Biology of Phytophthora Zoospores

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In the genus *Phytophthora,* one group of species which invades the underground parts of plants may be distinguished from a second whose members attack foliage and whose caducous sporangia, in a number of species, are an important vehicle of dissemination. Much of my experience has been gained with the soil-borne species, and much of my discussion of zoospore biology will be about them. I shall also refer to other Phycomycetous plant pathogens inhabiting the soil which possess zoospores resembling those of *Phytophthora,* particularly *Aphanomyces* and *Pythium* species.

Morphology and anatomy.—The laterally biflagellate zoospores of species of *Phytophthora* and other genera are often described as reniform, bearing the flagella on the concave side. Variations in shape and relationship of flagella and groove are seen in Fig. 1-A. The basic characteristics of the flagella (posteriorly directed whip-lash, anteriorly directed tinsel) have been illustrated, [Cunningham & Hagedorn (8)] for *Aphanomyces euteiches.*

Variation in shape as illustrated by different authors undoubtedly reflects differences in zoospore position when drawings were made. Zoospores of *Phytophthora megasperma* var. *sojae* are ovoid, bluntly pointed at one end, flattened on one side, or reniform, according to the position in which they were photographed (18). Fixation of zoospores as a preliminary to electron microscope study, coupled with interference contrast microscopy, gave good resolution under high power magnification and provided more detail of zoospore morphology in this fungus (21). Zoospores are ovoid, bluntly pointed, and flattened on one side at the anterior end. Along this side of the zoospore, the groove runs from end to end, parallel to the long axis of the spore. At each end the groove is very shallow; thus, zoospores appear reniform in shape when viewed end-on. In the center the groove is relatively deep, appearing as if arched over by outgrowths of the zoospore body. The flagella arise from a common point within the deep part of the groove, slightly nearer to the anterior than to the posterior end. These features were also seen in zoospores of *P. fragariae, Pythium aphanidermatum,* in secondary zoospores of *Aphanomyces cochlioides,* and in a species of *Saprolegnia.*

Electron microscope observations (21) confirmed these morphological features, and (12, 40, 54) provided details of morphology and internal anatomy, the functional significance of which is largely undetermined. A feature of zoospore cytoplasm in the Saprolegniales and Peronosporales is the abundance of vesicles. In *Sclerophthora macrospora,* Fukutomi & Akai (12) identified their content as lipid. Reichle (40) described lipid droplets in addition to membrane-bounded vesicles in zoospores of *Phytophthora parasitica,* and both he and our group have noticed many vesicles with crystalline content. In *P. megasperma* var. *sojae,* the physical nature of this material in young zoospores differs from that in old ones. The vesicle contents may serve as energy sources for sustaining zoospore motility.

Motility.—Depending on the species and temp, zoospores can remain motile for periods up to several days (17) in the absence of external food. The extent to which external nutrients may influence zoospore motility is as yet not clear. A 1% dextrose solution prolonged the motility of zoospores of *P. parasitica* var. *nicotianae* (14). Duration of motility of *Olpidium brassicae* zoospores was greatly increased by addition of amino acids (glycine, L-proline, L-histidine, D-isoleucine, L-isoleucine) at concentrations of 0.05-0.1 M, by rabbit serum (0.5-5% v/v), bovine serum albumin (0.2-2%, w/v) and by a 0.01 M mixture of phosphate buffers (51). In none of these examples was evidence provided that the active materials were actually metabolized, though this was inferred by Teakle & Gold (51). They also suggested that factors other than nutrition were involved —osmotic pressure, pH, and surface membrane stability. In contrast, Barash et al. (2) found that *Phytophthora drechsleri* can metabolize a variety of substances: sugars, amino, organic and fatty acids; but various sugars (glucose, fructose, sucrose) and amino acids (L-glutamine, L-asparagine, L-histidine, L-leucine, glycine, L-proline) at 0.05 M, did not affect duration of motility. They observed that the rate of utilization of certain compounds, e.g., D-glucose, was much lower during the motile stage than during cyst germination, and suggested that motile zoospores are less dependent on external nutrients than germinating cysts.

