# Comparative Germination of Culture-Produced and Plant-Produced Sporangia of *Pythium ultimum* in Response to Soluble Seed Exudates and Exudate Components

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## ABSTRACT

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Sporangia of *Pythium ultimum* were produced in vitro on culture media commonly used for the cultivation of *Pythium* spp. and on media amended with germinating seeds and excised radicles of various plant species. Their responses to seed exudates and selected sugars and amino acids were determined. On synthetic media, sporangia germinated in response to certain sugars and amino acids as well as cotton seed exudate. However, when produced in association with plant tissue or on media amended with α-phosphatidyl choline, sporangia failed to germinate in response to any sugar or amino acid tested individually or in combination, despite their ability to germinate in response to cotton seed exudate. Because sporangia reared in vitro more closely reflect the nature of those in soil when produced on plant-tissue-amended media than when produced on other conventional media, we concluded that molecules other than sugars or amino acids are responsible for activating sporangia of *P. ultimum* and establishing host-pathogen interactions under natural conditions.

Sporangia of *Pythium ultimum* Trow are exogenously dormant propagules that form in and on infected plant tissue during pathogenesis and can serve as major survival propagules in soil (29,32). When exposed to the appropriate stimulus, sporangia germinate rapidly and infect susceptible seed and seedling tissue within a few hours after planting (17,20–23,32,33). Although seed and root exudates are the principal source of stimulants for propagules of *Pythium* spp. in soil (5,10,14,30,32,33), the nature of the compounds responsible for stimulating propagule germination of *Pythium* spp. in soil under natural conditions currently is not known. Nevertheless, from a number of in vitro (1–4,6,8,11,12,16,26,28) and in vivo studies (30,32,33), it has been suggested that sugars and amino acids are the most likely stimulants of propagule germination among various *Pythium* spp.

Many of the previous studies of propagule germination among *Pythium* spp. involved assays that used propagules produced on synthetic media. Although it is not known whether propagules of *Pythium* spp. produced on synthetic media respond to exudates and exudate components in a manner similar to those produced on plant tissue in soil, it has been shown that sclerotia of *Sclerotium rolfsii* Sacc. produced in culture can be physiologically quite different from those produced under natural conditions (18). Conclusions on the nature of exudate stimulant molecules based on assays with fungal propagules produced in vitro on synthetic media cannot necessarily be extended to natural conditions in the spermosphere and rhizosphere.

Therefore, the purpose of this study was to determine whether sporangia of *P. ultimum* produced on synthetic media germinate in response to seed exudates and exudate components in a manner similar to sporangia produced on plant tissue.

## MATERIALS AND METHODS

**Media and culture conditions.** Media used to produce sporangia of *P. ultimum* were the following: water agar (WA) (Difco Laboratories, Detroit, MI); grass extract agar (GEA) (20); cornmeal agar (CMA) (Difco); lima bean agar (LBA), prepared by boiling 15 g of lima beans in 1 L of $H_2O$, filtering, adjusting volume to 1 L with sterile distilled water, and adding agar before autoclaving; V8 juice agar (V8) (27); and a defined mineral salts medium (SM + L), modified from Ruben, Frank, and Chet (25), containing the following ingredients per liter: 180 mg of D-glucose, 1.3 mg of L-asparagine, 1 g of α-phosphatidyl choline, 5.3 mg of $(NH_4)_2SO_4$, 2.4 mg of $MgSO_4 \cdot 7H_2O$, 1.1 mg of $CaCl_2$, 3.1 mg of $K_2HPO_4$, 1.6 mg of $KH_2PO_4$, 1.7 μg of thiamine HCl, and 30 g of agar. This last medium also was used without the addition of α-phosphatidyl choline (SM), and agarose was substituted for Bacto agar. Agar or agarose was added to all media at a concentration of 3%. Several of the above media were chosen because they commonly are used for the culture of *Pythium* spp. and have been used in previous investigations of propagule germination.

