Components in Alfalfa Root Extract and Root Exudate that Increase Oospore Germination of *Phytophthora megasperma* f. sp. *medicaginis*

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ABSTRACT

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The germination of oospores of *Phytophthora megasperma* f. sp. *medicaginis* was three- to four-fold higher in root extracts (86%) or root exudates (61%) of both the susceptible alfalfa cultivar, Moapa 69, and resistant germ plasm A77-10B, than in water (23%). Oospore germination occurred at pH 3.5–10.5 with optimum at pH 6.0. Oospores germinated by germ tube only in water, but in root extract or exudate up to 50% germinated by germ sporangia. Increased concentrations of root extract or exudate (up to 92 mg of solids per 100 ml) increased germination by germ sporangia, but at higher concentrations (138–275 mg/100 ml) the percentage of oospores germinating by germ sporangia decreased. The factors in alfalfa root extract or exudate that increased oospore germination were stable to heat and proteolysis and were not restricted to a certain molecular size. Lyophilization of alfalfa root extract and exudate prior to rehydration increased the percentage that germinated by germ sporangia but did not increase total oospore germination. Charcoal treatment of root extracts and root exudates, which reduced carbohydrate and amino acid content, also reduced the percentage of total oospore germination, but increased the percentage of oospores that germinated by germ sporangia. Oospore germination was also increased by root exudate and extract of pea and tomato plants (nonhosts). The cationic fraction of alfalfa root extract and exudate, which contained amino acids, stimulated oospore germination (58%) and also increased the percentage that germinated by germ sporangia (about 30%). Amino acids and sugars in root extract were analyzed and tested for effect on germination. Aspartic acid, singly and in combination with certain other amino acids, increased oospore germination when added to dilute root exudate but were not active in water. Ribose from the neutral fraction of alfalfa root extract or exudate increased germination slightly.

Oospores, produced by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan and Erwin, the causal agent of Phytophthora root rot of alfalfa (*Medicago sativa* L.), survive in soil and serve as primary inoculum (20). Plant root extracts and exudates contain a wide variety of compounds such as amino acids, sugars, glycosides, organic acids, vitamins, enzymes, alkaloids, nucleotides, and inorganic ions (18). Since root exudates increase oospore germination (5–8), and microbial activity in the rhizosphere has been attributed to the release of organic substances by plant roots (1), different components in alfalfa root extract and exudate were analyzed to determine which component(s) were responsible for increased germination of oospores. Root extracts and exudates from alfalfa were fractioned, characterized chemically, and assayed for effects on germination and subsequent formation of sporangia on germinated oospores. This research has previously been reported in an abstract (6) and in a thesis (5).

MATERIALS AND METHODS

Oospore germination and cultural conditions. Methods of obtaining and germinating oospores of *P. m.* f. sp. *medicaginis*, isolate P1057, were the same as previously reported (7). Solutions of sugars and amino acids were tested for effect on germination of oospores at concentrations corresponding to those determined by chemical analysis to be in alfalfa root extract. Preliminary experiments indicated that none of the pure compounds affected oospore germination when tested in water. Since they were inactive in water, they were tested in root exudate diluted to a concentration (0.1 mg total solids per milliliter) that was either only slightly or not stimulatory to oospore germination. Sugars were tested singly, but amino acids were tested both singly and in mixtures with aspartic acid by adding one acid at a time to aspartic acid.

Preparation of root exudate and extract. Alfalfa seeds of the *P. m.* f. sp. *medicaginis*-susceptible cultivar Moapa 69 (M69) and of the resistant germ plasm A77-10B were surface sterilized by exposure to chlorine gas for 24 hr (24) and grown aseptically in sterile water after which root exudate was collected as described previously (7). Root extract was obtained from alfalfa plants grown in steamed soil (Hanford coarse sandy loam) in the greenhouse for 2 mo as described previously (7). The pH of alfalfa root extract and exudate (naturally pH 6.0) was adjusted to various levels (from pH 3.5 to 10.5) with either 1 N NaOH or 1 N HCl for testing the effect on germination of oospores. All experiments were conducted with root extracts and exudates of both M69 and A77-10B, but since results were similar (5), most of the data reported here will be those from tests with M69.

Seeds of tomato (*Lycopersicon esculentum* Mill. 'Homestead FM-61') were wetted for 5 min with 75% (v/v) ethanol, washed in sterile water, soaked for 10 min in 0.5% (v/v) sodium hypochlorite, washed several times in sterile water, and incubated for 2 days in darkness at 24 C on 1% Difco nutrient agar to detect contaminated plants which were discarded. One hundred uncontaminated seedlings were incubated in 40 ml of sterile water in deep petri dishes (90 mm in diameter) under fluorescent lamps (12 hr light:12 hr dark), and after 3 days, root exudate was collected as described previously for alfalfa seedlings (7). The root extract was prepared by blending 10 g (fresh weight) of roots of 20-day-old tomato plants for 10 min in 100 ml of water as described previously (7).