Recent photographs (7, 18, 20) showing planar, sinusoidal waves along both flagella confirm the observations made by Couch (6) in 1941 that both are active in propelling the zoospore. These waves progress from base to apex along both flagella, and at first consideration it would seem that their actions must be mutually selfcancelling, the posteriorly directed flagellum propelling the zoospore forward, the anteriorly directed one, backward. The flagellate *Ochromonas* (Chrysophyceae) possesses two apically attached flagella (22). One is short, smooth and inactive, the other is long, bears mastigonemes and displays wave movement. Although the wave movement along the latter, anteriorly directed flagellum, is from base to tip, the mastigonemes cause a reversal of the direction of propulsion. Consequently the organism moves forward. If this is also true of the anterior, tinsel flagellum of the laterally biflagellate zoospore, then both flagella combine to propel it forward, the posterior whiplash flagellum pushing and the

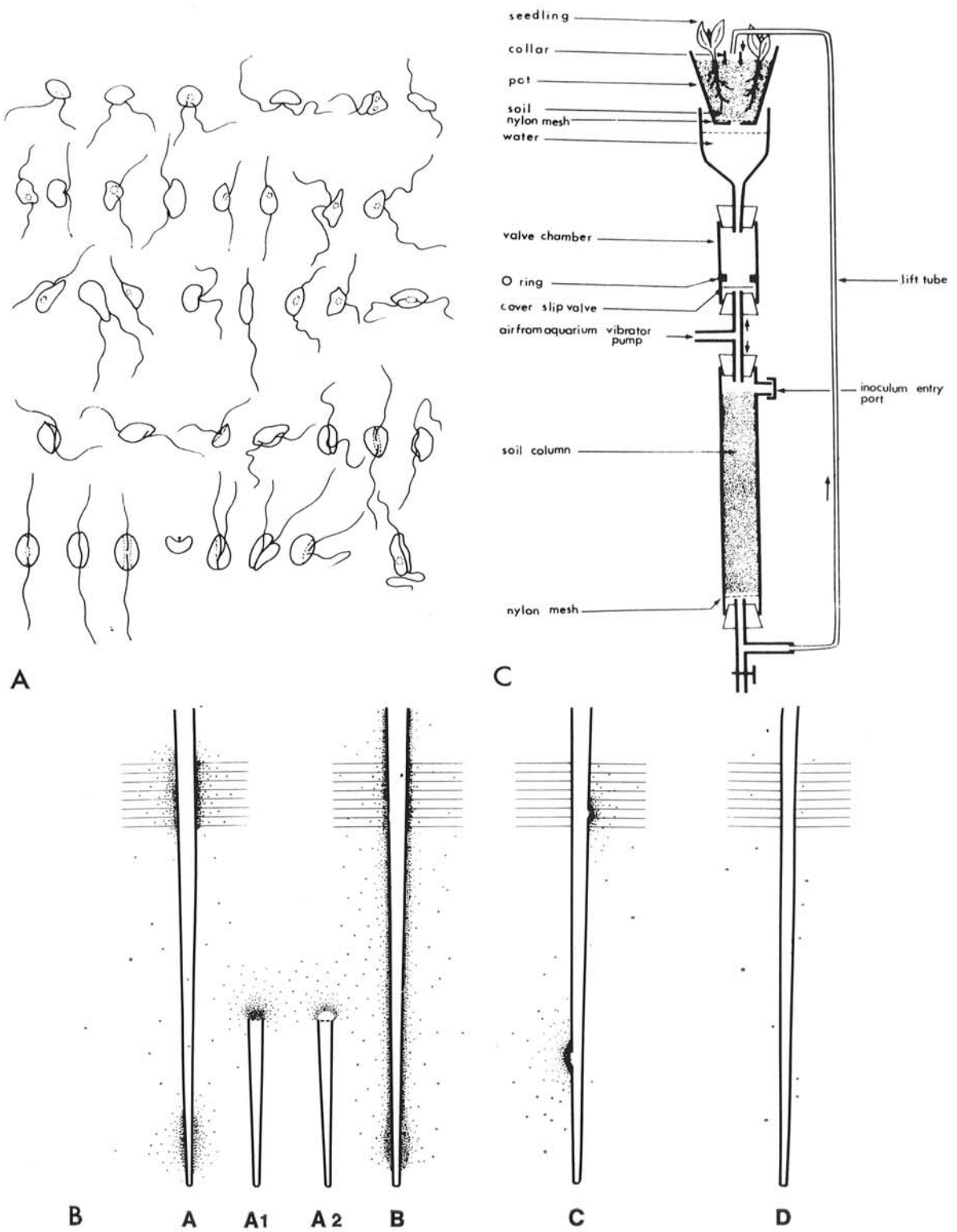

Fig. 1. **A)** Variation in shape of laterally biflagellate zoospores in *Phytophthora* and other genera. **B)** Diagrammatic representation of zoospore accumulation patterns in the presence of roots. For explanation see text. **C)** Diagram of soil perfusion apparatus used in infection studies with zoospores and cysts.

anterior pulling. Relevant here are Couch's observations (6) that secondary zoospores of species of the Saprolegniales with damaged, inactive, posterior flagella, swam forward.

During its forward movement, the laterally biflagellate zoospore executes a smooth path along an extended spiral combined with rotation about its axis. Couch's studies of primary zoospores of *Saprolegnia* (6) lacking either the anterior or posterior flagellum, suggest that it is the latter that provides the basic rotational movements, the former providing fine control. Zoospores with only posterior flagella swam in an irregular spiral, rotating awkwardly about their axes. Those possessing only anterior flagella swam smoothly, but without describing spiral paths or axial rotation.

