The Effect of Organic Compounds on the Encystment, Viability and Germination of Zoospores of *Phytophthora cinnamomi*

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(Received 25 November 1981; revised 8 March 1982)

A number of organic compounds were tested to determine their ability to induce encystment or germination of zoospores of the fungus, *Phytophthora cinnamomi*. The basic amino acids, lysine and arginine, induced encystment at concentrations in the millimolar range. Pectin ($500 \mu\text{g ml}^{-1}$) also induced encystment and in addition stimulated the encysted cells to germinate. A wide range of carbohydrates and the amino acids, glutamate and aspartate, stimulated cysts to germinate although they did not themselves induce encystment.

The polypeptide, poly-L-lysine and the basic proteins, histone 1, histone 4 and lysozyme together with the lectin, concanavalin A, immobilized swimming zoospores at concentrations between 0.3 and $10 \mu\text{g ml}^{-1}$. At concentrations below $1 \mu\text{g ml}^{-1}$ cysts were formed but at higher concentrations cells lysed and the viability of the population was reduced. These compounds did not induce germination of the encysted zoospores. Ethanol, isovaleraldehyde and hexanal also induced encystment but only at concentrations in the millimolar range.

These data, together with previous work on cations show that the triggering of motile cells to encyst does not in this species invariably result in the cells being committed to germination.

INTRODUCTION

The infection of plant roots by motile zoospores of the fungus, *Phytophthora cinnamomi* Rands, involves in its initial stages a chemotactic attraction to the root surface or its immediate neighbourhood, followed by encystment (cystospore formation), germination and penetration of host tissue by the emerging germ tube. This behaviour is similar to that of other species of root infecting *Pythiaceae* (Goode, 1956; Hickman & Ho, 1966; Hickman, 1970). The orderly progression through this sequence is essential to colonization of a new host.

To maximize the probability of an individual zoospore infecting susceptible tissue, there must be recognition and response to signals emanating from root surfaces. This response should allow motility to be retained until the spore is within a few millimetres of the root surface and in a locality where, after germination, sufficient nutrient is available to carry it through to the stage where it has access to the contents of living cells.

Simulated root models, complex mixtures such as V8 broth, and some pure compounds and defined mixtures have been used to demonstrate various parts of the sequence *in vitro* (Dukes & Apple, 1961; Royle & Hickman, 1964*a, b*; Zentmyer, 1966; Bimpong & Clerk, 1970; Ho & Hickman, 1967; Khew & Zentmyer, 1973; Halsall, 1976). In general no pure compound approached the activity of root exudate and most were only effective at high concentrations. An exception to this was found in the volatile fatty acids, alcohols and aldehydes, some of which were effective attractants at low concentrations (Allen & Newhook, 1974; Cameron & Carlile, 1978) of the order measured in root exudates (Young *et al.*, 1977). The greater part of the work cited has dealt with the aspect of chemotaxis and less information is available on the triggering of encystment and germination.

A simple hypothesis is that those compounds which induce positive chemotaxis in the motile zoospore act as triggers to encystment and germination once a threshold concentration has been reached. An example of this behaviour was reported with *Aphanomyces cochlioides* (Rai & Strobel, 1966) and with *Allomyces* sp. (Machlis, 1969). An alternative is that the stimuli required for positive chemotaxis, encystment and cystospore germination are different and that the processes are independent of each other. Some mono- and disaccharides attract zoospores without triggering encystment (Royle & Hickman, 1964*b*; Rai & Strobel, 1966; Khew & Zentmyer, 1973) while others cause encystment but do not act as attractants (Khew & Zentmyer, 1973; Bimpong & Clerk, 1970; Tokunaga & Bartnicki-Garcia, 1971; Allen & Harvey, 1974). These observations appear to support the second hypothesis.

Previous work with cation additions (Byrt, 1980, Byrt *et al.*, 1982) distinguished the following responses by populations of motile *P. cinnamomi* zoospores.

