Characterization of *Aphanomyces euteiches* Isolates Recovered from Alfalfa in Wisconsin

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ABSTRACT

Delwiche, P. A., Grau, C. R., Holub, E. B., and Perry, J. B. 1987. Characterization of *Aphanomyces euteiches* isolates recovered from alfalfa in Wisconsin. Plant Disease 71:155-161.

Aphanomyces-like fungal isolates recovered from alfalfa seedlings (alfalfa isolates) were morphologically similar to isolates of *A. euteiches* recovered from pea and green bean but were distinctly different from *A. cochlioides*. All alfalfa isolates were highly pathogenic on alfalfa, and one isolate was statistically more pathogenic to pea than other alfalfa isolates. All alfalfa isolates caused essentially no symptoms on hypocotyls and a low level of disease on roots of green bean. Green bean, lima bean, soybean, table beet, radish, oat, and tomato were determined to be nonhosts to alfalfa isolates of *A. euteiches*. Sweet clover was moderately susceptible, red clover and white clover expressed low susceptibility, and birdsfoot trefoil was highly resistant to *A. euteiches*. All alfalfa isolates but one grew slower than pea and bean isolates when incubated at 16–28 C. However, growth of alfalfa isolates, like that of pea isolates, was good at 32 C compared with poor growth by bean isolates at 32 C.

Biotic and abiotic factors can influence the establishment of forage legumes (23,32). Alfalfa seedling diseases are generally attributed to pathogens such as *Pythium* spp. (5,10,28), *Fusarium* spp. (28), *Rhizoctonia solani* Kühn (10,28), *Phytophthora megasperma* Drechs f. sp. *medicaginis* (*P. m.* f. sp. *megasperma*) (Kuan & Erwin) (10,14,21,27), and the root lesion nematode (*Pratylenchus penetrans* Cobb, Filipjev, and Schur.-Stek.) (20,30). The sensivity of alfalfa to flooded soil conditions is well documented, as is the fact that some root diseases are favored by such an environment (1–4,14).

We frequently recover an *Aphanomyces*-like fungus from field soils where the use of *Phytophthora*-resistant cultivars or metalaxyl fungicide does not improve the health of alfalfa seedlings. *Aphanomyces* sp. was previously recovered from alfalfa in Ohio (28) and Ontario (18). Schmitthenner (28) described isolates that were morphologically similar to *A. euteiches* Drechs., an important pathogen of pea (*Pisum sativum* L.) (22,26) and green bean (*Phaseolus vulgaris* L.) (25). However, McKeen and Traquair (18) reported their isolates to be similar to *A. cochlioides* Drechs., a destructive pathogen of beets (*Beta vulgaris* L.) (22). Isolates of *Aphanomyces* recovered from alfalfa have not been directly compared with isolates recovered from other hosts. Thus, we report on: 1) in vitro characteristics of isolates of *Aphanomyces* recovered from alfalfa, 2) host ranges of several of these isolates, and 3) comparative pathogenicity of alfalfa isolates with isolates of *A. euteiches* from pea and bean and with *A. cochlioides*.

Accepted for publication 18 September 1986 (submitted for electronic processing).

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MATERIALS AND METHODS

Isolation of *Aphanomyces* sp. Soil was obtained from six Wisconsin alfalfa fields in which *Phytophthora*-resistant cultivars were not successfully established. Thirty Vernal alfalfa seeds were planted in each soil in plastic 600-cm^3 containers, which were placed in a controlled-environment chamber at 24 C. After 3 days (early seedling emergence), soils were flooded for seven consecutive days. Alfalfa seedlings were selected from each soil, surface-disinfested with a 0.5% NaOCl solution, plated on metalaxyl-benomyl-vancomycin sulfate agar (MBVA) (24), a semiselective medium for *Aphanomyces* sp., and incubated at 22 C. Colonies with mycelium typical of *Aphanomyces* spp. were transferred to cornmeal agar (CMA) and incubated at 22 C.

Morphological characteristics. Twenty-eight isolates of *Aphanomyces* recovered from alfalfa, two isolates of *A. euteiches* (*A. e.*) f. sp. *pisi* (S11 and P14, W. F. Pfender), two isolates of *A. e.* f. sp.

phaseoli (C1 and S2, W. F. Pfender), and one isolate of *A. cochlioides* (ATCC AC8122, C.L. Schneider) were cultured on Difco CMA for 14 days at 24 C. The diameters of oogonia and the number of antheridia per oogonium were determined by examining 50 oogonia per isolate. Isolates were induced to form sporangia by floating agar plugs (5 mm diameter), cut from margins of colonies grown on CMA, in sterile Emerson water (19) (1:1, filtered water from Lake Mendota:glass distilled water) and incubated at 25 C.

Temperature relations. Twelve isolates of *Aphanomyces* recovered from alfalfa (isolates 317, 349, 418, 461, 627, 1301, 1517, 1518, 1528, 1529, A015, and A027), two isolates of *A. e.* f. sp. *pisi* (S11 and P14), and one isolate of *A. e.* f. sp. *phaseoli* (C1) were incubated on CMA at 4, 8, 12, 16, 20, 24, 28, 32 and 36 C. Radial growth was measured and expressed as millimeters of growth within 24 hr.

Pathogenicity studies. The pathogenicity of 28 isolates from alfalfa was compared with that of two isolates each of *A. e.* f. sp. *pisi* and *A. e.* f. sp. *phaseoli* and one isolate of *A. cochlioides*. Seed of the alfalfa cultivars Vernal, Answer, and Apollo II were planted in plastic seed-cavity trays (48 cavities per tray); each individual cavity measured 2.5 × 2.5 × 7 cm and was layered with a 1-cm layer of vermiculite followed by 5 cm of sand. Eight seeds were planted per cavity and covered with 1 cm of vermiculite. Trays were set in plastic boxes, and tap water was added to a depth of 3 cm. Three-day-old alfalfa seedlings, three cavities per isolate, were inoculated by placing a mycelial plug (5 mm diameter) from each isolate in each cavity 1 cm below the surface. After inoculation, the water level was raised to 2 cm from the top of the tray. Seedlings were incubated for 14 days at 25 C. Disease severity was recorded based on the following scale: 0 = no necrosis of roots, hypocotyls, and cotyledons; 1 = minimal necrosis of lateral roots, hypocotyls, and cotyledons; 2 = necrosis of roots and lower hypocotyls and chlorosis and minimal necrosis of cotyledons; 3 = extensive necrosis of roots, hypocotyls, and cotyledons and plants stunted; and 4 = plants dead.

