasite. It is often mentioned in insect pathology because of its distinctive pigments. The fact that a colony of \textit{E. coronata} was also obtained would indicate that the \textit{Serratia} was probably not the primary cause of death. This mixture of
E. coronata with a bacterium seems to be characteristic of E. coronata growing on culture medium. As soon as a colony of E. coronata becomes a little old bacteria seem to attack and destroy it, even to the spores.

On 9 March 1966 all the symphylans were dead but a few collembolans were present. Here again collembolans were able to survive in an environment contaminated with E. coronata.

On 15 April 1966 a few symphylans were added which were all dead by the end of the month. Fifteen symphylans were again introduced on 13 July. Ten days later they were all dead. E. coronata was isolated from two dead ones. On 10 August 1966 15 new symphylans were added. One of these survived until 18 January 1967.

On 14 February 1967 all symphylans were dead. The plate was repopulated with ten symphylans from the Entomology Farm on 15 February. Two of these were dead and two more showed symptoms of illness in 24 hours. In 48 hours only one survived. An examination on 18 March 1967 showed all to be dead.

Fifteen symphylans from the Entomology Farm were added 22 March. On 4 April all were dead and ten more were added. By 7 April all were dead.

The history of this culture shows E. coronata surviving a period of more than three years in an environment of varying but usually high humidity with a varying number of susceptible hosts present.
At one time (30 April to 14 July 1967) the plate was uninhabited for 76 days, but symphylans became infected when they were again introduced. It also showed the ability of a single symphylan to survive for a considerable period in a contaminated environment. The explanation may be that the single symphylan confined its movements to uncontaminated areas. An immunity which protected an individual against all future exposure was not observed.

**Colony Number VI**

The plate into which this series of introduction was made consisted of a layer of sand about 5 mm thick in the bottom of a 100 mm × 15 mm Petri dish. A microscope slide was laid on the sand. This plate had much the same history as Number V. It had been colonized and inoculated with *E. coronata* some months before it was selected for this observation. The last introduction of symphylans prior to the beginning of this study was on 7 September 1965. After this colony had died some time before 1 October 1965 the plate was allowed to dry up and no symphylans were added until 9 December 1965 when this history began. At this time ten symphylans were introduced, of which two survived until 23 December, when nine more were added. Five days later, 29 December, all but one were dead. A sporulating specimen was transferred to a jump-slant with milk agar. *E. coronata* was recovered. On 30 December, ten
symphylans were introduced. Two days later on 1 January 1966 all were dead but one. This one was dead on 13 January.

Fifteen symphylans from the Entomology Farm were added on 12 January 1966. The next day three were found dead but may have been injured during introduction. No _E. coronata_ was recovered. At the inspection on 2 February three were still alive. A dead one was transferred to a jump-slant where _E. coronata_ was isolated. By 10 February all symphylans had been killed.

The plate was left uninhabited and allowed to desiccate for a month, until 15 April, when the sand was again moistened, beet leaf added for food, and an undetermined number of symphylans added. Two weeks later, 2 May, all were dead.

Again the plate was left uninhabited for seven weeks this time, from 2 May to 13 July. On 13 July 1966 15 symphylans were introduced. On 26 July seven were still alive. An inspection on 10 August revealed seven adults and three second instar nymphs. This point marks a break in the infectiousness of this plate. Due to the poor water holding capacity of sand this plate went dry and the inhabitants were found dead on 12 October. After this occasion more symphylans were added and the last introduction showed no infection by 1 April 1967 when this observation ended.

The history of this plate seems to indicate that infectivity of _E. coronata_ may be lost from an environment once contaminated.
In this case the loss of infectivity followed seven weeks of desiccation.

**Colony Number VII**

The substrate in this Petri dish was plaster of Paris darkened with lampblack. The surface was grooved and a microscope slide was laid over the grooves to form runways. This plate did not support a colony of symphylans during the time of observation. Instead it was inhabited by a colony of subterranean collembolans. They were white and lacked a "spring". Their requirements were about the same as those of symphylans. They were maintained to see if they would be attacked by the fungus. They did not seem to be susceptible to *E. coronata*.

One item of interest took place on 13 July 1966. Upon inspection the collembolans all seemed to be dead from desiccation. They were shriveled and motionless but seemed to be intact. Moisture was added to the plate and at the inspection 13 days later, 26 July, a good number had survived. No dead were seen. If any had died the others had destroyed them.

The colony was healthy when this observation was discontinued.

**Colony Number VIII**

The substrate in this 100 mm × 15 mm Petri dish was plaster
of Paris colored black by a layer of India ink on the surface. This is not a very absorbent substance and while it is new the symphyllans do not thrive but it is not toxic enough to eliminate the inhabitants. As it ages in a moist condition the surface becomes more permeable. This was an old plate that had been uninhabited and desiccated for months. On 10 December 1965 it was moistened and ten symphyllans added. On 23 December all were dead but one. Ten more were added on this date. On 13 January 1966 all were dead but one. Two were in good enough condition to transfer to slants. One was laid on the agar while the other was set up in a jump-slant. *E. coronata* was isolated from both. The jump-slant appeared to be pure *E. coronata* but the slant upon which the dead symphyllan was laid developed a mixed culture of *E. coronata* and a bacterium which stained the agar greenish-yellow. This color of pigment is characteristic of certain *Pseudomonas* species. Bucher (1963) lists several species of *Pseudomonas* as pathogens or potential pathogens of insects and related forms. In this case the bacterium was probably a secondary invader as no observations were made of cases where symphyllans showed primary infection by bacteria. The fact that the fungus and bacterium grew together indicates that *E. coronata* does not produce antibiotics. Burges (1958) says that this is characteristic of the *Phycomycetes*. They compete by rapid growth rather than chemical warfare.
Subsequently eight introductions of symphylans were made after total or partial (one or two survivors) depletion of the populations. *E. coronata* was isolated three times, along with the characteristic appearance of the dead symphylans observed.

This was one of the most virulently infected plates. The pathogen must have been in the plate from a former experiment because it was not infected after this experiment started. This plate developed epizootic potential when new introductions were made in rapid succession. For example, an introduction of ten symphylans was made on 13 February 1967. All were dead by 15 February. On this date ten new symphylans were added and all were dead in 30 hours. When the plate was allowed to remain uninhabited for some time the infection of a new introduction of symphylans was slower.

**Colony Number IX**

This plate had a plaster of Paris and soil mixture as a substrate with a microscope slide as a hiding place. The container was a 100 mm × 15 mm Petri dish.

The first introduction was made on 10 December 1965 and consisted of 56 symphylan eggs. They were found in heavily infected soil held for a week at room temperature. Symphylans taken during the winter and early spring seem to be stimulated to lay eggs rapidly when the soil in which they are living is warmed. The eggs turned a
little brownish before hatching but only one molded and did not hatch. The young, fed pieces of beet and carrot, seemed to grow satisfactorily until 21 February 1966 when there was a big drop in the number of young symphylans. Before this time there had been too many to get an accurate count. By 9 March 1966 there were only six survivors. The cause of the decrease seemed to be a little more water than the plate could absorb, leaving a film of free water on the surface in which many became entangled. By May the survivors were of approximately mature size.

Due to the low water retention of this type of plate, colonies were killed by desiccation 24 June 1966 and 18 January 1967. New introductions were made after each of the colony failures. The plate was inhabited when this experiment was terminated at the end of March 1967.

This is a case of an uncontaminated plate remaining so over a long period of time. In spite of environmental difficulties the three successive colonies showed no symptom of disease.

**Colony Number X**

This colony was in another 100 mm × 15 mm Petri dish with a plaster of Paris and lampblack substrate with a microscope slide added as a hiding place for symphylans. It had a previous record of infection with *Entomophthora*. Ten symphylans introduced on
17 July 1965 had died in ten days.

On 10 December 1965 it was chosen for further observation. At this time ten symphylans were added along with food (a piece of beet leaf and a chip of beet root) and moisture. By 23 December all the symphylan were dead.

This plate continued to be heavily contaminated. Seven further introductions of symphylans were made. Each time before more symphylans were added the colony had become completely depleted or had only one or two survivors. The colony was found to be uninhabited in November 1966 but whether desiccation or \textit{E. coronata} was responsible for the death of the symphylans could not be determined when the examination was made. The plate was furnished with new symphylans which again began to die. \textit{E. coronata} was isolated again. Over the period of this experiment \textit{E. coronata} was isolated altogether four times from dead symphylans.

There were quite a few "slime mites" at times feeding on dead symphylans and fungus spores. \textit{Entomophthora} spores could at times be seen on the hairs of the mites. If the mites became infected the infection was not serious enough to eliminate the population. No cases of infection of mites was observed.

In this case one or two symphyylan survived after the rest of the colony had died but did not develop an immunity which protected them from infection when new individuals were introduced, and were then
overcome by the fungus.

**Colony Number XI**

The container for this colony was an enamel pan 12" × 16" × 2" with \( \frac{1}{4} \)" of ground hemlock bark as a substrate. A pane of glass was laid on the chips and the whole pan covered by a larger pane. This culture was set up late in 1964 and has been continuously inhabited until the end of this investigation—a period of about three years.

