The Influence of *Pratylenchus penetrans* and Temperature on Black Root Rot of Strawberry by Binucleate *Rhizoctonia* spp.

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ABSTRACT

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Strawberry plants (cultivar Honeoye) exhibited symptoms characteristic of black root rot when grown in soil infested with binucleate *Rhizoctonia* spp. (*R. fragariae*) anastomosis groups (AG) A, G, and I. Monoxenically cultured lesion nematodes, *Pratylenchus penetrans*, added to soil at rates of 170, 90, and 17 nematodes per cubic centimeter of soil increased the severity of Rhizoctonia root rot at both 10 and 24 C. Higher nematode levels were more effective in increasing root rot caused by these fungi than were lower levels. Feeder root length was reduced by both pathogens. Root rot in the presence of both pathogens was higher at 24 than 10 C. Binucleate *Rhizoctonia* AG-I was more virulent than AG-A or AG-G at 10 C, whereas AG-G caused more disease at 24 C than did the other anastomosis groups.

Black root rot of strawberry (*Fragaria* × *ananassa* Duchnesne) is generally regarded as a disease of complex etiology (9,15,21). Whereas many fungi have been implicated as causal agents, binucleate *Rhizoctonia* spp. (*Rhizoctonia fragariae* Husain & McKeen) have been the fungi most convincingly demonstrated as causing the disease (3,7,10,12,18,23,24). Binucleate *Rhizoctonia* spp. have been separated into at least 15 anastomosis groups (AG) (14), with isolates of *R. fragariae* assigned to three of these (AG-A, AG-G, and AG-I) in Japan (13) and Connecticut (10). The differentiation of these fungi, first into binucleate species and then into anastomosis groups, explains much of the variation previously seen in experiments concerned with *Rhizoctonia* spp. pathogenic to strawberry. In Connecticut, AG-I was generally more pathogenic to strawberry than AG-A and AG-G (10).

Finally, the interaction of binucleate *Rhizoctonia* spp. with other soil organisms may be necessary to consistently reproduce the disease syndrome as seen in the field. The lesion nematode *Pratylenchus penetrans* (Cobb) Filip. & Schur.-Stek has been regarded as the primary agent causing the disease (6,8), as a predisposing factor necessary for fungal infection (2,21), or as a component of a disease complex (2,9,18). Surveys have implicated the nematode in the disease (5,19), and the pathogenic effect of *P. penetrans* to strawberry has been demonstrated (2,22). However, the lack of sterile nematodes available in quantity for experimental purposes or the lack of controlled experiments using both nematodes and specific fungi leave the role of *P. penetrans* in the development of black root rot open to question.

In the course of field surveys for binucleate *Rhizoctonia* spp. on strawberry roots, we often found both severely stunted plants and adjacent healthy plants infected with the same anastomosis group of binucleate *Rhizoctonia* spp. *P. penetrans* numbers were consistently higher in stunted plants than in the healthy plants (*unpublished*). The objective of the present study was to determine the effects of *P. penetrans*, temperature, and binucleate *Rhizoctonia* spp. on the severity of strawberry root rot.

MATERIALS AND METHODS

Sixteen-week-old cultivar Honeoye strawberry runner plants were heat-treated to free roots of fungal contaminants (11), trimmed to 5-cm lengths, and repotted in 200 cm^3 of a coarse field soil (86.8% sand, 10.0% silt, 3.2% clay, 1.6% organic matter, pH 5.4) previously fumigated with methyl bromide.

P. penetrans inoculum was prepared by blending monoxenic carrot cultures of *P. penetrans* for 15 sec, decanting and sieving to remove carrot pieces, pelleting the nematodes by centrifuging for 5 min at 2,000 rpm, and resuspending in water.

Binucleate *Rhizoctonia* spp. AG-A (isolate R-190), AG-G (isolate R-95), and AG-I (isolate R-92) were grown on autoclaved fescue seeds for 2 wk at room temperature (10). Autoclaved seeds alone served as an uninoculated control. The binucleate *Rhizoctonia* spp. isolates used in these experiments have been deposited with the ATCC.

Randomly chosen plants were inoculated with six sterile or *Rhizoctonia* spp. infested fescue seeds per pot (equivalent to approximately 1 g of infested seed per 4 kg of soil). A suspension of *P. penetrans* was added to the soil surface at rates of 0, 17, or 170 adults, juveniles, and eggs per cubic centimeter of soil. A small amount of moist, screened soil was added to cover the nematode and fungus inoculum. The plants were incubated 8 wk in growth chambers with 12 hr of light per day at 10 or 24 C. Each treatment was replicated five times in a factorial design.

