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THE EFFECT OF ORGANIC COMPOUNDS ON THE ENCYSTMENT AND GERMINATION OF ZOOSPORES OF THE CINNAMON FUNGUS, *PHYTOPHTHORA CINNAMOMI*

Pauline N. Byrt, Helen R. Irving and Bruce R. Grant*

Russell Grimwade School of Biochemistry,

University of Melbourne,

Parkville, Victoria 3052, Australia.

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*To whom correspondence should be addressed.

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2. Zoospores, encystment and germination.
3. Zoospores, effects of organic compounds on.
4. Encystment of *Phytophthora* zoospores.
5. Germination of *Phytophthora* zoospores.

SUMMARY

A number of organic compounds were tested to determine their ability to induce encystment or germination of zoospores of the cinnamon fungus, *Phytophthora cinnamomi*. The basic amino acids, lysine and arginine, induced encystment at concentrations in the mM range. Pectin (500 $\mu g\ ml^{-1}$) 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, lysozyme and cytochrome c, together with the lectin, concanavalin A, immobilized swimming zoospores at concentrations between 0.3 and 10 $\mu g\ ml^{-1}$. At concentrations below 1 $\mu g\ ml^{-1}$ 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 mM range.

This data, together with that obtained from previous work on cations (Byrt *et al.*, 1982), shows 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 cinnamon 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 millimeters 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 V-8 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, 1964a,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. It has been shown that some mono- and disaccharides attract zoospores without triggering encystment (Royle & Hickman, 1964b; 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. Flagellae 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 asynchronous. 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 papillum or germinated by production of a hyphal germ tube. Papillated cysts could, under conditions as yet undefined, release a single swimming zoospore or form a hyphal germ tube. The addition of Ca^{2+}/V-8 broth to cell populations resulted in the differentiation of all viable cells into germinating cysts and allowed the assessment of the viability of the populations.

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° ± 1°C and used within 2 h.

The methods used to assay the effectiveness of individual compounds as stimulators 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% neutralised glutaraldehyde. The assay for encystment was carried out for 30 min at 19° ± 1°C except as noted. The viability assay extended over 2 h at 22° ± 2°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 Chemical Co., Mo., U.S.A. 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 hours against 5 mM EDTA in 20 mM Tris-HCl, pH 7.3, followed by a further 48 hours against glass distilled water. The solutions were then freeze dried. L-Alanine, L-asparagine, L-glutamic acid, L-histidine hydrochloride and L-leucine were Cal-biochem, grade A; L-arginine and L-lysine dihydrochloride were Mann Research Analytical grade. Poly-L-aspartic acid was Mann assayed lot B2977, MW 15,000, DP 130, poly-L-lysine hydrobromide Mann assayed lot B2979, MW 340,000, DP1680. Poly-L-alanine was obtained from Schwartz Biochemicals. Bovine serum albumin fraction V, fat-free, came from Commonwealth Serum Laboratories and β-crystallin (human) was a generous gift from Dr. R. Augusteyn, Melbourne University. The lectins were obtained from P-L Biochemicals, Wis, U.S.A.

RESULTS

Amino Acids, Peptides and Proteins

The effects of a range of amino acids are listed in Table 1. Of these, only three, L-lysine, L-arginine and L-histidine, stimulated encystment. They were effective at 20 mM but not at 1 mM. Although L-glutamic acid was ineffective as an inducer of encystment, it did increase the germination of encysted zoospores as did L-aspartic acid and L-histidine (Table 1). Here they were effective at 1 mM.

Three polypeptides, poly-L-lysine, poly-L-alanine and poly-L-aspartate, were tested, and compared with L-lysine (Table 2). Poly-lysine at a concentration of 1 $\mu g\ ml^{-1}$ caused immobilization of 90% of swimming zoospores in 30 minutes 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 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 $\mu g\ ml^{-1}$. 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. 1a and b.