(a) Cells underwent encystment. The elliptical cells became spherical and an alkali-resistant cell wall was secreted. The transformation was rapid and synchronous. This response was seen following the addition of Ca^{2+} or Sr^{2+} .

(b) Cells became immobilized and settled to the bottom of the container. They retained their elliptical shape or assumed a spherical form. Flagella were retained and no cell wall was formed. Immobilized cells sometimes developed large vesicles, became swollen and lysed. This response followed the addition of La^{3+} or Cs^+ .

(c) Cells remained motile, but with the passage of time some cells formed cysts. Encystment was slow and if the observations were continued over several hours, motile zoospores, cysts and germinating cysts could all be seen together in the cell population. This response was observed in distilled water or with low concentrations of some ions such as Na^+ . The responses listed under (a) and (b) were concentration dependent and in some cases, e.g. Fe^{3+} , low concentrations induced encystment, while higher concentrations induced immobilization and lysis. Once formed, cysts either remained as spherical refractile cells or formed a cyst papilla or germinated by production of a hyphal germ tube. Papillated cysts could, under conditions as yet undefined, release a swimming zoospore or form a hyphal germ tube. They could therefore be recognized as viable without further treatment. The viability of spherical cells in the population could only be assessed by the addition of Ca^{2+} /V8 broth, which lead to the production of germ tubes by all viable cells in the population including any motile zoospores present which had not encysted.

In this paper we report the effect of a variety of organic compounds on zoospore populations. The compounds were chosen initially on the basis of their reported presence in root exudate or their capacity to induce positive chemotaxis in the motile cells.

METHODS

The isolate of *P. cinnamomi* used was of the A-2 compatibility type isolated originally from the roots of *Isopogon ceratophyllus* by Dr Gretna Weste. It is lodged with the Commonwealth Mycological Institute, Kew (IMI 252489). The growth of the organism and the release of zoospores from axenic culture has been described previously (Byrt & Grant, 1979). This method yielded 10^4 – 10^5 cells ml^{-1} . After release, the zoospores were held in 1 mM-Tris-succinate buffer, pH 6.5. Leachings from mycelial mats prepared with glass-distilled water in acid washed glassware resulted in some ions always being present in the final zoospore suspension, e.g. Ca^{2+} was of the order of 100 μM . Zoospore suspensions were held at $16 \pm 1^\circ\text{C}$ and used within 2 h.

The methods used to assay the effectiveness of individual compounds as stimulants of encystment or germination, together with the precautions required in the handling of the zoospores have been described in detail elsewhere (Byrt *et al.*, 1982). The percentage of cells encysted or germinated was measured using an inverted microscope, after killing with 1% neutralized glutaraldehyde. The assay for encystment was carried out for 30 min at $19 \pm 1^\circ\text{C}$ except as noted. Viability of the cell population following a treatment was measured by recording the percentage of cells in the total population producing germ tubes following addition of Ca^{2+} /V8 broth. The viability assay extended over 2 h at $22 \pm 2^\circ\text{C}$. Where the ability of a specific compound to stimulate germination of cysts was tested, the cysts were induced either by the addition of Fe^{3+} (Byrt *et al.*, 1982) or by vigorous shaking (Tokunaga & Bartnicki-Garcia, 1971).

Chemicals. With the exceptions listed below chemicals were obtained from Sigma and were the purest grade offered. They were used without further purification. Polygalacturonic acid and pectin (7.5% methoxy content) were purified by dissolving in buffer and dialysing for 48 h against 5 mM-EDTA in 20 mM-Tris/HCl, pH 7.3,

followed by a further 48 h against glass distilled water. The solutions were then freeze dried. L-Alanine, L-asparagine, L-glutamic acid, L-histidine hydrochloride and L-leucine were from Calbiochem, grade A; L-arginine and L-lysine dihydrochloride were from Mann Research, analytical grade. Poly-L-aspartic acid was Mann assayed lot B2977, mol. wt 15000, DP130, poly-L-lysine hydrobromide was Mann assayed lot B2979, mol. wt 340000, DP 1680. These chemicals together with poly-L-alanine were obtained from Schwartz-Mann Biochemicals, Orangeburg, N.Y., U.S.A. Bovine serum albumin fraction V, fat-free, came from Commonwealth Serum Laboratories, Parkville, Victoria and β -crystallin (human) was a generous gift from Dr R. Augusteyn, Melbourne University. The lectins were obtained from P-L Biochemicals, Milwaukee, Wis., U.S.A.

