Epidemiology of Pythium Damping-off and Aphanomyces Root Rot of Peas After Seed Treatment with Bacterial Agents for Biological Control

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ABSTRACT

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Field plots were established in 1989 and 1990 to investigate the efficacy of seed treatments with *Pseudomonas cepacia* strain AMMD, *P. fluorescens* strain PRA25, *Corynebacterium* sp. strain 5A, and captan on the epidemiology of root rot of pea caused by *Aphanomyces euteiches* and preemergence damping-off caused by *Pythium* spp. Disease incidence was assessed every 2–3 days, and soil-water matric potential and soil temperature were recorded. Strains AMMD and PRA25 significantly ($P < 0.05$) suppressed initial disease 23–70% and the area under the disease progress curve 15–45% over both years compared to the nontreated control. Strain AMMD also suppressed the final incidence of disease at harvest by 11–19% over both years and PRA25 by 19% in 1989 compared to the nontreated control. Piecewise regression identified two distinct phases of the epidemics during each year, in which changes in the rate of symptom development were associated with periods of change in soil-water matric potential and increasing soil temperature. Disease incidence increased very little during the first phase of the epidemics and increased in rate significantly ($P < 0.05$) in the second phase of the epidemics during both years. There were no significant differences ($P > 0.05$) in rates among epidemics in either the first or second phases of the epidemics during both years. Multiple cycles in the average rate of increase of disease incidence were more apparent when expressed in terms of soil water status than chronological time. High negative cross-correlation coefficients at a temporal lag of 2–3 days were obtained for increases in the average rate of change in mortality expressed in terms of soil water status and the average rate of change in soil water status over time. The bacterial strains may be an environmentally sound and effective means of controlling Pythium preemergence damping-off and Aphanomyces root rot in peas.

Aphanomyces root rot, caused by the soilborne fungus *Aphanomyces euteiches* Drechs. (4,5), is the most serious disease of processing peas in the Great Lakes states and is a main factor limiting production. *A. euteiches* causes a cortical rot in the epicotyl and hypocotyl regions with subsequent root decay. Aboveground symptoms include a general wilting followed by yellowing and eventual death. Currently, there are no commercially available pea varieties with resistance to Aphanomyces root rot nor fungicides effective in suppressing disease.

Pathogenic *Pythium* spp. also are common in pea field soils and may contribute to root rot. Pythium seed rot and preemergence damping-off are controlled by treating seeds with the protective fungicide captan. However, there are potential restrictions on the continued use of captan in agriculture. Alternative seed treatments, particularly those that also reduce the severity of Aphanomyces root rot, are needed.

Treatment of pea seeds with certain bacteria resulted in significant increases in plant emergence and yield over several years in field plots infested with *A. euteiches* and *Pythium* spp., regardless of whether the seeds also were treated with the fungicide captan (11). Disease severity at 10% bloom also was lower than the control when the seeds were treated with the bacteria.

Results of experiments with biological control agents often are reported at fixed periods of time after planting or after the introduction of the organism(s) into the system. Although this methodology may indicate the efficacy of the biocontrol agent(s), little information is obtained on the progress of the disease or the biological processes involved (mechanisms of biological control). The dynamics of epidemics in which biological control agents are present should be considered. For example, a significant reduction in the rate of disease progress of Fusarium wilt of cucumber occurred with one nonpathogenic *Fusarium* sp. but not with another (7). This difference occurred even though both nonpathogenic strains significantly reduced germination of chlamydospores of the pathogen. Much can be learned by investigating epidemic development with biological control agents to gain insight into the interaction among pathogen, plant, biological agent, and environment. Observations may lead to new hypotheses that can be tested specifically with regard to the mechanism of control.

Our objective was to compare seed-applied bacterial biological control agents and the standard commercial seed treatment (captan) from an epidemiological perspective. In particular, rates of disease progress were calculated, compared among treatments, and related to environmental factors. The research reported here is but a portion of a larger experiment; results not relating to disease dynamics have been reported previously (10,11). Portions of this research also have been reported previously (1).

MATERIALS AND METHODS

Three bacterial species—*Pseudomonas cepacia* (strain AMMD, ATCC 52796), *P. fluorescens* (strain PRA25, ATCC 53794), and *Corynebacterium* sp. (strain 5A, ATCC 53934) (11)—were used to treat seeds of pea (*Pisum sativum* L. 'Perfection 8221') that had been treated commercially with captan (Captan 400-D, 38.2% a.i.) or that had not been treated. Each bacterial strain was grown in nutrient broth yeast extract (NBY) (15) shake culture at 20–22 C. After 48 h, 2.5 ml of the turbid suspension was plated on NBY agar and incubated for 24 h at room temperature. The bacteria were scraped from one agar plate and mixed thoroughly with 25 pea seeds. The seeds were air-dried for 18–24 h in a laminar-flow hood and stored for less than 24 h before being planted in the field plots. For each group of seeds treated with the same bacterial strain, three seeds were assayed for bacterial populations before the remainder was sown. Each seed was placed in 10 ml of sterile deionized water, sonicated for 20 s (Bronsonic ultrasonic cleaner model B-220, Bronson Cleaning Equipment Co., Sheldon, CT), and dilution plated onto NBY agar in petri dishes (11). Dishes were incubated at room temperature for 36–48 h, and colonies were counted. Population densities ranged from 10^7 to 10^8 colony-forming units per seed.

