The Relationship of Infection by *Pythium* spp. to Root System Morphology of Alfalfa Seedlings in the Field

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ABSTRACT

Larkin, R. P., English, J. T., and Mihail, J. D. 1996. The relationship of infection by *Pythium* spp. to root system morphology of alfalfa seedlings in the field. Plant Dis. 80:281-285.

Alfalfa root system morphology was evaluated in relation to root infection by *Pythium* spp. in developing seedlings in the field. Metalaxyl treatments (soil drench, seed treatment, or nontreated) were used to produce differing levels of root infection in the field. Experiments were conducted at an upland site on a research farm in Missouri in two consecutive years. Quantitative assessments of root system morphology were made using morphometric and topological classification techniques. A metalaxyl soil drench treatment reduced root colonization by *Pythium* spp. per unit root length compared with either the metalaxyl-treated seed treatment or untreated control in 1991 through the first 4 weeks after emergence. Root colonization by *Pythium* spp. was low in 1992 and no differences among treatments could be detected. In the 1991 field test, the metalaxyl soil treatment resulted in increased root system growth and complexity (greater length, more branches, faster growth rates) compared with the seed and control treatments over the first 4 weeks of seedling development. However, no effect of root infection on root system branching structure could be detected in either year. These results suggested that, under favorable environmental conditions, sublethal root infections of alfalfa seedlings by *Pythium* spp. can cause reductions in root system size and complexity under natural field conditions.

Poor establishment and development of alfalfa stands, as well as stand decline, are common problems in forage alfalfa (*Medicago sativa* L.) production (4,22,27). These problems have often been associated with soilborne fungal pathogens such as *Pythium* and *Phytophthora* spp. (4,11,12, 18,22). Several species of *Pythium*, including *P. ultimum* Trow, *P. irregulare* Buisman, and *P. sylvaticum* W. A. Campbell & J. W. Hendrix, can cause severe seed rotting, and preemergence and postemergence damping-off of alfalfa seedlings (11,12, 20,21,25,27). These pathogens also cause sublethal infections of actively growing feeder roots and other succulent or juvenile tissue of plants of all ages (11,12,15, 20,21). Sublethal infections can reduce root and shoot growth (11,12,20,21) and may affect yield (12,13,15).

Root system morphology encompasses structural characteristics of a root system, such as size, shape, and architecture. These characteristics largely determine the efficacy of a root system in acquiring and transporting resources and in anchoring the plant in soil (6–9). Root system architecture, or branching structure, in particular, directly affects the exploration and exploitation of soil resources, nutrient transport efficiency, and the energy requirements of the root system (6–9). Alterations in root system morphological characteristics related to sublethal infections by *Pythium* spp. during seedling development ultimately may influence the ability of a plant to withstand various environmental stresses during plant establishment and beyond. Thus, the relationships between these pathogens and root growth may be important for the understanding and management of such root diseases (3).

In previous greenhouse studies, Larkin et al. (20,21) demonstrated that infection of alfalfa seedlings by species of *Pythium*, particularly *P. irregulare* and *P. ultimum*, altered root system growth and morphology. These modifications were detected by using the morphometric and topological assessment techniques developed by Fitter (5–9), which enabled the quantitative analysis of root system structure and branching patterns. In that previous work (21), infections by *Pythium* spp. reduced root system growth and altered branching patterns. The objective of the present study was to characterize the responses of root systems of alfalfa seedlings to infection by *Pythium* species under field conditions using these assessment approaches. Preliminary results have been published previously (19).

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Missouri Agricultural Experiment Station Journal Series No. 12302

Accepted for publication 28 November 1995.

Publication no. D-1996-0108-04R

MATERIALS AND METHODS

Establishment of field plots. Field plots were established at the University of Missouri Horticulture and Agroforestry Research Station in New Franklin in 1991 and 1992. This site is located in central Missouri on a deep loess soil (Marshall silt loam, pH 6.0 to 6.9, clay layer at 25 cm depth) near the Missouri River. The experimental site was previously fallow or cropped to fescue prior to planting alfalfa, and contained natural levels of various *Pythium* spp., including *P. ultimum*, *P. irregulare*, *P. sylvaticum*, and *P. torulosum* Coker & F. Patterson (20). The population densities of these species were shown to be sufficiently high to cause damping-off and to reduce root growth of alfalfa in previous greenhouse experiments (20,21). Identification, distribution, and changes in species composition of *Pythium* spp. colonizing alfalfa roots throughout the season at this site have been reported elsewhere (20).

