# Evaluating Peas for Resistance to Damping-off and Root Rot Caused by Pythium ultimum

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ABSTRACT

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Six pea breeding lines developed at Minnesota proved to be resistant to damping-off and root rot caused by *Pythium ultimum* whereas two commercial cultivars were susceptible. Evaluating resistance by soaking seeds in hyphal suspensions before planting them was as effective as, and more rapid than, planting seeds in infested soil. The resistance of genotypes to damping-off was not lost by increasing exposure time of seeds in inoculum from 6 to 24 hr, by increasing inoculum concentration, or by testing in autoclaved or nonautoclaved sand or soil, but it was lost by injury to seed coats; however, each of these treatments accentuated the susceptibility of susceptible lines or cultivars. In a pea-field disease nursery, the percentage survival of plants of the nine breeding lines was greater than that of cultivar New Era, and the yield of six lines was significantly greater than that from either cultivar Alaska or New Era.

Breeding peas for resistance to damping-off or root rot has not always proved successful. Escobar et al (2) found that all of 40 commercial pea cultivars were susceptible to root rot caused by *P. ultimum*. On the other hand, McDonald and Marshall (9) reported that 83% of 105 strains of pea with colored flowers and 7% of 345 strains with white flowers were resistant to root rot. Similarly, Kraft and Roberts (7) identified 58 of 520 selections to be more resistant than a commercial control, when tested against *Pythium* spp. or *Fusarium solani*. Ohh et al (11) observed that cultivars resistant to *P. ultimum* were superior in stands and yields compared with cultivars that were susceptible but treated with captan. Additional experiments with nine Minnesota breeding lines of peas needed to be made since some of them showed promise of resistance better than that currently available. Thus, experiments were made (i) to ascertain if resistance to *Pythium ultimum* Trow existed in certain breeding lines of peas, and (ii) to identify factors that may affect that resistance.

## MATERIALS AND METHODS

Cultures of *Pythium* sp. were isolated from decaying seeds or diseased roots of peas (*Pisum sativum* L.) growing in a field plot at St. Paul, in which peas had been grown each season for several decades (a disease nursery). Cultures also were isolated from a commercial pea field near LeSueur, Minnesota. Isolates were maintained on cornmeal agar (CMA) in vials stored at 4 C. Subcultures were made every 2 mo; no loss in virulence was detected following successive subculturing.

Inoculum for tests in the greenhouse was prepared by growing the isolates either in a corn decoction broth or in a vermiculite-V-8 juice medium. The corn decoction broth consisted of 10 kernels added to 50 ml of distilled water, which then was autoclaved at 121 C for 20 min. A vermiculite-V-8 juice medium was prepared by adding one part V-8 liquid (30%) to two parts solid vermiculite (v/v); when the pH departed from the desired pH of 6.8, it was adjusted either by adding 1N NaOH or 25% lactic acid. Five-mm-diameter disks from 4 to 5 day-old cultures grown on PDA were added to each culture vessel. Cultures were allowed to grow in the corn decoction broth for 5 days and in the vermiculite-V-8 juice medium for 2 wk.

Except for field tests, seeds were surface disinfested by immersing them in 5% NaOCl and 95% ethanol (1:1, v/v) for 10 min; then they were washed three times with sterile distilled water.

Pea seeds were inoculated either by planting seeds in infested soil or by soaking seeds in a suspension of mycelial fragments for 24 hr, unless stated otherwise. Infested soil was prepared by adding 14-day-old cultures grown on the vermiculite-V-8 juice medium to a steamed silt loam or sand. The mycelial suspensions were prepared by mincing fungus mats from a 5-day-old culture (grown on decoction broth) in 50 ml of sterile distilled water for 2 min in a Waring Blendor. A standard suspension consisted of approximately 10,000 fragments per milliliter of water.

