# Electrotactic Response of Zoospores of Seven Species of Phytophthora

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ABSTRACT

Electrotaxis of zoospores of seven species of *Phytophthora* (*P. cactorum, P. capsici, P. cinnamomi, P. citrophthora, P. megasperma* var. *sojae, P. palmivora,* and *P. parasitica*) was studied under standardized conditions. In deionized water, zoospores exhibited three basic types of electrotactic response to currents of 0-5 $\mu$A. Type-A (attraction) was observed at the anode at currents usually of $<0.5$ $\mu$A ($<1.2$ V/cm). Zoospores exhibited an active, oriented attraction to the anode, followed by encystment and germination. No positive orientation of the germ tubes to the electrode occurred. Type-B (repulsion) was observed at the anode at currents usually $>0.5$ $\mu$A. Attraction of zoospores at the boundary to the electrode was active and oriented as in the case of Type-A. Type-C (immoblization) occurred at the cathode at currents usually $>0.5$ $\mu$A. Responses of zoospores ranged from decreasing swimming velocity and rotation to cessation of motion and bursting. All three types of electrotactic responses followed the equipotential lines very closely when the current was flowing.

No significant difference was observed in the basic patterns of electrotactic responses among different species of *Phytophthora*. The presence of various organic acids, sugars, metabolic inhibitors and surface-active agents in the zoospore suspension did not alter or prevent electrotaxis at chemical concentrations that did not affect motility of zoospores. Basic patterns of electrotaxis did not change among zoospores of various intermediate physiological stages before encystment. These results suggest that there might not be a direct relationship between electrotaxis and metabolic activity of the zoospores.

Microelectrophoresis and staining behavior of both motile and encysted zoospores of *Phytophthora* indicated that they were negatively charged. Electrokinetic properties of zoospores suggest the presence of a preponderance of acidic surface groups.

In nature, along with many complex factors of soil, tactic response of zoospores (both chemotaxis and electrotaxis) may serve as an important way to cause accumulation of zoospores on plant roots.

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The fact that motile organisms can respond to an electric stimulus has been known since the end of the 19th century (21, 29). Most of the early reports were concerned with various members of infusoria (e.g., *Paramecium*). Among these groups of organisms, cathodic electrotaxis (i.e., movement toward cathode) appears to be more common than anodic electrotaxis (i.e., movement toward anode).

Brokaw (4) demonstrated that when an electric field was established in a suspension of bracken spermatozoids containing bimalate or other chemotactically active ions, the spermatozoids oriented and swam toward the anode. The electrotaxis of fungal zoospores had received relatively little attention until recently. Troutman and Wills (28) reported that when zoospores of *Phytophthora parasitica* var. *nicotianae* were subjected to an electric current of 10-40 $\mu$A in deionized water or dilute NaCl solution, they migrate toward the cathode. They correlated this observation with zoospore accumulation on roots and concluded that zoospores were directed to the root surface by weak electric currents and attached on the root surface by electrostatic forces.

Using a fixed potential gradient of 2 V/cm, Katsura et al. (12, 13) showed that in deionized water and various sugar solutions, zoospores of *Phytophthora capsici* moved toward and accumulated at the cathode. However, in the presence of $10^{-2}$M of various organic acid solutions, zoospores aggregated markedly at the anode with a repulsion zone forming around the anode at more dilute concentrations of the organic acid solutions. Ho and Hickman (10) however, noted no active attraction in an electric field of zoospores of *Phytophthora megasperma* var. *sojae* toward either pole. Rather, zoospores were trapped and rapidly encysted around the cathode in response to a current of 0.1 - 0.8 $\mu$A, with a subsequent suppression of cyst germination.

Because of the apparent lack of agreement among the limited number of reports on electrotaxis of fungal zoospores, this investigation was initiated in order to provide a more comprehensive study of the electrotactic response of zoospores of seven species of *Phytophthora*. A brief report of this work has been published (14).

MATERIALS AND METHODS.—Nine isolates representing seven species of *Phytophthora* (Table 1) from the culture collection of the Department of Plant Pathology of the University of California at Riverside were used. In addition, two auxotrophic mutants of *P. capsici* [L-10 (arg-), and P-505-6(met-)] previously isolated by Castro (5, 6) and Timmer (25, 26) were also included in the study. The methods of culturing *Phytophthora* and obtaining zoospores have been described in a previous paper on chemotaxis (15).

All electrotaxis experiments were carried out using an observation cell similar to that used for chemotaxis (15). Two platinum electrodes (0.13 mm in diam) were introduced, one from each side of the specimen chamber and maintained at 1 cm distance between the two tips. A portion of the apparatus for electrotaxis study is shown in Fig. 1.