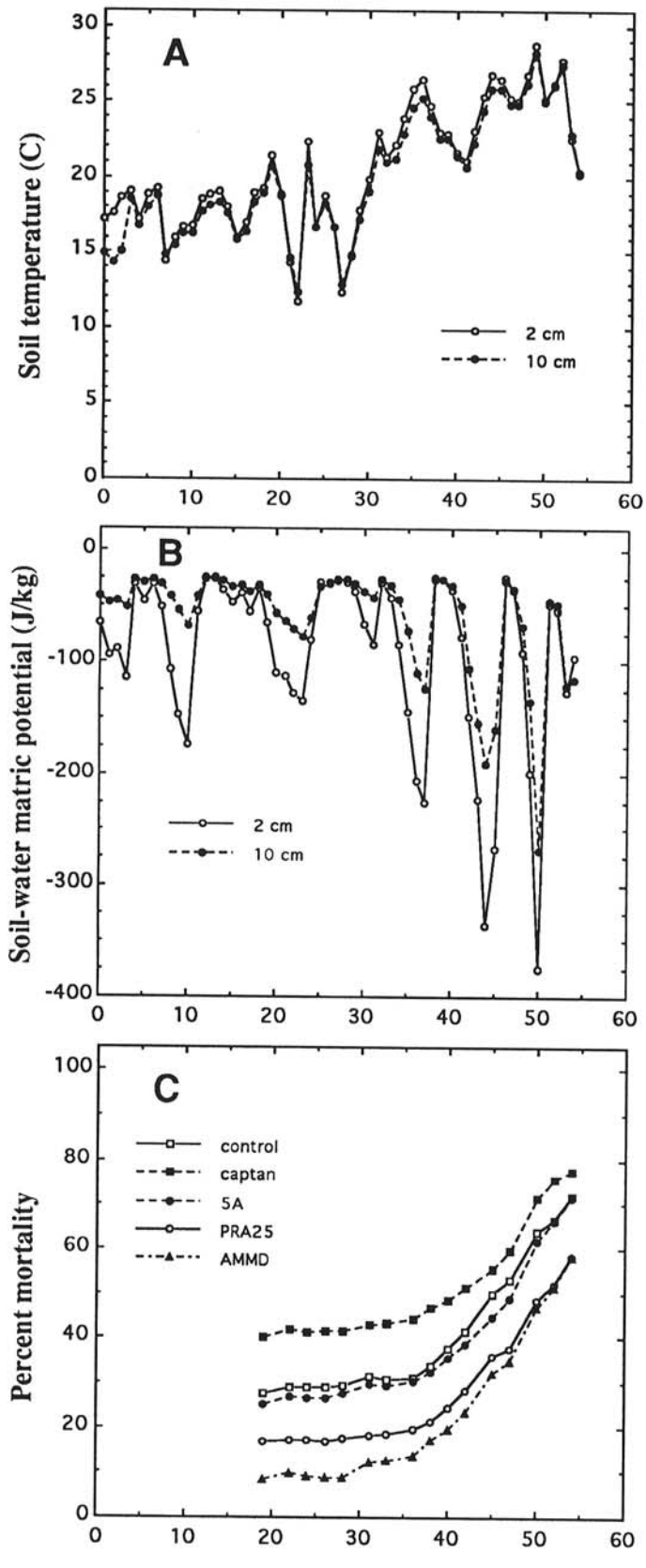

Fig. 1. A, Soil temperature; **B,** soil-water matric potential; and **C,** mortality of pea plants caused by *Aphanomyces euteiches* and *Pythium* spp. over time during 1989 in Arlington, WI. Each point in **A** and **B** represents the daily average of two readings at each depth, and each point in **C** represents the average of 20 replications on each date.

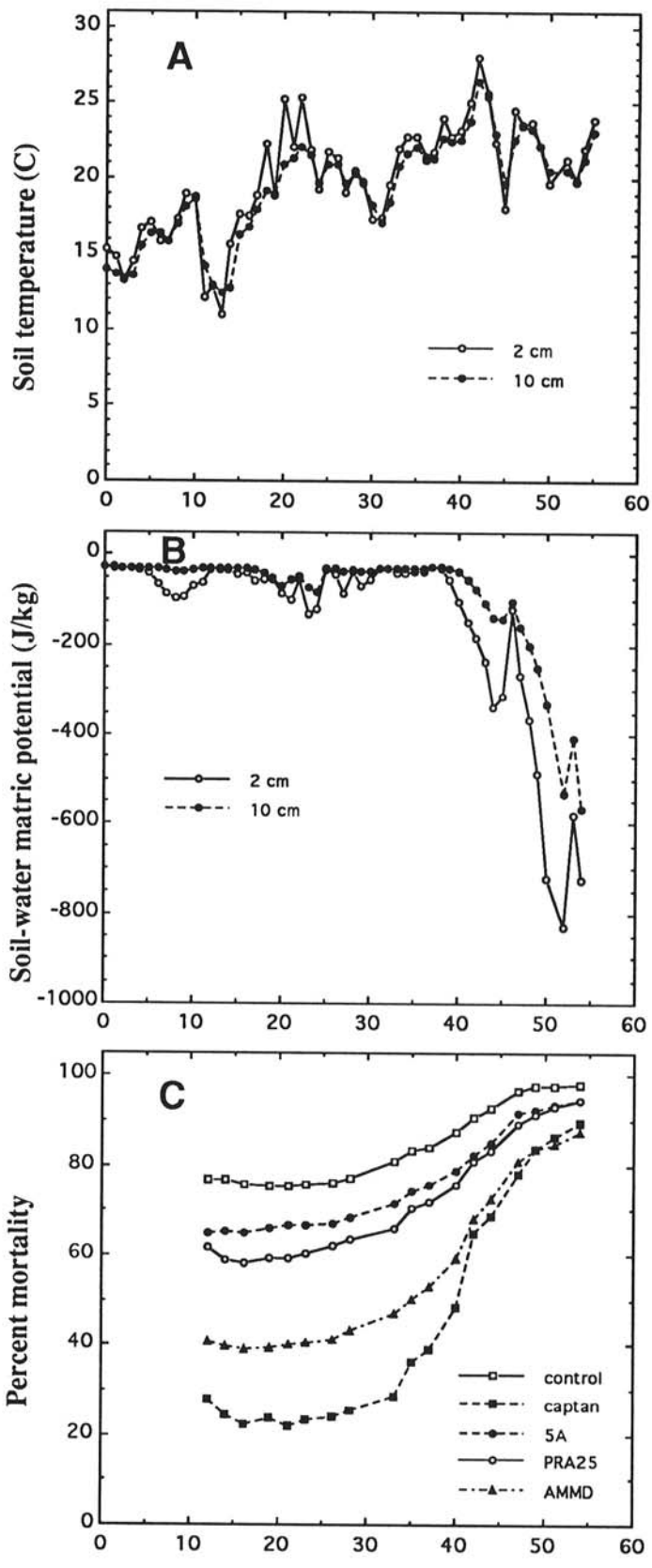

Fig. 2. A, Soil temperature; **B,** soil-water matric potential; and **C,** mortality of pea plants caused by *Aphanomyces euteiches* and *Pythium* spp. over time during 1990 in Arlington, WI. Each point in **A** and **B** represents the daily average of two readings at each depth, and each point in **C** represents the average of 20 replications on each date.