RESULTS

Amino acids, peptides and proteins

The effects of the following amino acids were studied: L-glutamic acid, L-aspartic acid, L-glutamine, L-asparagine, L-alanine, L-leucine, L-lysine, L-arginine and L-histidine stimulated the cells to encyst. They were effective at 20 mM but not at 1 mM. L-Glutamic acid, L-aspartic acid and to a lesser extent L-glutamine, at 1 mM, stimulated the germination of cells encysted by shaking, although they had no effect on encystment by themselves.

Three polypeptides, poly-L-lysine, poly-L-alanine and poly-L-aspartate, were tested, and compared with L-lysine (Table 1). Poly-lysine at a concentration of $1 \mu\text{g ml}^{-1}$ caused immobilization of 90% of swimming zoospores in 30 min and reduced viability to less than 50%. Some cells were transformed into cysts but the majority were not. At higher concentrations the total population lysed. In contrast poly-aspartate ($100 \mu\text{g ml}^{-1}$) immobilized some 58% of the swimming spores (cf. controls 36%), but no lysis was observed, and the cells remained fully viable. Poly-L-alanine had no effect at the highest concentration tested, $50 \mu\text{g ml}^{-1}$. On the basis of equivalent monomer units, poly-L-lysine was at least 10^4 times more effective in immobilization of motile zoospores than L-lysine but since it caused lysis of cells at low concentrations it resembled La^{3+} in its effect (Byrt *et al.*, 1982) rather than acting as an inducer of encystment.

The effects of lysine and poly-lysine suggested that proteins rich in lysine and arginyl residues might also be effective in triggering differentiation in zoospores. The results obtained with a series of these are shown in Fig. 1.

Histone 1, histone 4 (not shown) and lysozyme, all with high isoelectric points (pI about 11) and rich in lysine and arginine residues were effective in immobilizing the swimming zoospores at concentrations in the $\mu\text{g ml}^{-1}$ range, and some true cysts were formed. In the presence of these proteins at concentrations above $0.3 \mu\text{g ml}^{-1}$ the viability of the cell population decreased rapidly. However, myoglobin, a protein with pI of 7, was more effective than cytochrome c, pI 10.6 (not shown) in the induction of encystment, suggesting that interaction with the zoospore was not determined solely by the isoelectric point of the protein. None of the proteins tested stimulated germination of the encysted zoospores although bovine serum albumin and β -crystallin caused agglutination of both zoospores and cysts. Samples which contained $30 \mu\text{g ml}^{-1}$ of either of these latter proteins contained clumps of zoospores or cysts containing from 3 to 60 cells.

Another protein, the lectin concanavalin A, has been shown to induce lysis both in zoospores of the monoflagellate *Blastocladiella emersonii* (Jen & Haug, 1979), and in zoospores of *Phytophthora palmivora* (Sing & Bartnicki-Garcia, 1975*b*). Receptors to concanavalin A have been demonstrated on *P. palmivora* zoospores and cysts (Sing & Bartnicki-Garcia, 1975*a*) and more recently on *P. cinnamomi* zoospores (Hardham *et al.*, 1981).

To extend these observations, five proteins with lectin activity were tested for their effects on the motile zoospores. The results are shown in Table 2. Concanavalin A, Castor bean lectin and Phytohaemagglutinin P induced encystment and reduced the viability of the zoospore population. Concanavalin A was active at $0.3 \mu\text{g protein ml}^{-1}$, but Castor bean and Phytohaemagglutinin P lectins required $30 \mu\text{g ml}^{-1}$ to show any effect. At this concentration all three lectins reduced viability and in the case of concanavalin A induced lysis of a significant number of cells in addition to inducing encystment. The response of *P. cinnamomi* zoospores to concanavalin A appears to differ from that of *P. palmivora*, in which concentrations of $25\text{--}30 \mu\text{g ml}^{-1}$ induced lysis but no effect was reported at lower concentrations (Sing & Bartnicki-Garcia, 1975*b*).

