Susceptibility of American Grapevine Scion Cultivars and French Hybrid Rootstock and Scion Cultivars to Infection by Peach Rosette Mosaic Nepovirus

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ABSTRACT

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Peach rosette mosaic virus (PRMV), a nepovirus prevalent in Michigan, causes severe crop loss and death to Concord grapevine cultivars. Because of the high cost of soil fumigation, combined with the removal of most soil fumigants from the market, three types of grapevine cultivars (American scion cvs. Concord, Delaware and Niagara; French-American hybrid rootstock cvs. Couderc 1202, C. 1616, Teleki 5A, and Teleki 5C; French-American hybrid scion cvs. Chancellor, Foch, Seyval, and Vignoles) were tested for resistance to PRMV. In 1986, 44 vines of each cultivar were planted into a field that had contained mature, uniformly PRMV-infected Concord vines and a uniform population distribution of the dagger nematode vector (*Xipinema americanum*). From 1988–1991 each vine was annually tested for PRMV infection by enzyme-linked ammunosorbent assay in the spring and summer. By 1991, the final year of testing, PRMV was detected in less than 5% of the vines of Chancellor and Couderc 1616, 7% of the vines of Couderc 1202 and Foch, 18.2% of the vines of Niagara and Delaware, 20% of the vines of Teleki 5C, and 50% or more of the vines of Vignoles, Teleki 5A, and Concord. Seyval remained uninfected for the duration of the experiment. The greatest reduction in yield and growth (up to 40 and 60%, respectively) was in Concord. Chancellor, Couderc 1202, Couderc 1616, Foch, Teleki 5A, and Vignoles also showed reduced yield, growth, or both, when PRMV-infected and healthy vines were compared.

Peach rosette mosaic virus (PRMV), a member of the nepovirus group (4) is found only in Michigan (5,11,12) and Ontario, Canada (1). *Xiphinema americanum* Sensu Lato (7) and *Longidorus diadecturus* Eveleigh and Allen (1) are vectors of PRMV. In Michigan, the latter vector is not important. Grape decline caused by PRMV is present in more than 50 vineyards of cvs. Concord and Niagara in southwestern Michigan. The disease causes growth malformations and berry shelling, resulting in disease losses of up to 50-fold compared with healthy vines (D. C. Ramsdell, *unpublished*). The disease is limited primarily to cvs. Concord and Niagara (4,410 ha) both of which are American grape (*Vitis labrusca* L.) cultivars. However, there is concern that PRMV may be a threat to French-American hybrids as well.

Earlier studies have shown that the vector *X. americanum* can be found on grape roots to a depth of 2.13 m in the typical sandy soils of Michigan's vineyards (2,11). Shallow plus deep soil fumigation with gaseous soil fumigants was examined over an 8-yr period and found to control the vector and prevent reinfection of replanted virus-free Concord vines (9). However, because of the relatively high cost of such treatments (>$2,000/ha), the loss of all of the effective fumigants except D-D (a mixture of dichloro propane and dichloro propene), resistance to PRMV has been sought.

When 28 scion and rootstock cultivars of American, French hybrid and European grape (*V. vinifera* L.) were grown for 10 yr beneath mature PRMV-infected. Concord vines, 13 of these cultivars were found to be PRMV-infected, but effects on growth or yield were not measured (10). Grape cultivars resistant to grapevine fanleaf virus in California have also been identified (13). In 1986, we set out to test on a large scale the resistance/susceptibility of many important French-American hybrid scion and rootstock cultivars to infection by PRMV, and to measure the relative disease severity caused by PRMV. We also sought to discover an immune rootstock upon which to graft Concord, a juice grape that comprises 95% of Michigan's 11,000 acres. We included only those cultivars that did not become infected in the earlier test (10). We also eliminated further testing of European

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grapes due to their cold intolerance in most southwestern Michigan grape sites.

