Disease Progress and Yield Loss in Aphanomyces Root Rot of Peas

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ABSTRACT

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The epidemiology of Aphanomyces root rot of pea was studied in field plots on sandy soil. We found that the pathogen, *Aphanomyces euteiches* f. sp. *pisi*, can spread from an infected plant to a limited number of neighboring plants in the pea stand. In small field plots with soil infested at various inoculum levels, the disease incidence (percentage of plants infected) increased rapidly early in the season at high inoculum levels; at lower inoculum levels, the increase in disease incidence was similarly rapid, but occurred later in the season. Yield varied inversely with logarithm of soil inoculum level. The inoculum level of *A. euteiches* f. sp. *pisi* in soil decreased about 50% per year in the absence of a pea crop.

In Wisconsin, and in several other major pea-growing regions of the USA, common root rot caused in peas by *Aphanomyces euteiches* f. sp. *pisi* is a principal limiting factor in production. The quantitative epidemiology of this disease has received little attention. No studies have been published concerning quantification of disease progress of pea root rot, and a quantitative relationship between inoculum level of *Aphanomyces* and disease severity or yield loss has not been established. There has been one report in which soil inoculum level was measured quantitatively (11); in that study, however, estimates of disease severity in the field were not made. In another study (15), yield and root rot severity were compared, but no estimates of soil inoculum levels were made.

Quantification of disease progress for some other diseases incited by soilborne pathogens has been reported; for example, quantitative studies of the increase in disease incidence over a season have been published for onion white rot (6), tobacco black shank (4,9), wheat root rot (20), and Verticillium wilt of cotton (14). In these studies, disease incidence data were based on symptoms or signs of disease. The degree of disease development necessary for inclusion of a plant in the "infected" category ranged from the presence of lesions on roots (eg, 20) to a whole-plant response representing an advanced state of the disease (eg, 4). In still other studies, the change in disease *severity* with time, either in addition to disease *incidence* data (20) or alone (3,17), has been measured.

One other aspect of the epidemiology of root diseases for which there is little experimental evidence concerns the nature of the disease cycle in the field, ie, whether all infections occur from the reservoir of soilborne propagules, or whether inoculum produced on one infected plant can infect other plants within the same season. Scott (16) and Crowe et al (5) obtained direct evidence for plant-to-plant spread of the pathogen in white rot of onion. Huang and Hoes (7) found similar plant-to-plant spread of *Sclerotinia* in sunflower stands. Thus, for both of these diseases, a single infection from a soilborne propagule can potentially become the focus for the infection of several plants due to spread of the pathogen.

There has been much discussion of the relationship between inoculum density and final incidence of diseases caused by soilborne pathogens (1), but little has been published concerning the effect of inoculum levels on characteristics of the development of these diseases during the season. Notable exceptions are the work by Crowe et al (6), who documented the change in onion white rot incidence over time at several initial soil inoculum levels, and the work of Pullman and DeVay (14), who found a direct dependence of the rate of disease progress on inoculum levels, up to a saturation inoculum level, for cotton wilt.

The objectives of our research were to determine: the ability of *Aphanomyces* to spread from plant to plant, the longevity of inoculum of *Aphanomyces* in the soil, and the influence of inoculum level on disease progress and yield loss.

MATERIALS AND METHODS

All experiments were conducted in a region of intensive, irrigated vegetable production located on extremely sandy soil (Plainfield sand) in central Wisconsin, where pea root rot is particularly severe.

