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Comparison of *Pratylenchus penetrans* Infection and *Maladera castanea* Feeding on Strawberry Root Rot

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Abstract: The interaction of lesion nematodes, black root rot disease caused by *Rhizoctonia fragariae,* and root damage caused by feeding of the scarab larva, *Maladera castanea,* was determined in greenhouse studies. Averaged over all experiments after 12 weeks, root weight was reduced 13% by *R. fragariae* and 20% by *M. castanea.* The percentage of the root system affected by root rot was increased by inoculation with either *R. fragariae* (35% more disease) or *P. penetrans* (50% more disease) but was unaffected by *M. castanea. Rhizoctonia fragariae* was isolated from 9.2% of the root segments from plants not inoculated with *R. fragariae.* The percentage of *R. fragariae*-infected root segments was increased 3.6-fold by inoculation with *R. fragariae* on rye seeds. The presence of *P. penetrans* also increased *R. fragariae* root infection. The type of injury to root systems was important in determining whether roots were invaded by *R. fragariae* and increased the severity of black root rot. *Pratylenchus penetrans* increased *R. fragariae* infection and the severity of black root rot. Traumatic cutting action by Asiatic garden beetle did not increase root infection or root disease by *R. fragariae.* Both insects and diseases need to be managed to extend the productive life of perennial strawberry plantings.

Key words: Asiatic garden beetle, black root rot, *Fragaria* × *ananassa,* interaction, lesion nematode, *Maladera castanea, Pratylenchus penetrans, Rhizoctonia fragariae,* scarab, strawberry.

A complex of root diseases and root-feeding insects adversely affects perennial strawberry (*Fragaria* × *ananassa*) culture in the northeastern United States by compromising root health and plant vigor. The most important of these are strawberry black root rot and root damage by white grubs and root weevils (Schloemann, 2002). Black root rot is a debilitating root cortical disease caused by the binucleate fungus *Rhizoctonia fragariae* (Husain and McKeen, 1963; Wilhelm and Nelson, 1970; Wilhelm et al., 1972). This root disease results in severe losses due to a reduction in plant vigor, fruit yield, and the productive life of a perennial planting. The lesion nematode, *Pratylenchus penetrans,* has been shown to increase the severity of black root rot under field conditions (Goheen and Bailey, 1955; Goheen and Smith, 1956; LaMondia, 1994, 1999) and in controlled experiments (Chen and Rich, 1962; LaMondia, 2003: LaMondia and Martin, 1989). The mechanism by which *P. penetrans* increases black root rot appears to be local within the root rather than systemic in the plant, and at least partially due to direct effects of nematode feeding such as cortical cell damage and death. Indirect effects included discoloration of the endodermis and early polyderm formation, resulting in the isolation of the cortex from the stele and leading to early secondary growth of structural roots and senescence of the cortex. This secondary growth ultimately results in woody perennial roots without cortical tissues (Esau, 1977). Weakened or dying cortical cells caused directly or indirectly by *P. penetrans* are more susceptible to *R. fragariae,* leading to increased disease (LaMondia, 2003).

In previous work (LaMondia et al., 2002), and in field surveys of growers' fields, we determined that oriental beetle, *Exomala orientalis,* and Asiatic garden beetle, *Maladera castanea,* were the most commonly injurious white grub pests of strawberry in Connecticut. White grubs are indiscriminate feeders, grazing on the root system and surrounding soil. Root feeding by scarabs such as Asiatic garden beetle may also open infection courts for pathogens such as *R. fragariae.* The objective of this research was to determine whether (i) root damage by *M. castanea* increased the severity of black root rot and (ii) *P. penetrans* and *M. castanea* had similar influence on disease and *R. fragariae* infection.