Seeds of pea (*Pisum sativum* L. 'Little Marvel') were wetted for 5 min with 75% (v/v) ethanol, washed in sterile water, and surface sterilized for 30 min in 0.5% (v/v) sodium hypochlorite. After six washes in sterile distilled water, seeds were incubated for 2 days in darkness at 24 C on 1% Difco nutrient agar and contaminated plants were discarded. Seeds were transferred to sterile water (25 seeds in 50 ml of water) in deep petri dishes and incubated at 24 C under fluorescent lights as previously described (7). After 8 days, when the radicle was about 5–6 cm long, the culture fluid was

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centrifuged for 5 min at 1,200 *g* to remove sloughed-off cells. The supernatant was stored at −5 C. Before use, the exudate was passed through a Nalgene filter (0.2 μm). Pea root extract was prepared by blending 10 g (fresh weight) of roots of 15-day-old pea plants for 10 min in 100 ml of water in a Sorvall Omnimixer. The homogenate was processed as described previously (7).

Preparation of anionic, cationic, and neutral fractions and different molecular size components of alfalfa root extracts and exudates. To narrow the search for types of compounds in root exudate and extract, samples (20 ml) of root extract and exudate were passed through a column (1 × 10 cm) of Ag1-X8 acetate-form anion exchange resin (Bio-Rad Co., Richmond, CA). Neutral and cationic fractions were washed from the resin with 130 ml of water. The anionic fraction was eluted from the column with 100 ml of glacial acetic acid, dried in a rotary evaporator at 40 C, and redissolved in 20 ml of water.

The cationic and neutral fractions were lyophilized, dissolved in 20 ml of water, and passed through a column (1 × 10 cm) of Bio-Rad Ag 50-X8 cation exchange resin. The neutral fraction was washed from the resin with 130 ml of water, lyophilized, and used for analysis and oospore germination studies. The cationic fraction was eluted from the column with 100 ml of 1 N ammonium hydroxide, dried in a rotary evaporator at 40 C, and the residue was dissolved in 20 ml of water. The cationic and neutral fractions of the root extracts and exudates were freeze-dried, weighed, stored at −5 C, and reconstituted with sterile water for germination tests. Root extracts and exudates were dialyzed with membranes (Spectrapore, Fisher Co., Pittsburgh, PA) with different porosities that separated compounds into molecular sizes of 6,000–8,000 and 14,000–16,000 daltons.

To determine whether active components were proteinaceous, 10 ml of alfalfa root extract or exudate were mixed with 1 ml of pronase solution (0.22 g/ml) and incubated at 24 C for 20 hr. A control solution containing 1 ml of pronase in 10 ml of water was also tested. To determine the effect of heat, samples of root extracts and exudates were autoclaved at 121 C for 15 min.

Samples of root exudate and extract (25 ml) were mixed with activated charcoal (0.5 g) for 24 hr at 3 C and filtered through Nalgene filters (0.45 μm). To determine loss of carbohydrates and amino acids, quantities were determined in both the original and charcoal-treated root extracts and exudates. To eliminate volatile materials, root exudate and extract were freeze-dried and redissolved in water at the same original volume.

Determination of total carbohydrates and sugars, amino acids, and organic acids. Total carbohydrates were determined quantitatively in alfalfa root extract, exudate, and in the neutral fraction by using the anthrone-sulfuric acid method (14). Sugars were determined qualitatively and quantitatively by using descending paper chromatography. Samples as well as standards of known sugars were cochromatographed and separated by paper chromatography with ethyl acetate, pyridine, and water (8:2:1, v/v) as a solvent (17). After 18 hr in the solvent, chromatograms were air-dried, and sugars were detected by using either *p*-anisidine spray (9) or the silver nitrate-sodium hydroxide method (25). For quantitative analysis, the corresponding areas containing each sugar on chromatograms which were not sprayed were excised from the chromatogram. Sugars were eluted from each section with 5–10 ml of water, filtered, and aliquots were removed for sugar determination by the anthrone method (14).

The carboxylic acid composition of the anionic fractions was determined by high performance liquid chromatography (Varian 5000 HPLC, Downey, CA). Acids were separated on a Bio-Rad HPX-87 column, with 0.01 N H_2SO_4 as the solvent, flowing at a rate of 0.5 ml/min at ambient temperature (27). Peaks were detected with an ultraviolet detector at 210 nm and tentatively identified by cochromatography with known standards.

