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Plant-parasitic Nematodes Associated with Cherry Rootstocks in Michigan

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Abstract: In two field trials, 10-year-old sweet and tart cherry rooted on 'Mazzard', 'Mahaleb', 'MXM 2', 'MXM 14', 'MXM 39', 'MXM 60', 'MXM 97', and 'Colt' showed 10-203 *Pratylenchus penetrans* per g fresh root from all tart rootstocks, and up to 46 *Pratylenchus, Criconemella,* and *Xiphinema* spp. per 100 cm^3 soil. Infestation of soil containing 1-year-old Mazzard, Mahaleb, MXM 60, 'GI148-1', and 'GI148-8' with 625/100 cm^3 soil of either *P. penetrans* or *C. xenoplax* resulting in final nematode population densities of 123–486 and 451–2,496/g fresh root plus 100 cm^3 soil, respectively, and had little effect on plant height or dry weight after 157 days in a greenhouse. Population densities of neither nematode differed among the five rootstocks. In a second greenhouse experiment, soil containing the same rootstocks was infested with *P. penetrans* (1,250/100 cm^3 soil), maintained for 8 months in a greenhouse, 4 months in a cold room (2–4 C), and 3 additional months in a greenhouse. The number of *P. penetrans* recovered at the end of 475 days was approximately 10% of those recovered in the first experiment, probably due to the cold treatment. The ability of *P. penetrans* and *C. xenoplax* to infect the cherry rootstocks may be of concern in cherry management programs.
Key words: cherry, *Criconemella xenoplax, Helicotylenchus, lesion nematode, management, Meloidogyne,* nematode, *Pratylenchus penetrans, Prunus avium,* ring nematode, root-knot nematode, spiral nematode, rootstock, *Xiphinema americanum.*

As part of a Michigan stone fruit decline project, a survey of plant-parasitic nematodes was conducted in 26 sweet cherry (*Prunus avium*) orchards. *Pratylenchus, Criconemella, Xiphinema, Meloidogyne,* and *Paratylenchus* spp. were found in 24, 11, 15, 17, and 17 orchards, respectively, with *P. penetrans* as the most prominent species (14). Further analysis of five, 7- to 15-year-old orchards propagated on 'Mazzard' rootstock showed over 1,000 *P. penetrans*/g fresh root weight; however, there was no difference in nematode numbers between apparently healthy and unhealthy trees (14). Thus, nematodes may not be the only factor affecting tree health, or there may be some level of tolerance to nematodes in Mazzard cherry rootstock.

Mazzard and 'Mahaleb' are the rootstocks most commonly used for propagation of sweet and tart cherry trees (18). Significant resources are being invested in the development and evaluation of new rootstocks with desirable horticultural qualities for Michigan and elsewhere (3). The lack of information on the reactions to nematodes of the new rootstock materials, along with the known associations of *P. penetrans* (11,12,15), *C. xenoplax* (8,9,16,17,21), and *X. americanum* (20) with stone fruit decline, prompted us to evaluate new rootstocks as hosts for plant-parasitic nematodes. The objectives of this study were to determine: i) the association of plant parasitic nematodes with established cherry rootstocks and ii) the reaction of selected cherry rootstocks to infection by *P. penetrans* or *C. xenoplax.*

MATERIALS AND METHODS

Established cherry rootstock trials: Two cherry rootstock trials were established in 1980 at the North West Michigan Horticulture Research Station near Traverse City. Soil at the station site is a sandy loam to a depth of 55 cm, with soil pH varying from 4.8 at 0.0–20 cm to 5.9 at 20–40 cm deep (5). The trials were adjacent to each other and consisted of either 'Montmorency' tart cherry propagated on Mazzard, Mahaleb, 'MXM 2', 'MXM 14', 'MXM 39', 'MXM 60', 'MXM 97', and 'Colt'; or sweet cherry scion cultivars 'Hedelfingen', 'Gold', and 'Napoleon' propagated on the same rootstocks. Trees were pruned annu-

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ally to the modified central leader system, trickle irrigated, and fertilized at recommended rates. Ammonium nitrate was the source of nitrogen (4).