Alfalfa variety Pioneer 5432 (dormancy class 4, moderate resistance to *Phytophthora megasperma*, resistant to Fusarium and Verticillium wilt) was used in both years, and seeds were sown in treatment blocks of approximately 8 by 24 m at a rate of 17 kg/ha. Three treatments were imposed on the alfalfa plants to maximize differences in levels of root infection by *Pythium* species. These treatments were a metalaxyl (Apron) seed treatment (commercial preparation, 0.33 ml a.i./kg seed), nontreated seed with metalaxyl applied as a soil drench (Ridomil 2E applied at 250 μl a.i./m^2 in 60 ml of H_2O/m^2) at the time of planting, and nontreated seed and soil (control). Seed were sown on days of year 114 (24 April) and 121 (1 May) in 1991 and 1992, respectively. Before planting in each treatment block, 45 1-quart, open-bottom milk cartons were placed in the ground in each treatment block and filled with soil from the top 15 cm of the soil profile (20). Cartons were seeded and treated with metalaxyl at the same time as the remainder of each block. After seedling emergence, plants were thinned to leave only one or two plants per carton.

Plant densities were determined over time within 10 permanent, 0.09-m^2 plots located arbitrarily in each treatment block. Soil samples were collected from the upper 10 cm of soil of each block 1 week after planting and were assayed for the presence of metalaxyl using a metalaxyl-sensitive bioassay (1). Additionally, soil populations of *Pythium* spp. were determined periodically through the growing season by dilution plating of soil samples from each block on a selective medium as described previously (20). During the course of these experiments, environmental factors including air and soil temperatures (measured at 7.5 cm depth), relative humidity, rainfall, and soil moisture were monitored in each treatment block using a CR21X micrologger (Campbell Scientific, Inc., Logan, UT).

Extraction of root systems. Each week through the first 4 to 5 weeks after initial seedling emergence, six to eight replicate cartons from each treatment block were removed from the field. Each carton was cut away, and the individual root system was extracted intact from the soil by careful rinsing under a fine mist spray (20). Each extracted root system was then washed thoroughly in running water for 20 min, carefully untangled, and spread out under a film of water. A record of each intact, complete root system was made by making tracings onto an acetate sheet. After being traced, each root system was plated in sections by embedding in molten agar of a modified pimaricin-ampicillin-rifampicin (PAR) medium selective for *Pythium* and *Phytophthora* spp. (23). The medium was amended with rose bengal, benomyl, and penicillin, which assisted in the selective recovery of species of these genera (23). Plates were incubated in the dark at 24°C for 3 days before assessing colony density. Infection density of root systems was estimated as the number of colonies of *Pythium* spp. per unit root length. Identification, distribution, and comparative pathogenicity of representative isolates from these roots was determined and has been reported previously (20). There was no significant effect of treatment on the type or composition of *Pythium* spp. colonizing these roots. *Pythium irregulare, P. ultimum, P. sylvaticum,* and *P. torulosum* were isolated most frequently from root systems of all treatments, and accounted for more than 65% of all *Pythium* spp. isolated in 1991 (20).

Assessment of root system morphology. Tracings of the six to eight replicate root systems per treatment at each sampling date were evaluated in relation to selected morphological characteristics. Total root system length was estimated from tracings using a modified line-transect method (28). An image analysis system was used to determine the lengths of all individual root segments in 1991 only. For structural analyses, root systems were classified by morphometric and topological schemes (5–9) as described previously (21). In the morphometric system, any root segment that terminates in an apical meristem, or terminal branch, is defined as a first-order root. Where two first-order roots merge, there begins a second-order root. Where two second-order roots merge there begins a third-order root, and so forth. The morphometric system provides a convenient method of dividing a root system into regions of increasing root maturity, in which root segments of similar physiology, age, and function are classified together. English and Mitchell (2) demonstrated the utility of this system in quantifying the effects of root pathogens on root development.

