Specificity of Resistance to *Aphanomyces euteiches* in Seedling Alfalfa

E. B. HOLUB, Former Graduate Research Assistant, and C. R. GRAU, Professor, Department of Plant Pathology, University of Wisconsin, Madison 53706

ABSTRACT

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A bioassay was used to evaluate the reaction of alfalfa seedlings to *Aphanomyces euteiches*. Four-day-old seedlings were inoculated with zoospores, and disease severity was rated 10 days later on a five-class scale where 1 = healthy plant and 5 = dead plant. Plants were selected within classes, self-pollinated, and evaluated by progeny testing for reactions to *A. euteiches*. After progeny tests, 33 of 34 class 2 parent plants were rated as resistant to *A. euteiches*, compared with three of four class 3 parents and none of four class 4 parents. The parents were derived from alfalfa cultivars that yielded no class 1 plants when assayed against *A. euteiches*. When class 2 and class 3 plants were selected from these cultivars and self-pollinated, however, 30–60% of the progeny were rated as class 1. Experimental populations of alfalfa were created to determine the specificity of resistance to *A. euteiches*. Resistance to *A. euteiches* in these populations was not effective against disease caused by *Phytophthora megasperma* f. sp. *medicaginis*, and resistance to *P. m.* f. sp. *medicaginis* was similarly ineffective against *A. euteiches*. In another experiment, plants resistant to isolates of *A. euteiches* recovered from alfalfa were resistant to isolates from peas. However, pea isolates were less virulent than isolates from alfalfa, even on alfalfa populations previously selected against isolates from pea.

Aphanomyces euteiches Drechs. is known primarily for its destructiveness on pea (*Pisum sativum* L.) (8,13) and green bean (*Phaseolus vulgaris* L.) (20), but accumulating evidence suggests that it may also be an important pathogen of alfalfa (*Medicago sativa* L.) (2,3, 11,16,22). The earliest evidence was reported by researchers who investigated pea root rot and cited alfalfa as an additional host for *A. euteiches* (16,19,22). Recently, studies have associated this oomycete with seedling blight and poor establishment of alfalfa (3,17,21). Additional evidence is needed, however, to determine whether *A. euteiches* can cause root disease of alfalfa in the field and on plants beyond the seedling stage.

Disease resistance that specifically protects alfalfa against *A. euteiches* could be useful for determining the importance of root disease caused by *A. euteiches* relative to that caused by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan & Irwin (14). The latter oomycete is recognized as a major root pathogen of alfalfa in wet, poorly drained soils (1,4,9,15). Therefore, research was conducted to determine whether resistance to *A. euteiches* exists in alfalfa germ plasm and, if present, whether such resistance is effective against *P. m.* f. sp. *medicaginis* and isolates of *A. euteiches* recovered from pea. Resistance to pea isolates was of interest because host-specialized strains have been described within the species (20).

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MATERIALS AND METHODS

Bioassay for reaction of alfalfa seedlings to *A. euteiches* or *P. m.* f. sp. *medicaginis*. A bioassay (12) was used to test the reaction of alfalfa seedlings to *A. euteiches* or *P. m.* f. sp. *medicaginis*. Seedlings were grown in plastic cavities (2.5 × 2.5 × 7 cm deep) containing sand. Each seedling was inoculated with 100 zoospores 4 days after planting. The methods of Mitchell and Yang (18) and Irwin et al (12) were used to produce zoospores of *A. euteiches* and *P. m.* f. sp. *medicaginis*, respectively. The inoculated seedlings were incubated at 24 C in a growth chamber. Photoperiod was a 12-hr day (250 $\mu E \cdot m^{-2} \cdot s^{-1}$) and a 12-hr night. Seedlings were rated for severity of symptoms 10 days after inoculation on a five-class scale where 1 = healthy plant and 5 = dead plant (Fig. 1). Plants were selected within classes and self-pollinated, and progeny were evaluated for reactions to *A. euteiches* and *P. m.* f. sp. *medicaginis*. The progeny test was used to determine the phenotype of the parent plant.