Materials and Methods

The effects of *P. penetrans, R. fragariae,* and *M. castanea* on strawberry black root rot were investigated in a 2 × 2 × 2 factorial design (infested or not infested for each of the three organisms) in three greenhouse experiments. Single 1-year-old 'Honeoye' strawberry crowns obtained from a commercial nursery (Nourse Farms, S. Deerfield, MA) were planted in 10-cm-diam. pots containing 450 cm^3 pasteurized Merrimac sandy loam field soil (73.4% sand, 22.3% silt, 4.3% clay, pH 6.0). Crowns were transplanted to pots on 2 December 2000 for the first experiment, 15 March 2001 for the second experiment, and 24 September 2001 for the third experiment. One week after transplanting, plants were inoculated with *P. penetrans, R. fragariae,* and(or) *M. castanea* to provide eight factorial treatments with six replications arranged in a randomized complete block design. Inoculum of *P. penetrans* was extracted from carrot disk cultures. For experiment 1, 4 ml of a suspension containing 1,800 nematodes was applied in 1-ml quantities to four 2-cm-deep holes per treated pot, 3 cm from the crown of the plant. In the second and third experiments, 800 or 1,200 nematodes, respectively, were applied in 3-ml total volume per treated pot in 1-ml quantities to three 2-cm-deep holes per treated

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pot. This inoculum level is a small fraction of the nematode population (up to 2,500 nematodes per gram of root tissue) that can be recovered from field-grown strawberry roots, but represented a population that would be expected to cause root injury (50 to 150 nematodes per gram of root; LaMondia, unpubl. data). Non-inoculated plants received water in four 2-cm-deep holes per pot. Inoculum of *R. fragariae* was prepared on autoclaved rye seeds (Martin, 1988). Anastomosis groups (AG) A, G, and I were introduced on one colonized rye seed/AG for each infested pot. Inoculation into the pot was conducted in the same manner as for introduction of *P. penetrans,* but the new holes were placed in a staggered pattern to prevent carrying the previously introduced *P. penetrans* inoculum across treatments. Non-inoculated plants received three sterile (autoclaved) seeds per pot.

Third instar Asiatic garden beetle larvae were obtained from residential lawns in Mansfield, Connecticut, by using a shovel or motorized sod cutter to undercut the fescue turf, then shaking the larvae out of the roots and soil. Larvae were collected on 1 December 2000 for the first experiment, 14 March 2001 for the second experiment, and 23 September 2001 for the third experiment. Larvae were placed in adequate soil to prevent aggressive encounters and fed perennial ryegrass seed until inoculated into pots (1 day after field collection). Larvae were placed in pots containing strawberry plants by making a 2-cm-deep depression with a pencil and allowing 1 (first experiment) or 2 (second and third experiments) larvae per pot to burrow into the soil. Larvae not digging into the soil within 1 minute were replaced. The numbers of larvae inoculated into pots were similar to the population densities observed in heavily infested strawberry fields (Cowles, unpubl. data). Although larvae were collected at different times of the year, the feeding activity in each experiment reflected the springtime feeding leading up to pupation. By conducting these experiments during the winter and early spring, the potential for additional egg laying by scarabs was avoided, as this normally would take place in late July or early August.

The greenhouse conditions varied with each experiment due to the time of year. The greenhouses were heated to maintain temperatures between 18 °C and 29 °C. Overhead irrigation provided water as needed.

After 12 weeks in the greenhouse, the root system was washed free of soil, and roots were individually evaluated for black root rot. The percentage of the total root system with cortical rot was estimated visually by two persons and the independent ratings were averaged. Insect larvae were sifted from the soil and counted. Fresh shoot and root weights were recorded. Isolation of *R. fragariae* was attempted from ten 0.5-cm sections of surface-sterilized structural and perennial roots. Roots were exposed to 0.5% NaOCl for 30 seconds, rinsed with sterile distilled water, and placed on acidified water agar for 96 hours. Percentage root infection by *R. fragariae* was calculated from the number of infected root sections as a percentage of the total. Nematodes were extracted from 2 g of structural root tissue placed in a flask containing 50 ml water, shaken for 7 days using a wrist-action shaker, and then counted.

Insect larvae were still present in soil at the end of experiments 1 and 3. Larvae had pupated and emerged as adults during experiment 2. Experiments 1 and 3 were therefore analyzed using a general linear model nested ANOVA with the randomized complete block design nested within experiment. Data from experiment 2 were analyzed separately using a randomized complete block design ANOVA (SAS Institute, Cary, NC).

