Mechanism of Electrotaxis of Zoospores of Phytopathogenic Fungi

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ABSTRACT

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Plant roots generate weak electrical fields in the rhizosphere that may stimulate electrotactic swimming of zoospores of plant pathogenic fungi. Here we show that in vitro electrotaxis of zoospores of *Pythium aphanidermatum* was cathodic, whereas those of two other *Pythium* species and *Phytophthora palmivora* were anodic. Electrotaxis occurred in electrical fields comparable in magnitude to those generated by plant roots. Electrical fields of a physiological magnitude had little effect on the velocity of swimming but increased the turning frequency of the zoospores of *P. palmivora* over threefold. Reagents that affected calcium-ion transport or calmodulin function had an apparent effect on electrotaxis, but most also induced premature encystment or cell lysis. The surface charge of the posterior flagellum of an anodotactic zoospore of *P. palmivora* was positive, whereas the anterior flagellum was relatively electronegative. In contrast, the posterior flagellum of a cathodotactic zoospore of *Pythium aphanidermatum* was negatively charged, and the anterior flagellum was relatively electropositive. Results suggest that electrotactic swimming of zoospores is mediated by the combined effects of modulation of the turning frequency and electrophoretic orientation of the zoospore in the electrical field. Electrotaxis may be used, in conjunction with chemotaxis, to identify and locate the plant root regions most susceptible to infection.

Additional keywords: electrochemistry, root infection.

Phytophthora and *Pythium* species are responsible for many plant diseases of commercially important crops. These oomycetous fungi are dispersed primarly by asexual, flagellated zoospores released into water films and are attracted to the susceptible regions of plants. Zoospores have a limited time in which to contact a viable host that will enable them to complete their life cycle. Evolution of tactic responses by zoospores to host roots has conferred selective advantages to these fungi; tactic responses include directed movements in chemical gradients (chemotaxis), in water currents (rheotaxis), due to gravity (geotaxis), and in electrical fields (electrotaxis) (7,8,12). Chemotaxis enables zoospores to target plant roots by swimming toward regions of nutrient exudation, such as the root apex and wound sites (26,28,29). Rheotaxis and geotaxis are advantageous because the zoospore population stays in the aerobic surface layers of soil (7,8,28). The electrotactic response of zoospores has received comparatively little attention but may act synergistically with chemotaxis in facilitating host location (23,27,30,46).

Plant roots generate electrical fields in the rhizosphere as a consequence of spatial heterogeneities in electrogenic ion-transport systems in the root (2,35,47). In the species investigated to date, the current flow is carried mainly by protons. The shape of the electrochemical profile of a root varies in different plant species (17,32) and is influenced markedly by endogenous and exogenous factors, including plant-growth regulators, soil acidity, salinity, matric potential, and source of nitrogen (17,32,33,35,39). In most cases, however, positive electrical current enters the meristematic tissue and zone of cell elongation and exits basipetally in the mature tissue (32). Inward currents also are found at sites of wounds and emerging lateral roots (24,34).

The sites at which zoospores infect plant roots are electrically active. Roots generate electrical fields in the order of 1–100 mV cm^{-1} (17). The first reports of electrotaxis were based on intense electrical fields that were at least an order of magnitude greater than this (23,27,30,46). Furthermore, interpretation of these reports is complicated by possible experimental artifacts arising from the use of bare-wire electrodes that generate toxic electrode products and rapid changes in the ambient pH value adjacent to the electrodes (36). Recently, we showed that zoospores of *Phytophthora palmivora* (E.J. Butler) E.J. Butler exhibit electrotaxis in electrical fields in vitro that are comparable in strength to the electrical fields measured at the root surface (36). In the study (36), we used a chamber with agarose bridges separating the electrodes and zoospores to protect the cells from the products of electrolysis. Here we describe the use of this system to extend our studies of electrotaxis to examine the zoospores of three *Pythium* species and to determine the mechanism of electrotaxis in *P. palmivora*.

