The Effect of Cations on Zoospores of the Fungus *Phytophthora cinnamomi*

By PAULINE N. BYRT, HELEN R. IRVING AND BRUCE R. GRANT*

*The Russell Grimwade School of Biochemistry, University of Melbourne, Parkville,
Victoria 3052, Australia*

(Received 10 August 1981; revised 24 September 1981)

Swimming zoospores of the fungus *Phytophthora cinnamomi* exposed to a range of ions were much more sensitive to cations than anions. One cation, Ca^{2+} , induced zoospores to encyst and subsequently germinate, but most cations induced encystment only and were toxic at higher concentrations. In general, the sensitivity of zoospores to a cation increased with its charge density. At $0.3 \mu\text{M}$, La^{3+} reduced the viability of a zoospore population to 50%, while Fe^{3+} ($20 \mu\text{M}$) and Mn^{2+} ($50 \mu\text{M}$) induced encystment with only a slight decrease in viability. The other divalent and monovalent cations tested (Mg^{2+} , Ca^{2+} , Ba^{2+} , Li^{+} , Na^{+} , K^{+} , Cs^{+} , NH_4^{+}) were effective in inducing encystment or reducing viability at higher concentrations. Each ion showed a distinctive concentration–response curve. Only F^{-} and $\text{CH}_3\text{COO}^{-}$ among the anions tested (Cl^{-} , NO_3^{-} , F^{-} , $\text{H}_2\text{PO}_4^{2-}$, SO_4^{2-} , $\text{CH}_3\text{COO}^{-}$) had any effect on zoospores, and at 20 mM (pH 6.0) they reduced viability.

The cysts (cystospores) of the fungus were generally less sensitive to cations than were swimming zoospores, and only Cs^{+} and K^{+} (50 mM) reduced viability to the same extent in each population. Both zoospores and cysts of this fungus had a broad tolerance to pH and temperature, but cysts were more resistant to low temperatures than were motile zoospores.

INTRODUCTION

The biflagellate zoospores of the Oomycetes go through the process of cyst (cystospore) formation and cyst germination after their release from sporangia and before the formation of a new mycelial state. The changes in ultrastructure during this transformation have been described for a variety of species and genera (see Lunney & Bland, 1976). The details of the ultrastructural changes during the process of cyst formation and germination have been well documented in the genus *Phytophthora* (Hemmes & Hohl, 1971; Tokunaga & Bartnicki-Garcia, 1971; Bimpong & Hickman, 1975; Sing & Bartnicki-Garcia, 1975). However, there is less information on the mechanisms which trigger these developmental changes.

The external ionic environment affects zoospore differentiation in both the monoflagellate *Blastocladiella emersonii* and the biflagellate *Aphanomyces astaci* (Soll & Sonneborn, 1972; Svensson & Unestam, 1975). There are indications that *Phytophthora* zoospores are similarly affected since the motility of *Phytophthora palmivora* (Butl.) Butl. is reduced by Ca^{2+} and Mg^{2+} salts (Bimpong & Clerk, 1970). In addition, *P. palmivora* zoospores exhibit negative chemotaxis in response to a range of cations (Cameron & Carlile, 1980). In the case of the plant pathogen *Phytophthora cinnamomi* Rands, zoospores are repelled by inorganic cations (Allen & Harvey, 1974), and there is evidence that the disease pattern is influenced by soil cation levels (Broadbent & Baker, 1974; Halstall & Forrester, 1977; Boughton *et al.*, 1978).

We show here that in *P. cinnamomi* the transformation of swimming zoospores to germinating cysts is affected by the external concentration of cations and suggest that specific ion effects are involved rather than changes in osmotic pressure. The significance of the

results in relation to the potential behaviour of the parasite in soil and its capacity to act as a pathogen is discussed.

