Negative Geotaxis of Zoospores of the Fungus *Phytophthora*

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INTRODUCTION

Ho & Hickman (1967*a*) found that zoospores of *Phytophthora megasperma* in an aqueous suspension accumulated just below the surface. A plausible explanation for such an effect would be positive aerotaxis, which is widespread in micro-organisms (Carlile, 1975). However, *P. megasperma* zoospores showed no attraction to air or repulsion from carbon dioxide when capillaries containing these gases were placed in zoospore suspensions, suggesting that aerotaxis, either in the form of attraction to oxygen or repulsion from carbon dioxide, was not responsible for the effect (Ho & Hickman, 1967*b*). We, too, have found that in aqueous suspension *Phytophthora* zoospores tend to accumulate near the top of the liquid and conclude that the effect is due to negative geotaxis – active swimming in opposition to the attraction of gravity.

METHODS

Organisms. *Phytophthora cactorum* (Lebert & Cohn) Schroeter, strain P264, from bark lesions of Horse Chestnut (*Aesculus hippocastanum*) was provided by Dr C. M. Brasier, Forest Research Station, Alice Holt Lodge, Farnham, Surrey. *Phytophthora palmivora* (Butler) Butler, strain P50, a small chromosome variety (Sansome, Brasier & Griffin, 1975) from pods of Ghanaian cocoa (*Theobroma cacao*) was also supplied by Dr Brasier, and used under licence from the Ministry of Agriculture, Fisheries and Food. *Phytophthora nicotianae* Van Breda de Haan, Commonwealth Mycological Institute strain 158721, from Black Pepper (*Piper nigrum*) in Western Malaysia, was provided by Dr R. M. Jackson, Department of Microbiology, University of Surrey, Guildford.

Maintenance of strains and zoospore production. Cultures were maintained on slopes and Petri dishes of clear V8 juice agar (Khew & Zentmyer, 1973). Stock cultures were prepared approximately monthly by inoculation with zoospore suspensions to avoid possible deterioration of the ability to produce zoospores. The method for preparing zoospore suspensions was a modification of that of Khew & Zentmyer (1973). Petri dishes containing 20 to 25 ml V8 juice liquid medium (pH 5.2 to 5.5) were inoculated with 12 mm discs cut from agar cultures. After 3 days at 22 to 24 °C the mycelium was washed twice with sterile distilled water and then exposed in Petri dishes of sterile distilled water in the laboratory (about 20 to 22 °C) to the normal alternation of light and darkness for 48 h. The mycelium, which now bore abundant sporangia, was washed again, placed at 10 °C for 30 min, and returned to room temperature. Zoospores were released within 30 min. Zoospore densities were estimated using a haemocytometer, counting being deferred for a few minutes until the zoospores in the counting chamber had become sluggish. Zoospore densities were commonly $10^5 \text{ ml}^{-1} \pm 50\%$, and only suspensions within these limits were used for experiments. The concentration was not adjusted, as it was found that attempts to dilute or concentrate zoospore suspensions sometimes led to encystment of many zoospores. Aseptic procedures

were used in zoospore production, but not in experiments with capillary tubes, which rarely lasted more than 15 min.

Capillary test for geotaxis. Melting point tubes (internal diam. 0.45 to 0.50 mm) were trimmed to 40 mm length, and cleaned by boiling first in 2 M-HCl and then twice in distilled water. A zoospore suspension was drawn into a tube by capillarity so that it occupied the middle 25 mm. The tube was then plugged at both ends with silicone high vacuum grease, leaving an air gap of about 5 mm at each end of the zoospore suspension. Tubes were mounted either vertically by inserting into a plasticine base or horizontally with the aid of plasticine on a microscope slide. Zoospore behaviour in the vertical tubes was observed, if necessary, with a horizontal travelling microscope; that in horizontal tubes with a microscope with a mechanical stage. Zoospores in a tube were counted after transfer to -20°C for 2 min (which immobilized and killed them) at a $30\times$ magnification, which enabled zoospores to be seen clearly while giving a depth of field about equal to that of the internal diameter of the tube.

RESULTS AND DISCUSSION

Preliminary experiments were carried out in which capillary tubes filled with air, oxygen, nitrogen or carbon dioxide were placed horizontally in zoospore suspensions of *P. cactorum* and *P. nicotianae*. Neither attraction nor repulsion of zoospores was observed, and it was concluded, in agreement with Ho & Hickman (1967*b*), that aerotaxis was not responsible for the accumulation of zoospores at the surface. It was also observed, with the same two fungi and with *P. palmivora*, that predominantly unilateral illumination did not have a perceptible effect on the distribution of zoospores in a Petri dish, so it is unlikely that positive phototaxis caused the observed accumulation at the surface. Experiments were therefore carried out with all three species to ascertain whether negative geotaxis occurred.

When capillaries containing zoospore suspensions were mounted vertically, suspensions of *P. cactorum* and *P. nicotianae* showed some zoospore accumulation near the upper meniscus within 1 min; *P. palmivora* took a little longer to show an effect. In 15 min all three species showed a striking accumulation in the top 3 mm of the tube and a depletion in the lowest 10 mm. Inversion of the capillary at this stage led to dispersal of the accumulated zoospores and, within 15 min, to renewed accumulation at the new upper end, indicating that the effect cannot be explained in terms of fortuitous differences in the gas phase at the two ends. Moreover, no accumulation was seen at the ends of tubes placed horizontally, thus excluding an interpretation in terms of aerotaxis. Accumulation at the upper surface of vertical tubes took place both in normal laboratory illumination and in darkness, so light was not influencing zoospore distribution.