To detect resistance of peas in the greenhouse, peas were inoculated in three different ways. (i) Seeds were planted in soil artificially infested with the fungus. Two inoculum concentrations were used by mixing infested sand with nonsteamed sand; the fungus was cultured on vermiculite-V-8 juice medium and added to sand (v/v) at 10% for the high, and at 1% for the low concentration. (ii) Seeds were soaked in a suspension of mycelial fragments for 24 hr at 24 C. Hyphal suspensions contained about

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1,000 fragments/ml water for the high and 50 fragments/ml water for the low inoculum concentrations. Fifteen to 20 seeds were soaked in 50 ml of a hyphal suspension contained in a glass storage dish (100 mm diameter and 80 mm deep); only seeds that imbibed water were planted. (iii) A 20-ml suspension of hyphal fragments (1,000 fragments/ml water) was injected 3 cm deep into sand in each pot using a hypodermic syringe at planting time.

Where a rapid laboratory method was needed for detection of resistance, a variation of this method was used. Seeds were surface sterilized and soaked in a suspension of *P. ultimum* hyphal fragments for 24 hr at 24 C. Then seeds that had imbibed water were planted 1 cm deep in storage dishes (five seeds per dish) containing 300 cc of sand and 50 ml of tap water (autoclaved 40 min at 121 C) and incubated at about 25 C. There were two inoculum concentrations of three replicates per concentration in this test. The relative amount of mycelial growth on the soil surface was determined visually after 24, 48, and 72 hr, as shown in Fig. 1.

In studies on factors affecting damping-off, surface-disinfested pea seeds were immersed in a suspension of hyphal fragments (1,000 fragments/ml water) for 0, 6, 12, 18, and 24 hr in glass storage dishes containing 50 ml of suspension per dish. Ten seeds that had imbibed water and thereby had become inoculated in the moisture dish were transplanted into each of five, 10-cm-diameter clay pots of steamed sand for each treatment and genotype. Controls consisted of seeds soaked in sterile distilled water for the same time periods and planted.

The effects of inoculum concentration were studied in the greenhouse at 24 ± 4 C. Ten seeds that had been soaked in a hyphal suspension were planted in each of five clay pots (10-cm diameter) containing nonautoclaved sand. Twenty-one days after planting, percentage emergence, amount of root rot, and plant dry weights were determined. Plants were dried overnight at 60 C and the dry weight of each inoculated plant was divided by that of each noninoculated plant of the corresponding genotype. Experiments were repeated twice.

The following values were given for estimating root rot damage: 0 = healthy plants, no infection visible; 1 = slight browning of secondary roots; 2 = severe browning of secondary roots; 3 = firm tap root, secondary roots rotted; 4 = soft tap root, secondary roots completely rotted; 5 = plants dead (decay of roots and cotyledons).

The Minnesota breeding lines were developed by T. H. King and were increased several times per year from 1969 to 1972 in the greenhouse. The cultivars used, their sources, and their salient characteristics are as follows: Alaska, Northrup King & Co. (white flowered, early, short, smooth- and green-seeded); New Era and No. 447, both from Green Giant Co. (white flowered, medium maturity, short, wrinkled- and green- and white-seeded); Minnesota breeding lines 353-1, 353-1-G, and 353-1-B, all derived from USDA plant introductions (P.I.) 164417 × 164971 (all three lines purple-flowered, medium maturity and height, smooth- and green- and brown-seeded); Minn. 377-15, 377-15-G, 377-15-B derived from P.I. 171816 × 164971 (all purple-flowered, medium maturity and height, smooth-seeded–377-15-G was green- and brown-seeded and 377-15-B was green-seeded with purple spots); Minn. 378-A-3, 378-A-3-G, 378-A-3-B, 378-A-3-Bwr, and 378-A-3-W derived from P.I. 173057 × 164417 BH (all five lines of medium maturity and height; all but one purple flowered–378-A-3-W was white; all but one were smooth seeded–378-A-3-Bwr was wrinkled; all had green seeds–378-A-3-G had some brown, 378-A-3-Bwr had purple spots, and 378-A-3-W had some yellow seeds); Minn. 494-A-9 derived from P.I. 174923 × 167250, was purple flowered, medium maturity and height, seeds smooth and brown with purple spots.