Sporangia also were produced on agarose amended with surface-disinfested seeds of the following plant species: cotton (*Gossypium hirsutum* L. 'Acala SJ-2'), wheat (*Triticum aestivum* L. 'Geneva'), barley (*Hordeum vulgare* L. 'Hudson'), snapbean (*Phaseolus vulgaris* L. 'Bush Blue Lake 53'), oat (*Avena sativa* L., cultivar unknown), and soybean (*Glycine max* (L.) Merr. 'Pride B117'). In some experiments, agarose was amended with excised cotton radicles or heat-killed cotton seeds. Seeds and excised radicles were surface disinfested by soaking for 10 min in a 10% solution of sodium hypochlorite containing two to three drops of Tween 20 (Sigma Chemical Co., St. Louis, MO) as a wetting agent. Seeds and radicles were removed, rinsed thoroughly with sterile distilled water, and added to molten (45 C) 3% agarose in petri plates (five per plate). Heat-killed seeds were autoclaved in water for 20 min, rinsed, and added to plates of molten agarose.

All plates were inoculated with a 24- to 48-hr culture of *P. ultimum* and incubated at 27 C. After 4 days, all plant tissue was heavily colonized and rotted. Disks (2 mm in diameter) then were cut with a cork borer from the colony margin of *P. ultimum* growing on synthetic media and from the colony area around the periphery of rotted radicles or seeds in plant-tissue-amended media. Disks were leached as described below to induce sporangium formation.

**Production of sporangia.** Isolate P4 of *P. ultimum*, obtained from snapbean (20,24), was used throughout this study. To induce sporangium formation, colonized agar disks were placed in sterile petri plates and leached for two consecutive 10-min periods in approximately 20 ml of a leaching buffer (pH 5.8) containing 0.01 M $Ca(NO_3)_2 \cdot 4H_2O$, 0.004 M $MgSO_4 \cdot 7H_2O$, and 0.005 M $KNO_3$ (9). Buffer was replaced with fresh buffer after each 10-min leaching period. Finally, disks were leached for 3 hr. Then the buffer was removed, and disks were rinsed with sterile distilled water and incubated overnight at 24 C. This leaching procedure resulted in a rapid, synchronous formation of sporangia, and

all sporangia evaluated were of approximately the same age.

**Collection and preparation of seed exudates.** Seeds of cotton (cultivar Acala SJ-2) collected in 1987 and of good quality were sorted to remove damaged and deformed seeds and then were surface disinfested as previously described. One hundred seeds (12.5 g dry weight) were added to flasks containing 100 ml of sterile distilled water and placed on a rotary shaker at 27 C. After 24 hr, flask contents were filtered through Whatman #1 filter paper to remove seeds and large particulates, and the exudate was passed sequentially through 0.8- and 0.2-$\mu$m filters. The filtrate was evaporated in vacuo at 40 C to a volume of approximately 5 ml, then transferred to small preweighed test tubes and freeze-dried. Exudate residues were weighed, reconstituted in either sterile distilled deionized water (pH 5.5) or a 0.1 M phosphate buffer (pH 6.3) to a concentration of 100 mg/ml, and passed through a 0.2-$\mu$m filter before use in germination assays.

**Germination assays.** To assess germination of sporangia in response to a stimulant, leached disks containing sporangia were placed on acid-washed sterile glass slides (three to five replicate disks per slide). Germination stimulants consisted of the following: exudates from germinating cotton seeds collected as described, D-galactose, D-glucose, D-fructose, lactose, sucrose, L-aspartic acid, $\beta$-alanine, L-asparagine, L-glutamic acid, and L-serine. The specific sugars and amino acids were chosen because they are major components of cotton seed exudates (13). Cotton seed exudate was dissolved either in 0.1 M phosphate buffer or in sterile distilled deionized water, whereas all sugars and amino acids were dissolved in 0.1 M phosphate buffer. All stimulants were filtered through a 0.2-$\mu$m filter before use in assays. In most experiments, sugars and amino acids were used at concentrations of 10 mM. However, in some experiments, lower concentrations of D-glucose were used. Ten microliters of the appropriate stimulant was added to each disk, and cultures were incubated at 24 C. After 3 hr, germination of sporangia was assessed by staining disks with 0.03% acid fuchsin in 85% lactic acid, examining microscopically (×250), and counting the number of germinated and ungerminated sporangia. All sporangia within a random 1.5-mm$^2$ area of the disk were assessed for germination on each replicate disk for a total of 150–500 sporangia counted per treatment. Sporangia were considered germinated if a developing germ tube was visible.