The experiment was conducted during 1989 and 1990 in the Aphanomyces root rot nursery at the Arlington Agricultural Experiment Station near Arlington, WI (Columbia County). Soil at the site is a Plano silt loam (Typic Argiudoll, fine silty mixed mesic) and is naturally infested with *A. euteiches* and *Pythium* spp. The inoculum density of *A. euteiches* at the time of planting was 7.3 and 8.1 infective propagules per gram of soil during 1989 and 1990, respectively, and the inoculum density of *Pythium* spp. was 429 and 535 propagules per gram of soil during 1989 and 1990, respectively (11). Factorial seed treatments included two levels of fungicide (with and without captan) and four bacterial treatments (none, *P. cepacia* AMMD, *P. fluorescens* PRA25, and *Corynebacterium* sp. 5A) arranged in a randomized complete block design. The fungicide and bacterial effects were considered as fixed effects in the analysis. There were 20 blocks each year, with one replicate of each treatment per block. Each replicate consisted of two adjacent 1.3-m rows planted with 25 seeds each. One row was destructively sampled for disease severity ratings at 10% bloom (approximately 6 wk after planting), and the other row was used for disease assessments throughout the growing season and for yield determinations at harvest (10,11). The plots were planted on 19 and 23 May 1989 and 1990, respectively. Emergence was determined 19 days after planting in 1989 and 12 days after planting in 1990. Healthy plant stand (i.e., no observable symptoms) was assessed every 2–3 days until harvest, and percent mortality was calculated based on 25 seeds planted per row. Plants were harvested on 12 and 18 July 1989 and 1990, respectively.

Initial disease, final disease at harvest, and area under the disease progress curve (AUDPC) were analyzed with a two-way analysis of variance. Data for initial and final disease were transformed by the arcsine-square root transformation (14) before analysis, and AUDPC was calculated by the method of trapezoidal integration (13). A series of nonorthogonal, single-degree-of-freedom contrasts were examined to compare disease parameters from specific treatments (nontreated seed [control], seed treated with captan only, and seed treated with each bacterial strain) to answer questions regarding the efficacy of the bacterial biological control agents (8). Significance was determined by the construction of 95% confidence intervals (6,8).

Disease progress data were transformed logistically as $\ln(y/[1-y])$, in which y is the proportion of dead plants (12). Based on initial observations of the disease progress curves for the different treatments, piecewise regression (8) was performed on logistically transformed data to determine if various segments of the disease progress curves could be analyzed separately, i.e., if statistically significant segments of each epidemic existed based on the slope parameter. Comparison of slope parameters in each phase of the various epidemics was accomplished by first difference regression to account for serial correlation (6) and single-degree-of-freedom contrasts to compare estimates of the slope parameter. Differences in logits were regressed on differences in time, and significance was determined by constructing 95% confidence intervals for the estimates.

Soil-water matric potential (ψ_m) and soil temperature (C) were monitored at 2- and 10-cm depths in the field plots with gypsum blocks and thermistors, respectively (two probes per variable per depth), and precipitation (both rainfall and irrigation) was monitored with a tipping-bucket rain gauge, all connected to a CR21X datalogger (Campbell Scientific, Inc., Logan, UT). The datalogger recorded hourly averages of soil moisture and temperature, and the average value for each day was calculated, whereas the amount of precipitation was totalled for each day. The daily average of the two probes at each depth was averaged for analysis. The relationship of the increase in the proportion of disease incidence (y), expressed in terms of the average rate of change in disease with respect to chronological time t ($\Delta y/\Delta t$), soil water status ($\Delta y/\Delta\psi_m$), and soil temperature ($\Delta y/\Delta C$), was examined with respect to the average rate of change in soil water status ($\Delta\psi_m/\Delta t$) and soil temperature ($\Delta C/\Delta t$) between successive disease-evaluation dates by calculating the cross-correlation coefficients (2,3). A soil water-soil temperature interaction term (ψ_m*C) also was included in the analyses.

RESULTS

First experiment (1989). Soil temperature, soil-water matric potential, and plant mortality caused by *A. euteiches* plotted over time for 1989 and 1990 are presented in Figures 1 and 2, respectively. During 1989, the initial level of disease was influenced

TABLE 1. Selected single-degree-of-freedom contrasts for the effect of various seed treatments on several epidemiological parameters for infection of pea by *Aphanomyces euteiches* and *Pythium* spp. at Arlington, WI, in 1989 and 1990

Contrast and seed treatment[a]	Initial disease[b]		Final disease[c]		AUDPC[d]	
	1989	1990	1989	1990	1989	1990
Control vs. Captan	*[e]	*	ns	*	*	*
Control vs. 5A	ns	*	ns	ns	ns	ns
Control vs. PRA25	*	*	*	ns	*	*
Control vs. AMMD	*	*	*	*	*	*
Captan vs. 5A	*	*	ns	ns	*	*
Captan vs. PRA25	*	*	*	ns	*	*
Captan vs. AMMD	*	*	*	ns	*	*
5A vs. PRA25	ns	ns	ns	ns	*	ns
5A vs. AMMD	*	*	ns	ns	*	*
PRA25 vs. AMMD	*	*	ns	ns	ns	*
Control[f]	27.4	75.8	71.8	98.0	14.02	35.27
Captan[f]	40.2	22.4	77.6	89.6	17.75	18.52
5A[f]	25.0	64.8	71.4	94.4	13.26	31.80
PRA25[f]	16.6	58.4	58.4	94.6	9.48	30.11
AMMD[f]	8.2	39.0	58.2	87.6	7.66	23.27
Error mean square[g]	0.017	0.019	0.026	0.023	12.40	15.52

[a] Control = nontreated pea seed (cv. Perfection 8221); Captan = the fungicide captan; 5A = *Corynebacterium* sp. strain 5A; PRA25 = *Pseudomonas fluorescens* strain PRA25; AMMD = *P. cepacia* strain AMMD.