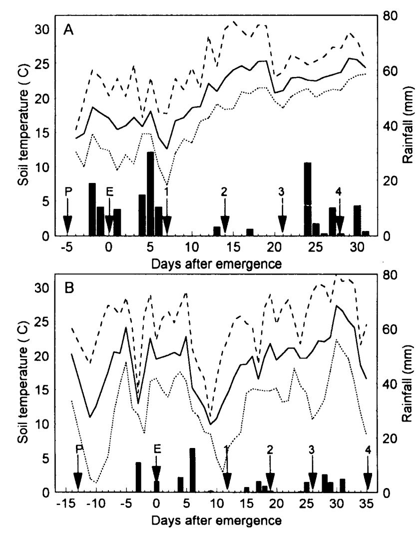

Fig. 1. Minimum (....), maximum (----), and average (——) daily soil temperature and rainfall in relation to planting date (P), seedling emergence (E), and alfalfa root sampling dates (1 to 4) during field evaluations in the spring of **(A)** 1991 and **(B)** 1992.

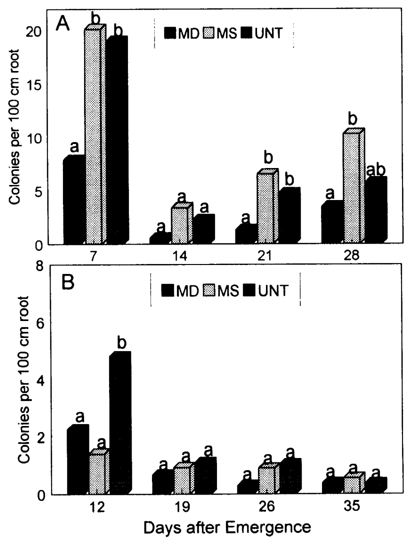

Fig. 2. Root colonization of developing alfalfa seedlings by *Pythium* spp. over time and as affected by metalaxyl seed and soil treatments in **(A)** 1991 and **(B)** 1992 field tests. MD = metalaxyl applied as a soil drench, MS = metalaxyl applied as a seed treatment, and UNT = untreated seed and soil. Bars topped by the same letter at each sampling date are not significantly different ($P < 0.05$) according to Fisher's least significant difference test.

The topological system is an extension of the morphometric approach that enables more intensive analysis of root system structure (7–9,21). In the topological system, the position of each root branch, or "link," is included in the classification, which provides comprehensive information on branching structure. The topological system provides greater sensitivity than other methods concerning both the degree and the pattern of root branching (7,8).

Root systems were characterized by morphometric orders and the values of topological parameters, including magnitude, altitude, path length, and total exterior path length. Magnitude is defined as the number of exterior links (first-order roots) that feed into the root system or into any individual link. Path length is the number of links between any given link and the shoot base. Altitude is the longest individual path length of a root system from one exterior link to the shoot base, and total exterior path length (P_e) is the sum of all path lengths from all exterior links to the base (21). Since the calculation of P_e values requires numerous summations based on the exact positioning of every link, a computer program based on equations adapted from Fitter (6,7) and Werner and Smart (29) was used to derive P_e values for all root systems (21).

Values for P_e and altitude are directly related to branching structure and co-vary with magnitude. The slope of the regression line from double-logarithmic ($\log_e$) plots of P_e against magnitude was used as a topological index (7,8) to define the branching structure of a root system. The maximum value for this topological index is 1.92, which corresponds with a herringbone pattern of root system development. In such a system, branching is restricted to the main axis (or taproot, in the case of alfalfa). The minimum theoretical value of the index is about 1.2 and corresponds to a dichotomously branched root system. Such a system branches with equal probability on all exterior links. Finally, a pattern of random growth, or one in which branch initiation is equally likely on all links, would produce a topological index of 1.52 (7).

Statistical analysis. Root system length, total numbers of root segments of all morphological orders, and values of topological parameters were compared among root systems of plants treated with metalaxyl or not treated. Statistical analyses were conducted using the general linear models procedures of Statistical Analysis Systems ver. 6.04 (SAS Institute, Cary, NC). Repeated measures analyses were conducted using a split-plot design on stand density, root colonization, and root morphology measurements made over time. Differences among treatments were detected by analysis of variance (ANOVA) and orthogonal contrasts at each assessment date. Topological indices for root systems of plants of varying levels of root infection by *Pythium* spp. were compared with each other and the topological extremes in branching by analysis of covariance (7,8,21) of ln P_e versus ln magnitude. All tests of significance were conducted at $P < 0.05$.

