Detection of *Aphanomyces euteiches* in Field Soil from Northern Idaho by a Wet-Sieving/Baiting Technique

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ABSTRACT

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Aphanomyces euteiches was detected at three locations in northern Idaho by a wet-sieving technique. Five-day-old pea seedlings were used to bait the fungus from the sieved organic fraction from 10 soil samples per field. Inoculated seedlings in rolled germination paper were incubated and observed at weekly intervals for 3 wk. Almost all fields sampled in the Grangeville area were severely infested, and fields in the Kendrick area and two out of six fields in the Genesee area were heavily infested with *A. euteiches*. This wet-sieving technique gave results similar to the greenhouse-pot technique currently used to determine Aphanomyces root rot potential and required no greenhouse space or special watering requirements. This technique revealed that infective oospores of *A. euteiches* were as deep as 60 cm in the soil profile and were present in areas with poor drainage in fields with low inoculum levels overall. A hierarchical statistical analysis was used to separate differences in inoculum levels within areas and between fields.

Common root rot of peas (*Pisum sativum* L.), caused by *Aphanomyces euteiches* Drechs., is a very destructive disease first described in the United States in 1925 (6,12). Since that time, the disease has been reported in England, Europe, Scandinavia, Australia, New Zealand, and Japan, and wherever peas are grown in short rotations (5,6,12). There are currently no control practices that are economically feasible other than avoidance of heavily infested fields (4,6,12,16,20). The use of dinitrophenol and dinitroaniline herbicides and cruciferous soil amendments has given some control (5,17), however, these controls are not sufficient when inoculum levels are high. No commercial varieties are available that have resistance sufficient enough to avoid economic loss in severely infested fields (6). Recently, advanced breeding lines that approach commercial type and are resistant or tolerant to *A. euteiches* have been released (7,8,10). The two factors that complicate the development of commercially acceptable pea cultivars with resistance to *A. euteiches* are the presence of strains or races of the pathogen that vary in pathogenicity and virulence (1,21), and the fact that *A. euteiches* often interacts with other root disease pathogens, which confuses field disease screening trials (12).

Previous research has demonstrated that the severity of common root rot is directly related to population densities of the pathogen and the macro- and microenvironment (4,14,15). *Aphanomyces euteiches* was reported to be predominant in the plowed layer of soil and favored by heavy clay soils with increased soil moisture retention compared to light sandy soils (12,14). The exception is the occurrence of the disease in sandy soils under sprinkler irrigation (J. M. Kraft, *personal observation*).

No quantitative techniques have been developed to directly determine the inoculum density of *A. euteiches* under field conditions. As a result, a greenhouse technique was developed that utilizes soil samples from test fields. Good correlations are found between the severity of root rot on peas planted in soil samples from test fields and the severity of root rot that develops in those fields (6,16,20).

Mitchell et al (11) determined that pea roots develop typical Aphanomyces root rot symptoms when inoculated with organic debris from infested fields. They concluded that wet-sieving the organic debris and inoculating pea seedlings with

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this debris could be used to determine the relative infective inoculum level of a given soil. However, no one has reported use of the wet-sieving technique to determine inoculum levels in field soils.

In 1985, *A. euteiches* was reported to be present at several locations in northern Idaho (2), and it has continued to be a serious problem in that area. However, no studies have been conducted to determine the distribution of *A. euteiches* in northern Idaho. This study was conducted to determine if the procedure of Mitchell et al could be used to detect *A. euteiches* and to describe its distribution in northern Idaho.

MATERIALS AND METHODS

A field survey was initiated in September 1988 to determine the extent and severity of Aphanomyces root rot in northern Idaho. Six fields near Grangeville and Genesee and five fields near Kendrick were assayed with the help of local agricultural-chemical fieldmen. Fields with both good and poor pea production records were sampled.

A Giddings hydraulic coring machine (Giddings Machine Co., Fort Collins, CO) attached to a two-wheel trailer was used to sample each field. Ten sites per field were sampled. Each field was sampled in a circular pattern, with two samples collected from the approximate center. In each field, samples were taken at sites representative of that field's topography. (For example, if 20% of a field was in a low area, then two out of 10 samples were collected from a low area.) Each sample consisted of bulking three 7.6 cm × 30 cm cores taken from a 1 m^2 area. All samples were placed in plastic-lined air sickness bags over ice and transported directly to Prosser, Washington. Samples were stored at 4 C until assayed.