[b] Disease incidence at emergence 19 and 12 days after planting in 1989 and 1990, respectively. Values are percent mortality and represent the average of 20 replications; analyses were performed after arcsine-square root transformation of the corresponding proportion data.

[c] Disease incidence at harvest 54 days after planting in 1989 and 1990. Values are percent mortality and represent the average of 20 replications; analyses were performed after arcsine-square root transformation of the corresponding proportion data.

[d] Area under the disease progress curve. Values are proportion-days and represent the average of 20 replications.

[e] * = significant differences at $P = 0.05$, ns = not significant at $P = 0.05$; significance was determined by the construction of 95% confidence intervals (6).

[f] Values are means.

[g] Error mean square from the two-way analysis of variance with the full complement of factorial treatments and 133 degrees-of-freedom (10,11).

by the various seed treatments. Initial disease was significantly less ($P < 0.05$) among plants from seeds treated with any bacterial strain than with the standard captan seed treatment (Fig. 1C; Table 1). Treatment with PRA25 or AMMD, but not 5A, resulted in significantly less initial disease than was found in the nontreated control. Furthermore, treatment with AMMD resulted in significantly less initial disease incidence than did treatment with PRA25. The high level of disease associated with the captan treatment was attributed to phytotoxicity during periods of hot, dry weather (10).

Piecewise regression identified two distinct phases of the epidemics, with the change in the rate of disease increasing, on average, 36–38 days after planting for plants from all seed treatments in 1989 (Fig. 3A). Disease incidence increased very little in the first phase of the epidemic regardless of seed treatment. The estimated rate of the increase of disease incidence early in the epidemics during 1989 was significantly different from zero ($P < 0.05$) only when 5A or PRA25 was applied to seeds (Table 2). There were no significant differences ($P > 0.05$) in the rate of disease increase among all treatments in the first phase of the epidemics based on single-degree-of-freedom contrasts.

Disease incidence increased in plants regardless of seed treatment beginning 36–38 days after planting in 1989 and was associated with an increase in the daily average temperature from 18 to 25 C (Fig. 1A) and a decrease in ψ_m from an average of −77 to −250 J/kg (Fig. 1B) 30–35 days after planting. The rate of disease incidence increased significantly in the second phase of the epidemic as compared to the first phase for plants from all seed treatments (Fig. 3A; Table 2); however, there were no significant differences ($P > 0.05$) in the rate of disease increase among plants from all seed treatments in the second phase of the epidemic.

Plants from seeds treated with PRA25 or AMMD, but not 5A, had a significantly lower level of final disease at harvest and less AUDPC than did plants from the nontreated control but were not significantly different from each other by either measure (Table 1).

Second experiment (1990). During 1990, soil temperatures were slightly cooler (Fig. 2A) and the soil was consistently wetter (Fig. 2B) than during 1989. These conditions favored Pythium seed rot and preemergence damping-off. As in 1989, each bacterial seed treatment resulted in significantly less initial disease ($P < 0.05$) than in the nontreated control, but the captan treatment was effective in suppressing initial disease under these conditions and was significantly better than the bacterial treatments in limiting initial disease incidence (Fig. 2C; Table 1). Also as in 1989, the AMMD treatment limited initial disease incidence more than did the other two bacterial treatments.

Piecewise regression once again identified two distinct phases of the epidemics, with the change in the rate of disease increasing, on average, 30–33 days after planting (Fig. 3B). As in 1989, disease increased very little over the first phase of the epidemic. The rate of disease increase was not significantly different from zero ($P > 0.05$) for plants from any seed treatment (Table 2), and there were no significant differences in the rate of disease increase regardless of seed treatment.

Disease increased in plants from all seed treatments beginning 28–33 days after planting and corresponded with an increase in soil temperatures and a decrease in ψ_m 22–26 days after planting. The rate of disease incidence once again increased in the second phase of the epidemic for plants in all treatments (Fig. 3B; Table 2), and as in 1989, there were no significant differences ($P > 0.05$) in the rate of disease increase.

Final incidences of disease at harvest in 1990 were generally higher than in 1989, which may be explained by higher levels of initial disease. The rate of disease increase during the second phase of the epidemics during 1989 and 1990 were similar (Table 2). Plants from seeds treated with AMMD, but not 5A or PRA25, had significantly less disease incidence ($P < 0.05$) at harvest than did plants in the nontreated control (Table 1). Disease incidence in plants from seeds treated with captan also was significantly different from the control but was not significantly different in plants from seeds treated with AMMD. Although captan suppressed initial disease, the final incidence of disease after the captan treatment was not significantly different from that after the bacterial treatments. Plants from seeds treated with PRA25 or AMMD, but not 5A, had significantly less AUDPC ($P < 0.05$) than plants from the nontreated control, and plants from seeds treated with AMMD had significantly less AUDPC than plants from seeds treated with PRA25 (Table 1). Plants from seeds treated with captan also had significantly less AUDPC than plants from the bacterial seed treatments, but this may be associated with the lower level of initial disease in the first phase of the epidemic.

Analysis of the parameters. The nearly parallel disease progress curves and the apparent effect of soil-water matric potential and soil temperature on the increase in the rate of disease progress prompted an investigation of the correlation between these environmental factors and the rate of change of disease progress for plants in each treatment. For brevity, only data from 1989 will be presented. Similar results were obtained with the 1990 data, except the correlations were not as large as those from