Plant-to-plant spread. Captan-treated seeds of *Pisum sativum* L. 'Perfection 8221' (Canners Seed Corporation, Lewisville, ID) were planted in a noninfested field in rows 17 cm apart at a rate of 30 seeds per meter of row. On the same day, peas were planted in the greenhouse in pasteurized soil reinfested with oospores of two isolates of *A. euteiches* f. sp. *pisi*, and watered heavily. After 1 wk, a 2.5-cm-diameter core of soil centered on a planted pea seed was removed from each of several widely spaced sites in the field planting. One of the greenhouse-grown plants, its roots having been washed free of all adhering soil, was transplanted into each hole, and noninfested soil was used to firm the plant in the hole. During the season, shoot symptoms of root rot developed on the transplanted peas. Forty-seven days after the planting date, plants within 35 cm of each of the transplanted peas were dug, in both the same and adjacent rows, and their locations relative to the transplanted pea plants were recorded. Control plants were dug from other areas in the planting at least 1 m from the introduced plants. Isolations were made from roots to determine whether they were infected with *Aphanomyces*. All but four of the oldest lateral roots were removed from each taproot, and this remaining root system was washed with a soft brush under running water to remove all adhering soil. The washed roots were immersed in 0.5% NaOCl for 30 sec, followed by 30 sec in 2% $Na_2S_2O_3$ and two rinses of sterile distilled water. They were then cut into segments (~2 cm long) before being plated on 2% Bacto water agar. Isolates of *Aphanomyces* growing from the root pieces were identified by growth habit and hyphal characteristics. Tissue from roots yielding no *Aphanomyces* on plates was examined microscopically for the presence of oospores resembling those of *Aphanomyces*.

Effect of inoculum level on disease progress. In 1979, 1980, and 1981, inoculum levels of *Aphanomyces* in small field plots were adjusted by spreading various amounts of soil from a naturally

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infested field over the plot surface and rototilling. Inoculum level of *A. euteiches* f. sp. *pisi* in each plot was determined on the day after infestation by taking soil samples (a composite of at least ten 2.5-cm-diameter cores per 7 m^2 plot), and testing them with a host bioassay according to the most-probable-number method (13). Captan-treated peas (cultivar Perfection 8221) were planted in late April or early May, at 30 seeds per meter of row, in rows 17 cm apart. Overhead irrigation was used throughout the season. At intervals of 7–10 days during the season, plants selected according to a predetermined randomized plan were dug from the plots for examination. Disease symptom severity on root, epicotyl, and shoot was rated by using 0–4 scales (12). Isolations were also made from the roots, as described above, to determine the proportion of plants infected by *Aphanomyces*. In 1979, there were four inoculum levels (0.006, 0.09, 0.5, and 3.6 infective propagules per gram [ippg]), and three 1.7 × 1.7-m replicate plots per inoculum level. A nearby block of peas planted on noninfested soil served as the control treatment. Isolations were made from 10 plants per replicate plot per sampling time. In 1980 and 1981, there were two inoculum levels (0.2 and ~0.02 ippg), plots were 1.5 × 9 m, and there were three and one replicate plots per inoculum level, respectively. In 1980, isolations were made from 10 plants per replicate plot, and, in 1981, from 25 plants per plot, at each sampling time.

Effect of cropping sequence on inoculum level. Inoculum level of *Aphanomyces* was assessed before and after field plots were planted with peas, oats, or left fallow. Soil samples were taken, as previously described, from the plots before planting and after harvest, and tested for inoculum level of *Aphanomyces* according to the host bioassay most-probable-number method (13). Data for pea crops was taken from the plots used for disease progress studies. In addition, some plots infested with *A. euteiches* either were left fallow (without weed control) or were planted to oats during the growing season. In these cases, there were three replicate 2 × 6-cm plots per treatment.

RESULTS

In plots where plants infected with *Aphanomyces* were transplanted into rows of young healthy peas in noninfested soil, the pathogen was recovered from roots of adjacent plants at the end of the season. In all cases, *Aphanomyces* spread from the initially infected plant to several other plants (from the second to the fifth nearest neighbors) in each direction within the row, and to plants in the next adjoining rows. No *Aphanomyces* was recovered from the roots of plants more than five plants (18 cm) away from the transplants, or at locations within the plot that were isolated from introduced plants.

Disease progress studies were made on peas grown in field plots of soil infested at varying inoculum levels in 1979, 1980, and 1981. In all plots, infection by *Aphanomyces* (as determined by plating root tissue on agar medium) was found to have occurred 7–14 days before visible root symptoms appeared. Disease symptoms progressed from roots to epicotyls to shoots, as exemplified in the 1979 epidemic (Table 1). Epicotyl symptoms were typical of infection by *Aphanomyces*; root symptoms were typical of combined infection by *Pythium* and *Aphanomyces* (12). Symptoms of Fusarium root rot or wilt did not develop.

