Prevalence and Distribution of *Aphanomyces euteiches* and *Phytophthora medicaginis* in Iowa Alfalfa Fields

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ABSTRACT

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Soil and/or root samples from 122 alfalfa stands in 45 of 99 Iowa counties were collected to determine the presence of *Aphanomyces euteiches* and *Phytophthora medicaginis*. Collections were made from sites believed to have a high probability of root disease problems. The presence of these fungi was assayed by baiting soil and root samples with healthy alfalfa seedlings (cv. Vernal) in water or metalaxyl (5 µg/ml) in petri dishes. *A. euteiches* and *P. medicaginis* were recovered from 74 and 35% of soil samples and 17 and 8% of root samples, respectively. Of the soil samples infested with *P. medicaginis*, 81% also were infested with *A. euteiches*. *A. euteiches* and *P. medicaginis* were detected in 31 and 26 counties, respectively. *A. euteiches* was recovered from 89% of sites in Northeast Iowa (the area with the greatest alfalfa production). *A. euteiches* may be more prevalent than *P. medicaginis* in poorly drained or diseased fields in Iowa.

Additional keywords: *Medicago sativa*, root rot, seedling blight

Aphanomyces euteiches Drechs. has long been known to be pathogenic to pea and other crops (15), and it was associated with alfalfa (*Medicago sativa* L.) during the 1920s (12). Proof of pathogenicity and further knowledge of its role as an alfalfa pathogen have been reported more recently (21). *A. euteiches* is now recognized as an important pathogen of alfalfa, causing seedling blight and root rot of mature plants, especially in poorly drained soils (2,14,18,19,24,27). *A. euteiches* is known to be distributed widely in the United States, and its geographic range includes Iowa (4,24). However, its overall impact on alfalfa production in Iowa and many other areas of the United States is unknown. Surveys have been conducted in some states to determine the distribution of *A. euteiches* (2,4,26), but the prevalence and distribution of *A. euteiches* within Iowa have not previously been investigated. Prior to this study, the fungus had been isolated very infrequently from alfalfa or soil samples received by the Iowa State University Plant Disease Clinic.

Some isolates of *A. euteiches* have a limited host range, and those pathogenic to alfalfa differ in their virulence to specific alfalfa cultivars (5,6,10). Knowledge of the prevalence and distribution of this pathogen in Iowa would be very helpful in diagnosing potential root rot and seedling problems and recommending appropriate cultivars and disease management practices for alfalfa in Iowa. Moreover, this information will broaden our knowledge of *A. euteiches* in general. Specific information on populations of this fungus is available from limited areas, particularly Wisconsin (2,5) and Kentucky (26). Information from Iowa and additional states will be useful in determining if consistent patterns of prevalence and population composition exist among different states.

Phytophthora medicaginis Hansen et Maxwell (=*Phytophthora megasperma* Drechs. f. sp. *medicaginis* T. Kuan & D.C. Erwin) is a well-known seedling blight and root rot pathogen of alfalfa (3,9). It is considered to be one of the most important pathogens of alfalfa, and most recently released cultivars have some resistance to this fungus. In spite of the availability of resistant cultivars, this disease remains a concern due to the susceptibility of some individual plants in resistant populations and the high percentage of growers using older, less expensive cultivars lacking disease resistance. Information on the occurrence of this pathogen is a valuable tool for developing appropriate disease management recommendations.

The objective of this work was to determine the prevalence and distribution of alfalfa-infecting strains of *A. euteiches* and *P. medicaginis* in Iowa in fields where alfalfa is grown.

MATERIALS AND METHODS

Soil and/or root samples were collected from 45 counties throughout the state, with the number of samples from each crop district approximately proportional to the alfalfa hectarage in that district (Table 1). Iowa is divided into nine crop districts, with the most intensive alfalfa production occurring in the northeast and south central crop districts. Within each district, fields with a history of stand establishment or root rot problems were selected for sampling. If potential root rot symptoms were present, two to four plants and soil were collected. If no symptoms were evident, samples consisted of soil only. A soil sample consisted of six to eight cores, 2 cm in diameter and 15 to 20 cm deep, collected from symptomatic portions or the most poorly drained portions of each field. A total of 122 sites was sampled in 1994. Of these, 49 samples consisted of soil and roots, 56 consisted of soil only, and 17 consisted of roots only. The number of sites sampled per district ranged from 1 to 34; the number of sites sampled per county ranged from 0 to 8.

