Alfalfa Seedling Resistance to *Phytophthora megasperma*

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ABSTRACT

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A reliable seedling assay is described which allows rapid differentiation of alfalfa cultivars resistant or susceptible to *Phytophthora* root rot (caused by *Phytophthora megasperma*). Fifty-nine of 62 clones, selected as resistant 7 days after the sowing of pregerminated seeds in infested soil, were resistant at maturity. Seedling resistance depended on inoculum concentration and duration of environmental conditions conducive to disease development. At 0.58 g dry weight of mycelium per kilogram dry weight of planting mix, almost all seedlings of cultivars Agate (resistant) and Vernal (susceptible) were killed within 3 days after saturation of the planting mix with water 4 days after sowing. When the inoculum concentration was lowered to 0.08–0.12 g dry weight of fungus per kilogram dry weight of mix, nearly all seedlings of susceptible cultivars were killed within 3–4 days after the planting mix was saturated, but a significantly higher percentage of seedlings from resistant cultivars survived more than 7 days under the same conditions. More seedlings of resistant cultivars than of susceptible cultivars survived 5 days after inoculation at the full cotyledon growth stage when zoospores (3×10^3 to 3×10^5 zoospores per container) were used as inoculum. The seedling assay was used to screen 20 alfalfa cultivars and breeding lines for which reaction to the fungus previously had been determined by assay of mature plants. Disease ratings of all seedlings tested were similar to the disease ratings of the mature plants. Possible use of the method in alfalfa breeding is discussed.

Phytophthora root rot of alfalfa (*Medicago sativa* L.) is incited by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* (as proposed by D. C. Erwin and Ta-li Kuan, *personal communication*) and limits yield and persistence of alfalfa on fine textured, poorly drained soils (2,4,9). Development of a reliable seedling assay would offer an efficient means for rapid detection of germplasm resistant to *P. megasperma* f. sp. *medicaginis* and also would be of particular value for screening plants for resistance to more than one disease.

A technique for selecting alfalfa seedlings resistant to *P. megasperma* f. sp. *medicaginis* first was described in 1973 (5). After 14 days in saturated soil, 79% of the seedlings of commercial cultivar Hayden were dead, whereas only 20% of the seedlings of a first generation root rot-resistant selection from Hayden were killed. In the same trial, only 3% of U. C. 47 (a *Phytophthora*-resistant line released by the University of California) were dead.

Since then other workers have reported that alfalfa seedlings have no resistance to *P. megasperma* f. sp. *medicaginis* (3,10). All seedlings of cultivars Agate and Apalachee, resistant and susceptible (respectively) to P. *megasperma* f. sp. *medicaginis*, died before emergence when exposed to continuous water saturation immediately after being planted in infested soil (10). Seed of these cultivars sown in infested soil and subjected to 3-day periods of alternate wetting and drying, showed no significant difference in the amount of postemergence damping-off.

A postemergence damping-off technique was used to identify *Phytophthora*-resistant phenotypes in seedlings of Australian cultivar Hunter River (1). The assay helped identify resistant plants as seedlings, but often differed widely in percentage of seedlings killed between known resistant and susceptible cultivars in different trials, and variability always was greater in seedling assays than in mature plant assays.

This paper describes studies undertaken to develop a reproducible method of screening alfalfa seedlings for resistance to *P. megasperma* f. sp. *medicaginis.* The effects on seedling resistance of planting mix, type and amount of inoculum, and alfalfa cultivar were investigated. Data also are presented on the relationship between seedling resistance and subsequent mature plant reaction.

MATERIALS AND METHODS

Isolation and sources of the pathogen. Isolates of *P. megasperma* f. sp. *medicaginis* were obtained by baiting from soil (7) collected from the Phytophthora root rot nurseries in St. Paul, MN (isolates 5b, 5c, 7b, and 7x) and Ames, IA (isolate 20), and by direct isolation from diseased alfalfa roots collected in Wisconsin (isolate 21). Isolates DA and B12 were supplied, respectively, by J. E. Mitchell, University of Wisconsin, Madison, and K. Leath, The Pennsylvania State University.