## RESULTS

**Resistance to Pythium in breeding and commercial pea lines.**—Resistance of six Minnesota breeding lines and two commercial cultivars was evaluated in the greenhouse at 24 ± 4 C, using emergence, root rot index, and dry weights of plants as criteria of resistance. The breeding

TABLE 1. Incidence of pre-emergence damping-off and severity of root rot in eight cultivars and lines of pea planted in the greenhouse in soil infested with *Pythium ultimum*

| Cultivar or Minnesota breeding line | Emergence[a] | | Root rot index[a,b] | | Dry weight relative to control[c] (%) |
|---|---|---|---|---|---|
| | Infested soil (%) | Control (%) | Infested soil | Control | |
| Alaska | 0 | 93 | 5.0 | 0 | 0 |
| New Era | 0 | 100 | 5.0 | 0 | 0 |
| 377-15-B | 80 | 90 | 2.2 | 0 | 77 |
| 377-15-G | 80 | 80 | 2.1 | 0 | 76 |
| 378-A-3-B | 100 | 100 | 2.0 | 0 | 88 |
| 378-A-3-G | 87 | 90 | 2.1 | 0 | 77 |
| 353-1-G | 100 | 100 | 2.0 | 0 | 88 |
| 353-1-B | 100 | 100 | 2.0 | 0 | 91 |

[a]Data are mean values from two experiments done at different times and each experiment had four replicates of 10 plants per replicate.
[b]Root rot index is on a scale of 0-5, where 0 = healthy plants and 5 = dead plants.
[c]Dry weights were taken 21 days after seeds were planted in infested soil.

lines proved resistant to damping-off (measured as emergence) and root rot (measured as an index and as plant dry weight) caused by *P. ultimum* (Table 1). Percentages of damping-off of breeding lines were less than 12 and the root rot index as 2.2 or less, whereas the root rot index was 5 (plants dead) for the two commercial cultivars. The differences in emergence, root rot index, and plant dry weights were not significantly different among the six breeding lines ($P = 0.05$).

The percentage emergence was greater and the root rot index was lower in Minnesota breeding line 353-1-G than in New Era even at high concentrations of inoculum (Fig. 1 and Table 2), and regardless of method of inoculation (Table 2). Pre-emergence damping-off and root rot were more severe where sand was infested with *P. ultimum* grown on the vermiculite-V-8 juice medium and least where inoculum was injected directly into soil. Between each comparison of inoculation method, there was a significant ($P = 0.05$) difference in the amount of pre-emergence damping-off in plants of cultivar New Era.

The breeding lines consistently were more resistant than Alaska and New Era to damping-off and root rot caused by *P. ultimum*. Cotyledons of susceptible cultivars were completely decayed, and those of resistant lines were healthy. Resistant lines usually had more adventitious roots on hypocotyls than susceptible cultivars did when plants were grown in soil infested with *P. ultimum*.

**Factors affecting damping-off.**—*Exposure time.*—In the susceptible genotypes (Minn. 378-A-3-W and New Era), the percentage of plants that died from pre-emergence damping-off increased rapidly as the time of exposure to inoculum increased from 6 to 24 hr. In contrast, in the resistant genotypes (Minn. 378-A-3-Bwr and Minn. 353-1-G), there was little effect on damping-off following prolonged exposure to inoculum (Fig. 2-A). Almost 100% of the susceptible genotypes died when they were exposed to inoculum for 18-24 hr but less than 10%

