A STUDY OF PLASMODIOPHORA BRASSICAE WOR.

by

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ABSTRACT

Reports concerning the life history of *Plasmodiophora brassicae* Wor. are highly conflicting. The contradictions in the literature on the subject and the difficulties encountered in the initial phase of the present investigation brought about an inquiry into the techniques employed by previous workers. Repetition of these techniques indicated that many previous experiments had not taken into consideration the problems inherent in an *in vitro* study of *Plasmodiophora brassicae*. Conclusions drawn from such experiments are thus of doubtful value.

Methods described as successful by earlier investigators were employed in an attempt to stimulate germination. The germination process was not observed, and contaminants introduced with the resting spore material made it impossible to designate any one motile organism as *P. brassicae*.

Attempts were made to obtain root hair infection. Four methods described by previous investigators proved unsuccessful. A limited degree of infection was obtained by placing young cabbage and cauliflower seedlings in a buffered nutrient solution containing washed *P. brassicae* resting spores. In spite of the fact that artifacts were readily produced, early infection stages were observed and photographed and the existence of a zoosporangial stage in the root hair was confirmed. The infection rate was too low to permit intensive
observations of the development of the parasite within the root hair.

A proteolytic enzyme preparation was somewhat successful in increasing the infection rate. This was taken as an indication that a combination of enzymes could provide the necessary germination stimulus.

Decay of the host tissues is apparently necessary to the maturation of the resting spores. Present experiments indicated that it is not feasible to separate the contaminants from these spores. As it was not possible to draw conclusions from contaminated cultures, it is concluded that the only approach of value is to provide an artificial germination stimulus to spores from clean clubs, so that the development of the parasite may take place under sterile conditions.
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Figure I. Resting Spores of *Plasmodiophora brassicae*.

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Figure III. Young Plasmodia of *Plasmodiophora brassicae* in Root Hair.

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A STUDY OF Plasmodiophora brassicae Wor.

INTRODUCTION

Club root of crucifers, caused by Plasmodiophora brassicae Wor., is undoubtedly one of the greatest problems now facing growers of cruciferous crops. The organisms attack cabbage, cauliflower, turnip, Brussels sprouts, rutabaga, rape, and mustard, as well as wild cruciferous hosts, causing the plant to die, or fail to form a marketable product.

The occurrence of this disease has been recognized for several centuries, and during this time many and varied hypotheses have been put forward to explain its cause and development. In spite of the extensive attention which has been given to this problem, a truly effective control measure is yet to be found. Contemporary workers are coming to be of the opinion that clues to the control of the disease lie in the life cycle of the parasite itself. The life cycle of P. brassicae, however, is the subject of much controversy and at the present time there are many conflicting hypotheses and few observed facts. The search for an effective control measure has been directed chiefly at the resting spore stage, and it could be, in the words of Grace M. Waterhouse (144),
"that too much fungicide is chasing too many spores." There must surely be a much more vulnerable phase in the developmental sequence of the organism towards which control measures could be effectively directed. Until the life cycle of the organism is clearly outlined, however, this approach to the control of the disease cannot be successfully utilized.

It was initially the intention of the author to offer a contribution to the clarification of the problem of the life cycle of *P. brassicae*. However, so many contradictions were found in the literature and so many difficulties in technique not mentioned by previous workers were encountered in the course of the investigation, that it was decided that the chief purpose of the investigation should be to point out the contradictions, to seek the reasons for the conflicting evidence, and to suggest possible sources of error. It is to be hoped that this analysis will provide a guide to future work on the problem and thus prevent the repetition of errors which have contributed to the confusion which surrounds the problem of *Plasmodiophora brassicae* today.

**REVIEW OF THE LITERATURE**

**Economic Significance of Plasmodiophora brassicae**

Club root may be considered as the most important disease of cruciferous crops in the temperate zone (10). The disease is widespread geographically and because the causal organism can persist in the soil for years, it presents a real problem to the market gardener and to others who grow crucifers intensively.
In 1869 half the cabbage crop in the St. Petersburg region of Russia was destroyed by club root and it was a plea on the part of the Royal Russian Gardening Society in 1872 which instigated the classic investigation by Woronin (48) into the cause of the disease. Similar difficulty was experienced in Great Britain during the nineteenth century causing inquiries to be made into the cause and control of the disease. As early as 1893 Halstead (18) estimated the loss in the United States as being represented by millions of dollars annually.

*P. brassicae* attacks cruciferous crops in Canada in more or less serious proportions each year. The disease has become widespread in the coastal regions of British Columbia. In 1947 particularly severe infections of club root were found on both cabbage and cauliflower in Chinese market gardens on the Fraser River delta (4). In 1950 heavy losses were again sustained in the lower Fraser Valley, especially in delta truck gardens (5). Since that time the Fraser Valley and the delta region have suffered yearly losses of cabbages, cauliflowers, and turnips. The disease has almost eliminated the production of crucifers in home gardens in the lower mainland. In 1955 club root was reported for the first time in the Terrace district of the province (7).

Growers in Fort William, Ontario, reported in 1954 that they were unable to raise cabbages because of the disease (6).

In recent years, the disease has severely limited crucifer production in southern Quebec and is considered the worst problem facing vegetable growers in the area. A special
survey made in 1946 revealed club root in every cabbage field inspected (3). In 1947 at Rivière des Prairies, Quebec, every turnip in a ten acre planting was affected and the crop almost a complete loss in spite of the application of powdered cyanamide as a control measure (4). Club root is general in the Montreal district, causing losses of from five to sixty per cent of the crop in cabbage, cauliflower, chinese cabbage, turnip, and radish (4).

A thirty per cent reduction in yield, as a result of loss of individual plants, as well as general growth retardation, is common yearly in the Maritime provinces, where common crop rotation of crucifers, hay, and potatoes does not permit the heavy application of lime that would discourage club root but encourage potato scab (6).

Symptoms of the Host Plant

All symptoms on the above ground portions of the plant are a result of hypertrophy and hyperplasia caused by the development of the parasite within the root tissue. The severity of clubbing and hence the severity of the above ground symptoms depends on the species and variety of the host, age of the host at the time of infection, and soil conditions such as temperature and moisture (10). The first indication of infection seen on the above ground portions of the plant is usually temporary wilting during warm periods, generally followed by stunted, unproductive growth. If infection occurs early in the development of the plant, death may result before maturity is
reached. These symptoms are a result of the disruption of the tissues which effect the absorption and translocation of water and mineral salts. Infected roots enlarge relatively rapidly to form excrescences which take on a variety of shapes. This rapid growth of clubbed tissue is inimical to the normal development of cork cambium and secondary invasion by low grade parasites always results in early decay (10).

