Epidemiology of Peach Rosette Mosaic Virus in a Concord Grape Vineyard

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Michigan State Agricultural Experiment Station Journal Series Article No. 8075.

This research was supported in part by a grant from the Michigan State Grape Research Committee.

The authors thank A. R. Putnam for weed identification and G. R. Bird, Natalie Knobloch, and John Davenport for nematode extractions and counts. Technical assistance by P. E. Parker and graphic art work by Tia Maxwell is appreciated.

Accepted for publication 12 September 1977.

ABSTRACT

RAMSDELL, D. C. and R. L. Myers. 1978. Epidemiology of peach rosette mosaic virus in a Concord grape vineyard. Phytopathology 68: 447-450.

A block of 165 *Vitis labrusca* 'Concord' grapevines was sap indexed to *Chenopodium quinoa* on an individual vine basis for 4 yr to determine the rate of spread of peach rosette mosaic virus (PRMV). The first indexing in 1974 revealed 15 infected vines. In 1975, 1976, and 1977, there were 14, 3, and 8 newly infected vines, respectively. Spread was slow and mostly to vines adjacent to previously infected vines. The pattern of spread was elliptical. Indexing the roots of 16 weed species associated with diseased vines revealed that curly dock (*Rumex crispus*), Carolina horsenettle (*Solanum carolinense*), and common dandelion (*Taraxacum officianale*) were infected. The virus was found to be seedborne in 5/137 (3.6%) dandelion seedlings assayed from mother plants. Grape seedlings from fruit of infected vines contained PRMV in 4/38 seedlings (9.5%) assayed onto *C. quinoa*. Pollen apparently did not contain virus. The presumed vector *Xiphinema americanum* was found to depths of 152.4 cm (5 feet) and 213.4 cm (7 feet) beneath PRMV-infected vines in two vineyards.

Additional key words: nematodes, NEPO virus, disease spread, weed hosts.

Peach rosette mosaic virus (PRMV), a NEPO virus, causes a very serious disease of *Vitis labrusca* L. 'Concord' grapevines and *Prunus persica* Batsch peach in southwestern Michigan. This disease has been described previously (1, 4, 5, 6, 8, 10, 11).

To date, 17 vineyards in southwestern Michigan's 18,000 acres of Concord juice grapes have been shown by the authors, using sap-indexing on *Chenopodium quinoa* Willd. and serology, to contain PRMV-diseased vines. Based upon visual observations of symptoms in nonindexed vineyards, it appears that this disease is present in a large number of additional vineyards in the state.

Studies of the rate of spread of tomato ringspot virus (TomRSV) recently have been conducted in grapes (13) and raspberries (2) for a 2-yr period. In our study, we decided to individually sap-index all vines around an infection focus over a 4-yr period, because recently infected vines are often symptomless.

Peach rosette mosaic virus is continuing to appear in heretofore healthy and productive vineyards. The disease is widespread and the virus is disseminated in some manner from vineyard to vineyard. This spread is not accounted for solely by the experimental vector, *Xiphinema americanum* Cobb, 1913 (6). Although some vineyards containing PRMV-infected vines have been planted on old peach sites which formerly contained infected trees, many vineyards with infected vines are planted on ground known not to have been previously planted with peaches.

Weeds have been implicated as being important in the ecology and spread of NEPO viruses in small fruit crops (9). Some viruses infecting grapevines have been shown to be seed- and pollen-borne in the grape host (3). Peach rosette mosaic virus has been shown to be seed-borne in *C. quinoa*. (6).

Vegetative propagation via rooted cuttings is responsible for the greatest amount of spread involving grape leaf roll virus in *Vitis vinifera* (14). All of the preceding factors were investigated by us as possible reasons for the extensive and widespread dissemination of this virus in Michigan vineyards.

This research was undertaken: (i) to determine the rate and extent of spread of PRMV under natural vineyard conditions; (ii) to detect possible weed host virus reservoirs and seed transmissibility in weeds; (iii) to ascertain if PRMV is pollen-and/or seed-borne in grape; (iv) to evaluate the possible role of vegetative propagation of cuttings taken from diseased vines in virus spread; and (v) to determine the populations of *X. americanum* beneath diseased vines at various depths in the soil and to evaluate how deep soil fumigation applications should be made for vector control.

We have published a preliminary report of this work elsewhere (11).