A 6-V battery was used for a current source and the output was controlled through a series of precision resistors to give a range of current intensity from 0.01 to 50 microamperes ($\mu$A), which in turn was amplified and recorded on a recorder. A reversing switch was introduced for polarity reversal. The potential gradient between the two electrodes was measured by a voltmeter

TABLE 1. Sources of species and isolates of *Phytophthora* used in this study

| *Phytophthora* spp. | Isolate number | Host | Geographic origin |
|---|---|---|---|
| *P. cactorum* (Leb. & Cohn) Schroet. | P-472 | Pear | California |
| *P. capsici* Leonian | P-504 | Pepper | Mexico |
| *P. cinnamomi* Rands | SB-216-1 | Avocado | California |
| *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian | P-316 | Lemon | Australia |
| *P. palmivora* (Butl.) Butl. | P-255 | Cacao | Costa Rica |
| *P. parasitica* Dast. (*P. nicotianae* var. *parasitica* Dast.) | P-480 | Citrus | California |
| *P. parasitica* (Dast.) var. *nicotianae* (B. de Haan) Tucker (*P. nicotianae* var. *nicotianae*) | P-580 | Tobacco | Kentucky |
| *P. megasperma*(Drechs.) var. *sojae* Hilde. | P-405 race 1 | Soybean | Mississippi |
| | P-406 race 2 | Soybean | Mississippi |

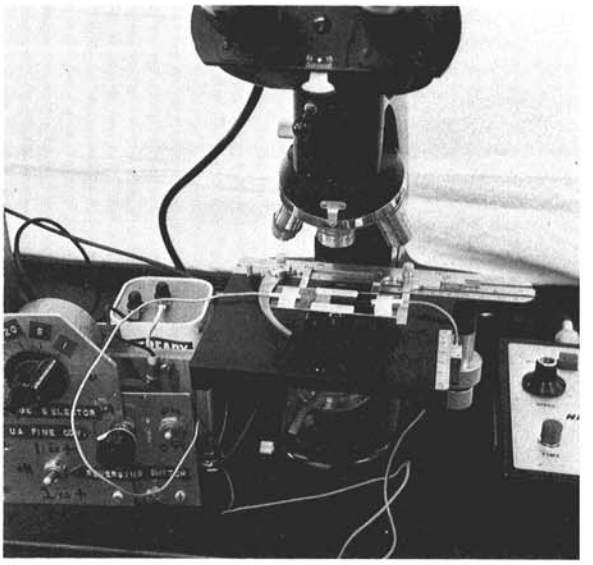

**Fig. 1.** Cell used for electrotaxis study, with two platinum electrodes, in place on microscope stage. The two platinum electrodes are attached to the current generating unit at the left.

(Silver Model 900). A fully automatic Nikon Microflex AFM with camera attachment was mounted on a Leitz microscope for photomicrography. When dark field photomicrography was necessary, an electronic flash (Point Source Strobex Model 136, Chadwick-Helmuth Co., California) was attached and synchronized to illuminate the field.

Electrotaxis was observed under ×56 magnification. An ocular micrometer (100 divisions) was used for the measurement of the zone formed by different types of electrotactic response. All measurements were made under ×56 magnification at a fixed reference point on the electrode 240 $\mu$m from the tip. The vertical distance at that point of the electrotactic response was recorded and expressed as a unit of electrotactic response. Under ×56 magnification, each unit of electrotactic response is equivalent to 24 $\mu$m. A higher magnification (×200) was frequently used for observation of behavior of individual zoospores near the electrode.

Unless otherwise stated, electrotaxis experiments were carried out with the zoospores suspended in deionized water. A stepwise increment of 0.1 $\mu$A current was applied successively at 1-min intervals over a range of 0 - 5.0 $\mu$A current intensity. The unit of electrotactic response was measured at each increment until it reached 100. To avoid any possible difference in the two platinum electrodes, the polarity was frequently reversed, with each of the experiments repeated at least twice. The concentration of zoospores was maintained at 4-5 × $10^5$/ml.

*Microelectrophoresis of zoospores.*—The cell for the microelectrophoresis study was made from a modified glass slide. The zoospore suspension was applied into an electrophoretic chamber of 30 × 1 × 1 mm. Two reversible Ag/AgCl electrodes, each of 50 $\mu$m diam maintained at 3 cm distance were used. Two 45-V batteries in series (90 V) were employed and the ampere output was measured with an ammeter (Simpson Model 270). Zoospores were suspended either in deionized water or in various pH buffer solutions of the same ionic strength (1: 0.05) (18). Conductivity of the suspension was measured on a conductivity meter (Type CMD 2d, Radiometer, Copenhagen). Movement of zoospores upon introduction of the current was timed with a stopwatch (precision 0.1 sec) over a distance of 214 $\mu$m in both directions (with current reversal). Electrophoretic mobility was calculated according to the method of Gittens and James (8). Each mean mobility was obtained from at least 10 observations. The electrophoresis experiments were carried out at room temperature (24 ± 2 C).

RESULTS.—*Types and patterns of electrotactic response.*—On the basis of numerous microscopic observations in which the behavior and motility of individual zoospores were carefully analyzed, the following three types and patterns of electrotactic response were recognized for zoospores of all species of *Phytophthora* studies (Fig. 2):

—(i).—Type-A is an accumulation of zoospores due to attraction. It occurred as a rule at the anode at a low current intensity (usually <0.5 $\mu$A). Zoospores remained actively motile as they approached the electrode. Within the zone of influence of the electric field, they became very excited, accelerated their swimming velocity and oriented themselves actively toward the electrode. Eventually, the motion ceased and the zoospores encysted around the electrode. Germination of encysted zoospores occurred after a prolonged period of time. However, there was a lack of tropic orientation of the germ tubes toward the electrode.