During the first 2 weeks of August 1990, 132 sweet cherry trees (9-20/rootstock) and 48 tart cherry trees (6/rootstock) were selected arbitrarily for sampling. In the sweet cherry trees, approximately 500 cm^3 composite soil samples of 4-6 cores were collected 15- to 20-cm-deep with a soil sampling tube with 2.5 cm opening between the canopy edge and trunk of each tree (14). In the tart cherry plots, a similar volume of soil was collected with a small shovel from an area of approximately 0.5-m deep × 1 m wide of 2 m long open trenches on one side of each tree. The trenches were dug to conduct a root distribution study. Approximately 10–15 g root tissue were collected in the same trench area of three trees/rootstock.

Nematode population densities were estimated from 100-cm^3 subsamples of soil extracted by centrifugal flotation (6) and by shaking 2 g fresh root for 48 hours in a solution of 10 μg/ml ethoxylethyl mercuric chloride (1). Nematodes were identified to genus (10) or species (7). Data were analyzed by ANOVA, and means were separated by Tukey's studentized range test (19) with SAS (SAS Institute, Cary, NC) programs.

Greenhouse studies: Two experiments were conducted. In the first experiment, 36 1-year-old bud breaking seedlings each of Mazzard, Mahaleb, MXM 60, 'GI148-1', and 'GI148-8' were individually planted into 800 cm^3 sandy loam (87% sand, 8% silt, and 5% clay) soil (pH 6.5) in 20 cm × 8-cm-d black plastic tubes. Soil in 12 pots of each rootstock was infested with either 0 nematodes (check), 5,000 (625/100 cm^3 soil) *P. penetrans,* or 5,000 *C. xenoplax* mixed stages from greenhouse cultures of peas and hairy vetch, respectively. Plants were arranged randomly on greenhouse benches, maintained at 25 ± 2 C and 16-hour day length, watered daily with tap water to saturation, and fertilized twice weekly with 20:20:20 N:P:K Peters (Grace-Sierra, Milpitas, CA) all-purpose fertilizer. At 49, 89, and 157 days after soil infestation, three seedlings/treatment from each rootstock (45/sampling date) were harvested to determine nematode infection levels and effect on plant growth. Nematodes were extracted from 100 cm^3 soil and 2 g root as described for the rootstock trials. Changes in plant growth and number of nematodes extracted over sampling dates were analyzed by slope comparison with a SAS procedure (General Linear Models). The total plant height, dry weight, and number of nematodes at 157 days after infestation were calculated from the slopes and analyzed by ANOVA; means were separated by Tukey's test.

Rootstock material and greenhouse conditions in the second experiment were identical to those of the first experiment, except that *C. xenoplax* was not included and *P. penetrans* was added at 10,000/tube (1,250/100 cm^3 soil), as described for the first experiment. Each treatment was replicated five times, and the experiment lasted for 475 days. After 8 months in the greenhouse, plants were transferred into a cold room (2–4 C) for 4 months to complete dormancy. Afterwards, plants were maintained in the greenhouse for an additional 3 months before they were harvested. Data were collected and subjected to analysis by ANOVA; means were separated by Tukey's studentized range test as described for the first experiment.

Results

Established cherry rootstock trials: Pratylenchus penetrans, X. americanum, C. xenoplax, and other less frequently observed genera (*Meloidogyne, Helicotylenchus, Tylenchus,* and others) were found in soils surrounding almost all of the rootstocks (Table 1). The nematode population densities in soil from the tart cherry rootstocks averaged 43% lower than in soils from the sweet cherry (Table 1). The numbers of *P. penetrans* per g root ranged from 10 to 203; however, there were no significant differences among the rootstocks in either soil or root nematode population densities (Table 1).

TABLE 1. Numbers of *Criconemella xenoplax*, *Pratylenchus penetrans*, *Xiphinema americanum*, and other species in soil from sweet and tart cherry rootstocks, and the number of *P. penetrans* per g fresh root of tart cherry rootstocks in field trials in northwestern Michigan.

Rootstock	No. trees sampled†	Number of nematodes/100 cm³ soil or /g fresh root¶				
		C. xenoplax	*P. penetrans*	*X. americanum*	Others	*P. penetrans*
			Sweet cherry			
Mazzard	20	17	37	28	3	—
Mahaleb	9	19	33	24	9	—
MXM 2	16	19	32	64	1	—
MXM 14	18	24	20	10	8	—
MXM 39	15	25	26	9	1	—
MXM 60	18	11	45	26	5	—
MXM 97	18	18	13	9	3	—
Colt	18	1	21	15	1	—
			Tart cherry			
Mazzard	6	12	15	8	2	113
Mahaleb	6	8	15	13	5	203
MXM 2	6	2	0	3	0	10
MXM 14	6	0	18	17	3	85
MXM 39	6	0	7	2	0	170
MXM 60	6	12	45	2	2	150
MSM 97	6	3	47	12	7	53
Colt	6	5	20	12	7	187

Data are means of 6–24 trees. Results are not statistically ($P \leq 0.05$) different according to Tukey's studentized range test. — indicates data not collected.
† Soil from the sweet cherry trees was collected with a soil probe on 1 August 1990; soil from the tart cherry trees was collected with a small shovel on 17 August 1990.
¶ Extracted from three trees per rootstock.