A. euteiches and *P. medicaginis* were detected by baiting with alfalfa seedlings (cv. Vernal) using the methods of Vincelli et al. (25). Vernal does not have resistance to *A. euteiches* or *P. medicaginis* (1). Soil samples were forced through a coarse screen (3-mm mesh), thoroughly mixed, and air-dried for 48 h. For each sample, approximately 25 g of air-dried soil was placed in each of two sterile petri dishes. Approximately 4 ml of sterile distilled water or a solution of metalaxyl (5 µg/ml) in sterile distilled water was added to the soil until the soil became damp. After 72 h, the dishes were flooded with approximately 20 ml of sterile water or 5 µg/ml metalaxyl solution, and three or four 5- to 10-day-old alfalfa seedlings (cv. Vernal) grown in sterile vermiculite were placed in the dishes.

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Table 1. Area planted to alfalfa, number of samples collected, and number of samples from which *Aphanomyces euteiches* and *Phytophthora medicaginis* were recovered for each crop reporting district in Iowa

District	Hectares[a]	Soil samples[b]				Root samples[c]		
		Number	*A. euteiches*	*P. medicaginis*	Both	Number	*A. euteiches*	*P. medicaginis*
Northwest	30,350 (6.0%)	1	0	1	0	0	...	...
North central	20,640 (4.1%)	4	4	0	0	1	0	0
Northeast	125,860 (24.9%)	28	25	11	11	19	4	2
West central	48,970 (9.7%)	14	10	3	3	16	1	1
Central	39,660 (7.8%)	10	8	4	3	7	1	2
East central	72,040 (14.2%)	16	9	5	4	0	...	...
Southwest	44,520 (8.8%)	6	5	2	2	0	...	...
South central	78,920 (15.6%)	22	17	9	7	19	4	0
Southeast	44,920 (8.9%)	5	1	2	0	4	1	0
State totals	505,870 (100%)	106	79	37	30	66	11	5

[a] Data for area planted from 1994, Iowa Agricultural Statistics Service, Des Moines. Numbers in parentheses are percentage of state total.
[b] Columns labeled *A. euteiches* and *P. medicaginis* include samples from which both fungi were recovered.
[c] Both pathogens were not recovered from the same root sample.

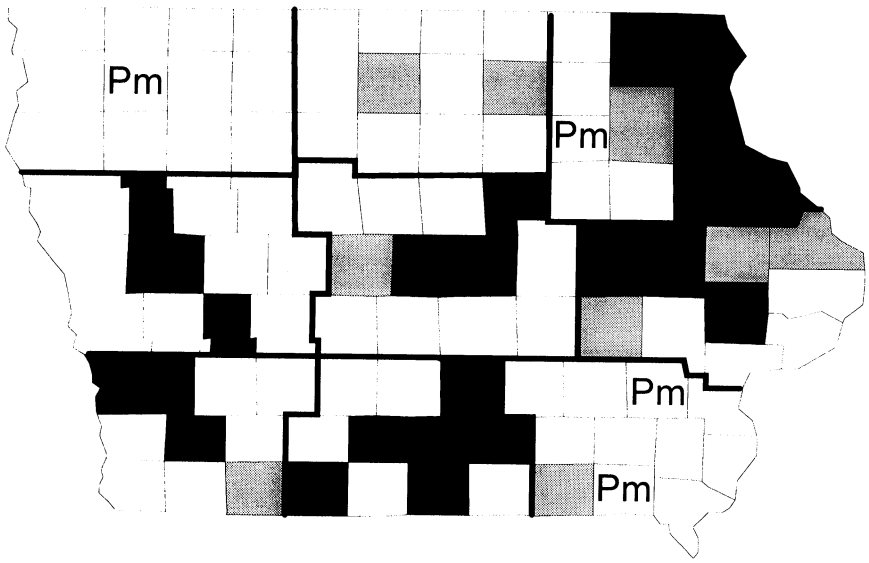

Fig. 1. Iowa counties in which *Aphanomyces euteiches* and *Phytophthora medicaginis* were detected in soil or root samples from alfalfa fields. Both fungi were detected in black-shaded counties; *A. euteiches* was detected in dark gray shaded counties; and *P. medicaginis* was detected in counties labeled **Pm.** Bold lines delimit the crop reporting districts.