Samples of root extracts and exudates were analyzed for individual amino acids on a single column (AA-20 ion exchange resin) Beckman automatic amino acid analyzer (Beckman Co., Fullerton, CA). Lithium citrate buffer system (pH 2.83, 3.70, and 3.75) was used to elute amino acids in a 4.5-hr run.

RESULTS

Germination of oospores in alfalfa root exudate and extract at varying pH values. Oospores germinated at pH values ranging from 3.5 to 10.5 in root extract or exudate with the optimum at pH 6.0 which is the pH of the original root extract and exudate. In root exudate, the percentage of germ sporangium formation was optimal at 6.0, but in root extract different pH values had no effect (Table 1).

Oospore germination in alfalfa root extract and exudate. Total oospore germination (86% for M69 and 78% for A77-10B), was greater in root extract than in root exudate (61% for M69 and 56% for A77-10B). Germ sporangia occurred only in root extract or exudate but not in water. Generally, root exudate stimulated a higher percentage of germ sporangium formation than root exudate (Fig. 1).

The increase in total percentage of oospore germination over that in water was directly proportional to concentration (total solids) in either root exudate (Fig. 2A) or root extract (Fig. 2B). However, the percentage of germination by germ sporangia varied with concentration of root extract in a bell-shaped curve response (Fig. 2B) with a maximum at 92 mg total solids per 100 ml of root extract. In parallel experiments, the response to concentration of root extract and exudates of A77-10B was similar to that of M69 (5).

Oospore germination in tomato, pea, and alfalfa root extracts and exudates. Root extracts and exudates from tomato and pea (neither are hosts) increased the percentage of total oospore germination (Fig. 3A) and germination by germ sporangia (Fig. 3B) over that in water. The degree of enhancement of germination by germ sporangia was greatest in pea, intermediate in alfalfa, and least in tomato (Fig. 3B).

Oospore germination in components of alfalfa root extract and exudate (M69) of different molecular sizes. The stimulatory effect of root exudates and extracts was not limited to a certain molecular size fraction. All molecular size fractions (above and below 8,000 daltons and 16,000 daltons) supported oospore germination in varying degrees (39–62%), but the effect of each was less than that of the original root extract (86%) and exudate (61%), but greater than in water (23%). Similar results were obtained with exudate and extract of A77-10B roots (5).

Oospore germination in heat- and pronase-treated alfalfa root extract and exudate. Total oospore germination was similar in autoclaved and unautoclaved root extract (about 80%) or exudate (about 58%). In distilled water the percentage was 21% (differences significant $P = 0.01$). Data for root extracts and exudates of both M69 and A77-10B plants were similar (5).

TABLE 1. Germination of oospores of *Phytophthora megasperma* f. sp. *medicaginis* in alfalfa (Moapa 69) root extract and exudate at different pH values

Oospores germinated (%)[y]					
Root extract			Root exudate		
	Total	With germ sporangia		Total	With germ sporangia
pH[x]	Mean[z]	Mean	pH[x]	Mean[z]	Mean
3.5	26.3 f	5.7 f	3.5	15.9 e	3.7 e
4.5	56.3 d	14.7 d	4.5	30.2 c	10.6 d
6.0	84.3 a	24.5 a	6.0	45.9 a	33.6 a
7.5	70.5 b	19.8 b	7.5	39.1 b	28.1 b
8.5	64.6 c	17.8 c	8.5	31.9 c	18.5 c
9.5	54.5 d	14.2 d	9.5	29.6 ed	8.8 d
10.5	47.3 e	11.3 e	10.5	27.0 d	5.3 e

[x] pH was adjusted with either 1 N HCl or 1 N NaOH. Root extracts were prepared from the water extracts of the homogenates from 10 g (fresh weight) of tap root tissue in 100 ml of water. Root exudate was collected from water in which seedlings were incubated aseptically for 8 days.

[y] Each value is the mean of three replicates of 450 oospores each.

[z] Means in one column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Total oospore germination was increased slightly but significantly ($P = 0.05$) by pronase treatment of M69 root extracts (86–93%) or exudate (61–68%); the effect of pronase alone (24%) was similar to that of water (23%). The percentage of germ sporangium formation was increased by pronase-treatment of root extract from 26 to 41% and in exudate from 34 to 47% (significant $P = 0.01$). Germination by germ sporangia did not occur in water.

Oospore germination in lyophilized and reconstituted root extract and exudate of alfalfa. Total oospore germination was similar in lyophilized and unlyophilized root extract (81%) or exudate (59%); germination in water was 27%. However, lyophilization of root extract markedly increased the formation of germ sporangia from 26 to 81% and of exudate from 26 to 81% of the total germinated. No germ sporangia formed in water. Differences were significant ($P = 0.05$).