The increase of Aphanomyces root rot incidence (percentage of plants infected) over time in the inoculum level plots is summarized in Fig. 1. Linear regression of disease incidence on time was performed in order to compare the curves to one another with respect to slope and intercept. For regression analysis, several transformations of disease incidence (10) were examined for their ability to linearize the data. The transformations used were: the logistic; the Gompertz; and one form of the Richards, $\ln[y^2/(1-y^2)]$. We did not use the Weibull distribution function,

TABLE 1. Influence of initial inoculum level of *Aphanomyces euteiches* f. sp. *pisi* on occurrence of infection and symptom development of pea root rot in field plots[a] in 1979

Inoculum level[b]	Time after planting when infection was first detected (days)	Days between planting and development of symptom severity rating >1.0[c]		
		Root	Epicotyl	Shoot
0.006	24	47	60	...
0.09	24	41	45	46
0.5	24	34	39	43
3.6	12	26	28	38

[a] All values are averages of three replicate plots per treatment.
[b] Inoculum level determined by a most-probable-number method (13), and expressed in number of infective propagules per gram.
[c] Symptom severity ratings for each plant part range from 0 (healthy) to 4 (maximum severity). Time values determined by interpolation from curves of symptom severity rating over time.

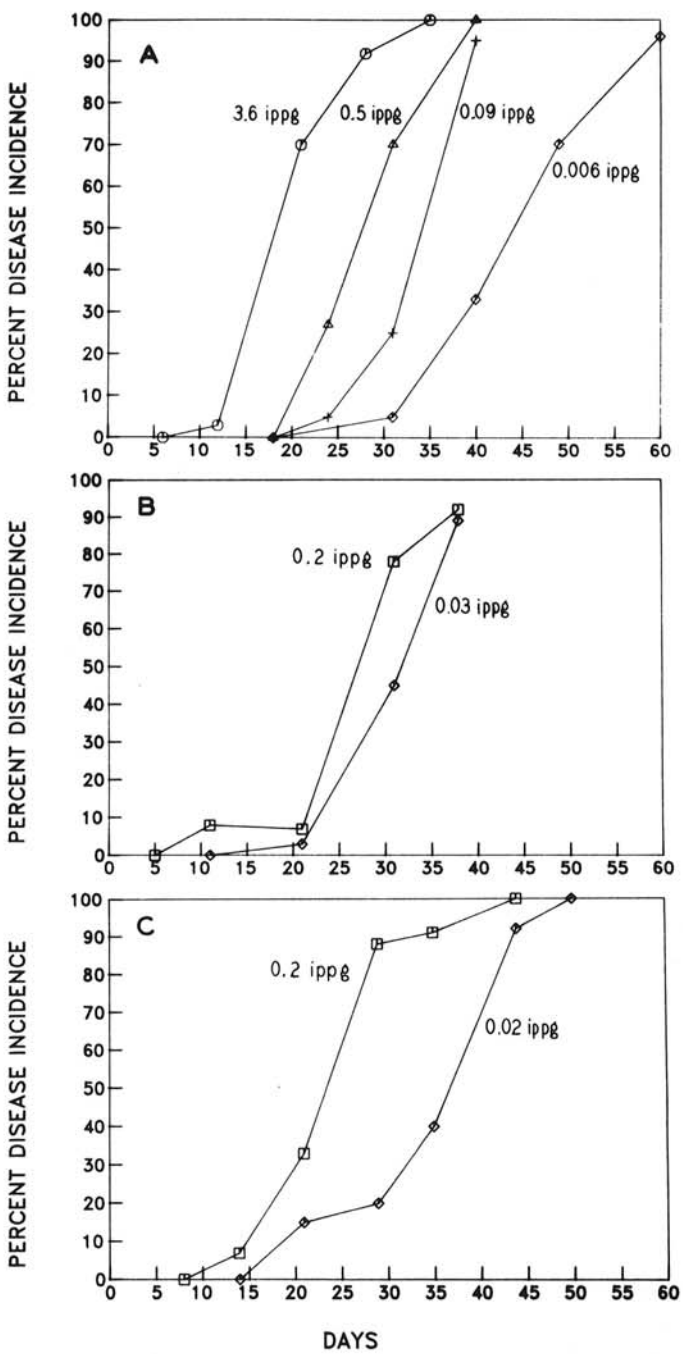

Fig. 1. Disease progress curves for pea root rot at various preplanting inoculum levels of *Aphanomyces euteiches* f. sp. *pisi* **A**, in 1979; **B**, in 1980; and **C**, in 1981. The percentage of plants infected was determined by plating roots on agar. Inoculum levels, expressed as infective propagules per gram (ippg), determined by a host bioassay most-probable-number method (13).