—(ii).—Type-B is an accumulation of zoospores at the boundary adjacent to a zone of repulsion. It occurred also

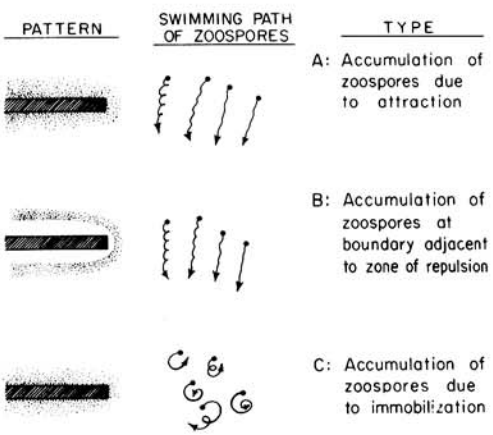


**Fig. 2.** Types of electrotactic response of zoospores of *Phytophthora*.

at the anode, but at a relatively higher current intensity (usually >0.5 μA) than that for Type-A. A distinct vacant area (a repulsion zone) was formed between the zoospore and the electrode with the zoospores accumulating at the boundary. The clear zone was not formed by a negative taxis of zoospores, rather the zoospores appeared to orient themselves actively toward the electrode, meeting a boundary which deterred them from approaching any nearer, they then turned around for a short distance and repeated the forward motion toward the electrode. Encystment of zoospores eventually occurred and germination was observed as in the case of Type-A.

—(iii).—Type-C is an accumulation of zoospores due to immobilization. As a rule, this was observed at the cathode. The zoospores appeared to be arrested, trapped, or immobilized inside a zone of influence of the electric field. This was shown first by a decrease in their swimming velocity, followed by rotation of the zoospore body and finally cessation of the motion. Subsequent bursting of the zoospores was frequently observed. There was little or no germination in the remaining intact zoospores.

Figure 3 shows the three types (A, B, and C) of electrotactic response as exhibited by zoospores of *P. palmivora* in deionized water. Dark-field tracing photomicrography (2-sec exposure) was taken to illustrate the continuous swimming paths of zoospores (shown as white traces in Fig. 3). Patterns of zoospore accumulation can be distinguished from the pictures.

Depending on the intensity of the current, zoospores of all the species of *Phytophthora* studied exhibited these three basic types of electrotactic response. The dimension of the zones of attraction (as in Type-A), repulsion (as in Type-B) or immobilization (as in Type-C) appeared to be a function of the current intensity.

*Electrotactic response of zoospores of several species of Phytophthora.*—Nine isolates representing seven species of *Phytophthora* (*P. cactorum, P. cinnamomi, P. citrophthora, P. palmivora, P. capsici, P. parasitica,* and *P. megasperma* var. *sojae*) and two auxotrophic mutants of *P. capsici* were included in this study. The results, plotted as units of electrotactic response vs. current intensity, are presented in Figs. 4-14.

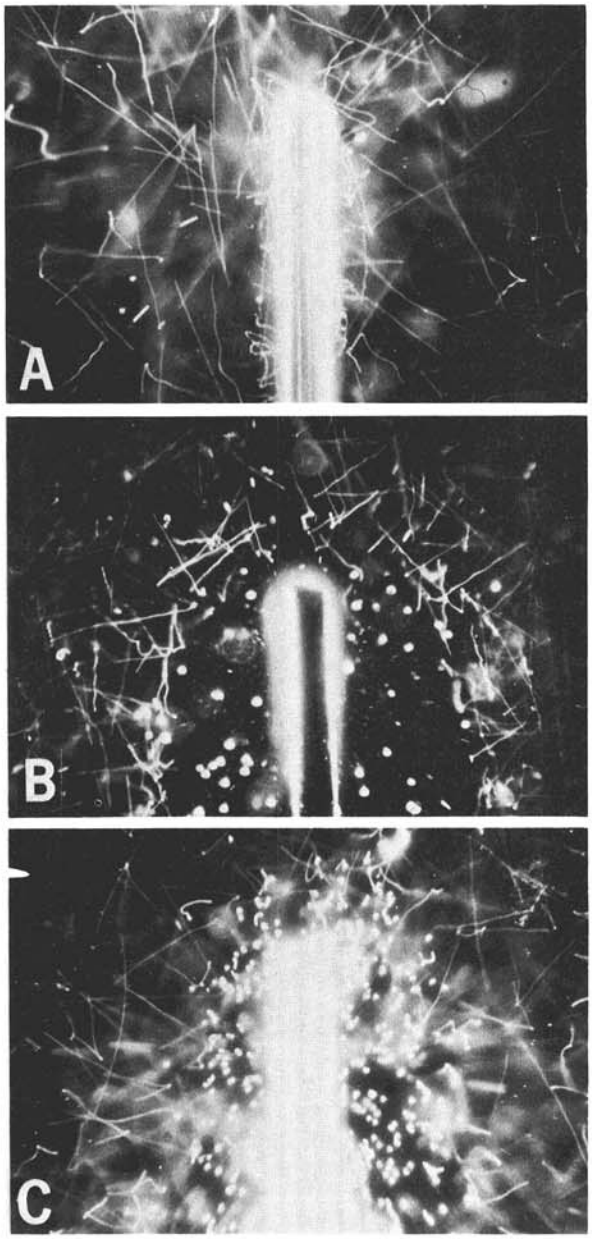


**Fig. 3-A, B, C.** Types of electrotactic response of zoospores of *Phytophthora palmivora* in deionized water. Traces produced by movement of zoospores were taken with 2-sec exposures under dark field illumination. **A)** Type-A response, showing accumulation of zoospores due to active attraction at the anode at 0.3 μA; **B)** Type-B response, showing accumulation of zoospores at the boundary adjacent to zone of repulsion at the anode at 1.0 μA; **C)** Type-C response, showing accumulation of zoospores due to immobilization at the cathode at 1.0 μA.