Anatomically the parasite affects the cortical parenchyma most conspicuously. One of the most striking appearances in sections of diseased tissue is the presence of more or less isolated groups of hypertrophied cells which Nawaschin (34) named "Krankheitsherde". The medullary ray cells become hypertrophied and form large bands of pathological tissue which split and force the xylem tissues apart, until the latter become distorted and shifted out of position. Separated from each other in this manner the vascular bundles are no longer able to function normally. The parasite very seldom invades the differentiated vascular tissue, but in young roots the cambium is a center of abnormal growth.

Distribution and Host Range of Plasmodiophora brassicae

Although club root disease is regarded as reaching its most serious proportions in the temperate zone, it is by no means confined to these regions. It is known to occur with considerable severity in tropical regions of Asia, Africa, and South America (11). The disease appears to have been first recorded on cultivated members of the Cruciferae, but by the end
of the last century it was recognized that various wild cruciferous plants could also be attacked by \textit{P. brassicae}. This latter group serves as perpetuating hosts of the parasite.

Up until 1949 all the plants proved to be susceptible were members of the Cruciferae. At this time it was reported that \textit{P. brassicae} was capable of infecting the root hairs of some non-cruciferous plants although the typical swellings did not occur (45). Colhoun (10) presents an extensive list of susceptible and non-susceptible cruciferous plants as well as a list of ten non-cruciferous plants in which zoosporangia have been observed.

**Relationships of Plasmodiophora brassicae**

\textit{Plasmodiophora brassicae} belongs to the family Plasmodiophoraceae in the order Plasmodiophorales. The relationships of this order and of the genera and species within it are uncertain, and the group has been assigned various positions in schemes of classification. Because of inadequate data concerning the order these assignations have been largely speculative, and a review of the literature shows that few workers have agreed on the systematic position of \textit{P. brassicae}.

Woronin (48) considered that the genus \textit{Plasmodiophora} was intermediate between the Myxomycetaceae and the Chytridiaceae, possessing some of the characters of each group. DeBary (3) described \textit{P. brassicae} as a doubtful member of the myxomycetes. Saccardo (39) included the Plasmodiophorales as a main subgroup under the Myxomycetes. Fitzpatrick (17) assigned the group to
the rank of a family which he included in the order Chytridiales of the class Phycomycetes. Sparrow (43), and Clements and Shear (9) also included them among the phycomycetous fungi.

Zoologists have also claimed the Plasmodiophorales and have included this order as a subclass of the Myxomycetes among the Protozoa (21).

The Plasmodiophorales appear to have, therefore, some developmental phases and cytological characteristics in common with the Myxomycetes, the Chytridiales, and the Protozoa. Whether this order has originated directly from such groups or developed along parallel lines with them from a distant common ancestor, however, is still uncertain. Our knowledge of the critical stages in the life cycles of the Plasmodiophorales is too incomplete to warrant definite conclusions at present. Further intensive study of the life cycles will doubtless invalidate many of the presently held beliefs concerning this group and point to more definite lines of relationships.

**Life History of Plasmodiophora brassicae**

**Germination of the resting spore.** The reports in the literature of the germination process and factors affecting it are highly contradictory. The lack of accurate information on this phase of the development of the organism appears to be the result of two factors. The chief problem appears to be that workers have found it extremely difficult to germinate the resting spores under conditions which allow observation of the germination process. Secondly, the spores are so minute that
they must be observed under an oil immersion lens with the accompanying disadvantages of a small field and shallow depth of focus. Slight movement on the part of the germinating spore removes it from view.

Several workers have given accounts of their attempts at in vitro germination of *P. brassicae* resting spores. Woronin (148) gave a brief description and a series of illustrations of the germination process. His technique consisted of growing cabbage seedlings in shallow watch glasses with a quantity of mature spores. Woronin's observations and drawings have provided a basis for all later studies of *P. brassicae*. Wellman (146), however, suggested that Woronin's observations and illustrations were partly based upon his knowledge of the saprophytic myxomycetes.

Eycleshymer (15) placed pieces of tissue containing spores under a cover glass and tapped the glass so as to free spores. The slide was then placed in a moist chamber for five to twenty-four hours, after which he claimed to have observed swarm cells.

Chupp (8) observed at least part of the process of germination. He found that spores would not germinate in distilled water, and recommended germinating spores obtained from frozen roots in a muck soil filtrate and incubating them at 28°C. He reported that germination dropped rapidly as the incubation temperature was lowered and he was unable to get germination at room temperature. He found that in the presence of a young seedling, infection took place at a temperature of from 16 to .
20°C. In contrast to this, Honig (23) found that germination occurred below 21°C in the absence of seedlings.

Cook and Schwartz (12) found results in germinating the spores to be most satisfactory in Knop's solution with one and two per cent glucose. They admitted that it was not possible to say definitely that the amoeboid bodies seen had originated from the germination of *P. brassicae* spores.

Wellman (146) teased spores out of previously frozen clubbed roots into sterile distilled water. Excess debris was removed, and the suspension centrifuged. Spore suspensions were made into hanging drop cultures in distilled water or tap water and the author reported seeing hundreds of swarm spores, and in very few cases, the actual germination process.

Ellison (14) experienced considerable difficulty in inducing germination of *P. brassicae* resting spores. He attempted to obtain germination by many methods, including those recommended as successful by previous workers. Sterile excised root tips were placed with resting spore suspensions in the hope that the presence of the living host might have a stimulatory effect on the spores. Rain and snow water were tried, the pH was manipulated, the media oxygenated. Results were either entirely negative or germination so very slight that it was impossible to obtain zoospores. Some degree of germination was obtained from infected roots which had been repeatedly frozen and thawed over a two month period, then macerated in a mortar. Water was added, and the spore suspension was repeatedly centrifuged in order to reduce the bacterial population. The washed spores were left to germinate in a Syracuse watch glass at 25°C.
On the third day, Ellison reported, a "fair" amount of germination had taken place. Only once was a zoospore observed escaping from the spore case. He found from his experience with the resting spores of many of the lower fungi that there are no generalized methods which are effective in inducing production of the swarm spores. Each specimen must be treated by the laborious process of trial and error.

Germination of spores in tap water was reported to occur readily by Ayers (1). He found that it took place at room temperature in from one to ten days depending on the maturity of the spores. He found that the best spores from the standpoint of germination were obtained from old clubs, heavily invaded by secondary organisms. Successive washings of the spore material in tap water apparently was satisfactory in removing contaminants.