METHODS

The isolate of *P. cinnamomi* (A-2 compatibility type) was obtained from the Brisbane Ranges, Victoria, by Dr G. Weste and designated by her as isolate PC110 (Commonwealth Mycological Institute Collection, IMI 252489). Zoosporangia were induced and zoospores released from axenic cultures as described previously (Byrt & Grant, 1979). After release the zoospores (10^4 – 10^5 cells ml^{-1}) were held in Tris-succinate buffer, pH 6.5 (1 mM) at 16 ± 1 °C and used within 2 h. All glassware with which zoospores came into contact was prewashed with 2 M-HCl overnight. Operations in which zoospores were transferred or mixed required care to minimize mechanical or pressure shock. Zoospores were exposed to the various ions in plastic tissue culture trays (Linbro, Conn., U.S.A.) for up to 120 min. Unless stated otherwise the pH was 6.5. When cyst populations were to be tested, they were induced from swimming zoospores by 2 min vigorous shaking (Tokunaga & Bartnicki-Garcia, 1971). Cells were examined directly or after fixation with 2% glutaraldehyde, pH 6. The numbers of cells and their developmental stage were determined using an inverted microscope with a calibrated grid in the ocular. A volume of 1.5 or 2.0 ml per chamber was used, and all incubations were carried out at 19 ± 2 °C. After fixation, cells were stable for at least a week at room temperature, but counting was always completed within 4 d of an experiment.

When the experimental procedure required that cells be transferred from one container to another, cells at different stages of development were not recovered with equal efficiency. Thus, the protocol was designed so that the entire experimental procedure could be carried out in one vessel. All experiments contained controls in which zoospores were held in 1 mM buffer during the period of treatment. All results are reported as percentages obtained from counts of 100–200 cells. Percentage encystment was calculated as: $100 \times (\text{mean no. of encysted cells per field in test sample})/(\text{mean no. of encysted cells per field in shaken sample})$. The percentage of encysted cells in the controls varied from one experiment to another but was usually in the region of 20%. All allocations of treatments were made on the basis of random number tables (Fisher & Yates, 1963) and the samples were counted blind.

It was possible to estimate the percentage of damaged cells by a direct count. However, the distinction between a damaged and an undamaged cell was not always clear-cut. Another method of assessing the viability of cells was to test their ability to germinate under optimal conditions. The addition of a mixture of V8 broth (5%) and Ca^{2+} (4 mM) was suitable for this purpose. The percentage of germinated cells was then used as an estimate of the viable cells in a population. All assays for viability were carried out at 22 ± 2 °C for 180 min, before the cells were killed by adding glutaraldehyde. Viability assays were carried out in the presence of the ionic species under test. It was shown in preliminary experiments that if the ions under test were added to swimming zoospores at the same time as the Ca^{2+} /V8 medium only Cs^+ had any effect on germination: with Cs^+ at 50 mM, germination was reduced from $98 \pm 1\%$ to $92 \pm 2\%$.

RESULTS

Three stages of zoospore development were distinguished.

1. *Motile zoospores*. After fixation, these were ovoid to elliptical in longitudinal view, and elliptical with a well-defined groove when viewed end on. No cell wall was visible and the cells had a clear or translucent appearance. Flagella were visible when the cells were viewed under phase-contrast or Nomarski interference microscopy. The cells appeared refractile under bright field microscopy.

2. *Cysts or cystospores*. These were round, with a clearly defined outline.

3. *Germinated cysts*. These cells had clearly visible germ tubes. However, some treatments gave rise to cyst papillae (Palzer, 1976; Ho & Zentmyer, 1977). The cyst papillum appeared as a clearly defined swelling or protuberance on the surface of the cyst.

In addition, damaged cells were present after certain treatments. These were sometimes swollen, and lacked the clear outline of undamaged cells. Some treatments also induced a high proportion of lysed cells and cell fragments were visible. The extent of lysis was measured by the difference between the initial and the final cell concentration.