Preparation of mycelial inoculum. Six pathogenic isolates of *P. megasperma* f. sp. *medicaginis* (DA, 7b, 7x, 5b, 5c, and 20) were grown as still cultures in 100 ml of liquid V-8 juice medium (100 ml Campbell's V-8 juice and 2.0 g $CaCO_3$/L deionized water) in 500-ml Erlenmeyer flasks at 25 C on a laboratory bench. After 10–13 days, mycelial mats were harvested, washed, and pressed between sterilized, brown paper towels to about 75–80% moisture (dry weight determinations were made on a subsample of the mycelial mats). The mats were chopped in distilled water in a Waring Blendor for 10 sec. Equal weights of each of the six isolates were used to prepare a composite inoculum. Four of the isolates failed to produce oospores in the V-8 juice liquid culture medium; two isolates (5b and 7x) produced only a few oospores.

Preparation of zoospore inoculum. Cultures of each isolate were grown on V-8 juice agar in 9-cm-diameter petri dishes for 8 days at 24 C and then transferred for 2–3 days at 28 C. Agar was removed from around the edge of each colony and the plates were flooded with 15 ml of sterilized, distilled water and incubated for 12–20 hr at 16 C (L. C. Davidse, *personal communication*).

Sources of alfalfa cultivars and breeding lines. Eighteen cultivars or breeding lines, ranging in reaction to *P. megasperma* f. sp. *medicaginis* from susceptible to moderately resistant, were tested (Table 1).

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Effects of planting mix, cultivars, and seedling growth stage on disease development induced by mycelial inoculum. Two heat-pasteurized planting mixes were tested, a peat-sand mix (1:1, v/v) (PS) and a sand-vermiculite-perlite mix (1:2:1, v/v) (SVP). The PS had the following characteristics: pH 6.4, and a water retention of 78, 36, 35, and 30% (w/w) at 0, −0.1, −0.33, and −1.0 bars matric potential (ψ_m), respectively. The SVP had the following characteristics: pH 7.9, and a water retention of 81, 30, 29, and 28% at 0, −0.1, −0.33, and −1.0 bars ψ_m, respectively. The experiment was conducted in 946-ml watertight plastic cups. Each cup contained either 775 g dry weight of PS or 690 g dry weight of SVP. These mixes were infested with *P. megasperma* f. sp. *medicaginis* at the rate of 0.116 g dry weight of the fungus per kilogram dry weight of planting mix. The inoculum was mixed into the soil by hand. Water (without nutrients) and 150 ml of nutrient solution (Hoagland's solution) per cup were added to the mixes to give final moisture contents of 36 and 30% (w/w) for the PS and SVP, respectively. In each cup, 40 alfalfa germlings of either Agate or Vernal (pregerminated for 24 hr) were distributed uniformly over the surface of the mix, then covered with 50 g of the infested mix. After the seeds were planted, the cups were covered with plastic bags and placed in a growth room with a 10-hr dark period at 16 C and with a 14-hr light period at 10,070 lux and 21 C. The bags were removed after 72 hr, when the majority (>90%) of the seedlings had emerged. Control cups, without the fungus, also were included in the experiment.

Two watering programs, hereafter referred to as saturated and unsaturated, were imposed. In the saturated treatment, water was added daily to each cup until 0.5–1.0 mm of free water remained on the surface. This treatment was begun at two seedling growth stages: the germling stage (time of seeding) and the full cotyledon development stage (4 days after sowing). In the unsaturated treatment, cups were watered daily to 36 and 30% (w/w) moisture retention (determined by weighing) for the PS and SVP, respectively.

After total emergence was determined 4 days after sowing, daily counts were made of the number of healthy seedlings in each pot. Seedlings were classified as healthy when cotyledons and hypocotyls showed no discoloration or collapse.

The experiment was completely randomized with three replications. Only treatments involving saturation at the full cotyledon stage and the unsaturated treatment for SVP and PS were repeated.

Inoculum dilution studies with mycelial fragments. Methods for preparation of inoculum, infestation of the planting mix, and sowing of Agate and Vernal germlings were as described above, except that only the SVP was used, and treatments were saturated only at the full cotyledon growth stage. A twofold serial dilution series, beginning with a concentration of 0.145 g dry weight of the fungus per kilogram dry weight of mix, through a 1:512 dilution was prepared. A noninoculated control treatment was included. Daily counts were made of the number of healthy seedlings. The experiment was replicated three times, with a completely randomized design, under the environmental conditions described above and was repeated once with an inoculum concentration four times greater than reported above for the highest concentration.

