Effects of Infection by *Pythium* spp. on Root System Morphology of Alfalfa Seedlings

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ABSTRACT

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Root system morphology of alfalfa seedlings infected by various *Pythium* spp. was evaluated in greenhouse experiments using morphometric and topological assessment methodologies. Infection of alfalfa seedlings by *Pythium* spp., particularly *P. ultimum* and *P. irregulare*, resulted in reductions in root system growth and changes in root system architecture. Reductions in root system morphological parameters, including total root system length and total numbers of root orders, as well as the topological parameters root system magnitude, altitude, and total exterior path length, indicated overall smaller root system size and complexity in infected plants compared with noninfected plants. Root system branching structure also was affected, with infected root systems developing with a lower degree of branching than noninfected root systems, as represented by fewer orders of branching and a more monopodial type of branching pattern. Although isolates of *P. ultimum*, *P. irregulare*, and *P. sylvaticum* all caused substantial pre- and postemergence damping-off, *P. sylvaticum* caused no measurable change in root system morphology. In contrast, *P. ultimum* caused severe reductions in root system size and branching complexity, and *P. irregulare*, which caused less-severe damping-off, also caused substantial changes in root system morphology. Isolates of *P. dissotocum* and *P. torulosum* had no effect on root system growth or morphology. Metalaxyl applied to the soil was effective in reducing the effects of these pathogens, whereas metalaxyl seed treatment was less effective. Use of architectural analysis methods enabled the quantitative assessment of the impact of root pathogens on root system branching structure for the first time.

Additional keywords: *Medicago sativa*, root topology.

The architecture, or branching structure, of a root system is fundamental to its functions of resource acquisition, transport, and anchorage (5–8). Root system architecture is determined primarily by topology and the distribution of branches within a root system and secondarily by its geometry, which includes the lengths, branching angles, and diameters of root branches (7). Root system architecture can affect the exploration and exploitation of soil resources, nutrient transport efficiency, and energy requirements of the root system (7,8,10,13).

Root system morphology encompasses all aspects of the structural characteristics of a root system, including size, shape, and architecture. The morphological development of a root system is directed primarily by plant genetics but also can be greatly affected by edaphic factors, including soil temperature, structure, aeration, water content, nutrient status, and pH (25,29). Soil microorganisms, such as fungi that form ecto- and endomycorrhizae, induce morphological changes in the root systems of several plant species (2,17,18,27). Nonsymbiotic rhizosphere organisms may affect root growth through the production of antibiotics and hormones (28). Interrelationships between root pathogens and root growth also have been acknowledged as playing important roles in root development, plant health, and the epidemiology of root disease (3,19,29); however, little quantitative information is available concerning the effects of root pathogens on root system structure.

Root pathogens, such as *Pythium* and *Phytophthora* spp., may affect root system development by modifying growth or altering branching patterns (16,21). Changes in root system morphology could reduce overall plant growth and vigor and might have important implications for the ability of the plant to respond to other physical and environmental stresses. Currently, relatively little is known about the relationships between these root pathogens and root growth, and yet, these relationships may be very important for the understanding and management of root diseases (3).

Traditionally, information on root growth in relation to root infection has been based primarily on data from soil and root cores (3,15,16). Soil cores enable the determination of root colonization by soil microflora, specific root length, and root length densities but provide virtually no information on root system architecture (5). Techniques for the assessment of root system morphology, developed recently by Fitter (4,6,7), permit the quantification of root system architecture, as well as the measurement of the degree to which architecture may be affected by edaphic factors and soil microflora. These techniques, based on morphometric and topological assessment systems, enable analysis of branching structure that is not possible with traditional root classification methodology (26). These methods were developed from geomorphologic techniques used for rivers and other natural branching systems and are based on the mathematics of rooted trees (6-8,26). They have been used to assess the effects of changes in water and nutrient supply (6,9,10), mycorrhizal associations (2,17,18,27), and plant ecological characteristics (9,14) on root system architecture.

Root pathogens of alfalfa (*Medicago sativa* L.), particularly *Pythium* and *Phytophthora* spp., often have been associated with difficulties in developing and establishing alfalfa stands (21,22,30). Poor stand quality and decline often occur despite the use of metalaxyl-treated seed. In a previous study (21), we determined that infections of alfalfa roots by several species of *Pythium*, including isolates of *P. irregulare* Buisman, *P. ultimum* Trow, *P. sylvaticum* W.A. Campbell & J.W. Hendrix, and *P. dissotocum* Drechs., caused reductions in total root system length. The major

objective of the current study was to use morphometric and topological methods to evaluate the influence of infection by these *Pythium* spp. on root system development and morphology during the early stages of alfalfa seedling growth. In addition, the efficacy of metalaxyl treatments in controlling the effects of these root pathogens was studied. Preliminary results have been published (20).