since three degrees of freedom are lost in the analysis, leaving few degrees of freedom for error in some of our data sets. A value of 0.01 was substituted for disease incidence = 0, and values of 100% disease incidence were omitted. Transformed values of disease incidence were also regressed against soil heat units (computed as soil temperature degree days based on 4 C) instead of time units, since the rate at which soil warmed during the spring differed in each of the 3 yr during the study, and we thought the soil temperature could influence rate of infection.

Based on coefficients of determination and examination of residuals plotted against expected values, the logistic transformation gave a somewhat better linear fit for most of the curves than did the other transformations. Use of soil heat units in place of days as the independent variable did not improve the R^2 values significantly. The R^2 and slope values for the regression of $\ln[y/(1-y)]$ on time are shown in Table 2. The R^2 values are significant ($P < 0.05$) for all curves. A comparison of slope (average rate of disease increase over the season) with the natural logarithm of inoculum level for experiments in all 3 yr shows a correlation ($r = 0.79$, significant at $P < 0.05$) of these two variables (Fig. 2). But this correlation is largely due to the data from 1979, since in 1980 and 1981 the inoculum levels did not have a significant effect on slope. A clearer relationship was found when inoculum level was compared to the time required to reach 50% disease incidence ($\ln[y/(1-y)] = 0$) in the pea stand, shown in Table 2. In each of the years 1979 and 1981, there were statistically significant delays in the occurrence of 50% disease incidence for each decreasing inoculum level tested. Correlation of days to 50% disease incidence versus natural logarithm of soil inoculum level for the combined 3 years' data showed this relationship to be statistically significant ($r = -0.89$, 6 df).

The largest range of inoculum levels of *Aphanomyces* was examined in 1979. In Table 3, disease severity and yield for the 1979 experiment are shown as a response to initial inoculum level of *Aphanomyces*. Severity of disease symptoms at harvest increased with increasing levels of *Aphanomyces*. Yield generally showed an inverse relationship with inoculum level. In comparison with the noninfested plot, pea yield was decreased by 42, 72, and 86% when the preplant inoculum level of *Aphanomyces* was 0.006, 0.5, and 3.6 infective propagules per gram (ippg), respectively. With the data from the noninfested plot omitted from the analysis, there were highly significant correlations between pea yield and area under the disease severity curve ($r = -0.97$); pea yield and days to 50% disease incidence ($r = 0.96$); and pea yield and logarithm of soil inoculum level ($r = -0.93$), with 10 degrees of freedom in each case.

Inoculum level of *Aphanomyces euteiches* f. sp. *pisi* in soil increased greatly in plots where peas were grown (Table 4). Whether the initial inoculum level was low (0.003 ippg) or high (3.6 ippg), the level was very high (13–34 ippg) by the end of the growing season. In one plot examined again the following spring, the inoculum level was found to have dropped about 45% during the winter. A similar inoculum loss (from 0.2 to 0.1 ippg) was observed from spring 1980 to spring 1981 where no peas were grown in the soil in the intervening 1980 season. Where peas were grown, however, the inoculum level increased from 0.2 ippg to 5.3 ippg the following spring.

TABLE 2. Linear regression of proportion plants infected (transformed to $\ln[y/(1-y)]$) versus time for Aphanomyces root rot of peas at several inoculum levels[a]

Inoculum level[b]	Year	R^2	Slope ± standard deviation	X-axis intercept of 50% disease incidence[c]
3.6	1979	0.87	0.40 ± 0.05	20
0.5	1979	0.90	0.41 ± 0.05	28
0.2	1980	0.60	0.25 ± 0.06	30
0.2	1981	0.97	0.26 ± 0.02	24
0.09	1979	0.89	0.34 ± 0.04	33
0.03	1980	0.79	0.27 ± 0.04	31
0.02	1981	0.89	0.21 ± 0.04	34
0.006	1979	0.80	0.18 ± 0.02	47

[a] Number of sampling dates, replicate plots per inoculum level, and plants per replicate varied for different years and inoculum levels. See Materials and Methods.
[b] Inoculum level determined by a most probable number method (13), and expressed as infective propagules per gram.
[c] Time in days at which $\ln[y/(1-y)] = 0$.