Histone 1, histone 4 (not shown) and lysozyme, all with high isoelectric points (pI ∿11) and rich in lysine and arginine residues were effective in immobilizing the swimming zoospores at concentrations in the $\mu g\ ml^{-1}$ range,

and some true cysts were formed. In the presence of these proteins at concentrations above 0.3 μg 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, 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 both BSA and β-crystallin caused agglutination of both zoospores and cysts. Samples which contained 30 μg 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, 1975b). Receptors to concanavalin A have been demonstrated on *P. palmivora* zoospores and cysts (Sing & Bartnicki-Garcia, 1975a) 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 3. Concanavalin A, Castor Bean Lectin and Phythaemagglutin P induced encystment and reduced the viability of the zoospore population. Concanavalin A was active at 0.3 μg protein ml^{-1}, but Castor Bean and Phythaemagglutin P lectins required 30 μg ml^{-1} to show any effect. At this concentration all three lectins reduced viability and induced lysis of a measurable 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 25-50 μg ml^{-1} induced lysis but no effect was reported at lower concentrations (Sing & Bartnicki-Garcia, 1975b).

Carbohydrates

Preliminary experiments showed that few carbohydrates had any effect on zoospore motility, even at concentrations as high as 50 mM (Table 4). Two polysaccharides, pectin and polygalacturonic acid, were effective in inducing encystment at the highest concentrations tested (500 µg ml^{-1}) (Table 5). Both polymers were neutralised 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 pectin and polygalacturonic acid solutions against EDTA removed the capacity of the latter to induce encystment. Dialysis reduced the ash content of the pectin from 7.1 ± .3% to 3.5 ± .6% and of the polygalacturonic acid from 6.9 ± .1% to 2.0 ± .7%. Analysis of the ash showed that it contained less than .15% calcium as determined by EDTA titration (Vogel, 1953). Pectin is known to have a high affinity for Cu^{2+}, Zn^{2+} and Fe^{3+} (Fogarty & Ward, 1974). Since it was previously shown that free Fe^{3+} ions were effective inducers of encystment at 10 µM, if Fe^{3+} made up only 10% of the ash present it would provide this concentration. However the effectiveness of Fe^{3+} ions complexed with pectin relative to the free ion is not known.

The lack of any effect of fucose in eliciting encystment is interesting since it has recently been demonstrated 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 therefore seems that adhesion and encystment are independent processes and that 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 in Tables 3 and 4 were screened and from them selected compounds

studied in more detail (Table 6). Glucose was the most effective stimulator of germination at 50 mM, while galacturonic acid was least effective at this concentration. In the case of 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 highly synchronous, and similar in its time course to cysts germinated in the presence of V-8 broth or Ca^{2+} (Fig. 3). This synchronous germination was in contrast to germination in distilled water, where germination was asynchronous and the percentage germination over a 2 hour period was low, and 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. 4 were obtained with sucrose, 150 mM, but similar results were obtained using glucose. If added after 10 minutes 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, listed in Tables 4 and 6, 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 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 by 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's 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).

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 7 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 on the cell population. While this does not completely rule out their role as inducers of encystment in theoretical terms, it is difficult to envisage these concentrations being reached in the rhizosphere. Hexanal, at 30 mM, completely inhibited germination, and even at 10 mM both hexanal and isovaleraldehyde reduced germination significantly. These results differ from those of Allen & Newhook (1974) who showed that ethanol was an effective inducer of encystment when tested in a capillary system. In our system there was no exposure to a concentration gradient, and this may in part explain the differences observed. In addition five organic acids were tested: ascorbic, acetic, oxalic, malic and succinic. 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 neutralise 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+} ions 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 also ensure that soluble carbohydrates were present, adding their stimulus, as necessary, to ensure that the motile cell differentiated to the stage of germ tube formation.

The formation of cyst papillae deserves a final comment. The existence of papillated cysts in this genus has been known for some time (Ho & Hickman, 1967, Hemmes & Hohl, 1971; Palzer, 1976). However their importance in the differentiation strategy of the fungus has not been fully recognised and the factors which control their formation and germination are not known. The formation of the papillated cyst may represent the true alternative to germination, allowing the cell to remain viable and subsequently recommence germination by germ tube or to de-differentiate to a swimming zoospore.

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TABLE 1. EFFECTS OF L-AMINO ACIDS ON ZOOSPORES

Addition	Time for 80% encystment (20 mM)	% Germination at 2 h* (1 mM)
-	> 3 h	13 ± 4
L-glutamic acid pH 6	> 3 h	74 ± 8
L-aspartic acid pH 6	n.d.	52 ± 9
L-glutamine	> 3 h	25 ± 1
L-asparagine	n.d.	13 ± 6
L-alanine	> 3 h	16 ± 3
L-leucine	> 3 h	16 ± 6
L-lysine pH 6	20'	15 ± 5
L-arginine pH 6	20'	14 ± 4
L-histidine pH 6	90'	48 ± 3

n.d. indicates no data. Germination percentages represent the means of five replicates ± the standard deviation.

