

# Wild and Cultivated Strawberries Can Tolerate or Resist Root-lesion Nematode

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**Abstract.** Intraspecific crossing of 'Guardian' and 'Midway' cultivated strawberry (*Fragaria ×ananassa* Duch.) produced a family of genotypes, some of which suppressed root-lesion nematode [*Pratylenchus penetrans* (Cobb)] population counts and produced large berries and high yield. Unlike 'Midway', 'Guardian' also suppressed *P. penetrans*. Among several beach strawberry [*Fragaria chiloensis* (L.) Duch.] and woodland strawberry (*Fragaria virginiana* Duch.) genotypes, variation was found in resistance and tolerance to root-lesion nematodes. Three *F. chiloensis* genotypes showed tolerance, and at least two genotypes may be somewhat resistant. Three *F. virginiana* genotypes also were tolerant, and three were resistant. Also, one ('Little Catawaqui 4') combined root growth vigor with nematode resistance. We concluded that exploitable genetic diversity in vigor and reaction to root-lesion nematodes exists in wild *Fragaria* and in *F. ×ananassa*.

The cultivated strawberry, *Fragaria ×ananassa* is a woodland (*Fragaria virginiana*) and beach strawberry [*Fragaria chiloensis* (L.)] hybrid. All three species are root-lesion nematode [*Pratylenchus penetrans* (Cobb)] hosts (Goheen and Braun, 1956; Hildebrand, 1934; McElroy, 1977; Steiner, 1931).

Hildebrand and West (1941) first suggested that root-lesion nematodes were involved in strawberry root rot. Initially, many researchers regarded root-lesion nematodes as synergizing with other pathogens, especially *Verticillium*, which itself is of questionable pathogenicity. However, Chen and Rich (1962) and Townshend (1963a) demonstrated that *P. penetrans* was pathogenic on strawberry.

Several nematologists have published host association reports of root-lesion nematodes with selected varieties of *F. ×ananassa* (Chapman, 1956; Goheen and Smith, 1956; Townshend, 1962). Others have observed differences in root-lesion nematode reproduction among the species varieties (Adams and Hickman, 1970; Kimpinski, 1985; Szczygiel, 1981; Vrain, 1985). Szczygiel (1981) evaluated 28 strawberry varieties in pots for *P. penetrans* susceptibility and concluded that root necrosis and growth reduction should be considered indicators of nematode-induced damage. Various edaphic and biotic factors

may affect the nematode damage threshold (Potter and Dale, 1991), which may be >500 nematodes per kilogram of soil (Szczygiel, 1983).

In this paper, we follow Cook and Evans' (1987) terminology, which considers resistance and susceptibility to relate to nematode reproduction and tolerance to describe host injury. Plant sources that are resistant (support few lesion nematodes or suppress lesion nematode counts) or are tolerant (do not exhibit growth reduction or root necrosis) are necessary to improve the commercial strawberry reaction to nematodes. Our objectives were to evaluate 1) the improvement of resistance or tolerance to nematodes through intraspecific crosses between *F. ×ananassa* varieties and 2) resistance or tolerance sources among *F. chiloensis* and *F. virginiana* genotypes.

## Materials and Methods

**Intraspecific *F. ×ananassa* crosses.** This experiment was conducted in two phases: 1) evaluation in the greenhouse of nematode multiplication on two *F. ×ananassa* parents and crosses of these parents and 2) fruit yield evaluation in the field from the parents and crosses. Based on Szczygiel's (1981) work, 'Midway' and 'Guardian' (which supported large and small populations of *P. penetrans*, respectively) strawberries were selected as parents. These varieties were crossed and selfed to produce three families.

