Incidence of *Aphanomyces euteiches* and *Phytophthora medicaginis* in Kentucky Alfalfa Fields

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ABSTRACT

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Aphanomyces euteiches was detected in soil or root samples from fields cropped to alfalfa in 47 counties from most major alfalfa-producing regions in Kentucky. Soil samples from 121 fields in 30 counties were tested for the presence of *A. euteiches* and *Phytophthora medicaginis* using a baiting technique. *A. euteiches* and *P. medicaginis* were detected in 57 and 9.9%, respectively, of samples collected. Given the prevalence of alfalfa-infecting strains of *A. euteiches*, studies are needed to determine whether the use of *Aphanomyces*-resistant cultivars will result in enhanced performance of alfalfa in Kentucky.

Aphanomyces euteiches Drechs. was first associated with diseased alfalfa (*Medicago sativa* L.) in the 1920s (11). Pathogenicity of this oomyceteous fungus to alfalfa was clearly established in the 1960s (19,20). Although it has been recognized as a pathogen of alfalfa since these studies, few data have become available on the relative importance of *A. euteiches* in causing loss to this forage crop. However, interest in this pathogen has grown in recent years, as researchers have occasionally associated it with serious disease outbreaks of alfalfa in the field (1,3,14,18).

In Kentucky, isolations and pathogenicity tests indicated that alfalfa-infecting strains of *A. euteiches* were present in soils or necrotic roots from plants in some alfalfa fields where stands failed to establish or yields were poor (W. C. Nesmith and P. Vincelli, *unpublished*). Growth chamber studies indicated that the inoculum potential of *A. euteiches* was destructively high in a representative naturally infested soil in Kentucky (22). Based on these observations and data, an ongoing research effort was initiated to evaluate the role of this pathogen on alfalfa production in the state.

Phytophthora medicaginis Hansen et Maxwell (=*Phytophthora megasperma* Drechs. f. sp. *medicaginis* Kuan & D.C. Erwin) is widely recognized as a destructive root pathogen of alfalfa in slowly drained soils (10). Knowledge of the relative incidence of *P. medicaginis* in Kentucky alfalfa fields could be useful in formulating disease-control recommendations.

The objectives of this research were to assess the prevalence and distribution of alfalfa-infecting strains of *A. euteiches* in regions of Kentucky with substantial alfalfa production, and to compare the incidence of *A. euteiches* with that of *P. medicaginis* in field soils cropped to alfalfa in Kentucky.

MATERIALS AND METHODS

During 1984–1989, more than 50 alfalfa fields were sampled during routine field visits by an extension plant pathologist. All fields sampled had problems with stand establishment and/or maintenance during the first few months following a spring seeding of alfalfa. Alfalfa-infecting strains of *A. euteiches* were detected using the following methods. For plant tissue, soil was removed from symptomatic roots, and these were floated in sterile deionized water in individual 10-cm polystyrene petri dishes, along with a healthy alfalfa seedling (cv. Arc) grown in the laboratory. These were incubated on a laboratory bench (temperature generally 20–22 C) for 1–5 days. Symptomatic roots and bait seedlings were examined microscopically (100–400×) for sporulation. Occasionally, direct isolations of necrotic alfalfa root tissues were made on half-strength cornmeal agar or MBV medium (16); sporulation was induced by incubating colonized plugs approximately 1 cm in size in petri dishes containing sterile deionized water for 1–5 days on a laboratory bench. In a number of fields, soil samples were tested for *A. euteiches* by the technique described by Parke and Grau (15). Duplicate soil samples were tested with and without metalaxyl fungicide; metalaxyl was included to permit detection in certain soils where aggressive growth of *Pythium* spp. might have precluded detection of *A. euteiches*.

Following the informal survey described above, a more formal survey of alfalfa fields was conducted. A total of 121 alfalfa fields in 30 counties was sampled during 1990–1992. Fields were selected by county extension agents as typical production fields for their counties. Representative counties were included from almost all major alfalfa-producing regions of the state. Sampling was most intensive in central Kentucky, where the counties having the highest alfalfa production were located. No samples were collected from the southeastern coal region of the state, where alfalfa production is very limited.

Soil samples were obtained by collecting at least five cores (15 cm deep) at random from the field using a soil probe (2 cm diameter). Most samples were collected between the months of March and October, inclusive. Samples were tested for the presence of *A. euteiches* and *P. medicaginis* by using a modification of the extended bioassay baiting technique reported by Stack and Millar for *P. medicaginis* (21), as follows. Soil samples were mixed thoroughly, and two approximately 20-ml subsamples were placed in separate petri dishes with lids removed for 1–2 days while soil air-dried on a lab bench. After drying, one dish was moistened to about field capacity (approximately 5 ml) with sterile deionized water, the lid was replaced, and the sample was incubated for 2–3 days on a lab bench. The duplicate dish was moistened with a metalaxyl solution of 5 μg/ml (prepared from an appropriate dilution of Ridomil 2E fungicide in sterile deionized water) but was otherwise treated identically. Following incubation, each subsample originally moistened with sterile deionized water or metalaxyl solution was flooded with 30 ml of the same solution, and three to four healthy alfalfa seedlings were placed in each dish. Seedlings for these tests were produced by placing untreated seeds (cv. Arc) into wax-coated paper cups containing 70 ml of perlite, moisten-

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ing with deionized water, and incubating on a lab bench under fluorescent lights for 4–7 days. Seedlings floated above soil samples were inspected daily at 3–5 days after flooding, and those exhibiting any evidence of decay were examined (100–400×) for fungal sporulation.