* Cysts were prepared by shaking.

TABLE 2. EFFECT OF POLY-L-LYSINE, POLY-L-ASPARTATE AND L-LYSINE ON ENCYSTMENT AND VIABILITY

Concentration		% Immobilization at 30 min 19°C	% Viability at 30 min
µg ml^{-1}	mM		
Poly-L-lysine			
20	(0.14)ø	99 ± 1	0*
10	(0.068)	99 ± 1	0*
3	(0.02)	96 ± 3	0*
1	(0.007)	90 ± 9	< 50**
0.3	(0.002)	85 ± 10	n.d.
Poly-L-aspartate			
100	(0.75)ø	58 ± 5	89 ± 6
Poly-L-alanine			
50	-	36 ± 5	89 ± 6
L-lysine	20	50 ± 6	84 ± 4
	10	49 ± 3	n.d.
	5	37 ± 9	n.d.
	1	45 ± 12	89 ± 2
	0.5	32 ± 6	n.d.
Control	-	36 ± 5	89 ± 6

ø mmoles of L-lysine present as poly-L-lysine or L-aspartate present as poly-L-aspartate.

* Total lysis.

** Partial lysis. Approximately 50% of remaining cells germinated.

n.d. no data. Values are means of five replicates ± the standard deviation.

TABLE 3. THE EFFECT OF LECTINS ON *P. CINNAMOMI* ZOOSPORE ENCYSTMENT AND GERMINATION

Lectin concentration $\mu g\ ml^{-1}$		% of cell population			
		0.0	0.3	3.0	30.0
Phythaemagglutin P.	E	39 ± 3	40 ± 6	39 ± 9	87 ± 7*
	V	93 ± 3	94 ± 4	89 ± 2	58 ± 5*
Concanavalin A	E	38 ± 7	86 ± 3*	85 ± 7*	100 ± 1*
	V	89 ± 3	62 ± 11*	64 ± 12*	15 ± 2*
Castor bean (native)	E	43 ± 5	45 ± 7	52 ± 6	63 ± 6*
	V	80 ± 6	78 ± 5	78 ± 3	71 ± 2*
Anti-H lectin	E	35 ± 5	36 ± 6	36 ± 2	39 ± 6
Type 1 (Ulex europaeus)	V	93 ± 2	88 ± 3	90 ± 2	91 ± 2
Potato lectin	E	35 ± 2	39 ± 4	48 ± 2	43 ± 7
	V	93 ± 2	95 ± 2	93 ± 2	90 ± 3

* Means significantly different at 0.05 probability level using student 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 ± 2°, rather than the usual 19 ± 2°.

E = encystment; V = viable.

TABLE 4 THE EFFECT OF CARBOHYDRATES ON ENCYSTMENT

ADDITION	% ENCYSTMENT AT 60 MIN.
-	20 ± 5
D-glucose	16
D-mannose	15
D-galactose	13
D-arabinose	12
L-arabinose	17
D-xylose	17
D-ribose	13
D-fucose	17
L-fucose	13
L-rhamnose	11
Galacturonic acid	28
Sucrose	17
Maltose	13
Cellobiose	15
Raffinose	17
Arabinogalactan	26
Amylose	27
Amylopectin	26
Dextrin	22

All tests carried out at 19° at a sugar concentration of 50 mM, except for raffinose (17 mM), arabinogalactan and amylopectin (10 mg ml^{-1}), dextrin (5 mg ml^{-1}) and amylose (saturated solution). All values are the means of two estimates except the control where the standard deviation is shown.

TABLE 5. THE EFFECT OF PECTIN AND POLYGALACTURONATE ON ENCYSTMENT OF ZOOSPORES

	% Motile zoospores
Control	70 ± 8
+ Pectin	22 ± 5
+ Pectin (dialysed)	32 ± 5
+ Polygalacturonate	45 ± 5
+ Polygalacturonate (dialysed)	63 ± 4*

All treatments were for 20 minutes at 20 ± 2°. The final concentrations of pectin and polygalacturonate were 500 $\mu g\ ml^{-1}$. The pectin contained 7.5% methyl ester. During the 20 minute treatment the percentage of swimming zoospores fell from 85 ± 3 to 70 ± 8% in the control samples.