Congruence between disease severity classes and resistance to *A. euteiches*. Seedlings of the cultivar Apollo II were inoculated with *A. euteiches* isolate Ae139 (collected from the University of Wisconsin Research Station at Marshfield) and assigned to disease severity classes 10 days later. Plants from classes 2, 3, and 4 were transplanted into well-drained soil (field soil and coarse sand [1:1, w/w] steamed at 100 C for 1 hr) and fertilized weekly with Hoagland's nutrient solution (10). No class 1 plants were found for this study. Class 4 plants were weak, but four of approximately 30 plants survived and eventually

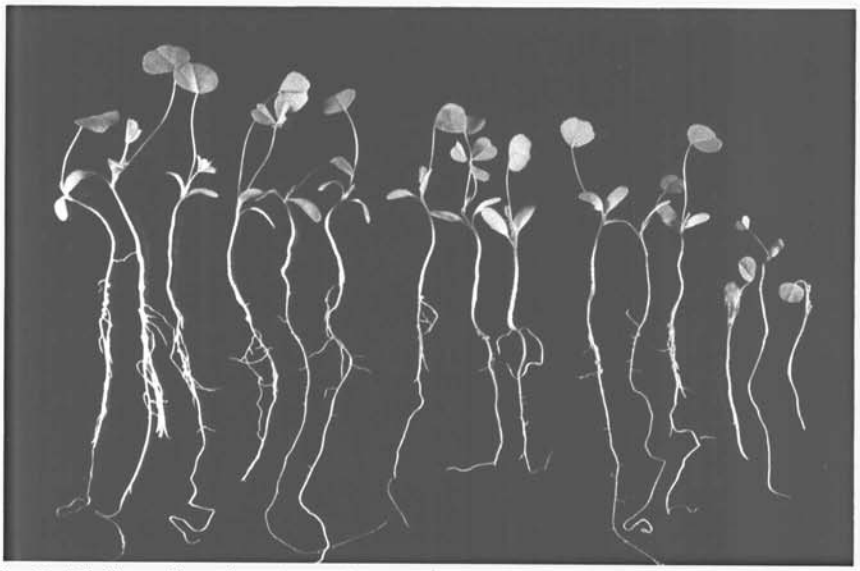

Fig. 1. Alfalfa seedlings inoculated with *Aphanomyces euteiches* and arranged, from left to right in groups of three, according to the five-class rating of disease severity: 1 = no macroscopic symptoms, pink hypocotyl and green cotyledons, white roots; 2 = minor brown discoloration confined primarily to lateral roots, overall stunted growth; 3 = brown discoloration of taproot and lateral roots, healthy hypocotyl and green or slightly chlorotic cotyledons; 4 = dark brown discoloration of roots and hypocotyl, necrosis of cotyledons; 5 = dead plant.

produced seed. Four plants from class 2 and and four from class 3 were also grown to produce seed. Each parent was self-pollinated, and 100 progeny from each parent were assayed subsequently for their reaction to isolate Ae139 using the seedling assay described above.

Specificity of resistance to *A. euteiches*. A test was conducted using experimental alfalfa populations to determine whether resistance to *A. euteiches* also conferred resistance to *P. m.* f. sp. *medicaginis*. The populations were derived by selecting plants against an isolate of either *A. euteiches* (Ae139) or *P. m.* f. sp. *medicaginis* (Pm2019, also collected from Marshfield) or combined inoculum of both oomycetes using the seedling assay. Twelve class 2 plants per population were polycrossed by hand to produce seed. The populations included two derived from Vernal plants selected against either Ae139 or Pm2019, three from Apollo II plants selected against either oomycete or combined inoculum of both oomycetes, and two from Apollo II or Vernal plants grown without inoculation. Progeny from each population were assayed for reaction to Ae139, Pm2019, and combined inoculum of both isolates.

