Peach Rosette Mosaic Virus, Symptomatology and Nematodes Associated with Grapevine 'Degeneration' in Michigan

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ABSTRACT

Peach rosette mosaic virus (PRMV) was present in five out of 21 (23.8%) of surveyed mature *Vitis labrusca* 'Concord' grapevines at two of seven locations exhibiting grapevine 'degeneration' in Michigan. All 'degeneration' vines were delayed 2-3 wk in breaking dormancy, and exhibited weak growth and berry cluster shelling compared to healthy vines. The presence of PRMV was detected by sap inoculation from surveyed vines to herbaceous indicator hosts and confirmed serologically. Vines infected with PRMV exhibited abnormalities in leaf morphology; short, crooked cane internodes; narrow angles of cane branching and an 'umbrella-like' vine growth habit. Yield of PRMV-infected vines was drastically reduced, compared to apparently healthy PRMV-free vines. No other sap-transmissible virus was detected in any of the surveyed vines. PRMV-positive vines had medium populations of *Xiphinema americanum* or high populations of *Criconemoides xenoplax* nematodes associated with root zones. PRMV-negative 'degeneration' vines at five of the seven locations surveyed had mean vine populations of 134 *X. americanum* and 152 *C. xenoplax* per 100 cc soil per vine. Vines at these locations also yielded low populations of four other plant pathogenic nematodes. Nonsap-transmissible viruses not yet detected, and/or high levels of *X. americanum* or *C. xenoplax* associated with nonPRMV-infected 'degeneration' vines could account for symptoms expressed by these vines.

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A disorder of *Vitis labrusca* L. 'Concord' grapes referred to locally in southwestern Michigan as 'grapevine degeneration' has been observed for at least 30 yr; however, symptoms described for 'degeneration' have been variable. Delayed bud break, foliation, and bloom in the spring; shattering of berry clusters; poor yield; weak growth; and sometimes vine death have been commonly listed as symptoms of the disorder (4, 9, 11). Cation (1, 2) described peach rosette mosaic as a soil-borne disease affecting peach (*Prunus persica* L. Batsch) and plum (*Prunus domestica* L.). Extreme shortening of internodes, causing a whorling of leaves, leaf distortion, and sometimes tree death were the most prominent symptoms. Klos et al. (9) reported that healthy, young peach seedlings inoculated with *Xiphinema americanum,* Cobb, 1913, and *Criconemoides* sp. nematodes separated from the root zone of field-infected peach trees developed typical peach rosette mosaic symptoms, and budding experiments indicated that Concord grape is a potential host. Plunge (11) also investigated the causes of 'degeneration.'

Dias and Cation's (4) recent publication described some symptoms in grape and peach caused by a Michigan culture of peach rosette mosaic virus (PRMV), physicochemical properties of the virus, and transmission studies. PRMV was shown to be a two-component polyhedral virus ca. 28 nm in diam and unrelated to other polyhedral viruses.

Cation (2) reported in 1951 that PRMV of peach occurred only in Michigan and New York. As this publication went to press, PRMV had been found in grapes only in Michigan.

The purpose of this research was to determine whether viral, and possibly nonviral, agents are involved in grapevine 'degeneration' in Michigan and to further describe the range of symptoms associated with PRMV of grape in the field.

MATERIALS AND METHODS.—*Virus purification.*—A culture of PRMV from a Michigan Concord grapevine (obtained from J. E. Bath) was mass-inoculated to *Chenopodium quinoa* Willd. plants growing under natural light in a greenhouse at 18-21 C, and supplemented with 15 h of fluorescent light of 5,380 lx. Purification was done according to the method of Dias and Cation (4), which involved buffer extraction, dialysis, differential centrifugation, Sephadex gel filtration and sucrose density-gradient centrifugation in a SW 25.1 rotor at 105,000 *g* for 2.5 h. Density gradients (10-40%) were fractionated and scanned (A_{254}) using an ISCO density-gradient fractionator (Instrument Specialties Co., Inc., Lincoln, Nebraska). One-ml fractions were collected and each fra tion was inoculated to individual *C. quinoa* plants to assay for infectivity.

Electron microscopy.—After residual sucrose was removed by dialysis, the purified virus preparation was applied to Formvar-coated copper grids, negatively stained in 2% phosphotungstic acid (PTA, pH 7), and observed with an electron microscope (Philips 300 TEM).