MATERIALS AND METHODS

Test site and plot design. The test site consisted of a 30-yr-old planting of cv. Concord grapevines that had been uniformly infected by PRMV. This was previously determined by extensive enzyme-linked immunosorbent assay (ELISA) testing of vines. The vines were removed in 1985, the soil was tilled, and soil samples were taken at a 15-cm depth in a diagonal pattern across the 1-ha site to determine if there was a uniform distribution of the nematode vector. The Jenkins (6) sugar flotation and screening method was used to isolate *X. americanum*. In the spring of 1986, a total of 44 1-yr-old rooted cuttings of virus-tested certified vines of each of 11 cultivars were planted, one cultivar per row. American scion cultivars Concord, Delaware, and Niagara were included as internal standards, because they are known to become infected by PRMV. French-American hybrid rootstock cultivars were Couderc 1202, Couderc 1616, Teleki 5A, and Teleki 5C; French-American hybrid scion cultivars were Chancellor, Foch, Seyval, and Vignoles. Vines were planted 2.44 m within rows × 3.1 m between rows. Vines were trained to an Umbrella Kniffin system on a two-wire trellis. Beginning the third year of growth, the vines were balanced pruned to a 30+10 system, which means that for the first 454 g of prunings, a total of 30 buds were left, and for each subsequent 454 g of prunings, a total of 10 buds were left. This system balances crop load versus cane growth, so that more exacting yield comparisons can be made between vines of a given cultivar.

ELISA tests. Beginning in 1988, vines were tested twice annually in late spring and summer by double antibody sandwich–ELISA (3,8). At each testing date, vines were sampled at six locations for young leaf tissue. One gram of leaf tissue was ground in 5 ml of extraction buffer (0.05M phosphate buffer, pH 7.2, containing 0.05% Tween 20 [v/v] and 0.2% [w/v] egg albumin) using a Tekmar Tissuemizer (Tekmar Co. Inc., Cincinnati, OH). Anti- PRMV-IgG (1 mg/ml of coating buffer) was applied in a volume of 0.2 ml/well of Immulon-I Microtiter plates (Dynatech Co. Inc., Alexandria, VA). Samples were applied at a volume of 0.2 ml/well. The alkaline phosphatase enzyme conjugate was used at a dilution of 1:1000 (v/v in extraction buffer) and plated at a volume of 0.2 ml/well. Substrate buffer containing *p*-nitro phenyl phosphate was used at the rate of 1 mg/ml of substrate buffer and plated at a volume of 0.2 ml/well. Plates were read after 30 min to 1 hr at A_{405nm}, using a Biotech Model 307 ELISA reader (Biotech Co. Inc., Winooski, VT). The mean value of healthy controls plus three standard deviations was used to determine the positive negative threshold. Duplicate plates were used in all tests.

Growth and yield measurements. Beginning in the winter of 1989, equal numbers of PRMV-diseased and healthy vines in near vicinity to each other were selected for pruning weight comparisons. Vines were balanced pruned and the prunings were weighed as a measure of vegetative growth. In 1991 and 1992, yield measurements were taken from cultivars that normally produce a crop (all scion cultivars and some rootstock cultivars). Analysis of variance (ANOVA) was done on all measurement data.

Nematode sampling in the test plot during growth of test vines. During the years 1988, 1989, and 1990, a soil sample was taken at a depth of 15 cm beneath every third vine in each row, advancing one vine down the row per year, in order to determine vector nematode populations. Nematodes were extracted as described earlier and enumerated.

Nematode survival test on four cultivars. To determine if cultivar resistance to PRMV was due to lack of nematode colonization or due to inherent resistance within the vine itself, 1-yr-old rooted cuttings of cvs. C. 1202 and C. 1616 (relatively resistant), Concord (known susceptible), and Delaware (intermediately resistant) were planted into 12-L clay pots containing sterilized soil amended with 1 L of soil containing a monoculture of ca. 150 *X. americanum*.