Smith (42) in an extensive study of the host-parasite physiology of the disease, was uniquely fortunate in obtaining uncontaminated spore suspensions. He reported that the germination of resting spores isolated from minced tissues of surface sterilized galls varied greatly from one spore preparation to the next, depending in part on the age of the gall tissue from which spores were isolated. Significantly better germination was obtained in non-sterile soil extracts, and he felt that the factor responsible for increased germination was associated with the microorganisms in the soil and was of a rather unstable nature. His work indicated that digestion of the resting spore preparation by proteolytic enzymes gave an
increase in the infection rate. No observations of the germination process or the resultant zoospores were made.

Partially disintegrated clubbed roots of cabbage stored in a frozen condition for several weeks were used by Seaman et al (41) in an attempt to obtain P. brassicae zoospores. The clubs were macerated, the coarse debris removed, and the spore suspension repeatedly washed by centrifugation. After the final washing, spores were resuspended in sterile tap water and incubated at 24°C or at room temperature. The occurrence of flagellated cells in such suspensions was unpredictable and variable. Seaman (personal communication) acknowledged the fact that possession of heterokont flagella is not confined to any one type of motile cell found in the spore supernatant. The germination block is, in his opinion, physiological although he believes that the need for "fully mature" spores from rotted clubs may not be as necessary as some have believed.

First motile stage. The majority of workers who have reported seeing the zoospore of P. brassicae do not give a complete description of the essential points: morphology of the flagella, their point of attachment to the zoospore, their position while the cell is moving freely in suspension. This lack of detail is likely due to the difficulty with which zoospores are obtained, and to their small size and great motility. It is to be hoped that the present day use of the phase contrast and electron microscopes will result in a more clearly documented record of zoospore morphology.
Most investigators agree that each resting spore gives rise to one zoospore, however, the description given for the zoospore varies widely. Woronin (148) described the myxamoeba when released from the spore wall as possessing a somewhat elongated spindle shaped body, provided with a rather long, whip-like flagellum at its beaked, sharply pointed anterior end. The motion was stated to be due in part to the lively weaving from side to side of the supple beak and also by the characteristic movement resembling creeping, the shape changing constantly. Chupp (8) considered the shape of the zoospore to be more or less pyriform, although he concurred with Woronin on many other morphological details. The presence of flagella was disputed by Honig (23) who was of the opinion that only amoebae were produced. He believed that such amoebae could survive for a long time outside the plant and increased in size during that time. It has been suggested (10) that he may have confused some other organism with certain stages in the life history of *P. brassicae*. The same criticism has been leveled at the investigations of Jones (24) who claimed that in addition to the single zoospore, other spores germinate to give rise to as many as twenty smaller bodies which may be uniflagellate or amoeboid. Karling (25) suggested that spores germinating under certain conditions may, instead of producing flagellate zoospores, give rise to bodies in an amoeboid state, however Ledingham (28), Ayers (1), and Ellison (114) reported that the zoospores are consistently biflagellate and heterokont. Ayers (1) found the fixed and stained primary zoospores to vary considerably in size. The smallest were as small as the resting spore, but the majority
were intermediate to moderately large. This variation was taken as an indication that growth of the zoospores occurs after their emergence from the resting spores.

Seaman et al (41) examined zoospores found in association with club root tissue. Observations with a phase contrast microscope indicated that the zoospores vary from elongate to pyriform or globose in shape and are anteriorly biflagellate and markedly heterokont. Examination of the zoospores with the electron microscope showed that the longer flagellum was of the tinsel type, with the shorter flagellum bearing no appendages. Seaman later granted (personal communication) that it would be highly unusual, phylogenetically for the primary zoospore to possess a tinsel-type flagellum if the secondary zoospore possesses a trailing whiplash flagellum as reported (12).

Considerable variation exists in the recorded sizes of zoospores. The following measurements have been given: 1.7-3.5μ (8); 4.0x2.0μ or 1.0x0.5μ (24); 3.0-3.5μ (12); 2.8-5.9μ (1); 3.0x3.0-3.9x4.8μ (41).

Penetration and zoosporangial formation. The difficulties involved in observing the actual process of host penetration have resulted in much conflicting evidence concerning the event. Although it is generally conceded that penetration takes place through the root hair (8, 12, 40, 1), there is disagreement as to the possibility that infection takes place through the epidermal cells as well. Although Chupp (8) believed that seldom if ever does direct penetration of epidermal cells occur, a
number of investigators (26, 20, 1) have stated that infection is not limited to the root hair region of the root. The work of Samuel and Garrett (40) indicated that infection takes place only when the root hair is young and full of protoplasm. It would appear unlikely that penetration of older tissues of the plant takes place (27).

According to most investigators the zoospores come to rest on the host and enter as amoebae through the root hair. Only twice has the penetration process been observed and described, and these two reports are rather discrepant. Honig (23) reported that the amoeba resulting from the resting spore became closely applied to the root hair, and then passed through a hole which appeared at the point of attachment. The hole disappeared immediately following penetration. Rochlin (38) showed that the cell wall of the root hair became swollen where a spore became attached. Treatment with chloroiodide of zinc indicated the absence of cellulose in the wall at this deformed spot. The penetrating amoeba assumed a spherical plasmatic form and was slightly larger than the spore. The protoplast passed through the swollen and gelatinized region and entered the host cell.

Small colorless, uninucleate bodies in the root hairs and epidermal root cells are taken as evidence that penetration has been effected. Ayers (1) found that when multiple infection of a root hair occurs these bodies show no tendency to fuse with one another.

It was believed by Chupp (8) that resting spores develop
from the plasmodia in the root hairs. This conclusion was later shown to be incorrect by Cook and Schwartz (12) when they recorded a hitherto unknown phase in the life history of *P. brassicae* and showed that zoosporangia are formed in the root hair at this stage. These investigators reported that the nucleus of the infecting body divided so that a small plasmodium was formed. This plasmodium may contain over one hundred nuclei (16). The multinucleate plasmodium, according to Cook and Schwartz, then cleaved into thin walled zoosporangia, each containing one nucleus. The nucleus of each divided to give four, or sometimes six, nuclei and a small mass of protoplasm collected around each. The zoospores eventually were thought by Cook and Schwartz to make their way to the exterior. The diameter of the zoosporangium was recorded as from 6.0-6.5μ while the zoospores were about 1.5μ in length and 0.5 to 0.7μ in diameter. No flagella could be seen. Ayers (1) confirmed the existence of the zoosporangial stage and provided further information regarding its development. He found that small plasmodia formed only a few zoosporangia, but large ones were transformed into a large number which may be arranged in compact, irregular aggregations. Ayers reported that shortly after formation of zoosporangia, the nucleus of each divided to produce four to eight zoospores which were shown to be biflagellate and heterokont with a diameter of from 1.9 to 2.3μ. Zoospores were observed escaping from the zoosporangia only three times. Apart from their smaller size, these secondary zoospores closely resembled those thought to
escape from the resting spores. The mature zoosporangia, according to Ayers, became attached to the cell wall and openings developed at the point of attachment. Apparently the zoospores escaped to the exterior through these openings for their discharge into the root hair was never observed nor were they ever found free within the root hair. Under moist conditions the zoospores were completely discharged. It was found that zoospore discharge from mature zoosporangia took place in a short time in tap water. According to Ayers, from two to eight days, depending on conditions of moisture and temperature, may elapse between the occurrence of infection and the final zoospore discharge. Nothing has been ascertained of the subsequent role of the discharged zoospores.