**Experimental design and data analysis.** All experiments were established as a completely randomized design with at least three replicates. All experiments were performed at least three times with similar results. Percentage data were transformed (arcsin of square roots) before analysis of variance. All experiments were analyzed as factorials. In cases where significant interactions between media and germination stimulants were observed, data on germination of sporangia produced on different media in response to each stimulant were analyzed separately. Means were separated with Waller-Duncan Bayesian least significant difference tests. In experiments designed to test germination of sporangia in response to different levels of glucose, linear regression analyses were performed. All tabular data represent results from one representative experiment.

## RESULTS

**Germination of culture-produced sporangia.** Sporangia of *P. ultimum* germinated readily in response to cotton seed exudate regardless of the medium on which sporangia were produced (Table 1). Germination was complete by 3 hr after addition of seed exudate. Water did not induce germination of sporangia produced on any of the culture media tested. However, the medium on which sporangia were produced strongly influenced the germination response toward sugar and amino acid exudate components. For example, germination of sporangia in response to D-glucose ranged from 0.8 to 78.7% among the media tested. Sporangia produced on GEA, CMA, WA, and SM (hereafter referred to as broad-response media) germinated most readily in response to 10 mM D-glucose, whereas less than 1% of the sporangia germinated when produced on a glucose-asparagine medium containing 0.1% $\alpha$-phosphatidyl choline (SM + L) (hereafter referred to as a narrow-response medium), despite nearly 100% germination in response to cotton seed exudate. Less than 10% of the sporangia produced on broad-response media (WA and GEA) germinated when exposed to D-glucose concentrations below $10^{-4}$ M (Fig. 1), whereas less than 2% of the sporangia produced on SM + L medium germinated in response to any of the D-glucose concentrations tested. The culture medium on which sporangia were produced affected their germination in response to sucrose in a manner similar to that of D-glucose (Table 1), whereas germination in response to L-asparagine was considerably lower than that of D-glucose on all media tested, except for SM + L.

**Influence of sugar and amino acid seed exudate components on sporangium germination.** In addition to glucose, sucrose, and asparagine, other monosaccharides, disaccharides, and amino acids commonly found in cotton seed exudate (13) were evaluated as germination stimulants of sporangia produced on either broad-response or narrow-response media (Table 2). Substantial levels

TABLE 1. Germination of culture-produced sporangia of *Pythium ultimum* (P4) in response to cotton seed exudate and exudate components

| Culture medium[x] | Percent germinated sporangia after addition of:[y] | | | | |
|---|---|---|---|---|---|
| | $H_2O$ | D-glucose | Sucrose | L-asparagine | Exudate[z] |
| GEA | 0.0 a | 78.7 a | 16.2 b | 7.9 b | 96.0 a |
| CMA | 0.0 a | 76.0 a | 77.0 a | 31.8 a | 87.8 a |
| SM | 0.0 a | 71.1 a | 73.2 a | 4.9 b | 95.2 a |
| LBA | 0.0 a | 68.6 a | 21.5 b | 1.5 b | 98.0 a |
| V8 | 0.0 a | 35.4 b | 10.5 bc | 12.5 ab | 93.5 a |
| SM + L | 0.0 a | 0.8 c | 2.3 c | 1.5 b | 98.0 a |

[x]GEA = grass extract agar; CMA = cornmeal agar; SM = glucose-asparagine mineral salts agar; LBA = lima bean agar; V8 = V8 juice agar; SM + L = SM + 0.1% $\alpha$-phosphatidyl choline (soy lecithin).