After 8 wk, roots were gently washed free of soil, placed on a 35 × 40 cm piece of plastic that was divided into 1-cm^2 grids, and photographed. Nematodes were extracted from a 2-g subsample of root tissue by means of shaking or modified Baermann funnels.

Slides of photographed roots were projected and the number of healthy and rotted root intercepts with the grid were counted to determine the length of healthy and rotted roots (20). Main and lateral roots were distinguished from fine feeder roots. Percent of rotted roots was determined for main and lateral root length data.

The previous experiment was repeated using 16-wk-old cultivar Honeoye runner plants that were not heat-treated, root-trimmed, removed from soil, or wounded in any way. Plants were inoculated with the same binucleate *Rhizoctonia* spp. isolates using the same inoculum levels. A suspension of *P. penetrans* obtained from monoxenic carrot culture was used at rates of 0 or 90 adults, juveniles, or eggs per cubic centimeter to infest the soil as described previously. Plants were again incubated at 10 or 24 C with 12 hr of light per day, with five replications per treatment. After 8 wk, data was collected as described previously.

RESULTS

Cortical root rot developed on heat-treated, root-pruned strawberries inoculated with binucleate *Rhizoctonia* spp.

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Symptoms were similar to those observed for field-grown plants affected by black root rot (Table 1). Root infection by *P. penetrans* consistently increased the severity of root rot caused by binucleate *Rhizoctonia* spp. High nematode inoculum levels of 170 *P. penetrans* per cubic centimeter of soil were more effective in this respect than were 17 *P. penetrans* per cubic centimeter of soil. Fungal root rot in combination with nematode infection was consistently higher at 24 than 10 C. Binucleate *Rhizoctonia* spp. AG-I was more virulent than AG-A and AG-G at 10 C, whereas the AG-G isolate was more virulent at 24 C than AG-I and AG-A.

Main and lateral root length was less at 10 than at 24 C for all fungal treatments. Root length was further reduced at both temperatures by root infection with nematodes and fungi. Reduction in root lengths in nematode-infested treatments was more pronounced at 24 than 10 C, with 26.3 and 8.2% reductions in root length at high nematode levels compared with nematode-free roots at 24 and 10 C, respectively. Feeder root length was not affected by temperature, but was reduced 7.0 and 35.5% by root infection with low and high nematode levels, respectively, when averaged over fungus treatments.

Root rot severity in runner plants inoculated with nematodes and fungi without root pruning or heat treatment was much less than in the previous experiment (Table 2). Percent of root rot of roots infected with binucleate *Rhizoctonia* spp. alone was not significantly different from uninoculated roots. However, when roots were infected with both *Rhizoctonia* spp. and *Pratylenchus*, symptom severity was greater than for plants infected with fungi alone. This interaction was more pronounced at 24 than at 10 C. Nematode activity and reproduction was greater at the higher temperature, and fungal root rot in combination with nematode infection was consistently higher at 24 than at 10 C.

Root rot severity did not differ among plants inoculated with binucleate *Rhizoctonia* spp. at 10 C, but at 24 C AG-G induced more root rot than the other anastomosis group isolates, alone and in combination with *P. penetrans*.

Main and lateral root length was not significantly reduced by infection with nematodes or fungi. Feeder root length was reduced by infection with binucleate *Rhizoctonia* spp. but not by *P. penetrans* infection. Plants infected with each of the three anastomosis groups had 21.1–27.7% fewer feeder roots than did the uninoculated plants. There were no differences between anastomosis groups.

Root rot was much greater in heat-treated, pruned roots infested with *P. penetrans* and binucleate *Rhizoctonia* spp. than in untreated plants infested with the same pathogens. Roots grew more quickly after heat treatment and root pruning and were about 40% larger than the untreated plants. Nematode infection decreased main and lateral root systems by 8.2–26.3% at 10 and 24 C, respectively, for heat-treated, pruned plants, and had no significant effect on the size of untreated roots. Feeder roots were reduced by 35.5, 22.0, and 7.0% at inoculum levels of 170, 90, and 17 *P. penetrans* per cubic centimeter of soil regardless of root treatment.