Dispersal and inoculum units. Phytophthora fragariae does not spread as mycelium in soil, but sporangia are produced on infected roots under moist conditions. The observed pattern of disease extension follows that of water movement from natural drainage (15), suggesting spread by means of zoospores. Zoospores are effective inoculum in soil if provision is made for their transport in moving water (16). There are many other examples of water transport of inoculum, presumably as zoospores, in soil, stream, and irrigation water. Klotz et al. (27) isolated species of *Phytophthora* parasitic on citrus from irrigation water; the effect of irrigation on the spread and development of *P. cinnamomi* among avocados is documented by Zentmyer & Richards (57); Klein (26) demonstrated that sporangia and zoospores were produced abundantly by flooding soybean plants attacked in the field by *P. megasperma* var. *sojae*, and that inoculum was present in run-off water; McIntosh (33, 34) discovered *P. cactorum* and other *Phytophthora* pathogens in irrigation water supplying orchards in British Columbia, and associated irrigation practice with the presence of these fungi in soil.

Behavior of zoospores toward roots.—There are many examples of the influence of root exudates on host/parasite interactions (45), influences leading to stimulation or inhibition of the growth of the pathogen, either directly or indirectly via effects on other members of the soil microflora (37). The motility of zoospores provides opportunity for an additional, multiple response to occur, seen in vitro as rapid attraction, accumulation, and encystment in large numbers in the region of elongation immediately behind the root tip (Fig. 2-A).

This striking phenomenon was first reported by Goode (13) in 1956, and has since been observed among several species of *Phytophthora* and other Phycomycetes (Table 1). With one conspicuous exception (55, 56, and Zentmyer, *unpublished data*) the response is nonspecific, occurring alike to roots of host and nonhost plants. The zoospore attraction phenomenon is readily reproduced using capillary root models containing root exudate or extract (43, 44); the root models provide the advantage of standardizing conditions for experimental work.