Fig. 1. Effect of proteins on zoospores of *P. cinnamomi*. (a) Immobilization. ●, Casein (pI 5); ○, myoglobin (pI 7.0); □, lysozyme (pI 11); △, histone 1 (pI 11). X is the value obtained with the maximum concentration of β -crystallin tested, and ▲ is the value obtained with the maximum concentration of bovine serum albumin. The data points for histone 4 are not shown, but gave a curve superimposable upon that recorded for histone 1. (b) Viability, the symbols used are the same as for (a). The curves for bovine serum albumin and β -crystallin were superimposable on that for casein. In this and subsequent figures the error bars represent ± 1 S.D.

Table 1. *Effect of poly-L-lysine, poly-L-aspartate and L-lysine on encystment and viability*

The experiment was conducted at 19 °C. Viability and immobilization were recorded at 30 min. The values are means of five replicates $\pm$ the standard deviation.

Addition	Concentration		Immobilization (%)	Viability (%)
	$\mu\text{g ml}^{-1}$	mm*		
Poly-L-lysine	20	(0.14)	99 $\pm$ 1	0†
	10	(0.068)	99 $\pm$ 1	0†
	3	(0.02)	96 $\pm$ 3	0†
	1	(0.007)	90 $\pm$ 9	<50‡
	0.3	(0.002)	85 $\pm$ 10	ND
Poly-L-aspartate	100	(0.75)	58 $\pm$ 5	89 $\pm$ 6
Poly-L-alanine	50	—	36 $\pm$ 5	89 $\pm$ 6
L-Lysine	—	20	50 $\pm$ 6	84 $\pm$ 4
	—	1	45 $\pm$ 12	89 $\pm$ 2
	—	0.5	32 $\pm$ 6	ND
Control	—	—	36 $\pm$ 5	89 $\pm$ 6

ND, No data.

* The numbers in parentheses indicate mmol of L-lysine or L-aspartate present as poly-L-aspartate, or poly-L-lysine.

† Total lysis.

‡ Partial lysis; <50% of remaining cells germinated.

Table 2. Effect of lectins on *P. cinnamomi* zoospore viability and encystment

Addition		Proportion of cell population encysted and viable (%)			
		0.0	Concn of lectin ($\mu\text{g ml}^{-1}$):		
			0.3	3.0	30.0
Phytohaemagglutinin P	E	39 $\pm$ 3	40 $\pm$ 6	39 $\pm$ 9	87 $\pm$ 7*
	V	93 $\pm$ 3	94 $\pm$ 4	89 $\pm$ 2	58 $\pm$ 5*
Concanavalin A	E	38 $\pm$ 7	86 $\pm$ 3*	85 $\pm$ 7*	100 $\pm$ 1*
	V	89 $\pm$ 3	62 $\pm$ 11*	64 $\pm$ 12*	15 $\pm$ 2*
Castor bean (native) lectin	E	43 $\pm$ 5	45 $\pm$ 7	52 $\pm$ 6	63 $\pm$ 6*
	V	80 $\pm$ 6	78 $\pm$ 5	78 $\pm$ 3	71 $\pm$ 2*
Anti-H lectin Type 1 (<i>Ulex europaeus</i>)	E	35 $\pm$ 5	36 $\pm$ 6	36 $\pm$ 2	39 $\pm$ 6
	V	93 $\pm$ 2	88 $\pm$ 3	90 $\pm$ 2	91 $\pm$ 2
Potato lectin	E	35 $\pm$ 2	39 $\pm$ 4	48 $\pm$ 2	43 $\pm$ 7
	V	93 $\pm$ 2	95 $\pm$ 2	93 $\pm$ 2	90 $\pm$ 3

E, encysted; V, viable.