MATERIALS AND METHODS

Determination of rate of field spread in an infected

00032-949X/78/000 074$03.00/0
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vineyard.—A block of 165 mature Concord grape vines, encompassing an infection focus in a vineyard at Lawton, Michigan, was the site of this work. Six dormant cane cuttings were taken from each vine in the winter of 1974, 1975, 1976, and 1977. The cuttings were forced in moist sand in a glasshouse. One or 2 gm of immature vegetative tissue was ground in 2% nicotine alkaloid in water (NAW) at a 5:1 ratio (v/w), using a sterile mortar and pestle. Plastic disposable gloves were used to apply inocula from each vine to a single *C. quinoa* indicator plant which previously had been sprinkled lightly with 45-μm (320-mesh) Carborundum. Test plants were rinsed with water within a few minutes after inoculation. Gloves were changed between inoculations to avoid cross contamination. All plants were kept in the glasshouse at 18 to 30 C under fluorescent lights with a 15-hr day-length. Symptoms developed in 10-21 days. Infected leaf sap was confirmed serologically in double-gel-diffusion plates against a grape strain PRMV antiserum (titer 1/256) as previously described (10).

Assay of vineyard weeds as possible virus reservoirs.—During 1974 and 1975, weeds were dug from beneath PRMV-infected vines, bagged with soil around their roots,and transported to the glasshouse at Michigan State University (MSU). Root tissue from each weed was ground in NAW using a mortar and pestle and sap was inoculated to *C. quinoa*. Resulting infections were confirmed serologically. Tomato ringspot virus infection found in one weed species was confirmed serologically, using TomRSV antiserum (ATCC No. PVAS 25 having a titer of 1:2,048).

Plants that were found to be infected with PRMV were labeled and allowed to produce seeds, which then were collected and germinated in steamed soil. Whole seedlings were individually ground in NAW and inoculated to *C. quinoa*. All resulting infections were confirmed serologically.

Assay of Concord grape pollen and seeds for the presence of peach rosette mosaic virus.—Pollen from several infected vines was gathered in 1974 and 1975. Pollen grains were separated from maternal tissue using a stereoscopic dissecting microscope. About 100 mg of pollen was ground in NAW on two occasions and inoculated to *C. quinoa*.

Fruit from each of five infected and five noninfected vines was gathered in 1975 and 1976. Seeds were extracted and washed. Following stratification in moist sand at 4 C for 3 mo, seeds were germinated in steamed soil. Newly emerged seedlings were removed from the soil, the seed coat was removed in cases in which it had not become detached, and the seedlings were washed in water. Whole individual seedlings were ground in NAW and inoculated to *C. quinoa*. Serological tests confirmed the identity of PRMV.

Comparison of vegetative propagation and survival of PRMV-infected and healthy Concord grape cuttings.—In order to make a comparison of percentage rooting of dormant cuttings from healthy vs. diseased cane wood, dormant cuttings infected with PRMV, taken from vines indexed during February, 1977, and from virus-free vines from a MSU vineyard, were allowed to root in moist sand for a period of 2 mo. Percentage rooting was determined for virus-free vs. diseased cuttings. In previous years (1973, 1974, and 1975) a number of PRMV-infected and healthy rooted Concord cuttings were planted out in fumigated soil at MSU, to determine relative growth and survival of healthy vs. diseased propagants.

Soil sampling beneath peach rosette mosaic virus-infected vines to determine populations of Xiphinema americanum at various depths.—Soil samples were taken during the summer of 1974 at the test vineyard site and at a nearby vineyard which also contained PRMV-infected vines. Samples were taken at soil depths of 15.3 to 20.3-cm (6- to 8-inches), and thereafter at 30.5-cm (1-foot) increment depths to 243.8 cm (8 feet) to determine the vertical distribution of populations of the experimental vector. A 5.1-cm (2-inch) diameter auger with extension handles was used to sample both sides of the vine row at 5 vine sites at each location. Samples were taken at 61.0 cm (2 feet) from the trunk. Nematode extractions were made using the Jenkins' sugar flotation method (7). Counts were expressed as the number of nematodes per 100 cc of soil.

RESULTS

Rate of field spread.—The initial indexing done in 1974 revealed 15 PRMV-infected vines (Fig. 1). Reindexing in 1975, 1976, and 1977 revealed an additional 14, 3, and 8 infected vines, respectively. Newly infected vines were mostly adjacent to or directly or diagonally across the row from vines previously found to be infected. Of the 14 vines found newly infected in 1975, 10 were adjacent to previously nonfected vines. In 1976 and 1977, 3/3 and 6/8 newly infected vines were adjacent, respectively. The pattern of spread was elliptical and slow, which is indicative of a NEPO virus.