Plants were grown from the seed and planted in 50 × 35 × 10-cm (17.5-liter) rubber trays of potting soil, with 45 plants per tray and one tray per family in a greenhouse. At the four-

leaf stage, the plants were inoculated with 56 *P. penetrans* per plant. Nematodes were injected in 10 ml aqueous suspension into the root zone using a syringe; we obtained nematode inoculum from a greenhouse culture of timothy grass roots (*Phleum pratense* L.). Runner plants from the seedlings were rooted and retained. After 14 weeks, the inoculated plants were removed from the trays, and roots were washed. Roots were excised from the crowns, and nematodes were mist-extracted from each root system for 2 weeks (Ayoub, 1980). Following extraction, each root mass was oven-dried at 72C for 24 h. The extracted nematodes were counted with a stereoscopic microscope (Ernst Leitz GmbH, Wetzlar, Germany) at ×50. The number of nematodes per gram of dry root was calculated by dividing the number of extracted nematodes per root system by the dry root weight.

From the 45 plants in each of the three crosses, 30 genotypes were selected for further greenhouse testing after the number of nematodes in the roots was counted. Two genotypes of selfed 'Midway', 10 of selfed 'Guardian', and 18 of 'Guardian' × 'Midway' fitted the selection criterion of having a relatively low nematode count (<25 per gram of root). Five runner plants per selection were grown in 10-cm (600-ml) clay pots in 450 g of steam-pasteurized soil. They were inoculated with 1000 *P. penetrans* injected in a 10-ml aqueous suspension into the root zone. Pots were placed on a greenhouse bench in a completely randomized design and were grown for 14 weeks at 25C. Nematodes were extracted from the entire root system for 2 weeks by mistifier (Ayoub, 1980). The number of nematodes per gram of dry root was calculated as previously explained. Nematodes also were extracted from a randomly selected 50-g soil sample per pot using the Baermann pan method (Townshend, 1963b), and the soil per pot was weighed. The number of nematodes in the soil per pot was calculated from the number in a 50-g soil aliquot. The total number of nematodes in soil plus root was derived by adding the number extracted from the entire root and the number per pot of soil. The original and derived variables were transformed using a  $\log_{10}(x + 1)$  transformation and subjected to analysis of variance and least significant difference test (SAS Institute, 1989).

In 1988, the 30 selected genotypes were planted in the field at Horticultural Expt. Station, Simcoe, Canada, for fruiting evaluation. Each genotype was planted as a single 10-plant row, 0.6 m between plants, and 1.2 m between rows, with plots containing 'Bounty' and 'Vantage' as standards. In 1989, genotypes were evaluated for berry size and yield. All marketable fruit (>2 cm in diameter) were harvested twice weekly. A 50-berry sample per genotype was weighed, and a weighted berry weight was calculated.

*Fragaria chiloensis* genotypes. Washington State Univ. Western Washington Research and Extension Center, Puyallup, provided *F. chiloensis* plant material. Five plants each from 13 genotypes plus 'Guardian' and 'Redcoat' were potted in 10-cm (600-ml) pots

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with potting soil and kept in a greenhouse at 25C. Pots were placed in a completely randomized design. Plants were inoculated with 210 *P. penetrans* per plant, injected in 12 ml water into the potting mix, and were grown in the greenhouse. After 20 weeks, the plants were removed from pots and as much of the peat-based potting mix as possible was washed off the roots in a stream of tap water. The fresh roots were blotted dry on paper towels, fresh weights recorded, and nematodes extracted by mistifier as previously explained. Oven-dried roots were weighed, the extracted nematodes were counted, and statistical analysis was performed as previously explained.

*Fragaria virginiana* genotypes. Wild *F. virginiana* plants collected from 20 locations in southern Ontario (between 42°N and 46°N lat.) were grown at Simcoe. At the five-leaf stage, five plants of each genotype were potted in 1600 g of a Delhi sandy loam soil in 15-cm (1.6-liter) plastic pots and inoculated with 1000 *P. penetrans* injected in a 25-ml aqueous suspension into the root zone. Ten 'Redcoat' plants and eight each of 'Guardian' and 'Midway' were infested similarly for comparison. Pots were placed in a completely randomized design in a greenhouse at 25C. All plants were grown for 14 weeks, during which time the runners and blossoms were removed. Then the nematodes were recovered from complete root systems by mist extraction and randomly selected 50-g soil aliquots. Statistical analyses were performed as previously described.