* Not significantly different from the control value at the level P = 0.05, using the student t-test. The mean values for the dialysed polysaccharide were different from those of the crude material at the level of P = 0.01 using the student t-test. All data was the mean of five replicates.

TABLE 5. EFFECT OF SUGARS ON GERMINATION OF CYSTS

Addition	% Germination by germ tube at 2 h	
Sugar Concentration	1 mM	50 mM
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
-	12 ± 3	14 ± 7

Values in which standard deviations are shown are the means of five replicates. The other values are means of duplicates. The results in column 3 were obtained on a different batch of zoospores to those in column 2.

TABLE 7. EFFECT OF ETHANOL, PROPIONALDEHYDE, HEXANOL AND ISOVALERALDEHYDE ON ZOOSPORE ENCYSTMENT AND VIABILITY

Concentration (mM)		% Population		
		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. * Means different at the level of P = 0.05 using student's t-test. Five replicates 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 ± 2° rather than the usual 19 ± 2°.

FIGURE LEGENDS

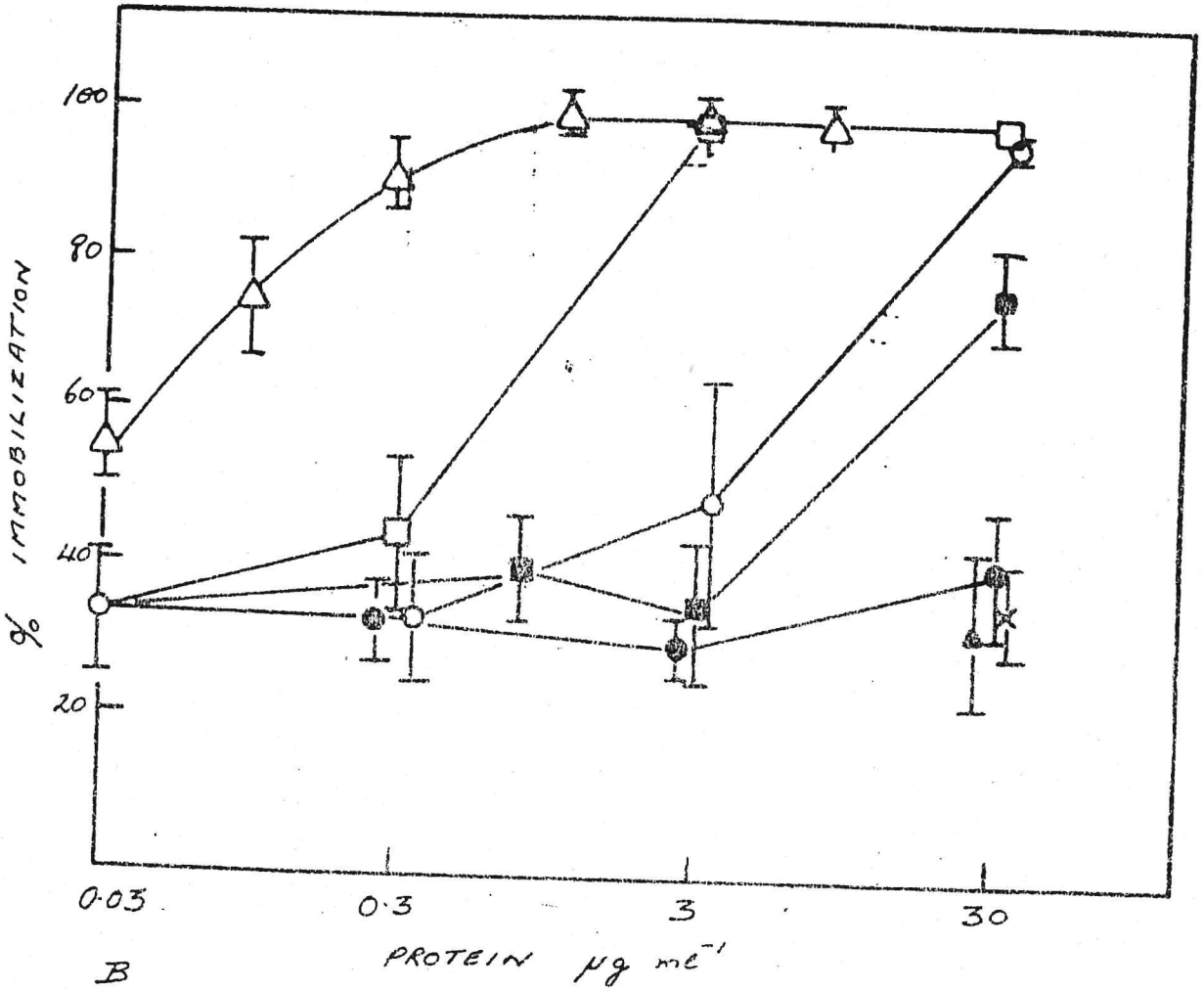
Figure 1. The effect of proteins on zoospores of *P. cinnamomi*. A. Immobilization. ●—●, casein (pI 5); O—O, myoglobin (pI 7.0); ■—■, cytochrome *c* (pI 10.6); □—□, lysozyme (pI 11); Δ—Δ, histone 1 (pI 11). -X- is value obtained with the maximum concentration of β-crystallin tested, and -Δ- value obtained with maximum concentration of BSA. The data points for histone 4 are not shown, but gave a curve superimposible upon that recorded for histone 1. B. Viability. Symbols used are the same as for A. The curves for BSA and β-crystallin were superimposible on that of casein. In both figures the bars represent two S.D. units.