Before any current was applied, zoospores were observed swimming freely at random in the specimen chamber with no reaction to the electrodes. As soon as the current was turned on, zoospores near the electrodes began to respond. At a current below 0.5 μA (or <1.2 V/cm), zoospores exhibited Type-A response toward the

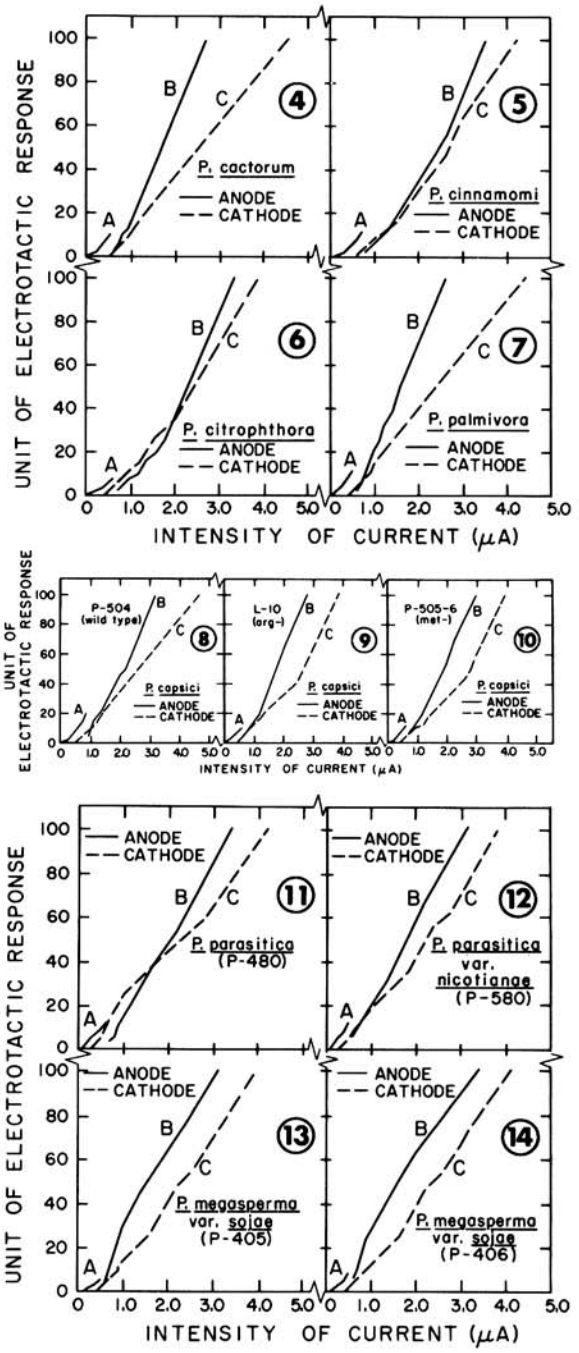


**Fig. 4-14.** Electrotactic response of zoospores of seven species of *Phytophthora* to various current intensities (d.c.) in deionized water. Letter A inidcates accumulation of zoospores due to attraction (Type A); B indicates accumulation of zoospores in boundary adjacent to zone of repulsion (Type B); C indicates accumulation of zoospores due to immobilization (Type C). Zones of attraction (as in A), repulsion (as in B) and immobilization (as in C) were measured with an ocular micrometer under ×56 magnification and expressed as unit of electrotactic response. Each unit is equivalent to 24 μm. In general, Type A response occurred at <0.5 μA and Type B response occurred at the anode at >0.5 μA while Type C response occurred at the cathode at >0.5 μA.

anode (i.e., a positively oriented attraction). A repulsion zone (Type-B) was detected at the anode generally at current above 0.5 μA. This response occurred when Type-A response ceased to appear, and the repulsion zone progressively increased with increasing current intensity.

In general, zoospores did not respond at the cathode until the current was increased to 0.5 μA and above. At such current intensities, a progressive immobilization of zoospores (Type-C response) was observed. The zone of immobilization increased with increasing current intensity. At a current above 2.0 μA, bursting of zoospores was common, beginning with zoospores located close to the electrode and progressing outward.

All three types (A, B, and C) of electrotactic response followed the equipotential lines very closely when the current was flowing. At a given current intensity, the time required for zoospores to achieve a maximal response was about 20-40 sec. Figure 15 shows the sequence of electrotactic response by zoospores of *P. palmivora* at the anode upon introduction of 0.5 μA current.