MATERIALS AND METHODS

Preparation of zoospores. *P. palmivora* strain P6390 was supplied by M. D. Coffey (University of California, Riverside) and grown under license from the Scottish Ministry of Fisheries and Food (license PH/26/1992). Cultures were grown, and zoospores were harvested in 2 mM sodium phosphate buffer at pH 7.2 (36). *Pythium* cultures were supplied by J. W. Deacon and S. Donaldson (University of Edinburgh, Scotland, UK). Zoospores were prepared from cultures grown on V8 medium (41). Agar blocks, 2 × 8 cm, were cut from a mature colony of either *Pythium aphanidermatum* (Edson) Fitzp., *Pythium catenulatum* Matthews, or *Pythium dissotocum* Drechs. and were immersed overnight in 15 ml of 2 mM sodium phosphate buffer at pH 7.2. Zoospore densities $>2 \times 10^5$ zoospores per milliliter were typically obtained by this method.

Measurement of electrotaxis. Electrotactic responses were compared with the method and apparatus of Morris et al (36). Zoospores (3×10^5 zoospores per milliliter in sodium phosphate buffer at pH 7.2) were placed in a chamber protected from platinum electrodes by agarose bridges and exposed to an electrical field for 60 min. At the end of an experiment, the chamber was physically partitioned into three sections, and the concentration of zoospores was determined in each section after fixing them with 0.2 ml of fixatives (0.4% [w/v] paraformaldehyde and 0.04% gluteraldehyde [v/v] in 10 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] buffer at pH 7.2).

The extent of electrotaxis was determined by a tactic response quotient (TRQ):

$$TRQ = (A - C)/(A + C + 2M)$$

for which A, M, and C are the densities of zoospores per square millimeter at the anode, center, and cathode of the chamber, respectively. The expression described the distribution pattern across an electrotaxis chamber, so the anode/cathode bias also was related to the number of zoospores at the center. Values between 0 and 1 indicated an anodic response, and those between 0 and −1 indicated a cathodic response. A *TRQ* value at or near zero indicated a reduction in the net accumulation of spores at either end of the chamber and, thus, no electrotaxis. Fields were 50 mV or 500 V cm^{-1}. The former represented a field comparable to that found in the rhizosphere. The higher field was tested to assess the inhibitory effects of a range of compounds in fields that normally saturated the electrotactic response. *TRQ* values were calculated from at least 10 replicates.

Measurement of turning frequency and direction of swimming. Zoospores of *P. palmivora* swam in a helical manner. The spiral motion followed a direct pathway interspersed with occasional turns. Turning frequencies of zoospores in the presence and absence of electrical fields were determined by recording their swimming pattern with time-lapse video microscopy. Paths of individual zoospores were traced from a video monitor during frame-by-frame playback and then digitized with an image-analysis system (18).

Zoospore suspensions were prepared in 2 mM sodium phosphate buffer (pH 7.2), and the swimming of individual zoospores was recorded over a period of 1 h. Video recordings were made with an Olympus CK2 inverted microscope (Olympus Optical Co., London) connected to a Panasonic time-lapse video recorder (model 6720-B, Matsushita Electric Industrial Co., Ltd., Osaka, Japan) at a final magnification of 300×. A turn was defined as an abrupt change in direction greater than 15° from a direct swimming pathway (described above). The number of turns per second was determined from the time display of the video recorder. These values were converted to turns per minute for ease of comparison. Experiments were carried out in duplicate, and the results were pooled.