MATERIALS AND METHODS

Inoculum and seedling preparation. Isolates of *Pythium* spp. were obtained and identified in a previous study (21). All isolates were collected from the roots of alfalfa seedlings grown in the field at the University of Missouri Horticulture Research Center at New Franklin. In experiment 1, 12 isolates of *Pythium* spp., representing six species previously observed to cause root symptoms on alfalfa, were tested for their effects on root system morphology. These species included: *P. irregulare* (three isolates), *P. ultimum* (three isolates), *P. sylvaticum* (three isolates), *P. torulosum* Coker & F. Patterson (two isolates), and *P. dissotocum* (one isolate). In subsequent repeated experiments (2 and 3), single isolates of *P. irregulare*, *P. ultimum*, and *P. sylvaticum* were used.

Inoculum was prepared by adding colonized agar blocks to a sterile medium composed of 30 g of fine vermiculite, 12 g of corn meal, and 80 ml of 10% clarified V8-juice broth in autoclavable bags (23). Cultures of individual pathogen isolates were incubated in the dark for 10–14 days at 20–24 C. Propagule counts were determined by dilution plating on a medium (RBPAR) selective for *Pythium* and *Phytophthora* spp. (24). Propagules consisted primarily of oospores mixed with mycelial fragments. Inoculum from these stock cultures was added to soil in amounts appropriate to produce an inoculum density of approximately 10^3 cfu per gram of soil. A volumetric mixture of New Franklin field soil and sand (2:1) was used in all experiments; the field soil provided a natural medium with its associated microflora populations, and the sand allowed easy removal of intact root systems. Inoculum cultures were mixed into soil for 15 min with a large mixer. Infested soil was placed into 950-ml milk cartons and planted with alfalfa variety Pioneer 5432 (Pioneer Hi-bred International, Des Moines, IA). In all tests, a natural, nonamended field soil, taken from the research fields at New Franklin, was included as a noninfested control treatment. The field soil contained natural populations of *Pythium* spp., including some that are pathogens.

Three fungicide (metalaxyl) treatments were imposed on each pathogen-infested soil as well as the noninfested field soil (control treatment): 1) metalaxyl-treated seed (Apron 25W commercial seed treatment, 0.33 ml a.i./kg), 2) metalaxyl applied as a soil drench (Ridomil 2E applied at seeding at a rate of 250 μl a.i./m^2), and 3) nontreated seed and soil. For each treatment-pathogen combination, four replicate milk cartons were prepared in the first experiment, whereas six to eight cartons were prepared in experiments 2 and 3. Ten to 12 seeds were planted per carton, and emerging seedlings were thinned to one to two plants per carton after about 14 days. Soil was irrigated daily, and plants were maintained at 16–24 C to provide a favorable environment for disease development.

In experiment 1, root systems were extracted for examination 18 days after seedling emergence; in experiments 2 and 3, roots were examined after 28 days of growth. To extract the roots, the cartons were cut open, and the soil was carefully washed away from the roots under a fine mist of water. Intact, extracted root systems were rinsed thoroughly in running water for 20 min, carefully untangled, and spread out under a film of water. A record of each complete root system was made by tracing onto an acetate sheet. Each root system was then embedded in molten RBPAR agar to determine the presence and incidence of each pathogen isolate within the root tissue. Tracings were used to evaluate root system morphological characteristics.

Assessment of root system morphology. Total root system length was estimated from tracings using a modified line-transect method (32). For structural analysis, root systems were classified by the morphometric and topological systems developed and described by Fitter (4–8). These systems differ from the traditional developmental model of root classification, in which roots are classified in the order that they appear, as axes that bear successively higher orders of laterals with fixed designations of primary, secondary, and tertiary laterals (26) (Fig. 1A). In the morphometric system, the direction of classification is reversed, and any root segment that terminates in an apical meristem, or terminal branch, is defined as a first-order root (Fig. 1B). 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 quick and 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. However, because root segments that are second-order or higher may contain multiple junctions of lower order root branches, this system does not provide complete information on branching structure.

The topological system is an extension of the morphometric system, but it is different in that it is link-based rather than segment-based (Fig. 1C). A link is defined as a length of root between two nodes or junctions of two root branches (6). In this system, links may be exterior or interior. An exterior link is one that ends in an apical meristem and, thus, is equivalent to a first-order root in the morphometric system. In the topological system, the position of each and every 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 pattern of root branching (6,7).