Inoculum dilution studies with zoospores. Alfalfa germlings of Agate and Vernal were sown into SVP prepared as for control treatments. At the full cotyledon growth stage, each cup was thinned to 32 seedlings and saturated. Motile zoospores from each of the six isolates of *P. megasperma* f. sp. *medicaginis* were combined in equal proportions in a composite inoculum. A tenfold dilution series from 3×10^5 to 3×10^2 zoospores in 30 ml of water was used to infest the cups. Thirty milliliters of water were added to each control cup. Three replications were used. The addition of the 30 ml of zoospore suspension produced a 4- to 5-mm layer of free water on the surface of the mix. Cups were saturated once each day for 5 days after inoculation, and the number of healthy seedlings was determined each day.

Screening of alfalfa cultivars as seedlings by applying mycelial and zoospore inoculum. A range of alfalfa cultivars (Table 1) was screened for resistance to *P. megasperma* f. sp. *medicaginis* by the methods outlined above for the mycelial and zoospore inoculations. To inoculate with mycelium, a concentration of 0.118 g dry weight of the pathogen (composite inoculum of equal wet weight of the six isolates) per kilogram dry weight of mix was used. To inoculate with zoospores, 5×10^4 zoospores of isolate 5b were added to each cup. The experimental design was a completely randomized block with three replications; the experiments were repeated once.

Relationship between resistance expressed in seedlings and in mature plants. Resistant seedlings of Agate, Apollo, LR, HR-P_3, 0310, and GS selected by the mycelial inoculation method and 15 seedlings of Vernal (susceptible) from the control treatments of the same experiment were planted individually in 10.3-cm-diameter clay pots containing about 400 g dry weight of PS. At 8 wk of age, these plants were inoculated with a composite inoculum (0.181 g dry weight of the pathogen per pot) by pouring 30 ml of inoculum uniformly over the surface of the mix, then incorporating the inoculum into the upper 2 cm of the mix. Control pots of noninoculated Vernal were included. Immediately after infestation, pots were placed in saucers kept filled with water for 2-day periods, then were allowed to drain for the next 2 days. This process of alternate wetting and drying was continued for 21 days, after which plants were removed from the pots and given a disease rating (see footnote c, Table 3). The experiment was conducted in a greenhouse at 19–22 C.

In another experiment, one rooted stem cutting from each of 18 resistant plants selected from Agate on the basis of seedling reaction were individually planted in 10.3-cm-diameter clay pots as described above. Similarly, rooted cuttings from 25 plants selected from Saranac (susceptible) were prepared. Two replicates of each clone (a specific plant) were used. Three months later, each pot was infested and subsequently watered by the alternate wetting and drying procedure described above. Noninfested control pots containing cuttings from one clone of Agate and Saranac were included. The plants grown from the cuttings were scored for disease reaction 21 days after inoculation.

TABLE 1. Sources of alfalfa cultivars and breeding lines and their field-disease reaction to *Phytophthora megasperma* f. sp. *medicaginis*

Cultivar or breeding line	Source	Disease reaction[a]
Agate	F. I. Frosheiser, Univ. of Minnesota	R
CA, A77-10B	D. C. Erwin, Univ. of California, Riverside, CA	R
Apollo	North American Plant Breeders, Ames, Iowa	MR
0321		MR
NAPB 72		R
0310		R
0320		R
0322		R
NY PopA	R. P. Murphy, Cornell Univ., Ithaca, NY	R
NY E32		R
LR	E. T. Bingham, Univ. of Wisconsin-Madison	R
GS		S
GV		S
W74-24		S
Hunter River - P_3	R. A. Bray and J. A. G. Irwin, C.S.I.R.O. and Q.D.P.I., Queensland, Australia, respectively	R
Saranac	Commercial	S
Vernal		S

[a]Abbreviations: R = resistant; MR = moderately resistant; and S = susceptible.