[y]Germination assessed 3 hr after addition of 10 $\mu$l of germination stimulant to each of three replicate disks per treatment; sugars and amino acids added at concentrations of 10 mM. Numbers in each column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan Bayesian least significant difference test.

[z]Equivalent of exudate from one 24-hr germinated seed added to each of three replicate sporangial disks per treatment.

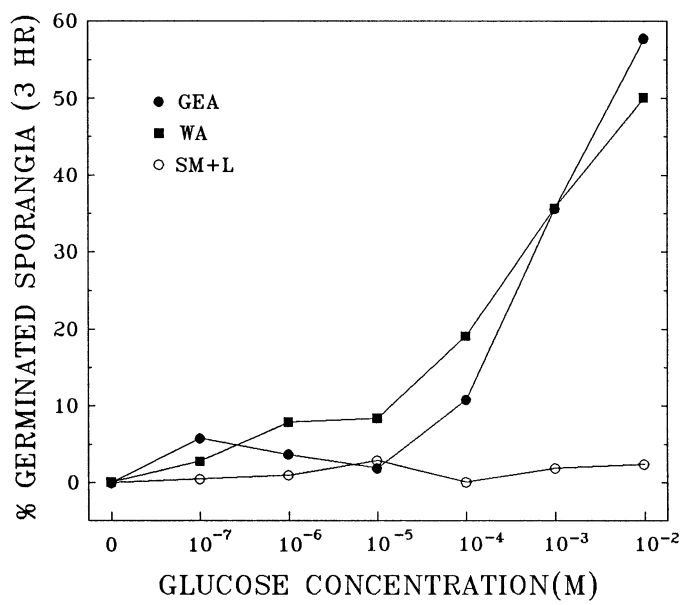


**Fig. 1.** Arithmetic plot of germination of sporangia of *Pythium ultimum* produced on three media in response to various concentrations of D-glucose. Each point represents the mean percentage of sporangia germinated 3 hr after addition of 10 $\mu$l of appropriate D-glucose solution. Linear regression coefficients for responses of sporangia on grass extract agar (GEA), water agar (WA), and glucose-asparagine mineral salts agar amended with 0.1% $\alpha$-phosphatidyl choline (soy lecithin) (SM + L) were 63.2 ($r^2 = 0.90$), 66.1 ($r^2 = 0.75$), and 4.1 ($r^2 = 0.93$), respectively.

of germination in response to six sugars and six amino acids were observed only when sporangia were produced on CMA (a broad-response medium), with percent germination ranging from 0 to 76% among treatments. D-Glucose, sucrose, and D-maltose induced the greatest levels of germination, whereas D-fructose and D-lactose were not effective as germination stimulants on broad-response media. Little or no germination of sporangia was observed in response to any of the individual sugars and amino acids tested when produced on SM + L or agarose amended with germinating cotton seeds (A + CS). Nonetheless, nearly 100% of the sporangia produced in this manner germinated after 3 hr in response to cotton seed exudate.

Levels of sporagium germination in response to combinations of D-glucose, D-maltose, and sucrose were similar to responses observed to individual sugars on all media tested (Table 3). However, combinations of individual amino acids or mixtures of both sugars and amino acids did not stimulate sporangium germination on any of the media tested (Table 3).

**Germination of plant-produced sporangia.** Sporangia produced on an agarose medium amended with heat-killed cotton seeds (A + DCS) germinated in response to sucrose and L-asparagine at levels similar to those produced on a broad-response medium (SM) (Table 4). However, germination of sporangia produced on A + DCS in response to D-glucose was significantly ($P = 0.05$) less than germination on broad-response media. Germination of sporangia produced on agarose amended with living, surface-disinfested seeds (A + CS) was dramatically reduced to levels that did not differ ($P = 0.05$) from those of sporangia produced on a narrow-response medium (SM + L) when exposed to D-glucose, sucrose, and L-asparagine. Sporangia produced on agarose amended with excised cotton radicles (A + CR) germinated to a much lesser degree in response to all stimulants tested than those produced on broad-response media (SM and A + DCS).

