Horizontal Distribution and Characterization of Rhizoctonia spp. in Tall Fescue Turf

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ABSTRACT

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A 12-yr-old stand of tall fescue (Festuca arundinacea) turf with a history of brown patch (induced by *Rhizoctonia solani*) was divided into 225 contiguous quadrats $(0.37 \text{ m}^2 \text{ each})$ and the soil sampled for propagules of Rhizoctonia in June 1980 and 1981 by direct assay of soil organic debris. Propagule number frequency distributions were described by the negative binomial frequency distribution in both years, indicating a horizontal

clumping of propagules. Fifty-three isolates in 1980 and 54 isolates in 1981 were recovered and identified as Rhizoctonia solani, R. zeae, or binucleate Rhizoctonialike fungi. R. solani was less frequently recovered (9% of the total in 1980 and 1981) than R. zeae (45.3% in 1980 and 48.2% in 1981) or binucleate Rhizoctonialike fungus (39.6% in 1980 and 42.6% in 1981).

Rhizoctonia solani Kühn induces brown patch, a foliar blight of tall fescue (Festuca arundinacea Schreb.) and other turfgrasses (9). This disease often causes severe damage to cool-season turfgrasses in the warm, humid areas of the United States, and frequently is severe during the summer months on tall fescue used throughout central North Carolina.

Fungi that closely resemble R. solani cause disease on turfgrasses and other crops (5,29). These fungi exhibit mycelial characteristics similar to those of R. solani, but they have predominantly binucleate hyphal cells, whereas cells of R. solani are multinucleate (24,25). Rhizoctonia cerealis van der Hoeven (1) is a binucleate species that causes "yellow patch" on turfgrasses (2), as well as sharp eyespot and root rot of wheat (26,30), but a perfect state has not been induced. R. cerealis is a pathogen on tall fescue under greenhouse and growth-chamber conditions (5). Certain other binucleate Rhizoctonialike fungi (RLF) have been induced to form a Ceratobasidium perfect state (3,4).

Little is known about the relative abundance and distribution of Rhizoctonia spp. in turf soils. Techniques utilizing frequency distributions have been applied to the study of population dispersion or distribution of many organisms, including insects (31), nematodes (13), bacterial lesions (32,37), and soilborne fungi (14). Lesion distribution of hypocotyl rot of snapbean (Phaseolus vulgaris L.), induced by R. solani, was characterized through frequency-distribution analysis, but the soil habitat was not sampled for direct assay of propagule level and distribution (7). These and other studies have demonstrated the value of characterizing organism dispersion in ecological and epidemiological investigations. The purpose of this study was to determine and to characterize the horizontal, or planar, distribution of propagules of Rhizoctonia spp. in organic debris of tall fescue soil, to identify the Rhizoctonia species that contribute to the overall population of Rhizoctonia propagules sampled, and to determine the relative proportion of each species or type.

MATERIALS AND METHODS

Site selection and sampling. An 83.6-m² area in a 12-yr-old stand of tall fescue (cv. 'Kentucky 31') maintained as home lawn turf with a history of brown patch was divided into 225 contiguous quadrats

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(0.37 m² each). The soil was a Cecil clay loam. Five soil cores (June 1980) and a single core (June 1981) were removed from each quadrat to a depth of approximately 5 cm with a standard soilsampling tube (2-cm bore diameter). Sample size was reduced to a single core per quadrat in 1981 because of difficulties in handling larger samples. Soil cores from each quadrat were placed in polyethylene bags, labelled with a quadrat number, and stored for 7 days at approximately 25 C before elutriation. Percent leaf area affected by typical Rhizoctonia lesions (24) within each quadrat was estimated visually with a scale of 1 to 5, in which 1 = no disease, 2 = slight, 3 = medium, 4 = severe, and 5 = very severe. Disease intensity was low at this time in both years.

Elutriation. Samples were weighed before separation of organic debris for assay of Rhizoctonia spp. Samples were processed through the method of Clark et al (8), using a semiautomatic elutriator (6). Air- and water-flow rates were approximately 50 cm³/sec and 80 ml/sec, respectively. Soil samples were broken up manually preceding elutriation, and elutriation time was 8 min to ensure proper separation of organic debris in various stages of decomposition from mineral components of soil.