Figure 2. The effect of glucose concentration on the germination of *P. cinnamomi* zoospores. Bars represent two units of standard deviation (S.D.).

Figure 3. Time course of zoospore germination in the presence of various additives. O—O, 10 mM $CaCl_2$; ●—●, 50 mM glucose; ▲—▲, V-8 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.

Figure 4. The effect of delaying addition of nutrient following the induction of encystment. The number of cysts germinated after 120 minutes. O—O, cysts germinated with germ tubes, O—O, cysts germinated as cyst papillae. The arrow indicates the number of germinated cysts in the absence of any addition.

A


100
80
60
40
20
% IMMOBILIZATION
0·03
0·3
3
30
PROTEIN μg mℓ⁻¹

B

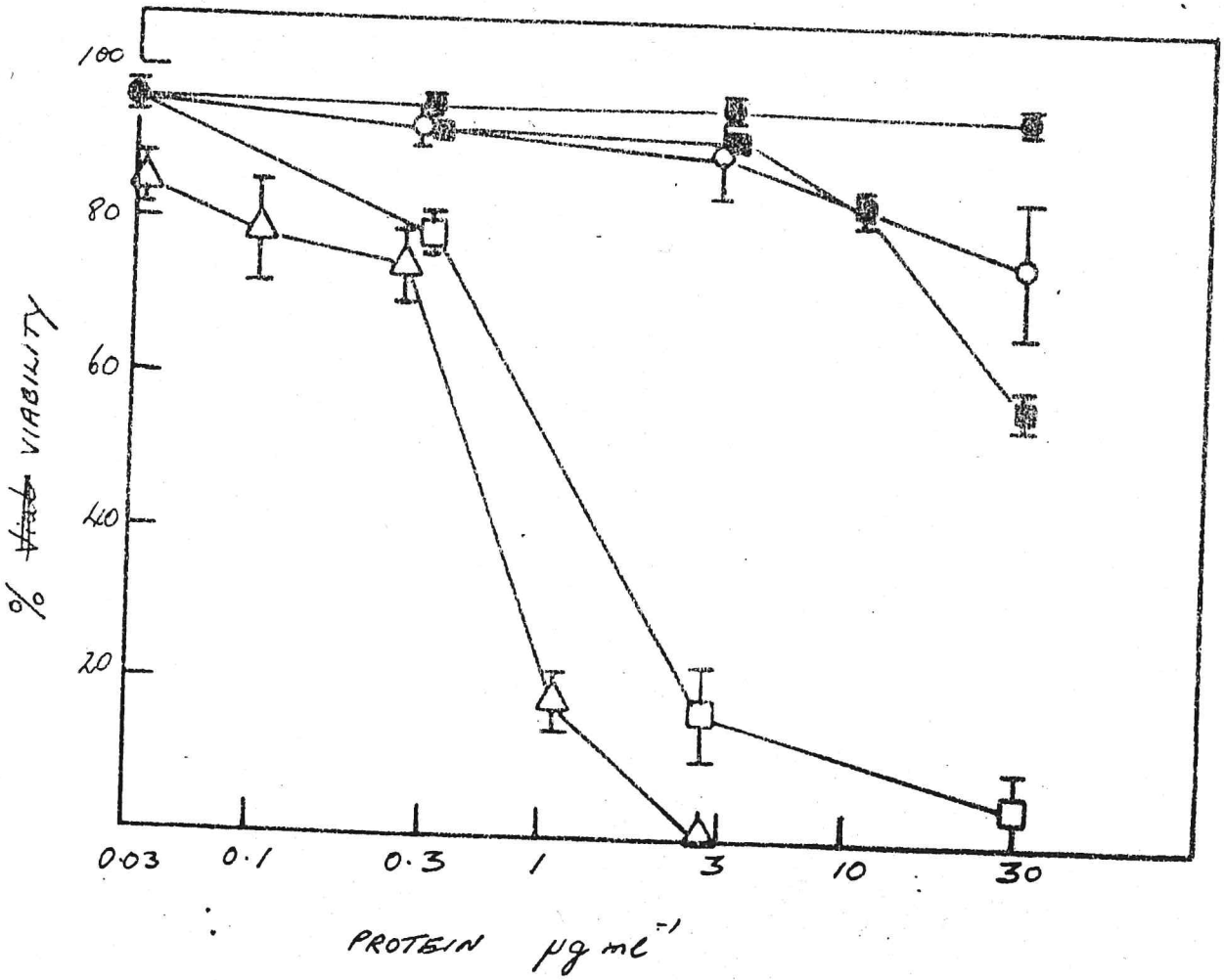

100
80
60
40
20
% VIABILITY
0·03
0·1
0·3
1
3
10
30
PROTEIN μg mℓ⁻¹

FIG 2.