In a second experiment, a test was conducted to determine whether resistance to *A. euteiches* from alfalfa was also effective against isolates from pea. Four experimental populations of alfalfa were created, each selected against a different isolate using the seedling assay described above. The isolates included two from alfalfa seedlings (Ae139 and Ae122) and two from pea seedlings (Ae111 and Ae121). Isolates Ae139 and Ae121 were recovered from field soil with a history of alfalfa root rot but no recent history (5–10 yr) of pea cultivation; isolates Ae111 and Ae122 were recovered from field soil with a history of pea root rot but no recent history (5–10 yr) of alfalfa cultivation. Seed from each population was assayed for reaction to each of the four isolates of *A. euteiches*.

RESULTS

Congruence between disease severity classes and resistance to *A. euteiches*. The original nonselected population of Apollo II yielded no class 1 plants when assayed against *A. euteiches*. When class 2 and class 3 plants were selected for progeny testing, a majority produced at least 30% class 1 progeny (Fig. 2). These plants were therefore considered resistant to *A. euteiches* because they produced a higher frequency of class 1 and class 2 progeny than observed in nonselected Apollo II. One class 3 plant was exceptional because it produced only 10% class 1 and 70% class 5 progeny. Thus, plants assigned to class 3 were more variable in reaction to *A. euteiches*. Each of the class 4 parents selected for progeny testing produced at least 90% class 4 and class 5 progeny and were therefore considered susceptible to *A. euteiches*.

A larger number of class 2 plants was evaluated to determine whether occasional susceptible plants might be assigned to this class, as was observed among class 3 plants in the previous experiment. Ten class 2 plants from Vernal and 20 from Apollo II were selected, and 100 self-progeny from each parent were assayed for reaction to isolate Ae139. The majority of class 2 plants were rated as resistant to *A. euteiches* and produced a range of 10–79% class 1 and class 2 progeny (Fig. 3). However, one Vernal plant was considered susceptible because progeny had a mean disease severity rating of 5.0, with less than 1% class 1 and class 2 progeny. The original nonselected Vernal population had approximately 3% class 2 progeny and 97% class 4 and class 5 progeny.