Root systems were characterized by morphometric orders as well as the topological parameters of magnitude, altitude, path length, and total exterior path length. Magnitude is defined as the number of exterior links 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. Total exterior path length (P_e) is the sum of all path lengths from all exterior links to the base.

The analysis of branching structure is complicated by the correlation of these parameters with root system size (6,7,9). Values for P_e and altitude are directly related to branching structure and covary with magnitude. At a given magnitude, P_e and altitude can vary between absolute minimum and maximum values that represent the topological extremes in branching (Fig. 2). Minimum values are produced by completely dichotomous branching, representing the highest degree of branching, and maximum values are produced by the herringbone or monopodial pattern of growth, representing the lowest degree of branching. The slope of the regression line from double-logarithmic ($\log_e$) plots of P_e or alti-

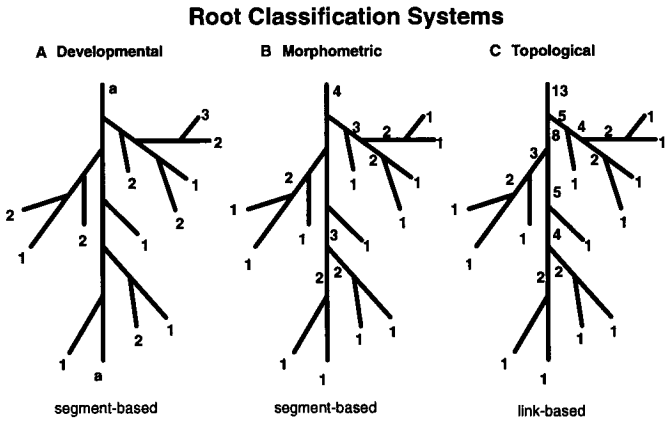

Fig. 1. Diagrammatic representation of a root system as classified by: **A,** the developmental system, in which "a" represents the axis and numbers refer to lateral root classes (26); **B,** the morphometric system, in which numbers refer to root-order classes (4); and **C,** the topological system, in which numbers refer to the magnitude of each link and are equal to the sum of magnitudes of its two daughter links (6).

tude against magnitude represents the relationship between these parameters and is an important topological index (6,7,9). This index characterizes the branching structure for groups of root systems and can be compared with other root systems and to the theoretical topological extremes in branching. Values for this topological index for P_e versus magnitude range from a maximum of 1.92 for the herringbone pattern to a minimum of about 1.2 for a completely dichotomous branching pattern (6). A topologically random growth pattern, or one where branch initiation is equally likely on all links, would produce a topological index of 1.52 (6,33). Therefore, high slope values imply a herringbone branching pattern, in which branching is restricted primarily to the main axis, whereas lower slopes imply a tendency toward more dichotomous branching, where branch initiation occurs with equal probability on all external links. Topological indices for root systems of plants infected by *Pythium* spp. were compared with noninfected plants and the topological extremes in branching by analysis of covariance.

Since calculating total exterior path length (P_e) values requires numerous summations based on the exact positioning of each and every individual link, a computer program written in BASIC was devised to estimate P_e values based on equations adapted from Fitter (5,6) and Werner and Smart (33). The program uses a limited number of parameters based on morphometric orders, including the numbers of first-, second-, and third-order roots, the average number of first-order per second-order root, second-order per third-order root, and so forth, as well as the distribution and placement of the various root orders, to estimate P_e values. In testing the program on over 100 root systems of various complexities, estimated values of P_e were always within 5% of actual values and usually within 2–3%. The program was accurate for root systems containing up to fifth-order root segments. This program was used to calculate P_e values for all root systems.

Statistical analysis was conducted using the general linear models procedures of the Statistical Analysis System, version 6.04 (SAS Institute, Cary NC). Analysis of variance was conducted on root system parameters using a completely randomized design and a two-way factorial analysis with interactions. Mean separation was accomplished using Duncan's multiple range test. A separate-slopes analysis of covariance model, with magnitude as the covariate, was used to detect differences in branching structure among sets of root systems. All tests for significance were conducted at $P < 0.05$.