Variation between elutriator chambers was tested by randomly selecting 20 quadrat samples and subdividing each of these into four equal subsamples per quadrat on a weight basis. Each of the four subsamples per quadrat was elutriated simultaneously and propagule counts made as before to identify possible bias in elutriation chambers. No consistent bias was found after analysis of these data.

Debris containing fescue leaves, stems, roots, and more decomposed debris was collected on 60-mesh sieves (240-µm openings), then washed through a 10-mesh sieve (1,700-μm opening) nested over another 60-mesh sieve. This allowed separation of live grass leaves, stems, and roots, which were discarded. Preliminary assay of these living substrates indicated propagules of Rhizoctonia were not present in numbers comparable to those in the more decomposed debris portions. Use of 60-mesh sieves allowed recovery of propagules of Rhizoctonia of a size capable of infection (15,38). Organic debris (240-1,700 μm) was washed with tap water from 60-mesh sieves onto coarse filter paper (9.0-cm diameter) in Buchner funnels (7.5-cm i.d.) attached to a vacuum pump with sufficient suction to remove excess water. Debris was then gently scraped from the filter papers into 100 × 15-mm plastic petri dishes. Small portions of debris were placed in each petri dish so that individual debris particles could be visualized after addition of approximately 20 ml of cooled (50 C) 1.5% water agar. Water agar was used as a nonselective medium to avoid possible bias in sampling of unknown *Rhizoctonia* species and because other media did not appreciably enhance recovery in other experiments (8).

Plates were incubated 24-48 hr at approximately 25 C before counting and recovery of colonies of *Rhizoctonia*. Because of the large number of plates in 1980, some debris plates were stored at 3 C for up to 2 days before the incubation period began to prevent overgrowth of *Rhizoctonia* by other organisms. No apparent differences in colony number were due to the cold storage.

Characterization of Rhizoctonia spp. Fifty-three isolates in 1980 and 54 isolates in 1981 were recovered by hyphal-tip transfer from debris plates onto acidified potato-dextrose agar (APDA) or plain PDA plates. Nuclear condition and the presence of dolipore septa of isolates was determined by staining young colonies (4–6 cm diameter) with 0.05% trypan blue in lactophenol (29). Gross morphological characteristics such as hyphal branching habit (the character considered for counting colonies of Rhizoctonia in debris plates), colony color, and presence or absence of sclerotia, were considered when identifying Rhizoctonia spp. (24).

Different morphological types of R. solani (three isolates), binucleate RLF (five isolates), and R. zeae Voorhees (five isolates) recovered from plates were paired with five anastomosis group (AG) tester isolates of R. solani (21,23) and binucleate Rhizoctonia spp. Ceratobasidium anastomosis group (CAG) tester isolates 1, 2, 3, 4, and 5 (4). Unknown isolates and AG and CAG tester isolates were grown on PDA approximately 48 hr, then 7-mm-diameter mycelial plugs were cut from margins of actively growing colonies and placed 4-6 cm apart on sterile glass slides coated with a thin layer of 1.5% water agar. Unknown isolates were paired with AG or CAG tester isolates and incubated in sterile, moist chambers in the dark at 28 C for 2-5 days. The hyphal contact area was stained with 0.05% trypan blue in lactophenol and examined microscopically at ×400 for presence of anastomosis (23).

Construction and analysis of frequency distributions. Total propagules per quadrat, propagules per gram of dry soil per quadrat, and foliar blight severity per quadrat were used to construct frequency tables. Data from 1980 were adjusted (by dividing by 5) for comparison with 1981 data in which single cores per quadrat were taken. Frequency data were tested for goodness-of-fit to several discrete frequency distributions, including the negative binomial (11), Neyman type A (20), and Poisson by chisquare analysis, using a computer FORTRAN routine developed by Gates and Ethridge (12).

RESULTS

Isolate identification and characterization. R. solani, R. zeae, and a high proportion of binucleate RLF were isolated from the sampled area in both years. Rhizoctonia species assayed were found occasionally as free sclerotia, but more commonly in infested debris as sclerotia or thick-walled hyphae.