Greenhouse studies: In the first experiment, dry weight increased ($P \leq 0.05$) in all treatments (Table 2). With the exception of GI148-8 (no nematodes added), Mahaleb (with *P. penetrans*), and Mazzard (with *C xenoplax*), plant height generally increased with time (Table 2). Although not statistically significant, the number of *P. penetrans* extracted from Mazzard and MXM 60 decreased, whereas it increased

TABLE 2. Regression analyses of the effect of *Pratylenchus penetrans* and *Criconemella xenoplax* on plant height, total dry weight, and the number of nematodes extracted from soil and roots of five cherry rootstocks at 49, 89, and 157 days after soil infestation with 625 nematodes/100 cm³ soil.

Treatment	Rootstock									
	Mazzard		Mahaleb		GI148-1		GI1480-8		MXM 60	
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
					Height (cm)					
Check	0.03	0.07	0.01	0.02	0.01	0.01	−0.02	0.10	0.05	0.13
P. penetrans	0.07	0.36	−0.02	0.02	0.03	0.07	0.00	0.00	0.11	0.52*
C. xenoplax	−0.02	0.03	0.01	0.01	0.03	0.01	0.01	0.01	0.10	0.45*
					Dry weight (g)					
Check	0.04	0.65**	0.05	0.83**	0.03	0.81**	0.02	0.84**	0.03	0.93**
P. penetrans	0.05	0.83**	0.05	0.85**	0.02	0.54*	0.02	0.76**	0.06	0.95**
C. xenoplax	0.04	0.86**	0.05	0.94**	0.03	0.82**	0.03	0.85**	0.04	0.69**
					Number of nematodes extracted†					
P. penetrans	−0.8	0.00	1.9	0.05	1.5	0.03	0.5	0.1	−0.1	0.0
C. xenoplax	12.0	0.60	61.5	0.59	22.7	0.40*	12.7	0.4*	21.1	0.4*

Data are means of three replications per sampling date. * and ** indicate slopes are significant at $P \leq 0.05$ and 0.01, respectively.
† Analysis based on sums of nematode population densities in soil (per 100 cm³) and roots (per g fresh weight).

on Mahaleb, GI148-1, and GI148-8 (Table 2). The number of *C. xenoplax* increased with time on all rootstocks with increases ($P \leq 0.05$) on GI148-1, GI148-8, and MXM 60 (Table 2). At the end of 157 days, however, there were no differences due to nematode treatment in plant height of all rootstocks or dry weights of Mazzard, Mahaleb, GI148-1, and GI148-8 (Table 3). The dry weight of MXM 60 was lower ($P \leq 0.05$) in the presence of *C. xenoplax* than *P. penetrans* (Table 3). The total number of *C. xenoplax* extracted were the highest in Mahaleb, followed by GI148-1, MXM 60, Mazzard, and GI148-8 (Table 3). None of the rootstocks differed in final *P. penetrans* or *C. xenoplax* population densities (Table 3).

In the second experiment, rootstocks' plant height was not affected. Dry weight of GI148-1 decreased, but dry weight of MXM 60 increased in the presence of *P. penetrans* (Table 4). The most nematodes were recovered from Mazzard rootstock (Table 4); however, the number of *P. penetrans* recovered in the second experiment was lower than in the first experiment (Table 3).

Discussion

Our study documents the association of *Pratylenchus, Criconemella,* and *Xiphinema* spp. with newly developed cherry rootstocks. *Pratylenchus penetrans* was reported on Mazzard and Mahaleb (11,12). *Pratylenchus penetrans* (11,12,15), *C. xenoplax* (8,9, 13,16,17,21) and *X. americanum* (20)—all with wide geographical distributions (14) and host ranges including plants other than stone fruit trees (2,7)—have been implicated as factors in *Prunus* decline, either by themselves or by interacting with other pathogens. The presence of these nematodes in the field and (or) their ability to parasitize newly developed cherry rootstocks may be of concern for future nematode management where the rootstocks are planted.