A 100-g subsample from each soil sample was screened through a 10-mesh screen (2-mm opening) and homogenized in a blender with 500 ml of sterile, glass distilled water for 3 min. The mineral and organic fractions were then collected together on an 200-mesh screen (75-μm opening) and washed under cold running tap water. The organic fraction was separated by resuspending this fraction in water and allowing the heavier particles to settle for 10–15 sec. The suspended organic fraction was then recollected on a 200-mesh screen and excess water was removed by vacuum. The moist organic debris was placed in a vial and stored at 3 C until assayed.

Five-day-old pea seedlings (cv. Bolero) were used to assay the organic debris for the presence of *A. euteiches*. Seeds were surface-disinfested (9) and germinated on sterilized germination paper in an incubator set at 21 C with a 16-hr day and 142$\mu E \cdot m^{-2} \cdot s^{-1}$ of illumination. Each soil sample was assayed by inoculating three replications of five seedlings each with approximately 10 mm^3 of debris on each seedling root. Inoculated seedlings were sufficiently isolated to prevent cross-contamination. Seedlings were observed at 1, 2, and 3 wk. At each harvest date, seedlings were counted and those that had Aphanomyces root rot were removed. When more than one pathogen was involved, infected tissue was placed on a semi-selective medium (13) to isolate *A. euteiches*. Data were recorded as the percent of seedlings that developed Aphanomyces root rot.

Because of the inability to collect random samples from each field and to randomly sample fields or geographical sites and because three measurements were made on each sample, a hierarchical, statistical analysis was conducted (3,18) followed with a mean comparison with a Duncan's multiple range test. A hierarchical analysis is an analysis of variance of stratified data. In this instance, geographic area was the first strata, fields the second, and sites within fields the third. The data was analyzed with the MSTAT (Integrated Microcomputer Program, Crop & Soil Sciences Department, Michigan State University, East Lansing) program.

RESULTS

Aphanomyces euteiches was detected from all 17 fields sampled. Differences in levels of *A. euteiches* between fields and geographic areas were evident when the procedure of Mitchell et al (11) was

Table 1. ANOVA table for hierarchical analysis of bioassay data from three geographic sites in central Idaho for the presence of *Aphanomyces euteiches* after a 1-wk incubation period

Source of variation	df	SS	MS	*F*	*P* (%)
Between geographic areas	2	8	4.13	7.27	0.006
Between fields within geographic areas	14	8	0.57	7.87	0.001
Error	153	11	0.07		

Table 2. Comparison of areas for inoculum levels of *Aphanomyces euteiches*

Geographic area	Sites (no.)	No. of seedlings with root rot[y] Incubation time 1 wk	2 wk	3 wk
Grangeville	6	6.5 a[z]	10.5 a	12.4 a
Kendrick	5	1.4 b	7.9 a	14.7 a
Genesee	5	0.9 b	2.5 b	6.8 b

[y] Total seedlings = 15.

[z] Data followed by same letter are not significant at $P = 0.05$ level. Data from each location were analyzed on a weekly basis and were converted to an arcsine transformation of percentage of seedlings infected with *A. euteiches* for statistical analysis. Each figure given is the average of 15 seedlings tested per field sample from a total of 750 or 900 seedlings examined per location.

Table 3. *Aphanomyces euteiches* inoculum potential for different fields in three areas estimated by the wet-sieving technique

Area	Field no.	No. of seedlings with root rot[y] Incubation time 1 wk	2 wk	3 wk
Grangeville	1	1.4 e[z]	2.9 d	3.7 c
	2	13.7 a	15.0 a	15.0 a
	3	3.1 de	7.4 c	12.1 ab
	4	6.4 bc	13.6 ab	14.9 a
	5	8.6 b	13.9 ab	15.0 a
	6	5.9 cd	9.9 bc	13.4 ab
Kendrick	1	1.0 a	10.1 a	15.0 a
	2	0.8 a	8.0 ab	14.9 a
	3	0.9 a	6.7 ab	14.8 a
	4	1.7 a	5.1 b	14.5 a
	5	2.6 a	9.4 ab	14.3 a
Genesee	1	1.9 a	4.7 a	11.0 a
	2	2.0 a	5.0 a	12.7 a
	3	0.4 a	2.1 a	6.3 b
	4	0.2 a	1.3 a	2.8 bc
	5	0.3 a	1.5 a	5.9 bc
	6	0.3 a	0.6 a	2.2 c

[y] Total seedlings = 15.