Oospore germination in alfalfa root extract and exudate treated with charcoal. Charcoal treatment reduced the total carbohydrates in root extract by 80% and in root exudates by 71%. Charcoal also reduced the amino acid content in root extract by 52% and in root exudate by 56%. Charcoal treatment of root extracts reduced the percentage of total oospore germination to 52% but greatly increased the percentage of germination by germ sporangia from 25 to 47%. Charcoal treatment of root exudate reduced oospore germination from 61 to 39%, but germ sporangium formation was not affected (33%). In water, only 23% of the oospores germinated and no germ sporangia were formed. Differences were statistically significant ($P = 0.05$).

Oospore germination in fractions of alfalfa root extract and exudate. After fractionation of root extract and root exudate by ion exchange chromatography into cationic, anionic, and neutral fractions of the total solids, 73.5% of the total applied to the column were recovered from root extract and 83.9% from root exudate of M69. Recovery from root extract and root exudate of A77-10B was similar (5). The cationic fraction not only had the greatest stimulatory effect on oospore germination, but also was the only fraction that induced germination of oospores by germ sporangia. Oospore germination was least in the anionic fraction and intermediate in the neutral fraction. The combined cationic and neutral fractions induced the highest percentage of germination, but germination of oospores by germ sporangia occurred only when the cationic fraction was included. The highest percentage of total oospore germination occurred when all fractions were combined, but it was slightly less than in the original preparation of root exudate (Fig. 4A) or root extract (Fig. 4B). The fractions of root extract and root exudate from the susceptible M69 supported only a slightly higher percentage of oospore germination than fractions from the resistant A77-10B, but the trends were similar (5).

Analysis of the anionic, cationic, and neutral fractions of alfalfa root extract and exudate. In the anionic fraction of root extracts of both M69 and A77-10B alfalfa plants oxalic, malic, citric, malonic, and succinic acids were detected. The anionic fractions of the root exudate of the same cultivars also contained these acids with the exception of malonic acid. Since the anionic fraction did not increase oospore germination, these compounds were not tested singly.

The total carbohydrate content was higher in alfalfa root extract than in exudate. In root extract maltose, galactose, glucose, and mannose were identified, and in root exudate maltose, sucrose, and mannose were identified (Table 2).

The concentrations of amino acids were generally lower in root

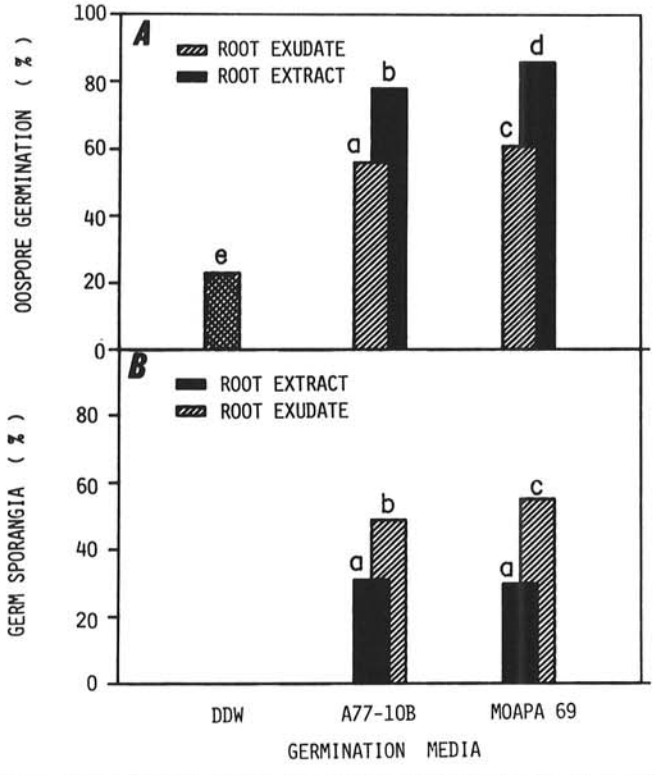

Fig. 1. A, Total oospore germination and **B,** germination by germ sporangia of *Phytophthora megasperma* f. sp. *medicaginis* in alfalfa root extracts and root exudates of susceptible cultivar Moapa 69 and a resistant germ plasm (A77-10B). Data for B were derived from the formula: [(percent oospore germination by germ sporangia)/(percent total oospore germination)] × 100. Root extracts were prepared from the homogenate from 10 g (fresh weight) of root tissue in 100 ml of water. Root exudate was collected from water in which seedlings were incubated aseptically for 8 days. DDW = double distilled water. Each value is the mean of three replicates with 450 oospores per replicate. Means designated by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