Accumulation is the result of a sequence of responses. Of these, two are all-important in the initial stages of aggregation, namely, attraction to the source of the stimulus (chemotaxis) and trapping within a zone close to this source.

After detecting the stimulus emanating from just behind the root tip (or from the mouth of the root model), the zoospore turns and swims towards it up a gradient of attracting factor. How does the zoospore perceive the stimulus? Does the surface of the zoospore as a whole act as a receptor or is a more specific sys-

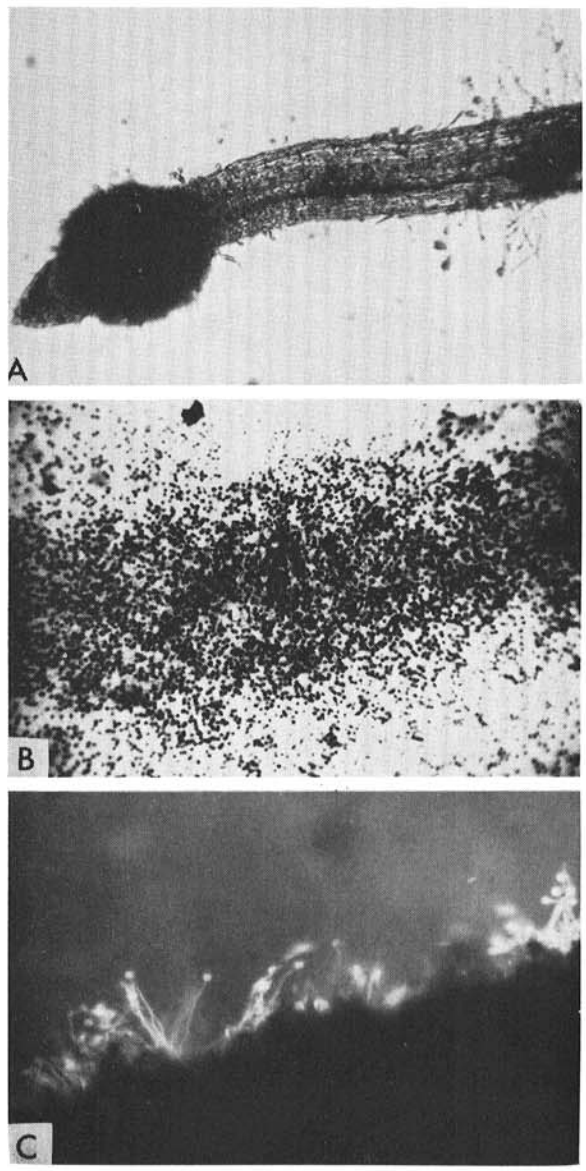

Fig. 2. A) Accumulation of zoospores of *Phytophthora capsici* behind root. (Approx. ×60) **B)** Accumulation of zoospores in nonsterile soil in relation to roots. Band of cysts of *Phytophthora drechsleri* on surface of cellulose acetate membrane marking line of contact between membrane and safflower root on other side. (Approx. ×50) **C)** Accumulation of zoospore cysts of *Phytophthora drechsleri* on safflower roots in soil. Note orientation of germ tubes toward root. (Approx. ×100)

TABLE 1. Phycomycete species in which accumulation of zoospores behind root tips has been observed