Field survey using herbaceous hosts and serology.—Four 20- to 30-yr-old Concord vineyards in

Van Buren County (labeled A, B, C, and D) and three in Berrien County (labeled E, F, and G) in southwest Michigan were selected for the survey because they exhibited a number of the previously described symptoms associated with 'degeneration.' Three vines which exhibited delayed bud break and weak growth during the 1972 year and the spring of 1973, were marked at each location. Immature leaf tissue was collected in early June, 1973, and transported in ice to East Lansing. One gram of tissue from each vine, and control tissue from virus-free Concord vines, was triturated in 15 ml of 2% nicotine alkaloid and a little 400-mesh corundum using a sterile mortar and pestle. Sap inoculations were made to two greenhouse-grown plants each of *Chenopodium quinoa, C. amaranticolor* Coste & Reyn., *Cucumis sativus* L. 'National Pickling', *Nicotiana tabacum* L. 'White Burley,' *Petunia hybrida* Vilm., and *Antirrhinum majus* L. 'Red Giant Crimson.' The latter has been shown to be a differential host for tomato ringspot virus (TomRSV) and tobacco ringspot virus (TRSV) (15) found causing disease symptoms in grape (5, 6). Cultures of TomRSV ('Baco Noir' grape isolate supplied by J. K. Uyemoto), TRSV (a muskmelon isolate obtained from D. de Zeeuw), and PRMV (a grape isolate from a southwest Michigan Concord vine which was purified by the authors as previously described) were similarly inoculated to sets of herbaceous indicators as standards. All plants were incubated in a Sherer-Gillett growth chamber programmed for 21 C and a 15-h photoperiod of 29,570-lx incandescent plus fluorescent light.

Symptom development at the end of 1 and 2 wk was compared among the vine-inoculated, standard culture-inoculated, and control plants.

Expressed sap from vine-inoculated plants showing symptoms and healthy controls was checked serologically against TomRSV, TRSV, grape fan leaf virus (GFLV), and PRMV antisera (the first two antisera were supplied by J. K. Uyemoto and the latter two by H. F. Dias). Agar gel double-diffusion tests were made in 9-cm diam plastic petri dishes containing 12 ml of 0.85% Ionagar No. 2S (Colab Laboratories, Inc., Glenwood, Ill.) in 0.85% NaCl solution containing 0.05% sodium azide. The center well containing the antiserum was 7 mm in diam. The six outer wells containing virus-laden sap or healthy sap were 5 mm in diam and were spaced equidistantly apart and 1 cm from the center well.

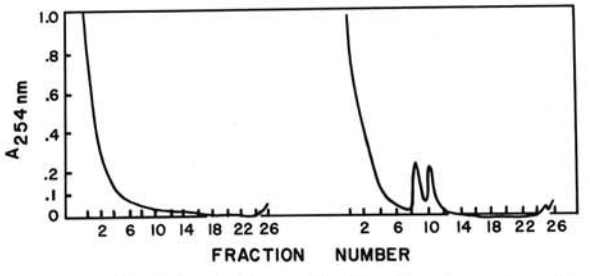

Fig. 1. (left) ISCO density-gradient fractionation pattern after sucrose density-gradient centrifugation of purified healthy *Chenopodium quinoa* sap. (right) Pattern showing Concord grape culture of peach rosette mosaic virus from purified infected *C. quinoa* sap showing presence of two components, both of which are infectious to *C. quinoa*.

Bud transmission studies.—Buds taken at early bud break from each vine at each location were chip-budded into virus-free indexed rooted Concord grape cuttings in pots. Two buds from each donor vine were budded to one recipient vine. Buds from virus-free vines were chip-budded to virus-free vines as controls. These vines will be observed in the field for a number of seasons to determine if any of the surveyed 'degeneration' vines are a result of nonsap-transmissible virus infection or if some are a result of a nonviral cause; i.e., high nematode populations.

Symptom expression and comparative yield of marked field vines.—Vines which were positive and negative for PRMV from the herbaceous host and serology tests were observed for symptoms throughout the balance of the growing season and compared with apparently healthy vines. Yield data from PRMV-infected and healthy vines were taken at harvest to determine the detrimental effect of PRMV upon yield. A spring frost killed primary shoots on all vines sampled. Yield was from secondary shoots, which is always less than from primary shoots.