Effect of electrical fields on flagellar orientation. The effect of electrical fields on the orientation of the anterior and posterior flagella of zoospores of *P. palmivora* and *Pythium aphanidermatum* were examined with a chamber constructed from a glass microscope slide with a central channel formed between two sections of glass (Fig. 1). Platinum-wire electrodes were glued to both ends of the channel, and molten agarose gel (1%, w/v) was poured over the electrodes and both ends of the channel to form a central well measuring 1 $cm^2 \times 0.1$ cm deep. Nonmotile, nonencysted zoospores were obtained by adding sodium azide (1 mM final concentration) to a suspension of zoospores in 250 mM PIPES buffer at pH 7.2. An aliquot of the suspension was placed in the central chamber and overlain with a coverslip prior to the application of an electrical field across the chamber. Azide-treated zoospores stuck to the glass chamber base and were not dislodged by moderate water currents applied with a Pasteur pipette or by drawing water through the chamber with capillary action from filter paper. Photographs were taken by dark-field optics before and after the application of electrical fields. Zoospores of *Pythium aphanidermatum* and *P. palmivora* were placed in fields of 100 and 500 mV cm^{-1}, respectively. The more intense fields could be applied to zoospores of *P. palmivora* because the zoospores could be prepared in high-resistance media that reduced the current, wattage, and subsequent heating in the chamber. The same high-resistance medium significantly inhibited zoospore production of *Pythium aphanidermatum*.

The extent of polarization of the anterior and posterior flagella was determined by image analysis (18) of dark-field photographs and was calculated from

$$P(\%) = [(\Sigma\cos\Theta)/n] \times 100$$

for which Θ is the angle of the flagellum relative to the anode-cathode axis, with the anode at 0° and the cathode at 180°. Positive values indicated anodic orientation, negative values were cathodic. The shorter anterior flagellum could be clearly differentiated from the longer posterior flagellum in these photographs (Fig. 2). Those specimens for which the anterior and posterior flagella could not be distinguished unequivocally were omitted from the analysis. Assays were carried out in triplicate, and the results from each were pooled.

Calcium and swimming behavior. Calcium ions are important in the electrotactic response of several cell types (4,11,13,15, 21,43,44). Therefore, we investigated the influence of Ca^{2+} on electrotaxis of a range of chemicals that effected Ca^{2+} availability or calmodulin function. Channel blockers, amiloride, TMB8 (3,4,5-trimethoxy benzoic acid 8-(diethylamino) octyl ester), and trifluoperazine (TFP) were obtained from Sigma Chemical Co. (Poole, Dorset, UK) and were prepared in 2 mM sodium phosphate buffer at pH 7.2. The calmodulin inhibitor R24571 (1-[bis-(4-chlorophenyl)methyl]-3-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]-ethyl]-1 H-imidazolium chloride; Calmidazolium, Sigma Chemical Co.) was prepared as a stock solution in dimethyl sulfoxide (DMSO). The final DMSO concentration was less than 0.1% (v/v) and did not affect swimming behavior or encystment. Ethylene glycol-bis-(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid (EGTA) was initially dissolved in 0.5 M NaOH, neutralized with 0.5 M HCl, and made

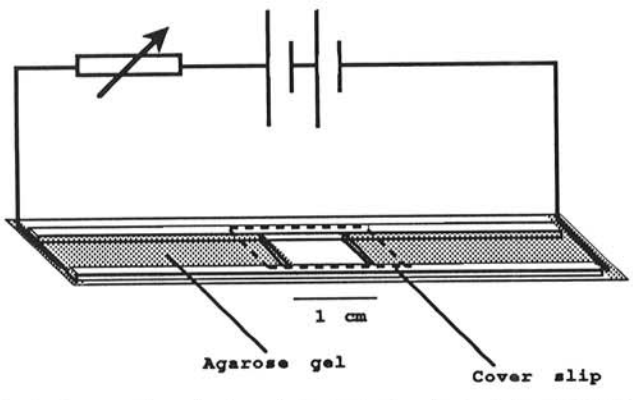

Fig. 1. Construction of micro-electrotaxis chamber to determine electrophoretic orientation of zoospore flagella. The base and sides were fabricated from glass microscope slides; the electrodes were platinum wire (0.5 mm in diameter). The central chamber measured 1 $cm^2 \times 0.1$ cm. Molten agarose was poured over the electrodes and ends of the chamber up to the position of the coverslip.