Host specificity studies. *Aphanomyces* sp. (alfalfa isolates 317, 349, 418, and 1528), *A. euteiches* (bean C1 and pea P14), and *A. cochlioides* (AC8122) were compared as pathogens of alfalfa (cultivars Vernal and Answer), pea (cultivar 8221), green bean (cultivar Eagle), table beet (cultivar Detroit Dark Red), radish (*Raphanus sativus* L. cvs. Red Prince and White Icicle), tomato (*Lycopersicon esculentum* Mill. cv. Bonnie Best), and oat (*Avena sativa* L. cv. Dal), all reported to be hosts of *Aphanomyces* spp. (29). Seeds of each crop species were planted in the culture system described earlier and maintained in a growth chamber at 24 C with a 12-hr photoperiod. Alfalfa, table beet, radish, tomato, and oat seeds were planted eight per cavity, and pea and green bean seed one per cavity.

Mycelial mats were produced in peptone-glucose broth, and the method of Mitchell and Yang (19) was modified by washing mycelial mats with Emerson water rather than a salt solution to induce zoospores. Zoospores (10^3 zoospores) of each isolate were dispensed into each cavity 4 days after planting for alfalfa, table beet, tomato, oat, and radish and 7 days after planting for pea and bean. Each host-isolate combination was distributed in one row of cavities (six cavities per row equals one replicate) and was replicated five times. One isolate was evaluated per plastic box. The planting medium was saturated at the time of inoculation and thereafter drained and resaturated on alternate days. Plants were incubated for 10 days at 24 C, then plants were evaluated for disease severity of roots, hypocotyl, or epicotyls on the scale described. Also, reduction in plant dry weight by *Aphanomyces* was determined by computing the proportion of dry weight of inoculated plants to that of uninoculated plants.

Symptomatic tissues of green bean hypocotyls, pea epicotyls, table beet

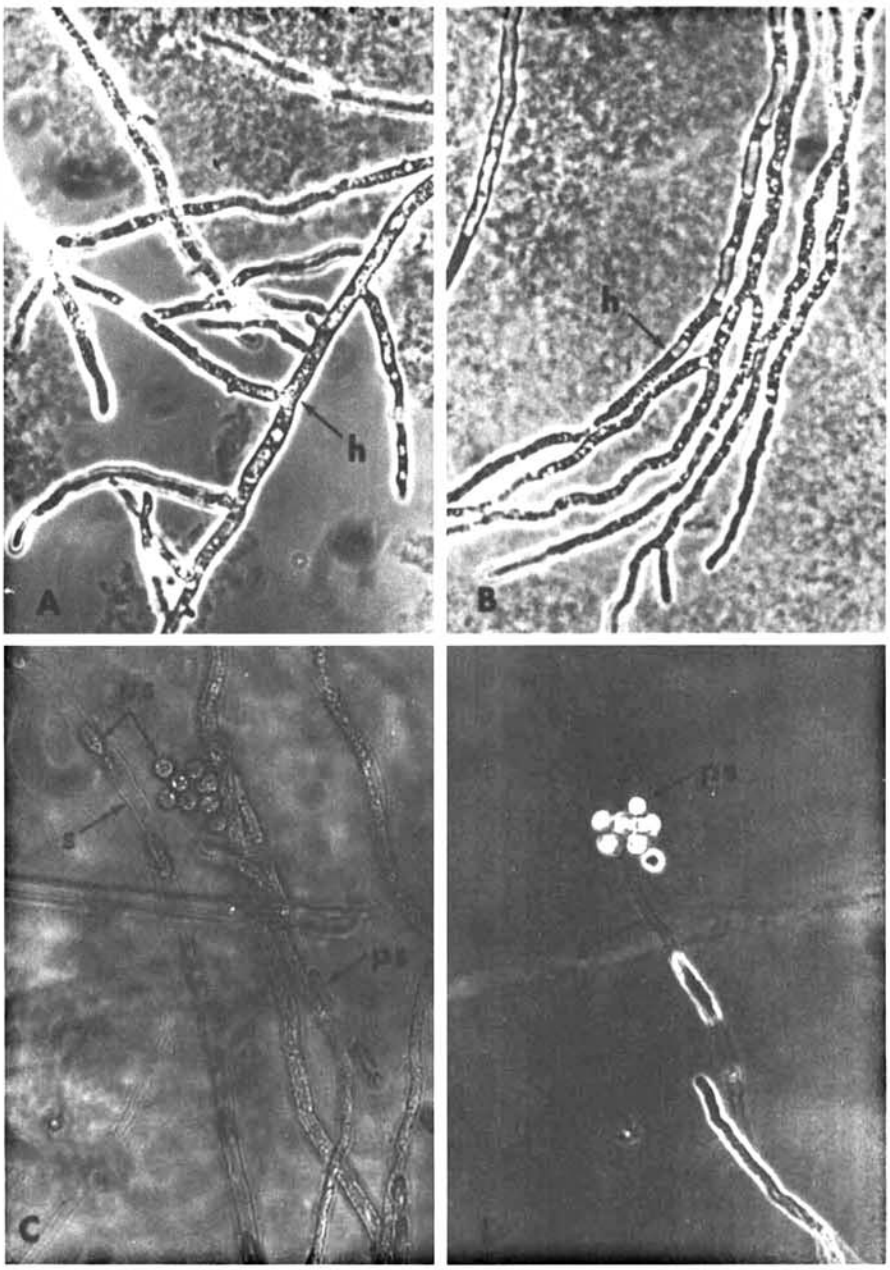

Fig. 1. Photomicrographs of vegetative and asexual reproductive structures of *Aphanomyces euteiches*: **(A)** Comparison of typical right-angle branching of hyphae (h) of *A. euteiches* isolates from alfalfa and **(B)** less evident for an isolate of *A. euteiches* f. sp. *phaseoli*. **(C)** Sporangia (s) and development of primary spores (ps) in sporangia, and **(D)** release of primary spores (ps) in clusters for an alfalfa isolate.