Table 1. Infection of grapevine cultivars by peach rosette mosaic virus over a 4-yr period in infested soil[a], Texas Corners, MI

Cultivar	Percentage of vines infected (cumulative)[b]			
	1988	1989	1990	1991
Chancellor (S)[c]	0.0	0.0	2.3	4.5
Concord (S)	25.0	31.8	50.0	52.4
Couderc 1202 (R)[d]	2.3	2.3	7.0	7.0
Couderc 1616 (R)	2.3	2.3	2.3	4.5
Delaware (S)	0.0	4.8	18.2	18.2
Foch (S)	7.0	4.8	7.0	7.0
Niagara (S)	0.0	9.1	15.9	19.2
Seyval (S)	0.0	0.0	0.0	0.0
Teleki 5A (R)	0.0	4.8	18.0	50.0
Teleki 5C (R)	2.3	7.0	15.9	20.0
Vignoles (S)	0.0	0.0	54.5	54.5

[a] All vines were tested by enzyme-linked immunosorbent assay twice per year for the 4 yr indicated. Six leaf samples per vine were collected at each sampling time. Vines were considered as positive for PRMV if the values at A_{405nm} exceeded the mean + 3sd of healthy samples.
[b] A total of 44 vines were sampled at each sampling.
[c] Scion cultivar.
[d] Rootstock cultivar.

Table 2. Effect of peach rosette mosaic virus infection on vine growth in 1991, 1992, and 1993, Texas Corners, MI

Cultivar	1991[a]				1992				1993			
	No. vines[b]	Healthy	Diseased	ANOVA[c]	No. vines	Healthy	Diseased	ANOVA	No. vines	Healthy	Diseased	ANOVA
Chancellor	2	172	105	−	2	105	72	−	2	250	159	−
Concord	6	479	172	+	8	1,254	650	+	7	1,336	1,034	+
Couderc 1202	2	285	300	−	3	218	103	−	2	250	363	−
Couderc 1616	2	377	300	−	2	3,746	590	+	1	1,816	545	NA[d]
Delaware	2	112	112	−	5	161	233	+	4	443	125	−
Foch	2	290	372	−	3	586	711	−	2	227	613	+
Niagara	5	365	300	−	6	517	466	−	6	257	310	−
Teleki 5A	4	219	154	−	7	1,181	697	−	4	1,617	624	+
Teleki 5C	...[e]	...	...	−	3	7,650	2,618	−	4	2,361	1,872	−
Vignoles	9	223	291	−	10	169	115	+	6	348	341	−

[a] Data collected as pruning weight (g) per vine.
[b] Number of vines in each category: healthy, diseased.
[c] (+) diseased vine pruning weight significantly different from healthy vine pruning weight, $P = 0.05$; (−) no significant difference.
[d] Not applicable due to insufficient replicates available.
[e] Not tested.

Table 3. Yield Effects: Healthy vines vs. peach rosette mosaic–infected vines in 1991 and 1992, Texas Corners, MI

Cultivar	1991[a] No. vines[b]	Healthy	Diseased	ANOVA[c]	1992 No. vines	Healthy	Diseased	ANOVA
Chancellor	...	...[d]	...[d]	−	2	5.4	0.05	+
Concord	6	6.13	3.57	+	6	5.53	2.87	+
Couderc 1202	2	3.65	2.15	−	3	8.1	5.93	+
Delaware	2	2.55	3.6	−	4	5.98	5.5	−
Foch	2	4.3	2.8	−	3	3.35	2.7	−
Niagara	5	3.37	2.17	−	...	...[e]	...[e]	−
Teleki 5A	...	...[f]	...[f]	−	7	0.19	0.27	−
Vignoles	9	2.09	1.15	−	7	2.13	0.99	+

[a]Data collected as kg fruit per vine.
[b]Number of vines in each category: healthy and diseased.
[c](+) diseased vine pruning weight significantly different from healthy vine pruning weight, $P = 0.05$; (−) no significant difference.
[d]Not tested; crop decimated by downy mildew.
[e]Not tested; grower harvested the crop by mistake.
[f]Not tested; insufficient crop on this rootstock cultivar.