Possibility of a sexual phase in the life cycle. The lack of information on sexuality in P. brassicae helps to explain the diverse interpretations of its life cycle. Nothing is known about sexual reproduction in P. brassicae, yet most workers have assumed it occurs. It is generally agreed that there is a reduction division in the formation of the spores, but no one is certain of the position of syngamy. The extremely small size of the nuclei of the organism has imposed limitations on attempts to study nuclear phenomena. This, combined with the fact that primary and secondary zoospores are difficult to obtain, has caused the hypotheses set forward to support the existence of a sexual phase to be of a varied nature.

Prowazek (37) contended that the incipient spore segments fused in pairs following cleavage, after which the zygote or
binucleate spore began to encyst. One of the so-called gametic nuclei then underwent a reduction division and formed a variable number of reduction bodies. Meiosis was followed almost at once by karyogamy. Prowazek's account was refuted by Maire and Tison (31) who failed to find any evidence of plasmogamy and karyogamy following cleavage. They nevertheless believed that sexual fusion occurs in *P. brassicae* and postulated that it might take place between two amoebae from germinating spores. Winge (47) agreed with this view and assumed that the motile cells from resting spores copulate in pairs to form small "myxaplasma" which penetrate the host and develop into plasmodia. The diploid phase was assumed to persist until the second sporogenous division where reduction occurs. Lutman (29), Chupp (8), and Milovidov (33) although uncertain as to the time and place of plasmogamy and karyogamy, believed that the reduction in chromosome number which takes place during the first sporogenous division was evidence that fusion must occur at some stage. Nawaschin (34) postulated that fusion occurs outside the host cell between pairs of zoospores.

The unorthodox views of Jones (24) have been seriously questioned by later workers and his hypothesis of unequal-sized gametes arising from the resting spores has been refuted by most investigators.

Cook and Schwartz (12) suggested a fusion of the secondary zoospores in the very young cortical cells, or in the root hair. Their hypothesis was based entirely on the observation of zoospores lying side by side in pairs, and the presence of
binucleate amoebae. Fedorintschik (16) postulated a fusion of gametes from zoosporangia or gametangia after a period of vegetative budding within the host. He also believed that two reductions occur in *P. brassicae*, one during the first division of the sporangium nucleus and another at sporogenesis. However, he reported only one nuclear fusion in his account.

Heim (19) after extensive studies of the nuclear behaviour of *P. brassicae* concluded that fusion of nuclei takes place in the older plasmodia in the root tissues, plasmogamy having occurred upon fusion of zoospores. These plasmodia, according to Heim, represent the diploid stage and do not ramify. It is this stage, during which the chromatin is barely discernible upon staining, which corresponds to the akaryote stage of some authors. The diploid nuclei, after a resting period, divide three times to provide the nuclei for future spores. She noted that the first division had all the characteristics of meiosis and had the same appearance as the first nuclear division in an ascus or basidium. The divisions within a single plasmodium were simultaneous. After the first division of the fusion nucleus the cytoplasm condensed around the daughter nuclei, and the pleurinucleate plasmodium became a mass of uninucleate plasmodia which in turn divided mitotically. Heim claimed that the tiny cells resulting from the second division rounded up and were capable of becoming amoeboid. The third and final division gave rise to the resting spores. These findings have not yet been confirmed by other workers.
The question of the existence and position of plasmogamy, karyogamy and reduction division thus remains in an uncertain state and will doubtless remain so until extensive monospore studies have been made.

**Growth and distribution of the parasite within the host tissues, and formation of resting spores.** Woronin (148) was uncertain as to whether amoebae fuse to form plasmodia, or whether each plasmodium arises from a single amoeba but he felt the first possibility was the more plausible. Nawaschin (34), Prowazek (37), and Fedorintschik (16) supported the theory that plasmodia or myxamoebae flow together within the host, but Maire and Tison (31) and Chupp (8) did not agree. According to Cook and Schwartz (12) amoebae in their early stages may combine to form large plasmodia although this is in no way a conjugation but only a simple joining up of protoplasts.

Some workers (29, 26, 16) believed the amoebae or young plasmodia to be capable of dividing repeatedly in the host cell so that their numbers may be rapidly increased. A few workers (12, 24) contended that these plasmodia may encyst under unfavourable conditions but this is thought unlikely (10).

It is now generally agreed that the spread of the parasite in the host tissues occurs in two ways: by migration of amoebae and young plasmodia from cell to cell, and by passive distribution of the parasite through repeated divisions of infected cells. Lutman (29), Chupp (8), Kunkel (26), Honig (23), and others have demonstrated in fixed and stained tissue the passage of small plasmodia from cell to cell. Cook and
Schwartz (12), more than a decade later, expressed doubt as to this occurrence and postulated that only the secondary zoospores, which in their opinion function as gametes, possess the faculty of passing from one cell to another. Fedorintschik (16) believed that in the early stages of the disease migration of amoebae is the principal method of distribution in the host tissues, but, after the plasmodia have formed and begun to mature, further spread is by division of infected cells. While it is now generally believed that division of the host cell greatly increases the number of infected cells, it nevertheless appears to play a minor role in the distribution of the parasite throughout the root.

Chupp (8) stated that the fungus is distributed horizontally and vertically through the cortex, cell division taking place as fast as invasion occurs. Studies made by Kunkel (26), Larson (27), and others showed that the parasite can reach the cambium and thereafter cells formed from the invaded cambium are also infected as they are formed. Kunkel (26) has shown also that many of the cortical cells become infected by the movement of the parasite outwards from the cambium. The cells of the medullary rays are usually severely attacked but only rarely is the parasite found in the xylem elements (26, 12).

When a plasmodium has become established in a cell suitable for development, the amoeboid movement ceases. Rapid growth, accompanied by nuclear divisions takes place at the expense of the food reserves of the cell. According to Woronin (48), the mature plasmodium becomes vacuolate and the protoplasm is divided by a fine lattice-work of vacuoles.
The vacuoles then begin to disappear and the granular protoplasmic substance lying between them collects into small sphaeroid aggregations which are the future spores. Woronin noted that at first a spore was naked, but soon a thin, unmarked wall was laid down around it. At no time was the spore mass ever enveloped by a common membrane. The resting spore walls contain chitin but no cellulose (36).