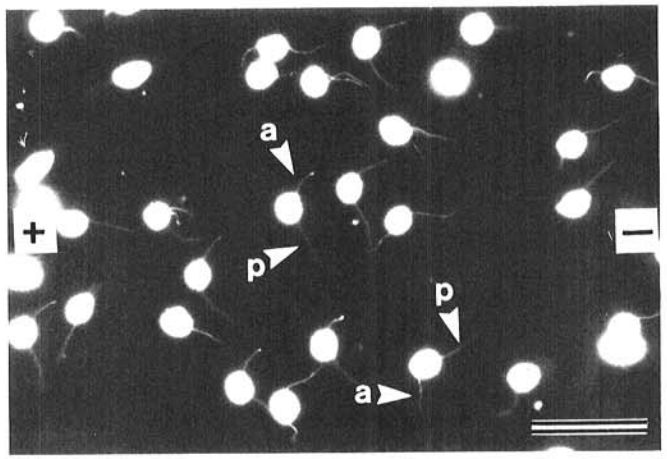

Fig. 2. Dark-field photomicrographs of nonmotile, nonencysted zoospores of *Phytophthora palmivora* showing the orientation of the anterior (a) and posterior (p) flagella in an applied electrical field of 500 mV cm^{-1}. The scale bar = 30 μm. The direction of the anode (+) and cathode (−) are indicated.

up to the required concentration with sodium phosphate buffer at pH 7.2. The concentration of Ca^{2+} in a zoospore suspension was measured directly with a Ca^{2+}-selective electrode. The EGTA concentrations required to give a specific Ca^{2+} concentration for a given temperature and pH value were calculated by the equations of Caldwell (6). These equations were incorporated into a computer program supplied by W. Schreurs (Colorado State University, Fort Collins, CO).

Analysis of encystment. Comparisons of the extent of encystment induced by the reagents were conducted in microtiter, multiwell plates (Dynatech Laboratories, Billinghurst, UK). A qualitative assessment of comparative encystment of 0.1-ml samples of zoospores in suspension was made. Observations were made every 10 min up to 1 h. Changes in behavior were scored in three groups, a–c. Group a included treatments causing an increase in the number of encysted spores of less than 30% of the population, compared to controls. In group b, encystment was ≥30%. Treatments causing some degree of spore lysis were assigned to group c. Controls contained either 2 mM buffer or 0.1% (v/v) DMSO minus the test compound. At least 10 replicates were assayed for each treatment.

RESULTS

Electrotaxis of zoospores of different species. At a physiological field strength of 50 mV cm^{-1}, only zoospores of *Pythium aphanidermatum* and *P. palmivora* showed significant electrotaxis ($P < 0.01$) (Table 1). The response of zoospores of *Pythium aphanidermatum*, in contrast to zoospores of *P. palmivora*, was toward the cathode. The tactic responses of all the species tested were greater at 500 mV cm^{-1} than at 50 mV cm^{-1} ($P < 0.01$). At a field strength of 500 mV cm^{-1}, greater than the electrical field normally generated by plant roots, both *Pythium catenulatum* and *Pythium dissotocum* showed significant taxis ($P < 0.01$) to the anode. Significance was calculated by comparing zoospore densities at the anode and cathode according to a Student's *t* test.

TABLE 1. Electrotaxis of zoospores of four pythiaceous fungi[a]

Organism	Tactic response quotient[b]	
	50 mV cm^{-1}	500 mV cm^{-1}
Phytophthora palmivora	+0.23* ± 0.04	+0.44* ± 0.02
Pythium aphanidermatum	−0.20* ± 0.04	−0.31* ± 0.05
Pythium catenulatum	0.0 ± 0.05	+0.17* ± 0.04
Pythium dissotocum	+0.02 ± 0.05	+0.18* ± 0.04

[a]Each value is the mean ± 95% confidence interval based on at least 10 replicate experiments. Means followed by * indicate the zoospore densities at the cathode and anode ends of the electrotaxis chamber were significantly different ($P < 0.01$) according to a Student's *t* test.
[b]Tactic response quotient is defined in text. Value determined for zoospores (density of $2.5 \pm 0.5 \times 10^5$ zoospores per milliliter) in 2 mM sodium-phosphate buffer (pH 7.2, 22–24 C).