Species		Reference
—[a]	*Aphanomyces cochlioides*	Rai & Strobel (39)
—	*A. euteiches*	Cunningham & Hagedorn (9)
?	*Olpidium brassicae*	Teakle (50)
?	*Phytophthora cactorum*	McIntosh (33)
?	*P. cambivora*	McIntosh (33)
—	*P. capsici*	Katsura & Hosomi (23)
		Miyata et al. (36)
?	*P. capsici*	Mehrotra (*unpublished data*)
+	*P. cinnamomi*	Zentmyer (55, 56, and *unpublished data*)
—	*P. citrophthora*	Zentmyer (56, and *unpublished data*)
?	*P. drechsleri*	Mehrotra (*unpublished data*)
?	*P. erythroseptica* var. *pisi*	Bywater & Hickman (4)
—	*P. fragariae*	Goode (13)
?	*P. infestans*	Lacey (29)
—	*P. megasperma* var. *sojae*	Ho & Hickman (19)
—	*P. palmivora*	Turner (53)
—	*P. palmivora*	Zentmyer (56, *unpublished data*)
—	*P. parasitica*	Mehrotra (*unpublished data*)
?	*P. parasitica* var. *nicotianae*	Troutman & Wills (52)
?	*Pythium aphanidermatum*	Kraft et al. (28)
—	*P. aphanidermatum*	Royle & Hickman (43)
?	*P. aphanidermatum*	Spencer & Cooper (49)
?	*P. debaryanum*	Spencer & Cooper (49)
?	*P. polytylum*	Spencer & Cooper (49)

[a] + = Host specific; — = nonspecific; ? = specificity not examined.

tem involved? Carlile (5) suggested the possible existence of specific chemoreceptors located at the cell surface uniformly around the front end of the zoospore, which turns until the receptors receive equal stimulation, i.e., until the zoospore faces the source of the stimulus. Studies of zoospore ultrastructure (12, 21, 40) have provided no evidence of structures identifiable as chemoreceptors, though it is difficult to visualize what form these might take. Modified cilia and flagella are associated with sensory perception (e.g., light, pressure, vibration, olfaction) in some protista and in Metazoa (48); it is perhaps not inconceivable that zoospore flagella may also function in a similar capacity, particularly the anterior, tinsel flagellum.

In the immediate vicinity of the source of the stimulus, the typical spiral path of zoospore movement alters profoundly. It becomes irregular, with rapid changes of direction that effectively trap zoospores in this zone. Trapping is followed, in turn, by rapid encystment, believed due to the abrupt change in the chemical-physical environment in the trapping zone. Germination of cysts quickly follows. During germination, the germ tubes emerge from points facing the stimulus source, and germ tube growth is chemotropically directed in the same direction.

Though the pattern of zoospore accumulation just behind root tips is of wide occurrence, others are known which differ from it in greater or lesser degree. The range of zoospore:root relationships is summarized in Fig. 1-B and in the following notes: Fig. 1-B: (A) represents the typical pattern of accumulation. Differing from it slightly is the accumulation of zoospores in the region of maturation (28) rather than in the elongation region. Sometimes zoospores also encyst amongst root hairs, probably because sluggish zoospores near the end of their period are trapped among the tangle of root hairs (13). Zoospore attraction was not involved, germ tube origin and growth was quite at random, and penetration of root hairs or the epidermis did not occur. Zoospores may mass on the exposed surface of a cut root (Fig. 1-B [A1]). A high concentration of active factor occasionally repulses zoospores immediately after immersion of cut roots in a zoospore suspension (Fig. 1-B [A2]) (43). This repulsion soon gives way to accumulation as seen in (A1).

Sometimes zoospores form a sheath completely surrounding the root with the highest concentration just behind the root tip (Fig. 1-B [B]). On both pea and soybean roots, zoospores of *Pythium aphanidermatum* display pattern A, whereas those of *Phytophthora megasperma* var. *sojae* display pattern B. This difference is most simply explained on the basis of differences in degree of response of the two fungi to the stimulatory factor(s) exuding from the roots of the two plants (19).

With some fungi, accumulation occurs only on the sites of wounds (Fig. 1-B [C]); e.g., *Pythium aphanidermatum* on chrysanthemum (31). This suggests that root exudates from different plants may differ in concentration and/or composition of stimulatory substances.