hypocotyls, alfalfa hypocotyls and roots, oat roots, tomato hypocotyls and roots, and radish hypocotyls were surface-disinfested in 0.5% NaOCl for 30 sec, rinsed twice in sterile distilled water, blotted on paper towels, plated on MBVA, and incubated at 22 C for 5 days. Ten pieces of host tissue were assayed per plate.

Susceptibility of legumes to alfalfa isolates. *Aphanomyces* (alfalfa) isolates 317, 349, 418, 1517, 1528, and A015 were tested for pathogenicity on the following legume hosts: alfalfa (cultivars Answer and Vernal), white sweet clover (*Melilotus alba* L. Med. cv. Common), red clover (*Trifolium pratense* L. cv. Arlington), white clover (*T. repens* L. cv. Ladino), birdsfoot trefoil (*Lotus corniculatus* L. cv. Dawn), lima bean (*Phaseolus lunatus* Macf. cv. Kingston), green bean (cultivar Eagle), pea (cultivar 8221), and soybean (*Glycine max* (L.) Merr. cv. Corsoy). Seeds were planted in the aforementioned culture system, and seedlings were inoculated with zoospores (10^3/cavity) 4 and 7 days after planting for the forage legumes and other legumes, respectively. Each host-isolate combination consisted of six cavities per tray and was replicated five times. After 10 days of incubation at 24 C, roots, hypocotyls, epicotyls, and cotyledons were assessed for disease by the previously described methods.

Statistical analysis. A randomized complete block design was used for each experiment. Analysis of variance and Fischer's least significant difference ($P = 0.05$) were used to compare treatment means.

RESULTS

Morphological traits. *Aphanomyces* isolates from alfalfa were morphologically similar to *A. euteiches* isolates recovered from pea in terms of hyphae (Fig. 1A,B), sporangia and primary spores (Fig. 1C,D), and oogonial characteristics (Table 1, Fig. 2A,B). However, alfalfa and pea isolates had more right-angle branching of hyphae than bean isolates (Fig. 1A,B). Isolates from alfalfa, pea, and bean were similar in colony appearance. Sporangia, primary spores, and zoospores were readily observed and were characteristic of the genus (Fig. 1 C,D). Oogonia of alfalfa isolates averaged 28.5 μm in diameter and ranged from 27 to 31 μm. Oogonia of alfalfa isolates were slightly smaller than oogonia of *A. e.* f. sp. *phaseoli* but were very similar to oogonial diameter of *A. e.* f. sp. *pisi* (Table 1). Most oogonia of alfalfa isolates had two antheridia per oogonium but ranged from one to four. Morphology of oogonia and antheridia was similar for alfalfa and pea isolates. However, oogonia isolates from alfalfa (Fig. 2A,B) were more plerotic than isolates from bean (Fig. 2C,D). Also, the antheridial stalks of bean isolates were more distinct than those of alfalfa isolates. Morphological characteristics for *Aphanomyces* isolates from alfalfa were distinctly different from our isolate of *A. cochlioides*. The mean diameter of oogonia for our isolate of *A. cochlioides* was 22.5 μm, and up to six antheridia were observed per oogonium. We conclude that the *Aphanomyces* isolates recovered from alfalfa belong to the species *A. euteiches*, based on morphological criteria set forth by Scott (29).

Effect of temperature on radial growth. Differences in radial growth among alfalfa isolates 317, 461, 627, 1301, 1517, 1529, A015, and A027 were not statistically significant and are reported as a combined mean of the "alfalfa group." Radial growth rates of alfalfa isolates 349 and 418 were statistically different from each other and other isolates, thus, their means are reported separately. Radial growth of both isolates from pea is reported as a combined mean. The rate of radial growth for all isolates of *A. euteiches* increased as temperatures increased from 8 to 28 C (Fig. 3). In general, isolates of *A. e.* f. sp. *pisi* grew faster than alfalfa isolates, except isolate 349 and *A. e.* f. sp. *phaseoli*. Pea and alfalfa isolates grew much faster than bean isolates at 32 C, indicating a closer similarity between pea and alfalfa isolates than between alfalfa and bean isolates. Alfalfa isolate 349 grew faster than all other isolates from alfalfa, and its growth rate was similar to those of pea isolates. Alfalfa isolate 418 grew the slowest of all isolates evaluated.

Pathogenicity to alfalfa. Most *A. euteiches* isolates recovered from alfalfa were highly pathogenic to alfalfa (Table 1), with the exception of isolates 723 and 863. Isolate 863 produced hyphae typical of *A. euteiches* but did not form oogonia or sporangia. Alfalfa cultivars did not differ in their reactions to individual isolates, and combined means are reported (Table 1). Isolates from green bean caused minimal symptoms on alfalfa, but isolates from pea caused disease levels similar to those caused by alfalfa isolates. The isolate of *A. cochlioides* caused no symptoms on alfalfa seedlings.