Effect of temperature

Both the viability and motility of zoospores were affected by temperature. The highest proportion of motile cells was maintained between 10 °C and 20 °C, although even in this

Fig. 1

Fig. 2

Fig. 1. Effect of temperature on zoospores and cysts. Cell suspensions were held in 1 mM-Tris-succinate buffer, pH 6, for 2 h at the temperatures indicated before assay: cyst viability ($\square$), zoospore viability ($\circ$) and motility ($\bullet$). Results are expressed as the percentage of the total population which was viable or motile. The error bars represent ± 1 s.d.

Fig. 2. Effect of pH on zoospore viability. Zoospore suspensions in 1 mM-Tris-succinate buffer, pH 6, were held for 30 min at 19 °C at the pH values shown and then assayed for motility ($\bullet$) and viability ($\circ$).

temperature range a high proportion of cells encysted during the 2 h incubation period (Fig. 1). Below 10 °C and above 25 °C both the number of motile cells and the number of viable cells decreased rapidly. Temperature also affected the behaviour of the cells in more subtle ways. For example, above 25 °C, the zoospores were much more liable to encyst as a result of mechanical shock. Cyst populations were similar to zoospores in their response to temperatures above 25 °C. By contrast, cysts were much more resistant than zoospores to temperatures below 10 °C (Fig. 1).

Effect of pH

Zoospores remained motile over the pH range 4.5–8.5 and viability did not vary over this range. At the extremes of pH, the number of motile zoospores fell markedly (Fig. 2). As the H^+ concentration increased above 10 μM (pH 5), an increasing proportion of zoospores were immobilized. They rounded up, but only a small proportion (less than 30% at pH 4.1) formed cyst walls resistant to 1 M-KOH. After exposure to pH 4.1 for 1 min the immobilized cells could not form alkali-resistant walls on shaking, nor when returned to pH 5.8 and incubated for 30 min. The response of cysts in viability tests at the extremes of pH was indistinguishable from that of zoospores.

Effect of cations

Zoospores of *P. cinnamomi* were affected by both the species of cation and its concentration. The effects observed ranged from transitory changes in swimming pattern to an increase in the rate of encystment and/or a decrease in the viability of cells. Here we report only the quantifiable effects on the transitions from zoospore to cyst and cyst to germinating cyst, as well as loss of viability. The rates at which ions induced changes in zoospores varied widely. Some ions, such as K^+ and Ca^{2+} acted rapidly: exposure to K^+ (10 mM) immobilized 80% of the zoospore population in 7 min and Ca^{2+} (25 mM) had the same effect after 20 min.

Fig. 3

Fig. 4

Fig. 3. Effect of KCl (O) and NaCl (●) on zoospore encystment. Salts were added to zoospore suspensions to give the final concentrations indicated and the cells were fixed with glutaraldehyde after 20 min at 19 °C. No cell loss was detected in any sample.

Fig. 4. Effect of NH₄Cl (O), CsCl (●) and LiCl (□) on zoospore encystment. Conditions were as for Fig. 3 except that the incubation period was extended to 40 min.

On the other hand, Mg²⁺ (25 mM) acted much more slowly, requiring 90 min to immobilize 80% of the zoospores. Na⁺ had a partial effect only, as exposure to Na⁺ (25 mM) for 15 min immobilized 50% of the zoospore population but there was then no further immobilization in the next 45 min.

The effect of various concentrations of Li⁺, Na⁺, K⁺, Cs⁺ and NH₄⁺ on immobilization of the swimming zoospores is shown in Figs 3 and 4. There were marked differences in the response to the different ions, with Li⁺ at up to 50 mM inducing no encystment while 5 mM-K⁺ induced essentially complete encystment. The order of effectiveness in the induction of encystment was K⁺ > NH₄⁺ > Na⁺ > Cs⁺ > Li⁺. The effect on viability was rather different (Fig. 5). Exposure to K⁺ at 14 mM reduced viability to 60% but an increase in K⁺ concentration above this level did not decrease viability further. Exposure to Na⁺, NH₄⁺, Li⁺ and Cs⁺ all resulted in a decrease in viability with increase in concentration.