In Fig. 1-B (D), no attraction or accumulation occurs: (i) zoospores encyst by chance on or near to host roots and origin, and orientation of germ tubes is at random as observed with *Pythium ultimum* and *Phytophthora cactorum* in the presence of peach roots (35). The reaction of zoospores of these fungi to wounds is not known. (ii) Lack of attraction and accumulation of zoospores of *P. cinnamomi* to roots of nonhost plants, contrasting with an A response to host (avocado) roots, may possibly be associated with the presence of inhibitors (Zentmyer, *unpublished data*).

In addition to the responses described above, different results have been obtained in separate tests using the same plant and fungus. Thus, Dukes & Apple (10) observed response C with *P. parasitica* var. *nicotianae* zoospores on tobacco roots, whereas Troutman & Wills (52) described a typical (A) response. Such differences as these may be related to differences in root exudation from plants grown under different environmental conditions.

Nature of the stimulus.—The most widely accepted explanation of zoospore accumulation is that it is induced by one or more chemical compounds present in root exudates. The sites of maximum accumulation, immediately behind root tips and over wounds, are those of maximum exudation (38, 46) and, as described above, zoospores respond in typical fashion to root

exudates or extracts diffusing from capillary root models. Several workers (19, 36, 44, 53) have shown that the active factor(s) are dialyzable and heat-stable. In detailed studies designed to identify the substance(s) involved, two general approaches have been followed: (i) examination of zoospore responses to known compounds likely to occur in roots and exudates; and (ii) fractionation of root exudates, identification and testing of the products, and subsequent tests with pure compounds.

The most common response-inducing substances are amino acids and sugars (Table 2). Rai & Strobel (39) and Chang-Ho & Hickman (*unpublished data*) found that the stimulating effect of exudates always exceeded that of recombined fractions, even though concentrations of the fractions were comparable with their concentration in natural materials. This may be explained by loss of other active factors (or failure to identify them) during fractionation. That we are still far from understanding the nature of zoospore response to plant roots is emphasized also by differences in response of particular fungi to different plants. For example, of the compounds occurring in sugarbeet roots, gluconic acid is the most effective in attracting zoospores of *Aphanomyces cochlioides* (39). However, though zoospores of this fungus respond very readily to pea roots and to pea root exudates, Chang-Ho & Hickman (*unpublished data*) were unable to find gluconic acid either in extracts or exudates. Zoospores of *Phytophthora cinnamomi* accumulate on host plant roots, including avocado, in response to their exudates, but are rarely attracted to roots of nonhosts (55, 56, and Zentmyer, *unpublished data*). This fungus displays strong and consistent chemotaxis to glutamic and aspartic acids which are present, however, in roots of both host and nonhost plants. This unusual example of chemotactic specificity may be associated with the existence of an inhibitor, as Zentmyer suggests, in roots of nonhosts. Alternatively, it might be a substance specific to host plants and inhibitory to nonpathogens. Zentmyer has suggested also that the balance of particular compounds in roots, e.g., amino acids, may contribute to the differences in root:zoospore relationships that have been observed with different plants.

TABLE 2. Substances inducing zoospore accumulation

Species	Substances inducing response	Reference
Aphanomyces cochlioides	Gluconic acid Glucose, fructose	Rai & Strobel (39)
Phytophthora capsici	Dilute solutions of hydrochloric acid, sulphuric acid, nitric acid, aspartic acid, glutamic acid Maltose, sucrose, glucose	Katsura & Hosomi (23)
P. citrophthora and *P. palmivora*	Glutamic acid, aspartic acid, asparagine, glutamine, glycine, methionine, histidine, glutaric acid Several sugars	Zentmyer (56)
P. cinnamomi	Glutamic acid, aspartic acid 4-aminobutyric acid	Zentmyer (56, & *unpublished data*)
P. parasitica var. *nicotianae*	Casamino acid Sucrose, dextrose, fructose, rhamnose, maltose	Dukes & Apple (10)
Pythium aphanidermatum	Glutamic acid Mixture of certain sugars and certain amino acids Ammonium glutamate	Royle & Hickman (44)
P. aphanidermatum	Glutamine, serine Sucrose, glucose	Chang-Ho and Hickman (*unpublished data*)
P. aphanidermatum and *P. debaryanum*	Glutamic acid	Spencer & Cooper (49)