RESULTS

Effect of planting mix, cultivars, and seedling growth stage on disease development induced by mycelial inoculum. When cups containing germinated seeds of Vernal (susceptible) or Agate (resistant) were unsaturated for 4 days after sowing, no significant differences in seedling emergence between infested (92%) and noninfested (93%) mixes were found. However, when cups were

saturated immediately after sowing, significantly fewer seedlings (average for both cultivars, Vernal and Agate) emerged in both noninfested (44%) and infested (3%) mixes, compared to those that were kept unsaturated. With saturated, noninfested mixes, significantly fewer seedlings emerged in the PS (33%) than in the SVP (54%).

Disease progress curves for all saturated, inoculated treatment combinations beginning 4 days after seeding (cotyledon stage), and for the unsaturated treatment combinations are shown in Fig. 1 and 2, respectively. On day 0 (first day of saturation), data averaged over mixes and water regimes showed that significatnly more ($P = 0.003$) seedlings of Vernal (13%) than of Agate (4%) had been killed. Three days after saturation significant differences (based on the percentage of seedlings killed) were found between cultivars (76 and 38% for Vernal and Agate, respectively) (Fig. 1), mixes (73 and 44% for PS and SVP, respectively), and water regimes (Fig. 1 and 2) (64 and 53% for the saturated and unsaturated regimes, respectively). None of the interaction terms in the analysis was significant.

At day 7, disease had progressed significantly more slowly in the unsaturated SVP, where only 57% of all seedlings were killed, compared to 83–95% killed in the unsaturated PS and saturated PS and SVP (the potting mix and water regime interaction was significant for day 7).

At day 7 significantly fewer seedlings of Agate than of Vernal had been killed for all treatments. However, as the period of exposure increased, more Agate seedlings were killed. By day 14, 93% of Agate seedlings (averaged over both mixes at saturation) had been killed, whereas all the Vernal seedlings had been killed within 5 days after saturation.

Inoculum dilution studies. Seedling emergence compared to that of the noninoculated controls was not significantly reduced ($P = 0.05$) at any mycelial inoculum concentration. The percentage of seedlings killed after emergence always was higher for Vernal than for Agate at all concentrations of inoculum tested (Fig. 3); and, as the period of saturation increased from 3 to 7 days, the percentage of seedlings killed increased in both cultivars. When this experiment was repeated with an inoculum concentration of 0.58 g dry weight of the fungus per kilogram dry weight of mix (four times greater than that used for the experiment reported in Fig. 3) all seedlings of Agate had been killed within 7 days after saturation.

Fewer seedlings of Agate than of Vernal were killed when these cultivars were inoculated with several different concentrations of zoospores (Fig. 4). Five days after saturation, 46% of the Vernal seedlings were killed compared to 16% of the Agate seedlings at 3×10^3 zoospores per cup. When the number of zoospores per cup was increased to 3×10^4, 97 and 55% of the Vernal and Agate seedlings, respectively, had been killed 5 days after saturation. Variability within treatments was slightly greater when zoospores (Fig. 4) rather than mycelial fragments (Fig. 3) were used as inoculum.

Resistance to *P. megasperma* f. sp. *medicaginis* by seedlings of alfalfa cultivars and breeding lines. The cultivars and breeding lines resistant to *P. megasperma* f. sp. *medicaginis* in mature plant tests also were the most resistant in the seedling assays in which mycelial inoculum was used (Table 2). The breeding line HR-P_3, which is known to be resistant to Australian isolates of the pathogen, also was resistant to North American isolates. The cultivars and breeding lines which were resistant in the mycelial inoculum assay also were resistant when inoculated with zoospores. Five days after saturation and inoculation with zoospores of *P. megasperma* f. sp. *medicaginis*, the percentage survival in the resistant cultivar, Agate, and the resistant breeding lines 0360, 72, and CA was 44, 66, 59, and 57%, respectively, whereas in susceptible cultivar Vernal and susceptible breeding line