The percentage of soil samples infested with each pathogen was determined, and confidence intervals were calculated for these estimates of percentage infestation. The calculation of confidence intervals for a probability in a binomial sampling (infested vs. not infested) is described by Conover (2). Values for sample size and proportion of samples infested were inputted into Table A4 of Conover (2) to derive confidence intervals.

RESULTS AND DISCUSSION

Alfalfa-infecting strains of *A. euteiches* were detected in soils and/or plant tissues collected from 47 counties in Kentucky, representing nearly all major alfalfa-producing regions of the state (Fig. 1). In the survey of soils collected from 121 Kentucky alfalfa fields, *A. euteiches* was detected in soil from 57% of fields sampled, with a 95% confidence interval of 48–66%. This incidence is somewhat higher than that observed in a previous survey (4). In that survey, alfalfa-infecting strains of *A. euteiches* were detected in 39 of a total of 111 soil samples collected from 13 states.

These data provide evidence that alfalfa-infecting strains of *A. euteiches* are common in alfalfa fields in Kentucky, infesting between 48 and 66% of alfalfa fields in the state. *A. euteiches* was detected in fields in 47 counties, including 23 of 25 with a countywide production exceeding 33,600 mt of alfalfa hay per hectare in 1991 (23). Samples were not collected from the remaining two high-production counties, so it is possible that alfalfa-infecting strains of *A. euteiches* are present in some fields in those counties, as well. As of 1991 (23), alfalfa was being grown on 96,400 ha in the 47 counties where *A. euteiches* was detected in this study. Our estimate of an incidence of 48–66% in field soils suggests that between 46,300 and 63,600 ha planted to alfalfa are infested with alfalfa-infecting strains of *A. euteiches* in Kentucky. This figure is probably conservative, however, since it is likely that *A. euteiches* will be found in some of those counties that were not sampled in the present study.

Although alfalfa-infecting strains of *A. euteiches* appear to be widespread in Kentucky alfalfa fields, it is still unclear whether this pathogen is an important limiting factor for alfalfa production in the state. It has been clearly established that *A. euteiches* is a pathogen of alfalfa seedlings (1,3,8,19,20,22). In Kentucky, we have implicated *A. euteiches* in a few outbreaks of serious loss of alfalfa seedlings in the field, although we have no data suggesting it is a widespread problem at stand establishment. *A. euteiches* also has been associated with necrotic tertiary roots of mature alfalfa plants (11). In Kentucky, we typically find relatively few healthy tertiary roots on alfalfa plants growing in slowly drained soils, where yields are often low. We have isolated a number of fungi from necrotic tertiary roots in these sites, including *A. euteiches* (P. Vincelli and W. C. Nesmith, *unpublished*). Hancock has shown that infection of tertiary roots by *Pythium* spp. can reduce the growth of alfalfa under controlled conditions (6,7). The same may be true for *A. euteiches,* but studies have not yet been conducted to test this hypothesis. In any case, the widespread occurrence of alfalfa-infecting strains of *A. euteiches* in Kentucky indicates the need for research to determine if the use of *Aphanomyces*-resistant cultivars will result in enhanced performance of alfalfa.

P. medicaginis was detected in 9.9% of 121 fields sampled, with a 95% confidence interval of 6–17%. Prior to this study, many agriculturalists believed *P. medicaginis* to be ubiquitous in Kentucky alfalfa fields. We suspected that *P. medicaginis* was less frequent in Kentucky soils than commonly believed. This was based on diagnostic work, replicated demonstration trials with *Phytophthora*-resistant varieties, and the observation that alfalfa varieties susceptible to Phytophthora root rot have been successfully grown on many farms in Kentucky. Results of the current study suggest that *P. medicaginis* is present in less than 20% of alfalfa fields in Kentucky.

Baiting techniques were used for detection of these pathogens in the present study. Previous studies have shown that these pathogens can be reliably detected in soil using such techniques (3,9,12,13,15, 17,21). Also, use of alfalfa seedlings as a bait assured that isolates of *A. euteiches* detected in this study were alfalfa-infecting strains of this pathogen (5).

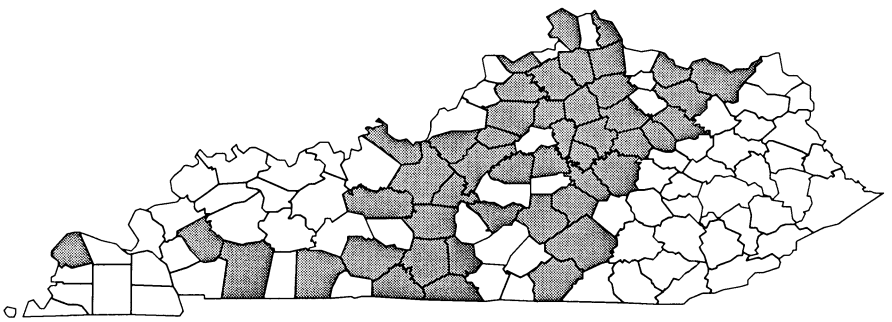

Fig. 1. Counties in Kentucky where alfalfa-infecting strains of *Aphanomyces euteiches* were detected as of 1992.

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