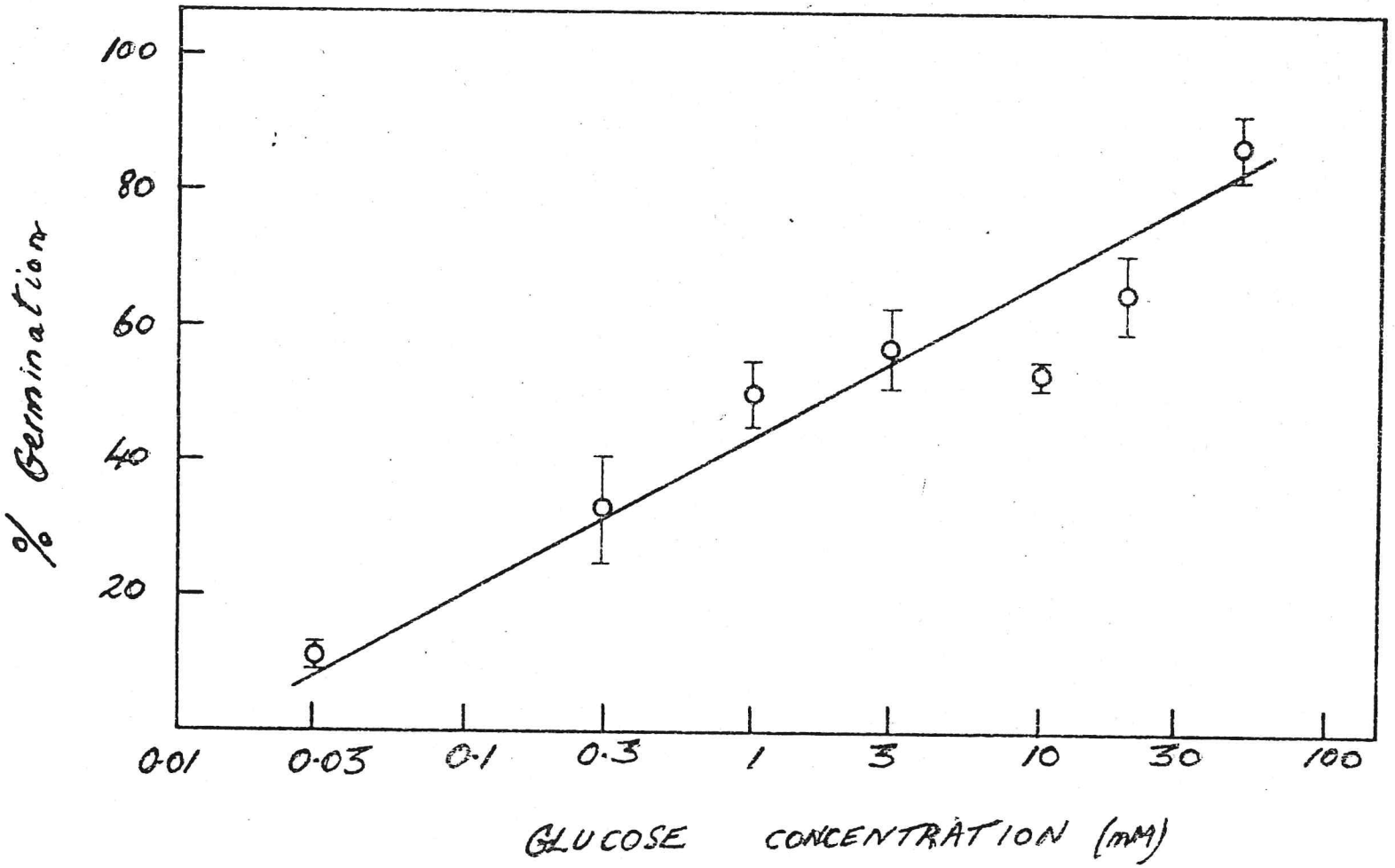

% Germination
100
80
60
40
20
0·01
0·03
0·1
0·3
1
3
10
30
100
GLUCOSE CONCENTRATION (mM)