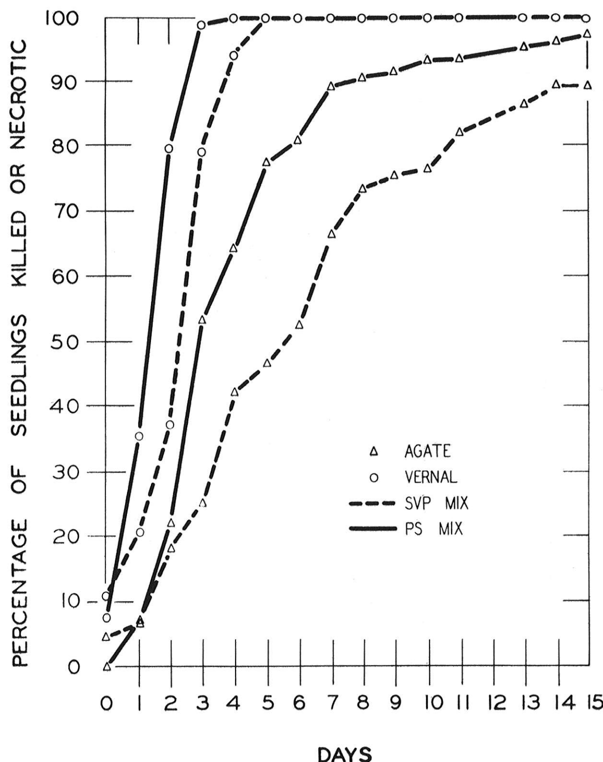

Fig. 1. Effects of planting mixes (peat-sand [1:1, v/v] [PS] and sand-vermiculite-perlite [1:2:1, v/v][SVP]) on disease development in two alfalfa cultivars (Agate and Vernal). Germlings were sown into the mixes infested with mycelium of *Phytophthora megasperma* f. sp. *Medicaginis* (0.116 g dry weight of fungus per kilogram dry weight of each mix). Each planting mix was saturated daily for 15 consecutive days, commencing 4 days after sowing, when the seedlings were at the full cotyledon growth stage.

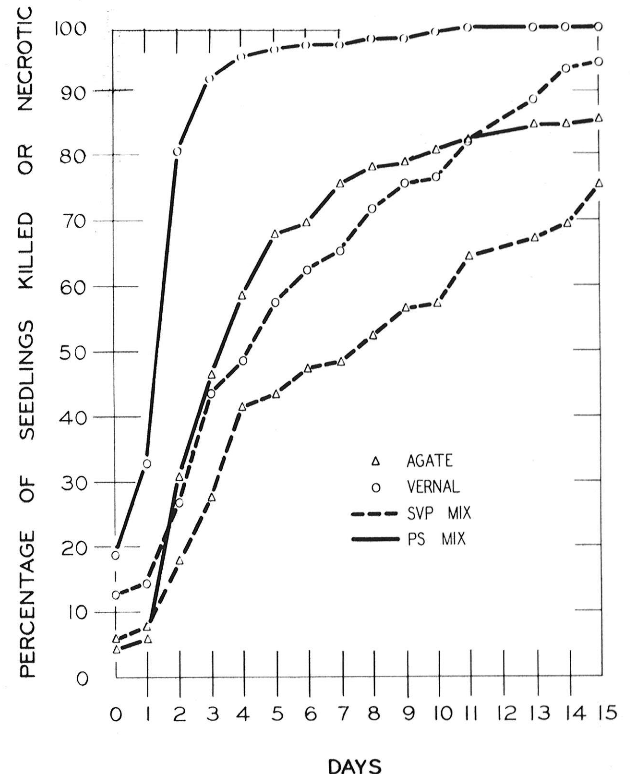

Fig. 2. Effects of planting mixes (peat-sand [1:1, v/v][PS], and sand-vermiculite-perlite [1:2:1, v/v][SVP]) on disease development in two alfalfa cultivars (Agate and Vernal). Germlings were sown into the mixes infested with mycelium of *Phytophthora megasperma* f. sp. *medicaginis* (0.116 g dry weight of fungus per kilogram dry weight of each mix). Each planting mix was watered daily by weight to a moisture retention of 36 and 30% (w/w) for the PS and SVP, respectively, (unsaturated treatment) for 15 consecutive days, commencing 4 days after sowing, when the seedlings were at the full cotyledon growth stage.

W74-24 only 2 and 0%, respectively, of the seedlings survived.

The relationship between the resistance expressed in seedlings and in mature plants. Clones known to be resistant as seedlings also were resistant when reinoculated as mature plants (Table 3). In another experiment, in which 2-mo-old rooted cuttings from clones of Agate, which were resistant in the seedling test, and Saranac, which is highly susceptible, were inoculated, 15 of the 18 Agate clones were highly resistant, and the two Saranac clones were highly susceptible. These results show that seedling assay provides a reliable prediction of the disease response of a mature plant.