* The means are significantly different from the control at the level $P = 0.05$ using Student's *t*-test. All values are means of four replicates. The mean level for zoospore encystment in the absence of any addition is higher than usual since the experiments were carried out at $22 \pm 2^\circ\text{C}$ rather than the usual $19 \pm 2^\circ\text{C}$.

Carbohydrates

A range of carbohydrates were tested for their effect on zoospore motility. None had any effect at concentrations as high as 50 mM. Compounds tested were D-glucose, D-mannose, D-galactose, D-arabinose, L-arabinose, D-xylose, D-ribose, D-fucose, L-fucose, L-rhamnose, galacturonic acid, sucrose, maltose, cellobiose, raffinose, arabinogalactan, amylose, amylopectin and dextrin. Two other polysaccharides, pectin and polygalacturonic acid, were effective in inducing encystment at the highest concentrations tested ($500 \mu\text{g ml}^{-1}$) (Table 3). Both polymers were neutralized by addition of NaOH prior to use, but the amount of Na^+ present was insufficient to account for the immobilization recorded (Byrt *et al.*, 1982). Pectin solutions also induced germination in a high percentage of cells without addition of a further stimulus.

Dialysis of solutions against EDTA removed the capacity of polygalacturonic acid but not pectin to induce encystment. Dialysis reduced the ash content of the pectin from 7.1 ± 0.3 to $3.5 \pm 0.6\%$ and of the polygalacturonic acid from 6.9 ± 0.1 to $2.0 \pm 0.7\%$. Pectin is known to have a high affinity for Ca^{2+} , Cu^{2+} , Zn^{2+} and Fe^{3+} (Fogarty & Ward, 1974). However, analysis of ash samples from the dialysed pectin by energy dispersive X-ray fluorescence showed none of these ions to be present at concentrations above $1 \mu\text{g ml}^{-1}$. Therefore the action of pectin is unlikely to be due to metal ions retained as impurities.

The inability of fucose to elicit encystment is interesting since it has recently been suggested that fucosyl residues on plant root surfaces mediate adhesion of the zoospore to specific sites on maize roots (Hinch & Clarke, 1980) and it has also been shown that the zoospores of *P. cinnamomi* contain specific fucosyl binding sites on their surface membranes (Hinch, 1981). It seems therefore that adhesion and encystment are independent processes since free fucose is not an effective inducer of encystment.

Despite their lack of effectiveness as inducers of encystment, all carbohydrates stimulated germination to some extent. The range of compounds listed above were screened and from them selected compounds were studied in more detail (Table 4). Glucose was the most effective stimulator of germination at 50 mM, while galacturonic acid was least effective at this concentration. With glucose the stimulation was shown to be concentration dependent, detectable at 0.3 mM, but requiring 50 mM to approach saturation (Fig. 2). Germination of encysted zoospores in the presence of added sugar was similar in its time course to cysts germinated in the presence of V8 broth or Ca^{2+} (Fig. 3). In distilled water, germination was slow and the percentage germination over a 2 h period was low; a large percentage of the cysts had formed papillae. If the sugar was to be effective in the stimulation of zoospore germination, it had to be present at the time encystment was initiated in order to obtain maximum effect (Fig. 4). The data presented in

Fig. 2. Effect of glucose concentration on the germination of *P. cinnamomi* cysts.

Table 3. *Effect of pectin and polygalacturonate on encystment of zoospores*

All treatments were for 20 min at $20 \pm 2^\circ\text{C}$. The final concentrations of pectin and polygalacturonate were $500 \mu\text{g ml}^{-1}$. The pectin contained 7.5% methyl ester. During the 20 min treatment the percentage of swimming zoospores fell from 85 ± 3 to $70 \pm 8\%$ in the control samples. All results are the mean of five replicates $\pm$ the standard deviation.