TABLE 2. Analysis of the swimming behavior of zoospores of *Phytophthora palmivora* in applied electrical fields[a]

Swimming behavior	Field strength		Control
	50 mV cm^{-1}	500 mV cm^{-1}	0 V cm^{-1}
Velocity ($\mu m\ s^{-1}$)	168.8* ± 6.4	140.2 ± 6.1	132.2 ± 6.2
Turns min^{-1}	17.0* ± 3.0	20.7* ± 3.7	5.4 ± 1.5
Velocity to anode ($\mu m\ s^{-1}$)	170.4 ± 8.8	140.2 ± 8.3	...
Velocity to cathode ($\mu m\ s^{-1}$)	166.5 ± 9.3	140.3 ± 8.8	...
Turns min^{-1} to anode	18.5 ± 3.9	20.5 ± 4.4	...
Turns min^{-1} to cathode	14.6 ± 4.8	21.2 ± 6.8	...

[a]Each value is the mean ± 95% confidence interval based on at least 117 measurements in duplicate experiments. Control values are a mean of four experiments ($n = 267$). Means followed by * are significantly different from controls ($P < 0.01$) according to a Student's *t* test.

Swimming behavior of zoospores of *P. palmivora* in electrical fields. Zoospores in electrical fields swam slightly faster than those in the control (no field) (Table 2). The average swimming speed also varied slightly between experiments, however, and possibly reflected small differences in ambient temperature. The rate of turning in electrical fields of 50 and 500 mV cm^{-1} was increased by a factor of three to four compared to controls. No significant difference in the rate of turning or of swimming velocity was found for zoospores moving toward the anode compared to those moving toward the cathode ($P > 0.05$) according to a Student's *t* test.

Effect of an applied field on zoospore flagellar orientation. Because electrical fields aligned the direction of zoospore swimming, we investigated the effect of electrical fields on the alignment of the flagella of zoospores that swam in opposite directions in an electrical field. Anodotactic zoospores of *P. palmivora* and cathodotactic zoospores of *Pythium aphanidermatum* were paralyzed in a sodium-azide solution and adhered to the base of the chamber before application of the electrical fields. The angular distribution of the two flagella of *Pythium* and *Phytophthora* zoospores were random in the absence of the electrical field. Due to the field, the anterior flagellum of zoospores of *P. palmivora* was oriented randomly, while the posterior flagellum was pointed toward the cathode (Table 3). In contrast, the posterior flagellum of *Pythium aphanidermatum* was pointed toward the anode, and the anterior flagellum was oriented randomly. Thus, the direction of electrotactic swimming reflected a charge dipole of the cells that may have been due to the relative surface charge of the anterior and posterior flagella.

Calcium and electrotaxis. Calcium ions are important in the regulation of motility, electrotaxis, and electrotropism of a variety of cell types (5,11,13,15,21). To determine whether Ca^{2+} influenced electrotaxis of zoospores, compounds that caused Ca^{2+} deprivation, calcium-channel blockade, alteration of Ca^{2+} transport, and inhibition of calmodulin function were investigated (Table 4).

EGTA is a divalent cation chelator with a high affinity for Ca^{2+} at neutral or alkaline pH values. The calcium concentration of the zoospore suspensions was reduced from a measured value of 50 μM to calculated values of 0.18, 1.7, and 2.6 nM in the presence of 1 mM, 0.5 mM, and 250 μM EGTA, respectively. A decrease in the electrotactic response of the zoospores was found as the EGTA concentration increased (Table 4), although this also was related to an increased adverse effect on the swimming behavior of the zoospores. EGTA caused concentration-dependent lysis and encystment of the zoospores. Early encystment of zoospores, induced by EGTA or other compounds described below, may account for the decrease in the TRQ value. In these cases, the swimming period, and hence the opportunity of the cells to respond to the electrical field, would be decreased concomitantly.