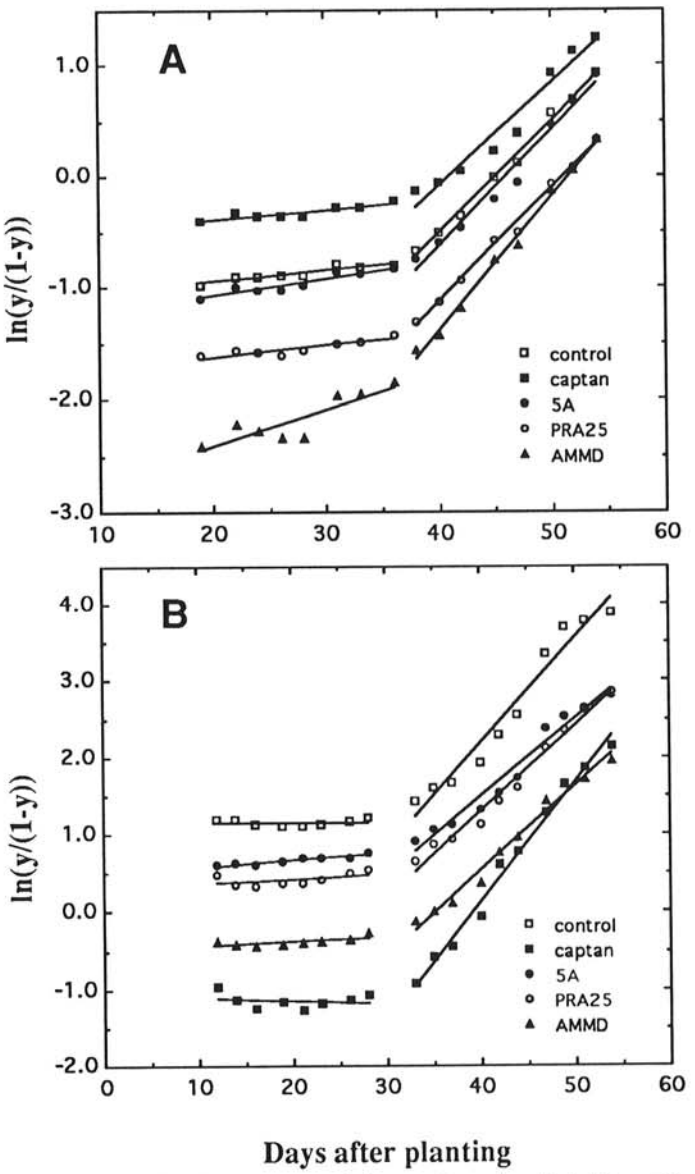

Fig. 3. Relationship between the logistic transformation ($\ln(y/[1 - y])$) of the proportion of mortality of pea plants (y) and time after planting for selected seed treatments for the control of the pea root rot complex caused by *Aphanomyces euteiches* and *Pythium* spp. in **A**, 1989 and **B**, 1990 in Arlington, WI. Breaks in the plots separate different phases of the epidemics as identified by piecewise regression.

the 1989 data due to the low rate of change in soil moisture during 1990.

Temporal patterns for the average rate of change in disease incidence expressed in terms of chronological time ($\Delta y/\Delta t$), soil water status ($\Delta y/\Delta \psi_m$), and soil temperature ($\Delta y/\Delta C$) and the average rate of change in soil water status ($\Delta \psi_m/\Delta t$) and soil temperature ($\Delta C/\Delta t$) over time are presented in Figures 4 and 5. Multiple cycles in the increase in plant mortality were observed in association with plants from all seed treatments and were more apparent when expressed in terms of soil water status (Fig. 4C) than in chronological time (Fig. 4A) or soil temperature (Fig. 5C). Similar patterns were observed for plants from all seed treatments throughout the 1989 and 1990 growing seasons regardless of how the data were expressed, thus, the nearly parallel disease progress curves in Figures 1C and 2C. The constant rate values estimated for the two phases of the epidemics were found to represent average rates over time.

High negative cross-correlation coefficients at a temporal lag of 2–3 days were obtained between the average rate of increase in plant mortality with respect to soil water status ($\Delta y/\Delta \psi_m$) and the average rate of change in soil water status with respect to time ($\Delta \psi_m/\Delta t$) for plants from each seed treatment (Table 3). Lower negative cross-correlation coefficients were obtained between the average rate of increase in plant mortality with respect to chronological time ($\Delta y/\Delta t$) and the average rate of change in soil water status with respect to chronological time ($\Delta \psi_m/\Delta t$) than in the relationships based on soil water status. Relationships based on chronological time were generally variable and not consistent with respect to temporal lag in both years (data not shown), indicating that expressing the data solely in terms of chronological time may not adequately describe the various epidemics. High positive cross-correlation coefficients were obtained between the average rate of increase in plant mortality with respect to soil temperature ($\Delta y/\Delta C$) and chronological time ($\Delta y/\Delta t$) and the average rate of change in soil temperature with respect to chronological time ($\Delta C/\Delta t$). The inclusion of the interaction term (ψ_m*C) in the analysis did not increase the correlation between variables significantly over those of ψ_m and soil temperature individually (Table 3). Furthermore, expressing the data in terms of soil heat units (soil-temperature degree days based on 4 C) (12) did not significantly increase the cross-correlation coefficients. Thus, regardless of seed treatment, the change in the rate of disease progress may be described as a function of soil water status and soil temperature more so than of chronological time.

DISCUSSION

Analysis of the data indicated that the various disease progress curves essentially were parallel in 1989 and 1990. Disease progressed in plants at similar rates regardless of seed treatment and phase of the epidemic. Disease progressed very little, if at all, in plants from all seed treatments in both years during the first part of the season, and then the rate of symptom development increased significantly in the second part of the season. However, at no time were there significant differences among plants from different seed treatments with regard to the rate parameter.

The epidemics could be described, in large part, by the amount of initial disease at emergence, and the level of disease incidence was related to the ability of the various seed treatments to control seed rot and preemergence damping-off. Seed treatment with *P. cepacia* strain AMMD and *P. fluorescens* strain PRA25 significantly suppressed initial disease incidence on the average of 59.3 and 31.2%, respectively, over both years compared to the nontreated control. Seed treatment with *Corynebacterium* sp. strain 5A significantly suppressed initial disease only during 1990 by 15% compared to the nontreated control. Moreover, all three bacterial seed treatments significantly suppressed initial disease compared to the standard captan treatment in 1989 when the soil was drier with higher temperatures but not in 1990 when the soil was wet and cool. In other studies, AMMD applied to pea seeds suppressed preemergence damping-off caused by *Pythium ultimum* and *Pythium sylvaticum* by 47% and suppressed the incidence of seed infection 44–60% in growth-chamber tests (9). Thus, strains AMMD and PRA25 may be viable alternative seed treatments to control preemergence damping-off.