## Results and Discussion

*Intraspecific F. xananassa crosses.* In the greenhouse evaluation, 'Guardian' supported the fewest nematodes per gram of dry root and the third fewest nematodes in soil plus root; 'Midway' was near the ranking midpoint for each criterion (Table 1). Within the 'Guardian' x 'Midway' family, the genotypes designated 6.01, 6.03, 6.15, 6.31, and 6.40 had low counts, but 6.05, 6.07, 6.27, 6.38, and 6.45 had high counts. Of the former group, all produced high dry-root weights, whereas among the latter group, only clone 6.05 produced a high dry-root weight.

Although berry size and yield were not correlated with the total nematodes in soil plus root ( $r=0.16$  and  $0.01$ , respectively;  $P \leq 0.05$ ), some genotypes that carried low nematode populations (6.01, 6.03, and 6.15) also had large berries and high yield.

*Fragaria chiloensis* genotypes. A high degree of variability was evident in the number of nematodes per gram dry root ( $cv = 104$ ); each *F. chiloensis* genotype had one plant in five with a low nematode population. On the assumption that the low count might have resulted from death of the nematode inoculum, the data were reanalyzed using the remaining four replicates. HMI and 'Del Norte' supported the highest nematode populations in their root systems, whereas CL5, CA11, and CA12 supported few (Table 2). Using a general linear-model contrast analysis (SAS Institute, 1989), 'Guardian' and 'Redcoat' roots were significantly ( $P \leq 0.05$ ) heavier than

those of the *F. chiloensis* genotypes. The two varieties also supported significantly fewer lesion nematodes per gram dry root than DL40, HMI, FS20, BSP14, LCM10, or CL2 (Table 2).

*Fragaria virginiana* genotypes. Although 'Guardian' and 'Midway' had the lowest total lesion nematode population in soil plus root and 'Redcoat' also ranked low, the *F.*

Table 1. *Pratylenchus penetrans* populations in genotypes of selfed, parental, and crossed 'Guardian' and 'Midway' strawberry (*F. xananassa*) grown in greenhouse pots for 14 weeks (N = 5).

Genotype designation <sup>x</sup>	Nematodes <sup>z</sup>		Dry root wt (g)	Berry <sup>y</sup>	
	No./gram dry root	Total no. in soil + root		Wt (g)	Yield (t·ha <sup>-1</sup> )
6.45	1430	680	0.4	10.0	7.7
6.38	990	600	0.5	7.7	4.6
6.27	490	660	1.2	7.6	18.5
7.15 (G)	480	700	1.2	17.1	1.8
6.07	460	590	0.9	9.2	14.6
6.44	440	250	0.6	10.7	9.2
6.20	380	260	0.5	9.5	12.8
7.05 (G)	340	700	3.1	12.1	6.2
6.05	260	510	2.5	9.6	9.4
7.20 (G)	210	480	2.8	8.3	10.9
6.11	200	280	2.1	13.4	15.6
6.02	180	330	3.9	6.4	7.2
6.43	180	440	1.4	11.2	20.6
6.30	170	530	2.4	9.2	11.3
7.35 (G)	150	470	1.8	10.3	13.9
6.16	150	260	0.6	9.5	13.9
6.41	130	590	4.3	11.9	13.3
7.27 (G)	110	470	3.0	9.9	9.5
Midway	110	390	1.6	---w	---
5.01 (M)	80	380	2.5	7.4	4.6
6.40	70	220	2.5	8.7	10.2
7.45 (G)	70	240	3.2	6.6	9.0
7.30 (G)	60	490	4.0	---	---
6.15	60	280	1.4	11.6	9.5
7.08 (G)	60	330	5.3	7.9	2.8
6.01	60	180	1.8	10.7	9.4
6.31	30	190	5.1	7.6	16.3
7.41 (G)	20	260	3.1	10.3	9.2
6.03	20	290	3.5	11.7	11.5
7.12 (G)	20	150	6.1	6.2	14.5
5.44 (M)	10	70	1.7	4.6	7.0
Guardian	10	170	1.7	---	---
Vantage <sup>v</sup>	---	---	---	10.8	18.7
Bounty	---	---	---	9.9	22.1
LSD <sub>(0.05)</sub>	589	422	2.7		