RESULTS

Plant density and root system colonization. In 1991, numerous rainfall events and cool temperatures coincided with planting and seedling emergence (Fig. 1A), providing conditions favorable for root growth as well as infection by *Pythium* spp. Plant densities were not significantly different among treatments at any time throughout the season and did not change significantly over the first 4 weeks after emergence according to repeated measures ANOVA (data not shown). Plant densities averaged 340 to 510 plants per m^2 for all treatments.

Seven days after initial seedling emergence, colonization of alfalfa roots averaged 19.0 and 7.8 infections per 100 cm of root for plants extracted from nontreated soil and metalaxyl-treated soil, respectively (Fig. 2A). Metalaxyl applied as a soil drench significantly reduced colonization of alfalfa roots by *Pythium* spp. compared with Apron-treated seed or control treatments over the first 4 weeks of plant growth in 1991, as determined by repeated measures ANOVA. The concentration of metalaxyl in the upper 10 cm of the soil in the metalaxyl soil drench treatment at this time was estimated at 1 to 2 μg per g of soil. In all treatments, numbers of infections per unit root length declined by 14 days after emergence and then increased slowly. By 21 and 28 days after emergence, significantly fewer infections were observed in metalaxyl-treated than in nontreated soil and/or with treated seed. There was no detectable pattern regarding the location of infection sites on the roots. Infections occurred with equal frequency throughout the root system on first, second, and higher order roots. At the time of plating, root systems generally were asymptomatic and apparently healthy, regardless of soil treatment.

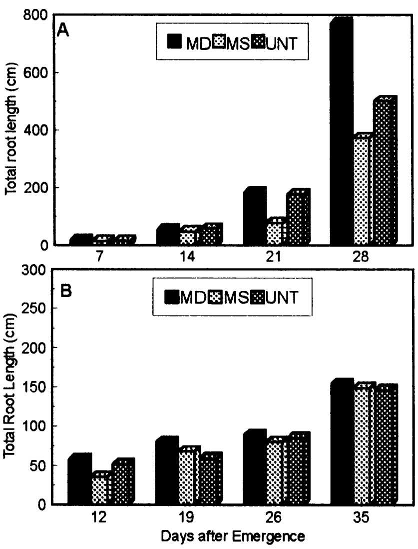

Fig. 3. Total root system length of developing alfalfa seedlings over time and as affected by metalaxyl seed and soil treatments in **(A)** 1991 and **(B)** 1992 field tests. MD = metalaxyl applied as a soil drench, MS = metalaxyl applied as a seed treatment, and UNT = untreated seed and soil. No significant differences among treatments were detected at any one sampling date in either year according to Fisher's least significant difference test ($P < 0.05$), but significant differences were observed over time for most parameters in 1991 according to repeated measures analysis of variance (see Table 1).

Alfalfa stand establishment occurred under more erratic environmental conditions in 1992, as characterized by wildly fluctuating temperatures and little rainfall following planting and seedling emergence (Fig. 1B). In association with these less desirable environmental conditions, root growth and infection by *Pythium* spp. were both much more limited than in 1991. Plant density was substantially less for all treatments than in 1991, averaging 200 to 330 plants per m^2, but stand counts did not change substantially over time and were not significantly different among treatments (data not shown).

Infection of alfalfa roots by *Pythium* spp. also was substantially lower in 1992 throughout the study period than in 1991 for all treatments, and differences among treatments were observed only at the first sampling date (Fig. 2B). Metalaxyl applied as a soil drench or seed treatment reduced colonization compared with nontreated soil at 12 days after emergence. Colonization, however, averaged less than 1 infection per 100 cm of roots throughout the remaining sampling dates for all treatments.

Soil populations of *Pythium* spp. were also lower in plots receiving the metalaxyl soil drench treatments than in nontreated plots throughout the 1991 season as determined by repeated measures ANOVA. In metalaxyl drench plots, total populations declined from 552 to 187 CFU per g of soil during the 1991 growing season. Populations in the nontreated control plot did not decline during the growing season, remaining at between 540 and 790 CFU per g of soil. Soil populations in the seed-treated plots also declined somewhat during the season, but populations were never significantly lower than in the nontreated control plots.