RESULTS

Experiments 1 and 3: Maladera castanea larvae were consistently recovered from pots at the conclusion of experiments 1 and 3 and both root and shoot weights were reduced ($P = 0.001$ and 0.01, respectively) as a result of larval feeding (25.8% and 9.1%, respectively) (Table 1). *Rhizoctonia fragariae* also reduced ($P = 0.004$) root weight (13%) but not shoot weight over the 12-week duration of the experiments. The introduction of *P. penetrans* to pots did not affect root or shoot weights. The percentage of the root system affected by black root rot was increased ($P = 0.0001$) by inoculation with either *R. fragariae* (51% more disease) or *P. penetrans* (58% more disease). The presence of Asiatic garden beetles did not increase the severity of black root rot. *Rhizoctonia fragariae* was isolated from 9.2% of the root segments from plants not inoculated with *R. fragariae* (averaged over all three experiments). The percentage of *R. fragariae*-infected root segments was increased 4-fold by inoculation of the soil with *R. fragariae* on rye seeds. Lesion nematodes were recovered from all inoculated plants in similar numbers and were not present in non-inoculated plants. Inoculation with *R. fragariae* or Asiatic garden beetles did not affect *P. penetrans* numbers per 2 g root. The interaction ($P = 0.005$) of all three pathogens or pests on percentage black root rot resulted from the low level of disease that occurred on plants not inoculated with any of these organisms. The *P. penetrans* by *R. fragariae* interaction ($P = 0.004$) was the result of higher levels of *R. fragariae* recovery for treatments with *P. penetrans* compared to those without, when *R. fragariae* was not inoculated into pots (17.5% vs. 8.3%, respectively).

Experiment 2: Maladera castanea larvae were inoculated into but not recovered from any pots at the conclusion of experiment 2. Therefore, we analyzed the data from experiment 2 separately from experiments 1 and 3. The timing of experiment 2 (March to June, 2001) provided conditions resulting in early pupation and adult emergence. As a result, any larval root feeding was confined

TABLE 1. The effects of lesion nematode *Pratylenchus penetrans* (Pp), fungal pathogen *Rhizoctonia fragariae* (Rf), and Asiatic garden beetle *Maladera castanea* (AGB) on root disease, *R. fragariae* infection, nematode extraction from roots, and plant vigor (experiments 1 and 3).

Treatment factors[a]			Percent BRR[b]	Percent Rf isolation[c]	Pp per 2g root	Root wt (g)	Shoot wt(g)
Pp–	AGB+	Rf–	12.5	8.3	0.0	9.4	13.9
Pp–	AGB+	Rf+	16.2	56.7	0.0	8.4	11.7
Pp–	AGB–	Rf–	7.4	8.3	0.0	13.0	14.5
Pp–	AGB–	Rf+	16.8	45.0	0.0	11.7	13.3
Pp+	AGB+	Rf–	17.5	12.5	57.4	10.7	14.0
Pp+	AGB+	Rf+	25.0	48.3	81.3	8.5	12.5
Pp+	AGB–	Rf–	16.9	22.5	88.3	13.4	14.5
Pp+	AGB–	Rf+	24.1	55.8	73.1	11.6	14.7
	Main Effects						
	Pp+		20.9	34.8	75.0	11.0	13.9
	Pp–		13.2	29.6	0.0	10.6	13.4
			P = 0.0001	ns[d]	0.0001	ns	ns
	Rf+		20.5	51.5	38.6	10.0	13.1
	Rf–		13.6	12.9	36.4	11.5	14.2
			P = 0.0001	0.0001	ns	0.004	ns
	AGB+		17.8	32.9	34.7	9.2	13.0
	AGB–		16.3	31.5	40.3	12.4	14.3
			P = ns	ns	ns	0.001	0.01
	Interactions (P value)						
	Pp × AGB		ns	0.05	ns	ns	ns
	Pp × Rf		ns	0.004	ns	ns	ns
	AGB × Rf		ns	ns	ns	ns	ns
	Pp × AGB × Rf		0.005	ns	ns	ns	ns

[a] A 2 × 2 × 2 factorial with six replicates performed two times (experiments 1 and 3). Data were analyzed by general linear model nested analysis of variance with the randomized complete block design nested within experiment.

[b] Percentage black root rot (BRR) determined as a percent of the root system.

[c] Percentage of ten 0.5-cm root segments from which *R. fragariae* was recovered on water agar.

[d] ns = not significant ($P > 0.05$).

TABLE 2. The effects of lesion nematode *Pratylenchus penetrans* (Pp), fungal pathogen *Rhizoctonia fragariae* (Rf), and Asiatic garden beetle *Maladera castanea* (AGB) on root disease, *R. fragariae* infection, nematode extraction from roots, and plant vigor (experiment 2).