Growth characteristics are some of the traits for which the rootstocks are selected. Thus, comparisons of the growth of different rootstocks independent of nematode treatment is not valid for this study. Comparison of the numbers of nematodes on the different rootstocks can be used to determine host efficiency or the nematodes' preferences among the rootstocks. The higher numbers of *P. penetrans* on Mazzard and Mahaleb than those on GI148-1, GI148-8, and MXM 60 rootstocks suggest that the nematode may have preference among the rootstocks. The fact that there was little significant effect on growth parameters, however, suggests that the

TABLE 3. Effects of *Pratylenchus penetrans* and *Criconemella xenoplax* on plant height, total dry weight, and number of nematodes extracted per 100 cm^3 and per g fresh root in five cherry rootstocks at 157 days after inoculation with 625 nematodes/100 cm^3 soil.

Treatment	Rootstock				
	Mazzard	Mahaleb	GI148-1	GI148-8	MXM 60
			Height (cm)		
Check	27.9 a	22.0 a	18.6 a	17.2 a	26.3 a
P. penetrans	30.3 a	25.9 a	17.6 a	19.6 a	25.6 a
C. xenoplax	25.1 a	21.7 a	18.7 a	15.0 a	20.3 a
			Dry weight (g)		
Check	4.15 x	3.88 x	2.70 x	2.32 x	3.29 xy
P. penetrans	4.01 x	3.97 x	1.89 x	2.39 x	4.38 x
C. xenoplax	3.56 x	3.61 x	2.11 x	2.06 x	2.91 y
			Number of nematodes extracted†		
P. penetrans	485.5	421.0	196.2	151.8	123.1
C. xenoplax	692.6	2497.6	1059.8	451.4	889.2

Data are means of three replications. Numbers followed by different letters in each column are statistically different ($P \leq 0.05$) according to Tukey's studentized range test.

† Numbers are sums of soil (100 cm^3) and root (per g fresh weight) population densities. There were no statistical differences in the number of nematodes among the rootstocks for each nematode species across columns according to Tukey's range test.

TABLE 4. The effect of *Pratylenchus penetrans* treatment on plant height, total dry weight, and nematode reproduction in five cherry rootstocks at 457 days after inoculation with 1,250 nematodes/100 cm^3 soil.

	Rootstock				
Treatment	Mazzard	Mahaleb	GI148-1	GI148-8	MXM 60
		Height (cm)			
Check	45.9 a	59.1 a	45.9 a	50.1 a	38.7 a
P. penetrans	44.1 a	42.7 a	42.8 a	38.2 a	49.3 a
		Dry weight (g)†			
Check	11.40 x	18.50 x	10.50 x	7.63 x	11.10 y
P. penetrans	12.70 x	14.00 x	6.80 y	4.76 x	23.20 x
		Number of nematodes extracted†			
P. penetrans	4.0 m	36.6 mn	16.0 n	12.5 n	4.6 n

Data are means of five replications.
Numbers followed by the same letters in each column are not statistically different ($P \leq 0.05$) according to Tukey's studentized range test.
Numbers followed by the same letter across the columns are not statistically different ($P \leq 0.05$) according to Tukey's studentized range test.
† Numbers are sums of soil (100 cm^3) and root (g fresh weight) population densities.

nematode population densities were either low or there may be some undefined tolerance level in some or all of the rootstocks. Economic thresholds for *P. penetrans* and *C. xenoplax* numbers for each of the rootstocks under orchard conditions should be determined.

It is not unusual to find high numbers of *P. penetrans* after the onset of cherry tree dormancy in the winter under orchard conditions where the topsoil is either frozen or covered with snow, and roots are insulated from direct cold injury (14). The low numbers of *P. penetrans* recovered in the second greenhouse experiment, despite the high inoculum level, may have been due to the cold temperature that uniformly affected the potted soil and the entire root system.

In view of the limitations of nematicide use and the lack of effective nematode control alternatives, there is a need to develop an integrated approach to nematode management. Although the reaction of the rootstocks to all of the nematodes is unknown, the fact that *P. penetrans* and *C. xenoplax* can infect the cherry rootstocks may be of concern in future nematode management programs.

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