Nematode sampling.—Soil samples from beneath all surveyed vines and three aparently healthy vines at locations C through F were taken at a depth of 10.2-20.3 cm (4-8 in) in the fall to determine the identity and number of nematodes associated with the vines. Jenkins' sugar flotation method (7) was used for extraction of nematodes.

RESULTS.—*Purification of PRMV.*—Following sucrose density-gradient centrifugation, two opalescent bands were observed. The gradient tube was fractionated into 26 one-ml fractions. The resulting ISCO density-gradient scan indicated that the two bands corresponded to the two peaks collected in fractions 8 through 12 (Fig. 1). Both peaks were infectious to *C. quinoa,* which agrees with the data of Dias and Cation (4). The purified virus reacted positively with an antiserum prepared against PRMV. The purified virus is an isometric particle ca. 28 nm in diam (Fig. 2), which confirms previous work (4).

PRMV symptoms on herbaceous indicator plants.—Symptoms were first evident on *C. quinoa* within 10 days after inoculation. New leaves exhibited a yellow-green mottle (Fig. 3-A), while mature inoculated leaves had irregular yellow blotches 2-5 mm in diam (Fig. 3-B). Terminal epinasty was also evident, and as time after inoculation progressed, terminal death and leaf yellowing increased until leaves dropped and the plants died.

Symptoms on *C. amaranticolor* were first noted 16 days after inoculation. Terminal epinasty, terminal necrosis, and mottling of new leaves were the initial symptoms observed (Fig. 3-C). *C. amaranticolor* did not die, but older plants became very stunted with much lateral proliferation and small leaves.

PRMV did not cause any detectable symptoms on other herbaceous hosts tested.

Results of field survey using herbaceous hosts and serology.—Sap inoculations from three of three vines at location A and two of three vines at location B south of Lawton, Michigan, in Van Buren County, caused symptoms typical of PRMV on *C. quinoa* and *C. amaranticolor.* Agar gel double-diffusion tests were positive for PRMV in all five cases; however, sap from

these plants failed to react with TomRSV, TRSV, or GFLV antisera. Inoculations from vines at the remaining five locations (C through G) did not cause any symptoms in the herbaceous hosts. Herbaceous indicator plants inoculated with cultures of TRSV and TomRSV developed symptoms typical of those described for these viruses (13, 14) within 7-11 days.

Symptomatology of PRMV-infected vines and effects upon yield.—All PRMV-infected vines were delayed 2-3 wk in breaking dormancy, compared to apparently healthy vines in the same fields (Fig. 4-A). Late and uneven bloom on infected vines resulted in small, uneven clusters which shelled off most berries (Fig. 4-B). After foliation was complete, leaf deformities became evident; the petiolar sinuses of many leaves were flat rather than concave as in healthy leaves (Fig. 4-C). Other leaves were puckered or deformed (Fig. 4-D), and some were mottled. In general, overall leaf color of PRMV-infected vines was a lighter green than noninfected vines. Leaf symptoms were too variable to be a dependable symptom for field diagnosis of PRMV infection. Cane symptoms described in the following text are better criteria for diagnosis of PRMV.

Cane growth of PRMV-infected vines is typified by short, crooked growth of the first four to six internodes with odd angles of cane branching (Fig. 4-D,E). The overall effect of this altered cane growth gives an infected vine an 'umbrella-like' growth habit (Fig. 4-F).

The 'degeneration' vines at locations C through G, which were negative for PRMV, did not show all of the symptoms associated with PRMV. Delayed bud break and somewhat poor growth occurred, but as the season developed, these vines did not exhibit extremely short internode development or crooked cane growth. Leaf color and shape were mostly normal and crop yield was not impaired to the degree that it was with PRMV-infected vines.

Vines which have been infected with PRMV for a number of years die, but the grower usually removes the vine prior to death because of poor production. There were elliptical areas of missing vines at both PRMV-positive vineyards, but not at the other locations (Fig. 4-G). Growers at locations A and B reported a slow spread of the disease and both said that attempts at replanting these areas resulted in stunted, sick vines within 1-2 yr.

The berry yield of PRMV-infected vines was markedly lowered as compared to apparently healthy vines which indexed free of the virus. The mean yield of five PRMV-infected vines was 86.4 gm, while the mean yield of three PRMV-free vines was 4,346.7 gm. Berries from infected vines at maturity contained only one seed and were insipid to the taste.