Biocontrol of seed rot and preemergence damping-off by the bacterial seed treatments does not appear to be the sole mode of action in this system. The rate of Aphanomyces root rot might be expected to be greater in plants from seeds treated with the bacteria than in plants from nontreated seeds because a larger number of surviving plants would be available for infection by *A. euteiches* in plants from the bacterial seed treatments. The final level of disease incidence then would be approximately the same for plants from all seed treatments. *A. euteiches* can spread from an infected plant to neighboring plants, increasing the probability of a plant becoming infected and resulting in an increase in the rate of disease progress (12). This, however, was not the case. Statistically significant rate parameters were not apparent in the first or second phase of the epidemic in either year. Furthermore, disease-severity data at 10% bloom indicated that Aphano-

TABLE 2. Estimated rate parameters, their standard deviations, and probability values for epidemics after selected seed treatments for control of the pea root rot complex caused by *Aphanomyces euteiches* and *Pythium* spp. at Arlington, WI, in 1989 and 1990

Treatment[a]	$\hat{r}_1$[b]	$s(\hat{r}_1)$[c]	P_1[d]	$\hat{r}_2$[b]	$s(\hat{r}_2)$[c]	P_2[d]
1989						
Control	0.013 (0.010)[e]	0.0065	0.0998	0.105 (0.102)	0.0129	0.0002
Captan	0.012 (0.009)	0.0051	0.0525	0.091 (0.093)	0.0205	0.0044
5A	0.018 (0.015)	0.0073	0.0489	0.107 (0.106)	0.0163	0.0006
PRA25	0.013 (0.011)	0.0051	0.0473	0.108 (0.103)	0.0134	0.0002
AMMD	0.042 (0.033)	0.0211	0.0946	0.125 (0.015)	0.0146	0.0001
1990						
Control	0.001 (−0.000)	0.0053	0.9133	0.119 (0.136)	0.0292	0.0036
Captan	−0.003 (−0.004)	0.0180	0.8864	0.143 (0.154)	0.0241	0.0003
5A	0.010 (0.009)	0.0044	0.0589	0.095 (0.101)	0.0211	0.0021
PRA25	0.007 (0.007)	0.0105	0.5483	0.106 (0.111)	0.0157	0.0001
AMMD	0.006 (0.006)	0.0070	0.4066	0.100 (0.109)	0.0152	0.0002

[a] Control = nontreated pea seed (cv. Perfection 8221); Captan = the fungicide captan; 5A = *Corynebacterium* sp. strain 5A; PRA25 = *Pseudomonas fluorescens* strain PRA25; AMMD = *P. cepacia* strain AMMD.

[b] Estimated rate parameter for a particular phase of the epidemic. Subscript denotes the first or second phase of the epidemic as identified by piecewise regression (Fig. 3).

[c] Standard deviation of the estimated parameter from first difference regression of logistically transformed data.

[d] Probability that the predicted rate is not significantly differently from zero.

[e] Values are rate parameters from first difference regression (to correct for serial correlation) of logistically transformed data. Rate parameters in parentheses are from linear regression of uncorrected logistically transformed data.

myces root rot also was controlled by the bacterial seed treatments (10,11). Strains AMMD and PRA25 significantly suppressed the severity of root rot over both years compared to the nontreated control. Final disease incidence also was significantly suppressed 15% over both years by AMMD compared to the nontreated control and 19% by PRA25 in 1989. Biocontrol by the bacterial strains kept the level of disease incidence throughout the growing season lower than expected if *A. euteiches* was not controlled to any extent. The AUDPC, which takes into account the entire epidemic, also indicated control of Aphanomyces root rot in addition to control of Pythium preemergence damping-off.