Sporangia produced on media amended with germinating seeds of various plants did not differ in their response to cotton seed exudate from those produced on a broad-response medium (Tables 4 and 5). However, germination of these sporangia in response to individual exudate components was significantly ($P = 0.05$) reduced as compared with germination of sporangia produced on the broad-response medium CMA (Table 5). Percent germination of sporangia produced on all seed-amended agarose media in response to D-glucose and L-asparagine did not differ from germination levels on the narrow-response medium SM + L. Responses to sucrose were similar.

TABLE 2. Influence of sugars and amino acids on germination of sporangia of *Pythium ultimum* produced on three different media

| Germination stimulant[w] | Sporangium production medium[x] | | |
|---|---|---|---|
| | CMA | SM + L | A + CS |
| Sugars | | | |
| D-fructose | 0.5[y] | 0.0 | 0.4 |
| D-galactose | 16.4 | 0.0 | 0.0 |
| D-glucose | 75.6 | 2.7 | 0.0 |
| D-lactose | 0.0 | 0.0 | 0.0 |
| D-maltose | 64.9 | 0.0 | 0.8 |
| Sucrose | 66.6 | 0.0 | 0.6 |
| Amino acids | | | |
| L-alanine | 21.1 | 0.0 | 0.0 |
| L-arginine | 2.0 | 0.0 | 0.2 |
| L-asparagine | 37.6 | 0.6 | 0.5 |
| DL-aspartic acid | 5.0 | 0.8 | 0.3 |
| L-glutamic acid | 37.2 | 0.0 | 0.7 |
| L-serine | 43.9 | 0.0 | 0.3 |
| $H_2O$ | 0.0 | 0.0 | 0.0 |
| Seed exudate[z] | 93.0 | 96.8 | 90.4 |

[w]Ten microliters of 10 mM solutions of each stimulant added to each of five replicate disks containing sporangia.
[x]CMA = 3% cornmeal agar; SM + L = a glucose-asparagine mineral salts agar amended with 0.1% α-phosphatidyl choline (soy lecithin); A + CS = 3% agarose amended with five surface-disinfested cotton seeds.
[y]Numbers represent percent germinated sporangia assessed 3 hr after addition of stimulant.
[z]Equivalent of exudate from one 24-hr germinated seed added to each of five sporangial disks (10 μl) per treatment.

TABLE 3. Influence of sugars and amino acids applied individually and in combination on germination of sporangia of *Pythium ultimum* produced on three different media

| Germination stimulant[w] | Sporangium production medium[x] | | |
|---|---|---|---|
| | CMA | SM + L | A + CS |
| D-glucose (Glu) | 93.3[y] | 0.0 | 6.3 |
| D-maltose (Mal) | 93.5 | 0.0 | 1.0 |
| Sucrose (Suc) | 84.3 | 0.0 | 5.1 |
| L-alanine (Ala) | 50.3 | 0.0 | 4.4 |
| L-asparagine (Aspn) | 70.7 | 2.6 | 0.6 |
| L-glutamic acid (GlutA) | 5.5 | 0.6 | 0.4 |
| L-serine (Ser) | 79.7 | 2.3 | 0.0 |
| Glu + Mal + Suc | 78.2 | 0.0 | 0.0 |
| Ala + Asp + GlutA + Ser | 0.0 | 0.0 | 0.0 |
| Glu + Mal + Suc + Ala + Asp + GlutA + Ser | 0.0 | 0.0 | 0.0 |
| $H_2O$ | 0.0 | 0.0 | 0.0 |
| Seed exudate[z] | 99.3 | 88.9 | 90.1 |

[w]Ten microliters of 10 mM solutions of each stimulant added to each of three replicate sporangial disks per treatment.
[x]CMA = 3% cornmeal agar; SM + L = a glucose-asparagine mineral salts agar amended with 0.1% α-phosphatidyl choline (soy lecithin); A + CS = 3% agarose amended with five surface-disinfested cotton seeds.
[y]Numbers represent percent germinated sporangia assessed 3 hr after addition of stimulant.
[z]Equivalent of exudate from one 24-hr germinated seed (10 μl) added to each of three replicate sporangial disks per treatment.