This has been a good colony comparing well with Colony Number I (on diatomaceous earth in a Petri dish). It soon built up to a maximum population and has stayed there since. There seems to be a density beyond which a symphylan population goes with difficulty. General observation indicates that where symphylans are confined to one area, as they were in cultures such as these, that they come to rest at intervals of \( \frac{1}{2} \)" to 1" apart in all directions. They do not seem to congregate in bunches. (Figure 13, page 109). Perhaps this has something to do with population restriction. Ailing or undersized symphylans are seldom seen. They are healthy normal individuals or they disappear.

**Colony Number XII**

This colony was set up with \( \frac{1}{2} \)" of sand as a substrate in a 12" × 16" × 2" pan. A glass was laid on the sand and a larger glass
over the top of the pan. This container was colonized in the spring of 1965 and at first the symphylans became well established. On 8 May material from an experimental culture using bran, which had been autoclaved and inoculated with *E. coronata*, was deposited around the edges of the colony. No kill was noted so small blocks from a sporulating culture of *E. coronata* on milk agar were placed around the edges of the pane of glass which was laid on the sand. This inoculation was done on 22 May. Dead symphylans were observed on 25 May. Several clutches of eggs were laid during this early period.

On 11 December 1965 when this colony was added to the group for further observation about eight symphylans were still alive. To check the possibility that the sand was not a good substrate about \( \frac{1}{4} \)" of bark chips were spread over the sand. No more symphylans were added. On 1 February 1966 one second instar symphylan was seen in addition to the eight adults. From here on the colony dwindled until no life was seen by 10 May 1966. However, two symphylans were found when the bark chips were examined. The old substrate was discarded and the pan and glass panes were washed and stored until 4 August 1966.

On the above date \( \frac{1}{2} \)" of bark chips were deposited in the pan as substrate and the glass panes put in place. Symphylans were introduced by placing clods of soil containing symphylans on a
screen to prevent the soil from scattering and laying this in the pan until desiccation drove the symphyllans out of the clods into the bark chips. This method produced a good number of undamaged symphyllans but it also captured about three centipedes as well as numerous collembolans and millipedes. The millipedes and collembolans caused no damage but the centipedes were predacious on the symphyllans. The centipedes were removed when observed.

This second colonization was only moderately successful. The centipedes, no doubt, tended to keep the culture down and also a thick growth of a white mycelium of a fungus that smelled like a wood-rot organism over-grew the chips. While this did not seem to be poisonous to the symphyllans it curtailed their movements. The symphyllans were observed mostly confined to trails made through the fungus by pill bugs. No evidence of *E. coronata* could be detected. A few symphyllans survived to the end of the experiment.

**Colony Number XIII**

This colony was set up in a 12" × 16" × 2" enamel pan on a substrate of sand from the greenhouse. It was set up originally in 1964 and was quite prosperous for awhile. Several masses of eggs were observed in February and April of 1965. This culture was never voluntarily exposed to *E. coronata* nor was any isolated from dead symphyllans. But by the time this culture was included in the
special observation experiment on 11 December 1965 the colony was not doing well. Only 12 symphylans could be counted when there should have been at least 100. A thin layer of bark chips was added at this time to allow the symphylans to move around under the glass with greater ease.

For a while in early 1966 the surviving symphylans seemed to succeed fairly well. But by 9 March all seemed to have disappeared. The pan was set aside until 14 February 1967 when 25 more symphylans were added. They survived until the end of the experiment and seemed to be healthy.

This was an unsatisfactory colony. About the only conclusion to be drawn from its history is that sand is not a good substrate for symphylans. It desiccates rapidly and as Martin (1948) has reported, symphylans are unable to penetrate a layer of fine sand.

**Colony Number XIV**

This colony was set up in an enamel pan 12" × 16" × 2" using diatomaceous earth for a substrate. As with other colonies in this type of pan a sheet of glass was laid on the substrate and a larger one covered the pan (Figure 2, page 103). Fifty symphylans from Plate I were introduced on 17 December 1965. This plate had always been healthy and remained so after the transfer.

But by January 1966 severe mortality was noted in the newly
transferred population. Observation did not furnish a clue as to the cause. On 9 January slants were set up with four dead symphyllans. One tube showed a greenish bacterial colony. Two tubes were overrun with a species of Rhizopus and the fourth tube produced no identifiable culture.

On 26 January three still survived and ten more were added. On 27 January 30 symphyllans were added and on 29 January ten more were added. This made a total introduction of 50 symphyllans. On 1 February four dead symphyllans were found. Three were set up in "jump-slants" with milk agar. All three showed E. coronata by 7 February.

On 10 February five or six symphyllans were still alive. On 21 February only one symphyllan was seen. On 9 March one was again seen. It is a common occurrence to have a few symphyllans survive in a contaminated environment for a considerable period of time unless the level of infective material is high, in which case the colony is depleted in the course of a few days. Nothing more was observed on the colony until 5 July 1966 when 50 more symphyllans were introduced. They seemed to survive fairly well for a while, but by 24 December no more could be found.

On 23 February 1967, 25 to 50 more symphyllans were added. By the first of April 1967 these were gone.

The big question with this substrate is how it became
contaminated with *E. coronata*. It seems unlikely that the symphylans could have been infected because the first population which showed infection had been transferred from the colony in Plate I which had never shown any mortality before or after the transfer.

The pan and the glass sheets were new and clean. The diatomaceous earth was from the same lot as that used in Plate I and had been stored in a very dry warm place.

The infection must have been due to accidental laboratory introduction but how this came about could not be determined. The transfer utensils were routinely cleaned with alcohol. *E. coronata* spores are large, sticky and inactivated by desiccation. Aerial infection by this organism has never been reported.

This situation would seem to indicate that the fungus is more contagious than generally supposed. It is possible that there is a resistant stage which could be disseminated under ordinary laboratory conditions, but which has not yet been recognized.

The mortality in this container was evidently caused by infection with *E. coronata*. The fact that the first attempt at isolation made on 7 January 1966 did not produce the expected fungus did not eliminate the probability that *E. coronata* was the cause of mortality, in view of the fact that the organism was isolated from dead symphylans one month later. *E. coronata* is easily overgrown by secondary invaders which may make its isolation difficult or
impossible because the fungus itself is destroyed by some of these organisms.

**Observation of Additional Colonies**

From time to time *E. coronata* showed up in a culture where it was not expected. On 9 October 1963 all the symphylans held in a Petri dish were found to be dead. More symphylans were added as the plate became depopulated. The symptoms observed identified the disease as *E. coronata*. Later isolation on agar plates confirmed the identification. This plate had not been purposely inoculated.

A similar thing occurred in one of three Petri dishes with substrates of diatomaceous earth and soil, which had been autoclaved and colonized with 30 symphylans each on 22 March 1967. In a few days the symphylans were all dead apparently with *Entomophthora* although it was not isolated. Later another plate in this series developed similar trouble.

*E. coronata* gained entry to these populations was not determined. There is the possibility that the culture had been contaminated when the symphylans were being introduced but this does not seem likely considering the fact that the spores of this fungus are not known to be airborne nor could they be expected to withstand the desiccation they would encounter if they had been carried on laboratory instruments even if these had not been cleaned. The infection
might have been carried on food. The carrots used had been in the ground all winter and showed some decay but these areas were discarded. The fungus mites which showed up regularly in the old culture plates might have carried the pathogen from an infected plate or an infected symphylan may have been introduced from the soil.

All that can definitely be said is that this pathogen sometimes shows up in laboratory cultures as reported by Getzin (1964) and Swenson (1967).

**Discussion**

The colonies under observation fell into three groups: those in uncontaminated environments, those in contaminated environments, and those which showed variable symptoms.

**Colonies in Uncontaminated Environments:**
**Numbers I, III, IX, and XI**

The substrates included in the group were diatomaceous earth (Plate I), soil-plaster of Paris mixture (Plates III and IX) and ground hemlock bark (Pan XI). The diatomaceous earth and ground bark seemed to provide the best environment to judge from reproductive rates and long uninterrupted colonization by symphylans.

Original colonies in Plates III and IX, with soil-plaster substrates, were lost at one time or another due to desiccation and had
to be replaced with new symphylans.

The symphylans in the colonies showed no disease and reproduction took place up to the environmental limit. Symphylans seem to have a space requirement (Figure 13, page 109) which prevents crowding beyond a certain point. Savos (1958) and Michelbacher (1938) noted the long life of symphylans in captivity. The findings of this experiment agree with their work. Colonies I and II survived without interruption for two and one-half years. No doubt some of the original inhabitants were still alive at the end of that time, however, individuals were not marked.

The histories of these colonies on uncontaminated substrates and the experiments of the above authors would indicate that symphylans do not ordinarily carry latent pathogens in their populations which may show up under stress and destroy the colonies.

Colonies in Contaminated Environments:
Numbers II, V, VIII, X, and XIV

The history of these colonies is of an original population of symphylans becoming infected with _E. coronata_, artificially or otherwise, and infective material from them contaminating the environment. Uninfected symphylans introduced into such environments became infected and died whereupon the fungus sporulated and added to the contamination. Infection and death took place in as short
a time as 30 hours (Colony VIII). Symphylans became infected on substrates which had been uninhabited with symphylans for several weeks (Colony II) to several months (Colony V for two and a half months and Colony X for five months).