The patterns (or types) of electrotactic response by the two auxotrophic mutants (L-10 and P-505-6) of *P. capsici* (Fig. 9 and 10) were basically similar to those of the wild type (P-504) (Fig. 8). Zoospores of *P. parasitica, P. parasitica* var. *nicotianae* and *P. megasperma* var. *sojae* (Fig. 11-14) behaved similarly to other species of *Phytophthora* (Fig. 4-8) except for some differences in the degree of their responses. Overall, there appeared to be no basic difference in the patterns (or types) of electrotactic response among different species of *Phytophthora*.

*Effect of various organic acid and sugar solutions on electrotaxis.*—To determine whether the presence of

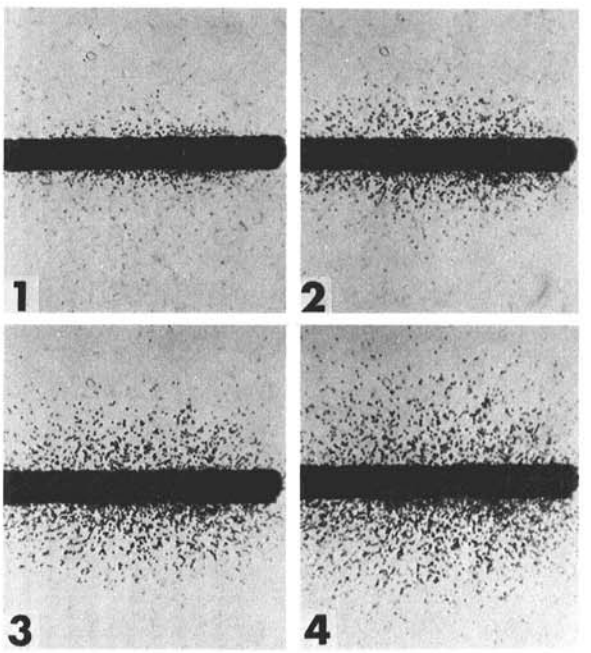


**Fig. 15.** Sequence of electrotactic response of zoospores of *Phytophthora palmivora* at the anode upon introduction of current (0.5 μA). A complete sequence of events (from 1-4) can be accomplished in 20-40 sec.

metabolites such as organic acids and sugars affect electrotaxis, six organic acids (cis-asconitic, citric, fumaric, α-ketoglutaric, malic, and succinic, all L-form), four monosaccharides (arabinose, ribose, glucose, and mannose, all D-form), and three disaccharides (lactose, maltose, and sucrose) were incorporated into zoospores suspensions at $10^{-3}$M (this concentration did not affect motility of zoospores), and the electrostatic response was studied. None of these chemicals affected or altered the basic patterns (or types) of electrotactic response of zoospores of *P. cactorum, P. capsici* and *P. palmivora* toward the respective electrode as compared with those in deionized water (controls). Actively oriented attraction of zoospores (Type-A response) was observed at the anode at 0.5 μA current. At currents of 1.0 μA and above, a repulsion zone was formed at the anode with actively swimming zoospores accumulating at its boundary (Type-B response). Immobilization of zoospores at the cathode (Type-C response) was observed at 1.0 μA and above.

*Electrotaxis in the presence of various antibiotics, metabolic inhibitors, and surface-active agents.*—The following compounds were incorporated into zoospore suspensions to determine whether they affect electrotaxis of zoospores: bacitracin, chloramphenicol, *p*-chloromercuribenzoate, cycloheximide, 2,4-dinitrophenol, EDTA·$Na_2$, filipin, iodoacetic acid, N-methylmaleimide, neomycin $SO_4$, nystatin, penicillin G, pimaricin, polymyxin, sodium azide, sodium barbital, sodium dodecyl $SO_4$, streptomycin $SO_4$, tetracycline HCl, Tween 80, urea and vancomycin HCl. At a concentration (the highest one selected from a series of 10-fold increments) which showed no adverse effect on motility of zoospores, none of the chemicals prevented or altered the basic patterns of electrotaxis of zoospores.

*Electrotaxis of zoospores at different stages before encystment.*—Complete encystment of zoospores can be induced within 1-2 min by subjecting the zoospore suspension to continuous mechanical agitation in a Vortex mixer (17, 27). To determine whether the possible physiological changes in zoospores at various intermediate stages before encystment affect their electrotactic response, zoospores were subjected to mechanical agitation in a Vortex mixer for various periods of time (0-40 sec). Immediately after the treatment, electrotaxis was studied at various current intensities. Zoospores exhibited electrotactic response only as long as the motility was retained. Also, there appeared to be no significant influence on the basic patterns of zoospore accumulation over a period of 0-40 sec of mechanical agitation. Types A and B response were observed toward the anode with the latter at 1.0 μA current or higher. Type-C response (immobilization of zoospores) was observed at the cathode at 0.5 μA current and above. A study of zoospores subjected to more than 40 sec of mechanical agitation was not conducted because most of the zoospores had encysted.