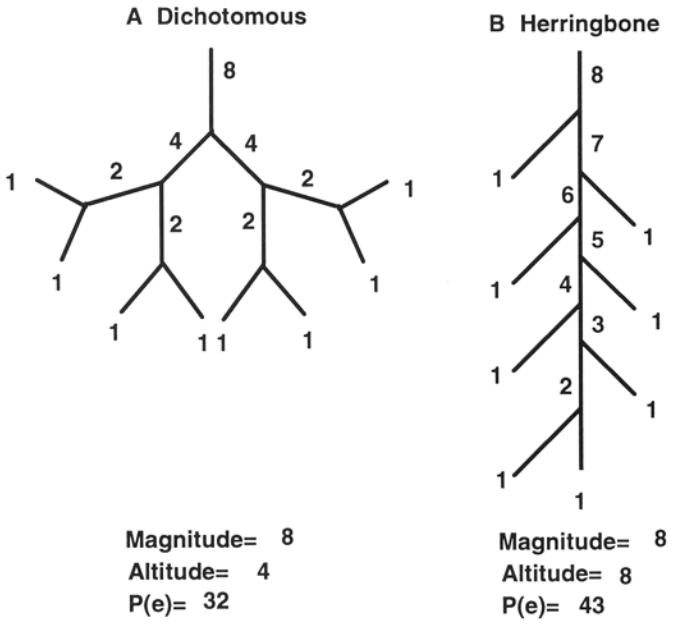

Fig. 2. Comparison of topological extreme types of branching patterns and their characteristics according to the topological classification system of Fitter (6,7). **A,** Dichotomous branching and **B,** herringbone or monopodial branching. Numbers refer to the magnitude ordering system for each link. Root system magnitude is the same as the largest link magnitude, altitude is the longest path length from an exterior link to the shoot base, and total exterior path length (P_e) is the sum of the values of all links in the system.

RESULTS

Infection of alfalfa roots by *Pythium* spp. had significant effects on root system development of surviving seedlings at 18 days after emergence in experiment 1 (Fig. 3A). Multiple isolates of *P. irregulare* and *P. ultimum* significantly reduced total root system length and total numbers of root segments of all morphometric orders (first-, second-, and third-order roots), as well as the topological parameters of root system magnitude, altitude, and P_e, compared to plants grown in noninfested field soil. Total root system length was reduced by an average of 43% in plants infected by *P. irregulare* compared to control plants. P_e and root system magnitude were reduced by 56.5 and 41%, respectively, with infection by *P. irregulare* in the same test. A single isolate of *P. dissotocum* also reduced total root system length, magnitude, altitude, and P_e. Isolates of *P. torulosum* and *P. sylvaticum* had no consistent effect on root system development. All *Pythium* spp. tested were recovered from roots of surviving plants, regardless of the presence or absence of symptom development. Treatment of seed or soil with metalaxyl significantly increased root system size and complexity compared to plants in infested, nontreated soil, represented by increases in all root system parameters measured over all *Pythium* spp. tested (Fig. 3B). Interactions between fungicide treatments and *Pythium* spp. were not significant for any parameter.