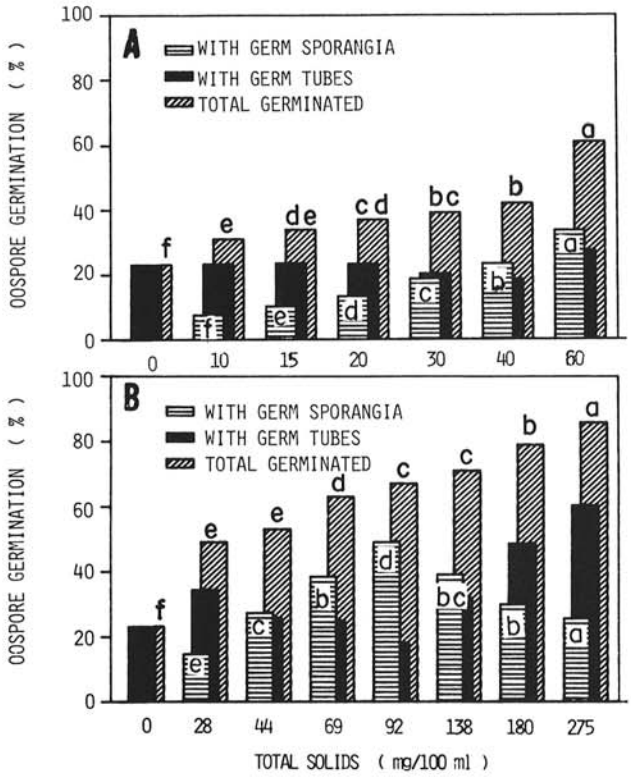

Fig. 2. Total oospore germination and germination by germ sporangia of *Phytophthora megasperma* f. sp. *medicaginis* in varying concentrations of **A,** root exudate and **B,** root extract of susceptible alfalfa cultivar Moapa 69. Root extracts were prepared from the homogenate from 10 g (fresh weight) of root tissue in 100 ml of water. Root exudate was collected from water in which seedlings were incubated aseptically for 8 days. Concentrations, expressed as total solids per 100 ml, were determined gravimetrically after lyophilization. Each value is the mean of three replicates with 450 oospores per replicate. Means designated by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

exudate than in root extract but qualitatively, the amino acid content was similar (Table 3).

Effect of sugars and amino acids on germination of oospores. When solutions of individual sugars and amino acids, at concentrations similar to those occurring in alfalfa root extract (Table 3), were tested in water, no increase in oospore germination occurred. Subsequently individual compounds were tested in dilute root exudate (0.1 mg of total solids per milliliter), a concentration at which germination was either not or only slightly increased (Fig. 2). Of the sugars tested, ribose induced the highest percentage of oospore germination (38.4%) while maltose induced the lowest (31.3%) compared to the dilute root exudate control (29.0%). Galactose, glucose, and sucrose decreased the percentage germinated by germ sporangia slightly (from 7 to 3%) (significant $P = 0.05$).

Aspartic acid was the only single amino acid that stimulated germination. When mixtures were made by adding one amino acid at a time to aspartic acid, the total oospore germination increased from 29% in water to 41% for aspartic acid and to 59% for some of the mixtures; germination by germ sporangia increased from 7% in water to 19% for aspartic acid and to 39% for some of the mixtures (Table 4).

DISCUSSION

Sussman and Douthit (23) distinguished two types of dormancy in fungi: constitutive dormancy "a condition wherein development is delayed due to an innate property of the dormant stage such as a barrier to the penetration of nutrients, a metabolic block or the production of a self inhibitor," and exogenous dormancy, "a condition wherein development is delayed because of unfavorable chemical or physical conditions of the environment." Cochrane (4) questioned the necessity for use of the term "exogenous dormancy" and considered oospores of *P. cactorum* (3) to be constitutively dormant.

Beakes (2) speculated that one of the keys to breaking oospore dormancy of *Saprolegnia ferax* may be the mobilization of wall glucans and that environmental conditions such as light and temperature might be the triggers for induction of glucanase production. In our previous report (7), heat shock at 33 C and oxidation by dilute potassium permanganate activated dormant oospores of *P. m.* f. sp. *medicaginis* (tetrazolium bromide vital stain test) and induced a high percentage of germination in water. However, the role of these external factors is not known.

Although oospore dormancy has generally been considered to be constitutive (4,28), oospores of some species of *Phytophthora* germinate soon after their formation if the proper external conditions exist (15). Förster et al (8) reported that the percentage of oospore germination of *P. m.* f. sp. *medicaginis* in water increased with age from about 3% at 11 days of age to 28% at 50 days of age. However, the percentage of germination of 11-day-old oospores was increased by root extract to 65% and that of 50-day-old oospores was increased to 85%. These data suggest, as do ours, that endogenous constraints (constitutive dormancy) were overcome by nutrients in root exudates.