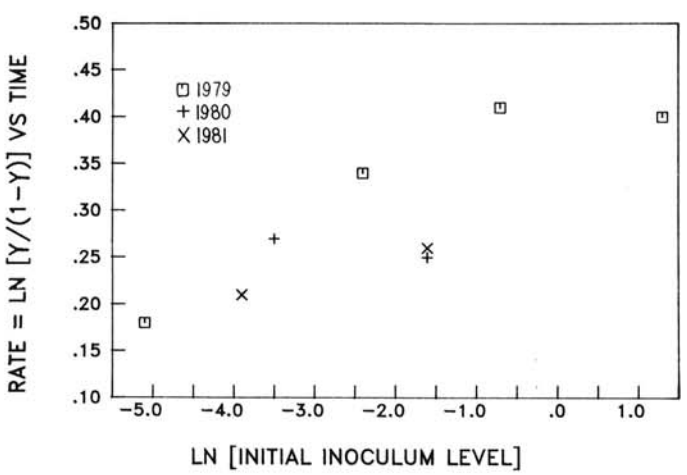

Fig. 2. Relationship of initial inoculum level of *Aphanomyces euteiches* f. sp. *pisi* to rate of disease increase. Rate was calculated as the average slope of the transformed disease incidence curve [ln $(y/(1-y))$ versus time].

TABLE 3. Influence of initial inoculum level of *Aphanomyces euteiches* f. sp. *pisi* on final disease severity and yield in 1979 field plots[a]

Inoculum level[b]	Final disease severity rating[c]	Total yield from 40 plants	
		Pods (no.)	Pea weight (g)
<0.006[d]	...	...	166 w
0.006	5.1 x[e]	72 x	96 x
0.09	7.3 y	52 y	83 x
0.5	8.6 y	40 z	47 y
3.6	10.0 z	...	23 z

[a] All values are averages of three replicate plots per treatment.
[b] Inoculum level determined by a most probable number method (13), and expressed as infective propagules per gram.
[c] Disease severity rating ranges from 0 (healthy) to 12 (maximum severity), and is the sum of ratings (0–4) for root, epicotyl, and shoot.
[d] Inoculum level in check plot was below the level of detection.
[e] Values within a column followed by the same letter do not differ, $P = 0.05$, according to Duncan's new multiple range test.

TABLE 4. Change in inoculum level of *Aphanomyces euteiches* f. sp. *pisi* in the presence or absence of a pea crop

Year	Summer crop	Soil inoculum level of *Aphanomyces*[a]		
		Preplant	Harvest	Following spring
1979	Peas	0.003	13	...
	Peas	0.09	34	...
	Peas	0.4	24	...
	Peas	0.7	19	...
	Peas	3.6	16	9.1
1980	Peas	0.2	...	5.3
	Oats[b]	0.2	...	0.1
	Fallow[b]	0.2	...	0.1

[a] Inoculum level determined by means of a most-probable-number method (13), and expressed as infective propagules per gram.
[b] Averages for three replicate plots. All other values in the table are from single plots.