The mode of action or the physiological and ecological processes

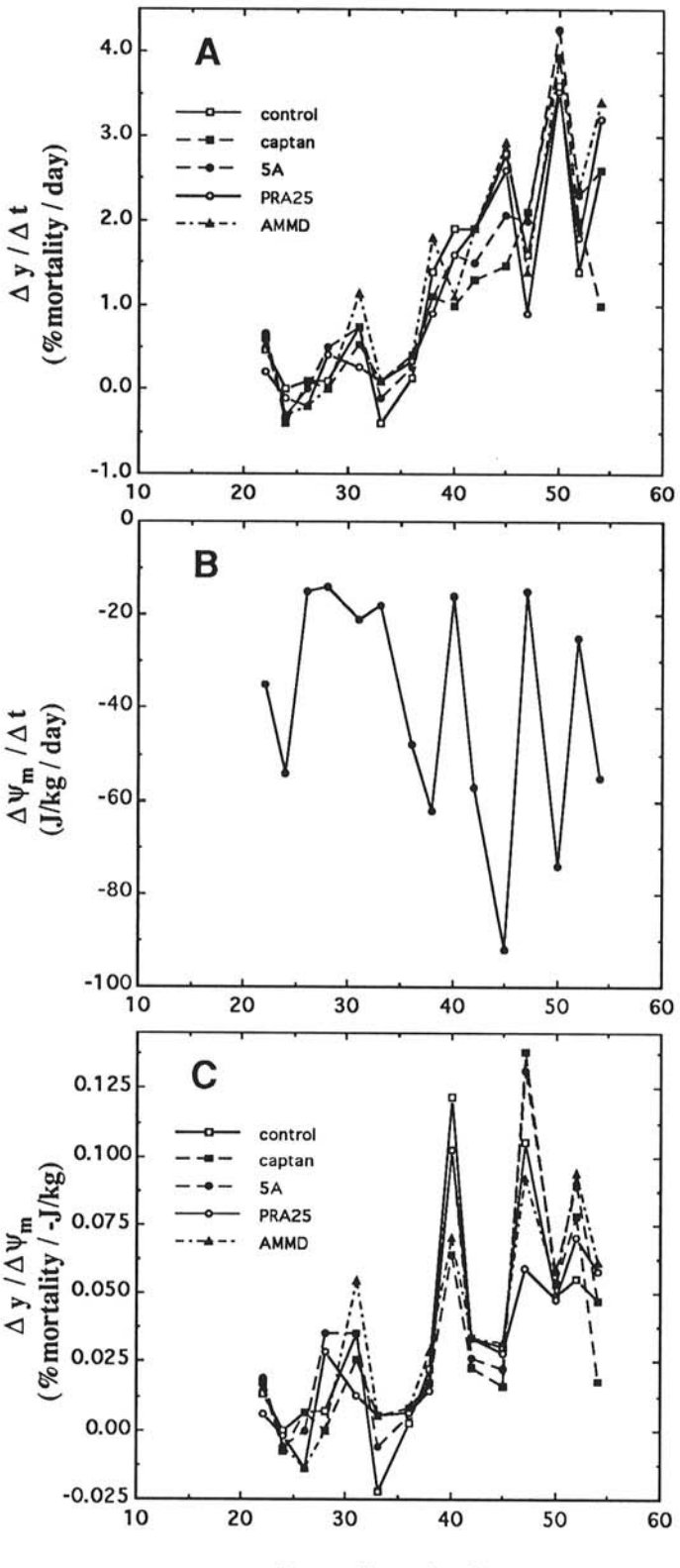

Fig. 4. Average mortality rates of pea caused by *Aphanomyces euteiches* and *Pythium* spp. with respect to **A,** chronological time ($\Delta y/\Delta t$), **C,** soil water status ($\Delta y/\Delta \psi_m$), and **B,** the rate of change in soil water status ($\Delta \psi_m/\Delta t$) calculated between successive disease-assessment dates during 1989 in Arlington, WI.

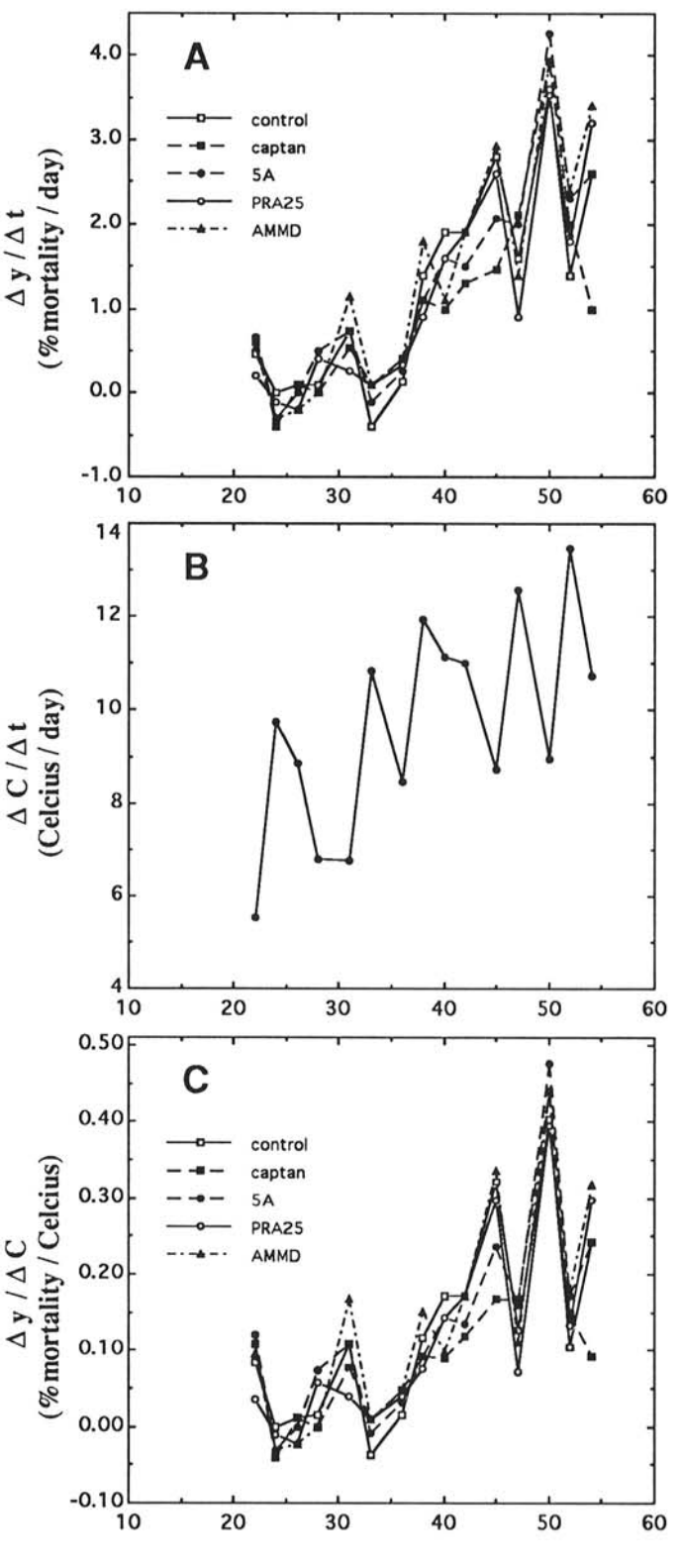

Fig. 5. Average mortality rates of pea caused by *Aphanomyces euteiches* and *Pythium* spp. with respect to **A,** chronological time ($\Delta y/\Delta t$), **C,** soil temperature ($\Delta y/\Delta C$), and **B,** the rate of change in soil temperature ($\Delta C/\Delta t$) calculated between successive disease-assessment dates during 1989 in Arlington, WI.

responsible for disease control in this system are unknown. Seed infections by *Pythium* spp. under conducive conditions take place within 12 h of planting (9) and may be the cause of seed rot or preemergence damping-off. However, *A. euteiches* was isolated from symptomless pea epicotyls 10 days after planting (11). Infection of pea plants by *A. euteiches* may have occurred very early in the epidemic and may have been partially responsible for preemergence damping-off or may have predisposed the plants to infection by *Pythium* spp. Similarly, infection by *Pythium* spp. may have predisposed the plant to infection by *A. euteiches*. Symptoms of Aphanomyces root rot did not appear, however, until plants were stressed by high temperatures and extreme changes in soil-water matric potential 2–3 days before a disease-assessment date. The distinction between latent and new infections and the relationship to symptom expression during an epidemic in this system has not been made. It is not known if changes in the physical soil environment are related to periods of infection with rapid symptom development, or if symptom development is a result of latent infections expressed as a result of plant stress. A combination of both probably is involved, but this is a crucial distinction regarding the effective management of the disease. The timing and site of biological activity cannot be determined without further, detailed experimentation.