Root exudates and extracts increased the rate of germination of oospores of *P. m.* f. sp. *medicaginis* five times, and the incidence of germination three to four times over that in water (8). Germination of oospores of *P. megasperma* f. sp. *glycinea* was increased by incubation in soil extract with and without soybean seedlings and

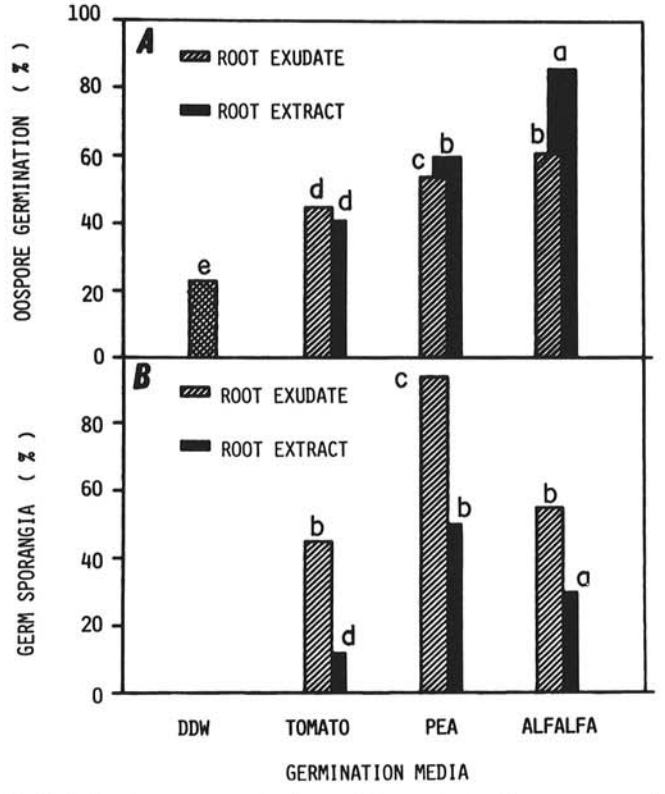

Fig. 3. A, Total oospore germination and **B,** germination by germ sporangia of *Phytophthora megasperma* f. sp. *medicaginis* in tomato (nonhost), pea (nonhost), and alfalfa root extracts and root exudates. Data for B are derived from the formula: [(percent oospore germination by germ sporangia)/(percent total oospore germination)] × 100. Root extracts were prepared from the homogenate from 10 g (fresh weight) of root tissue in 100 ml of water. Root exudate was collected from water in which seedlings were incubated aseptically for 8 days. DDW = double distilled water. Each value is the mean of three replicates with 450 oospores per replicate. Means designated by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

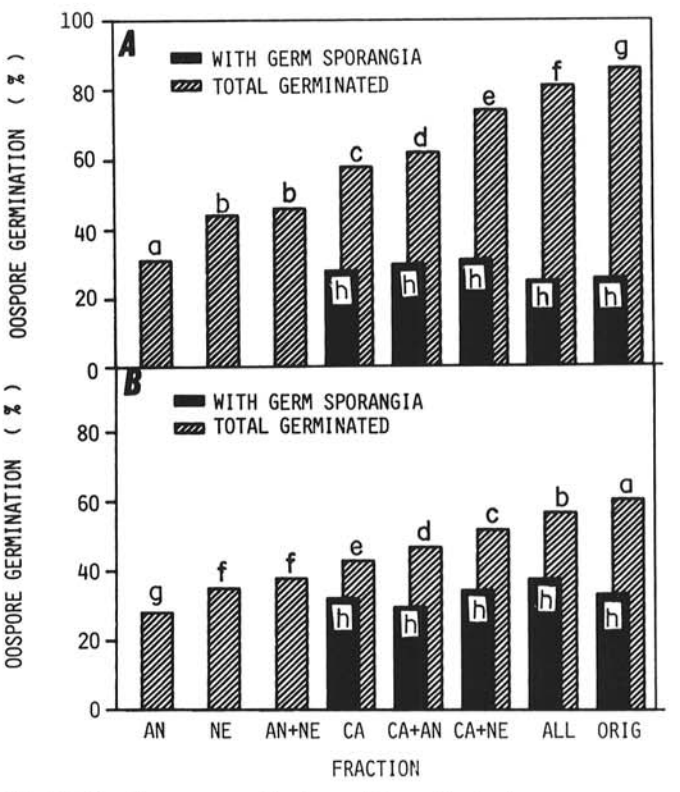

Fig. 4. A, Total oospore germination and **B,** germination by germ sporangia of *Phytophthora megasperma* f. sp. *medicaginis* in neutral (NE), cationic (CA), and anionic (AN) fractions of root exudate and of root extract from susceptible alfalfa cultivar Moapa 69. Root extracts were prepared from the homogenate from 10 g (fresh weight) of root tissue in 100 ml of water. Root exudate was collected from water in which seedlings were incubated aseptically for 8 days. Fractions were obtained by passing the root extract or exudate through columns of ion exchange resins. Each value is the mean of three replicates with 450 oospores per replicate. Means designated by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Quantitative analysis of sugars in the neutral fraction of root extract and exudate[w] of a susceptible cultivar (Moapa 69) and a resistant (A77-10B) germ plasm line of alfalfa