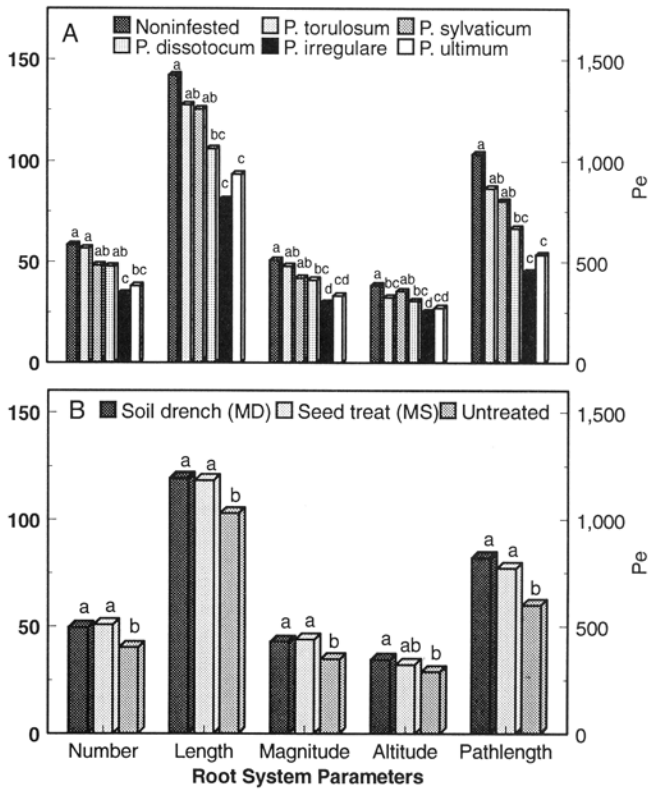

Fig. 3. Root system morphological parameters of alfalfa seedlings in experiment 1 after 18 days of growth as affected by **A,** soil infestation by various *Pythium* spp. and **B,** fungicide treatments of metalaxyl applied to the soil (MD) or seed (MS) or untreated soil (averaged over all *Pythium* spp. tested). Interactions between pathogen and fungicide treatment factors were not significant for any parameter. Root system parameters included the total number of root segments of all morphometric orders, total root system length (in centimeters), and the topological parameters of magnitude (first-order roots), altitude, and total exterior path length (P_e). Bars topped by the same letter are not significantly different from each other for each parameter according to Duncan's multiple range test.

In experiment 2, conducted with single-pathogen isolates, infection by *P. irregulare* and *P. ultimum* resulted in significant reductions in root system parameters after 28 days of root growth (Fig. 4). *P. ultimum* reduced total root system length, total numbers of root orders, root system magnitude, altitude, and P_e. *P. irregulare* reduced total numbers of root orders, root system length, and magnitude, whereas *P. sylvaticum* generally did not significantly affect root system morphology. Similar results were observed in experiment 3, with both *P. irregulare* and *P. ultimum* isolates reducing all root system parameters measured (data not shown). In addition to these root system parameters, *P. ultimum* and *P. irregulare* significantly reduced stem height and number of nodules formed per root in experiment 2 (data not shown).

Substantial pre- and postemergence damping-off was observed with isolates from all three species as well as in the noninfested field soil. The combined effects of pre- and postemergence damping-off are shown in the percent survival relative to the noninfested, metalaxyl-drenched control soil 14 days after emergence in experiment 2 (Table 1). The most severe damping-off was caused by *P. ultimum* and *P. sylvaticum*, with less than 20% seedling survival in the untreated control and metalaxyl seed treatments. Metalaxyl applied as a soil drench effectively reduced seedling loss due to damping-off with all *Pythium* spp. tested, whereas seed treatment generally did not. Similar results were observed in experiment 3, although damping-off caused by *P. sylvaticum* was not as severe (58% survival).

Application of metalaxyl as a soil drench enhanced the development of root systems of seedlings grown in pathogen-infested soil with all *Pythium* spp. tested in experiment 2 (treatment-pathogen interaction was not significant) (Fig. 5A). Soil drench treatments resulted in significantly higher values for most root system parameters, including the total numbers of roots of all orders, total root system length, root system magnitude, altitude, and P_e than those resulting from the seed treatments or untreated, infested controls. Differences between the soil drench treatments and untreated controls were greatest when soil was infested with *P. ultimum* (Fig. 5B). Application of metalaxyl had no significant effect on root system development when soils were infested with *P. sylvaticum*.

Root system branching patterns of plants infected by *P. irregulare* or *P. ultimum* were significantly altered compared to plants in noninfested soil, as determined by analysis of covariance of P_e with root system magnitude in experiment 1. This difference is represented by a significant change in slope (b) when P_e is regressed against magnitude for root systems infected by these pathogens, with $b = 1.86$ and 1.72 for *P. irregulare* and *P. ultimum*, respectively, compared to $b = 1.36$ for plants in the noninfested field soil (Table 2, *Pythium* spp.). The higher topological index for roots infected by *P. irregulare* and *P. ultimum* is closer to the value produced by the monopodial, herringbone pattern (1.92), whereas root systems in noninfested soil had a low topological index, which was closer to that produced by the more highly

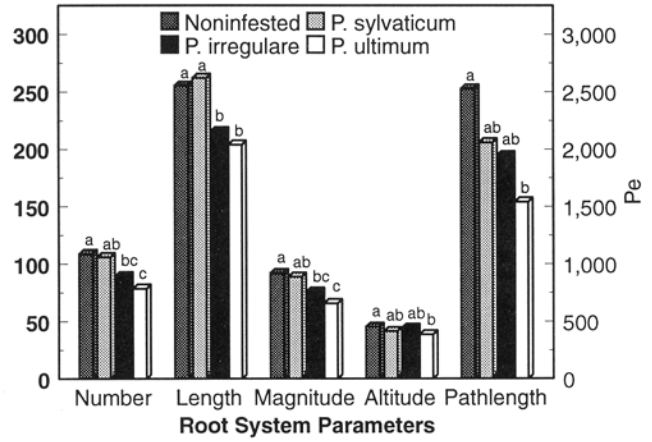

Fig. 4. Root system morphological parameters of alfalfa seedlings after 28 days of growth as affected by soil infestation by various *Pythium* spp. in experiment 2. Root system parameters included the total number of root segments of all morphometric orders, total root system length (in centimeters), and the topological parameters of magnitude (first-order roots), altitude, and total exterior path length (P_e). Bars topped by the same letter are not significantly different from each other for each parameter according to Duncan's multiple range test.