At maturity the resting spores are nearly spherical. Considerable variation in their size has been reported. The following spore diameters have been recorded: 1.6μ (18); 1.9-4.3μ (8); 1.7μ (46); 2-3μ and 4.6x6.0μ (12); 3.9μ (23).

The mature spores remain in the clubbed tissue until they are released to the soil by decay.

EXPERIMENTAL WORK

Materials

Severely clubbed cauliflower roots were obtained in the fall of 1960 from a garden in Vancouver. These clubs were air dried and kept in this condition until experimental work began the following spring. Three races of the organism were obtained from the Canada Department of Agriculture Experimental Farm at Charlottetown, Prince Edward Island. Greenhouse tests with soil grown cabbage and cauliflower plants showed the Vancouver race to be much more pathogenic than the three eastern races, so the latter were not used in the following experiments. When it became necessary to grow infected plants, the dried roots were soaked in distilled water and macerated in a Waring blender.
The macerate was mixed into loam soil prepared for greenhouse use at the rate of approximately five grams of macerate per kilogram of soil. This soil was put in six-inch pots. Six-week old cabbage and cauliflower seedlings were transplanted from flats to the pots of infested soil.

Varieties used during the course of the experimental work were Snowball X, a susceptible variety of cauliflower, and Winter King Savoy, a susceptible variety of cabbage.

Methods

Source of clubbed tissue. Seedlings were grown in pots of infested soil in the greenhouse during the summer months with a temperature range of 65 to 95°F. As it was found that club development was at a minimum in the greenhouse during the winter, the infected plants were, from October to May, grown in growth chambers at 70°F, with about 1000 foot candles of white fluorescent light. The clubbed tissue was then kept frozen until used. The majority of clubs obtained from these plants were badly decayed by the time they reached a size suitable for use. This invasion by secondary organisms was hastened by the maintenance of a high level of moisture in the soil, a precaution which was made necessary as a result of the inefficiency with which the distorted root systems conducted water.

Because the soil grown clubs became decayed at an early stage in their development, attempts were made to grow clubbed plants in soil-less culture in the hopes of obtaining large, clean clubs from which to prepare inoculum. Two-week old
cabbage seedlings which had been germinated on moist filter paper and then exposed to a heavy suspension of resting spores in watchglasses for four days were supported above half-pint jars containing Knop's nutrient solution (2) with microelements added (22). Only the root system of each plant was submerged. Aeration was provided to each jar for two-hour periods three times daily. The nutrient solution was renewed twice weekly. In spite of the aeration, the roots of the seedlings became covered by a heavy growth of phycomycetous water molds, introduced with the spore material. This mold growth rapidly destroyed the root system with the consequent death of the plant. "Moldex" (methyl parahydroxybenzoate) was added in an attempt to retard mold growth but was ineffective. The application of a higher concentration of this inhibitor was not attempted because it was thought that this could inhibit the development of both \textit{P. brassicae} and the host plant.

Another series was set up in the laboratory, the plants being grown in silica sand with nutrient solution being supplied by a subirrigation system. Gallon crocks, connected at the base to a large carboy containing nutrient solution, were filled with washed silica sand. The nutrient solution saturated the sand when the carboy was raised, and drained upon lowering the vessel, drawing air through the sand at the same time. Half the seedlings transplanted to this sand had been germinated and grown for two weeks in infested soil, the remaining plants had been germinated on moist filter paper and exposed in watchglasses to a heavy spore suspension, consisting of washed, macerated, decayed club tissue. The plants were grown in the sand for 120 days.
All seedlings initially exposed to infested soil developed clubbed roots, however none of those exposed to spores in aqueous suspension did. Either infection did not take place at all, or the root hairs once infected were killed by the action of contaminant microorganisms. Although at an early stage of development the sand grown clubs were clean, decay soon set it and the clubs at the age of two months were not much cleaner than those grown in soil. This was likely due to a carry-over of microorganisms on transplanting from the soil and to the necessity of keeping the sand nearly saturated with nutrient solution in order to prevent the clubbed plants from wilting.

Preparation of resting spore inoculum. Two methods were used in the preparation of resting spore inoculum for in vitro studies of P. brassicae. Initially the method of Ayers (1) was followed, using soil grown clubs which were heavily invaded with secondary organisms. These clubs were macerated for three minutes in a Waring blender and, after allowing the coarse debris to settle out, the supernatant was centrifuged and the material which had been spun down washed three times in tap water. The resulting spore paste was stored in glass flasks at 5°C. Secondly, in an attempt to obtain cleaner spores, the following procedure was carried out. Young clubs which had a minimum amount of decay were used. All fibrous roots and any decayed areas were cut away and the clubs washed in running tap water for one hour to remove any soil particles which had lodged in crevices of the hypertrophied tissue. The clubs were
then soaked in a ten per cent solution of "Chlorox" (5.25 per cent commercial calcium hypochlorite) for fifteen minutes to reduce the surface bacteria. After being rinsed three times in sterile distilled water, the clubbed tissue was macerated in a sterile Waring blender for three minutes. The macerate was filtered through cheesecloth and the filtrate centrifuged at 3300 r.p.m. for fifteen minutes. The material which was spun down was resuspended in sterile distilled water and recentrifuged three times. The wet spore paste was then stored in sterile flasks at 5°C. Care was taken to minimize aerial contamination but it was not feasible to carry out the entire procedure under completely aseptic conditions because the required equipment was not available.

Germination of resting spores. As very little information exists as to the germination requirements of \emph{P. brassicae} resting spores, as many methods as possible were employed in the hope that one would produce the necessary germination stimulus. Spores, from both clean and decayed clubs, were suspended in sterile tap water, sterile distilled water, heat sterilized crude soil extract, and non-sterile crude soil extract. The soil extract was prepared by pouring water through a clay pot filled with garden loam. The pH of these media was adjusted to 4.0, 6.0, and 8.0. Half the flasks containing the spores were kept at room temperature and half kept in an incubator at 25°C. Of those kept at room temperature, half were wrapped in aluminum foil to exclude light, the remainder were exposed to daylight. During the day small amounts of the suspension were withdrawn at four-hour intervals and examined under
a Leitz phase contrast microscope. A solution of methyl cellulose was used as an alternative mounting medium to slow down the movements of the motile organisms. A 0.1N solution of iodine in a two per cent solution of potassium iodide in water was introduced under the cover slip after examination of the living organisms, and this was found to fix and stain flagella in such a manner that the number and approximate length could be determined, although distortion was too severe to observe any further morphological details.