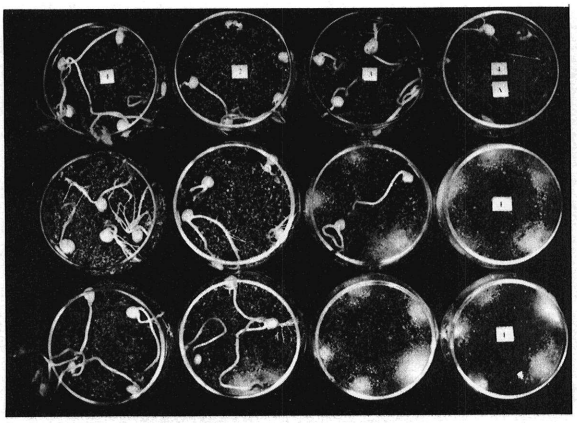

**Fig. 1.** Rapid laboratory method of evaluating pre-emergence damping-off of four pea cultivars 5 days after inoculation by soaking seeds in a suspension of hyphal fragments of *Pythium ultimum* for 24 hr and planting them in steamed soil. Inoculum concentrations were: top row, control; middle row, low concentration (50 fragments/ml); bottom row, high concentration (1,000 fragments/ml). From left to right: Minn. 378-A-3-Bwr, Minn. 353-1-G, Minn. 378-A-3-W, and New Era.

TABLE 2. Effect of inoculation method and inoculum concentration of *Pythium ultimum* on pre-emergence damping-off and root rot of New Era and Minnesota breeding line 353-1-G of peas in the greenhouse

| Inoculation method | Concentration | Emergence and root rot index per cultivar[a] | | | |
|---|---|---|---|---|---|
| | | New Era | | Minn. 353-1-G | |
| | | (%) | Index[b] | (%) | Index[b] |
| Sand[c] | High[d] | 0 | 5.0 | 94 | 2.8 |
| | Low[e] | 22 | 4.6 | 96 | 1.4 |
| | Control | 98 | 0 | 100 | 0 |
| Soak[f] | High[g] | 36 | 4.8 | 100 | 2.0 |
| | Low[h] | 78 | 4.0 | 100 | 0.3 |
| | Control | 100 | 0 | 100 | 0 |
| Injection[i] | High[g] | 85 | 2.9 | 90 | 0 |
| | Control | 100 | 0 | 92 | 0 |

[a]Data are means of two experiments, three replicates per experiment, 10 plants per replicate.
[b]Based on a scale of 0-5, where 0 = healthy plants and 5 = dead plants.
[c]Sand mixed with vermiculite-V-8 juice.
[d]10% vermiculite-V-8 juice inoculum in sand (v/v).
[e]1% vermiculite-V-8 juice inoculum in sand (v/v).
[f]Seeds soaked in hyphal suspension for 24 hr before planting.
[g]1,000 hyphal fragments/ml.
[h]50 hyphal fragments/ml.
[i]Hypodermic injection of 20 ml of hyphal suspension per pot at planting.