R. zeae was the most frequently isolated species, and binucleate RLF were isolated more frequently than R. solani (Table 1). R. zeae isolates conformed to the description given by Voorhees (36) and temperature growth response of selected R. zeae isolates on PDA indicated growth optima in vitro at approximately 32 C, in agreement with other workers (19,28,35). Hyphal cells of R. zeae isolates were multinucleate, although septal visualization was difficult because the mycelium appeared grainy. Isolates of R. zeae did not anastomose with R. solani AG tester isolates or with binucleate RLF CAG tester isolates.

Isolates of binucleate RLF were fairly uniform in appearance and had a tan or light brown pigmentation in 2-wk-old PDA cultures grown in light at 20–25 C. Dark brown sclerotia were formed in older cultures. These binucleate isolates were visually distinguishable from isolates of *R. cerealis* recovered from diseased bentgrass (*Agrostis palustrus* Huds.) in North Carolina during cool, wet weather (S. B. Martin and L. T. Lucas, *unpublished*), and none of the isolates tested anastomosed with CAG 1 (2). Thus, the binucleate RLF in this study were probably not *R. cerealis*. Some of these isolates were pathogenic but only slightly virulent on tall

fescue in greenhouse tests (S. B. Martin, unpublished).

R. solani was the least frequently isolated species from debris, even though it was frequently isolated from lesions on diseased foliage of tall fescue affected with typical brown patch symptoms at this site and other areas in North Carolina. Of three morphologically dissimilar R. solani isolates tested, two isolates were AG I, and the other did not fuse with any of the AG tester isolates.

Propagule distribution. Total propagule density ranged from 0 to 26 propagules per quadrat sample in 1980 and from 0 to 22 propagules per quadrat sample in 1981. Frequency class 22 (Fig. 1A) represents a combined class and includes samples with propagule numbers greater than or equal to 22. The variance (s^2) exceeded the mean (\bar{x}) in all observed frequency distributions tested including total propagule number per quadrat and those observed frequencies from data converted to propagules per gram of dry soil in both 1980 and 1981. The variance to mean ratio (s^2/\bar{x}) greatly exceeded unity in all these frequency distributions, and data were not described by a Poisson distribution, which assumes that the variance is equal to the mean (Table 2). The observed frequency data are positively skewed, although differences in skewness are apparent between 1980 and 1981 (Fig. 1).

Observed frequencies of total propagules per quadrat in both 1980 and 1981 were best described by the negative binomial distribution, with k=4.87 and 1.10 for the 1980 and 1981 data, respectively. The k value is an index of dispersion where clumping or aggregation increases as the value of k decreases (11,31,33). When propagule numbers were adjusted to propagules per gram of dry soil for both years, none of the tested statistical distributions described the data well (Table 2). These frequency data show divergence in the higher frequency classes when observed and expected values are compared (Fig. 1B and D). This may be because the soil weight had relatively little to do with the amount of organic debris present in a sample, since this debris was confined almost entirely to the surface of the samples.

Distribution of foliar disease. Foliar disease distributions were dissimilar for 1980 and 1981 (Table 3). In 1980, most of the theoretical distributions, with the exception of the Poisson and Poisson with zeros, were not fitted because the mean exceeded the variance. The Poisson and Poisson with zeros did not adequately describe the observed frequency data. In 1981, the variance exceeded the mean, and the observed frequency distribution was best described by the negative binomial and Neyman type A distributions (Table 3).

There was no significant correlation between propagule level (total per sample) within a quadrat to degree of foliar blight in that quadrat in either 1980 or 1981.

DISCUSSION

Rhizoctonia spp. propagules were not randomly distributed horizontally in tall fescue organic debris. Observed frequency distributions for total propagules per quadrat sample were better described by the negative binomial or Neyman A. This indicated a horizontal clumping of propagules in the organic debris in soil. Sampling was performed for only 2 yr and was not performed sequentially within years; thus, the distributions described could

TABLE 1. Frequency of isolation of *Rhizoctonia* species recovered from tall fescue organic debris in June 1980 and 1981

	Percent is	
Source	1980	1981
Rhizoctonia zeae	45.3	48.2
Binucleate Rhizoctonialike fungi	39.6	42.6
Rhizoctonia solani	9.4	9.2
Unknown ^b	5.7	0

^a Fifty-three isolates recovered in 1980 and 54 in 1981.