In none of the treatments were P. brassicae zoospores positively identified. Neither temperature, light, nor pH had any apparent effect on the stimulation of germination. No one treatment had a high concentration of a heterokont biflagellate organism of a size that would cause it to be considered as a P. brassicae zoospore. There were markedly more flagellate organisms in flasks containing spores obtained from soil-grown, decayed clubs. In all the non-sterile soil extracts, as well as in all media containing spores from decayed tissue, a biflagellate heterokont zoospore was observed frequently, of a size ranging from 3.0 to 10.0µ in length.

Of particular interest is the observation that several of these zoospores were tetraflagellate, each cell bearing two long and two short flagella at its anterior end. The position of the flagella, however, indicated that the tetraflagellate organism was a result of incomplete cleavage of protoplasm in spore formation rather than of fusion. Although it was difficult to make out the internal structure and details of flagellar morphology,
the biflagellate form of the zoospore matched the description
given by previous authors (1, 28) except that the wide variation
in size would seem to lessen the possibility of its being a
P. brassicae zoospore. Within two days bacterial contamination
was so high as to preclude accurate observation with the phase
contrast microscope.

In an attempt to eliminate the bacterial contamination,
two bacteriocidal agents, rose bengal at .067 per cent as reco­
mmended by Martin (32), and streptomycin sulfate at 500 parts
per million were added to the non-sterile soil extract contain­
ing spores from decayed clubs. Although both substances retarded
bacterial growth, the growth of water molds was extremely rapid
as a result of the elimination of competition for the substrate.
Because of the possibility of the phycomycetous organism
releasing biflagellate zoospores which could be confused with
the P. brassicae zoospores, attempts to reduce bacterial con­
tamination by the use of bacteriocidal agents were not repeated.

Although the number of motile organisms and bacteria was
considerably less in flasks containing spores from clean clubs,
the non-sterile soil extract medium contained several biflagel­
late organisms. None of these, however, resembled those seen in
flasks containing spores in non-sterile soil extracts and spores
from decayed clubs. In all the flasks containing clean spores
there were heavy concentrations of starch grains which hampered
observations. It would appear that these starch grains are
broken down by the microorganisms responsible for the decay of
the clubbed tissue. Attempts to separate the starch grains
from the resting spores were unsuccessful as the two are of a similar size and density. Microscopic examination of these spores revealed them to have a less dense cytoplasm than those obtained from decayed club tissue, and as this was taken as an indication of immaturity, the spores from young, clean clubs were not used in the experiments which followed. Resting spores from old clubs are shown in Figure I.

The parasite within the root hair. On the basis of the above observations, and in view of the difficulties involved in germination of the resting spores under in vitro conditions, it was felt that attention should be directed at that portion of the life cycle which takes place in the root hair of the plant. It was also hoped that an examination of the micro-environment of the root hair growing in a liquid medium would reveal something of the processes of germination and penetration. Consequently, several attempts were made to obtain root hair infection in young cabbage and cauliflower seedlings.

Initially, the method for obtaining root hair infection described by Samuel and Garrett (40) was employed. Seedlings were germinated and grown in tumblers of infested soil for one week. They were then rinsed in distilled water and stained in acetocarmine. At the same time attempts were made to obtain root hair infection following the method described by Palm and McNew (35). Seedlings were sown in tumblers of infested silica sand supplied with Knop's nutrient solution (2). Seedlings were removed after six days and placed in one per cent acetocarmine. No infections were observed in either method. In
both methods, particularly that of Samuel and Garrett, it was extremely difficult to remove adhering particles of soil and sand without at the same time removing or damaging the root hairs containing the parasite. It was found that adhering particles of sand and soil prevented the coverslip from lying evenly on the stained root and thus hampered observations. Another disadvantage to these two methods is that the damage caused to the root hairs upon removal from the soil and on prolonged rinsing resulted in changes in the cytoplasm and consequent disruption and obliteration of any stages of the parasite present.

A solution culture technique described by Macfarlane (30) was tried. Spores obtained from decayed clubs were suspended in a modified Hoagland and Snyder's solution as given by Macfarlane. Small vials were used to contain the spores and cauliflower seedlings were supported in the vials on fine mesh nylon gauze, in which holes for the roots had been punched. Macfarlane claims to have obtained abundant infections in these vessels within four to six days. In the present experiment, however, the seedlings died within forty-eight hours, apparently from attack of the root system by bacteria and water molds introduced with the spores. This decay of the roots was hastened by lack of aeration in the narrow vials.

An attempt was made to obtain infection under aseptic conditions following the method of Chupp (8). Diseased roots that contained spores but were not far enough invaded by bacteria were surfaced sterilized in .001 per cent mercuric chloride, rinsed, transferred to agar slants in test tubes, and
minced finely. These were incubated for four days and any showing bacterial contamination were discarded. Then a few drops of sterile soil filtrate and a young cabbage seedling, which had been grown under sterile conditions in a petri plate, were added. After one week the seedlings were stained in acetocarmine and examined. Drops of the liquid surrounding the root hairs were also examined microscopically. This procedure proved to be very laborious and the great majority of slants were contaminated. No root hair infection was observed, nor were any motile cells seen. It was apparent that very few spores were released upon mincing and it was possible that there were insufficient spores to cause infection.

Attempts were then made to obtain root hair infection of seedlings under conditions of minimum contamination. Spores were deposited on filter paper using a Buchner funnel and suction flask and washed several times with sterile, distilled water. One-third of these spore-laden filter paper disks were soaked in .001 per cent mercuric chloride for five minutes and rinsed three times with sterile, distilled water, and the remaining third were used directly. Week-old cabbage and cauliflower seedlings, grown under sterile conditions in petri plates, were placed on the filter paper bearing the spores in sterile petri plates. A few drops of sterile, distilled water were added to provide the necessary moisture. In ten days time the seedlings were removed for examination. Prior to examination the seedlings were stained, then mounted in a semi-permanent fashion to preserve the roots for comparison to seedlings grown under similar conditions but not exposed to *P. brassicae* spores.
minced finely. These were incubated for four days and any showing bacterial contamination were discarded. Then a few drops of sterile soil filtrate and a young cabbage seedling, which had been grown under sterile conditions in a petri plate, were added. After one week the seedlings were stained in aceto-carmine and examined. Drops of the liquid surrounding the root hairs were also examined microscopically. This procedure proved to be very laborious and the great majority of slants were contaminated. No root hair infection was observed, nor were any motile cells seen. It was apparent that very few spores were released upon mincing and it was possible that there were insufficient spores to cause infection.