*Microelectrophoresis of Phytophthora zoospores.*—Since very little information was available concerning the nature of surface charge of zoospores of *Phytophthora,* such an investigation was conducted, using the microelectrophoresis technique. In an electric field of 90 Vdc, both motile and encysted zoospores of *P. cactorum, P. capsici, P. cinnamomi, P. citrophthora, P. palmivora, P. parasitica,* and *P. megasperma* var. *sojae* invariably moved toward the anode. When the polarity of the current was reversed, the movement of zoospores was also reversed, but always toward the new anode. This was especially evident in the case of motile zoospores. If the zoospores were moving away from the anode before the current was applied, they changed the direction and moved toward the anode once the current was introduced. This clearly indicated that both motile and encysted zoospores carried a net negative charge on their surfaces.

The electrophoretic mobility of zoospores of *P. cactorum, P. capsici, P. citrophthora* and *P. palmivora* was also studied in an attempt to determine the nature of the ionizable surface groups. Negative electrophoretic mobility was observed throughout the range of pH 2 to 10 for all four species of *Phytophthora*. The fact that no positive mobility was observed even at low pH suggests the presence of a preponderance of acidic surface groups.

*Staining behavior of zoospores.*—To provide additional evidence on the nature of the surface charge of zoospores, several biological stains were used in attempts to stain zoospores. These included both negative (anionic) stains: acid fuchsin, eosin, orange G, and Sudan IV; and positive (cationic) stains: basic fuchsin, crystal violet, fast green, neutral red, and safranin O. Regardless of the species of *Phytophthora* used, zoospores were readily stained by any of the positive stains but were not stained (or in some cases, only faintly stained after a prolonged period of time) by any of the negative stains. This staining characteristic supports the conclusion that zoospores carry a negative surface charge.

DISCUSSION.—There are discrepancies in the earlier reports on electrotaxis of fungal zoospores by various workers. Troutman and Wills (28) claimed that zoospores of *Phytophthora parasitica* var. *nicotianae* migrated actively toward the cathode in deionized water or dilute NaCl solution. This, coupled with the finding that zoospores did not stain with the negative stain eosin, led them to conclude that "spores (zoospores) and flagella probably possess a positive charge." However, the fact that "zoospores failed to stain with negative stain" should indicate that they are negatively charged since staining requires the interaction of opposite electrical charges between the stain and the object (2, 9). That eosin failed to stain zoospores has also been confirmed in the present study. In addition, we have tried a number of other negative (e.g., acid fuchsin, orange G, and Sudan IV) and positive stains (e.g., basic fuchsin, crystal violet, fast green, neutral red, and safranin O) and invariably only the positive stains readily stained the zoospores while negative stains failed to do so. This, together with the finding from the electrophoresis study (zoospores moved toward the anode in an electric field), supports the assumption that zoospores carry a negative charge.

Ho and Hickman (10) observed no active attraction of zoospores of *Phytophthora megasperma* var. *sojae* toward either anode or cathode in an electric field. However, they reported that zoospores were trapped and encysted rapidly around the cathode in response to currents of 0.1 - 0.8 μA, without stimulation of cyst germination or direct germ tube growth. This response is

similar to the Type-C response around the cathode observed in our study. Trapping, progressive immobilization of zoospores and lack of germination of the encysted zoospores are among some of the common features found in the Type-C electrotactic response. In addition, we also observed frequent bursting of zoospores around the cathode.

Using a fixed potential gradient of 2 V/cm, Katsura et al. (12) reported that in deionized water and sugar solutions, the zoospores of *P. capsici* swam toward and accumulated at the cathode. Regarding the behavior of the zoospores around the electrode, they stated: "The swimming velocity of zoospores decreased as they came up close to the cathode, moved around the electrode with turn and rotation and finally ceased the motion. A repulsion zone was quickly formed at the anode." This description of zoospore behavior around the electrodes agrees very well with our observation of a Type-C response at the cathode and of Type-B response at the anode at a comparable potential gradient (an equivalent of $1.4 \pm 0.2$ $\mu$A in our study). In $10^{-2}$M of various organic solutions (e.g., malate, malonate, succinate, glutamate, and aspartate), they reported that zoospores were markedly attracted to and aggregated at the anode, with a repulsion zone formed at lower concentrations of the solutes. This is similar to our findings at the anode in which a Type-A (attraction) response was observed at low current intensities ($<0.5$ $\mu$A) and a Type-B (formation of a repulsion zone) response at higher current intensities ($>0.5$ $\mu$A in various organic acid solutions as well as in deionized water). We, however, could not confirm the observation of Katsura et al. (12) in the formation of a repulsion zone around the cathode in various organic solutions. Instead, a Type-C (immobilization) response was observed around the cathode.

After careful analysis and comparison of our results with the previous results of others, we have come to the following conclusions:

—(i).—The discrepancies in the earlier reports on the electrotaxis of *Phytophthora* zoospores are not due to the use of different species of *Phytophthora,* since the present study has demonstrated no basic differences in the patterns of type of electrotactic response among several species of *Phytophthora* including those species tested by previous workers: *P. capsici, P. megasperma* var. *sojae* and *P. parasitica* var. *nicotianae.*

—(ii).—Perhaps Troutman and Wills (28) had observed an accumulation of zoospores at the cathode as a result of trapping and immobilization rather than active attraction. Unfortunately, since they did not specify the exact current used (only the range of current was given) when such an observation was made, it is difficult to compare their results with ours.