Table 1. Effect of binucleate *Rhizoctonia* spp. (BNR), *Pratylenchus penetrans* (Pp), and temperature on percent root rot, root length, and nematode reproduction in heat-treated, root-pruned strawberries, spring 1987[a]

BNR AG[b]	Pi[c]	Root rot (%)[d]	Root length: Main + lateral (cm)	Root length: Feeder (cm)	Pf[e]
		Incubation temperature = 10 C			
A	0	25.8	459.8	1,257.7	0.0
A	17	30.6	647.4	1,492.5	10.3
A	170	36.1	517.6	1,071.7	87.3
G	0	27.1	531.7	1,259.9	0.0
G	17	27.0	497.8	958.3	12.7
G	170	36.3	521.4	1,098.7	91.2
I	0	36.4	527.3	1,124.6	0.0
I	17	31.4	520.1	1,178.2	14.2
I	170	51.7	406.7	712.8	111.0
None	0	22.8	581.1	1,214.4	0.0
		Incubation temperature = 24 C			
A	0	30.1	662.0	1,484.2	0.0
A	17	41.0	629.5	1,197.7	53.8
A	170	69.8	528.6	792.3	111.0
G	0	32.7	707.9	1,191.1	0.0
G	17	45.4	576.7	999.4	39.2
G	170	81.6	486.2	609.1	95.1
I	0	38.0	746.0	1,424.1	0.0
I	17	36.6	646.5	1,328.5	25.0
I	170	69.6	561.0	675.7	88.2
None	0	40.0	730.7	1,337.6	0.0
df = 74	Mean square error	0.01	9,257.3	106,213.5	1,153.1
Contrasts					
With BNR vs. without BNR		0.01	0.01	NS[f]	
BNR vs. BNR + Pp		0.0001	0.01	0.001	
BNR + Pp −24 C vs. BNR + Pp −10C		0.001	0.05	NS	
AG-I vs. AG-A + AG-G −10 C		0.01	NS	NS	
AG-G vs. AG-A + AG-I −24 C		0.05	NS	NS	
High Pp vs. low Pp		0.001	0.01	0.001	
AG-G + Pp vs. AG-A + AG-I + Pp −10 C		NS	NS	NS	
AG-I + Pp vs. AG-A + AG-G + Pp −10 C		0.01	0.05	NS	
AG-I + Pp vs. Ag-A + AG-G + Pp −24 C		NS	NS	NS	

[a] Data represent mean of four replications.
[b] Binucleate *Rhizoctonia* spp. anastomosis group.
[c] Initial population density of *P. penetrans* per cubic centimeter of soil inoculated in suspension.
[d] Data analyzed as arc sine (square root of percent healthy root).
[e] Final population density of *P. penetrans* per gram of root tissue.
[f] NS = not significant.

DISCUSSION

The black root rot complex of strawberries in Connecticut appears to involve both binucleate *Rhizoctonia* spp. and plant parasitic lesion nematodes. Both organisms have been implicated in the disease by other workers (2,17) and by surveys of strawberry fields affected by black root rot (5,10,19). Surveys of Connecticut strawberry fields associating *P. penetrans* root infection with severe black root rot symptoms in the presence of the same anastomosis group of binucleate *Rhizoctonia* spp. were the basis for the experiments reported here. Field surveys have demonstrated that binucleate *Rhizoctonia* spp. inoculum potential in field soils may be very high (10), and that *P. penetrans* recovery from strawberry roots is commonly in the range of 250–800 nematodes per gram of root (5,16). *P. penetrans* recovery from soil is typically much less than from roots (2). Experimental inoculum levels of 1 g of infested fescue seed per 4 kg of soil and 17–170 *P. penetrans* in suspension per cubic centimeter of soil represent realistic and common inoculum levels, as shown by final nematode densities in roots. High nematode mortality resulting from infestation of soil with a nematode suspension is reflected in *P. penetrans* root densities of 10.3–357.3 per gram of

root 8 wk after inoculation. Final root weights ranged from 4 to 16 g per plant.

P. penetrans has been demonstrated to be pathogenic to strawberry roots under aseptic conditions (2,22) and in the presence of other organisms (2,8). In these experiments, typical symptoms of *P. penetrans* infection were readily distinguished from black root rot symptoms caused by fungi. Binucleate *Rhizoctonia* spp. readily caused root rot symptoms on cultivar Honeoye strawberries at 10 and 24 C, regardless of nematode infection. However, *P. penetrans* infection dramatically increased the severity of root rot symptoms caused by these fungi in both heat-treated, root-pruned plants and in untreated plants. High *P. penetrans* populations were more effective than low populations in this respect. The severity of root rot symptoms increased for all three anastomosis group isolates (AG-A, AG-G, and AG-I) in the presence of *P. penetrans*, but was especially pronounced with AG-I at 10 C and AG-G at 24 C. This ranking appears to be a reflection of the ability of these anastomosis groups to cause disease at these temperatures, rather than a specific interaction of any one anastomosis group isolate with *P. penetrans*. AG-G was consistently more virulent at 24 C than was AG-A or AG-I. At 10 C, AG-I was the most virulent, confirming the results of previously reported pathogenicity studies (10).