Unknown isolates were morphologically similar to binucleate Rhizoctonialike fungi but possessed mostly three nuclei per cell.

not be claimed to be truly "contagious" (33), although spatial heterogeneity was indicated. Implications of nonrandom spatial population distributions to sampling precision and accuracy and to statistical analyses have been made by several authors (13,14,31,32).

The difference in k values between 1980 and 1981 for soil propagule distributions may have been due to differences in sample size between years (even though correction for this was attempted), a change in the apparent location of "clumps" due to pathogen movement over the disease season, and subsequent survival or mortality over winter, or differential rates of survival among individual sites of sampled area. The general conclusion of clumped

or nonrandom distribution of propagules, however, was upheld for both years.

With a closely aggregated perennial crop such as tall fescue turf, with pathogens that spread by rapid mycelial growth during periods favorable for disease, and with considerable mowing during disease development, the propagules might be expected to be randomly dispersed. Similar arguments have been proposed for distribution of Fusarium oxysporum f. cubense in soils (34). This was not the case for propagule distributions of Rhizoctonia spp. over the time frame studied. The fact that there were several species of Rhizoctonia sampled and that the relative levels of each contributing species were consistent between the years sampled

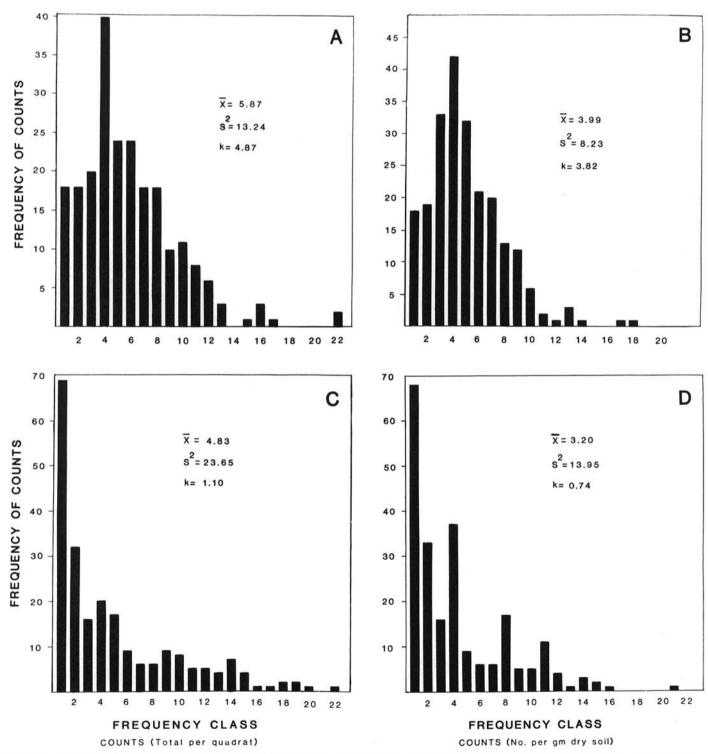


Fig. 1. Observed frequency distributions of *Rhizoctonia* propagule counts in 1980 (A = total, B = corrected to number per gram of dry soil) and 1981 (C = total, D = corrected number per gram of dry soil).

TABLE 2. Goodness-of-fit of theoretical frequency distribution to observed frequency data of *Rhizoctonia* propagules per quadrat sample in organic debris from tall fescue (*Festuca arundinacea*) turf

Year	Theoretical distribution		Probability of exceeding	
	Propagule no.	Distribution	χ^2 value	k^{b}
1980	Total	Poisson	0.000	
		Negative binomial	0.539	4.87
		Neyman type A	0.268	
	Corrected ^c	Poisson	0.0	
		Negative binomial	0.080	3.82
		Neyman type A	0.067	
1981	Total	Poisson	0.0	
		Negative binomial	0.229	1.102
		Neyman type A	0.0	
	Corrected	Poisson	0.0	
		Negative binomial	0.007	0.742
		Neyman type A	0.0	

^aOther theoretical distribution functions such as Thomas Double Poisson, Poisson-Binomial, and Poisson with zeros were tested but did not give a significant fit.

indicated the probable role of site microenvironment in propagule distribution. Many environmental factors—both biotic and abiotic—could influence inoculum density, including temperature, moisture, edaphic factors (13,17,18,22), and microbial antagonists, to mention a few. If such factors were not uniform over a field, differential inoculum levels could result.