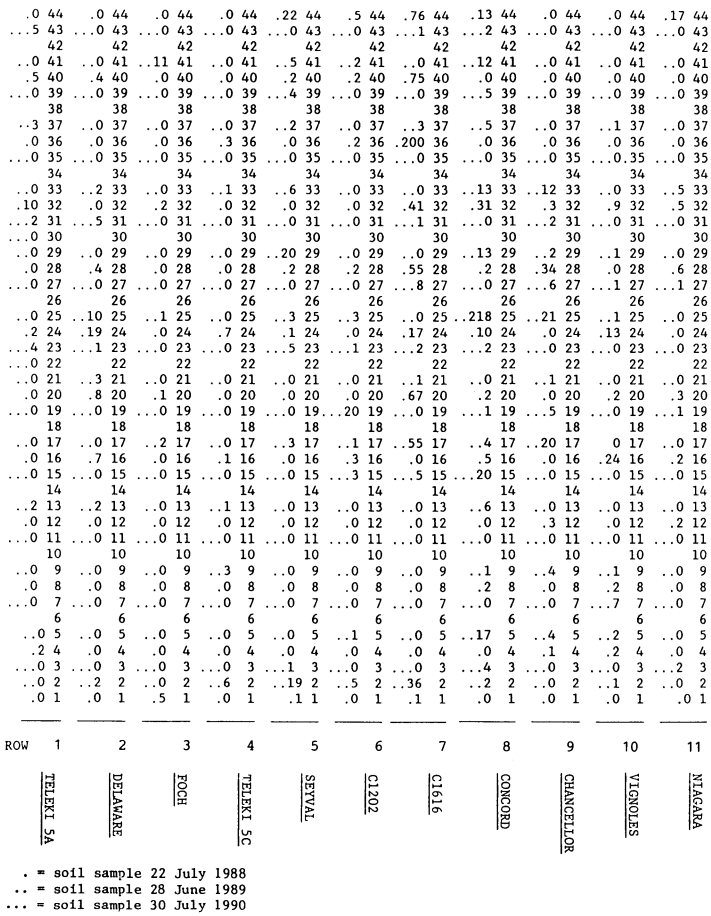

Fig. 1. Map of test plot area showing vector nematode (*Xiphinema americanum*) population distribution for the years 1988–1990. Consecutive numbers in each row are vine locations. Numbers with dots to the left represent nematode populations per 100 cm^3 soil. Populations are as follows by year of sampling: (.) = 1988; (..) = 1989; (...) = 1990.

Five replicate pots per cultivar were replicated in a randomized complete design and kept in the greenhouse at a temperature of 20–30 C, with a 14-hr daylength. Soil samples (100 g) were taken at 30, 90, 120, and 210 days after planting to determine nematode populations.

RESULTS AND DISCUSSION

Vine infection by cultivar over time, as determined by ELISA. By 1988, 2 yr after planting, 25% of the Concord vines were found positive for PRMV by ELISA (Table 1). Seven percent of the Foch vines, and 2.3% of the Teleki 5C, Couderc 1202, and C. 1616 vines were also infected. The percentage of vines found positive by ELISA was higher in 1989 for all cultivars except Chancellor and Seyval. In 1990, PRMV was also detected in Chancellor by ELISA. By 1991, the last year of ELISA testing, vines of Seyval remained negative for PRMV by ELISA. Chancellor had only 4.5% vine infection. Niagara and Delaware ended up with 19.2 and 18.2% of the vines infected, respectively, which is a higher level of susceptibility than previously thought. The rootstock Couderc 1616 had 4.5% infection by 1991, which was less than that of C. 1202, which had 7.0%.

Growth and yield measurements comparing healthy and PRMV-infected vines. Vine-pruning weight data from early spring 1991 (Table 2) indicated a significant reduction only for Concord; pruning weight was reduced by 64%. Pruning weight data from early spring 1992 shows highly significant reductions of 48, 84, and 32% for Concord, C. 1616, and Vignoles, respectively. In 1992, PRMV-infected Delaware had a significant (30%) increase in pruning weight. In 1993, PRMV-infected Concord and Teleki 5A had significantly less growth (23 and 61%, respectively), and PRMV-infected Foch had a significant increase in vine growth.

Yields were taken in 1991 and 1992. In 1991, only Concord had significantly less yield (42%) than healthy vines (Table 3). In 1992, Chancellor, Concord, C. 1202, and Vignoles had yield reductions of 99.9, 48, 26.9, and 53.5%, respectively.