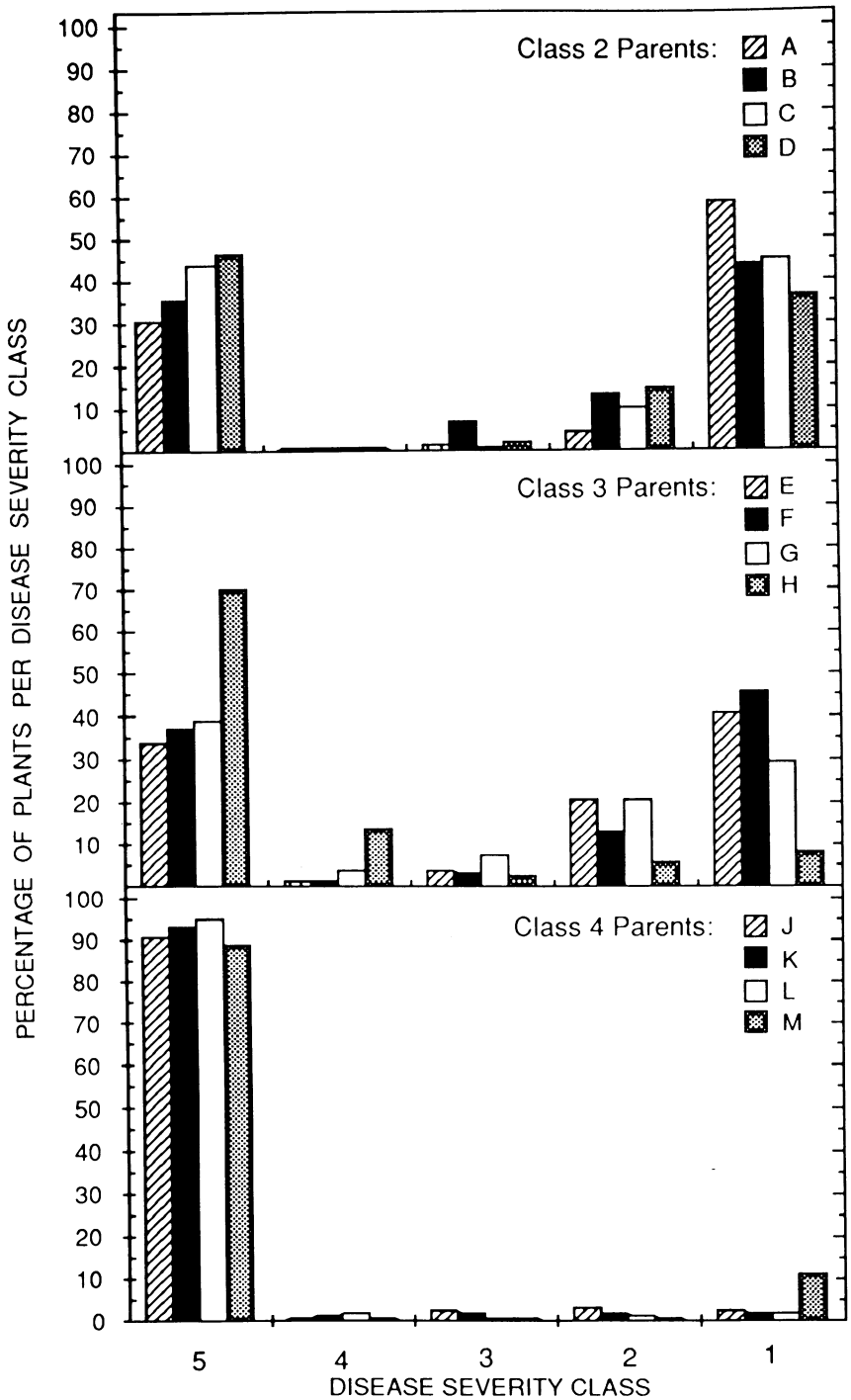

Fig. 2. Reaction of progeny from self-pollinated alfalfa plants to inoculation with *Aphanomyces euteiches* (isolate Ae139). Parents were selected initially for their reaction to Ae139 using the five-class rating of disease severity, and progeny were evaluated using the same rating scale to determine the phenotype of the parent. Included were four class 2 parents (A–D), four class 3 parents (E–H), and four class 4 parents (J–M). Approximately 100 progeny from each parent were assayed for reaction to Ae139.