Addition	Encysted zoospores (%)
Control	30 ± 8
Pectin	78 ± 5
Pectin (dialysed)	68 ± 5
Polygalacturonate	55 ± 5
Polygalacturonate (dialysed)	$37 \pm 4^*$

* Not significantly different from the control value at the level $P = 0.05$, using Student's *t*-test. The mean values for the dialysed polysaccharides were different from those of the crude material at the level of $P = 0.01$ using Student's *t*-test.

Table 4. *Effect of sugars on germination of cysts*

Values in which standard deviations are shown are the means of five replicates. The other values are the means of duplicates. The results with 1 and 50 mM sugars were obtained with different batches of zoospores.

Addition	Proportion of cysts germinated* (%)	
	Concn of sugar (mM):	
	1	50
D-Glucose	57 ± 1	81 ± 6
D-Galactose	50 ± 1	60 ± 8
L-Arabinose	22 ± 9	57 ± 2
D-Xylose	21 ± 3	66 ± 4
D-Ribose	22 ± 5	61 ± 10
D-Fucose	19 ± 2	58 ± 11
Sucrose	23 ± 4	79
Cellobiose	25 ± 10	94
Galacturonic acid	29	37 ± 9
Control	12 ± 3	14 ± 7

* As determined by observing presence or absence of germ tubes at 2 h.

Fig. 4 were obtained with sucrose, 150 mM, but similar results were obtained using glucose. If added after 10 min the sugar had no effect and there was a high proportion of cysts forming cyst papillae, an indication that cells were in nutrient poor conditions (Ho & Hickman, 1967; Hemmes & Hohl, 1971). In addition to the simple mono- and disaccharides, a wide range of polysaccharides with different glycosyl linkages, different sugar units and different molecular weights all stimulated germination of cysts. The compounds tested at concentrations of

Fig. 3

Fig. 3. Time course of cyst germination in the presence of various additives: ○, 10 mM-CaCl₂; ●, 50 mM-glucose; ▲, V8 broth; ■, no addition. In each case the cysts were induced by shaking and the addition made immediately after induction. Bar represents two S.D. units.

Fig. 4

Fig. 4. Effect of delaying addition of nutrient following the induction of encystment on the number of cysts germinated after 2 h. ○, Cysts germinated with germ tubes; ●, cysts forming papillae. The number of germinated cysts in the absence of any addition was 42%.

10 mg ml⁻¹ were pectin, amylose, polygalacturonic acid, arabinogalactan, agar, pachyman, lichenan, laminarin, pullulan and cellopentaose. Pectin, with 95% of the cells forming germ tubes, was the most effective and cellopentaose was least effective (60%). This widespread stimulation of germination which does not have any obvious relationship to structure or to metabolism was also shown with another group of compounds, the sulphonic acid buffers commonly used in cell-free systems (Good *et al.*, 1966). These buffers, at 10 mM and pH values within the range of 6–7.5, stimulated 50–80% of the cells to form germ tubes. They had no effect on encystment (Byrt, 1980). The formation of cyst papillae deserves comment. It has been known for some time that fungi of this genus form papillated cysts (Ho & Hickman, 1967; Hemmes & Hohl, 1971; Palzer, 1976). However, the factors which control their formation and germination are not known and their importance in the differentiation strategy of the fungus has not been recognized. In previous work we have not distinguished between cysts and cysts which have formed papillae, but these results together with more recent work (unpublished) suggest that the formation of a cyst papilla may be the true alternative to germination. Cysts with papillae remain viable and subsequently recommence germination by germ tube formation if conditions become favourable to the mycelial stage of the fungus. Alternatively they can be de-differentiated to release a swimming zoospore, which gives the organism an opportunity to attempt colonization in another location.