Hypothetically, TMB8 inhibits the release of Ca^{2+} from intracellular stores (16,37). The TRQ of zoospores in electrical fields was not affected by 20 μM TMB8. Increasing the concentration

TABLE 3. Polarization of the posterior and anterior flagella of *Phytophthora palmivora* and *Pythium aphanidermatum* in applied electrical fields[a]

Flagella	Polarization (%)[b]	
	Phytophthora palmivora[c]	*Pythium aphanidermatum*[d]
Anterior	−1.2 ± 9.5[e]	4.9 ± 7.8
Posterior	−23.4 ± 9.1	47.0 ± 6.4

[a]Each value is the mean ± 95% confidence interval. The results are pooled from three independent experiments.
[b]Defined in text.
[c]Zoospores placed in an electrical field of 500 mV cm^{-1} for 30 min at pH 7.2.
[d]Zoospores placed in an electrical field of 100 mV cm^{-1} for 30 min at pH 7.2.
[e]For *P. palmivora*, $n = 223$ and 246, respectively. For *Pythium aphanidermatum*, $n = 306$ and 302, respectively.

of this compound to 100 μM caused premature encystment and lysis of zoospores (Table 4). High concentrations of organic and inorganic calcium-channel blockers also affected motility or induced encystment or lysis. However, 50 μM verapamil and lanthanum stimulated electrotaxis (Table 4). Amiloride inhibited Na^+ and Ca^{2+} transport in mammalian cells and decreased uptake of Ca^{2+} and Na^+ into zoospores of *P. palmivora* (25). Electrotaxis was not affected by 100 μM amiloride but was reduced at 200 μM, which also caused zoospore encystment.

The calmodulin antagonists R24571 and TFP each caused encystment of zoospores. With R24571, but not with TFP, lysis of the spores occurred within 10 min. Perturbation of calcium transport and metabolism promoted zoospore encystment. With the exception of verapamil and lanthanum, these compounds reduced the TRQ value or had no effect. Measurement of electrotaxis, in the system presented here, requires a finite time for significant accumulation of zoospores to occur at the anode or cathode (36). Thus, treatments that stimulated encystment would be expected to reduce the TRQ and could not be linked specifically to the regulation of electrotaxis.

DISCUSSION

Zoospores of four species of oomycetous pathogens exhibited electrotaxis in vitro. The magnitude of the response varied significantly between species. However, the maximum electrical fields measured with vibrating microelectrodes around plant roots is 50–100 mV cm^{-1}, assuming a soil water resistivity of 5,000 Ω cm (17,32). Therefore, electrotaxis of *Pythium catenulatum* and *Pythium dissotocum* may not be sufficiently sensitive to be strongly influenced by the in vivo electrical fields generated by plant roots and other tissues. Our evidence suggests, however, that zoospores of *P. palmivora* and *Pythium aphanidermatum* are influenced by the natural electrical fields found around root tips, sites of wounds, emerging lateral roots, or stomatal guard cells (3).

TABLE 4. The effect of agents affecting calcium transport and calmodulin activity on electrotaxis of *Phytophthora palmivora* zoospores in an applied field of 500 mV cm^{-1}[a]

Compound	Mode of action	Concentration (μM L^{-1})	Electrotaxis (TRQ)[b]
Control	...	...	+0.44 ± 0.02
EGTA[c]	Calcium ion chelator	250	+0.43 ± 0.03[d]
		500	+0.12* ± 0.04[d,e]
		1,000	+0.08* ± 0.04[e,f]
TMB8[g]	Inhibitor of intracellular Ca^{2+} release	20	+0.46 ± 0.05[f]
		100	ND[h]
Verapamil	Ca^{2+} channel blocker	50	+0.71* ± 0.04[d,e]
Lanthanum	Ca^{2+} channel blocker (nonselective)	50	+0.62* ± 0.03
		100	+0.57* ± 0.07[f]
Cobalt	Ca^{2+} channel blocker (nonselective)	50	+0.44 ± 0.05[e,f]
		100	ND
Amiloride	Na^+ - Ca^{2+} exchange inhibitor	100	+0.45 ± 0.05
		200	+0.39* ± 0.03[f]
		300	ND
R24571[i]	Calmodulin inhibitor	2	+0.23* ± 0.04[d]
Trifluoperazine	Calmodulin inhibitor	2	+0.32* ± 0.05[d]
		10	ND[f]