Both Cs⁺ and NH₄⁺ treatments resulted in a high proportion of swollen and obviously damaged cells. The Li⁺ treatment produced cells which had the appearance of motile zoospores but which moved only sluggishly, and these cells did not encyst or germinate on addition of Ca²⁺/V8 broth. The order of effectiveness of Group I cations in causing cell damage was Cs⁺ > Li⁺ > K⁺ > Na⁺. The damage caused by NH₄⁺ relative to the other monovalent cations tested was very dependent upon concentration. Below 10 mM, NH₄⁺ had little effect on either motility or viability. However, at 50 mM it was second only to K⁺ in causing immobilization and to Cs⁺ in the reduction of viability of zoospore populations.

Cells incubated in solutions of mannitol or sorbitol at concentrations up to 100 mM did not show significant increases in the rates of encystment or decrease in viability. This, together with the differences in the effects of individual ions, makes it very unlikely that changes in osmotic pressure have a large effect on the processes observed. Even NaCl, the salt least effective in inducing encystment, was more effective than mannitol or sorbitol.

Fig. 5

Fig. 6

Fig. 5. Effect of monovalent cations K^+ ($\square$), Na^+ ($\circ$), NH_4^+ ($\bullet$), Li^+ ($\triangle$) and Cs^+ ($\blacksquare$) on zoospore viability. Conditions were as for Fig. 3 except that the incubation period was extended to 40 min for Li^+ - and Cs^+ -treated samples. Viability was assessed following the addition of Ca^{2+} /V8 and incubating at 24 °C for 3 h. No cell loss was detected in any sample.

Fig. 6. Effect of divalent cations Sr^{2+} ($\circ$), Ca^{2+} ($\bullet$), Mg^{2+} ($\square$) and Ba^{2+} ($\blacksquare$) on zoospore immobilization. Conditions were as for Fig. 4 except that Mg^{2+} -treated samples were incubated for 90 min at 20 °C prior to fixation. No cell loss was detected in Ca^{2+} -, Sr^{2+} - or Ba^{2+} -treated samples, but loss occurred in the presence of Mg^{2+} .

The effect of four divalent cations of Group IIA is shown in Fig. 6. All the ions in this series promoted encystment, with Sr^{2+} being the most effective. Mg^{2+} , the least effective, required 90 min exposure before detectable effects could be observed. At this time cells were rounded and often swollen. Some of the cells retained reduced flagellar activity resulting in slow rotary motion. At a concentration of 10 mM the order of effectiveness in inducing encystment was $Sr^{2+} > Ca^{2+} > Ba^{2+}, Mg^{2+}$. Both Ca^{2+} and Sr^{2+} induced encystment without any cell damage, and in Ca^{2+} encystment was followed immediately by germination. The germination rate was higher in $Ca(NO_3)_2$ than in $CaCl_2$ and this was one instance where the anionic species influenced the zoospore response. Germination did not immediately follow encystment after the addition of Sr^{2+} unless nutrient was added.

The trivalent cation La^{3+} has a similar ionic radius to Ca^{2+} and has been shown to act in a similar fashion in a number of biological systems although at lower concentrations (Takata *et al.*, 1967). When La^{3+} was added to zoospore suspensions it was found that cells were immobilized at 0.1–1 μM almost immediately (Fig. 7). Cells which had been treated with La^{3+} were not viable, and at La^{3+} concentrations above 1 μM there was complete lysis of the cell population. Therefore La^{3+} does not appear to act as an analogue of Ca^{2+} in this system, and mimics Ca^{2+} only to the extent of causing immediate immobilization.

Fe^{3+} and Mn^{2+} both induced encystment of the entire population of cells, Fe^{3+} at 15 μM and Mn^{2+} at 500 μM . However, cells which encysted in the presence of these ions were completely viable, and thus Fe^{3+} and Mn^{2+} resembled Ca^{2+} and Sr^{2+} rather than La^{3+} in their effect. The trivalent cation Al^{3+} had no effect on either viability or encystment at the highest concentration tested (30 μM , pH 5.5).