It should be emphasized that we are dealing with a series of responses; attraction, trapping, etc. While these may all be stimulated by one or a particular group of compounds, it may be possible that each separate response is controlled by a different factor. For example, Rai & Strobel (39) found that the amino-acid fraction of root exudate was not chemotactically effective, but promoted cyst germination and germ tube growth. In contrast, the organic acid and sugar fractions attracted zoospores but had no effect on cyst germination and subsequent growth. In addition to this complementation effect, there is also evidence that synergism may play a part in the accumulation phenomenon. Zoospores of *Pythium aphanidermatum* were only weakly attracted to amino acids and sugars when these were presented as separate mixtures, whereas combinations of the two groups of compounds resulted in an accumulation response resembling that to root exudates (44). Chang-Ho and I have recently obtained a similar result (*unpublished data*).

During the last 10 years or so, a great deal of information has been gathered on zoospore accumulation. Experiments have been carried out under a variety of conditions, with plants in different stages of development. Perhaps this is an appropriate time to remind ourselves of the fact that composition and quantity of root exudate changes with the physiological condition of the plant (25, 41, 42). It seems clear, now, that further studies will require more controlled conditions. Moreover, they should include more than one fungus: plant combination so that more valid comparisons can be made.

Ho & Hickman (19) used ionic resins to examine the influence of individual ions rather than of whole compounds on zoospore behavior. Particles of acetate, formate, chloride, and ammonium resins introduced into zoospore suspensions of *Phytophthora megasperma* var. *sojae* were without effect. Around particles of hydroxide and hydrogen resins, zoospores accumulated and encysted in a broad ring a short distance away, forming a halo. Attraction was not involved. The cysts surrounding hydroxide-resin particles did not germinate, but almost all of those surrounding the hydrogen-resin particles germinated. Moreover, the germ tubes emerged

from the cysts on the side facing the particles, and grew toward them. Thus, all the responses shown by zoospores to roots were elicited except attraction and encystment on the surface. The latter may reflect a concentration effect. The simplest explanation of these events is that H^+ ions may be involved in the series of responses shown by zoospores to roots.

Zoospores of *P. parasitica* var. *nicotianae* responded to the passage of a weak electric current through water or dilute NaCl by migrating to the cathode (52). The region of elongation immediately behind the root tip bears a negative surface charge (11, 47), suggesting that electrotaxis rather than chemotaxis may be responsible for zoospore responses to roots (52). Subsequent experiments with other fungi have failed to reproduce the effect. These include experiments by Royle (*unpublished data*) with *Pythium aphanidermatum*, with *P. aphanidermatum* and *P. debaryanum* (49), and with *Phytophthora megasperma* var. *sojae* (19). Royle and Ho & Hickman observed accumulation of cysts in the region of the cathode, but this was due only to encystment of zoospores that happened to be in that area; no attraction of zoospores toward the cathode was observed. The cysts of *Pythium aphanidermatum* in Royle's experiments failed to germinate within the period required for germination in water or in exudate. Young zoospores of *Phytophthora megasperma* var. *sojae* were often unaffected by the current, although they responded readily to the presence of roots. Older zoospores encysted around the cathode, but percentage germination was low and there was no orientation of germ tubes toward this electrode. Response of zoospores to electric current thus differs from that to roots and root exudates. While zoospores of *P. capsici* responded to an electric current, the direction of their movement, to cathode or anode, depended on the nature of the solution in which they were suspended (24). In deionized water they collected around the cathode; in weak solutions of sodium salts of various organic and amino acids, at the anode; and around the cathode in solutions of some mono- and disaccharides. Miyata et al. (36) commented on the ambiguity of these results, and could not reconcile them with the electrotaxis theory of zoospore accumulation.