Treatment factors[a]			Percent BRR[b]	Percent Rf isolation[c]	Pp per 2g root	Root wt (g)	Shoot wt (g)
Pp–	AGB+	Rf–	20.7	0.0	0.0	8.8	20.8
Pp–	AGB+	Rf+	21.8	8.3	0.0	5.8	18.0
Pp–	AGB–	Rf–	17.5	0.0	0.0	9.1	18.3
Pp–	AGB–	Rf+	22.7	30.0	0.0	7.3	16.0
Pp+	AGB+	Rf–	25.5	6.7	20.8	8.5	22.2
Pp+	AGB+	Rf+	37.2	50.0	53.8	8.5	16.6
Pp+	AGB–	Rf–	27.7	0.0	75.8	7.8	23.2
Pp+	AGB–	Rf+	24.3	10.0	43.3	7.4	23.1
	Main Effects						
	Pp+		28.7	16.7	48.5	8.0	21.3
	Pp–		20.7	9.6	0.0	7.7	18.3
			P = 0.01	ns[d]	0.0001	ns	ns
	Rf+		26.5	24.6	24.3	7.3	18.4
	Rf–		22.9	1.7	24.2	8.5	21.2
			P = ns	0.0001	ns	ns	ns
	AGB+		26.3	16.3	18.7	7.9	19.4
	AGB–		23.1	10.0	29.8	7.9	20.2
			P = ns	ns	ns	ns	ns
	Interactions (P value)						
	Pp × AGB		ns	ns	ns	ns	ns
	Pp × Rf		ns	0.0001	ns	ns	ns
	AGB × Rf		ns	ns	0.04	ns	ns
	Pp × AGB × Rf		ns	0.0005	0.04	ns	ns

[a] A 2 × 2 × 2 factorial with six replicates (experiment 2). Data were analyzed by a randomized complete block design analysis of variance.

[b] Percentage black root rot (BRR) determined as a percent of the root system.

[c] Percentage of ten 0.5-cm root segments from which *R. fragariae* was recovered on water agar.

[d] ns = not significant ($P > 0.05$).

to the initial stages of the experiment and there were no differences in root or shoot weights resulting from insect inoculation (Table 2). None of the treatments affected root or shoot weights. *Pratylenchus penetrans* increased ($P = 0.01$) the percentage of the root system with black root rot by 38.6%. *Rhizoctonia fragariae* inoculation did not increase disease but increased ($P = 0.001$) the recovery of *R. fragariae* from the root system. The presence of Asiatic garden beetles did not increase the severity of black root rot. Lesion nematodes were recovered from all inoculated plants in similar numbers and were not present in roots of non-inoculated plants. Inoculation with *R. fragariae* or Asiatic garden beetles did not affect *P. penetrans* numbers per 2 g root. The significant two- and three-way interactions of pathogens or pests on *R. fragariae* and *P. penetrans* recovery (Table 2) resulted from the numbers of zero values in the data sets for recovery of *R. fragariae* or *P. penetrans* in the non-inoculated treatments rather than biological reasons.

Discussion

Strawberry black root rot and root-feeding insects each impact perennial strawberry production in the northeastern United States. The resulting decline in plant vigor and fruit yield shortens the productive life of a perennial planting, often by several years. The loss of a fruiting year and additional costs required during replanting have a dramatic negative impact on economic return (DeMarree and Rieckenberg, 1998). In our experience, the combined effects of root disease and root-feeding insects such as white grubs and weevil larvae affect many declining fields. The impact of combined root feeding and black root rot in causing plant collapse suggested to us that root feeding by scarab larvae (white grubs) could enhance root infection in a similar manner to lesion nematodes (LaMondia, 2003) by opening up additional sites on roots where *R. fragariae* may enter the injured root system.