Host specificity studies. Mean disease severity values for isolates 317, 1528, and 418 were not statistically different from each other for each host, thus a combined mean (alfalfa 1) is reported for these isolates (Fig. 4). All alfalfa isolates were

Table 1. Comparison of *Aphanomyces* isolates from alfalfa, pea, bean, and sugar beet for morphological traits and pathogenicity to alfalfa

Isolate	Host	Oogonium diameter (mm)	No. antheridia per oogonium	*Aphanomyces*-type sporangium	Pathogenicity[a]
235	Alfalfa	31.0	1–4	+	3.0
238	Alfalfa	28.2	1–3	+	3.0
317	Alfalfa	27.6	1–3	+	2.7
349	Alfalfa	27.0	1–3	+	3.0
418	Alfalfa	...	...	+	3.0
460	Alfalfa	30.4	1–3	−	2.0
627	Alfalfa	...	...	+	3.0
722	Alfalfa	28.7	1–3	+	3.0
723	Alfalfa	27.9	1–2	−	1.3
727	Alfalfa	30.1	1–4	−	3.0
741	Alfalfa	28.2	1–2	+	3.0
1317	Alfalfa	27.0	1–4	+	3.0
1319	Alfalfa	27.5	1–3	−	2.7
1321	Alfalfa	28.4	1–4	+	2.7
1517	Alfalfa	29.8	1–4	+	3.0
862	Alfalfa	29.2	1–4	+	3.0
863	Alfalfa	...	...	−	2.0
864	Alfalfa	27.0	1–3	−	3.0
1302	Alfalfa	29.1	1–3	+	3.0
1303	Alfalfa	29.0	1–3	+	3.0
1305	Alfalfa	27.8	1–3	+	3.0
1317	Alfalfa	27.0	1–4	+	3.0
1529	Alfalfa	28.3	1–3	+	3.0
1539	Alfalfa	29.8	1–3	+	3.0
1700	Alfalfa	27.6	1–3	+	3.0
A015	Alfalfa	29.1	1–2	+	3.0
A027	Alfalfa	29.5	1–3	+	3.0
1528	Alfalfa	27.9	1–3	+	3.0
C1	Bean	31.8	1–4	+	0.7
S2	Bean	32.7	1–4	+	0.0
S11	Pea	26.7	1–4	+	2.7
P14	Pea	26.9	1–4	+	2.7
AC 8122	Sugar beet	22.5	1–6	+	0.0

[a] Pathogenicity assessed on a scale of 0–4: 0 = no symptoms; 1 = slight root discoloration; 2 = moderate to extensive root discoloration and/or cotyledon chlorosis and/or slight to moderate hypocotyl discoloration; 3 = extensive discoloration of roots and hypocotyls, cotyledon tissues collapsed; and 4 = plants dead.

highly pathogenic to alfalfa. Although similar in pathogenicity to alfalfa, isolate 349 (alfalfa 2) was statistically more pathogenic to pea than other alfalfa isolates and thus was reported separately (Fig. 4). All alfalfa isolates caused essentially no symptoms on stems and a low level of disease on roots of green bean (Fig. 4A,B). *A. e.* f. sp. *pisi* was more pathogenic to alfalfa than was *A. e.* f. sp. *phaseoli* but was less pathogenic than isolates from alfalfa. *A. cochlioides* caused minimal disease on alfalfa, pea, and bean but was very pathogenic to table beets (Fig. 4). Some degree of host specificity was also measured when assessed in terms of percent isolation from plant tissues (Fig. 4C). Alfalfa isolates were not readily isolated from other crop species, except alfalfa isolate 349, which was readily isolated from pea. All isolates of *A. euteiches* were recovered from alfalfa, but isolation of *A. cochlioides* was limited to beets. The effect of *Aphanomyces* species on plant weight (Fig. 4D) was similar to the trends for disease severity (Fig. 4A,B). Alfalfa isolates reduced plant weight of alfalfa seedlings much more than they reduced plant weights of pea, bean, and table beet. Alfalfa isolate 349 did not reduce plant dry weight of pea to the degree expected based on disease severity of stems and roots. However, pea and bean strains of *A. euteiches* caused fewer but significant reductions in plant weight of alfalfa plants. All isolates of *A. euteiches* from alfalfa, pea, and bean and *A. cochlioides* caused no symptoms of, were not isolated from, or caused no plant weight reduction of tomato, oat, and radish.

Susceptibility of legumes to alfalfa isolates. Root disease severity (Fig. 5A) and percent reduction in plant weight (Fig. 5B) were used to compare the relative susceptibility of several legume species to isolates of *Aphanomyces* recovered from alfalfa. Isolate means were not significantly different from one another, thus combined means are presented in Figure 5A,B. Both alfalfa cultivars (Vernal and Answer) were more susceptible to *A. euteiches* than to other forage species as determined by all measures of pathogenicity (Fig. 5A,B). Although less susceptible than alfalfa, sweet clover was more susceptible than other forage legumes. Lima bean, green bean, pea, and soybean were determined to be poor hosts to alfalfa isolates of *A. euteiches* based on statistically insignificant differences between inoculated and uninoculated plants for all measures of pathogenicity. Alfalfa isolate 349 was less pathogenic to pea in this study than in other studies (Figs. 4 and 5). *A. euteiches* was isolated from alfalfa, sweet clover, red clover, pea, green bean, lima bean, soybean, white clover, and birdsfoot trefoil at frequencies of 65, 65, 31, 21, 0, 0, 0, 0, and 0%, respectively.