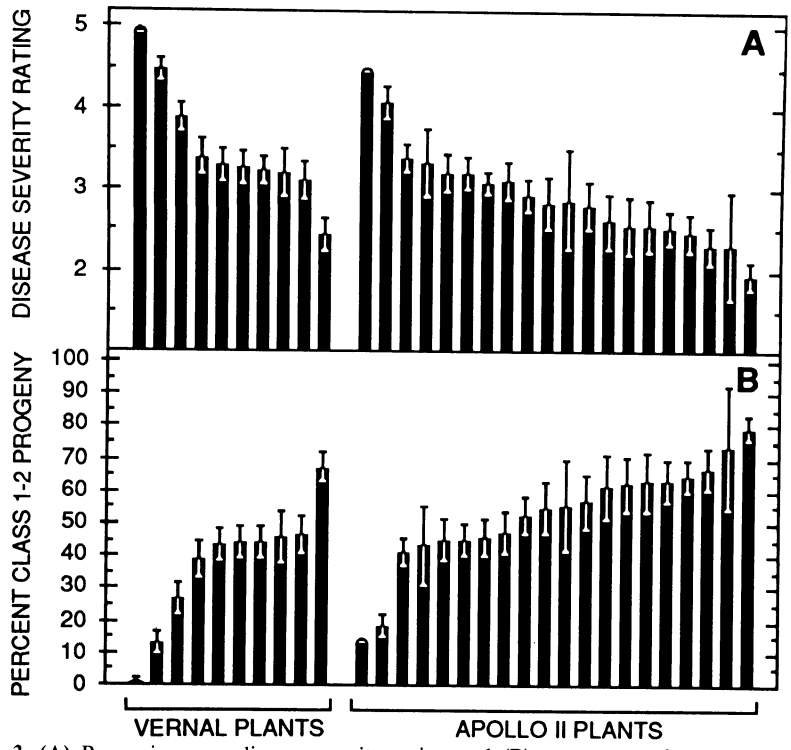

Fig. 3. (A) Range in mean disease severity rating and **(B)** percentage of class 1 and class 2 progeny from parent alfalfa plants selected for resistance to *Aphanomyces euteiches*. Ten class 2 parents were selected from Vernal alfalfa and 20 from Apollo II; 100 selfed progeny from each parent were subsequently assayed for reaction to Ae139. Each bar indicates the standard error of a mean.

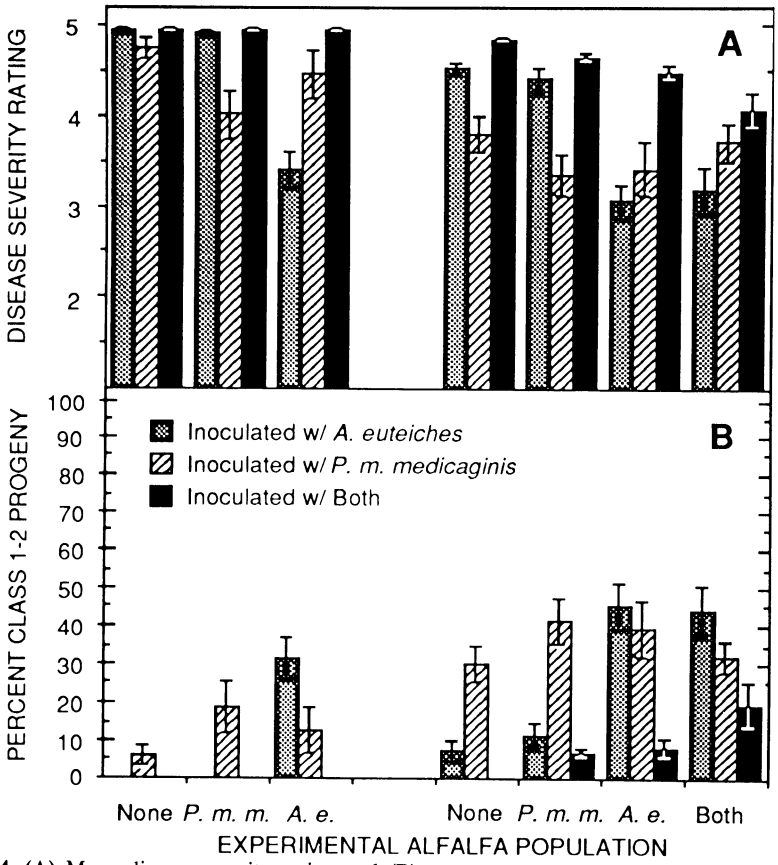

Fig. 4. (A) Mean disease severity rating and **(B)** percentage of class 1 and class 2 progeny from seven experimental alfalfa populations compared for reactions to *Aphanomyces euteiches* (Ae139) and *Phytophthora megasperma* f. sp. *medicaginis* (Pm2019). The populations were derived from Vernal and Apollo II alfalfa using selection against either oomycete (*A. e.* or *P. m. m.*) or neither (none); another population was selected from Apollo II against both oomycetes (both). Seed for each population was produced by polycrossing 12 selected plants, and approximately 100 progeny from each population were assayed for reaction to either or both oomycetes. Each bar indicates the standard error of a mean.