Root system development and morphology. Root system development was characterized by exponential increases in root system growth through the first 4 weeks in all treatments in 1991, as indicated by total root system length (Fig. 3A). Similar trends were observed for other root system characteristics, including the total number of root segments of all morphometric orders, root system magnitude (number of root apical meristems), altitude, and total exterior path length. Treatment effects were significant over time for all morphological parameters according to repeated measures ANOVA (Table 1). The metalaxyl soil drench treatment resulted in overall greater numbers of root segments, total root system length, magnitude, altitude, and total exterior path length over time than did metalaxyl seed treatments or the nontreated control and metalaxyl seed treatments combined. Thus, reductions in root infection by *Pythium* spp. corresponded with larger and more complex root systems. However, extreme variability among individual root systems precluded the detection of significant differences among treatments at any individual sampling date. Significant time effects included linear, quadratic, and cubic trends over all treatments, but no significant treatment × time interactions for any parameter (Table 1). This indicates that although there were complex polynomial trends over time with most parameters, these overall trends due to time were similar for all treatments. Root system parameters on the final assessment date (28

Table 1. Repeated measures analysis of variance for several root morphological parameters over time as affected by metalaxyl treatments in 1991 field test

Source of variation	DF	$P_r > F$[a]				
		Number	Root length	Magnitude	Altitude	P_e
Treatment[b]	2	0.037*[c]	0.032*	0.039*	0.004**	0.078
MD vs. MS	1	0.011*	0.010**	0.012*	0.026*	0.026*
MD vs. all	1	0.025*	0.017*	0.033*	0.565	0.058
Error (treatment)	18	0.624	0.313	0.629	0.865	0.612
Time (days)	3	0.001**	0.001**	0.001**	0.001**	0.001**
linear	1	0.001**	0.001**	0.001**	0.001**	0.001**
quadratic	1	0.004**	0.001**	0.003**	0.243	0.001**
residual	1	0.015*	0.023*	0.013*	0.338	0.022*
Treatment × time	6	0.083	0.061	0.106	0.293	0.146
Error (time)	54					

[a] Level of significance (probability of a greater *F*) for each source of variation for each root system parameter. Root system parameters included the total number of root segments of all morphometric orders (number), total root system length (length), and the topological parameters of magnitude, altitude, and total exterior path length (P_e).

[b] Treatments included a metalaxyl soil drench (MD), metalaxyl seed treatment (MS), and untreated seed and soil.

[c] Values followed by asterisks indicate significant effects at $P \leq 0.05$ (*) and $P \leq 0.01$ (**), respectively.

days after emergence) reflected the overall growth patterns among treatments (Fig. 4A).

Although morphological differences were detectable in the overall size and complexity of root systems, root system branching patterns did not differ significantly among treatments. The slopes of double-log plots of P_e versus magnitude varied to some extent among treatments, but differences were not statistically significant (Table 2). The slope values for the P_e plots ranged from 1.28 to 1.60 on the final sampling date, suggesting branching patterns that varied from a random branching pattern to a more dichotomously branched root system. A gradual shift in root branching structure from a herringbone-type pattern toward a more dichotomously branched pattern was observed with all treatments over time, as indicated by a decrease in the topological index (slope) over time (Table 2). This shift in branching structure was most apparent by the third and fourth week after emergence.

In contrast to 1991, root system growth was much slower during the first 5 weeks of seedling development in 1992 (Fig. 3B). Root systems were substantially smaller and less complex than in 1991, as indicated by all root system parameters measured at 35 days after seedling emergence (Fig. 4B). There were no significant differences among treatments for any root system parameter over time or at any individual sampling date in 1992. Similarly, there were no significant differences among treatments regarding root system branching patterns in 1992 (Table 2). The slightly higher slope values observed throughout the study period in 1992 compared with 1991 indicate a lower degree of branching and a simpler, more herringbone-like branching structure.