TABLE 2. The percentage survival of seedlings of alfalfa cultivars and breeding lines 7 days after saturation in a sand-vermiculite-perlite (1:2:1, v/v) mix (SVP) infested with mycelium of *Phytophthora megasperma* f. sp. *medicaginis*[y]

Cultivar	Mean percent Survival[z]
NY PopA	38 a
0310	35 a
NAPB 72	33 ab
Agate	30 ab
0322	30 ab
HR-P_3	29 ab
0320	29 ab
NY E32	22 b
LR	21 b
Apollo	11 c
0321	11 c
WL-318	9 c
GS	5 c
WL-312	5 c
GV	4 c
W74-24	1 d
Saranac	1 d
Vernal	0 d

[y]A composite inoculum (0.118 g dry wt of fungus per kilogram dry weight of SVP mix) containing eight isolates of the pathogen was used.
[z]Means followed by the same letter are not significantly different ($P = 0.05$).

DISCUSSION

The results of these experiments show that alfalfa cultivars can be screened in the early seedling stage for mature plant resistance to *P. megasperma* f. sp. *medicaginis*; however, both the inoculum concentration and the period of exposure to the pathogen under environmental conditions conducive to disease development have a profound effect on the expression of resistance. Therefore, critical control of these parameters is required to allow expression of resistance which correlates to that of mature plants. With mycelial inoculum at concentrations of 0.08–0.116 g dry weight of fungus per kilogram dry weight of planting mix, nearly all seedlings of susceptible cultivars were killed within 3–4 days after the mix was saturated (7–8 days after sowing). In contrast, significantly more seedlings of resistant cultivars survived when tested under these conditions. At 7 days after the mix was saturated, 20–30% of the Agate seedlings were still without visible necrosis of hypocotyls or cotyledons, although they appeared to be stunted compared with those of the noninoculated controls.

In our studies, inoculum levels were strictly controlled, whereas in previous work, control of that variable was insufficient to allow definite conclusions (3,10). A reproducible inoculum dosage, based either on dry weight measurements of hyphal mass or on specific numbers of zoospores, and a planting mix of specified dry weight and moisture regime, enabled our tests to be repeated with a high degree of reproducibility.

Nearly complete preemergence damping-off occurred when

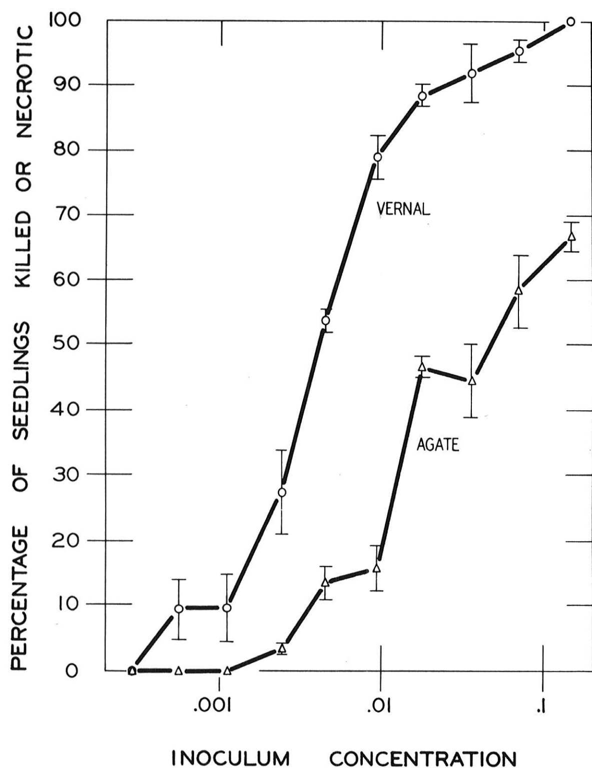

Fig. 3. Effect of various concentrations of mycelial inoculum of *Phytophthora megasperma* f. sp. *medicaginis* (grams dry weight of mycelium per kilogram dry weight of sand-vermiculite-perlite [1:2:1, v/v]) on disease development in seedlings of cultivars Agate and Vernal 7 days after the mix was saturated. Saturation was commenced 4 days after sowing, when the plants were at the full cotyledon growth stage.