A preliminary study of inheritance of resistance to *A. euteiches* was performed using reciprocal cross-pollinations, without emasculation, between two plants from Vernal that were considered resistant and susceptible to *A. euteiches*. Selfed progeny were assayed against isolate Ae139, and 3 and 67% were rated as class 1 or class 2 from the susceptible and resistant parents, respectively. Progeny from cross-pollinations were rated as 28% class 1 and class 2 progeny from a cross using the resistant parent as the female and 32% from a cross using the resistant parent as the male. Each percentage was calculated from 100 inoculated seedlings.

Specificity of resistance to *A. euteiches*. Alfalfa populations selected against *A. euteiches* produced progeny that had lower disease severity ratings and greater percentages of class 1 and class 2 progeny after inoculation with *A. euteiches* than did nonselected populations (Fig. 4). Increased resistance to *A. euteiches* in selected populations did not increase resistance to *P. m.* f. sp. *medicaginis*, and selection for resistance to *P. m.* f. sp. *medicaginis* did not increase resistance to *A. euteiches*. However, resistance to both oomycetes could be increased simultaneously if combined inoculum was used for selection.

No conclusive evidence was found for differential reactions among alfalfa populations, initially selected against different isolates of *A. euteiches*, when the progeny were tested against the same isolates (Fig. 5). All four isolates of *A. euteiches* were useful in selecting for resistance. However, each alfalfa population developed less disease when inoculated with either isolate from pea (Ae111 or Ae121) than with either isolate from alfalfa (Ae122 or Ae139), regardless of which isolate was used initially for selection.

DISCUSSION

This study provides evidence that resistance to *A. euteiches* is present in germ plasm of cultivated alfalfa. Of the 34 class 2 plants selected for progeny tests (Figs. 2 and 3), 97% were identified as resistant to *A. euteiches*; 75% (three of four) of the class 3 plants were also considered resistant (Fig. 2). Hence, the percentage of class 1 and class 2 plants could be used as a measure of resistance to *A. euteiches* in a given population, but this would likely underestimate resistance because resistant plants assigned to class 3 would be excluded. A conservative estimate may be justified when comparing results from numerous experiments because of differing environments, sources of germ plasm, and bias introduced by individual researchers from use of a subjective scale for rating disease. A more accurate estimate of the percentage of resistant plants in a population could be achieved by adding