Vineyard weeds as possible virus reservoirs.—Three of 16 weed genera and species associated with diseased vines were found to be infected with PRMV. The virus was isolated from a single curly dock plant (*Rumex crispus* L.), from 5 to 9 Carolina horsenettle plants (*Solanum carolinense* L.), and from 5 of 14 common dandelion plants (*Taraxacum officinale* Weber). All PRMV-infected weeds were symptomless. Tomato ringspot virus, but not PRMV, was isolated (and confirmed serologically) from all eleven common plantain plants (*Plantago major*) tested. Any weeds that tested positive for PRMV infection serologically were not tested for infection by TomRSV or other viruses. Other weeds tested but found noninfected were: quackgrass (*Agropyron repens* L. Beauv.), redroot pigweed (*Amaranthus retroflexus* L.), thymeleaf sandwort (*Arenaria serpyllifolia* L.), common milkweed (*Asclepias syriaca* L.), wild oat (*Avena fatua* L.), poverty brome (*Bromus sterilia* L.), orchard grass (*Dactylis glomerata* L.), henbit (*Lamium amplexicale* L.), Virginia pepperweed (*Lepidium virginicum* L.), perennial ryegrass (*Lolium perenne* L.), common chickweed (*Stellaria media* L. Cyrillo), and yellow goat's beard (*Tragopogon pratensis* L.).

Seed-borne aspects of peach rosette mosaic virus in weeds.—Dandelion seedlings from PRMV-infected mother plants were found to be PRMV-infected at a level of 3.6% (5 of 137 seedlings) when assayed individually on *C. quinoa*. Carolina horsenettle seedlings were not

PRMV-infected (0 of 96 seedlings) when assayed on *C. quinoa*. The curly dock plant did not form seeds, therefore seed transmission tests were not done.

Pollen and seed-borne aspects of peach rosette mosaic virus in Concord grape.—Inoculum prepared from hand sorted pollen grains failed to transmit PRMV to *C. quinoa* in two attempts. Germination of the 1975 crop of seeds from healthy and PRMV-infected vines was 4.8% (16/330) and 0.8% (3/390), respectively. All three seedlings from infected vines assayed negative for PRMV. The 1976 crop of seeds from healthy and diseased vines germinated at levels of 28.7% (86/300) and 11.7% (35/300), respectively. Seedlings from infected vines, when assayed on *C. quinoa*, were infected at a level of 11.4% (4/35). The overall level of seed-borne virus was 9.5% (4/38) for the combined 1975 and 1976 tests. The fact that PRMV was not recovered from pollen suggests that the virus is seed-borne via maternal tissue.

Vegetative propagation and survival of peach rosette mosaic virus-infected Concord grape cuttings.—A total of 34.3% (12/35) and 88.6% (31/35) dormant cuttings from PRMV-infected and virus-free Concord vines formed roots, respectively. Healthy cuttings had much better root development than did diseased cuttings. After 3 yr of attempts to establish 40 PRMV-infected vines in the vineyard at MSU, only 40.5% have survived after annually replanting diseased vines that winter-killed over a 3 yr period. Ninety-five percent of the healthy rooted cuttings planted in the same vineyard and never replanted have survived.

Vertical distribution of Xiphinema americanum populations beneath peach rosette mosaic virus-infected vines.—Populations of *X. americanum* at the vineyard site (location A, Table 1) varied from 0.3 to 2.5/100 cc of soil and were found to a depth of 213.4 cm (7 feet). At location B (a nearby PRMV-infected vineyard), populations varied from 0.3 to 10.5/100 cc of soil and were found to a depth of 152.4 cm (5 feet).

DISCUSSION

Concord grape vines infected with PRMV can exhibit severe symptoms when infection has been established for some time, but about one-third of the vines in this study which had been infected for less than 1 yr were symptomless. Thus, sap-indexing of individual vines around apparent infection foci is essential to ascertain the extent of the infection focus, prior to removal of diseased vines and soil fumigation as part of a control program. Control of the dagger nematode is imperative to stop the spread of the virus from the edge of the infection focus. The fact that PRMV is present in weeds and is seedborne in dandelion and grape, gives new insight into the possible increased incidence of this disease. These include: (i) Virus may be transmitted by nematodes from infected weeds to vines in addition to possibly being spread from vine to vine. (ii) Old peach orchard sites formerly containing trees infected with PRMV may not be the only source of PRMV infection of Concord grape. Any soil with PRMV-infected weeds could serve as an inoculum source. (iii) The discovery that PRMV is seed-borne in dandelion indicates that this weed could be a source of primary inoculum for the disease in vineyards heretofore containing only healthy vines, since both dandelions and *X. americanum* are ubiquitous in Michigan vineyards.