How infection was picked up by a symphylan from an environment which had been uninhabited for some time was not determined. The common avenue of attack is directly through the integument by the germ tubes from spores attached to the cuticle (Prasertphon, 1963) and (Yendol and Paschke, 1965). Figure 9 shows evidence of _F. coronata_ attacks on the cuticle of _Galleria_ larvae. This method would seem to be effective when the host is in range of a sporulating mycelium but when sporulation has stopped for want of a food source other methods of entrance would seem to be necessary.

Some possibilities suggest themselves. An old spore or hyphal body might be activated by a change in humidity or some effect of the presence of a host species and produce an infected structure. Other organisms such as collembolans and mites might harbor the disease undetected.

An interesting possibility is suggested by the work of Yendol and Paschke (1965) who found germ tubes from ingested _F. coronata_ spores penetrating the esophageal walls of termites. Spores remain in the carcasses of symphylans killed by this fungus. How long these spores remain viable is not known. Symphylans were reported
to eat spores of this fungus by Getzin and Shanks (1964a). The intestine showing through the surface of the back of symphylans turned black soon after they were introduced to a substrate containing lampblack, thus indicating ingestion of the substrate whether edible or not.

Colonies with Variable Histories:
Numbers IV, V, XII, and XIII

Colony IV (substrate plaster of Paris and lampblack), Colony V (substrate sand), and Colony XII (substrate and later covered with hemlock bark) were infected with *E. coronata* as evidenced by the history of introductions of the fungus and its isolation from dead specimens. After various periods of being uninhabited by symphylans new introductions into these containers no longer became infected. This indicates that *E. coronata* is fastidious in its survival requirements and will die out of an environment if conditions are not suitable.

Pan XIII had a sand substrate and did fairly well for a time but deteriorated as time went by. Due to the fact that symphylans cannot force their way through sand, living space under the glass became more and more restricted. No symptoms indicating specific disease were noted. The number of individuals did not increase, however.
Observations on Other Colonies

Once in a while *E. coronata* infections showed up in colonies where it was not expected. The two plates mentioned and the pan with diatomaceous earth (Colony XIV) fall into this class.

All that is known is that this pathogen shows up in symphylan cultures once in a while. Getzin and Shanks (1964a) and Swenson (1967) had this happen to their cultures. No survey has been run to see how often *E. coronata* could be recovered from field-collected symphylans or from local soils.

These observations indicate:

1. Symphylans are highly susceptible to *E. coronata* infection both from spores from active colonies attaching to the surface of the body and from residual stages of the fungus remaining in the environment.

2. In congested areas the infection can reach epizootic proportions.

3. The survival of the pathogen in the environment may be for at least several months without symphylans being present.

4. Immunity to the pathogen did not seem to develop. At times one or two symphylans survived on contaminated substrates but when newly introduced individuals were killed by the fungus, these original survivors became infected and died.
TEMPERATURE EXPERIMENTS

Temperature experiments were conducted to find out how well the growth of *E. coronata* coincided with the optimum temperatures for symphylan activity. Also involved with the matter of heat tolerance is the danger of phycomycosis of those coming in contact with *E. coronata*. Emmons, Chester and Charles (1961) reported mycotic infections of the skin and mucous membranes surrounding the nostrils and lips of horses in Texas. Bras et al. (1965) reported a case of mycosis in the nasal passage of an 11-year-old boy from Grand Cayman (an island close to Jamaica). These authors state that species of the genera *Absidia*, *Mucor*, *Rhizopus*, and *Basidiobolus* have previously been known to attack human skin and internal organs. These are all aplanate Phycomycetes as are the species of *Entomophthora*.

Preliminary experiments were made using milk agar as the nutrient medium.

On 29 December 1965 six plates were inoculated with a growing culture of *E. coronata*. Three plates were maintained at 7°C and three at 16°C.

After 14 days (12 January 1966) the plates which were incubated at 7°C showed almost no growth. After 40 days one plate showed no growth. The second plate showed a colony 10 mm in diameter. This
represents a radial growth of 0.125 mm per day. The third plate showed a radial growth of 4 mm or 0.05 mm per day. The average radial growth per day for the three plates was 0.58 mm.

Three plates were incubated at 16°C for 14 days. At this time the colonies of *E. coronata* had reached the edges of the 90 mm plates. This amounts to a daily radial growth of 3.2 mm.

Four plates of milk agar were similarly inoculated with *E. coronata* on 7 February 1966. Two plates were incubated at 15°C for five days. The first plate showed a colony of 20 mm in diameter indicating a rate of radial growth of 2 mm per day. The second plate developed a colony of 25 mm in diameter, indicating a radial growth of 2.5 mm per day. Average radial growth of both plates equals 2.25 mm.

Two plates were incubated at 22°C for five days. The first produced a colony 35 mm in diameter, indicating a radial growth of 3.5 mm per day. The second plate had developed a colony 45 mm in diameter indicating a radial growth of 4.5 mm per day. The average for the two plates was 4 mm radial growth per day. Table 1 gives this data in tabular form. Graph 1 (page 110) shows the growth curve for these plates.
Table 1. Combined results of milk agar growth data.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Plate</th>
<th>Average radial growth of colony per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>7°C</td>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2.6 mm</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.1 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7 mm Average</td>
</tr>
<tr>
<td>15°C</td>
<td>A</td>
<td>2.0 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.25 mm Average</td>
</tr>
<tr>
<td>16°C</td>
<td>A</td>
<td>3.2 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2 mm Average</td>
</tr>
<tr>
<td>22°C</td>
<td>A</td>
<td>3.5 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0 mm Average</td>
</tr>
</tbody>
</table>

Further investigations of temperature reactions were performed in early 1967 on Difco Bacto-Sabouraud Agar. Seven plates and 11 slants of Difco Bacto-Sabouraud Maltose Agar were inoculated 9 February 1967 with a spore smear of *F. coronata*. One plate and two slants were incubated at each of five different temperatures: 2°, 15°, 23°, and 30°C and two plates and three slants were incubated at 37°C.

The colonies to be incubated at 2° C were allowed to remain at 17°C for 12 hours to give the spores a chance to germinate and
begin invasion of the substrate. At the five-day inspection too little growth was evident to measure. Eleven days after inoculation the 2°C plate still showed almost no growth of *E. coronata* but a colony of contaminating mold was about 30 mm in diameter. Since it was not sporulating no identification was made. The slants showed little growth but some bacterial contamination.

The plate and slants incubated at 15°C produced colonies of about 25 mm in diameter in five days. This is an average of 2.5 mm per day radial growth.

At 23°C an average radial growth of 7 mm per day was obtained.

The transfers incubated at 30°C gave inconclusive results due to contaminating organisms. Graph 1, page 110, gives the curve for these plates. Table 2 shows this data in tabular form.

Five plates and ten slants of Bacto-Sabouraud Maltose Agar were inoculated 18 February 1967. One plate and two slants each were incubated at the following temperatures: 1°, 15°, 17°, 24°, and 37°C for three days.

The plate and slants incubated at 1°C showed no growth.

Those incubated at 15°C produced colonies of about 10 mm in diameter which showed an average radial growth of 1.7 mm per day.

The plate incubated at 17°C produced a colony of 20 × 40 mm
Table 2. Temperature experiment 9 to 14 February 1967.

<table>
<thead>
<tr>
<th>Temperature and colony</th>
<th>Diameter and condition after five days</th>
<th>Radial growth per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>2°C Plate</td>
<td>Spores beginning to germinate</td>
<td>0</td>
</tr>
<tr>
<td>Slant A</td>
<td>Spores beginning to germinate</td>
<td>0</td>
</tr>
<tr>
<td>Slant B</td>
<td>Spores beginning to germinate</td>
<td>0</td>
</tr>
<tr>
<td>15°C Plate</td>
<td>25 mm Good condition</td>
<td>2.5 mm</td>
</tr>
<tr>
<td>Slant A</td>
<td>25 mm Good condition</td>
<td>2.5 mm</td>
</tr>
<tr>
<td>Slant B</td>
<td>25 mm Good condition</td>
<td>2.5 mm</td>
</tr>
<tr>
<td>23°C Plate</td>
<td>? Overgrown with mold</td>
<td></td>
</tr>
<tr>
<td>Slant A</td>
<td>90 mm Good</td>
<td>8.0 mm</td>
</tr>
<tr>
<td>Slant B</td>
<td>? Contaminated</td>
<td></td>
</tr>
<tr>
<td>30°C Plate</td>
<td>? Contaminated</td>
<td>0</td>
</tr>
<tr>
<td>Slant A</td>
<td>? Contaminated</td>
<td>0</td>
</tr>
<tr>
<td>Slant B</td>
<td>? Contaminated</td>
<td>0</td>
</tr>
<tr>
<td>37°C Plate A</td>
<td>0 Overgrown with mold</td>
<td>0</td>
</tr>
<tr>
<td>Plate B</td>
<td>0 Overgrown with mold</td>
<td>0</td>
</tr>
<tr>
<td>Slant A</td>
<td>0 Not contaminated</td>
<td>0</td>
</tr>
<tr>
<td>Slant B</td>
<td>0 Overgrown with Penicillium</td>
<td>0</td>
</tr>
<tr>
<td>Slant C</td>
<td>0 Overgrown with Penicillium</td>
<td>0</td>
</tr>
</tbody>
</table>
(30 mm average) or a radial growth of 5 mm per day. Both slants had colonies of 20 mm diameter each or 3.3 mm radial growth per day. This gives an average of 3.8 mm radial growth per day.