## DISCUSSION

It generally has been accepted, but never conclusively proven, that sugar and amino acid components of seed and root exudates are the primary germination stimulants of propagules of *Pythium* spp. in nature. This conclusion has been derived primarily from speculative or incomplete correlative information obtained from in vitro studies with culture-produced propagules. For example, Agnihotri and Vaartaja (1,2,4) suggested that sugars, amino acids, and organic acids might be responsible for stimulating sporangial germination in soil because these compounds induced the greatest amount of germination of CMA-produced sporangia of *P. irregulare* Buis. and *P. ultimum* after 16 hr of incubation. Both Flentje and Saksena (11) and Keeling (15) observed more seed rot caused by *Pythium* spp. among pea and soybean lines that

TABLE 4. Germination of plant-produced sporangia of *Pythium ultimum* in response to cotton seed exudates and exudate components

| Culture medium[x] | Percent germinated sporangia after addition of:[y] | | | | |
|---|---|---|---|---|---|
| | $H_2O$ | D-glucose | Sucrose | L-asparagine | Exudate[z] |
| A + CS | 0.2 a | 8.1 c | 5.2 c | 0.6 c | 97.9 a |
| A + DCS | 0.0 a | 43.3 b | 80.7 a | 10.6 ab | 98.0 a |
| A + CR | 0.3 a | 26.1 b | 39.2 b | 3.9 abc | 96.1 a |
| SM | 0.0 a | 71.4 a | 71.7 a | 23.2 a | 100.0 a |
| SM + L | 0.0 a | 2.1 c | 1.5 c | 0.1 c | 98.8 a |

[x]A + CS = agarose amended with surface-disinfested cotton seeds; A + DCS = agarose amended with heat-killed cotton seeds; A + CR = agarose amended with surface-disinfested excised cotton radicles; SM = glucose-asparagine mineral salts agar; SM + L = SM + 0.1% α-phosphatidyl choline (soy lecithin).
[y]Germination assessed 3 hr after addition of 10 μl of germination stimulant to each of three replicate disks per treatment; sugars and amino acids added at a concentration of 10 mM. Numbers in each column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan Bayesian least significant difference test.
[z]Equivalent of exudate from one 24-hr germinated seed added to each of three sporangial disks per medium.

released more sugars and amino acids during germination than among those lines that released less. At the same time, they observed that sugars support the growth of *P. ultimum*. They concluded that sugars were responsible for stimulating *Pythium* and initiating these host-pathogen interactions in soil. Similarly, Matthews and Bradnock (19) observed a direct correlation between carbohydrate exudation in vitro and decreased emergence of pea seedlings in the field due to *Pythium* seed rot and, thus, speculated that carbohydrates were responsible for eliminating fungistasis and stimulating the activity of *Pythium* in soil.

We clearly demonstrated that sporangia of *P. ultimum* can behave quite differently in their response to sugars and amino acids present in seed exudates, depending on the substrates on which they are produced. We have observed that sporangia derived from diseased plant tissue fail to germinate in response to selected sugars and amino acids present in seed exudates despite their rapid response to unfractionated seed exudate. Combinations of sugars, amino acids, or mixtures of both also were not effective in stimulating germination. In contrast, sporangia produced on traditional synthetic culture media germinate quite readily in response to many of these stimulants. The inability of L-glutamic acid to support abundant germination when tested individually and its apparent suppression of germination when combined with sugars suggest that this amino acid actually may be inhibitory in the presence of other compounds. It appears, therefore, that molecules other than sugars and amino acids present in seed exudates also could be important in activating quiescent propagules of *P. ultimum* and establishing host-pathogen interactions in nature. This is in contrast to current beliefs about the exudate factors initiating *Pythium*-seed interactions, although molecules other than sugars and amino acids have been reported to be stimulants of sporangial germination (20).