Thus, *P. cinnamomi* zoospores were sensitive to all the cations tested. Different cations caused responses which differed both qualitatively and quantitatively and these are

Fig. 7. Effect of La^{3+} (O), Fe^{3+} (●), Mn^{2+} (□) and Ca^{2+} (■) on zoospore encystment. Conditions were as for Fig. 3 except that the incubation period was extended to 30 min for La^{3+} -, Fe^{3+} -, and Mn^{2+} -, and 40 min for Ca^{2+} -treated samples.

Table 1. *Summary of effects of inorganic cations on zoospores*

Cation	Concn (mM)	Response after 30 min	
		Immobilization (%)	Damage (%)
Control	—	15	5
La^{3+}	0.001	100	75
Fe^{3+}	0.015	92	20
Al^{3+}	0.030	<20	<10
H^{+}	0.1	70	11
Mn^{2+}	0.5	98	27
K^{+}	5	95	30
Ca^{2+} *, Sr^{2+}	20	90	5
NH_4^{+}	30	85	50
Mg^{2+}	30	50	60
Na^{+}	30	35	20
Li^{+}	30	20	65
Cs^{+}	30	35	90

* Ca^{2+} -induced cysts germinate spontaneously.

summarized in Table 1. The trivalent cations, Fe^{3+} and La^{3+} , were effective at concentrations several orders of magnitude lower than those required for the mono- and divalent cations, but Al^{3+} was without effect.

Effect of anions

In all of the experiments described above the Cl^{-} salts were used, except in the case of La^{3+} in which the NO_3^{-} salt was used. There was no difference between the Cl^{-} , NO_3^{-} , SO_4^{2-} and PO_4^{3-} salts when Na^{+} was the common cation (Table 2). Where there was an increase in the percentage of encystment it could be shown to be due to the variation in Na^{+} concentration. Acetate, and to a lesser extent fluoride, reduced zoospore motility and viability.

Effect of cations on cysts

When cysts which had been induced to form by shaking were incubated in the presence of the various cations it was found that they were less sensitive than the swimming zoospores (Table 3). Only Cs^{+} reduced the viability greatly, to 3% at 50 mM. Both K^{+} and NH_4^{+} also reduced viability but none of the other ions tested had significant effects ($P = 0.05$).

Table 2. *Effect of various salts on zoospore encystment and viability*Values are the means of five replicates $\pm$ standard deviation.

Addition	Concn (mM)	Encystment at 60 min (%)	Viability at 60 min (%)
Control	—	16 $\pm$ 5	95 $\pm$ 4
NaCl	20	18 $\pm$ 7	91 $\pm$ 7
NaCl	40	79 $\pm$ 7	64 $\pm$ 12
NaNO ₃	20	39 $\pm$ 11	91 $\pm$ 4
Na ₂ SO ₄	20	74 $\pm$ 11	70 $\pm$ 8
NaH ₂ PO ₄ /Na ₂ HPO ₄ , pH 6	20	51 $\pm$ 15	88 $\pm$ 7
NaF	20	33 $\pm$ 7	67 $\pm$ 8
Na(CH ₃ COO), pH 6.8	20	94 $\pm$ 12	48 $\pm$ 14
KCl	20	ND	56 $\pm$ 10
NH ₄ Cl	20	ND	45 $\pm$ 16
CaCl ₂	20	100 $\pm$ 12	96 $\pm$ 2

ND, No data.

Table 3. *Effect of cations on cyst viability*

Cysts were exposed for 40 min at 19 °C.

Addition	Concn (mM)	Viability (%)
Control	—	84 $\pm$ 6
Na ⁺	50	80 $\pm$ 7
K ⁺	50	58 $\pm$ 7*
Li ⁺	50	68 $\pm$ 10
Cs ⁺	50	3 $\pm$ 3*
NH ₄ ⁺	50	62 $\pm$ 4*
Ca ²⁺	30	100 $\pm$ 3
Mg ²⁺	30	97 $\pm$ 3
Sr ²⁺	30	80 $\pm$ 4
Ba ²⁺	30	90 $\pm$ 2

* Significant at level $P = 0.05$.