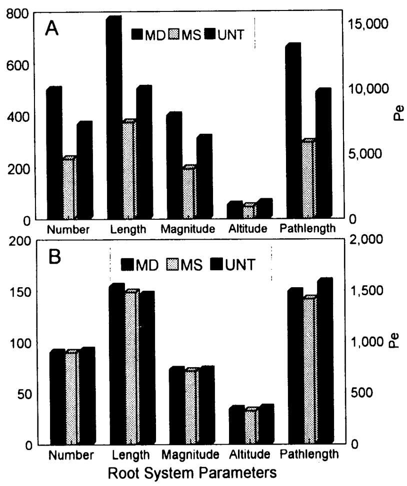

Fig. 4. Root system morphological parameters of alfalfa seedlings as affected by metalaxyl seed and soil treatments **(A)** after 28 days in 1991 and **(B)** after 35 days in 1992. Root system parameters included the total number of root segments of all morphometric orders (number), total root system length (length) in cm, and the topological parameters of magnitude, altitude, and total exterior path length (P_e = right y-axis). MD = metalaxyl applied as a soil drench, MS = metalaxyl applied as a seed treatment, and UNT = untreated seed and soil. No significant differences among treatments were detected for any parameter on these dates in either year according to Fisher's least significant difference test ($P < 0.05$), but significant differences were observed over time for most parameters in 1991 according to repeated measures analysis of variance (see Table 1).

DISCUSSION

This study demonstrated that sublethal root infections by natural populations of *Pythium* spp. can affect root system growth and morphology of alfalfa in the field. Under conducive environmental conditions, significantly different levels of infection were established by applying metalaxyl as a soil drench and seed treatment. The lower root infection density for seedlings from drenched soil was associated with significant increases in root system length, numbers of root segments of all morphometric orders, root system magnitude, altitude, and total exterior path length, compared with seedlings from the nontreated soils. These differences reflected the reduced size and complexity of root systems associated with infection by *Pythium* species. In contrast to these detectable differences in root system morphological traits, however, no significant effect of root infection on root system branching structure could be detected in these tests. Previous greenhouse experiments (20,21), as well as the 1992 field results, demonstrated that metalaxyl treatments have no direct effect on root system growth or morphology in the absence of infection by Pythiacious fungi.

Table 2. Comparison of regression slopes (topological index) from double-log plots of the root system topological parameters total exterior path length versus magnitude for alfalfa seedlings over time as affected by metalaxyl seed or soil treatments in 1991 and 1992 field tests

Day[a]	MD[b]	MS	UNT	r^2
1991				
7	1.74[c]	1.75	1.75	0.99
14	1.70	1.76	1.89	0.98
21	1.31	1.34	1.49	0.96
28	1.28	1.32	1.60	0.96
1992				
12	1.87	1.84	1.91	0.99
19	1.79	1.61	1.93	0.97
26	1.76	1.47	1.41	0.94
35	1.45	1.39	1.44	0.94

[a] Day = number of days after seedling emergence when root systems were measured.

[b] MD = Metalaxyl soil drench applied at seeding using Ridomil 2E at 250 µl a.i./m^2. MS = Metalaxyl seed treatment as a commercially prepared Apron treatment (0.33 ml a.i./kg seed). UNT = untreated seed and soil.

[c] Slope values among treatments were not significantly different ($P = 0.05$) at any assessment date in either year according to analysis of covariance. Slope values represent the topological index for characterizing root branching patterns. Slope values for topological extremes in branching pattern are 1.92 for the herringbone-type pattern and 1.20 for a completely dichotomous pattern. Values for r^2 indicate the coefficient of determination for each analysis of covariance.

Fluctuating temperatures and drier conditions in the second year of experiments were not conducive to plant growth or infection by *Pythium* spp. and, thus, root infection in metalaxyl-drenched soil could not be reduced significantly from the already low levels of infection in nontreated soil. The lack of substantial root colonization or any significant effects due to these root pathogens in the 1992 season also indicates the potentially sporadic nature of root disease caused by *Pythium* spp., and emphasizes the fact that these pathogens only cause disease problems when conditions are favorable, in particular cool, wet conditions (12–14).

Reductions in root infections by *Pythium* spp. per unit root length, which were observed following the second week after emergence in both years, indicate that the rate of root system growth was greater than the rate of new infections by *Pythium* spp. This represents a reduction in the infection rate relative to root growth, and not a decrease in total infections per root system. This decline was associated with the period of most active root growth, as well as environmental conditions not favorable for the development of *Pythium* spp.