The plate and slants incubated at 24°C showed a diameter of about 70 mm. Of course with the slants the expansion could only be lengthwise of the tubes. This amounts to about 11.7 mm radial growth per day.

The plate and two slants incubated at 37°C showed almost no growth. Table 3 summaries these data. Graph 1, page 110, shows this same data.

Sixteen slants of Sabouraud agar were inoculated on 2 March 1967 and incubated at the following temperatures: 1°, 15°, 17°, 24°, 30°, 32°, and 35°C.

The slants were incubated for eight days at which time one slant for each temperature was photographed (Figure 11, page 108). The cultures incubated at 24°, 27°, and 30°C covered almost the entire surface of the slant and had thus reached the limit of expansion before this reading was taken. This is shown when comparison is made with the daily growth rates of 11.1 mm, 10.7 mm, and 9.5 mm observed in the following experiment for similar colonies incubated at the same temperatures. The important consideration shown in this experiment is the difficulty of the mycelium invading the substrate at temperatures near 0°C or above 30°C. The
Table 3. Temperature experiment 18 February 1967.

<table>
<thead>
<tr>
<th>Temperature and colony</th>
<th>Diameter in 2 days</th>
<th>Diameter in 3 days</th>
<th>Radial growth per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 °C Plate</td>
<td>Trace</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Slant A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Slant B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Slant A   | 0            | 0             | 0            |
| Slant B   | 0            | 0             | 0            |

<table>
<thead>
<tr>
<th>Slant B</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>15 °C Plate</th>
<th>5 mm</th>
<th>10 mm</th>
<th>1.7 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slant A</td>
<td>5 mm</td>
<td>10 mm</td>
<td>1.7 mm</td>
</tr>
<tr>
<td>Slant B</td>
<td>5 mm</td>
<td>10 mm</td>
<td>1.7 mm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Slant B</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>17 °C Plate</th>
<th>10 mm</th>
<th>20 × 40 mm</th>
<th>5.0 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slant A</td>
<td>10 mm</td>
<td>20 mm</td>
<td>3.3 mm</td>
</tr>
<tr>
<td>Slant B</td>
<td>10 mm</td>
<td>20 mm</td>
<td>3.3 mm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Slant B</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>23 °C Plate</th>
<th>40 mm</th>
<th>70 mm</th>
<th>11.7 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slant A</td>
<td>40 mm</td>
<td>70 mm</td>
<td>11.7 mm</td>
</tr>
<tr>
<td>Slant B</td>
<td>40 mm</td>
<td>70 mm</td>
<td>11.7 mm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Slant B</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>34 °C Plate</th>
<th>0</th>
<th>0</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slant A</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Slant B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Slant B</th>
</tr>
</thead>
</table>

Average
growth is shown in the following table.

Table 4. Temperature experiment 2 March 1967.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Diameter of colony</th>
<th>Radial growth per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°C</td>
<td>3 mm</td>
<td>.2 mm</td>
</tr>
<tr>
<td>15°C</td>
<td>58 mm</td>
<td>3.6 mm</td>
</tr>
<tr>
<td>17°C</td>
<td>76 mm</td>
<td>4.7 mm</td>
</tr>
<tr>
<td>24°C</td>
<td>81 mm</td>
<td>5.1 mm</td>
</tr>
<tr>
<td>27°C</td>
<td>87 mm</td>
<td>5.5 mm</td>
</tr>
<tr>
<td>30°C</td>
<td>89 mm</td>
<td>5.6 mm</td>
</tr>
<tr>
<td>32°C</td>
<td>trace</td>
<td>0</td>
</tr>
<tr>
<td>35°C</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Seven plates and 14 slants of Difco Sabouraud Agar were inoculated with a small lump of spores from a growing colony of F. coronata at 4 p.m. on 8 March 1967. They were incubated at 24°C until 8:00 the next morning (16 hours) to give the spores opportunity to germinate. One plate and two slants were then transferred to incubators at each of the following temperatures: 1°, 17°, 24°, 27°, 30°, 32°, and 35°C.

At 10 p.m. 10 March (a total of 54 hours, including the 16 hour germination period) measurement was made and a photograph (Figure 12, page 108) was taken of one set of slants. Growth is shown in Table 5 and on Graph I (page 110).
To test further the relationship of temperatures near that of the human body (37°C) a series of slants of Sabouraud Agar were inoculated with F. coronata spores and held at 27°C for 48 hours at which time the spores had germinated and covered an area of about 5 mm in diameter. At this time the tubes were placed in an incubator and held near 37°C.

After 30 hours a sample was removed to laboratory temperature (23°C). Every 24 hours thereafter another sample was removed to the lower temperature until a total of four samples had been removed from the 37°C to the 23°C temperature where they were held for a week.

Soon after introduction to the 37°C incubator the area of the fungus colony increased about 1 mm and some sporulation took place and some spores germinated but after a few hours all growth stopped.

Table 5. Temperature experiment 9 March 1967.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Diameter of colony</th>
<th>Radial growth per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°C</td>
<td>5 mm</td>
<td>1.1 mm</td>
</tr>
<tr>
<td>17°C</td>
<td>20 mm</td>
<td>4.4 mm</td>
</tr>
<tr>
<td>24°C</td>
<td>50 mm</td>
<td>11.1 mm</td>
</tr>
<tr>
<td>27°C</td>
<td>48 mm</td>
<td>10.7 mm</td>
</tr>
<tr>
<td>30°C</td>
<td>43 mm</td>
<td>9.5 mm</td>
</tr>
<tr>
<td>32°C</td>
<td>25 mm</td>
<td>5.6 mm</td>
</tr>
<tr>
<td>35°C</td>
<td>8 mm</td>
<td>1.8 mm</td>
</tr>
</tbody>
</table>
In one tube removed after being incubated for 30 hours the fungus recovered, developed and eventually covered the slant.

None of the slants remaining at 37° C for 51, 75, or 99 hours showed any recovery.

This test was repeated beginning 4 August 1967 with four replicates of six slants each of Sabouraud Maltose Agar. The slants were inoculated with stabs of E. coronata spores from young colonies and incubated at room temperature for 30 hours at which time the colonies averaged about one centimeter in diameter.

At this time all slants except the checks were placed in an incubator at 37° C. At this temperature growth stopped.

After a period of 36 hours one replicate of six slants was taken from the 37° C cabinet and incubated at 20° C for seven days. The other replicates were removed at 24 hour intervals and treated likewise.

The six check slants that had been held at 20° C produced normal sporulating colonies of E. coronata.

On the six slants removed from 37° C after 36 hours one colony recovered. The five other colonies seemed to be dead, or at least they were inactivated. No recovery was noted on any of the slants held at 37° C for longer than 36 hours.
Discussion of Temperature Experiments

Michelbacher (1938) gives the optimum temperature for Scutigerella immaculata as lying between 12° and 20° C. However, one group of his symphylans survived for 24 months at a temperature of 28° C. The thermal death point he considered to be close to 37° C. Below 10° C their movements slowed down and little feeding took place below 4.5° C. Waterhouse (1963) agrees closely with Michelbacher.

These temperatures agree well with those shown in the growth curves in Graph 1 (page 110). From 1° to 10° C E. coronata grows more slowly than some of its competitors such as species of Penicillium, Aspergillus and Rhizopus. Optimum falls between 20° and 25° C. The death point, near 37° C for symphylans, seems to be very near the point at which E. coronata is inactivated. The temperature correlation between these two organisms would appear to be close.

The fact that this strain of E. coronata seems to be suppressed at temperatures slightly below body temperature (37° C) is encouraging evidence that it is probably harmless to mammals. At least it should be safe to experiment with it if reasonable precautions are taken. Hall and Bell (1961) report that the E. coronata, isolated from aphids at Riverside, California, grown in Sabouraud Dextrose
Agar, grew equally well from 24° to 33°C. Their culture could recover and grow after being incubated seven days at 36°C but when incubated at 39°C for seven days it was inactivated. This is a little higher than the Getzin strain but probably not significantly.

Emmons, Chester, and Charles (1961) have reported two strains of *E. coronata* isolated from mycoses in horses that grew well at 40°C. They suspect mutations have taken place in culture as one isolate that once grew at 40°C will no longer grow at temperatures above 33°C. Bras et al. (1965) incubated the isolates from the nasal tissue of the boy infected with *E. coronata* at 30°C. Apparently no attempt was made to determine the lethal temperature for this strain.

These reports seem to indicate that tropical strains of *E. coronata* which can grow at high temperatures do exist and that they can attack man and domestic animals. This is a matter that needs further study.
EXPERIMENTS WITH CULTURE MEDIA

High Protein Media Experiments

High protein media have been generally reported to be the most suitable for rearing entomogenous fungi in general. In line with this, media using insects as a basis was tried. Wax moth larvae (Galleria mellonella), cutworms (Peridroma saucia), and crickets (Acheta domestica) were used at various times. They were blended in a Waring Blender with one or two volumes of water until an homogenous milky mass resulted. Two percent agar by weight was added and the medium was autoclaved for 15 minutes and poured in plates. E. coronata did very well and sporulated profusely. Such concoctions while very effective are time consuming and expensive to produce under ordinary conditions. They might, however, be useful in culturing the more fastidious species.