Significance of zoospore accumulation.—Turning to more practical considerations of root:zoospore relationships, two questions arise. Is accumulation essential for disease development and, second, does the phenomenon occur in soil?

Zoospore accumulation appears to be important for some fungi but not for others. Thus, MacWithey (32) and Kraft et al. (28) found aggregation of zoospores to be necessary for disease establishment by, respectively, *Aphanomyces cochlioides* on sugarbeet and *Pythium aphanidermatum* on bentgrass. On the other hand, in tests by Spencer & Cooper (49), single zoospore infection by *P. aphanidermatum* and by *P. debaryanum* resulted in extensive colonization of cotton roots held under moist conditions. It should be emphasized that these experiments were not carried out in soil.

Bhalla (3) demonstrated a relationship between temp and number of zoospores required to initiate progressive root rot in 50% of pea seedlings (ED_{50}) growing in vermiculite. Average ED_{50} values at 28, 24, and 20 C were 39 (as low as 3), 710, and 985 zoospores, while at 16 C, infection did not reach 50% at any concentration of zoospores used. In natural soil, ED_{50} values at 28, 24, and 20 C showed a similar trend but were somewhat higher, 282, 537, and 2400 zoospores, respectively.

Recent unpublished experiments by Mehrotra demonstrated that zoospore accumulation on roots occurs in soil. In one type of experiment, safflower seedlings were grown in nonsterile soil in tubes made from cellulose acetate sheet. The tubes were held at an angle so that the growing radicle was in contact with one side of the tube membrane. After several days, when the radicles had grown to a length of 5-6 cm, each tube was surrounded by nonsterile soil in a larger glass cylinder closed at one end by a perforated membrane. Cylinders containing plant tubes were placed for 2.5 hr in a zoospore suspension of *Phytophthora drechsleri*. Afterward the plant tubes were carefully removed and adhering soil was gently dislodged in water. A strip of the cellulose acetate membrane covering the root was then cut out and stained with cotton blue. The position of the root was marked by a band of many thousands of cysts which extended from near the root tip to the root hair zone (Fig. 2-B).

A more stringent test of the capacity of zoospores to reach and accumulate on roots was carried out by adding zoospore suspensions to the surface of moist soil in small pots containing safflower seedlings growing in a circle near the periphery of the pots. Care was taken to avoid wetting the seedlings directly with zoospore suspension by applying it through a small glass collar pressed to a depth of a few mm into the soil. Approximately 22 hr after adding 20 ml suspension to the soil surface, cysts were observed near the ends of the roots (Fig. 2-C) after very careful removal of the plants from the pots and staining with a fluorescent dye (Calcofluor White RW). As seen in in vitro tests, the germ tubes of zoospores that had encysted a short distance away from the root were clearly orientated toward it.

In conclusion, we are beginning to explore another important aspect of zoospore biology in soil. How long do zoospores, motile or encysted, remain effective as inoculum units? Our main approach to this question

TABLE 3. Percentage root infection of *Carthamus tinctorius* 4 days after perfusion of soil column in which zoospores and cysts of *Phytophthora drechsleri* were previously held for various periods

Period in days inoculum held in soil column before perfusion begun	Zoospore inoculum		Cyst inoculum	
	Tap root	Lateral roots	Tap root	Lateral roots
0	100	100	100	60
1	100	88	50	38
2	100	76	50	28
3	50	44	25	26
6	50	48	50	30
15	12	0	0	0

was prompted by Atkinson's (1) recent success in the use of a modification of the soil perfusion technique (30) to study infection of *Chamaecyparis lawsoniana* by *P. lateralis*. A diagram of the apparatus as used in our laboratory is shown in Fig. 1-C, and results obtained by its use confirm the potential of these fragile propagules as inoculum units in soil (Table 3). Some at least remain viable for up to 15 days. Zoospores appear to be more successful than cysts as inoculum probably as a result of their motility.

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