<sup>z</sup>Data from greenhouse pot trials; nematodes per gram dry root = number nematodes extracted from root ÷ dry root weight.

<sup>y</sup>Field-grown for berry production data only.

<sup>x</sup>Genotypes designated "7.-- G" are selfed 'Guardian', "5.-- M" are selfed 'Midway', "6.--" are crosses of 'Guardian' x 'Midway'.

<sup>w</sup>Data not taken.

<sup>v</sup>'Vantage' and 'Bounty' = standards of berry size and yield only.

Table 2. *Pratylenchus penetrans* populations in genotypes of *Fragaria chiloensis* varieties and breeding lines and in 'Guardian' and 'Redcoat' (*F. xananassa*) grown in greenhouse pots for 20 weeks (N = 5).

Genotype designation	Nematodes		Dry root wt (g)
	No./gram dry root <sup>z</sup>	Total no./root	
DL40	1970	1220	0.6
HMI	1850	2870	1.5
FS20	1820	1160	0.6
BSP14	1720	840	0.5
LCM10	1640	1360	0.8
CL2	1630	1290	0.8
Del Norte	1610	2520	1.6
TR4	1270	1220	1.0
CA12	1140	620	0.5
RCP37	950	940	1.0
ZB4	800	1580	2.0
CA11	550	540	1.0
CL5	600	760	1.3
Redcoat	90	380	4.3
Guardian	30	170	5.3
LSD <sub>0.05</sub>	1526	1298	0.7

<sup>z</sup>Nematodes per gram dry root = number nematodes extracted from root ÷ dry root weight.

*virginiana* genotypes ‘Townsend 17’ and ‘St. Davids 1’ ranked between ‘Guardian’ and ‘Redcoat’ for this criterion (Table 3). Only the genotype ‘Little Cataqui 4’ supported fewer nematodes per gram of dry root than ‘Redcoat’ and slightly more than either ‘Guardian’ or ‘Midway’. The genotype ‘Little Cataqui 4’ also produced a higher root weight than any of the three *F. xananassa* varieties. Of 13 genotypes that supported more than the mean number of nematodes per gram of root (336), 10 also had more than the mean total number in soil plus root (1545). By both criteria, the genotype ‘Brockville 28’ supported the greatest number of nematodes.

Our work with *F. xananassa* genotypes (Table 1) indicates that there is variation in nematode tolerance and resistance (or suppressiveness). We found that ‘Midway’ supported a slightly larger total population than ‘Guardian’. Our ranking agrees with Szczygiel’s (1981) findings with ‘Guardian’ and ‘Midway’ and supports Kimpinski’s (1985) results with ‘Redcoat’. The variation range in ‘Guardian’ selfed and ‘Guardian’ x ‘Midway’ genotypes suggests that progress can be made to increase either tolerance or resistance. Some genotypes that suppressed nematodes and produced a relatively vigorous root system also had large fruit and good fruit quality (data not shown). This group of clones could be useful as parents for developing lesion-nematode resistant or tolerant varieties without resorting to wild genotypes.

The variability among *F. chiloensis* and *F. virginiana* genotypes precluded determining whether genetic resistance to *P. penetrans* in the hybrid *F. xananassa* is derived preferentially from one or the other wild species. Some suppressive genotypes were present in *F. virginiana* (Table 3); however, the slight dif-

ference between *F. chiloensis* genotypes CL5 and CA11 and ‘Guardian’ or ‘Redcoat’ (Table 2) supports the possibility that resistance also may be found in *F. chiloensis*. More *F. chiloensis* genotypes should be tested for resistance. In addition, the genotypes HMI, ZB4, and ‘Del Norte’ may be tolerant as they grow and produce reasonable root weights, despite a high nematode presence.