Ordinary cow's milk, solidified with two percent agar, made a very successful medium. The fungus grew on it almost as well as on the insect based agar and it was cheap and easy to prepare.

Sabouraud Maltose Agar (Difco Bacto-Sabouraud Agar)\(^2\) proved satisfactory although the fungus did not grow as profusely as on milk.

\(^2\)Difco Laboratories, Detroit, Michigan.
agar (Figure 7, page 106). Sabouraud Maltose Agar is one of the standard mycological media, is transparent so that growth can be easily seen, and is slightly acid, pH 5.6, which tends to select against bacteria.

Egg yolk has been used by many researchers (Getzin, 1964 and Gustafson, 1965b). *E. coronata* grew well on this medium but it was found to be harder to handle than agar media. It was easily contaminated and dried out rapidly.

Egg white was also used and supported growth of *E. coronata* well but it was harder to use than egg yolk as it tended to bubble and overflow when heated to sterilizing temperatures.

Knox Unflavored Gelatine, 3 a seven gram envelope dissolved in 350 milliliters of hot water, was tried. This material is almost pure protein without fat or carbohydrate. *E. coronata* grew more slowly than on the above agar media and liquefied the gelatine as it grew. At room temperature the whole container of gelatine soon becomes liquefied. The mycelium penetrated into the liquefied gelatine farther than into other media used. The growth appeared diffuse but more or less normal hyphal bodies were formed. When hyphal masses developed, which floated on the surface, sporulation took place. The addition of peptone at the rate of one gram to 100

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milliliters of gelatine gave more rapid growth.

*E. coronata* was able to grow after the gelatine had been liquefied. This indicates that the medium might be made the basis of a liquid type of culture by which large amounts of the fungus could be grown economically.

Knox Gelatine was mixed with two percent agar to prevent liquefication as the fungus grew. Growth was fair. A little better growth was obtained by adding peptone at the rate of one gram peptone to 100 milliliters of medium. At 23°C growth on gelatine-peptone agar was estimated to be only about one-fourth as rapid as on milk agar.

**Vegetable Media Experiments**

Boiled vegetable matter is known to support the growth of many parasitic fungi even though they may be unable to attack the living cells of uncooked plants. Autoclaved potato slices have been used by many workers as a medium for isolating fungi. For this reason several fleshy vegetable materials were examined: slices of potato, red table beet, sow-thistle (*Sonchus*) root, rutabaga, and pieces of cabbage leaves. These were autoclaved in Petri dishes and inoculated with *E. coronata*. Vegetative growth of the fungus was thin and crust-like but sporulation was fairly heavy. The cabbage leaves seemed to be the least suitable.
Table 6. Formulae for media used in culturing *E. coronata*.

<table>
<thead>
<tr>
<th>Media</th>
<th>Ingredients and directions</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sabouraud Maltose Agar</td>
<td>Peptone 10 g, Maltose 40 g, Agar 15 g, Water 1000 g</td>
<td>pH about 5.6. A standard medium for fungus culture. Acidity slows bacterial growth.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Whole Milk Agar</td>
<td>Whole milk 100 cc, 1-2% Agar, Autoclave and pour.</td>
<td>Growth and sporulation good.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A very handy medium as it can be bought in small quantities as needed. pH near 7.</td>
</tr>
<tr>
<td>3. Whole Egg Whole Milk Agar</td>
<td>Whole egg 40 cc, Whole milk 160 cc, Agar 2 g</td>
<td>Fair.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contaminates easily</td>
</tr>
<tr>
<td>4. Potato Agar</td>
<td>Potatoes 100 g, Boil in small amount of water, mash and drain. Add 1% Agar to remaining liquid.</td>
<td><em>E. coronata</em> grows slowly and does not sporulate much. Easily overgrown by bacteria.</td>
</tr>
<tr>
<td>5. Milk Agar</td>
<td>5% Dried skim milk 5 g, 1% Agar 1 g, Add water to make 100 cc.</td>
<td><em>E. coronata</em> grows but sporulation is not heavy.</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Media</th>
<th>Ingredients and directions</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Milk Pream Agar</td>
<td>Dried skim milk 5 g Pream 2 g Agar 1 g Water 100 cc</td>
<td>Growth good. Compares with Wax Moth Medium.</td>
</tr>
<tr>
<td>8. Milk-Egg Agar</td>
<td>Dried skim milk 4 g Egg yolk 3 g Agar 1 g Water 100 cc</td>
<td>Growth very good. Compares with Egg Yolk Medium.</td>
</tr>
<tr>
<td>10. Potato slices</td>
<td>Autoclave a slice of potato in bottom of Petri dish. If to be inverted as a jump plate, fasten with a drop of egg white.</td>
<td>Supports fairly good growth.</td>
</tr>
</tbody>
</table>

Continued on next page
<table>
<thead>
<tr>
<th>Media</th>
<th>Ingredients and directions</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Gelatine</td>
<td>Unflavored gelatine prepared according to directions on package.</td>
<td>E. coronata grows well. Proteolytic activity of the fungus liquefies the gelatine. Shows possibility both as a solid and liquid medium.</td>
</tr>
</tbody>
</table>

1 Bacto-Sabouraud Agar, made by Difco Laboratories, Inc. Detroit, Mich.
Experiments Involving the Ability of *E. coronata* to Grow on Materials Found in Natural Substrates

Four Petri dishes of garden soil were tested. Two were moistened with water only and the other two with Knox Gelatine solution at a dilution of seven grams of gelatine to 350 milliliters of water. They were autoclaved and inoculated with blocks of milk agar from an *E. coronata* culture.

The plates moistened with water only supported no growth beyond the germination of spores ejected by the fungus on the inoculating block. In the two plates enriched with agar the hyphae spread over much of the soil.

A similar experiment was carried out with dead leaves. In this case there was some spread of the fungus beyond the area of original inoculation but much greater spread when additional nutrient in the form of gelatine was added.

Ground hemlock bark was examined by the same methods. The two unenriched plates showed little growth of *E. coronata* but other contaminating fungi began to grow. In the plates enriched with gelatine the *E. coronata* grew and penetrated the spaces between the chips. The bottom of the plate was covered with spores.

These experiments suggest that *E. coronata* is unlikely to grow on most of the substrates found connected with soil unless they are
changed in some way, perhaps by a fungus capable of breaking down plant material. The following experiment was carried out with this possibility in mind with two species of fungus.

*Experiments on the Ability of E. coronata to Grow on Other Fungi*

Costantin (1897) reported finding *E. coronata* growing on the lamellae of *Psalliota* sp. Martin (1925) found it growing on a brown *Hypocnthus* on rotten wood. In view of these naturally occurring relationships of *E. coronata* with other fungi an experiment was set up to see if it could grow on commercial mushrooms and a wood-rot mycelium overgrowing a bark chip culture of symphyllans.

The mushroom experiment was set up 20 May 1967. Filter papers were placed in five Petri dishes. Cultivated mushrooms bought at a grocery market were sliced and arranged in two of the Petri dishes. All dishes were then autoclaved. After autoclaving uncooked mushrooms which had been surface sterilized in Clorox (sodium hypochlorite 5.25%) were sliced into the three remaining sterile Petri dishes. All five plates were then inoculated with cubes of agar from *E. coronata* colonies.

By 30 May some growth and sporulation was noticeable on both autoclaved and uncooked slices. Growth on the uncooked material was most noticeable on the gills.
Five more plates were prepared in the same way. Small pieces of infected mushroom, both autoclaved and raw, were transferred to the new plates. These showed infestation in three to four days.

Recovery of *F. coronata* was made from several pieces of infested mushroom using Sabouraud Agar slants.

The wood rot mycelium experiment was set up 22 May 1967. The mycelium growing over the surface of the bark chips on Colony XII (Page 38) was used. Two blocks of agar from an *F. coronata* colony were placed on the mycelial mat under the protecting pane of glass. The culture sporulated profusely and shot spores to distances of up to 5 mm around the blocks and onto the surface of the glass.

Collembolans attacked the *F. coronata* wherever they could reach it. They were specifically observed eating the spores lodged on the glass.

On 7 June 1967 samples of mycelium from positions immediately around the place of introduction of the *F. coronata* were selected. These samples were placed on blocks of Sabouraud Agar in the lids of sterile Petri dishes. The bottoms of the dishes, containing a layer of Sabouraud Agar, were inverted over the lids. No *F. coronata* was isolated.

This experiment was repeated after the collembolan population
had been reduced to a low level by exposure to Vapona. Still no
growth of *E. coronata* could be observed. The agar was eventually
overgrown by the hyphae of the wood rot fungus. No parasites were
evident on this type of fungus.

This experiment confirms the observation of others that
*E. coronata* can live on certain other fungi, but only under some
conditions. This may be one method of survival or even multiplication in soil.