DISCUSSION

Our results showing the sensitivity of *P. cinnamomi* zoospores to cations resemble those described by Svensson & Unestam (1975) for zoospores of the biflagellate Oomycete *Aphanomyces astaci*, although it is difficult to make direct comparisons as those authors did not distinguish quantitatively between encystment and germination. They found that LiCl and CsCl were toxic, that zoospores were more sensitive to KCl than to NaCl, and that germination rates were highest in the presence of CaCl₂. They concluded that the initiation of germination was partly due to an osmotic effect on the zoospores as mannitol and mannose were also effective. However, in their work, as in ours, mannitol was less effective than NaCl, and much less effective than KCl or CaCl₂, and it seems more likely that specific ion effects were involved.

Soll & Sonneborn (1972) also concluded that ion effects on the monoflagellate *Blastocladiella emersonii* zoospores were not due to osmotic shock, as sucrose had no effect in their system. In many respects, however, the ion selectivity patterns observed with *B. emersonii* differed significantly from those for biflagellates. K⁺, Na⁺, Cs⁺ and Rb⁺ salts were all found to elicit encystment and germination of *B. emersonii* zoospores, although Li⁺ was found to be toxic. Ca²⁺ and Mg²⁺ were less effective and germ tubes formed in response to Ca²⁺ appeared to be defective, and NH₄⁺ ions had little effect.

Soll & Sonneborn (1972) performed their experiments in the presence of low concentrations of other ions, and low concentrations of ions are thought to be essential for motility of both monoflagellate and biflagellate zoospores (Cameron & Carlile, 1980). In our experiments, no attempt was made to exclude low concentrations of ions either carried over as leachate from the washed mycelium or from the glass containers in which mycelium and zoospores were held. Therefore, we would expect that low concentrations of ions, in the micromolar range, would be present at all times. Such concentrations are apparently sufficient to maintain the motility and viability of *P. cinnamomi* zoospores. At the higher ion concentrations tested in our work, the effect of Ca^{2+} was unique. Although both Sr^{2+} and K^+ were more effective in inducing encystment, only Ca^{2+} triggered encystment followed by germination. Although our experiments do not point to any mechanism, they suggest that Ca^{2+} may well play a specific role in nature in the developmental cycle of this fungus.

It is well established that cations interact with biological membranes at concentrations comparable with those used in the present studies (Williams, 1972). The ability to distinguish between closely similar ions and molecules is a basic function of cell membranes. A similar selectivity is also shown in a range of non-biological systems. It has been shown that the relative order of activity of the alkali cations and cations of higher valencies with many biological and non-biological systems is restricted to a few sequences. These include the Hoffmeister lyotropic series and are related to the field strength of the ions under different conditions (Diamond & Wright, 1969). Thus, it may be predicted that if the effects of cations on zoospores involved a direct interaction of cation with anionic plasmalemma, the relative order of cation activity would correspond to one of these sequences.

The relative order of activity of the alkali cations on encystment was $\text{K}^+ > \text{Na}^+ > \text{Cs}^+ > \text{Li}^+$ which is either sequence V or VI of Diamond & Wright (1969). However, the order of effectiveness in causing cell damage did not correspond to any of the 11 possible sequences. Neither did the order of activity of the divalent cation series on encystment or viability correspond to any of the sequences proposed by Diamond & Wright (1969) for these ions, and the effects of Ca^{2+} differed qualitatively as well as quantitatively from the other divalent ions.