	Concentration (μg glucose/ml)[x]			
	Root extract		Root exudate	
Sugar	Moapa 69	A77-10B	Moapa 69	A77-10B
Raffinose	37.4	24.8	1.1	0.6
Isomaltose and maltose	51.6	71.6	2.1	0.9
Sucrose	17.8	16.0	2.9	0.4
Xylobiose	21.4	13.3	1.2	0.4
Galactose	97.9	24.4	0.4	0.6
Glucose	201.1	48.8	0.9	0.9
Mannose	158.4	149.9	0.3	2.7
Fructose	21.4	8.9	1.3	1.0
Arabinose	26.7	16.0	1.2	0.6
Ribose	5.3	9.8	...[y]	...[y]
Others[z]	40.9	42.6	1.0	3.1
Total	679.9	430.1	12.4	11.2

[w] Root extracts were prepared from the water extract of the homogenate by blending 10 g (fresh weight) of tap roots (45 days old) in 100 ml of water. Root exudate was collected from water in which seedlings were incubated aseptically for 8 days.

[x] Analysis by descending paper chromatography.

[y] Ribose was not detected by paper chromatography.

[z] The part of the neutral fraction that remained at the origin and did not travel with the solvent.

TABLE 3. Quantitative analysis of amino acids of alfalfa (Moapa 69) root exudate and extract and its cationic fraction

	Concentration (nmole/ml)			
Amino acid[x]	Root extract[y]	Cationic fraction[z]	Root exudate[y]	Cationic fraction[z]
Phosphoserine	trace		4.80	
Aspartic acid	66.4	51.0	3.18	2.80
Threonine	18.9	17.9	0.60	0.35
Serine	42.8	37.1	0.48	0.92
Asparagine	697.5	600.9	1.62	1.54
Glutamic acid	37.1	16.6	trace	0.32
Glutamine	13.8	6.7	0.38	0.32
Proline	trace	trace	0.22	0.20
Glycine	11.8	11.0	0.46	0.40
Alanine	60.8	55.7	1.56	1.52
Citrulline	5.3	2.6	2.98	2.76
Valine	9.7	8.0	0.56	0.52
Cystine	5.3	2.8	1.08	1.00
Methionine	trace	trace	0.28	0.20
Isoleucine	11.9	11.2	0.56	0.54
Leucine	30.1	25.2	1.00	0.96
Tyrosine	trace	trace	0.28	0.26
Phenylalanine	trace	trace	0.44	0.38
γ-aminobutyric acid	70.7	68.8	0.98	0.82
Tryptophane	6.4	5.1		
Ethanlolamine	21.1	17.5		
Ornithine	trace	trace	0.86	0.80
Lysine	22.0	21.4	0.78	0.70
Histidine	17.3	15.7	0.58	0.56
3-methylhistidine	trace	trace		
Arginine	33.8	32.7	0.38	0.36

[x] Amino acids are listed in order of their sequence as they were eluted from the Beckman amino acid analyzer column.

[y] Root extracts were prepared from the water homogenate of roots of 45-day-old alfalfa cultivar Moapa 69 plants in 100 ml of water. Root exudate was obtained by incubating aseptically grown seedlings in water for 8 days.

[z] Cationic fraction was obtained by passage of exudates and extracts through a Bio-Rad Ag 1-X8 acetate-form anionic exchange resin. Cationic and neutral fractions were washed from the resin with water, lyophilized, redissolved in double-distilled water and passed through a column of Bio-Rad Ag 50-X8 cation exchange resin. The cation fraction was eluted from the column with 1 N ammonium hydroxide, dried in a rotary evaporator and redissolved in the original amount of double-distilled water.

other plant tissues (10,19). Our work confirms the concept that germination of oospores is stimulated by nutrients. However, why some oospores (about 20%) germinated in water without nutrients can not be explained.

Amino acids, especially aspartic acid, were the most active nutritional components of root exudates that stimulated oospore germination and production of germ sporangia. No other reports on the effect of various fractions or components of root exudate or root extract on oospore germination of *Phytophthora* have appeared in the literature. Likewise, there are no other reports on the chemical components of root exudates from alfalfa. The similar effects of components having different molecular sizes and lack of an effect by heat treatment or the proteolytic enzyme, pronase, on alfalfa root extract or root exudate, strengthened the conclusion that the increase in oospore germination and/or germ sporangium formation was not due to a large protein molecule. Because aspartic acid, when tested singly, increased the total oospore germination by 40% and increased the percentage of germination by germ sporangia by 80% over the water control (Table 4), it is probably one of the most important components. However, since aspartic acid alone and combined with other amino acids was not stimulatory to oospore germination except when added to dilute root exudate, and since the complete mixture of all amino acids was required to induce a maximum increase in oospore germination, the system appears to be complex.