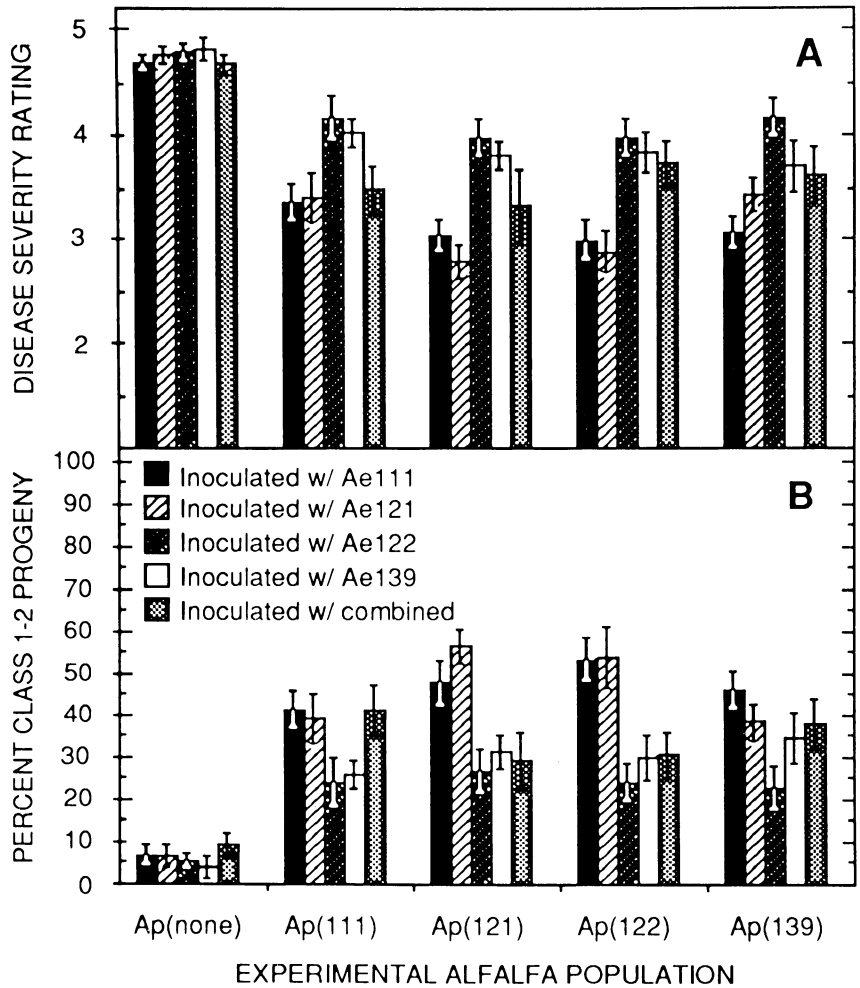

Fig. 5. (A) Mean disease severity rating and **(B)** percentage of class 1 and class 2 progeny from five experimental alfalfa populations compared for reactions to four isolates of *Aphanomyces euteiches*. The populations were derived from Apollo II alfalfa (Ap) using selection against one of four isolates: Ae111 or Ae121 from pea or Ae122 or Ae139 from alfalfa. The number in parentheses for each population name refers to the isolate used initially for selection of the parents. Seed was produced by polycrossing 12 selected plants for each population, and approximately 100 progeny from each population were assayed for reaction to each of the four isolates. Each bar indicates the standard error of a mean.

a portion of the percentage of plants assigned to class 3 to the percentage of class 1 and class 2 plants. However, a larger sample of class 3 plants from several sources of germ plasm should be evaluated to decide what proportion of such plants have genes for resistance to *A. euteiches*.

Breeding for resistance to *A. euteiches* in a commercial cultivar of pea has been a long and gradual process (D. J. Hagedorn, *personal communication*), so the discovery of highly effective resistance in alfalfa was unexpected. Breeding for resistance to *A. euteiches* could progress rapidly in alfalfa, as suggested by the past success of alfalfa breeders with rapid development and release of cultivars resistant to *P. m.* f. sp. *medicaginis* (5,6,12). This seems evident, because seedlings from nonselected populations of Apollo II or Vernal that were inoculated with *A. euteiches* were rarely assigned to class 1, yet symptomless plants with increased vigor were common among the progeny derived from the class 2 and class 3 parents. A 30% increase in resistance was observed between nonselected cultivars and *A. euteiches*-resistant populations that had been derived from those cultivars (Figs. 4B and 5B).

Resistance to *A. euteiches* should enable future studies on the effect of *A. euteiches* on alfalfa productivity to be done under field conditions. Greenhalgh and Merriman (7) used fungicide treatments to compare disease of subterranean clover caused by *A. euteiches* and *Phytophthora clandestina* Taylor, Pascoe, & Greenhalgh under field conditions. However, the specificity and efficacy of such chemicals may not be sufficient to reveal the relative importance of each pathogen in causing disease. Because resistance to *A. euteiches* is independent of resistance to *P. m.* f. sp. *medicaginis*, a potential disease complex between *A. euteiches* and *P. m.* f. sp. *medicaginis* could be examined by comparing the field performance of alfalfa lines that differ in resistance to both. Furthermore, resistance to *A. euteiches* was effective against isolates from pea, so it might also be useful for measuring the performance of alfalfa grown in field soil with a history of pea root rot.

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