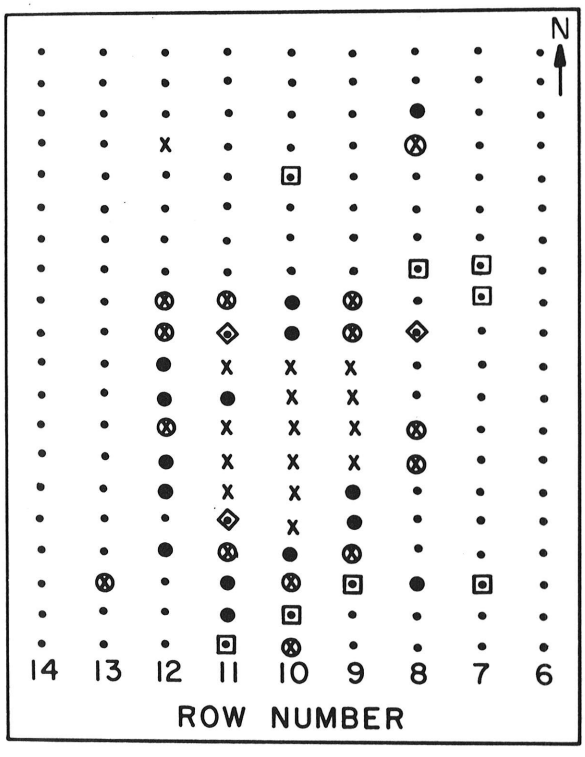

Fig. 1. Diagramatic representation of a block of mature cultivar Concord grapevines which has been sap-indexed on *Chenopodium quinoa* over a 4-yr period to determine the pattern of spread of peach rosette mosaic virus (PRMV); • = healthy vine, x = missing or dead vine. Vines which indexed positive for PRMV infection for the first time in 1974, 1975, 1976, and 1977 in that sequence are delineated by ●, ⊗, ◈, and ⊡, respectively.

TABLE 1. Populations of *Xiphinema americanum* at various depths beneath Concord grapevines infected with peach rosette mosaic virus (PRMV) at Lawton, Michigan in 1974

	Vineyard location and mean no. of nematodes/100 cc soil/vine[a]	
Soil-depth sampled	Location A[b]	Location B[b]
15.2 cm (6 inches)	1.0	9.3
30.5 cm (1 foot)	0.0	10.5
61.0 cm (2 feet)	0.3	3.3
91.4 cm (3 feet)	...[c]	1.7
121.9 cm (4 feet)	0.0	0.3
152.4 cm (5 feet)	1.0	1.0
182.9 cm (6 feet)	0.0	0.0
213.4 cm (7 feet)	2.5	0.0
243.8 cm (8 feet)	0.0	0.0

[a] Vines were core-sampled once on each side of five vines at 61.0 cm (2 feet) from the trunk at each location.

[b] Location A is the vineyard site where the rate of spread of PRMV was studied. Location B is at a PRMV-infected vineyard about 2 km (1.25 mi.) distant from location A.

[c] Sample not taken because of rocks.

(iv) The finding that PRMV is seed-borne in grape may also explain the widespread distribution and extent of the disease in Michigan. It is a practice among processors to give pomace to growers to spread in their vineyards to increase organic matter. Concord grapes are hot-pressed for juice extraction and are held at about 60 C (140 F) for 40 min. Seeds that have undergone this treatment germinate, but we have not yet assayed seedlings for seed-borne virus following this heat treatment.

Growers try to avoid propagating wood from infected areas, since diseased grape cuttings root poorly and survive very poorly following Michigan winters. However, since recently infected vines are symptomless, propagating wood could be a source of inoculum in new planting or replanting situations. In addition, this could be a dangerous source of virus in material exported to other states. The finding that *X. americanum* is present at depths of 152.4 cm (5 feet) to 213.4 cm (7 feet) poses some serious control problems. However, deep placement fumigation tests (12) indicate promise for control of dagger nematodes at those depths.

Work is in progress to determine the effect of such fumigation techniques on the population of *X. americanum* in Michigan soils and preliminary results are promising (D. Ramsdell and G. Bird, *unpublished*). The evaluation of American and French hybrid cultivars for tolerance or immunity to PRMV is also under way.

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