Larkin et al. (21) demonstrated in previous greenhouse studies that infection of alfalfa roots by *Pythium* spp. could reduce root growth and alter root branching patterns. For example, plants grown in soil infested with *P. ultimum* developed significantly smaller and less complex root systems than plants in infested soil drenched with metalaxyl. In those experiments, as well as in the present study, the patterns of change in morphological and topological parameters that occurred in association with infection suggested that the main effects of these pathogens are to reduce the overall growth of a root system, affecting size and complexity of root systems, but having only a minor effect on altering root system branching structure. Root system branching was affected to some degree in greenhouse tests, however (21). Although no significant differences in branching structure could be detected in these field tests, a similar trend as in the greenhouse tests was observed, with roots grown in metalaxyl-treated soil tending toward more complex branching than more heavily infected root systems (21). Infection levels of *Pythium* spp. in the greenhouse tests were somewhat higher (5 to 20 infections per 100 cm of root) than observed through much of these field tests, suggesting that higher infection levels in the field may also result in detectable effects on root branching structure.

Branching structures for root systems of all treatments were observed to evolve from a herringbone-type pattern to more randomly or dichotomously branched patterns over the first 4 weeks of seedling growth in the field. These changes were detected readily by the changes in topological index (slope) values over time. This appears to be a natural progression in the branching structure of the developing root system and corresponds with changes in root system function as the root system matures, as was previously noted (21).

Values of morphological parameters in these field experiments differed considerably from those derived in previous greenhouse experiments (21). These differences reflect the many differences between the experimental systems. For example, inoculum densities in the field and greenhouse soils differed, as did the composition of *Pythium* species in the soils. Additionally, soil and climatic differences between the systems were great. Despite this, the trends in root system development in treated and nontreated soils were similar. In the 1992 field season, all root system morphological parameters were substantially lower than observed in 1991. This disparity in root growth between the two years was probably due to a number of factors, including a later planting date in 1992, more severe competition from weeds, and substantial differences in environmental conditions, primarily lower rainfall and less conducive spring temperatures in 1992.

Hancock (13) previously noted the difficulty of detecting the impacts of root infection on plant root growth in the field. Although he was able to detect significant relationships between infection of alfalfa by *Pythium* spp. and root length density as a measure of root growth in the greenhouse (12), he could not detect significant relationships in field tests (13). Our present study indicates the importance of environmental conditions in evaluating these relationships in the field. Populations of *Pythium* spp. in soil and levels of root colonization by these pathogens are known to be associated closely with soil environmental conditions and to exhibit pronounced seasonal fluctuations (12–14,20). Similarly, root growth and development also are dependent on soil environmental conditions and can vary dramatically with changing conditions (7,8,24,26). Additional problems in detecting significant influences of sublethal infection are related to the extreme variability observed between individual root systems. This is a problem exacerbated by the highly variable nature of the synthetic varieties of alfalfa.

The morphological and architectural techniques for analyzing root system structure developed by Fitter (6–9) are well suited to the evaluation of the effects of root pathogens on root system morphology (21), and this study demonstrated that these assessments can also be applied in the field. Fitter (10) previously used these techniques to assess root architecture of some herbaceous perennial species under field conditions, and Hetrick et al. (16,17) also applied a variation of these techniques to examine the influence of mycorrhizae on root system structure. In field situations, there are numerous factors, including root and soil microflora (saprophytic, symbiotic, and pathogenic), nematodes, and other organisms, that are interacting with roots, and may affect root development. These techniques can be especially useful for examining the composite effects of the contributions of all of these interactions on root system growth and morphology under various environmental conditions.

Whether the reduced root system development observed in the present study would have put plants at a competitive disadvantage with surrounding plants is uncertain. Confinement of root systems in cartons enabled the ready extraction of plants from soil, but it precluded any interaction with other root systems during exploration of soil. Application of these morphological and topological methodologies to studies that allow root system interactions should help elucidate the roles that soilborne pathogens play in plant competitive processes.

ACKNOWLEDGMENTS

This research was funded in part by a gift from the Ciba-Geigy Corporation. The authors thank Greg Birk, Keith Emery, Eric Scherf, Susan Taylor, and Paul Verslues for their technical assistance.

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