[z] Data followed by same letter within an area and time are not significant at $P = 0.05$ level. Data were converted to arcsine transformation of percentage of seedlings infected with *A. euteiches* for statistical analysis. Each figure is the average of 15 baited seedlings exhibiting symptoms for each incubation time with 150 seedlings tested per field.

used. Table 1 is an ANOVA (analysis of variance) table for a hierarchical statistical analysis. The data illustrate highly significant differences among the areas sampled and between fields within areas sampled for levels of *A. euteiches*.

Differences between areas when data from all fields per site were pooled are illustrated in Table 2. After 1 wk, Grangeville appeared to have the hi est inoculum levels of *A. euteiches* as determined by numbers of seedlings infected. At the end of the second and third week of incubation, the Kendrick and Grangeville areas were similar in levels of infective inoculum of *A. euteiches*. The Grangeville and Kendrick areas had higher levels of infective *A. euteiches* inoculum than the Genesee area.

The relative inoculum level for each field sampled in all three areas is given in Table 3. It is apparent that field 2 in the Grangeville area was the most severely infested with *A. euteiches* because all seedlings baited with organic debris from this field were infected in 1 wk. This is in contrast to field 1, where the numbers of infected plants were low throughout the 3-wk incubation period. In the Kendrick area, all fields sampled were heavily infested with *A. euteiches* and were not statistically different from each other.

A. euteiches was detected in samples as deep as 60 cm in the profile when soil cores were collected at that depth. In addition, in fields in the Genesee area with low levels of *A. euteiches*, the pathogen was most often found in samples collected from low areas with a corresponding higher soil moisture level. For example, in field 3 from the Genesee area, *A. euteiches* was detected at a significant level only in the sample from a low area that was poorly drained.

Combined samples of field soil collected from fields 1, 2, and 3–6 in the Genesee area were assayed with the procedure of Reiling et al (16) and Sherwood and Hagedorn (20) to determine the common root rot potential of these fields. The combined samples from fields 1 and 2 had a disease index of 86.1, which is not considered safe to plant peas, while fields 3–6 had a disease index of 26.9, which is considered safe (20).

DISCUSSION

The method of Mitchell et al (11) effectively determined the relative inoculum level of *A. euteiches* in soil from several fields in northern Idaho. Statistical differences between geographic areas and between fields were evident when the data were subjected to a hierarchical analysis. It is significant that the Grangeville area was considered by the cooperating fieldmen to be the most severely infested, Kendrick moderately infested, and the Genesee area slightly infested. The data presented here agree with those observations. The results presented in Table 3, which illustrate a significant difference in inoculum levels in fields 1 and 2 as compared to fields 3–6 in the Genesee area, agree with the results from the grow-out procedure. The procedure described here for determining inoculum levels of *A. euteiches* takes considerably less space than the greenhouse-grow-out test (16,20). The simplicity of the procedure and the use of an available statistical analysis program makes this technique a workable one.

The survival structure of *A. euteiches* is the thick-walled oospore (11,12,19), which is embedded in infected cortical tissue and can be recovered on a 200-mesh screen (11). There is a positive correlation between oospore numbers and root disease severity (4,11,14). Consequently, the use of a procedure that separates out the organic fraction to assay for numbers of infective oospores of *A. euteiches* is realistic. Symptoms caused by *A. euteiches* are distinct from those caused by *Pythium* spp., *Thielaviopsis*, *Rhizoctonia*, or *Fusarium* (6). The latter organisms were occasionally recovered from the organic debris as evidenced by symptoms on pea seedlings. *Aphanomyces euteiches* was readily isolated from infected seedling tissue (13) to reconfirm its presence in infected tissue.

Chan and Close (4) determined that a curvilinear relationship exists between a disease severity index for root rot, caused by *A. euteiches*, and the number of oospores in soil. They determined that this relationship exists by counting numbers of oospores in organic debris, which is tedious and time consuming. They also found that the most probable number method for estimating inoculum density was less satisfactory than the greenhouse method of Reiling or Sherwood and Hagedorn (16,20). Neither Chan and Close (4) nor Pfender et al (15) assessed the feasibility of using the procedure of Mitchell et al (11) for determining inoculum levels of *A. euteiches* in field soil.

The procedure of Mitchell et al (11) allowed us to assay several sites in northern Idaho for the presence of *A. euteiches*. This procedure was sensitive enough to discern differences in inoculum levels between geographic areas, between fields within a geographic area, and even between sites within a field. This procedure also allowed us to determine that *A. euteiches* is present as deep as 60 cm in the soil profile.

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