[a] Assays were conducted at room temperature in 2 mM sodium-phosphate buffer at pH 7.2 ± 0.1. Each value is the mean ± 95% confidence interval based on at least 10 replicates. Means followed by * indicate a significant difference from the control ($P < 0.01$) according to a Student's t test.
[b] Tactic response quotient.
[c] Ethylene glycol-bis-(β-amino-ethyl ether) *N,N,N',N'*-tetraacetic acid.
[d] Slight increase in number of encysted spores (<30%) compared to a control.
[e] Lysis of cells.
[f] Marked increase in number of encysted spores (>30%) compared to a control.
[g] 3,4,5-trimethoxy benzoic acid 8-(diethylamino) octyl ester.
[h] ND = not determined.
[i] 1-[bis-(4-chlorophenyl)methyl]-3-[2-(2,4-dichlorophenyl)-2-[2,4-dichlorophenyl)methoxy]-ethyl]-1 H-imidazolium chloride; Calmidazolium.

The response of *P. palmivora*, *Pythium catenulatum*, and *Pythium dissotocum* was anodic in an electrical field of 500 mV cm^{-1}, whereas that of *Pythium aphanidermatum* was cathodic. The anodic and cathodic regions of plant roots vary according to the plant species (32), growth conditions (33), source of combined nitrogen (35), and presence of plant-growth regulators (33). The relationship between the direction of electrotactic swimming and the electrical polarity at the sites of zoospore accumulation around plant roots is now under investigation. Preliminary evidence suggests a positive correlation between the endogenous electrical polarity of a plant root and the zone of zoospore accretion (B. Reid, B. M. Morris, and N. A. R. Gow, *unpublished data*).

Zoospores can respond to fields as low as 5 mV cm^{-1} (36), equivalent to a voltage drop of only 5 μV across their diameter. We showed previously that electrotaxis was not the result of electrophoretic or electroosmotic displacement of the zoospore (36). We propose that electrotaxis is the result of two processes: orientation of zoospores in the field according to their electrical dipole (electro-topotaxis) and voltage-dependent stimulation of the turning frequency (electro-klinokinesis). The electrical dipole may be the result of the relative charge of proteins or glycoproteins in the anterior and posterior flagella. Our observations of the deflections of anterior and posterior flagella in electrical fields suggest that anodotactic and cathodotactic zoospores had natural charge dipoles oriented in opposite directions. The charge dipole was such that the posterior flagellum was positively charged relative to the anterior flagellum for zoospores of *P. palmivora* that swam to the positive pole. In contrast, the posterior flagellum of cathodotactic zoospores of *Pythium aphanidermatum* may be negatively charged compared to the anterior flagellum because it was deflected strongly toward the anode. The posterior and anterior flagella of both zoospore species deflected asymmetrically, suggesting they may bear opposite charges at neutral pH values (Fig. 3). The posterior flagellum of biflagellate zoospores is considered the steering organ of the cell (9). Because orientation of the posterior flagellum was affected markedly by the electrical field, this may play a dominant role in dictating the polarity of the electrotactic response. Differences in the