Alfalfa root samples were washed with tap water, and necrotic roots were used in the assay. If no necrotic roots were evident, apparently healthy fine root pieces were used. Root pieces were placed in petri dishes containing sterile distilled water or a 5 µg/ml metalaxyl solution, and bait seedlings were added as described above. After 3 days, bait seedlings were inspected daily for macroscopic evidence of decay and were inspected microscopically for fungal structures of *A. euteiches* or *P. medicaginis*. These fungi were identified by their characteristic sporangia and oospores, as described by Delwiche et al. (2), Scott (20), and Hansen and Maxwell (9). In the dishes containing sterile distilled water, bait seedlings often decayed rapidly and became extensively colonized by other fungi, particularly *Pythium* and *Fusarium* species. To better detect *P. medicaginis*, these seedlings were discarded, the water was carefully poured off and replaced with sterile water, and healthy seedlings were added. This procedure was continued until either *P. medicaginis* was detected or three sets of bait seedlings had been inspected. Relative virulence of both species recovered in the seedling assay was not determined, but isolates capable of infecting alfalfa seedlings in the assay were assumed to be capable of infecting alfalfa in the field, as reported previously (13,22,26). Some seedlings infected by *A. euteiches* during the assay were transferred to MBV medium (16) for isolation of the fungus. The presence of other known alfalfa pathogens on bait seedlings also was recorded.

RESULTS AND DISCUSSION

A. euteiches was recovered more frequently than *P. medicaginis* from both soil and roots, and recovery of both fungi was greater from soil than from roots. *A. euteiches* and *P. medicaginis* were recovered from 74 and 35% of soil samples and from 17 and 8% of root samples, respectively (Table 1). Of the 49 sites where both soil and root samples were collected, there was only one site from which one of the pathogens (*P. medicaginis*) was recovered from the roots but not the soil. There were 31 sites from which one or both pathogens were recovered from the soil but not the roots. From the 17 sites where only roots were collected, *A. euteiches* was recovered from only one sample, and *P. medicaginis* was recovered from two samples. Therefore, the plant samples did not contribute greatly to our knowledge of the distribution of these pathogens in Iowa. These results indicate that soil sampling is a more sensitive method for detection of these fungi in a field. Low frequency of detection in roots may be due to a combination of factors, including lack of root infection, insufficient plant sampling, lack of fine roots in the sample, and possible limitations of the baiting technique. One effect of *A. euteiches* infection may be a reduction in size of the root system (17), and infected roots may slough off easily; therefore assaying existing roots for presence of the fungus may not be the most appropriate method to evaluate the effects of *A. euteiches* on plant health. A more appropriate method might include evaluation of root length and structure, as reported for *Pythium* infection of alfalfa (7,8,11).

Of the 37 soil samples from which *P. medicaginis* was recovered, *A. euteiches* also was recovered from 31. However, this proportion was not significantly greater than the proportion of all samples that contained *A. euteiches* (chi-square, I = 0.05). *A. euteiches* and *P. medicaginis* were detected in 31 and 26 of the 45 sampled counties, respectively (Fig. 1). Both fungi were recovered from fields in seven of the nine crop reporting districts of the state; sampling was inadequate in the northwest and north central districts, which have less intensive alfalfa production. Undoubtedly the distribution of both fungi in Iowa includes counties from which they were not detected due to lack of sampling or low sampling intensity.

Our results indicate that *A. euteiches* is more prevalent than *P. medicaginis* in Iowa

alfalfa fields that are prone to root rot problems. However, the relative extent of damage by the two pathogens is still unknown. The more frequent recovery of *A. euteiches* compared with *P. medicaginis* is consistent with results from a Kentucky survey (26) in which alfalfa stands were selected arbitrarily, regardless of disease symptoms or drainage conditions. Our results indicate a higher prevalence of both fungi, but this may be due to the sampling criteria. The apparent predominance of *A. euteiches* in both surveys suggests that this fungus may be a more common pathogen than *P. medicaginis*, although some bias may result from the baiting technique. *P. medicaginis* is somewhat more difficult to recover than *A. euteiches* using this technique. Therefore, the prevalence of *P. medicaginis* may be underestimated. Forty-eight soil samples were tested repeatedly for *P. medicaginis* by replacing the water solution and bait seedlings in the assay dish. *P. medicaginis* was recovered after two or three tests from 20% of those samples that did not yield the fungus after the first test. If all samples that were initially negative had been tested three times, we might have recovered the fungus from additional samples.

The role of other seedling and root pathogens must not be overlooked. Bait seedlings used in both soil and root samples were commonly infected by species of *Fusarium, Pythium,* and *Rhizoctonia.* Fungi in each of these genera are pathogenic to alfalfa roots (7,8,11,23). Therefore, some of the observed symptoms on the plant samples may not be attributable to *A. euteiches* or *P. medicaginis.*

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