In both experiments reported here, fungus-free Honeoye runner plants were grown in the same soil type and were inoculated with the same rates of the same fungal isolates. The soil was infested with similar levels of the same nematode and was subjected to the same incubation conditions. Thus, the major difference between the two experiments was whether the runner plant roots were heat-treated and root-pruned or not. Presumably, the difference in root rot severity demonstrated between the two experiments was a function of these differences in root treatment. Chen and Rich (2) reported that root-pruned plants quickly produced new roots that were readily attacked by *P. penetrans*, resulting in a reduction in root length by the nematode. Plants that were not root-pruned had slower growth and no such reduction in root size after 6 wk. They reported no differences in root rot ratings, even though fungi were isolated more frequently from pruned than unpruned roots. Fast-growing young roots may be more susceptible to nematode and fungal attack than older roots, or the stress associated with heat treatment or root pruning may predispose roots to fungal infection.

Our data indicate that temperature appears to be the factor determining whether binucleate *Rhizoctonia* spp. AG-G or AG-I predominates. AG-I causes more disease and results in more root death with or without nematodes at cooler temperatures and may be more active in late fall to early spring. AG-G is more virulent under warmer conditions, and may be the predominant cause of black root rot during the summer. Survey results have indicated that AG-G is the predominant fungus isolated from strawberry roots in the spring and AG-I is most common in fall samples (10). Roots infected with AG-G during warm periods or AG-I during cool periods will tend to be destroyed more quickly and completely than roots infected with isolates causing less disease at those temperatures. The differential survival of roots colonized by these anastomosis groups may well account for these survey results.

P. penetrans is most active at warmer temperatures, but this nematode has been shown to be active and cause plant damage at cool temperatures down to 7 C (4). *R. solani* Kühn and *P. minyus* have been associated with a root rot of winter wheat in which root degradation by both nematodes and fungi continues through the winter leading to early stunting of plants (1). A similar situation may occur in perennial strawberries. Long-term studies of the interaction of these two organisms during the long periods of root inactivity associated with cold weather, as well as determination of the role of nematodes and fungi in root disease development over summer conditions, are needed.

Table 2. Influence of binucleate *Rhizoctonia* spp. (BNR), *Pratylenchus penetrans* (Pp), and temperature on percent root rot, root length, and nematode reproduction in undisturbed strawberry roots, fall 1987[a]

BNR AG[b]	Pi[c]	Root rot (%)	Root length: Main + lateral (cm)	Root length: Feeder (cm)	Pf[d]
		Incubation temperature = 10 C			
None	0	10.7	346.6	852.5	0.0
None	90	13.4	321.2	638.0	108.4
A	0	9.6	268.9	605.8	0.0
A	90	8.4	294.0	606.6	70.7
G	0	8.7	265.3	616.0	0.0
G	90	14.2	283.5	648.2	67.6
I	0	13.9	292.8	651.3	0.0
I	90	9.8	329.2	647.4	34.3
		Incubation temperature = 24 C			
None	0	10.4	322.4	1,110.5	0.0
None	90	11.8	300.3	695.4	230.9
A	0	9.6	260.8	740.9	0.0
A	90	15.6	311.0	613.6	357.3
G	0	12.4	278.9	672.5	0.0
G	90	26.5	298.1	612.0	235.4
I	0	9.1	305.8	678.1	0.0
I	90	14.2	284.5	622.7	250.8
df = 60	Mean square error	45.16	4,648.0	59,659.8	15,159.9
Contrasts					
With BNR vs. without BNR		NS[e]	NS	0.01	
BNR vs. BNR + Pp		0.05	NS	NS	
BNR + Pp −24 C vs. BNR + Pp −10 C		0.01	NS	NS	
AG-I vs. AG-A + AG-G −10 C		NS	NS	NS	
AG-G vs. Ag-A + AG-I −24 C		0.01	NS	NS	
AG-G + Pp vs. AG-I + AG-A + Pp −10 C		NS	NS	NS	
AG-I + Pp vs. AG-A + AG-G + Pp −10 C		NS	NS	NS	

[a] Data represent mean of five replications.
[b] Binucleate *Rhizoctonia* spp. anastomosis group.
[c] Initial population density of *P. penetrans* per cubic centimeter of soil inoculated in suspension.
[d] Final population density of *P. penetrans* per gram of root tissue.
[e] NS = not significant.

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