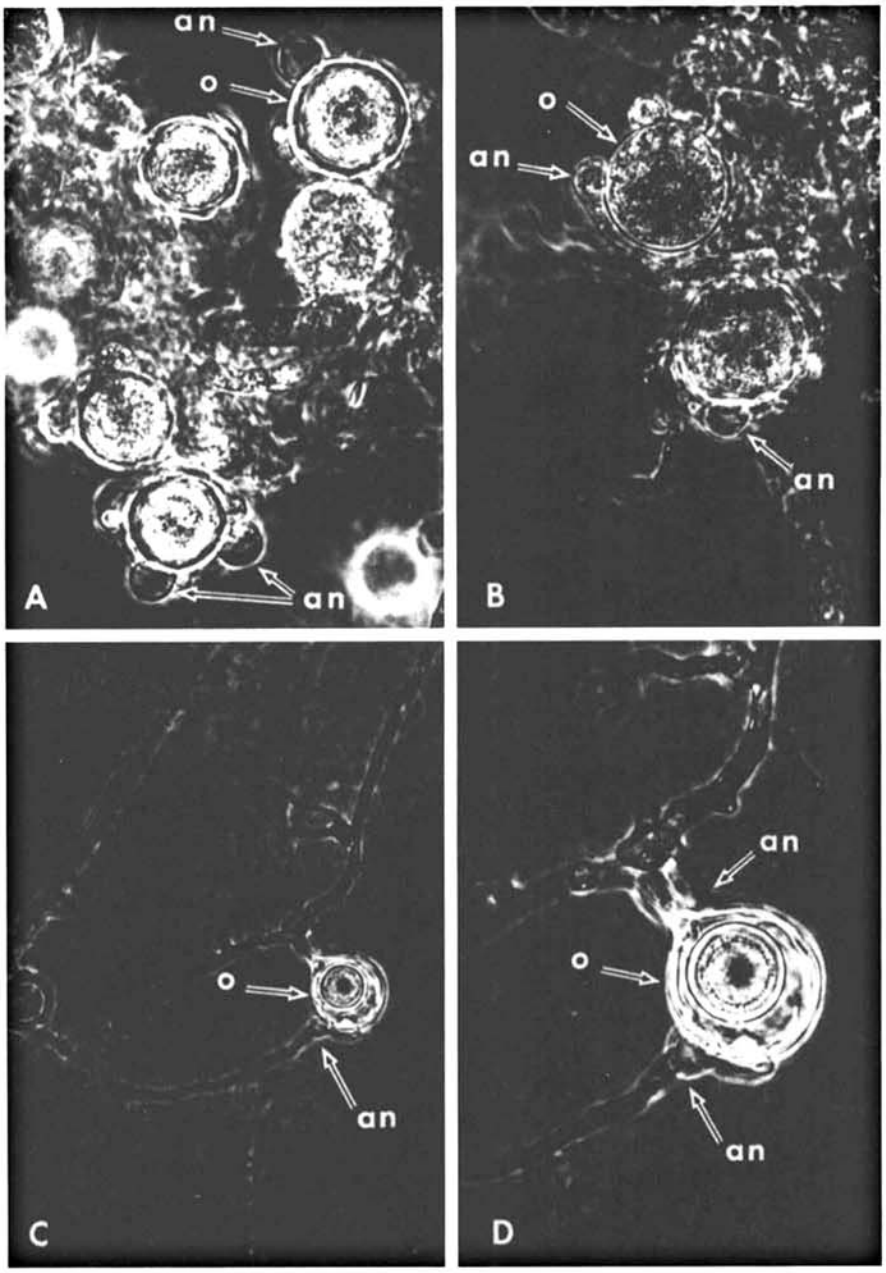

Fig. 2. Oogonia of *Aphanomyces euteiches* formed in culture. **(A)** Oogonia (o) of alfalfa isolates forming in clusters with attached antheridia (an). **(B)** Antheridial stalks of alfalfa isolates are short or not observed. **(C)** Antheridial stalks of *A. euteiches* f. sp. *phaseoli* are long, and many originate from different hyphae. **(D)** Aplerotic zone is quite large for isolates of *A. euteiches* f. sp. *phaseoli* compared with those of isolates from alfalfa.

DISCUSSION

Aphanomyces isolates recovered from alfalfa plants were identified as *A. euteiches* according to Scott's criteria (29). Oogonial measurements of our alfalfa *Aphanomyces* isolates fell within the same range (27–31 μm) as the oogonia of *A. e.* f. sp. *pisi* and *A. e.* f. sp. *phaseoli* (25), but we found *A. cochlioides* oogonia to be smaller (22.5 μm). We also found our alfalfa isolates to have oogonia diameters similar to that of McKeen and Traquair's (18) alfalfa isolate 460 (29 mm). Colony appearance of *Aphanomyces* isolates recovered from alfalfa also supports the designation of our alfalfa isolates as *A. euteiches*. Whereas *A. cochlioides* (AC 8122) produces superficial dense growth on CMA with a strongly directional pattern of growth (29), our isolates from alfalfa produce a less dense superficial colony with less directional growth patterns similar to *A. e.* f. sp. *pisi* and f. sp. *phaseoli*. Our data agree with McKeen and Traquair (18) that maximum radial growth of alfalfa isolates occurs at 28 C and that radial growth is less at 32 C. Radial growth was slightly faster for pea isolates, but pea and alfalfa isolates were similar in that radial growth was still pronounced at 32 C in contrast to the

bean isolate, which essentially stopped growth at 32 C (25).

Speciation of alfalfa *Aphanomyces* isolates also was supported by pathogenicity studies. Alfalfa seedlings were severely diseased by isolates of *A. euteiches* from alfalfa. The degree of host specificity that we found among *A. euteiches* isolates from pea, bean, and alfalfa for their respective hosts was less well defined than the specificity of *A. cochlioides* for beet. Our results showed for all measures of pathogenicity that *A. euteiches* isolates from alfalfa were more pathogenic on alfalfa than on pea or bean. However, alfalfa isolate 349 appeared, with respect to pathogenicity and radial growth on agar at high temperatures, more similar to pea isolates than did all other alfalfa isolates. Our results indicated a trend for pea isolates to be more aggressive on alfalfa and bean than were alfalfa and bean isolates on pea. Previous reports indicate that isolates from pea are moderately aggressive on alfalfa (6,16,31). Schmitthenner (28) isolated two types of *Aphanomyces* from alfalfa and designated the two types *A. euteiches* and alfalfa *Aphanomyces*. He regarded the alfalfa *Aphanomyces* as a strain of *A. euteiches*