Alcohols, aldehydes and organic acids

The effectiveness of certain volatile alcohols and aldehydes as attractants to *P. palmivora* zoospores (Cameron & Carlile, 1978) suggests that these might, at higher concentrations, act as inducers of encystment. The results presented in Table 5 showed that they were relatively ineffective, requiring concentrations very much higher than those at which they acted as attractants. Concentrations below 10 mM (not shown) had no effect. It is likely, therefore, that these compounds are not important as inducers of encystment in nature. Hexanal, at 30 mM, completely inhibited germination, and at 10 mM both hexanal and isovaleraldehyde reduced germination significantly. Our results differ from those of Allen & Newhook (1974) who found that ethanol was an effective inducer of encystment when tested in a capillary system. In

Table 5. *Effect of ethanol, propionaldehyde, hexanal and isovaleraldehyde on zoospore encystment and viability*

Addition		Proportion of cell population encysted and viable (%)		
		Concn of addition (mM):		
		0	10	30
Ethanol	E	37 ± 4	47 ± 6*	47 ± 9*
	V	88 ± 3	91 ± 3	88 ± 5
Propionaldehyde	E	42 ± 1	55 ± 8*	61 ± 3*
	V	86 ± 3	82 ± 4	80 ± 3*
Hexanal	E	35 ± 3	51 ± 6*	64 ± 8*
	V	79 ± 3	63 ± 8*	0
Isovaleraldehyde	E	39 ± 2	63 ± 7*	—
	V	87 ± 5	78 ± 2*	—

E, encysted; V, viable.

* The means are different from controls at the level of $P = 0.05$ using Student's *t*-test. Five replicates were used throughout. The levels of zoospore encystment in the absence of any addition is higher than usual, since these experiments were carried out at $22 \pm 2^\circ\text{C}$ rather than the usual $19 \pm 2^\circ\text{C}$.

addition ascorbic, acetic, oxalic, malic and succinic acids were tested. Of these, only acetate had any effect and at 20 mM, the maximum concentration tested, the entire population was immobilized, and the cells were not viable. Although some encystment and a reduction in viability was observed in the presence of other acids, this could be accounted for by the sodium ions added to neutralize the solutions. None of the acids tested stimulated germination, and oxalate was slightly inhibitory.

DISCUSSION

The object of this work is to contribute towards the understanding of the behaviour of the pathogen, *P. cinnamomi*, in the vicinity of plant roots. Each treatment was assessed in terms of immobilization of zoospores and germination of cysts. In some species of fungi a distinction between immobilization and encystment may be artificial, as the two processes are inseparable, with a single external stimulus inducing an ordered, rapid and irreversible change from a swimming zoospore to a germinating cystospore. This has been clearly established in *Blastocladiella emersonii* zoospores (Soll & Sonneborn, 1972).

In the presence of pectin (this study) and Ca^{2+} (Byrt *et al.*, 1982) the *P. cinnamomi* cells are transformed from swimming zoospores to germinating cystospores. This response to a single external trigger resembles that of *Blastocladiella*. However, with *P. cinnamomi* other possibilities exist. The presence of Fe^{3+} or basic amino acids within certain concentration ranges induces encystment but cells induced are not committed to germinate. In order to proceed to germ tube formation, other compounds such as simple sugars or the acidic amino acids must be present at the time the stimulus to encystment is received. These compounds, which allow differentiation to proceed to the stage of germination, have not by themselves any capacity to induce encystment. The possibility of separating encystment from germination in this way argues against a cascade effect in which one single external trigger irreversibly commits the cell to transform from the motile state to the germinating state.

Polycations, such as poly-L-lysine, and the basic proteins together with concanavalin A are toxic to motile cells at low concentrations. Our data suggest that within narrow concentration limits they may also trigger encystment, but their potency in disrupting the cell membranes is such that this is difficult to quantitate with the technique used.

In nature, at the root-soil interface, pectin from the root cap or root slime either alone or complexed with metal ions, may be an important natural trigger to germination. It is equally possible that free Ca^{2+} or H^+ present in the root zone is effective. Induction of encystment in this zone would mean that soluble carbohydrates were present and this would ensure that the motile cell differentiated to the stage of germ tube formation.

This work was supported by grants from the Ian Potter Foundation, the Dieback Research Committee, and the Australian Research Grants Committee.

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