TABLE 1. Survival of alfalfa seedlings after 14 days of growth in soil infested with various *Pythium* spp. and as affected by treatment of soil or seed with metalaxyl in experiment 2

	Survival (%)[z]			
Treatment[y]	Noninfested	*P. irregulare*	*P. sylvaticum*	*P. ultimum*
Metalaxyl soil drench	100 a	71.1 a	88.9 a	55.5 a
Metalaxyl seed treatment	28.9 c	46.7 ab	4.9 b	20.0 b
Nontreated field soil	53.3 b	24.4 b	15.5 b	11.1 b

[y] Metalaxyl soil drench was applied at seeding using Ridomil 2E at 250 μl a.i./m^2. Seed treatment was a commercially prepared Apron 25W treatment (at 0.33 ml a.i./kg of seed).

[z] Data are presented as the percentage of plants that survived relative to plant survival in the noninfested, metalaxyl drench treatment. Inoculum of each *Pythium* spp. was added to field soil at 10^3 cfu per gram of soil. Values followed by the same letter are not significantly different from each other for each *Pythium* spp. tested according to Duncan's multiple range test.

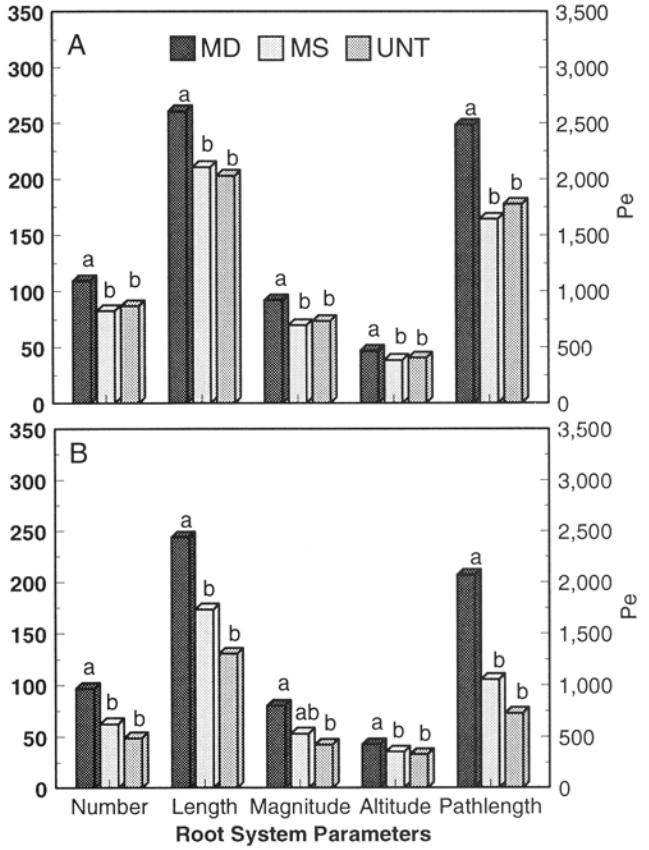

Fig. 5. Root system morphological parameters of alfalfa seedlings after 28 days of growth in experiment 2 as affected by fungicide treatments of metalaxyl applied to the soil (MD) or seed (MS) or untreated soil and grown in soils amended with **A,** *Pythium ultimum*, *P. irregulare*, *P. sylvaticum*, or a noninfested field soil, or **B,** *P. ultimum* only. Interactions between soil infestation by *Pythium* spp. and fungicide treatments were not significant for any parameter. Root system parameters included the total number of root segments of all morphometric orders, total root system length (in centimeters), and the topological parameters of magnitude (first-order roots), altitude, and total exterior path length (P_e). Bars topped by the same letter are not significantly different from each other for each parameter according to Duncan's multiple range test.

branched dichotomous pattern (1.2). Root systems colonized by *P. torulosum*, *P. sylvaticum*, and *P. dissotocum* had topological indices similar to plants in noninfested soil and indicated no change in branching structure. Comparison of regression slopes of P_e versus magnitude in experiment 2 also indicated an effect of infection by *Pythium* spp. on root system branching structure (Table 2, *Pythium* spp.). Root systems of plants infected by *P. ultimum* and *P. irregulare* tended toward a higher topological index compared to those in noninfested soil or soil infested with *P. sylvaticum*, although differences in slope were significant only for *P. irregulare* according to analysis of covariance ($P < 0.05$).