It is known that *Phytophthora* zoospores are repelled by many inorganic cations (Allen & Harvey, 1974; Cameron & Carlile, 1980). This negative chemotactic response occurs at different threshold concentrations for each cation. Comparison of our results with the studies on negative chemotaxis shows that for some cations, e.g. H^+ , Na^+ , K^+ , Li^+ and Ca^{2+} , the threshold concentration for inducing negative chemotaxis is close to the minimum concentration for inducing a detectable effect, either encystment or cell damage. For Mg^{2+} and La^{3+} there is no correspondence, Mg^{2+} being less effective and La^{3+} more effective in our system.

For the monovalent cation series, the negative chemotactic response follows the Hoffmeister lyotropic series for cation exchange reactions. Therefore, a general mechanism to explain the phenomenon of negative chemotaxis has been suggested whereby cations interact with the negatively charged zoospore membrane. This reduces the negative charge, thus altering the transmembrane potential and changing flagellar activity (Cameron & Carlile, 1980). However, our data and also that of Cameron & Carlile (1980) for divalent cations indicates that superimposed on such a general ion-membrane interaction there are specific ion effects. This is to be expected, as it is known that, even where binding constants are similar, different cations modify membrane structure in different ways (Williams, 1972; Papahadjopoulos *et al.*, 1979). Beyond this there is the further situation of site-specific binding of which that of Ca^{2+} has been most intensively studied (Whitfield *et al.*, 1980).

The studies reported in this paper also have practical implications. Although it is hazardous to extrapolate from the laboratory to the field, the results do suggest that soil cation content could influence the behaviour of *P. cinnamomi* zoospores directly. In soils with high K^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} or Fe^{3+} levels, the cells would not remain in the motile stage for long and this, in turn, could influence the rate of dispersion. The levels of the above cations

required to induce encystment are in the range of concentrations reported for soil solutions (Nye & Tinker, 1977), although the levels of NH_4^+ required for any effect on zoospore behaviour would not be expected in natural waters. Failure of Al^{3+} to produce any effect on zoospore motility or viability argues against this ion playing a role in the disease distribution, although it has been shown that this ion does limit mycelial growth of the related *P. capsici* (Muchovej *et al.*, 1980).

Although high cation levels reduce the viability of motile zoospores, once encystment has taken place in the absence of these cations the cells are more resistant to their effects. The results also show that zoospores of *P. cinnamomi* have a broad tolerance to pH. Only the extremes of the acid or alkaline range observed in soil might be expected to inhibit zoospore development. Similarly, zoospores and cysts tolerate a broad range of temperatures, although surface soil temperatures might be expected to exceed 25 °C and fall below 10 °C, the limits of tolerance, on many occasions. Here too, the cyst stage displays a greater tolerance of low temperatures than the motile stage, although a different spore form, the chlamydospore, is the survival stage of this organism.

This work was supported in part by grants from the Victorian Forests Commission and from the Potter Foundation to B. R. G. P. N. B. was supported by a Commonwealth Postgraduate Research Award.