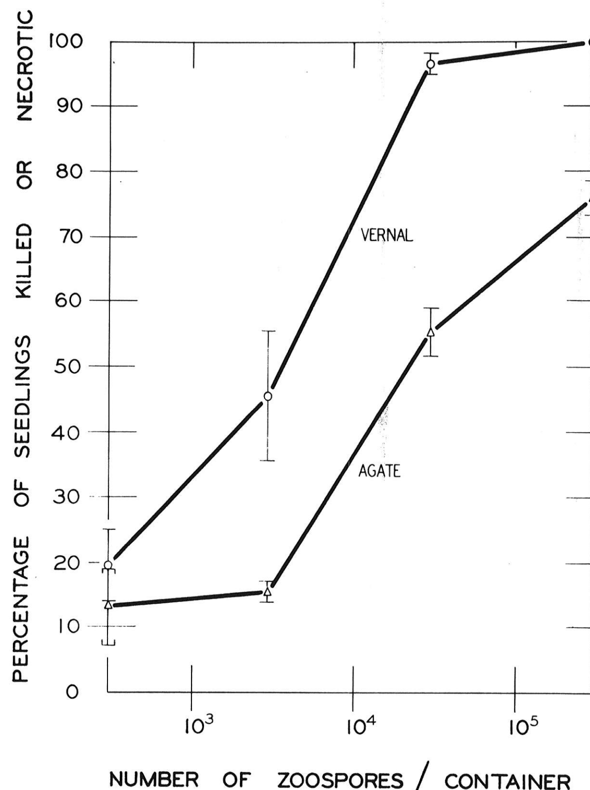

Fig. 4. Effect of concentration of zoospores of *Phytophthora megasperma* f. sp. *medicaginis* on disease development in seedlings of alfalfa cultivars Agate and Vernal grown in sand-vermiculite-perlite (1:2:1, v/v), 5 days after inoculation and saturation. Inoculation and saturation occurred 4 days after sowing, when the plants were at the full cotyledon growth stage.

germlings were sown into planting mixes infested with *P. megasperma* f. sp. *medicaginis* at 0.116 g dry weight of the fungus per kilogram dry weight of mix saturated immediately. Gray et al (5) reported very low levels of preemergence damping-off of seedlings from seeds sown into infested soil which was maintained at a high soil moisture content by standing the pots in saucers filled with water. These differences may be explained by differences in inoculum concentration, planting mixes, and watering regimes. We were able to eliminate preemergence damping-off at all inoculum levels tested by delaying saturation of the planting mixes until 4 days after sowing.

When seedlings classified as resistant were examined after 7 days in a saturated mycelium-infested mix or after 5 days in a saturated zoospore-infested mix, their roots usually showed slight necrosis of the cortical tissue and were shorter than were the roots of control plants. However, when the resistant seedlings were repotted into noninfested PS or SVP in pots with unimpeded drainage, almost all seedlings grew rapidly and at 6 wk of age could not be distinguished readily from control plants.

Fifty-nine of 62 clones from a range of cultivars selected as resistant in the seedling assay showed resistant root reactions when reinoculated as mature plants. Three clones which were resistant in the seedling assay were moderately susceptible to susceptible in the mature plant test, which suggested that different genetic mechanisms may control seedling and mature plant root reactions in these clones.

TABLE 3. A comparison of the reaction to *Phytophthora megasperma* f. sp. *medicaginis* of alfalfa seedlings and their subsequent reaction as mature plants or clonal cuttings

Cultivar or breeding line	No. of clones tested	Seedling reaction[a]	Method of evaluation	No. of clones with disease severity index of[c]				
				1	2	3	4	5
Agate	11	R	Mature plants[d]	5	6			
Apollo	2	R		1	1			
HR-P3	7	R		3	4			
GS	6	R		3	3			
LR	7	R		5	2			
0310	11	R		10	1			
Vernal	15	--[b]				1	8	6
Agate	18	R	Clonal propagules[e]	8	7	2	1	
Saranac	25	--[b]	Clonal propagules				18	7