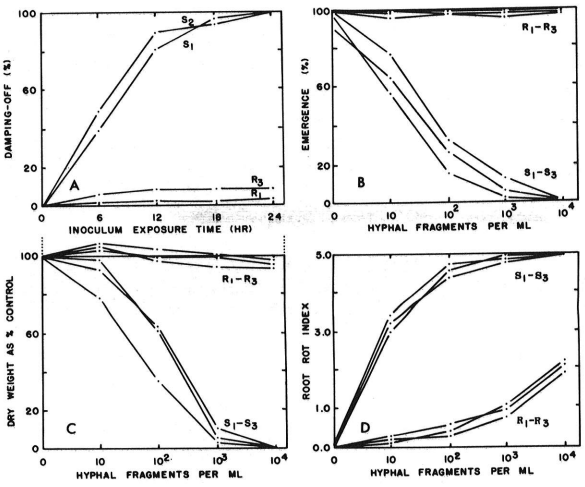


**Fig. 2-(A to D).** Factors affecting resistant (R) and susceptible (S) genotypes of pea which were exposed to inoculum of *Pythium ultimum*: **A)** Effect of inoculum exposure time on damping-off; **B)** Effect of inoculum concentration on seedling emergence; **C)** Effect of inoculum concentration on dry weight of 21-day-old plants; and **D)** Effect of inoculum concentration on root rot index. Resistant and susceptible breeding lines or cultivars were: $R_1$ = Minn. 378-A-3-Bwr, $R_2$ = Minn. 378-A-3-G, $R_3$ = Minn. 353-1-G, $S_1$ = New Era, $S_2$ = Minn. 378-A-3-W, and $S_3$ = Green Giant 447; data are averages of three trials of five replicates per trial and 10 plants per replicate.

of the plants of resistant genotypes died at the longest exposure period.

*Inoculum concentration.*—Values for emergence (Fig. 2-B) and dry weights of plants (Fig. 2-C) were progressively less with each increase in inoculum concentration from 0 to 10,000 fragments/ml for susceptible but not for resistant genotypes. However, the root rot index was greater for the three susceptible genotypes than for the three resistant ones (Fig. 2-D). Even at 10,000 fragments/ml, the resistant genotypes sustained a root rot index of approximately 2 compared with the susceptible genotypes with an index of 5 (plants dead). These results confirm some of the observations made earlier (Fig. 1 and Table 2). Also, there was a correlation ($r = -0.84$) between dry weight of plants and the amount of root rot.

Another experiment was done twice with three replicates per time, in which cultivars Alaska and New Era, and Minnesota breeding lines 378-A-3-W, 378-A-3-G, 378-A-3-Bwr, 377-15-G, 377-15-B, and 353-1-G were tested at high (1,000 fragments/ml) and low (50 fragments/ml) concentrations of inoculum. The results (data not shown) for cultivars common to those in Fig. 2 were nearly identical: the performance of Alaska was like that for New Era, and the performance of Minn. 377-15-B and -G was almost the same as that of Minn. 378-A-3-G shown in Fig. 2-B and 2-D.

*Seed coat injury.*—Seeds of the Minnesota breeding lines are hard and it is difficult to obtain uniform germination within certain time periods. To aid germination, seeds of New Era and four breeding lines were surface-disinfested and then the testae were cut with a sterile scalpel to make an incision 5 mm long. Injury to either cotyledons or embryo was avoided. Cut seeds were sown in steamed soil of a greenhouse mix which then was infested with *P. ultimum* grown on vermiculite-V-8 juice medium for 14 days at a ratio of 1:10 (v/v) of inoculum to soil. Percentages of emerged seedlings were determined 14 days after planting, and are reported below as mean values from two trials of four replicates per trial.

The percentage emergence of plants of the three breeding lines (353-1-G, 377-15-G, and 378-A-3-G) varied from 92 to 95% for intact seeds and 20 to 35% for cut seeds planted in infested soil and was 81 to 91% for intact seeds and 100% for cut seeds planted in noninfested soil. In contrast, plant emergence for Minn. 378-A-3-W and New Era was only 5 to 10% for intact seeds and 0 to 8% for cut seeds planted in infested soil compared to 85 to 98% for intact and 100% for cut seeds planted in noninfested soil. Thus, the cutting of testae in previously resistant breeding lines greatly increased their susceptibility to pre-emergence damping-off.

*Potting medium.*—To ascertain whether choice of potting medium was important in the evaluation of genotypes for resistance to damping-off and root rot, two resistant and two susceptible cultivars were tested in three different potting media (sand, silt loam,and a greenhouse potting mixture). No appreciable effect of medium was noted (Table 3). Little or no pre-emergence damping-off occurred in the two resistant genotypes in either autoclaved or nonautoclaved soil and 82 to 100% of the seedlings in the susceptible genotypes succumbed to damping-off, regardless of potting medium. The difference between growth in silt loam and either sand or potting mix was not significant ($P = 0.05$).