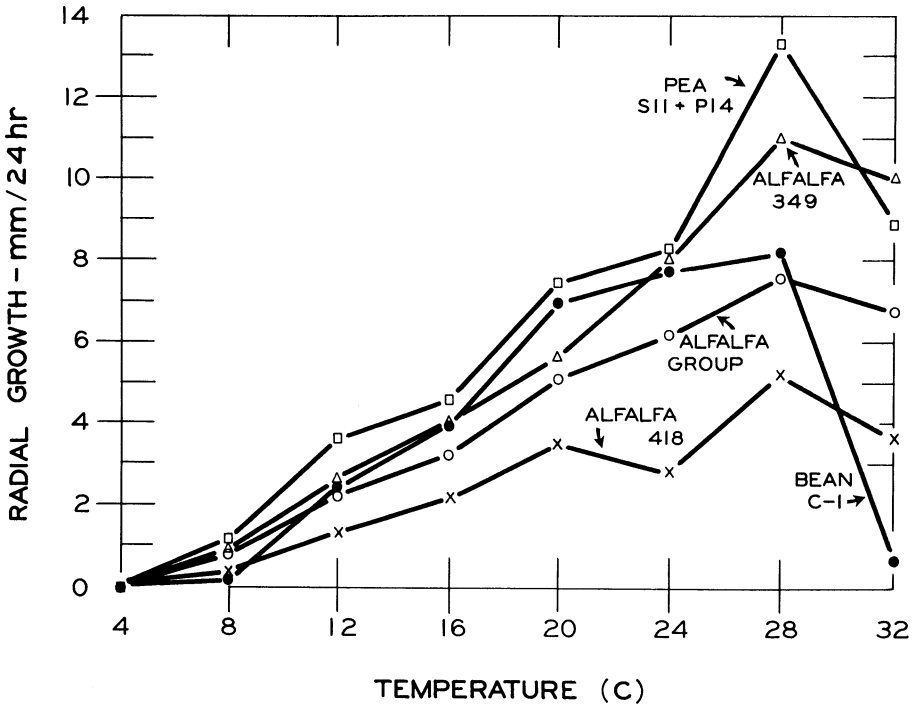

Fig. 3. Effect of temperature (C) on radial growth (mm/24 hr) of *Aphanomyces euteiches* alfalfa isolates 349 and 418 and combined means of isolates 317, 461, 627, 1301, 1517, 1518, 1529, A015, A027, *A. euteiches* f. sp. *pisi* (S11 and P14), and *A. euteiches* f. sp. *phaseoli* (C1).

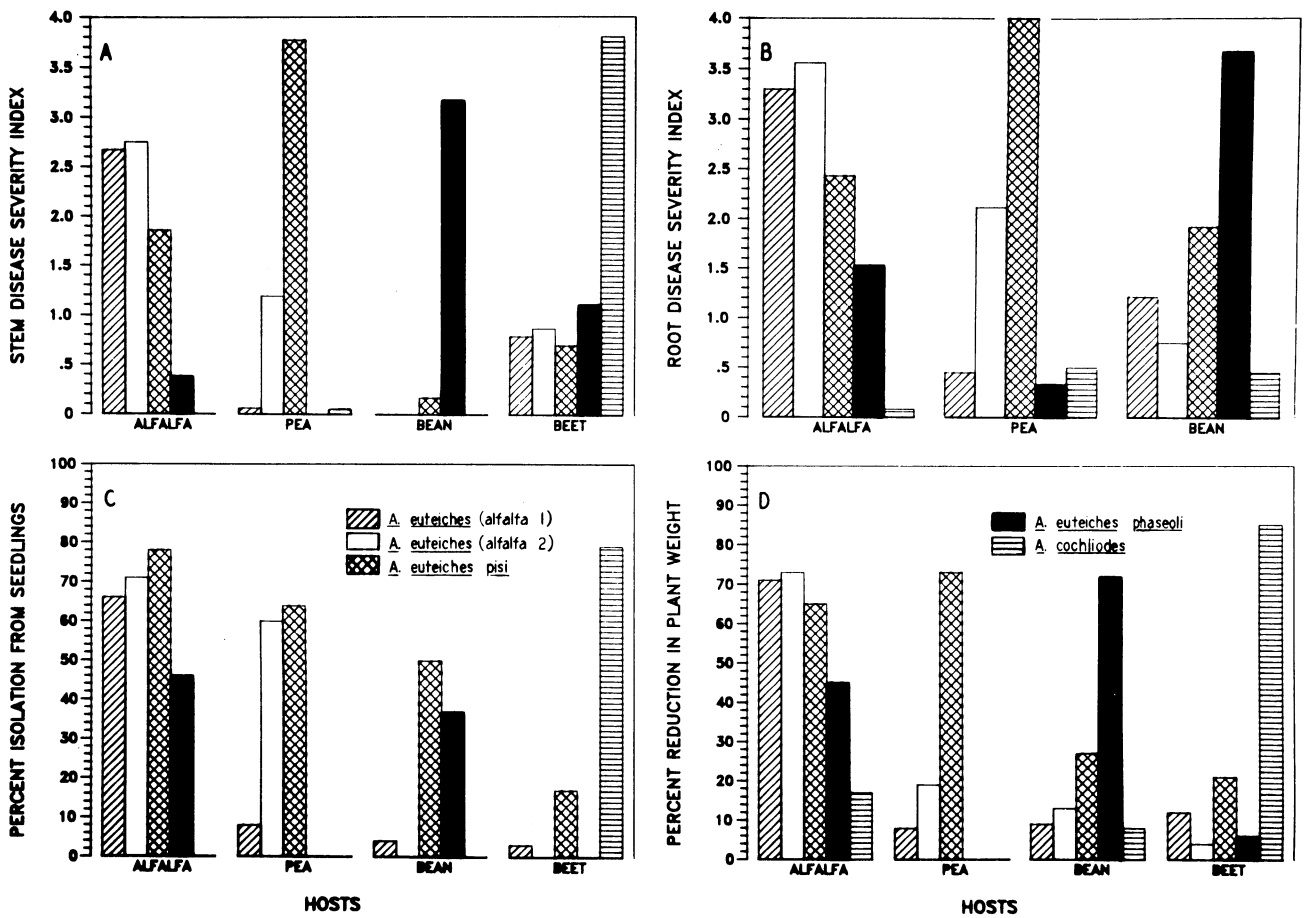

Fig. 4. Pathogenic effect of *Aphanomyces euteiches* isolates from alfalfa (alfalfa 1 = isolates 317, 418, and 1528; alfalfa 2 = isolate 349), *A. euteiches* f. sp. *pisi* (isolate P14), *A. euteiches* f. sp. *phaseoli* (isolate C1), and *A. cochlioides* on alfalfa, pea, green bean, and beets as measured by **(A)** stem disease severity, **(B)** root disease severity, **(C)** percent isolation from roots, and **(D)** percent reduction in plant weight.