**Growth on Dead Insects**

Experiments were carried out several times using insects
killed by boiling water, arranged in a Petri dish around an open
place in the center and autoclaved. After cooling, a block of sporulating culture of *E. coronata* was introduced into the space in the
center of the plate and allowed to shoot spores onto the sterilized insects.

Two species of insects, wax moth larvae, *Galleria mellonella* (Linn.) and the European house cricket, *Acheta domestica* (L.), were
used because of their availability. Both were kept in culture. On
both species *E. coronata* grew and sporulated. The growth, however,

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4 Made by Shell Chemical Company, Agricultural Chemical Division, 110 West 51st Street, New York, N. Y. 10029.
was of the surface type similar to that on an agar plate. The infected insects soon broke down and the fungus and spores seemed to be destroyed by bacterial growth in most of the experiments. However, if the cadavers were allowed to dry out soon after being infected they could be made to sporulate when moistened at a later date.

On 9 January 1966 an experiment was run using crickets which had been killed in boiling water but were not autoclaved. Decay set in and prevented growth of *E. coronata*. Five days later some crickets which had undergone some decay before autoclaving were inoculated. After three more days some surface growth was noted but contamination had already overtaken *E. coronata*.

Autoclaved insects can be considered a fairly satisfactory medium for culturing the fungus.

**Experiments with Overflow Plates**

Gustafsson (1965a) reported isolating *E. coronata* from greenhouse soil by mixing a small amount of soil with a nutrient agar and inverting this over a plate of nutrient material onto which the Entomophthora spores would land when they were discharged. To be effective the spores or other stages of the fungus found in the soil must grow through the surrounding agar and sporulate. The fact that *E. coronata* develops to sporulating stage rapidly and discharges its spores, enabled him to obtain a pure culture on the lower plate even
if the original growth was rapidly overgrown by other species.

To get an idea of the requirements for this method several experiments in overflowing *E. coronata* spores with Sabouraud Agar under varying conditions were carried out. Old cultures and Petri dish lids well covered with spores were used as sources of fungus material. These were flooded with Sabouraud Agar and allowed to set. The resulting growth could be followed under the dissecting scope through the transparent agar.

The results were not very conclusive. As could be expected contamination was often severe. This would also be true in the case of isolation from a soil sample.

Some trends were evident, however. One is that the newer the spores the more successfully they grow. This is a characteristic of the genus, *Entomophthora*, which is often mentioned in the literature. Gustafsson (1965b) emphasizes that insects which have been dead only a short time should be used in trying to isolate these pathogens.

Another characteristic is that the cooler the agar is at the time of pouring the better is the growth of spores. Clumps of hyphae withstand heat better than individual spores. In isolation work this heat sensitivity of the spores might be a point to keep in mind.
EXPERIMENTS WITH SELECTIVE MEDIA

Experiments with Antibiotics and Sulfonamides (Bacteriostats)

Screening with Difco Bacto-Unidisks

To find out the reactions of E. coronata to various inhibitors several tests were made. The purpose of this work was to get some idea of what materials could be used to protect E. coronata from competition when an attempt to propagate it might be desired and also to locate a substance which could be used to control the fungus if this became necessary.

Several Difco Bacto-Unidisks for Antibiotics No. 1, medium concentration, were employed. These test devices incorporated Penicillin 5 units, Streptomycin 5 mcg., Chloromycetin 10 mcg., Kanamycin 10 mcg., Erythromycin 5 mcg., Tetracycline 10 mcg., Novobiocin 10 mcg., and Neomycin 10 mcg. None of these compounds inhibited E. coronata to a measurable extent.

A similar series of Bacto-Unidisks for Sulfonamides, medium concentration, was also tried. These devices incorporated samples of Elkosin 150 mcg., Gantrisin 150 mcg., Sulfadiazine 150 mcg.,

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5 Difco Laboratories, Inc. Detroit, Michigan.
Sulfamerazine 150 mcg., and Sulfathiazole 150 mcg., Thiosulfil 150 mcg., Triple Sulfa 150 mcg., and Sulfamethoxypyridazine 150 mcg. No sensitivity was shown by *E. coronata* to any of these materials. This is not surprising as these materials are all bacteriostats. The indication is that any of these drugs can be used in considerable concentration in *E. coronata* cultures. If liquid cultures of large volume were desired such bacterial depressants would be of great help because absolute sterility becomes more difficult as cultures become larger and require agitation.

**Further Tests with Common Antibiotics and Sulfonamides**

**Penicillin.** Penicillin G. procaine at the rate of 1000 units per gram was incorporated into two plates of milk agar. *Entomophthora* grew on both plates. This is a very high concentration and the fact that the fungus became established on these plates shows that penicillin has little inhibiting effect.

**Streptomycin.** Strep-Cambiotic, an aqueous suspension of Penicillin and Streptomycin, at the rate of 200,000 units of Penicillin G. procaine and 0.5 gram Streptomycin per milliliter, was introduced into milk agar or Sabouraud agar at the rate of 0.1 milliliter in 100 milliliters of medium just before pouring. No inhibition

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6 Made by Charles Pfizer and Co., Inc., New York, N. Y.
was shown.

**Mycostatin.** Mycostatin\(^7\) is said by the manufacturer to be an antibiotic with antifungal activity. The U. S. P. sterile form is commonly used in tissue cultures to prevent growth of fungi (Squibb folder enclosed with product). Mycostatin Topical Powder was the product used because it is obtainable at drug stores. Each gram of powder contains 100,000 units of mycostatin dispersed in talc. The mycostatin was dissolved in alcohol and then mixed with milk agar or Sabouraud Maltose Agar before being poured. The talc was ignored. It was used at two concentrations, 25 units and 100 units per milliliter of medium. *E. coronata* did not seem to be inhibited at either concentration.

As a check spores from a contaminating colony of *Aspergillus niger* and mycelia from a colony of a species of blue *Penicillium* were inoculated onto two plates each of Sabouraud agar containing 25 units per milliliter and 100 units per milliliter. Both of the 25 units per milliliter plates showed no growth. Both of the 100 units per milliliter began to grow but in a week one plate became severely repressed and stopped growing. The other plate showed some growth and eventually both species sporulated. This result is not as conclusive as it could be hoped because the plates at the higher concentration showed more growth than at the lower concentration. This

\(^7\)(Squibb Nystatin) made by E. R. Squibb and Sons, New York, N. Y.
erratic result probably could be traced to lack of contact with the mycostatin. This material is insoluble in water and while alcohol was used to dissolve it, thorough mixing in cooling agar is difficult.

Both concentrations showed prevention or repression of Aspergillus sp. and Penicillium sp. while neither concentration seemed to inhibit E. coronata. This difference could be important in culturing E. coronata in large quantities as species of Penicillium and Aspergillus are among the most serious competitors.

Experiments with Chemical Inhibitors

Sorbic acid, methyl p-hydroxybenzoate, and formalin were chosen because they are used in artificial diets for mass rearing of cabbage loopers (Shorey, 1963). They were used in the same concentrations in this experiment. Sorbic acid and methyl p-hydroxybenzoate were dissolved in 2 milliliters of alcohol before being mixed with 100 milliliters of medium.

Sorbic acid at 0.1% concentration did not prevent the growth of Penicillium sp., Aspergillus niger, or E. coronata. Penicillium seemed to be held down more than the other two. At 0.5% concentration all three species were destroyed.

Methyl p-hydroxybenzoate at 0.02% prevented the growth of all three fungi.

Formalin at the rate of 0.2 ml of 37% formaldehyde solution
with 2 ml of alcohol was added to 100 ml of medium just before pouring. The alcohol was included as a check because this amount was used in the sorbic acid and methyl p-hydroxybenzoate solutions as a solvent. This concentration of formalin inhibited *E. coronata* and *Aspergillus niger* but had little effect on *Penicillium* sp.

Checks with alcohol at the rate of 2 ml per 100 ml of agar were provided because it was used at this rate as a solvent for the other chemicals. There was no inhibition of any species in these checks.

**Fat-Covered Media Experiments**

Shaerffenberg (1964) suggested that a common characteristic of entomogenous fungi is their ability to penetrate waxes and lipids which enables them to attack the cuticle of insects. For this reason fatty or fat-covered substrates are selective for this type of organism, since a mixing of the lipids with the substrates or covering them with fatty substances promotes the entomogenous fungi while retarding their aerobic competitors.

By way of testing this idea, Crisco, as a representative of solid fats, and Wesson Oil, as a representative for liquid fat, were added to several plates of milk agar as a surface covering and as enrichment which were autoclaved into the medium.

The results were not encouraging. While bacteria and other
contaminants may have been held down Aspergillus, two species, and Penicillium, one species, were not inhibited. This is not surprising since Aspergillus is listed by Madelin (1963) as somewhat pathogenic to insects and some species of Penicillium seem able to grow on almost anything.

**Experiments with pH of Media**

Experiments were performed using milk agar at pH values from 4.5 to 8 or above without noticeable effect on the growth of *E. coronata*.

**Discussion of Experiments with Selective Media**

This series of experiments points to the possibility of giving *E. coronata* a competitive advantage over many species of bacteria by the use of antibiotics and over some of the most troublesome molds by the proper concentration of fungus inhibitors, such as mycostatin or sorbic acid, or combinations of several. In a mass-production undertaking this might be important. Also upon introduction into soil these drugs might have a brief period of effectiveness.