In one of the more comprehensive studies of sporangial germination behavior of *P. ultimum*, Stanghellini and Hancock (32,33) found that sporangia produced on rolled oat broth or autoclaved oat seed germinated within 1–1.5 hr in infested soil when either bean seed exudate or various concentrations of glucose were added. This is consistent with our results because sporangia produced on heat-killed plant tissue behaved like those produced on synthetic media in their response to sugars and amino acids. However, in the study of Stanghellini and Hancock (32), sporangia recovered from naturally infested soil germinated in response to glucose as well as bean seed exudate. Although the origin of sporangia in naturally infested soil is not clear from their report, it is reasonable to assume that these sporangia originated from diseased plant tissue. If true, this observation of sporangial germination in response to glucose is in direct contrast to our results. Certainly, sporangia and oospores of some *Pythium* spp., presumably originating from decaying plant tissue, are capable of germinating in response to compounds present in cornmeal agar and water agar when extracted from soil (7,31). However, it is possible that, as sporangia and oospores age and weather in soil, they become more responsive to other compounds (such as sugars and amino acids) in their environment that allow them to germinate quite readily on isolation media.

TABLE 5. Germination of sporangia of *Pythium ultimum* produced on an agarose medium amended with seeds of various plant species in response to cotton seed exudate and exudate components

| Culture medium | Percent germinated sporangia after addition of:[w] | | | | |
|---|---|---|---|---|---|
| | $H_2O$ | D-glucose | Sucrose | L-asparagine | Exudate[x] |
| Controls[y] | | | | | |
| CMA | 0.0 a | 73.9 a | 56.0 a | 16.3 a | 96.1 a |
| SM + L | 0.0 a | 0.0 c | 0.0 d | 1.1 bc | 93.1 a |
| Agarose +[z] | | | | | |
| Wheat | 0.0 a | 1.0 bc | 1.6 bcd | 0.0 c | 100.0 a |
| Barley | 0.0 a | 5.9 bc | 0.0 d | 1.1 bc | 94.0 a |
| Oat | 0.0 a | 2.7 bc | 2.3 bc | 3.0 b | 100.0 a |
| Snapbean | 0.0 a | 0.0 c | 0.6 cd | 0.0 c | 97.5 a |
| Cotton | 0.0 a | 4.0 bc | 0.5 cd | 0.7 bc | 96.0 a |
| Soybean | 0.0 a | 3.7 bc | 3.2 b | 1.1 bc | 93.4 a |

[w]Germination assessed 3 hr after the addition of 10 μl of 10 mM solution of the stimulant to each of three replicate disks per treatment. Numbers in each column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan Bayesian least significant difference test.

[x]Equivalent of exudate from one 24-hr germinated seed added to each of three replicate sporangial disks per treatment.

[y]CMA = 3% cornmeal agar (broad-response control); SM + L = a glucose-asparagine mineral salts agar amended with 0.1% α-phosphatidyl choline (soy lecithin) (narrow-response control).

[z]Seeds of the various plant species surface disinfested and embedded in molten 3% agarose; five seeds added to each of three plates.

The reasons for limited responses of sporangia to sugars and amino acids when produced on living plant tissue are not completely understood. However, the ability of a soy-lecithin-amended medium to give rise to sporangia that mimic those produced on plant tissue implicates a potential role for complex lipid sources in regulating propagule responses to sugars and amino acids. In support of this hypothesis, lipids precipitated from chloroform/methanol extracts of germinating cotton seeds and added to a glucose-asparagine mineral salts medium also supported the production of sporangia that germinated in an identical manner to those produced on plant tissue or on a medium amended with soy lecithin (E. B. Nelson, *unpublished*).

Physiologically, sporangia of *P. ultimum* produced in association with diseased plant tissue appear to be quite different from those reared on conventional synthetic media. We believe that responses of propagules produced in this manner more closely resemble the responses one can expect from propagules produced in their natural environment. Therefore, in future in vitro studies of propagule germination behavior with *P. ultimum*, sporangia must be produced in such a way as to mimic those in nature if extrapolations to in vivo situations are to be meaningful and if the molecules important in eliciting these responses are to be identified.

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