Nematode distribution throughout the test area over time. In general, there was a reasonably uniform population of the vector as shown on a map of the test area (Fig. 1) with populations of *X. americanum*/100 cm^3 soil over a 3-yr period (1988, 1989, and 1990). As a result, the cultivars tested had similar chances for infection over the time period of the field test.

Nematode survival on four grapevine cultivars. There were no significant differences in nematode survival or colonization on the four cultivars throughout the 210 days of the test (ANOVA: $P = 0.05$) (Fig. 2). This information indicates that the resistance of cultivars C. 1202 and C. 1616 was not due to lack of colonization or survival by *X. americanum*.

In summary, C. 1616 would make an

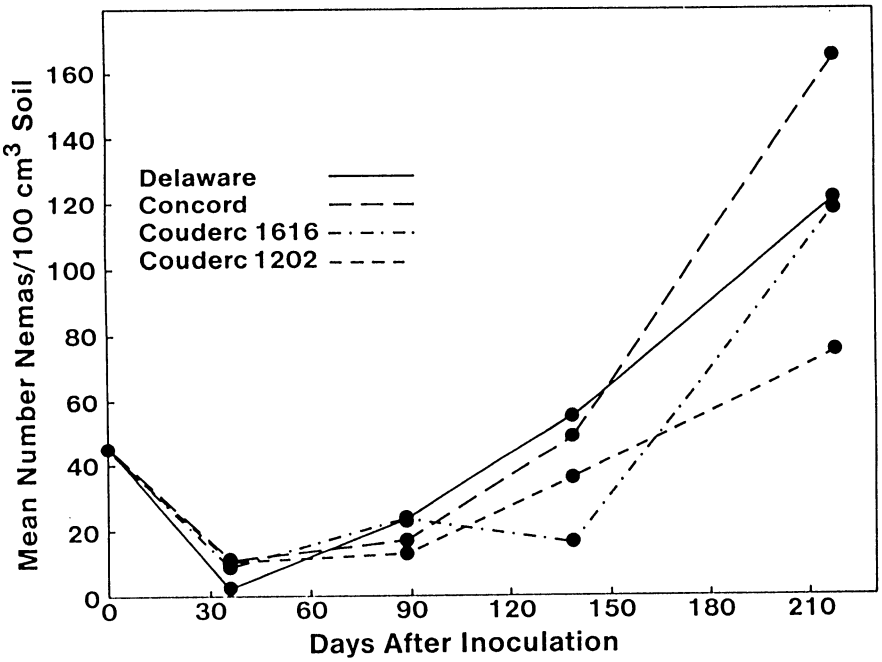

Fig. 2. Results of test of 1-yr-old rooted cuttings of four grapevine cultivars to determine level of survival and colonization on roots by PRMV vector *Xiphinema americanum*. None of the populations are significantly different from each other at any time interval ($P = 0.05$).

excellent rootstock on which to graft PRMV-susceptible Concord and Niagara. Neither of these two important juice-type cultivars would survive in PRMV/vector-infected soil on their own roots. Alternatively, C. 1202 could be used as a rootstock; however, it is not as cold hardy as C. 1616. The French-American hybrid Vignoles should not be planted in PRMV-infested soil on its own roots. It is quite susceptible to infection by PRMV and suffers heavy yield losses. Seyval, an excellent white wine grape cultivar, is virtually immune to PRMV infection. We did not test Seyval for nematode susceptibility in the pot tests done, because we were looking primarily for immune rootstocks. Seyval is not a rootstock type of grape. It could be planted into infested soil on its own roots and do well. The two excellent red-fruited wine grape cultivars Chancellor and Foch also showed good resistance to PRMV infection. The growth and yield of Foch was not affected by PRMV infection; however, yield of Chancellor was severely affected. Delaware, a champagne-type grape of minor importance to Michigan, could be planted on its own roots in infested soil. In fact, it has been observed to grow and produce well next to PRMV-infected Concord vines in commercial vineyards. However, Delaware would not make a good rootstock for Concord because of its relatively weak growth. The two rootstock cultivars Teleki 56A and 5C are of no value for grafting susceptible scion cultivars due to their susceptibility to PRMV infection.

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