None of eight common antibiotics or an equal number of sulfonamides showed inhibition of *E. coronata*.

Fungus inhibitors Mycostatin, methyl p-hydroxybenzoate, sorbic acid, and formalin inhibit *E. coronata* and most other fungi at low concentrations. There is, however, the possibility that some
of these chemicals at concentrations below that which inhibits

E. coronata might have inhibiting effects on certain other fungi and
thus reduce competition.

This fungus showed tolerance to a wide range of pH values.

This indicates that the number of competing species could be reduced
by manipulation of the acidity of the media.
INFECTION OF LIVING INSECTS

The experiments on infecting symphylans are recorded under the section Ecology and Epizoology of Symphylan Populations Under Controlled Environments. Observations of the process were as follows:

Infection of the symphylan by *E. coronata* takes place when a spore becomes attached to the integument where it germinates and a germ tube penetrates the cuticle. This requires a moist environment such as soil. Once inside the body, the mycelium branches into the various organs and eventually kills the host. The hyphae become thick and may separate into sections which are known as "hyphal bodies". Figure 6 (page 105) shows hyphal bodies in a cross section of a symphylan recently killed by the parasite.

Getzin and Shanks (1964a) reported conidia and hyphae in the mid gut of symphylans upon which they made histological studies but they found invasion to be only by way of the integument. However, Yendol and Paschke (1965) reported infection in termites to be through the walls of the esophagus as well as the integument.

The only early symptom of infection observed during the investigation consisted of excessive grooming of the antennae which in a case or two resulted in terminal sections being broken off. Usually this symptom was not evident, however. The terminal symptom was
tions on the effects of E. coronata on insects would be of value in gaining further knowledge of the potentiality of this pathogen. Entomophthora coronata is reported to be a pathogen of aphids (Hall and Dietrick, 1955), symphylans (Getzin and Shanks, 1964), and termites (Yendol and Paschke, 1965).

Prasertphon (1963) reported successfully infecting wax moth larvae, Galleria mellonella (Linn.), by forcing them to crawl over the surface of a sporulating culture of E. coronata and then confining them in Petri dishes on damp filter paper.

This method of exposure was tried several times. Within 12 hours dark spots would develop in the cuticle of a majority of the larvae. This was apparently a reaction to the penetration of germ tubes from spores (Figure 9, page 107). This was the only species...
showing visible evidence of attack. Only when the exposed larvae were stored at 15°C, which is much below optimum for Galleria, did as many as five to ten percent become infected.

Those succumbing to infection turned black and looked much like larvae dying from many other causes. Isolation of E. coronata was successful only if the cadavers were placed in close proximity to a nutrient material such as Sabouraud or milk agar soon after death. The older a specimen becomes the poorer becomes the possibility of isolating Entomophthora.

Attempts were made to inoculate green peach aphid colonies on leaves of cabbage or radish seedlings in the laboratory by putting a lamp chimney over the plants and inverting a sporulating plate over the upper end. Many of the aphids died but E. coronata was not isolated.

Variegated cutworms, Peridroma saucia (Hon.), were exposed in the same way as Galleria larvae but no symptoms of mortality could be determined. They did not seem to develop the spots on the cuticle as did Galleria.

European house crickets, Acheta domestica (Linn.) were exposed by being forced to crawl through E. coronata spores and then being stored under conditions of high humidity and low temperature. No case of infection or death was detected.

Mealworms, Tenebrio molitor (Linn.), larvae were treated
in the same way as the crickets and wax moth larvae but showed low mortality. Like wax moth larvae more of them became infected when confined to both cool (15°C) temperature and high humidity. Under such conditions ten to 15 percent have died and the fungus in the cadavers sporulated.

Damp-wood termites, *Zootermopsis*, were used in several experiments none of which produced recognizable infection.

On 12 February 1966 a series of materials that are found as natural substrates were tested for their ability to support *E. coronata*. These consisted of garden soil, fallen leaves, and bark chips. One quarter-inch layers of these materials were placed in Petri dishes and autoclaved for 20 minutes to destroy competing organisms and then inoculated with blocks of agar from an *E. coronata* culture. On 7 March 1966 ten damp-wood termites were introduced into these old cultures to determine whether they could be attacked by the *E. coronata* spores which were present in large numbers in the Petri dishes. The results after seven days are shown in Table 7.

While the substrates enriched with gelatine produced better growth of *E. coronata* there was no significant relation to the mortality of the termites. The missing ones were no doubt eaten by the remaining members of the colony. No *E. coronata* was isolated from the few dead ones recovered and no spots on the cuticle were observed.
A check experiment was set up 7 March 1966. One culture was composed of ten termites, *Zootermopsis*, on untreated dead leaves.

One culture was composed of leaves and ten termites over which a sporulating culture of *E. coronata* was inverted for 48 hours. At that time the leaves were white with spores. One week later, 14 March, only one termite in either culture was dead and this was caused by its being wedged against the glass by a leaf so that it could not free itself.

*Peridroma saucia*, variegated cutworms, were introduced into the autoclaved fleshy plant material experiment set up 7 February 1966.

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**Table 7. Experiments with materials found in natural substrates.**

<table>
<thead>
<tr>
<th>Natural material</th>
<th>Plate</th>
<th>Found dead or debilitated</th>
<th>Missing</th>
<th>Alive</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garden soil</td>
<td>A</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>Needed food</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>Needed food</td>
</tr>
<tr>
<td></td>
<td>C*</td>
<td>3</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D*</td>
<td>3</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Dead leaves</td>
<td>A</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Ground hemlock bark</td>
<td>A</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C*</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D*</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

* Enriched with Gelatine.
to test the growth of \textit{E. coronata} on various substrates. The fungus was growing well on the plant material and the surfaces were well covered with spores.

Three larvae were used in each plate. There were two plates each for cabbage, sawthistle, and rutabaga slices, and three plates each of beet and potato slices. The total number of cutworms introduced was 36.

After two weeks the 26 surviving larvae were transferred to fresh beet slices. None of the dead larvae showed any fungal growth suggestive of \textit{E. coronata}.

This series of experiments would seem to indicate that \textit{E. coronata} is restricted to hosts with thin cuticles. Infection through the gut was not observed but the possibility was not ruled out.

\underline{Injection of Insects with \textit{E. coronata} Spores}

Crickets, \textit{Acheta domestica}, and wax moth, \textit{Galleria mellonella}, larvae were injected with \textit{E. coronata} conidia suspended in Yeager's physiological saline solution (Yeager, 1939).

Spores were scraped from sporulating cultures or from the surfaces of Petri dish covers upon which spores had collected. The spores were suspended by shaking in ten milliliters of saline. Each individual was injected with 0.01 to 0.015 ml of this suspension.

An experiment using both crickets and wax moth larvae was
set up 2 March 1967. The injected insects and checks were incubated at three temperatures: 15°, 24°, and 32°C.

For each temperature, three lots of five crickets each were confined in Petri dishes with damp filter papers in the bottom to maintain humidity. One lot was injected with 0.01 to 0.015 ml of _E. coronata_ spore suspension. One lot was injected with the same amount of saline only. The other lot was exposed to the spores from a vigorous culture of _E. coronata_ on Sabouraud Agar.

Also for each temperature four lots of ten wax moth larvae were selected. One lot was injected with 0.01 to 0.015 ml of spore suspension. One lot was exposed to the spores by being forced to crawl on an _E. coronata_ culture and one lot was injected with physiological saline only. One lot was used as a check and was not injected or exposed to spores.

Each lot was confined to a Petri dish on damp filter paper.

**Results from the Cricket Experiment**

At 15°C all of the injected checks survived. All of the crickets exposed externally to spores survived. But all those injected with spores were dead in 48 hours.

At 24°C none of the injected checks nor those exposed to spores on the cuticle died but all five of those injected with spores were dead in 53 hours.
At 32°C none were dead at 53 hours. One of the injected crickets was dead at five days and two more were dead after seven days while two survived for two weeks.

The surface of a dead cricket that had been injected with spores was sterilized with Clorox and positioned in a test tube to within one-fourth inch of a slant of Sabouraud Agar. It sporulated and infested the slant with a typical _E. coronata_ colony. Also using the method of Gustafsson (1965b) a small piece of dead cricket from an injected specimen was stuck to a small piece of Sabouraud Agar and this was fastened to a Petri dish cover which was inverted over a bottom containing Sabouraud Agar. The fungus in the insect part sporulated and infected the agar below giving a fine culture of _E. coronata_.

This experiment would seem to indicate that insects at high temperatures may be able to withstand infection for a longer time, but subsequent experiment did not bear this out.

**Results of the Wax Moth Experiment**

At 15°C all spore injected larvae were dead in 52 hours. One injected check died six days later. One of the larvae exposed to spores on the surface died in six days. All developed spots on the skin in 24 hours (Figure 9, page 107). There was no mortality among the checks.

At 24°C all the larvae injected with spores were dead in 30
hours. There was no mortality in the lot injected with saline only. The surface contaminated lot developed spots but no mortality. The checks showed no mortality or spotting.