because alfalfa isolates were pathogenic to alfalfa only, whereas isolates from peas caused only minimal postemergence death of alfalfa. Haglund and King (9) suggested that alfalfa *Aphanomyces* isolates are strains of *A. euteiches* pathogenic to alfalfa but less aggressive on pea. McKeen and Traquair (18) reported their *Aphanomyces* isolates from alfalfa resembled *A. cochlioides* more than *A. euteiches*, although they failed to parasitize sugar beets. We support the conclusions of Schmitthenner (28) and Haglund and King (9) that isolates of *A. euteiches* from alfalfa are more aggressive on alfalfa than on pea. However, because of the small number of isolates from pea and bean, we conclude that the evidence is not sufficient to assign a new forma specialis to isolates recovered from alfalfa.

The pathogenicity of alfalfa *Aphanomyces* isolates to other forage legumes was of great interest. In most cases, red clover and birdsfoot trefoil are successfully established in sites naturally infested with *Aphanomyces* sp. Alfalfa was more susceptible to *A. euteiches* than other forage species, although sweet clover was more susceptible than red clover, white clover, and birdsfoot trefoil. The latter three forage species were concluded not to be good hosts for *A. euteiches*. Our results are supported by Cunningham and Hagedorn (6), who found red clover and birdsfoot trefoil to be poor host but white sweet clover to be a moderate host to *A. euteiches* isolates originally recovered from pea.

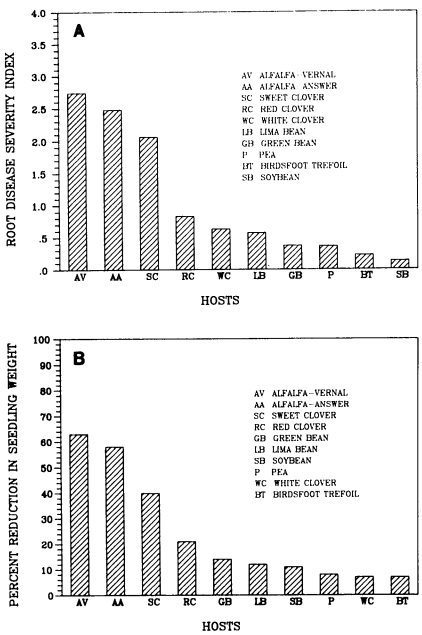

Fig. 5. Effect of *Aphanomyces euteiches*, composite of alfalfa isolates 317, 349, 418, 1517, 1528, and A015, on **(A)** root disease severity indices (scale of 1–4, where 0 = no necrosis of roots, hypocotyls, and cotyledons; 1 = minimal necrosis of lateral roots, hypocotyls, and cotyledons; 2 = necrosis of roots and lower hypocotyls and chlorosis and minimal necrosis of cotyledons; 3 = extensive necrosis of roots, hypocotyls, and cotyledons and plants stunted; and 4 = plants dead) and **(B)** percent reduction in seedling weights of alfalfa, sweet clover, red clover, white clover, birdsfoot trefoil, lima bean, green gean, pea, and soybean.

The impact of *A. euteiches* alone has not been investigated, but its frequent association with *P. m.* f. sp. *medicaginis* leads us to speculate that both pathogens act together to form a disease complex. *Phytophthora*-resistant cultivars establish and persist longer than susceptible cultivars in wet-soil environments (12,13,17) but still decline at an unsatisfactory rate when both *P. m.* f. sp. *medicaginis* and *A. euteiches* are present (11). Poor performance of *Phytophthora*-resistant cultivars can be attributed to differences in seedling resistance to Phytophthora root rot for alfalfa cultivars with equal adult plant resistance (21), the presence of highly virulent forms of *P. m.* f. sp. *medicaginis* capable of causing extensive disease on highly resistant cultivars (7,8), and lower expression of *Phytophthora* resistance at temperatures higher than 24 C (E. B. Holub, *unpublished*). In addition, abiotic stress associated with water-saturated soils can alone be a major cause of poor alfalfa growth in wet soils (1–4,33) or predispose plants to Phytophthora root rot (4,15). Our studies pursued another biotic factor, leading us to the frequent association of *A. euteiches* with poor productivity of alfalfa in wet soils in Wisconsin. Identification of an additional pathogen provides the basis to more thoroughly investigate the biotic and abiotic factors associated with poor alfalfa productivity in wet soils. Alva et al (1) refer to this situation as a "wet-soil syndrome." *Pythium* spp. and *P. m.* f. sp. *medicaginis* are traditionally regarded as major biotic factors associated with water-logged soil environments, but we conclude that *A. euteiches* is a neglected factor in the wet-soil syndrome and warrants further investigation. Current studies are directed at pathogen variability, the identification of resistance to *A. euteiches*, the impact of resistance on yield performance of alfalfa, and the direct comparison of seedling disease caused by *A. euteiches* and *P. m.* f. sp. *medicaginis*.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of S. A. Vicen and Judy Gosse for graphics and manuscript preparation, respectively. Research supported by the College of Agriculture and Life Sciences, University of Wisconsin-Madison, as Hatch Project 2798 and EPA/USDA/Texas A&M Research Foundation Subcontract L200048.