—(iii).—Although Ho and Hickman (10) failed to observe active migration of zoospores to either pole, they reported trapping and encystment of zoospores around the cathode; this is similar to Type-C response we observed at the cathode. Their failure to observe attraction at the anode might be due to unfavorable experimental conditions (e.g., unfavorable current intensity) or to different experimental conditions from those of our study.

—(iv).—The data of Katsura et al. (12) in general agree with ours. However, their observations were limited by the use of a fixed potential gradient throughout their study. Although their study did not distinguish three basic types of electrotactic response, their results did encompass the three types of electrotactic response we have found.

In the study of electrotaxis, as well as chemotaxis, it is important to differentiate types of response by carefully analyzing the behavior of individual zoospores under the microscope. Accumulation of zoospores may occur as a result of active attraction or trapping and immobilization which could be due to different mechanisms.

The advantage of using a range of controlled current intensities is that the behavior of zoospores can be scanned in an electric field over a wide spectrum of current intensities. If one judges electrotaxis by whether or not the zoospores have accumulated on the electrode, one could fail to observe a specific response at a certain current intensity. For instance, at a very high current intensity, one could easily overlook a response at the anode due to the formation of a large zone of repulsion which, in some cases, may extend beyond the range of a microscopic field even at a low magnification. Furthermore, it is not uncommon for a motile organism to alter its pattern of electrotactic response with different current intensity. Pearl (20) showed that with increasing current intensity *Paramecium* and *Chilomonas* changed from cathodic electrotaxis (moving toward cathode) to anodic electrotaxis (moving toward anode).

The following findings seem to indicate that active metabolism has no direct bearing on the electrotactic response of zoospores, though more definite experiments are needed to clarify this: (i) Additions of various organic acid and sugar solutions in the medium did not affect the basic patterns of electrotactic response toward the two electrodes. (ii) The presence of various metabolic inhibitors and surface-active agents at concentrations that did not inhibit the motility of zoospores neither prevented nor altered the basic patterns of electrotactic response. (iii) No apparent alteration in the basic patterns of electrotactic response toward the two electrodes was observed among the zoospores of various intermediate physiological stages before encystment.

Several hypotheses (1, 11, 19, 24) suggested that a change in membrane potential of motile organisms is involved either directly or indirectly in their tactic responses. Whether the electrical phenomenon is associated with tactic response of zoospores remains an interesting subject to be explored. Judging from the speed and precision of their tactic response, it is not inconceivable to assume the existence of a simple, well-coordinated "neurological system" in zoospores.

A common relationship seems to exist between chemotaxis and electrotaxis of zoospores. In chemotaxis, the positively charged (cationic) molecules were most effective in attracting zoospores (15) and in electrotaxis, the positive electrode (anode) exhibited active, oriented attraction for zoospores. It may be that positively charged particles are more effective in upsetting the membrane potential of zoospores than negatively charged particles (since zoospores possess a net negative surface charge). Related to this, Jeon and Bell (11) maintained that effective chemotactic agents for free-living amoebae are

positive polyions. Bingley and Thompson (3) noted that on applying electrical potentials to the rear of an amoeba cell, cytoplasmic streaming occurred in a direction away from the negative electrode and toward the positive electrode.

Two phenomenon reported by Troutman and Wills (28) in their electrotaxis study of zoospores of *P. parasitica* var. *nicotianae* were observed also in this study. Patterns of electrotactic response of *Phytophthora* zoospores followed the equipotential lines very closely when the current was flowing. Observations on the germinating zoospores in an electric field, revealed no conclusive evidence of tropic orientation of the germ tubes to the direction of the current flow. Possibly, in this case, tactic and tropic responses are due to different mechanisms.

On the relation of electrotaxis of zoospores to pathogenesis in nature, Troutman and Wills (28) noted that electrotaxis of zoospores could occur similarly in the rhizosphere of plants. Several studies have established the presence of weak currents around plant roots and the existence of areas of different surface charges (7, 22, 23). Furthermore, both cationic and anionic exchange properties of plant root surface have been demonstrated (30, 31). Conceivably, in nature, negatively charged zoospores can be attracted to the positive spots on the root surface, and by virtue of electrostatic forces, attach themselves on these surfaces. The total current around an actively growing plant root in $10^{-4}$N KCl at 25 C was found to be $3 \times 10^{-7}$A for bean (22) and $5\text{-}6 \times 10^{-7}$A for corn (16). As has been shown in the present study, such current intensities are, in fact, sufficient to initiate an electrotactic response for zoospores of *Phytophthora*. It is not unreasonable to assume that, in nature, both chemotaxis and electrotaxis play a contributing role in causing zoospore accumulation on plant roots. The interaction of other complex factors of soil should, of course, also be taken into consideration.