R. zeae was found most frequently in this study and has been reported as pathogenic, but only mildly virulent, as a foliar pathogen on several grasses (19), although grass species were not specified. Our investigations (S. B. Martin, unpublished) have suggested that R. zeae can, under certain conditions, severely blight tall fescue, but, under normal temperatures for brown patch development, most isolates of R. solani from diseased tall fescue were more virulent than R. zeae isolates. These same data also indicated a very low virulence of binucleate RLF from soil debris on tall fescue.

Overwintering of R. solani in infected but asymptomatic plants has been suggested to be more important than previously thought (10,16). Studies on survival of R. solani and associated parasitic and saprophytic habits over time indicate that inoculum levels of the pathogen in field soil depends on time of sampling (22,27). These studies have been with annual field crops, but a similar situation may exist in R. solani populations in turf. Papavizas et al (22) and Roberts and Herr (27) reported higher inoculum levels of R. solani in the fall, with lower levels in the spring. This phenomenon was attributed to decomposition of substrates during the spring, resulting in lack of nutrient availability for R. solani survival. Inoculum of R. solani might build up during the parasitic phase on turf in summer, and subsequent soil populations should reflect this. Concomitant with an inoculum buildup during a favorable disease season and spread by mowing and by active parasitism and saprophytism, apparent inoculum (propagule) distribution and the relative proportions of Rhizoctonia species may also change. The propagule levels could not be correlated with foliar disease levels, and the lack of correlation could have been due to the heterogeneity of species and their relative virulence. Exact counts of specific propagule levels by species in each quadrat might better relate to subsequent foliar disease foci (27). This situation is complicated because of the several species of Rhizoctonia in turf soils; these species all grow on agar media used for enumeration of R. solani, and trapping methods do not distinguish R. solani from other Rhizoctonia spp. that may be present (S. B. Martin, unpublished). Therefore, relation of soil inoculum densities of Rhizoctonia spp. as a group to foliar disease (induced primarily by R. solani) is difficult. Environmental parameters and inoculum potential are also equally important and relate to differential disease development.

TABLE 3. Goodness-of-fit of theoretical frequency distribution to observed frequency data of severity of foliar blight on tall fescue in each quadrat

Year	s ^{2b}	$ar{x}^{\mathrm{c}}$	Theoretical distribution	Probability of exceeding χ^2	k
1980 ^d	0.699	0.799	Poisson	0.007	
			Poisson with zeros	0.0027	
1981	0.5645	0.4776	Poisson	0.1198	
			Poisson with zeros	0.2856	
			Negative binomial	0.5082	2.38
			Neyman type A	0.6036	

^aDisease severity rated per quadrat with a 1 to 5 scale, with 1 = no disease,

These data indicated that populations of overwintered propagules of *Rhizoctonia* in tall fescue organic debris are diverse, consisting of at least three species, and are not randomly distributed horizontally, and that the soil propagule density of *R. solani* is quite low. These data imply that fungi that closely resemble *R. solani* may be quite common in some soils and, therefore, the nuclear condition of RLF should be routinely determined to aid in identification.

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^bDispersion parameter of negative binomial distribution.

^c Propagule numbers corrected for number per gram of dry weight soil.

 $^{2 = \}text{slight}$, 3 = medium, 4 = severe, and 5 = very severe. $b^3 c^2 = \text{the sum of the squared deviations of individual ratings per quadrat from the mean divided by the total minus 1.$

c = a arithmetic average, or mean of ratings per quadrat.

^dOnly the Poisson and Poisson with zeros was fitted in 1980 because the $\overline{x} > s^2$

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