[a]The abbreviation R = resistant.
[b]Plants of cultivars Vernal and Saranac generally are very susceptible; therefore, it was presumed that the seedlings of these plants would have been susceptible (see Table 2).
[c]Disease severity index:
1 = Tap root, secondary roots, and fine feeder roots white (healthy).
2 = Small lesions not encompassing more than 0.2 of the circumference of the tap root. They were present mainly at the junction of the tap and lateral roots. Girdling lesions permissible on all roots up to 1 mm in diameter.
3 = Lesions on the tap root encompassing 0.2–0.5 of the circumference. Girdling lesion permissible on all roots up to 2 mm in diameter.
4 = Lesions completely girdling the tap root and/or larger lateral roots. Almost all smaller secondary roots destroyed.
5 = Entire tap root rotted, above ground parts dead.
[d]Mature plants were 8 wk old when inoculated.
[e]Clonal propagules were 13 wk old when inoculated.

Pratt et al (8) described a cotyledon assay in which zoospore inoculum was used for screening for resistance to *P. megasperma* f. sp. *medicaginis* in alfalfa seedlings. Resistant alfalfa cultivars, selected by mature plant root inoculation tests, contained significantly more seedlings that showed a resistant cotyledon reaction than did susceptible cultivars. They also found that the susceptible cotyledon reaction in Vernal was expressed over a wide range of zoospore concentrations, but did not investigate the effect of inoculum concentration on the resistant cotyledon reaction.

The seedling method employed in this study does not allow the assessment of intermediate classes of resistance as have been established for mature root assays (4) and the cotyledon assay (8). Lu et al (6) hypothesized that inheritance of susceptibility to *P. megasperma* f. sp. *medicaginis* in alfalfa is controlled by a single gene with incomplete dominance, to explain the appearance of clones with intermediate levels of resistance. A specific phenotype which categorically could be defined as moderately resistant could not be determined by the seedling assay. However, 3–4 days after saturation of mycelium-infested planting mix, when seedlings were at the full cotyledon stage, almost all seedlings of susceptible cultivars had been killed, while considerably more seedlings of resistant cultivars survived than would be anticipated from mature plant root assays. We postulate that seedlings which succumb to the pathogen between 3–7 days after saturation of the mix may possess intermediate levels of resistance.

This postemergence damping-off seedling assay allows the evaluation of mature plant resistance in alfalfa to *P. megasperma* f. sp. *medicaginis* and is useful for the rapid comparison of resistance levels in alfalfa cultivars and for the selection of resistant phenotypes that could be used in a breeding program.

LITERATURE CITED

1. BRAY, R. A., and J. A. G. IRWIN. 1978. Selection for resistance to *Phytophthora megasperma* var. *sojae* in Hunter River lucerne. Austr. J. Exp. Agric. Anim. Husb. 18:708-713.
2. CHI, C. C. 1966. Phytophthora root rot of alfalfa in Canada. Plant Dis. Rep. 50:451-453.
3. ERWIN, D. C. 1966. Varietal reaction of alfalfa to *Phytophthora megasperma* and variation in virulence of the causal fungus. Phytopathology 56:653-657.
4. FROSHEISER, F. I., and D. K. BARNES. 1973. Field and greenhouse selection for Phytophthora root rot resistance in alfalfa. Crop Sci. 13:735-738.
5. GRAY, F. A., R. B. HINE, M. H. SCHONHORST, and J. D. NAIK. 1973. A screening technique useful in selecting for resistance in alfalfa to *Phytophthora megasperma*. Phytopathology 63:1185-1188.
6. LU, N. S.-J., D. K. BARNES, and F. I. FROSHEISER. 1973. Inheritance of Phytophthora root rot resistance in alfalfa. Crop. Sci. 13:714-717.
7. MARKS, G. C., and J. E. MITCHELL. 1970. Detection, isolation and pathogenicity of *Phytophthora megasperma* from soils and estimation of inoculum levels. Phytopathology 60:1687-1690.
8. PRATT, R. G., J. E. MITCHELL, and D. A. WILLIS. 1975. Resistance and susceptibility to *Phytophthora megasperma* expressed in alfalfa cotyledons. Phytopathology 65:365-369.
9. PURSS, G. S. 1959. Root rot of lucerne. Queensl. Agric. J. 85:767-770.
10. WELTY, R. E., and T. H. BUSBICE. 1976. Phytophthora root rot of alfalfa in North Carolina. Plant. Dis. Rep. 60:14-18.