The basis of nematode tolerance in *F. chiloensis* is not clearly understood. However, according to Hancock (1991), the genotype RCP37 had a higher photosynthesis and CO<sub>2</sub> assimilation rate, due in part to thicker spongy mesophyll in the leaf. Archbold and Zhang (1991) reported that the genotype BSP14 exhibited drought stress resistance through osmotic modification that resulted in increased soluble sugar concentration in the plant under drought stress. Because nematodes metabolize sugars while feeding (Jones, 1981), a plant with the capacity to produce excess sugars might be better able to withstand parasitic feeding by nematodes than one that lacks this capability. In our study, genotypes RCP37 and BSP14 were among the *F. chiloensis* genotypes that supported a medium number of nematodes on a lesser root system. Further studies on correlation of plant physiology with nematode tolerance among *F. chiloensis* genotypes seem warranted.

Among the wild *F. virginiana* genotypes examined thus far, great diversity exists in growth vigor (Table 3). The genotypes ‘Oro 11’, ‘Normandale 3’, and ‘HES 9’ that supported many nematodes also produced vigorous roots and may be examples of tolerance as are HMI, ZB4, and ‘Del Norte’.

Diversity, and hence resistance to *Pratylenchus penetrans*, in cultivated strawberries can be increased by introducing unim-

proved wild germplasm (Scott and Lawrence, 1975; Sjulín and Dale, 1987). Several genotypes might be useful in this regard. The genotype ‘Little Cataqui 4’ supported few nematodes and produced a higher root weight than *F. xananassa* varieties. The genotype ‘Normandale Fish Hatchery 2’, which ranked below the mean number of nematodes by both criteria and had the second highest dry root weight, could be useful as a growth vigor source, exceeding that of the *F. xananassa* varieties.

Our work, like that of Szczygiel (1981) and Vrain (1985), has considered only a limited selection of genotypes and varieties, but it has indicated diversity that may be exploitable. Further screening is needed to examine the potential usefulness of a wider range of *Fragaria* genotypes in developing root-lesion nematode resistance or tolerance.

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Table 3. *Pratylenchus penetrans* populations in genotypes of *Fragaria virginiana* and in ‘Guardian’, ‘Midway’, and ‘Redcoat’ (*F. xananassa*) grown in greenhouse pots for 14 weeks (N = 5).

Genotype and location no.	Nematodes		Dry root (g)
	No./gram dry root <sup>2</sup>	Total no. in soil + root	
Brockville 28	1010	3680	3.0
Packenhams 15	730	2780	3.3
Normandale 3	550	3240	4.5
Alfred 10	540	1640	2.6
Aubrey Lake 21	500	1470	2.0
St. Davids 1	450	530	0.7
St. Williams 24	430	2730	5.9
Oro 11	420	3040	4.9
H.E.S., Simcoe 9	420	2190	4.0
Hillier 16	390	1650	3.9
Balls Falls 23	360	1570	2.6
St. Andre 4	350	1910	2.6
Jordan 2	340	1390	3.3
Lions Head 22	240	1070	3.6
Normandale Fish Hatchery 2	240	1310	5.1
Saugeen River 20	230	1330	3.8
Sauble Beach 19	220	1140	3.8
Townsend 17	200	810	2.6
Silverlake 17	190	1140	3.2
Redcoat	190	980	3.9
Little Cataqui 4	80	1450	4.7
Midway	70	280	3.3
Guardian	60	290	3.6
LSD <sub>0.05</sub>	476	1875	0.9

<sup>2</sup>Nematodes per gram dry root = number nematodes extracted from root ÷ dry root weight.

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