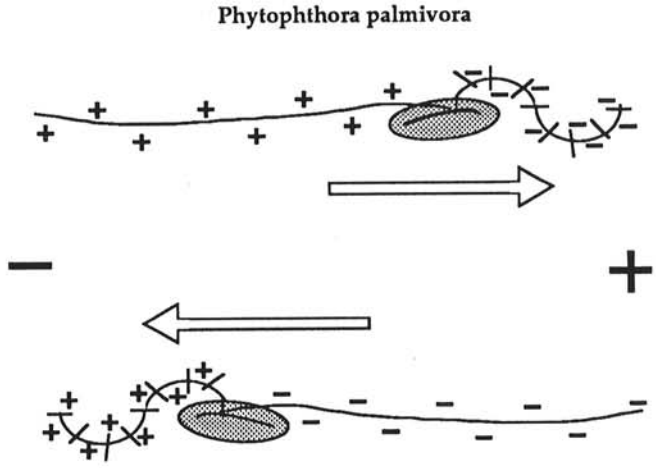

Fig. 3. Diagram of the charged dipole of zoospores of *Phytophthora palmivora* and *Pythium aphanidermatum* resulting in the orientation of the zoospore toward either the anode (+) or cathode (−) of an applied electrical field.

chemistry of the two flagella of a related pathogen, *Phytophthora cinnamomi*, is evident in the finding that monoclonal antibodies have been generated that have specific recognition for the anterior flagellum and its mastigonemes (20).

Field-dependent stimulation of the turning frequency of zoospores also may be significant in the electrotactic response of zoospores to the endogenous electrical fields of plants. The magnitude of these fields decreases with increasing distance from the root surface. Therefore, zoospores approaching a root surface will experience an increasingly large electrical field that can be estimated to be at least 250 mV cm^{-1} at 10 μm from the root surface, depending on the plant species. Frequent zoospore turning in the vicinity of a root has been reported previously (26) and could enhance the accumulation of zoospores at the root surface.

The mechanism by which electrical fields stimulate changes in turning frequency is not clear. One possible mechanism is modulation of the conductance of voltage-gated channels for calcium ions. Electrotaxis of the unicellular alga *Chlamydomonas* (13), bracken spermatozoids (4), *Paramecium* (31,38), and fish keratocytes (11) is dependent on the provision of an adequate supply of exogenous calcium ions and, in some cases, could also be inhibited by calcium-channel blockers. Moreover, calmodulin is localized in both flagella and, in particular, the basal region of the anterior tinsel flagellum of *P. cinnamomi* (19). We observed that calcium deprivation reduced the TRQ, but interpretation of these data was complicated by the fact that compounds affecting calcium-ion transport and almodulin function induced premature encystment, and, in some cases, lysis of the spores. Verapamil and lanthanum ions seemed to promote electrotaxis rather than inhibit it. Increased encystment led to a reduction in the TRQ by reducing the swimming time and, hence, the ability of the spores to respond to the field.

External electrical fields of 0.1–1.0 mV μm^{-1} caused cathode-localized membrane depolarization and calcium entry in mouse neuroblastoma cells (1). In our experiments, however, the strength of the electrical fields required to induce electrotaxis was less than that expected to influence voltage-gating of known calcium channels (40). The expected perturbation of membrane potential at the anode- and cathode-facing ends of the cell can be estimated at <10 μV for an exogenous field of 10 mV cm^{-1} (10). This small voltage may be insufficient to affect the probability that a voltage-gated channel is open (40,42). Because zoospores are motile and swimming involves frequent changes in direction, there would not be sufficient time for electrical fields to redistribute proteins in the cell membrane, thereby influencing ion transport and flagellar motion. Thus, although calcium ions are important in the normal swimming pattern of *Achlya* zoospores (45) and the adhesion and germination of cysts of zoospores of *Pythium* and *Phytophthora* species (14,22), we cannot provide any unequivocal evidence for a role for calcium in zoospore electrotaxis.

Our results suggest that attraction of swimming zoospores to host roots is related in part to the sensing of endogenous electrical gradients generated by growing roots or other plant tissues. Electrotaxis is nonspecific insofar as it should be stimulated equally by host and nonhost plants. Similarly, zoospore chemotaxis has only rarely been shown to be selective for the host species (48). Apparently, host location and identification are regulated independently. The former may involve concerted and synergistic chemotactic and electrotactic mechanisms.

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