Attempts were then made to obtain root hair infection of seedlings under conditions of minimum contamination. Spores were deposited on filter paper using a Buchner funnel and suction flask and washed several times with sterile, distilled water. One-third of these spore-laden filter paper disks were soaked in ten per cent "Chlorox" for ten minutes and rinsed three times in sterile, distilled water, one-third were soaked in .001 per cent mercuric chloride for five minutes and rinsed, and the remaining third were used directly. Week-old cabbage and cauliflower seedlings, grown under sterile conditions in petri plates, were placed in sterile petri plates on the filter paper bearing the spores. A few drops of sterile, distilled water were added to provide the necessary moisture. In ten days time the seedlings were removed for examination. Prior to examination the seedlings were stained, then mounted in a semi-permanent fashion to preserve the roots for comparison to seedlings grown under similar conditions but not exposed to *P. brassicae* spores.
Several stains were tried: one per cent solution of cotton blue in lacto-phenol, .05 per cent aqueous Giemsa tissue stain, aqueous aniline blue at a 1:500 dilution, seventy-five per cent lactic acid followed by one per cent cotton blue in lacto-phenol, and one per cent aqueous aceto-carmine. Of these, aceto-carmine proved most satisfactory, staining the mature zoosporangia brilliant red, and the young plasmodia faintly pink. Seedlings were placed in aceto-carmine for three days, after which the root systems were mounted in the stain and heated almost to boiling. Sufficient pressure was then placed on the cover slip to flatten the radicle without damaging the root hairs. This was found necessary in order to have the root hairs on a single plane for observation and photography. The coverslip was then sealed to the slide with a mixture of two parts vaseline to two parts lanoline to one part paraffin. This sealing compound was readily applied and kept the slides in good condition for up to two months.

Infections were extremely few and sporadic (Table I). Although the seedlings in the "Chlorox" and mercuric chloride treated plates showed no greater incidence of infection than those untreated, the former plates remained free from decay several days longer than those untreated. A major disadvantage of this method was that the root hairs tended to adhere to the filter paper and were consequently damaged when the seedlings were removed for examination.

The most successful method for obtaining root hair infection by *P. brassicae* was found to be one in which week-old cabbage and cauliflower seedlings were placed in sterile
petri plates containing a thin layer of Knop's solution with trace elements, to which was added a .5 molar phosphate buffer containing spores. The spores were obtained from decayed club tissue and washed thoroughly by centrifugation. The seedlings were removed daily, rinsed in an isotonic buffer solution, and stained in the usual manner. Over five hundred roots were mounted in the manner previously described. Infection took place sporadically, but the number of infections was much greater than in any other method tried (Table I). Unfortunately, infection stages were seldom observed before ten to fourteen days after exposure to the spores, and by this time the seedlings were no longer vigorous and healthy, the contaminants introduced with the spores having destroyed some of the root system. Phycomycetous water molds continued to be a problem as they obliterated the view of many root hairs.

Early infection stages appeared in root hairs until the seedlings died at about three weeks. The young plasmodia stained so faintly in aceto-carmine as to be indiscernible except in phase contrast, while mature zoosporangia stained very darkly and distinctly. It appeared as though the affinity for the stain increased with maturity. Nuclei of the host cell stained deeply, but were in most cases a distinctive spindle shape, although occasional spherical nuclei were observed. Infection was apparently confined to the root hair, never having been seen in the epidermal cells. In only a few instances was distortion of the root hair containing the parasite observed. Control seedlings grown under identical conditions but without
the spores were used throughout the observations for comparative purposes. The distortions seen periodically in infected seedlings were observed with equal regularity on the control seedlings.

Young plasmodia were seen chiefly in the tip and mid-section of the root hair (Figures II. and III). Anywhere from one to seven plasmodia could be found in a single root hair. They were at times somewhat irregular in outline, although the majority approached being spherical in shape. The size ranged from 3.7μ to 7.4μ in diameter. Because the nuclei of the plasmodia were not discernible it was not possible to determine the number of nuclei per plasmodium. No larger plasmodia were seen although occasionally clusters of overlapping, spherical, lightly staining bodies were observed. It was not possible to tell whether these groups were fusing plasmodia or incipient zoosporangia.

The mature zoosporangial clusters were unmistakable, consisting of from six to thirty spherical zoosporangia (Figure IV). Each zoosporangium measured from 5.0 to 8.4μ in diameter, and in many, zoospore cleavage lines could be readily seen. Measurements of stained zoosporangia were the same as those of living zoosporangia. An attempt was made to maintain a section of infected root in a buffer solution under the microscope in order that the escape of zoospores from the zoosporangia might be observed. However, too few infections were obtained to permit this to be repeated until successful.

A striking feature noted in all the mounted roots was the number of resting spores which adhered to each root hair in spite of the washing each root system received prior to
staining (Figure IV).

Because it had been noted that the spores obtained from clubbed tissue tended to adhere together, an attempt was made to break down the matrix which held them by the use of trypsin, a proteolytic enzyme. Accordingly, 1:100 trypsin powder was dissolved in 0.85 per cent sodium chloride at 5000, 500, 50 and 5 parts per million. Two milliliters of enzyme solution, two milliliters of .5 molar phosphate buffer of pH 6.2, and six milliliters of a heavy \textit{P. brassicae} spore suspension which had been repeatedly washed in a centrifugation process, were incubated for fourteen hours at 25°C in fifty milliliter flasks, the final enzyme concentration being 1000, 100, 10, and 1 parts per million. A check with no enzyme was run at the same time. At the completion of the digestion period, the spores in each treatment were centrifuged and washed three times in .5 molar buffer of pH 6.2. Following this they were suspended in eight milliliters of modified Knop's solution with two milliliters of .5 molar phosphate buffer of pH 6.2. Six-day old cabbage and cauliflower seedlings, grown under sterile conditions in a petri plate, were placed in petri plates containing ten milliliters of nutrient-spore-buffer mixture, with the shoots supported on one-quarter inch diameter glass rods. Seedlings were removed daily for examination in the usual manner.

Again infection was sporadic and infrequent, however there were indications that spores exposed to the strongest concentration of enzyme had a slightly higher rate of infection than did the controls (Table I), however, a more extensive experiment would have to confirm this finding.
The relative effectiveness of the various methods of obtaining root hair infection by \textit{P. brassicae}, as carried out in this investigation, is presented in Table I.

DISCUSSION

It is rather difficult to explain the confliction in reports which various workers have made concerning their success in germinating the resting spores of \textit{Plasmodiophora brassicae}. Several methods reported as successful by other authors were carefully repeated in these experiments with entirely unsatisfactory results. It would seem strange that many of the problems and complications which were encountered by the present author were not mentioned by the majority of workers reporting success. It is felt by this author that many of the techniques employed by previous investigators did not take into consideration the problems inherent in an \textit{in vitro} study of \textit{P. brassicae}.