Ruben et al (16) showed that, for *Pythium aphanidermatum*, an increase in nutrient concentration in a synthetic medium containing lecithin increased oospore germination by germ sporangium formation. In soil, however, oospores of *P. aphanidermatum* germinated by germ sporangia but only in the absence of added nutrients (21). Chlamydospores of *P. parasitica* germinated by germ hyphae in broth media, but in soil extracts, with or without asparagine (0.01 M), they germinated by germ sporangia (26).

Ribose increased oospore germination slightly (but significantly) from 29% (control) to 38.4%, but sucrose, galactose, and glucose suppressed germ sporangium formation. Glucose or sucrose (10 g/L) inhibited oospore germination of *P. m.* f. sp. *medicaginis* (8). However, fructose and mannitol (5 g/L) stimulated oospore germination of *P. erythroseptica* (11). Oospore germination of *P.*

TABLE 4. Effect of amino acids on germination of oospores of *Phytophthora megasperma* f. sp. *medicaginis*

	Oospores germinated (%)[y]	
Amino acids[x]	Total mean	With germ sporangia mean[z]
Asp	41.1 h	18.6 k
Asp + val	41.3 h	19.6 j
Asp + val + glutamine	43.5 g	21.4 i
Asp + val + glutamine + ser	46.2 f	22.6 h
Asp + val + glutamine + ser + asparagine	46.3 f	23.9 g
Asp + val + glutamine + ser + asparagine + gly	46.4 f	24.6 f
Asp + val + glutamine + ser + asparagine + gly + glut	49.6 e	26.6 e
Asp + val + glutamine + ser + asparagine + gly + glut + lys	52.2 d	27.8 d
Asp + val + glutamine + ser + asparagine + gly + glut + lys + leu	52.8 c	30.8 c
Asp + val + glutamine + ser + asparagine + gly + glut + lys + leu + prol	54.5 b	32.3 b
All amino acids	58.8 a	38.6 a
Dilute root exudate control	29.0 i	7.2 e

[x] The concentration of amino acids tested in dilute root exudate (0.1 mg total solids per milliliter) corresponds to that in the root extract (see Table 3).

[y] Each value is the mean of three replicates with 450 oospores per replicate. Values followed by a different letter varied significantly ($P = 0.1$) according to Duncan's multiple range test.

[z] Data are calculated from: [(percent oospore germination by germ sporangia)/(percent total oospore germination)] × 100.

m. f. sp. *glycinea* was reduced in an extract of natural soil supplemented with glucose (0.5%) (19). Conversely, oospores of *P. aphanidermatum* that had been passed through snails were markedly stimulated to germinate by several sugars (including glucose and sucrose) (22).

Charcoal, which adsorbs some organic molecules, decreased total oospore germination and increased germ sporangium formation when added to alfalfa root exudate and root extract. Since charcoal reduced the amino acid content of root extract by over 50% and oospore germination by over 70%, the effect of charcoal was most likely due to reduction of the amino acid content. Lyophilization of root exudate or extract, which would eliminate some volatile compounds, had no significant effect on total oospore germination but induced a large increase in germ sporangia.

Oospore germination over a wide range of pH values from 3.5 to 10.5 in alfalfa root extract or root exudate with an optimum at pH 6.0 (Table 2) is a typical response of most *Phytophthora* spp. (15). Conversion of dormant to germinable oospores of *Pythium ultimum* was optional at pH 7.0 (12).

Oospore germination by formation of germ sporangia is probably of epidemiological importance. Tsao (26) suggested that germ sporangia produced by chlamydospores in response to nutrients, ". . . would influence the inoculum potential of *Phytophthora parasitica*." Although germ sporangium formation increased with concentration of total solids in root exudate to a certain point, concentrations above that level decreased germ sporangium formation (Fig. 2). Therefore, oospore germination by germ sporangia may be a function of concentration of nutrients of which amino acids may be the most important.

Since root extracts and root exudates from both resistant (A77-10B) and susceptible (M69) alfalfa and from nonhost tomato and pea plants enhanced oospore germination and germ sporangium formation (Fig. 4), the nutrient effect is probably nonspecific. The lack of an effect by the resistant germ plasm A77-10B is not surprising since resistance in alfalfa to *P. m.* f. sp. *medicaginis* is expressed within root tissue (13).

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