Similarly, root rot was more severe in the two susceptible genotypes than in the two resistant ones and the difference between the resistant and susceptible genotypes was significant ($P = 0.05$). Also, the results were similar among the sand, silt loam, and potting

TABLE 3. The effect on emergence and root rot of peas in the greenhouse in two resistant cultivars (Minnesota breeding lines) and two susceptible cultivars (one a breeding line) of three potting media on seeds infested with *Pythium ultimum*

| Cultivar | Disease reaction[c] | Seed infested (+) or not (−)[d] | Emergence[a] | | | Root rot index[a,b] | | |
|---|---|---|---|---|---|---|---|---|
| | | | Non-autoclaved | | Autoclaved potting mix[f] (%) | Non-autoclaved | | Autoclaved potting mix |
| | | | Sand (%) | Silt loam[e] (%) | | Sand | Silt loam[e] | |
| 378-A-3-Bwr | R | − | 100 | 100 | 100 | 0.0 | 0.4 | 0.0 |
| | | + | 100 | 100 | 100 | 0.2 | 0.5 | 0.8 |
| 353-1-G | R | − | 100 | 92 | 100 | 0.1 | 0.2 | 0.0 |
| | | + | 92 | 100 | 100 | 1.5 | 0.9 | 1.2 |
| 378-A-3-W | S | − | 100 | 92 | 100 | 0.6 | 2.3 | 0.0 |
| | | + | 0 | 18 | 0 | 5.0 | 4.7 | 5.0 |
| New Era | S | − | 96 | 90 | 96 | 0.5 | 1.7 | 0.0 |
| | | + | 0 | 10 | 0 | 5.0 | 4.8 | 5.0 |

[a]Data based on two trials, three replicates/trial, 10 plants/replicate, 21 days after seeds planted.
[b]Values based on scale of 0 = healthy plants and 5 = dead plants.
[c]Disease reactions (R = resistant and S = susceptible) based on previous tests.
[d]Surface-disinfested seeds soaked in mycelial suspensions (1,000 fragments/ml) for 24 hr and planted in soil.
[e]Silt-loam soil from pea "disease nursery".
[f]Greenhouse potting mix of equal amounts (v/v) of silt-loam, sand, peat, and manure.

mixture (Table 3).

Thus, in evaluating resistance of pea genotypes to pre-emergence damping-off and root rot, any one of these three potting media would give equally satisfactory results. Results in Table 3 also confirm the existence of resistance in these genotypes found previously.

**Emergence, survival, and yield of genotypes in the field.**—Seeds of 11 pea genotypes were sown in a pea disease nursery in the field at St. Paul. Most of these genotypes had 75% or greater emergence, except for New Era in which 65% of the plants emerged (Table 4). At least 65% of the plants survived except for New Era where only 50% survived. Yields of six of nine breeding lines were significantly greater than yields of the two commercial cultivars in the nursery. Although 378-A-3-W proved to be susceptible to *P. ultimum* in the greenhouse, it emerged well and had the best survival and yield in the field (Table 4).

The "disease nursery" has been planted to peas every season for several decades and the following pathogens have been isolated consistently from roots of susceptible plants: *Aphanomyces euteiches* Brechs., *Fusarium oxysporum* Schl. emend. Snyd. & Hans. f. sp. *pisi, F. solani* (Mart.) App. & Wr. emend. Snyd. & Hans. f. sp. *pisi*, *Rhizoctonia solani* Kühn, and *Pythium* spp. Of 20 isolates of *Pythium* isolated from roots of peas, 16 were *P. ultimum* Trow, two were *P. debaryanum* Hesse, one was *P. vexans* de By, and one was *P. irregulare* Buisman. Pre-emergence damping-off of pea occurred by adding inoculum of either *P. ultimum* or *P. debaryanum* to soil; however, little damping-off or root rot occurred in similar tests with *P. irregulare* or *P. vexans*. Thus, *P. ultimum* was considered to be the predominant pathogenic *Pythium* species in this plot.