The principle factor which leads to confusion is the presence of a wide variety of contaminant microorganisms in the same micro-environment as the \textit{P. brassicae} resting spores. Several of these contaminants are similar in morphology to that reported for \textit{P. brassicae} zoospores. It is apparent that very few investigators have realized the significance of this factor and thus the majority of conclusions which have been made on the morphology of the germination product have been drawn from unsound evidence. Although in this study a biflagellate, heterokont organism resembling the description given by others was seen, it is, in the author's opinion, entirely impossible to state that an organism present in a highly contaminated medium
is a *P. brassicae* zoospore unless it has been observed escaping from a *P. brassicae* resting spore. This process has been observed and described only three times in the entire history of the study of the organism and the reports are highly contradictory (45, 46, 14). This failure to associate the zoospore with the resting spore could account in part for the conflicting descriptions of both morphology and size of the zoospore. It is granted that close examination of the organism under observation is made difficult by the small size and rapid movement of the motile organisms. It would seem, however, that if the high rate of germination obtained in various media by some workers (15, 46, 1) actually occurred, it would take only continued observation to see the germination process as frequently as was necessary to obtain an accurate description of both the germination process and the zoospore itself.

It is indeed difficult to reconcile the extremely high germination rate and long viability of *P. brassicae* resting spores under natural conditions in the soil, with the very low, inconsistent rate of germination in *in vitro* laboratory studies. The wide geographical distribution of the pathogen, its occurrence in many soil types, its high resistance to drastic control measures and extreme temperatures, would indicate that the spores of this parasite are remarkably tolerant of abuse. The failure to obtain a good rate of germination and subsequent infection under carefully controlled *in vitro* conditions would then seem to indicate the lack of some specific germination factor present under natural conditions in the soil, but lacking under the circumstances necessary for the study and observation
of germination and infection in the laboratory. It is entirely possible that the factor responsible for the maturation of the resting spore is closely concerned with the decay of the root tissue by the soil microorganisms which are ultimately responsible for contamination in culture. It is logical to suggest that the same process which aids in dispersing the organism should be effective in preparing it for germination, in somewhat the same manner that the spores of some coprophilous fungi are prepared for germination by passage through the digestive tract of an animal. The various enzyme complexes responsible for the breakdown of host tissues could readily act as germination promoters. That this germination stimulus may be an enzymatic process is suggested in these experiments by the increase, albeit slight, in the infection rate by spores exposed to the proteolytic enzyme, trypsin. Besides the proteolytic enzymes such as trypsin and pepsin, many others take part in the normal breakdown of plant material in the soil. Cellulase, pectinesterase, polygalacturonase, and the Q enzyme, all could conceivably have a role in the preparation of the resting spores for germination. It remains to be seen whether a synthetic complex of enzymes applied to the spores could successfully duplicate the natural germination factors and induce a high germination rate. If the germination stimulus could be duplicated in such a manner, it would then be possible to use spores from clean clubs, thus eliminating contamination by microorganisms.

Difficulties in technique are not confined to the germination of the resting spores. This investigation illustrated that plants grown and infected in soil and sand are of little
value in the study of P. brassicae, as it is not possible to remove particles from the root system without causing damage to the tissue. Damage to the root hairs cannot be tolerated if the living tissue is to be examined, and is to be avoided if stained tissue is to be used. Ideally, the examination of the parasite within the host plant, and in particular the root hairs, should be carried out on living tissue in order that artifacts brought about by fixing, sectioning, and staining techniques may be avoided. The use of living tissue also permits the examination and observation of the development of the parasite, if only for short periods of time. Although most workers have used staining techniques (12, 8, 16, 20) in order to study the parasite in the root hair, none have mentioned the ease with which artifacts are produced in these tissues. This investigator found that there is a pronounced tendency of the cytoplasm to pull away from the cell wall when the root hair is mechanically injured or exposed to a toxic substance such as any of the commonly used stains. Such coalesced clumps of cytoplasm take up stain to a greater or lesser degree and could be mistaken for a root hair parasite of a plasmodial nature. Although root hair nuclei are generally of a characteristic shape, the occasional abnormal nucleus seen in these experiments is of the same size and shape as a young plasmodium. The distortion of the root hair, taken by most authors (48, 1) to be a reaction on the part of the host plant to the fungus, is most probably caused by some other factor, as it occurred in these experiments with equal frequency in uninfected roots.

With regard to the elucidation of the life cycle of the
parasite, this study has done little more than confirm the existence of an early amoeboid or plasmodial stage and a later zoosporangial stage. Because no flagellate organisms arising from *P. brassicae* resting spores were observed, and because spores adhered in large numbers and with tenacity to the root hair exterior, the suggestion is put forth here that, in some cases at least, a protoplast is exuded from the spore directly into the host through the cell wall at the point of contact, without the existence of a primary flagellate stage. This, however, is no more than a hypothesis, and along with the hypotheses regarding the function of the so-called secondary zoospores and the existence of a sexual phase, remains to be substantiated by irrefutable evidence.

It is apparent from this study that such evidence will be obtained only when satisfactory techniques have been developed. A method of obtaining high germination rate of the resting spores under conditions of minimum contamination is essential in order that the product of germination may be studied. Necessary also is a technique which will allow the infection of the host plant and growth of the parasite under conditions favorable for observation of the living parasite. Until these problems in technique are overcome, further work on *Plasmodiophora brassicae* is likely only to add to the confusion which already surrounds the problem, without in any way adding to our knowledge of the life cycle of the parasite.
LITERATURE CITED


APPENDIX

TABLE I

Relative Effectiveness of Nine Methods of Obtaining Root Hair Infection by *Plasmodiophora brassicae*

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of Roots Examined</th>
<th>No. of Infections Seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. after Samuel and Garrett</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>2. after Palm and McNew</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>3. after Macfarlane</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>4. after Chupp</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>5. spores on filter paper washed with &quot;Chlorox&quot;</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>6. spores on filter paper washed with mercuric chloride</td>
<td>95</td>
<td>2</td>
</tr>
<tr>
<td>7. spores on filter paper washed with water</td>
<td>101</td>
<td>1</td>
</tr>
<tr>
<td>8. spores in petri plates without trypsin</td>
<td>531</td>
<td>35</td>
</tr>
<tr>
<td>9. spores in petri plates with trypsin</td>
<td>95</td>
<td>17</td>
</tr>
</tbody>
</table>
FIGURE I
RESTING SPORES OF *PLASMODIOPHORA BRASSICAE*
(X 2000)
FIGURE II

YOUNG PLASMODIUM OF PLASMODIOPORA BRASSICAe IN ROOT HAIR

(X 2000)
FIGURE III

YOUNG PLASMODIA OF PLASMODIOPHORA BRASSICAE IN ROOT HAIR

(X 3500)
FIGURE IV

CLUSTER OF ZOOSPORANGIA IN ROOT HAIR

(X 1400)