## LITERATURE CITED


1. AMBROSE, E. J., and J. A. FORRESTER. 1968. Electrical phenomena associated with cell movements. *In* Aspects of cell motility. Symp. Soc. Exp. Biol. No. 22:237-248.
2. BAKER, J. R. 1966. Cytological technique, the principles underlying routine methods (5th ed.). John Wiley and Sons, New York. 149 p.
3. BINGLEY, M. S., and C. M. THOMPSON. 1962. Bioelectric potentials in relation to movement in amoebae. J. Theor. Biol. 2:16-32.
4. BROKAW, C. J. 1958. Chemotaxis of bracken spermatozoids. Implications of electrochemical orientation. J. Exp. Biol. 35:197-212.
5. CASTRO, J. 1968. Genetical studies with the genus Phytophthora. Ph.D. Dissertation, Univ. Calif., Riverside. 115 p.
6. CASTRO, J., G. A. ZENTMYER, and W. L. BELSER. 1971. Induction of auxotrophic mutants in Phytophthora by ultraviolet light. Phytopathology 61:283-289.
7. FENSOM, D. S. 1959. The bio-electric potentials of plants and their functional significance. III. The production of continuous potentials across membranes in plant tissue by the circulation of the hydrogen ion. Can. J. Bot. 37:1003-1026.
8. GITTENS, G. J., and A. M. JAMES. 1960. An improved microelectrophoresis apparatus and technique for studying biological cell surfaces. Anal. Biochem. 1:478-485.
9. GURR, E. 1965. Rational use of dyes in biology and general staining methods. Williams & Wilkins Co. Press, Baltimore, Md. 422 p.
10. HO, H. H., and C. J. HICKMAN. 1967. Factors governing zoospore responses of Phytophthora megasperma var. sojae to plant roots. Can. J. Bot. 45:1983-1994.
11. JEON, K. W., and L. G. E. BELL. 1965. Chemotaxis in large, free-living amoebae. Exp. Cell Res. 38:536-555.
12. KATSURA, K., H. MASAGO, and Y. MIYATA. 1966. Movements of zoospores of Phytophthora capsici. I. Electrotaxis in some organic solutions. Ann. Phytopathol. Soc. Japan. 32:215-220.
13. KATSURA, K., and Y. MIYATA. 1971. Swimming behavior of Phytophthora capsici zoospores. Pages 107-128. *In* S. Akai, and S. Ouchi, ed. Morphological and biochemical events in plant-parasite interaction. Phytopathol. Soc. Japan Press, Tokyo.
14. KHEW, K. L., and G. A. ZENTMYER. 1971. The electrotactic response of zoospores of several species of Phytophthora fungi. Phytopathology 61:898 (Abstr.).
15. KHEW, K. L., and G. A. ZENTMYER. 1973. Chemotactic response of zoospores of five species of Phytophthora. Phytopathology 63:1511-1517.
16. LARSON, G. E. 1964. The bioelectric potentials surrounding the root of Zea mays. Ph.D. Dissertation, Rutgers—The State University, New Brunswick, N.J. 52 p.
17. MENYONGA, J. M., and P. H. TSAO. 1966. Production of zoospore suspension of Phytophthora parasitica. Phytopathology 56:359-360.
18. MILLER, G. L., and R. H. GOLDER. 1950. Buffers of pH 2 to 12 for use in electrophoresis. Arch. Biochem. 29:420-423.
19. NAITOH, Y., and R. ECKERT. 1969. Ionic mechanism controlling behavioral responses of Paramecium to mechanical stimulation. Science 164:963-965.
20. PEARL, R. 1901. Studies on electrotaxis. I. On the reactions of certain infusoria to the electric current. Amer. J. Physiol. 4:95-123.
21. PFEFFER, W. 1905. The physiology of plants. III. (Translated by A. J. Ewart, 2nd ed.) Clarendon Press, Oxford, England. 451 p.
22. SCOTT, B. I. H., and D. W. MARTIN. 1962. Bioelectric fields of bean roots and their relation to salt accumulation. Aust. J. Biol. Sci. 15:83-100.
23. SCOTT, B. I. H., A. L. MC AULAY, and P. JEYES. 1955. Correlation between the electric current generated by a bean root growing in water and the rate of elongation of the root. Aust. J. Biol. Sci. 8:36-46.
24. SMITH, J. L., and R. N. DOETSCH. 1969. Studies on negative chemotaxis and survival value of motility in Pseudomonas fluorescens. J. Gen. Microbiol. 55:379-391.
25. TIMMER, L. W. 1969. The inheritance of nutritional deficiencies, mating type, streptomycin resistance, and pigment-producing ability in Phytophthora capsici and P. drechsleri. Ph.D. Dissertation, Univ. Calif., Riverside. 84 p.
26. TIMMER, L. W., J. CASTRO, D. C. ERWIN, W. L. BELSER, and G. A. ZENTMYER. 1970. Genetic evidence for zygotic meiosis in Phytophthora capsici. Amer. Jour. Bot. 57:1211-1218.
27. TOKUNAGA, J. 1969. Fine structure and chemical composition of the cyst and hyphal walls of Phytophthora palmivora. Ph.D. Dissertation, Univ. Calif., Riverside. 97 p.
28. TROUTMAN, J. L., and W. H. WILLS. 1964. Electrotaxis of Phytophthora parasitica zoospores and its possible

role in infection of tobacco by the fungus. Phytopathology 54:225-228.
29. UMRATH, K. 1959. Galvanotaxis. Pages 164-167. *In* W. Ruthland, ed. Handbuch der Pflanzenphysiologie 17. Springer, Berlin.
30. WILLIAMS, D. E. 1962. Anion-exchange properties of plant root surfaces. Science 138:153-154.
31. WILLIAMS, D. E., and N. T. COLEMAN. 1950. Cation exchange properties of plant root surfaces. Plant Soil 2:243-256.