FIG 3

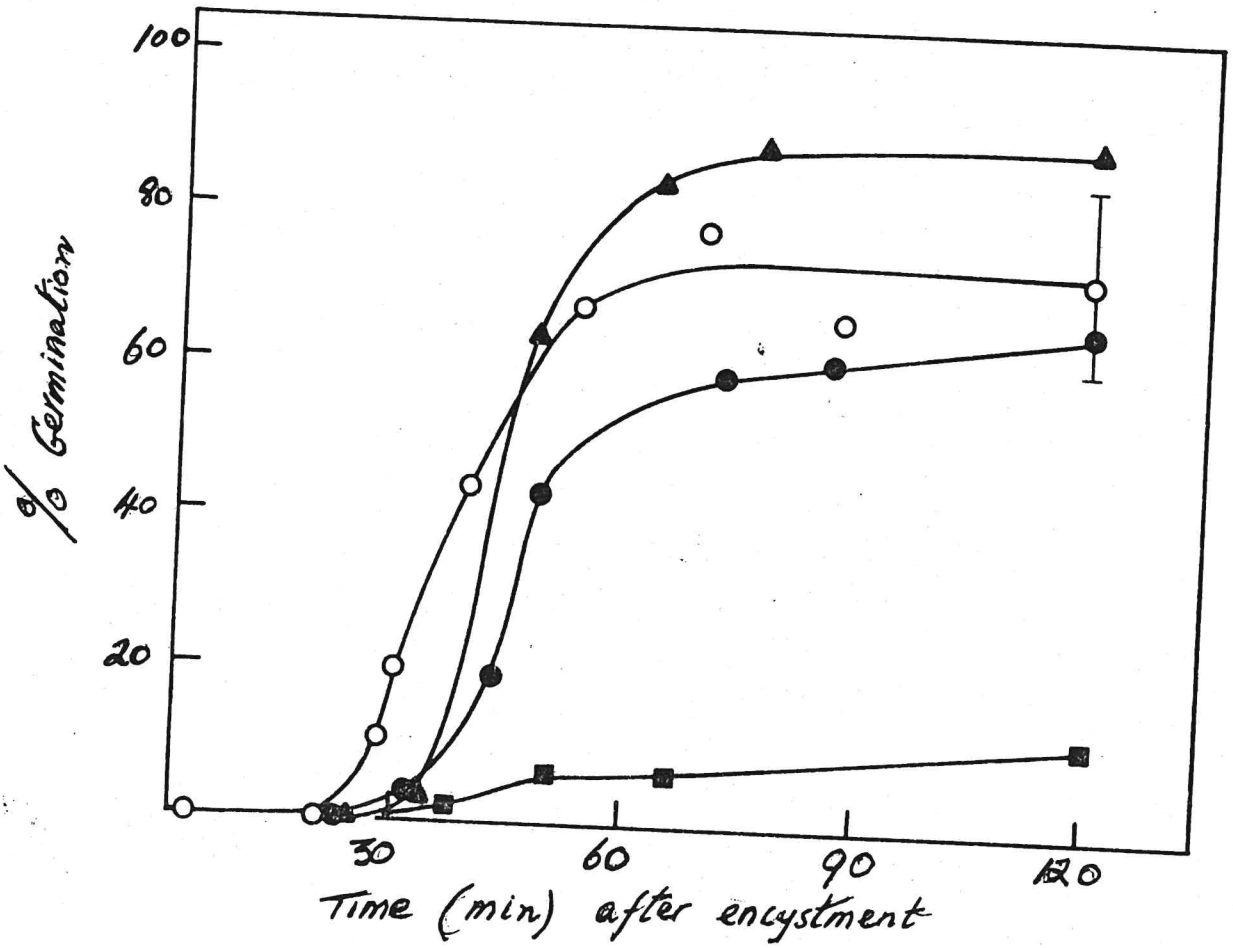
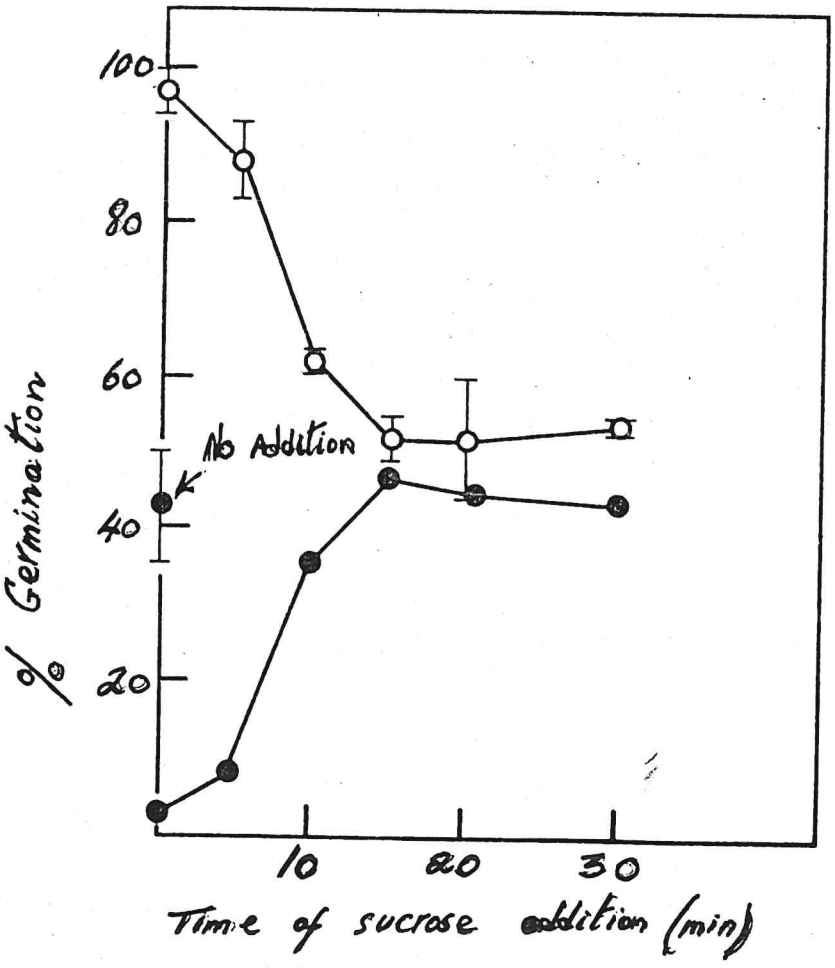

100
80
60
40
20
% Germination
30
60
90
120
Time (min) after encystment

FIG 4


100
80
60
40
20
% Germination
No Addition
10
20
30
Time of sucrose addition (min)