Observations were carried out on the speed at which *P. nicotianae* zoospores accumulated in the top 3 mm of a tube, by filling tubes from the same zoospore suspension and freezing and counting after various times. Counts for the top 3 mm were as follows (time, min, followed by the number of zoospores): 0, 41; 2, 82; 5, 101; 10, 216; 15, 220; 40, 212; 120, 198. Thus there was an approximately linear increase in count for 10 min, followed by little change for 2 h. Similar results were obtained for the other species. It was not clear why accumulation of zoospores in the upper part of the tube did not continue for longer than 10 min. Possibly a tendency to move upwards is counteracted by zoospore collisions and direction changes as zoospores move towards a more densely populated region. Alternatively, zoospores which do not migrate to the top may be ones which are becoming sluggish and are about to encyst, as a result of the mechanical agitation that they received when the

Table 1. *Zoospore distribution in vertical tubes*

The number of zoospores in the top and bottom 1 mm of suspensions of zoospores in capillary tubing placed vertically for 15 min, were counted as described in Methods. Five tests were carried out on each zoospore suspension.

Species	Zoospore counts		Zoospore ratios		
	Top	Bottom	Top	Bottom	Top
			Expected*	Expected	Bottom
<i>P. cactorum</i>	172	13	8.6	0.7	13.2
	72	3	3.6	0.2	24.0
	45	2	2.3	0.1	22.5
	78	0	3.9	0	∞
	98	4	4.9	0.2	24.5
	Average	93.0	4.7	0.2	21.1
<i>P. nicotianae</i>	39	2	2.0	0.1	19.5
	44	4	2.2	0.2	11.0
	36	2	1.8	0.1	18.0
	89	3	4.5	0.2	29.7
	58	3	2.7	0.2	19.3
	Average	52.2	2.6	0.1	18.6
<i>P. palmivora</i>	50	2	2.5	0.1	25.0
	26	11	1.3	0.6	2.4
	62	10	3.1	0.5	6.2
	42	4	2.1	0.2	10.5
	50	6	2.5	0.3	8.3
	Average	46.0	2.3	0.3	7.0

* The number of zoospores expected in 1 mm length of tube, on the basis of 10^5 zoospores ml^{-1} , an internal tube diam. of 0.5 mm, and uniform distribution of zoospores, is 20. Examination of horizontal control tubes showed that numbers of zoospores per 1 mm length of tube corresponded closely with the numbers predicted on the basis of haemocytometer counts of the suspension from which the sample was taken.

tube was being filled. The geotactic response was more marked in *P. cactorum* and *P. nicotianae*, in which after 15 min there were about 20 times more zoospores in the top than in the bottom 1 mm, than in *P. palmivora*, where the difference was less than 10-fold (Table 1).

Accumulation of zoospores at the upper surface of a suspension is due to active swimming and not flotation, as zoospores have a higher density than water; they sink on gentle centrifugation yet remain viable and motile. The geotaxis may not, however, be a true sensory response, i.e. one involving receptors and signal transduction. Phytophthora zoospores are asymmetrical in the position of flagella and in their shape; they are larger at the rear than at the front (Ho, Hickman & Telford, 1968). The zoospore may therefore tend to point slightly upwards as it swims, and show a net tendency to swim upwards. The swimming speeds of our zoospores were considerably in excess of $100 \mu\text{m s}^{-1}$, as were those of *P. megasperma* (Ho & Hickman, 1967a) and *P. cinnamomi* (Allen & Newhook, 1973), so a tendency towards upward swimming would be adequate to account for the observed negative geotactic response.

Geotaxis is known in eukaryotic micro-organisms, for example *Paramecium* (Dryl, 1974) and algae (Halldal, 1962), even though little studied. This is believed to be the first report of its occurrence in a fungus. It adds to the repertoire of responses known in *Phytophthora* (Carlile, 1975) – chemotaxis, electrotaxis and rheotaxis – which will need to be taken into account by plant pathologists considering the organism's life in the soil and infection of plants.

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REFERENCES

- ALLEN, R. N. & NEWHOOK, F. J. (1973). Chemotaxis of zoospores of *Phytophthora cinnamomi* to ethanol in capillaries of soil pore dimensions. *Transactions of the British Mycological Society* **61**, 287–302.
- CARLILE, M. J. (editor) (1975). *Primitive Sensory and Communication Systems: the Taxes and Tropisms of Micro-organisms and Cells*. London: Academic Press.
- DRYL, S. (1974). Behaviour and motor response of Paramecium. In *Paramecium: a Current Survey*, pp. 165–218. Edited by W. J. Van Wagtendonk. Amsterdam: Elsevier Scientific Publishing Co.
- HALLDAL, P. (1962). Taxes. In *Physiology and Biochemistry of Algae*, pp. 583–593. Edited by R. A. Lewin. New York: Academic Press.
- HO, H. H. & HICKMAN, C. J. (1967*a*). Asexual reproduction and behaviour of zoospores of *Phytophthora megasperma* var. *sojae*. *Canadian Journal of Botany* **45**, 1963–1981.
- HO, H. H. & HICKMAN, C. J. (1967*b*). Factors governing zoospore responses of *Phytophthora megasperma* var. *sojae* to plant roots. *Canadian Journal of Botany* **45**, 1983–1994.
- HO, H. H., HICKMAN, C. J. & TELFORD, R. W. (1968). The morphology of zoospores of *Phytophthora megasperma* var. *sojae* and other Phycomycetes. *Canadian Journal of Botany* **46**, 88–89.
- KHEW, K. L. & ZENTMYER, G. A. (1973). Chemotactic responses of five species of *Phytophthora*. *Phytopathology* **63**, 1511–1517.
- SANSOME, E., BRASIER, C. M. & GRIFFIN, M. J. (1975). Chromosome size differences in *Phytophthora palmivora*, a pathogen of cocoa. *Nature, London* **255**, 704–705.