At 32°C all the larvae injected with spores were dead in 48 hours. Two injected with saline only died in a week. The other eight pupated. The lot exposed to spores on the cuticle developed spots but no mortality. None of the checks died.

One of the larvae killed by injected spores is shown sporulating in Figure 10 (page 107).

_E. coronata_ injected at the rate used here is lethal to _Galleria_ larvae at all temperatures. The only larva to die after being exposed to spores on the cuticle was held at a temperature favorable to _E. coronata_ (15°C) but much below the optimum for _Galleria_ which is around 30°C.

Prasertphon (1967) found that a spore-free filtrate of _E. coronata_ was toxic to _Galleria_ larvae when injected. This factor may have contributed to the high mortality found in this experiment.

**Further Experiments with Injected Crickets**

An experiment was set up on 23 March 1967 to test further the relation of high temperatures to inhibition of mortality among injected crickets as the results of the 2 March 1967 experiment suggested.

Temperatures used for incubation were 15°, 24°, 27°, 30°, 87 hours.
32°, 35°, and 38°C. For each temperature two lots of ten crickets each were used. One lot was injected with 0.01 to 0.015 ml of Yeager's saline only, to act as a check. The other lot was injected with _E. coronata_ spores looped from the cover of sporulating plates so that only spores and no hyphal bodies were used. By the use of a blood counting chamber it was estimated that the spores ran about 260,000 per milliliter. At an injected dose of 0.01 to 0.015 ml each cricket should have received between 2600 to 3900 spores.

It must be admitted at the outset that getting a valid estimate of _E. coronata_ spores is difficult. They are sticky and cling together in bunches and sprout immediately in moist surrounding further complicating counts and dosages.

The results were very inconclusive as far as temperature relationships went. At the critical temperatures of 35° and 38°C ninety percent of the injected crickets died in two days. Altogether 81% of the injected crickets died in two days. None of the injected checks died until four days and then only six out of 70 individuals. They were not found dead but missing, apparently eaten by the others.

Perhaps more differential results would be obtained with lower dosages of spores. There is also the possibility that a bacterial infection was involved or that crickets are also susceptible to Prasertphon's toxic effect (Prasertphon, 1967). The experiment could not be repeated because of lack of time and the fact that the
cricket colony was severely depleted at this time by an insecticide accident.

There is one conclusion which might be drawn from this experiment and that is that *E. coronata* is deadly if it can gain access to an arthropod. The relationship between the cuticle and the invading germ tube of the fungus whether on the surface of the body or in the alimentary canal, if the spores have been eaten, is crucial.

**Discussion of Experiments with Infection of Living Insects**

Under humid cool conditions wax moth larvae and mealworms were infected through the cuticle by spores placed on the surface of the body.

The results with aphids were inconclusive.

The termites, cutworms and crickets were not infected through the integument.

When crickets and wax moth larvae were injected with *E. coronata* spores mortality was high. The only evidence of resistance was related to high temperature.

Apparently little infection could be expected from *E. coronata* in insects with heavy cuticles. If, however, the pathogen can gain entrance to the body of an insect, it causes death in a few hours at room temperature. Cool temperatures seem to favor the fungus at the expense of the insect, especially with insects which normally live
in areas of high temperature and low humidity, such as wax moth and mealworm larvae.

**Experiments with Humidity**

A few experiments were undertaken using sugar-saturated salt solutions to control humidity in an effort to obtain villous resting spores from sporulating symphylans. The villous conidia seem to be induced by high humidity or actual contact with water. No spores having the resistant characteristics of a true resting stage, such as heavy, dark cell walls, were observed under any conditions.

As the air in the spaces between soil particles is saturated except in very dry soil (Myer and Anderson, 1948) further experiments with lowered humidities did not seem worthwhile as the relationships between symphylans and the pathogen are normally confined to the soil.

It may be of interest to note that one of the symptoms of desiccation in symphylans is progressive collapse of the antennal segments beginning at the tips of the antennae. This is due to reduction in volume of body fluids and a resulting lowering of blood pressure. When this telescoping effect has involved from one-third to one-half the antennal segments, death takes place. A wound that permits escape of fluid produces a similar effect on the antennae.
Entomophthora coronata (Costantin) is an entomogenous Phycomycete which can parasitize the garden symphylan, Scutigerella immaculata (Newport). Because this fungus is one of the few natural enemies of the symphylan, a study of the biological relationships between the two organisms was made.

Populations of symphylans which were not exposed to the fungus remained healthy and reproduced to the limit of available living space if proper environmental conditions were provided. No outbreak of disease that could be explained as latent viral, bacterial or protozoon infection which had been activated by the stresses of confinement was observed. No resistance to E. coronata infection was evident.

To populations which had been exposed to the fungus it proved to be virulent. When unexposed individuals were added to infected populations as rapidly as the infected ones died, the rate of infection increased until new introductions were killed in as short a time as 30 hours.

E. coronata also shows some survival ability. Symphylans introduced into contaminated environments which had been uninhabited for periods of time up to five months in length became infected. Under these conditions, however, several days to weeks passed before symptoms of infection appeared.
Wax moth larvae and European house crickets were injected with *E. coronata* spores suspended in physiological saline solution. Over 95% died within a day or two. Controls injected with the saline only showed a loss of not over five or ten percent. There were some indications that temperatures of 35°C and above had some effect in slowing the progress of the disease.

Wax moth larvae and mealworms were infected by surface application of spores and subsequent storage in conditions of high humidity and cool temperatures. Dark spots developed on the integument of the wax moth larvae where germinating spores attacked the cuticle. But unless conditions were kept favorable to the fungus infection often went no further. The thin integument and restriction to damp habitats no doubt help to explain symphylan susceptibility to this parasite.

Penetration of the body wall by germ tubes from *E. coronata* spores lodged on the surface seems to be the common port of entry into the host. But the fact that symphyllans were able to pick up infection from environments in which sporulation has not taken place for months, due to lack of a food source for the fungus, suggests that infection may be possible by some other route, perhaps through the digestive tract from ingested spores.

Upon gaining entry to the body, by whatever route, the hyphae ramify through the tissues and bring death to the victim. After the
death of the host, conidiophores break through the integument and forcibly discharge spores into the surrounding area.

If a discharged spore falls onto a suitable host it may send a germ tube through the cuticle and infect a new individual. If the surface upon which a spore lands is not suitable for colonization it may produce a new mycelium, which grows until the food in the spore is exhausted, it may produce secondary spores or it may develop into a "villous resting spore". How it will develop depends largely on humidity and the presence or absence of free water.

The temperature range of the strain of *E. coronata* used in this investigation conforms closely to that recorded for the symphyllan. Activity for both organisms begins at a few degrees above freezing reaching optima between 20° and 30°C. Lethal temperatures for both organisms is below 37°C. The fact that the fungus becomes inactive at this temperature indicates that it is relatively safe to work with.

*E. coronata* grows well on a large number of media. Formulae high in protein from animal sources produce prolific growth but plant material can be used after it has been autoclaved. Both cooked and uncooked slices of mushrooms were colonized by the fungus. This wide range of food tolerance makes it an easy organism to experiment with.

Eight sulfonamides and the same number of bacteriostatic
antibiotics were tested against *E. coronata* but none showed inhibitory effects. Fungus inhibiting agents Mycostatin (an antibiotic), methyl p-hydroxybenzoate, sorbic acid, and formalin inhibited *E. coronata* at levels used in insect rearing media. There was no indication that *E. coronata* produced antibiotic substances against other microorganisms. It may, however, produce some product which is poisonous to insects if injected. Collembola and symphylans eat the fungus and spores without immediate toxic effects.

The effectiveness of *E. coronata* as a control for symphylans seems encouraging enough in laboratory tests to justify mass production and field application experiments.
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Figure 1. Plates used in rearing symphylans.

Figure 2. Enamel pan with ground bark substrate used in rearing symphylans.
Figure 3. The Garden Symphylan, *Scutigerella immaculata* (Newport). (Enlarged 8 times)

Figure 4. Collembolans, fungus-eating insects of the soil. (Enlarged 20 times)
Figure 5. Symphylan killed by Entomophthora coronata (Costantin) Kevorkian showing sporulation and germination of spores. (Enlarged 8 times)

Figure 6. Entomophthora coronata hyphal bodies (mycelium) in the tissue of a symphylan.
Figure 7. *Entomophthora coronata* (Costantin)
Kevorkian conidia and mycelium in Sabouraud Maltose Agar.
(Enlarged 100 times)

Figure 8. *Entomophthora coronata* (Costantin)
Kevorkian villous conidium, sometimes called a "resting spore".
(Enlarged 1000 times)
Figure 9. Discoloration of cuticle of wax moth larvae indicating attack by germinating Entomophthora coronata spores.
(Enlarged 2.25 times)

Figure 10. Entomophthora coronata sporulating on wax moth larvae.
(Enlarged 4 times)
Figure 11. Sabouraud maltose agar slants incubated eight days.

Figure 12. Sabouraud maltose agar slants incubated 54 hours.
Figure 13. Symphylans on ground hemlock bark showing characteristic spacing. (Slightly enlarged)
Graph 1. Growth Rate of Entomophthora coronata on Sabouraud Agar at Various Temperatures

* For the same information see Figure 11.
** For the same information see Figure 12.