LITERATURE CITED

1. Alva, A. K., Lanyon, L. E., and Leath, K. T. 1985. Excess soil water and Phytophthora root rot stresses of Phytophthora root rot sensitive and resistant alfalfa cultivars. Agron. J. 77:437-442.
2. Barta, A. L. 1980. Regrowth and alcohol dehydrogenose activity in waterlogged alfalfa and birdsfoot trefoil. Agron. J. 72:1017-1020.
3. Barta, A. L. 1984. Ethanol synthesis and loss from flooded roots of *Medicago sativa* L. and *Lotus corniculatus* L. Plant Cell Environ. 7:187-191.
4. Barta, A. L., and Schmitthenner, A. F. 1986. Interaction between flooding stress and Phytophthora root rot among alfalfa cultivars. Plant Dis. 70:310-313.
5. Chi, C. C., and Hanson, W. W. 1962. Interrelated effects of environment and age of alfalfa and red clover seedlings on susceptibility to *Pythium debaryanum*. Phytopathology 52:985-989.
6. Cunningham, J. L., and Hagedorn, D. J. 1962. Attraction of *Aphanomyces euteiches* zoospores to pea and other plant roots. Phytopathology 52:616-618.
7. Faris, M. A. 1985. Variability and interaction between alfalfa cultivars and isolates of *Phytophthora megasperma*. Phytopathology 75:390-394.
8. Faris, M. A., Sabo, F. E., and Barr, D. J. S. 1983. Studies on *Phytophthora megasperma* isolates with different levels of pathogenicity in alfalfa cultivars. Can. J. Plant Pathol. 5:29-33.
9. Haglund, W. A., and King, T. H. 1961. Inoculation technique for determining tolerance of *Pisum sativum* to *Aphanomyces euteiches*. Phytopathology 51:800-802.
10. Hancock, J. G. 1983. Seedling diseases of alfalfa in California. Plant Dis. 67:1203-1208.
11. Havey, M. J., and Grau, C. R. 1985. Decline of established alfalfa in soils naturally infested with *Phytophthora megasperma* f. sp. *medicaginis* and level of correlation by seedling assay. Plant Dis. 69:221-224.
12. Hine, R. B., Gray, F. A., Schonhorst, M. H., and Sanders, J. S. 1975. Resistance to Phytophthora root rot in selected lines of nondormant alfalfa. Phytopathology 65:840-844.
13. Irwin, J. A. G., Miller, S. A., and Maxwell, D. P. 1979. Alfalfa seedling resistance to *Phytophthora megasperma*. Phytopathology 69:1051-1055.
14. Kuan, T. L., and Erwin, D. C. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybeans and alfalfa. Phytopathology 70:333-338.
15. Kuan, T. L., and Erwin, D. C. 1980. Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. Phytopathology 70:981-986.
16. Linford, M. B. 1927. Additional hosts of *Aphanomyces euteiches*, the pea root rot fungus. Phytopathology 17:133-134.
17. Lueschen, W. E., Barnes, D. K., Rabas, D. L., Frosheiser, F. I., and Smith, D. M. 1976. Field performance of alfalfa cultivars resistant and susceptible to Phytophthora root rot. Agron. J. 68:281-285.
18. McKeen, W. E., and Traquair, J. A. 1980. *Aphanomyces* sp., an alfalfa pathogen in Ontario. Can. J. Plant Pathol. 2:42-44.
19. Mitchell, J. E., and Yang, C. Y. 1966. Factors affecting growth and development of *Aphanomyces euteiches*. Phytopathology 56:917-922.
20. Nelson, D. L., Barnes, D. K., and MacDonald, D. H. 1985. Field and growth chamber evaluations for root-lesion nematode resistance in alfalfa. Crop Sci. 25:35-39.
21. Nygaard, S. L. 1985. Factors affecting Phytophthoora root rot of seedling alfalfa (*Medicago sativa* L.). M.S. thesis. University of Wisconsin-Madison. 142 pp.
22. Papavizas, G. C., and Ayers, W. A. 1974. *Aphanomyces* species and their root diseases in pea and sugarbeet. U.S. Dep. Agric. Tech. Bull. 1485. 158 pp.
23. Parker, C. A., and Chatel, D. L. 1982. Factors determining success or failure in legume establishment. Pages 145-154 in: Nitrogen Fixation in Legumes. J. M. Vincent, ed. Academic Press, Sydney. 228 pp.
24. Pfender, W. F., Delwiche, P. A., Grau, C. R., and Hagedorn, D. J. 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. Plant Dis. 68:845-847.
25. Pfender, W. F., and Hagedorn, D. J. 1982.

Aphanomyces euteiches f. sp. *phaseoli*, a causal agent of bean root and hypocotyl rot. Phytopathology 72:306-310.
26. Pfender, W. F., and Hagedorn, D. J. 1983. Disease progress and yield loss in Aphanomyces root rot of peas. Phytopathology 73:1109-1113.
27. Pulli, S. K., and Tesar, M. B. 1975. Phytophthora root rot in seeding-year alfalfa as affected by management practices inducing stress. Crop Sci. 15:861-864.
28. Schmitthenner, A. F. 1964. Prevalence and virulence of *Phytophthora, Aphanomyces, Pythium, Rhizoctonia,* and *Fusarium* isolated from diseased alfalfa seedlings. Phytopathology 54:1012-1018.
29. Scott, W. W. 1961. A Monograph of the Genus *Aphanomyces*. Tech. Bull. 151. Va. Agric. Exp. Stn. Blacksburg. 95 pp.
30. Sheaffer, C. C., Rabas, D. L., Frosheiser, F. I., and Nelson, D. L. 1982. Nematicides and fungicides improve legume establishment. Agron. J. 74:536-538.
31. Sherwood, R. T., and Hagedorn, D. J. 1962. Studies on the biology of *Aphanomyces euteiches*. Phytopathology 52:150-154.
32. Tesar, M. B., and Jackobs, J. A. 1972. Establishing the stand. Pages 415-435 in: Alfalfa Science and Technology. Agronomy 15. C. H. Hanson, ed. American Society of Agronomy, Madison, WI.
33. Thompson, T. E., and Fick, G. W. 1981. Growth response of alfalfa to duration of flooding and to temperature. Agron. J. 73:329-332.