Injury or stress to strawberry root tissue is important for increasing infection by *R. fragariae*. It appears that *R. fragariae* commonly resides on the sloughed cortex of healthy perennial roots (LaMondia, 2003; Wilhelm and Nelson, 1970; Wilhelm et al., 1972). Our ability to recover *R. fragariae* from plants that had not been inoculated is consistent with our previous observations that plants obtained from nurseries often have a low incidence of infection by the fungus. From any source of inoculum, the fungus may then infect structural or feeder roots, especially when the plant is under stress or roots are damaged by lesion nematodes. Nematode feeding and movement result in direct cortical cell damage and death, and indirectly result in discoloration of the endodermis and early polyderm formation, followed by localized areas of secondary growth initiation in structural roots and cortical cell weakening or death (LaMondia, 2003; Townshend, 1963; Zunke, 1990). Weakened or dying cells resulting from direct or indirect effects of *P. penetrans* are more susceptible to *R. fragariae,* thereby increasing infection and cortical root rot.

Our initial hypothesis was that root feeding and cortical damage by scarabs such as the Asiatic garden beetle might also open infection courts for *R. fragariae.* Any differences in the development of black root rot between treatments inoculated with *M. castanea* or *P. penetrans* might give some insight into the mechanism of the interaction between *R. fragariae* and *P. penetrans.* The data suggest that whereas *M. castanea* damaged roots and reduced root weight, there was no increase in black root rot severity resulting from white grub feeding. The demonstration that lesion nematodes increased root disease and root infection by *R. fragariae* under the same conditions indicates that the root damage caused by a range of lesion nematode densities was more conducive to *R. fragariae* infection than the more significant root loss due to insect feeding. Apparently, the kind of injury sustained by root systems is important relative to invasion by *R. fragariae.* Traumatic cutting action by cortical-feeding insects may actually decrease the amount of tissue available for fungal colonization, perhaps leaving only the cut ends of roots available for immediate infection. Nematode feeding and movement through roots, on the other hand, cause individual cell death and lesions consisting of dead or damaged cortical cells without directly reducing the total root surface area. The areas of *P. penetrans* infection, consisting of both dead and weakened cells as well as the localized stimulation of early secondary growth resulting in senescence of cortical cells (LaMondia, 2003; Townshend, 1963), appeared to be more suitable for extensive *R. fragariae* infection than the cut ends of roots caused by *M. castanea* feeding.

Asiatic garden beetle was chosen as a representative of the larger complex of root-chewing insects, including the other scarab species mentioned previously, and root weevils (*Otiorhynchus sulcatus, O. ovatus,* and *O. rugosostriatus*). In all instances, these root feeders cut roots while feeding, and we might expect the interactions between *R. fragariae* and these chewing insects to be similar.

In previous work (LaMondia et al., 2002), we documented the abundance of white grubs in experimental plots of strawberry plantings, including oriental beetle. Japanese beetle, Asiatic garden beetle, and European chafer (*Exomala orientalis, Popillia japonica, Maladera castanea,* and *Rhizotrogus majalis,* respectively). Additional surveys of commercial fields in Connecticut determined that oriental beetle and Asiatic garden beetle are the most common injurious white grub pests in strawberry plantings. White grubs are indiscriminate feeders, grazing on both the plant root system and surrounding soil. Under crowded population conditions, these larvae can destroy not only the root system but will also consume the entire cortex of the strawberry crown. The recent registration of imidacloprid for management of white grubs in strawberries has been valuable for suppressing oriental beetle, Japanese beetle, and European chafer populations. However, imidacloprid is less effective against Asiatic garden beetles, and damaging populations can survive insecticide treatment (Cowles, unpubl. data). The reduced sensitivity of Asiatic garden beetle to imidacloprid (relative to other white grub species) makes understanding the impact of this species on strawberry growth and black root rot especially important.

Interestingly, we have observed in commercial fields that the response of different varieties of strawberries to combined root feeding and black root rot varies, with some varieties (e.g., 'Allstar' and 'Annapolis') remaining quite vigorous while nearby varieties (e.g., 'Honeoye' and 'Kent') collapsed. While root chewing by insects does not increase root rot, the varietal differences in response to the combined injuries suggest that tolerance to root feeding and disease may make the difference between the plant being able to recover by re-growing lost tissue versus dying. Variation among commercially available cultivars in susceptibility to black vine weevil (Cowles, 2004) and to black root rot organisms (Dale and Potter, 1998) appears to be independent, so certain cultivars are better able than others to tolerate these combined challenges. The overall ability of the strawberry plant to survive requires a critical level of root function, which dictates that both insects and diseases need to be managed to extend the productive life and increase the economic return on a perennial strawberry planting.

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