REFERENCES

- ALLEN, R. N. & HARVEY, J. D. (1974). Negative chemotaxis of zoospores of *Phytophthora cinnamomi*. *Journal of General Microbiology* **84**, 28–38.
- BIMPONG, C. E. & CLERK, G. C. (1970). Motility and chemotaxis in zoospores of *Phytophthora palmivora* (Butl.) Butl. *Annals of Botany* **34**, 617–624.
- BIMPONG, C. E. & HICKMAN, C. J. (1975). Ultrastructural and cytochemical studies of zoospores, cysts and germinating cysts of *Phytophthora palmivora*. *Canadian Journal of Botany* **53**, 1310–1327.
- BOUGHTON, T. J., MALAJCZUK, N. & ROBSON, A. D. (1978). Suppression of the infection of jarrah roots by *Phytophthora cinnamomi* with application of calcium carbonate. *Australian Journal of Botany* **26**, 611–615.
- BROADBENT, P. & BAKER, K. R. (1974). Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. *Australian Journal of Agricultural Research* **25**, 121–137.
- BYRT, P. N. & GRANT, B. R. (1979). Some conditions governing zoospore production in axenic cultures of *Phytophthora cinnamomi* Rands. *Australian Journal of Botany* **27**, 103–115.
- CAMERON, J. N. & CARLILE, M. J. (1980). Negative chemotaxis of zoospores of the fungus *Phytophthora palmivora*. *Journal of General Microbiology* **120**, 347–353.
- DIAMOND, J. M. & WRIGHT, E. M. (1969). Biological membranes: the physical basis of ion and non-electrolyte selectivity. *Annual Review of Physiology* **31**, 581–646.
- FISHER, R. A. & YATES, F. (1963). *Statistical Tables for Biological, Agricultural and Medical Research*, 6th edn. Edinburgh: Oliver and Boyd.
- HALSALL, D. M. & FORRESTER, R. I. (1977). The effects of certain cations on the formation and infectivity of *Phytophthora* zoospores. I. Effects of calcium, magnesium, potassium and iron ions. *Canadian Journal of Microbiology* **23**, 994–1001.
- HEMMES, D. E. & HOHL, H. R. (1971). Ultrastructural aspects of encystment and cyst germination in *Phytophthora parasitica*. *Journal of Cell Science* **9**, 175–191.
- HO, H. H. & ZENTMYER, G. A. (1977). Morphology of *Phytophthora cinnamomi*. *Mycologia* **69**, 701–713.
- LUNNEY, C. Z. & BLAND, C. E. (1976). Ultrastructural observations of mature and encysting zoospores of *Pythium proliferum* de Bary. *Protoplasma* **90**, 119–137.
- MUCHOVEJ, J. J., MAFFIA, L. A. & MUCHOVEJ, R. M. C. (1980). Effect of exchangeable soil aluminium and alkaline calcium salts on the pathogenicity and growth of *Phytophthora capsici* from green pepper. *Phytopathology* **70**, 1212–1214.
- NYE, P. H. & TINKER, P. B. (1977). *Solute Movement in the Soil-Root System*. Oxford: Blackwell Scientific Publications.
- PALZER, C. (1976). *Zoospore inoculum potential of Phytophthora cinnamomi*. Ph.D. thesis, University of Western Australia.
- PAPAHADJOPOULOS, D., POSTE, G. & VAIL, W. J. (1979). Studies on membrane fusion with natural and model membranes. *Methods in Membrane Biology* **10**, 1–121.
- SING, V. O. & BARTNICKI-GARCIA, S. (1975). Adhesion of *Phytophthora palmivora* zoospores: electron microscopy of cell attachment and cyst wall fibril formation. *Journal of Cell Science* **18**, 123–132.
- SOLL, D. R. & SONNEBORN, D. R. (1972). Zoospore

- germination in *Blastocladiella emersonii*. IV. Ion control of cell differentiation. *Journal of Cell Science* **10**, 315–333.
- SVENSSON, E. & UNESTAM, T. (1975). Differential induction of zoospore encystment and germination in *Aphanomyces astaci*, Oomycetes. *Physiologia plantarum* **35**, 210–216.
- TAKATA, M., PICKARD, W. F., LETTVIN, J. Y. & MOORE, J. W. (1967). Ionic conductance changes in lobster axon membrane when lanthanum is substituted for calcium. *Journal of General Physiology* **50**, 461–471.
- TOKUNAGA, J. & BARTNICKI-GARCIA, S. (1971). Cyst wall formation and endogenous carbohydrate utilization during synchronous encystment of *Phytophthora cinnamomi* zoospores. *Archiv für Mikrobiologie* **79**, 283–292.
- WHITFIELD, J. F., BOYNTON, A. C., MACMANUS, J. P., RIXON, R. H., SIKORSKA, M., TSANG, B., WALKER, P. R. & SWIERANGA, S. H. H. (1980). Role of calcium and cyclic AMP in cell proliferation. *Annals of the New York Academy of Sciences* **339**, 216–240.
- WILLIAMS, R. J. P. (1972). A dynamic view of biological membranes. *Physiological Chemistry and Physics* **4**, 427–439.