A similar comparison of regression slopes of P_e versus magnitude as affected by fungicide treatment over all pathogen species in experiments 1 and 2 indicated a significantly lower topological index for plants exposed to the metalaxyl drench treatment than for those in the untreated control (Table 2, metalaxyl). This lower index represents a significant change in branching pattern, indicating a higher degree of branching and a structure closer to the dichotomous branching pattern in the metalaxyl drench-treated root systems compared to the herringbone pattern of the infested but fungicide-untreated root systems. A significant reduction in slope also was observed in the metalaxyl seed-treated root systems in experiment 1 but not in experiment 2 or in subsequent repeated tests.

Comparison of regression slopes for the additional topological index of root system altitude versus magnitude indicated results similar to those shown using P_e versus magnitude for all experiments (altitude data not shown). Root systems infected by *P. ultimum* or *P. irregulare* tended to have higher slope values than those in noninfested soil, indicating a change in root system branching pattern due to pathogen infection.

DISCUSSION

This study represents the first time architectural analysis has been used to quantify the impact of infection by a root pathogen on root system morphology. In greenhouse tests, infection of alfalfa seedling roots by *Pythium* spp., particularly *P. ultimum* and *P. irregulare*, caused reductions in root system growth and changes in root system architecture. Reductions in root system morphological parameters indicated overall smaller root system size and complexity in infected plants compared with noninfected plants. Root system branching structure also was affected, with infected root systems developing with a lower degree of branching, represented by fewer orders of branching and a more monopodial or herringbone structure.

Isolates of *P. ultimum*, *P. irregulare*, and *P. sylvaticum* caused substantial pre- and postemergence damping-off, but the ability of a species to cause damping-off was not directly related to its ability to affect root system morphology. For example, although *P. sylvaticum* caused substantial damping-off, it resulted in little change in root system morphology in surviving plants when quantified by topological parameters. In contrast, *P. ultimum* caused both severe damping-off and severe reductions in root system growth of surviving plants, and *P. irregulare*, which caused less damping-off than the other two species, severely reduced root system growth.

Metalaxyl applied as a soil drench not only reduced damping-off but also substantially reduced the effects of these pathogens on root system growth and branching structure. Plants grown in metalaxyl-treated soil maintained higher numbers of root segments of all orders, greater root system length, and generally more complex branching patterns than did untreated plants in infested soil. Metalaxyl applied as a seed treatment generally was not as effective in controlling the effects of these pathogens. Overall, plants not treated with metalaxyl tended to have a branching pattern closer to herringbone, while those treated with metalaxyl developed with a branching pattern closer to that of a random or dichotomously branched system.

A primary effect of these pathogens was to reduce the overall size and length of infected root systems. This stunting may be caused either by a delay in the growth and development of infected root systems or by the loss of infected root segments and branches through necrosis and death. Changes in branching structure due to pathogen infection were more subtle than were the reductions in root system size but were detectable by topological analysis. These changes were distinct from the reductions in root system size. Differences in branching structure related to the degree and location of branch initiation and were not directly related to size of a root system (5,7,8). These changes in branching structure were represented primarily by a trend toward a herringbone branching structure in infected plants compared to a more dichotomously branched root system in healthy plants at the same stage of growth. This pattern indicates a tendency toward a lower degree of branching with infection by these *Pythium* spp., characterized by branching primarily along the main axis and few branches of second or higher morphometric order. Healthy root systems tended toward a higher degree of branching, where branch initiation occurred throughout the root system and more branches of higher orders were produced. Because these studies focused on seedling development through 4 wk after emergence, it is unknown whether this stunting of root system growth and change in structure caused by infection by *Pythium* spp. would continue throughout the growing season. There also have been reports of root-forking or an increase in lateral branching above the points of infection caused by some *Pythium* spp. and other root pathogens (15,16). This symptom was not observed on any individual root system or as a trend with infection in any of our tests.