The predominance of any one or several of these pathogens usually depends on the soil temperature and moisture. Peas were planted in May, and the rainfall in May, 1970, was 35 mm above normal for that month but in June it was 67 mm below normal. The wetter soil in May favors infection by *P. ultimum* but the drier soil in June favors infection by species of *Fusarium* and *Rhizoctonia*. Thus, it is not clear whether the differences in yield among the genotypes is due to the effect of *P. ultimum* in soil, or of any other root pathogen, or whether the differences in yield are due to the genetic potential of the genotypes.

TABLE 4. The emergence, survival, and yield of nine Minnesota breeding lines and two commercial cultivars (Alaska and New Era) of pea that differed in earlier tests in resistance (R) or susceptibility (S) to *Pythium ultimum* and which were planted in a pea disease nursery in St. Paul where the five important root pathogens of peas were known to occur

| Cultivar or line | Previous reaction to *P. ultimum* | Emergence[a] (%) | Survival[a,b] (%) | Yield[a] (g) |
|---|---|---|---|---|
| Alaska | S | 86 | 75 bc | 35 a |
| New Era | S | 65 | 50 a | 89 ab |
| 494-A-11[c] | S | 96 | 66 b | 96 bc |
| 377-15-G | S | 89 | 65 b | 108 bc |
| 377-15-B | S | 90 | 68 b | 131 bc |
| 353-1-B | R | 87 | 78 bc | 159 cd |
| 378-A-3-B | R | 76 | 68 b | 176 d |
| 353-1-G | R | 86 | 76 bc | 208 de |
| 378-A-3-G | R | 76 | 68 b | 223 de |
| 494-A-9 | R | 90 | 73 b | 225 de |
| 378-A-3-W | R | 80 | 89 c | 252 e |

[a]Data are means from seven replicates of 25 seeds per replicate in one year (1970). Different letters after each datum indicate significant differences by Duncan's multiple range test ($P=0.05$).

[b]Percentage survival is the product of number of plants at harvest multiplied by 100 divided by number of plants that germinated, used by King et al (4). The following root pathogens were present in soil: *Aphanomyces euteiches, Fusarium oxysporum, F. solani, Pythium ultimum,* and *Rhizoctonia solani.*

[c]A selection from material similar to Minn. 494-A-9.

## DISCUSSION

A high degree of resistance to pre-emergence damping-off and root rot caused by *P. ultimum* occurs in certain genotypes of peas. This resistance has been demonstrated repeatedly by a variety of testing methods in the laboratory, greenhouse, and in the field, although the field test involved exposure of plants to at least five different pea root pathogens. This resistance is superior to that in certain commercial cultivars as measured by emergence, root rot index, plant dry weights, and survival of plants through maturity. Kraft and Roberts (8) also demonstrated the feasibility of laboratory testing of pea cultivars for resistance to *P. ultimum,* but they recognized the need for further tests in the field.

Resistance appears to be stable in that it is not affected by long-time exposure to high concentrations in inoculum of *P. ultimum*. It can be evaluated reliably in the laboratory using dishes containing either steamed or nonsteamed soil or in the greenhouse using several different potting media. The only situation in which resistance was lost was after injury to testae. This suggests that resistance to damping-off resides in the seed and the nature of the seed exudate, as has been demonstrated by several workers (1, 3, 6, 10, 12), and probably not root exudates (5). Thus, one could test a considerable number of lines or cultivars of peas for resistance to *P. ultimum* in the laboratory and greenhouse with some confidence that cultivars to be resistant under controlled conditions would also be resistant in the field. The Minnesota breeding lines resistant to *P. ultimum* in laboratory and greenhouse trials grew well and yielded well in the field when seeds were planted in soil known to harbor at least five pea root pathogens.

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