The bacterial biocontrol agents are colonizers of the spermosphere and rhizosphere (9). Studies on root colonization in adjacent field plots with antibiotic-resistant strains indicated that AMMD and PRA25 persist in the rhizosphere and on the epicotyl in high populations throughout the growing season (J. H. Bowers and J. L. Parke, *unpublished data*; 10). Thus, the bacteria were present to interact physically or physiologically with *A. euteiches* whenever infection periods occurred. Furthermore, the presumed differences in rhizosphere communities imposed by the different seed treatments did not affect the response of symptom development to the environmental variables. The resolution of the ecology and etiology of the pathogen may be valid regardless of the differences in rhizosphere community structure.

TABLE 3. Cross-correlation coefficients calculated between the average rate of change in mortality of peas based on chronological time ($\Delta y/\Delta t$), soil water status ($\Delta y/\Delta\psi_m$), soil temperature ($\Delta y/\Delta C$), and the interaction of soil water and temperature ($\Delta y/\Delta\psi_m$*C) and the average rate of change in soil water status ($\Delta\psi_m/\Delta t$), soil temperature ($\Delta C/\Delta t$), and the interaction of soil water and temperature ($\Delta\psi_m$*C/Δt) between successive disease-assessment dates for selected bacterial seed treatments in 1989

Dependent variable	Seed treatment[a]	Cross-correlation coefficients with independent variables[b]		
		$\Delta\psi_m/\Delta t$	$\Delta C/\Delta t$	$\Delta\psi_m$*C/Δt
$\Delta y/\Delta t$	Control	−0.145[c]	0.710	−0.223
	Captan	−0.218	0.508	−0.322
	5A	−0.152	0.664	−0.261
	PRA25	−0.005	0.755	−0.100
	AMMD	−0.059	0.640	−0.165
$\Delta y/\Delta\psi_m$	Control	−0.584	...	...
	Captan	−0.687	...	...
	5A	−0.607	...	...
	PRA25	−0.403	...	...
	AMMD	−0.492	...	...
$\Delta y/\Delta C$	Control	...	0.686	...
	Captan	...	0.529	...
	5A	...	0.662	...
	PRA25	...	0.746	...
	AMMD	...	0.624	...
$\Delta y/\Delta\psi_m$*C	Control	...	...	−0.590
	Captan	...	...	−0.762
	5A	...	...	−0.608
	PRA25	...	...	−0.394
	AMMD	...	...	−0.536

[a] Control = nontreated pea seed (cv. Perfection 8221); Captan = the fungicide captan; 5A = *Corynebacterium* sp. strain 5A; PRA25 = *Pseudomonas fluorescens* strain PRA25; AMMD = *P. cepacia* strain AMMD.

[b] Data reported for a temporal lag of 2–3 days.

[c] Values greater than 0.514 and 0.641 in absolute value are significant at $P = 0.05$ and 0.01, respectively ($n = 15$).

Epidemics of Aphanomyces root rot reported previously (12) were quite different than those in our study in that a long lag phase was not observed, and disease incidence always reached 90–100% in each of 3 yr, even though inoculum densities were higher in our field plots than in the earlier study. Several factors account for this. In the previous study, disease incidence was reported as the percentage of plants infected by *A. euteiches*, determined by plating roots on agar medium (12). In our study, disease incidence was recorded as the percentage of plants with symptoms (percent mortality or the inverse of healthy stand). Field experiments in the previous study were conducted in an extremely sandy soil heavily and frequently irrigated (12). The sandy soil presumably drained rapidly and produced frequent and deep cycles in soil-water matric potential, which may be conducive to infection and/or symptom development. In contrast, the soil in our plots was a silt loam and not prone to rapid drying throughout the root zone. Infection periods may have lasted longer in our studies than in others but may not have been as frequent as in the previous study.

Changes in soil water status and soil temperature were strongly associated with the epidemiology of Aphanomyces root rot on pea, and plants from all seed treatments were similar in response to these variables. Disease increased slightly early in the epidemic during 1989 with frequent cycling of the soil water status. This appeared to stress the plants enough to show symptoms of infection by *A. euteiches*. Disease did not increase early in the epidemic during 1990 with less frequent and a suppressed magnitude of soil water cycles compared to the 1989 trial. The soil did not dry out, and plant growth was not stressed; thus, fewer symptoms developed during 1990 after initial disease. When temperatures increased in both years, with a decrease in soil-water matric potential (higher temperatures and less precipitation later in the season allowed the soil to dry), an increase in the rate of disease progress occurred. Warm temperatures and dry soil conditions may have enhanced symptom development.

Different temporal patterns for the increase in tobacco black shank caused by *Phytophthora parasitica* var. *nicotianae* were observed when mortality was expressed in terms of soil water status and chronological time (3). Multiple cycles in the increase of disease also were observed when the increase in mortality was expressed in terms of soil water status, similar to the data obtained in our study.

The use of a soil water status-soil temperature interaction term to describe the relationship between the average rate of change in disease incidence and environmental variables increased the cross-correlation coefficients only slightly. Changes in soil moisture and temperature may each have separate effects on the development of symptoms over time in that each may place different physiological stresses on the plant. Taken together as one term or variable, changes in the interaction of soil moisture and temperature may not describe the epidemic adequately. One variable may mask effects of the other variable. Disease progress should be expressed in terms of soil water status and soil temperature, but detailed research under controlled conditions may be needed to adequately describe the weight of each variable and its relationship to changes in the rate of disease progress.

Seed treatment with bacterial strains consistently suppressed disease incidence and AUDPC in this portion of the study and suppressed disease severity and increased emergence and yield in another portion of the study (10,11) compared to the nontreated control in 2 yr under different environmental conditions. In contrast, control of preemergence damping-off with a captan seed treatment was inconsistent and was dependent on soil moisture and soil temperature. Additionally, epidemics for the various seed treatments were similar in regard to the relationship between the average rate of change in disease incidence and environmental variables. We believe that these organisms represent a viable, environmentally sound, and effective means to control Pythium preemergence damping-off and Aphanomyces root rot of peas.

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