The branching structure of a root system is closely related to its functional attributes and abilities (5,8). Fitter (7,8,13) determined that a herringbone pattern is the most efficient branching structure for the exploration and exploitation of mobile soil resources, such as water and nitrogen. Because it also contains a larger number of high magnitude, and subsequently high volume, links and a greater exterior path length, herringbone patterns have higher energy requirements for their production and maintenance than other structures (7,8,13). The more compact dichoto-

TABLE 2. Comparison of regression slopes from double-log plots of the root system topological parameters total exterior pathlength versus magnitude for alfalfa seedlings in experiments 1 and 2 as affected by infection by various *Pythium* spp. and treatment of the seed or soil with metalaxyl

Treatment[x]	Experiment 1		Experiment 2	
	Slope[y]	r^2	Slope	r^2
Pythium spp.				
P. irregulare	1.86*	0.97	1.69*	0.88
P. ultimum	1.72*	0.96	1.56	0.90
P. sylvaticum	1.43	0.88	1.42	0.92
P. torulosum	1.39	0.95	...[z]	...
P. dissotocum	1.37	0.91	...	...
Noninfested field soil	1.36	0.92	1.43	0.92
Metalaxyl				
Nontreated field soil	1.71	0.95	1.63	0.89
Metalaxyl seed treatment	1.49*	0.94	1.48	0.91
Metalaxyl soil drench	1.43*	0.87	1.37*	0.87

[x]Inoculum of each *Pythium* spp. was added to each field soil at 10^3 cfu per gram of soil. Metalaxyl soil drench was applied at seeding using Ridomil 2E at 250 μl a.i./m^2. Seed treatment was a commercially prepared Apron 25W treatment (0.33 ml a.i./kg of seed). Interaction between pathogen and fungicide treatment factors was not significant in either experiment. Root systems were characterized at 18 days after emergence in experiment 1 and at 28 days after emergence in experiment 2.

[y]Values followed by an asterisk indicate slopes are significantly different ($P < 0.05$) from the noninfested or nontreated field soil control for that experiment 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 pattern and 1.20 for a completely dichotomous pattern. Values for r^2 indicate the coefficient of determination for each regression line.

[z]Root morphological characteristics were not determined for this pathogen in this test.

mously branched root system has a lower energy requirement and is more efficient at nutrient transport, but is less efficient at resource acquisition. Therefore, herringbone patterns are advantageous where soil-derived resources limit growth and are characteristic of plants in nutrient- or water-deficient habitats (7,9,10). This pattern has been verified for several plant species, in which root systems of plants grown under conditions of low soil moisture or low fertility respond by exhibiting a herringbone growth pattern, whereas at high water or fertilization rates, a more highly branched dichotomous branching pattern is preferred (7,9–11,14). Thus, the growth of alfalfa root systems infected with *Pythium* spp. in this study was similar to the response of plants under nutrient or water stress.

Our studies on the growth of alfalfa root systems indicated that early in development (first 1–2 wk) alfalfa roots adopted a herringbone branching structure, characterized by a high topological index (R. P. Larkin and J. T. English, *unpublished data*). This structure established the initial penetration, exploration, and anchorage needed by developing alfalfa seedlings. As the plants grew through 3–4 wk, root branching became more prolific, and root systems were shifted gradually from the herringbone pattern toward a more highly branched pattern, evidenced by a gradual drop in the topological index over time. This change in branching structure over time also can be seen in the generally lower topological index values observed in experiment 2 versus experiment 1 (Table 2), which was conducted at an earlier stage of seedling growth. This pattern also has been observed in several other plant species (10,11,14). In light of this natural progression from herringbone to a more dichotomously branched root structure with root system development, the effect of pathogen infection can be viewed as maintaining the root system at a more juvenile or immature stage of root structural development. This juvenile stage consists not only of smaller root system size, but also a less mature stage of root system architecture. Changes in root system branching may be a direct effect of infection by these pathogens or could be the result of plant adaptation to the disruption or decreased abilities in water and nutrient uptake caused by root infection. These stress-inducing changes in root system morphology in conjunction with other environmental stresses may contribute to plant decline over time.

Use of the morphometric and topological classification systems enabled the quantitative assessment of root system parameters and the characterization of root system branching structure. The only limitations to the use of these systems were their labor-intensive natures and the need to have complete root systems. However, recent innovations in the development of technologies for the acquisition and analysis of root systems, such as image analysis systems (10,11,29), computer-driven algorithms for the determination of root parameters (1,10,11), and fractal characterization of root systems (12,31), will make these techniques more appropriate, applicable, and accessible to investigators working with plant root systems under varying conditions. Fitter (11) and others (14,17,18) have applied these techniques in field situations on portions of large root systems with favorable results. Although much more research is needed to understand the interrelationships between root pathogens, root growth, and plant health, continued work with quantitative methods such as these has much potential for clarifying these important interactions.

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