DISCUSSION

We found that *Aphanomyces* can spread from an infected plant to roots of neighboring healthy plants. It is not known whether this pathogen spread occurs by mycelial growth between roots in contact with one another or by short distance zoospore movement between roots. In any case, we found the spread to be limited to a distance of five plants (~18 cm) from the initially infected plant, which is consistent with our observation (*unpublished*) that lateral roots of one plant grow far enough to contact the lateral roots of the fourth or fifth plant away from it in the row. It is not clear whether the disease spread occurs as a serial transfer through adjoining plants or whether each of the five neighboring plants becomes infected by root contact with the initially infected transplant (ie, we do not know whether there is only one secondary infection cycle, or multiple cycles, in the span of one season). In any case, Aphanomyces root rot does not appear to be a monocyclic ("simple interest" [19]) disease in which each infection must originate from the reservoir of soilborne inoculum. Rather, there is an increase in amount and spatial distribution of inoculum when an initial infection leads to the colonization of a root system by *Aphanomyces*, and this increase permits pathogen spread to a neighboring plant. The rate of this spread, however, is severely constrained by an apparent requirement for the contact (or at least proximity) of the plant's roots. The epidemiological importance of this constrained spread of the pathogen would depend on the density and spatial distribution of the initial inoculum in the field. At relatively uniform, high inoculum densities, many plants will contact inoculum early in the season as roots grow through the soil, resulting in 100% disease incidence early in the season. Here, the fact that secondary spread can occur is of no importance. At lower inoculum densities (eg, such that only one in 15 plants will encounter soilborne inoculum), the low number of primary infections could be increased 10- to 15-fold due to secondary spread. If the spatial distribution of the inoculum were approximately uniform, disease incidence could thus increase from less than 10% to almost 100% by the end of the season, through the occurrence of small contiguous disease foci. Finally, if only a very few disease foci are initiated in a field (whether due to a small amount of inoculum evenly distributed, or to clumped inoculum of either low or high concentration), the limited ability of the pathogen to spread would result in only a few small pockets of diseased plants, and thus a low disease incidence, by the season's end.

The major limiting process in this type of epidemic is probably root growth, as discussed by Huisman (8). Since, as he points out, in root diseases the root is much more mobile than is the inoculum, primary infections will occur at a rate determined largely by the rate at which roots grow through the volume of infested soil. In the case of pea root rot, root growth will similarly affect the rate at which secondary infections can occur. Thus, we may be able to understand pea root rot incidence curves at differing inoculum levels by considering the host-mediated rate of root-pathogen encounter together with the dynamics of plant-to-plant pathogen spread following primary infections. By understanding such aspects of infection and disease spread and the influence of environmental factors on them, we can develop models to describe and predict epidemics caused by soilborne pathogens, as has been done by Bloomberg (2) for root rot of Douglas fir seedlings.

We found that the rate of disease increase, averaged over the entire season, was dependent on initial inoculum level. In two other studies of epidemics caused by soilborne pathogens, a clear relationship was found between initial inoculum level and rate of disease increase. The rate of epidemic development in Verticillium wilt of cotton increased with increasing levels of inoculum, up to a saturation inoculum level of 40 propagules per gram (14). In onion white rot epidemics (6), rate of disease increase likewise was dependent on initial soil inoculum level. Although we also found a dependence of rate on inoculum level, the rates were quite similar at all inoculum levels after disease incidence reached about 5%. The lower rates calculated for the low inoculum level plots are due primarily to longer duration of low incidence values at the beginning of the epidemic, which have the effect of reducing the rate averaged over the whole season. Although the rates of disease increase are similar after the rapid increase begins, inoculum level clearly does have an effect on the initiation of rapid disease increase. The delaying effect of low inoculum levels on disease progress is manifested in the longer time required to reach 50% disease incidence.

We found a logarithmic relationship between initial inoculum level and yield of peas. At low inoculum levels, small increments of inoculum resulted in relatively large changes in yield; at higher inoculum levels, increasing increments of inoculum had less effect on yield. Although initial inoculum level had no effect on final disease incidence (all plots reached 100%), initial inoculum level did determine the length of time required to reach a given disease incidence (eg, time to 50% disease incidence was correlated with initial inoculum level). Time of 50% disease incidence was in turn strongly correlated with yield loss. Reiling et al (15) found that yield in commercial fields was correlated with symptom severity at the time of full blossom. We (12) also found that yield was correlated with symptom severity at blossom, but this correlation was weaker than the correlation of yield with the time to 50% disease incidence.

Inoculum level decreased about 50% in 1 yr in the absence of peas. If the half-life of inoculum of *Aphanomyces* in this sandy soil is considered thus to be 1 yr, 9 yr without peas would be needed to decrease inoculum level from 3.6 infective propagules per gram (which caused severe shoot symptoms and a yield loss of about 85%) to 0.006 infective propagules per gram (which caused mild shoot symptoms and about 40% yield loss). This agrees roughly with the earlier finding (18) that fields with a high root rot potential remained hazardous to pea production for at least 6–8 yr after the last pea crop.

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