Nematode genera and populations associated with surveyed vines.—Most of the vines surveyed had high populations of plant pathogenic nematodes. At location A, *Criconemoides xenoplax,* Raski, 1952, beneath PRMV-positive vines averaged 153 per 100 cc soil per vine. At location B, the two PRMV-positive vines had *C.*

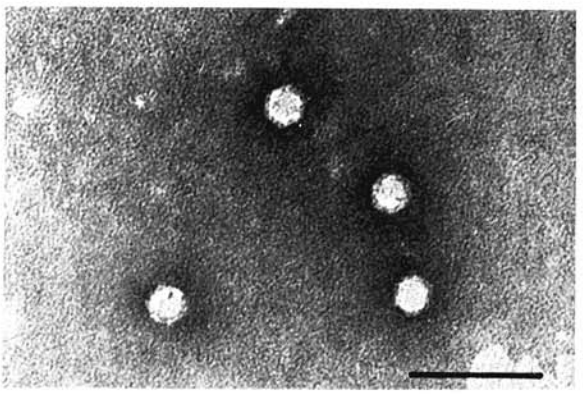

Fig. 2. Negatively stained peach rosette mosaic virus particles from purified Concord grape culture (× 300,000). Bar represents 100 nm.

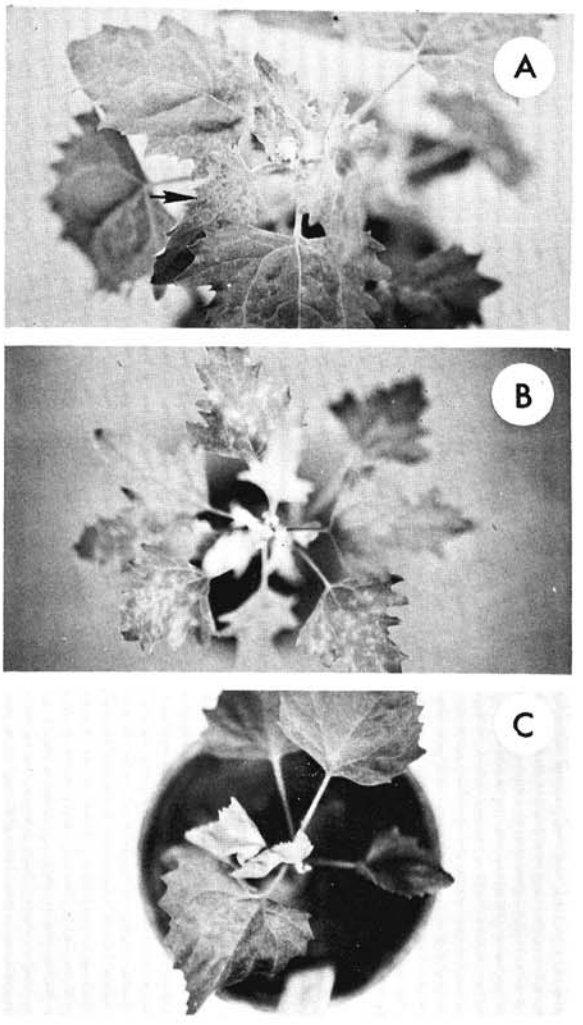

Fig. 3-(A to C). Symptoms of two *Chenopodium* spp. infected with peach rosette mosaic virus (PRMV). **A)** Systemic mottle (arrow) of uninoculated *Chenopodium quinoa* leaves 10 days after inoculation. **B)** Yellow blotches on PRMV-inoculated *C. quinoa* leaves 14 days after inoculation. **C)** Severe terminal epinasty and necrosis of new leaves of *Chenopodium amaranticolor* 16 days after inoculation with PRMV.

xenoplax and *X. americanum* populations of 55 and 35 per 100 cc soil per vine, respectively. Low populations of *Trichodorus* sp. were also present beneath the PRMV-positive vines at locations A and B. Nematode populations beneath nonPRMV 'degeneration' vines at locations C through G ranged from 30-240 *X. americanum* and from 37-280 *C. xenoplax* per 100 cc soil per vine. Nematode populations beneath apparently healthy vines at locations C through F (no apparently healthy vines at location G) ranged from 10-93 *X. americanum* and from 120-230 *C. xenoplax* per 100 cc soil per vine. *Hoplolaimus, Longidorus, Pratylenchus,* and

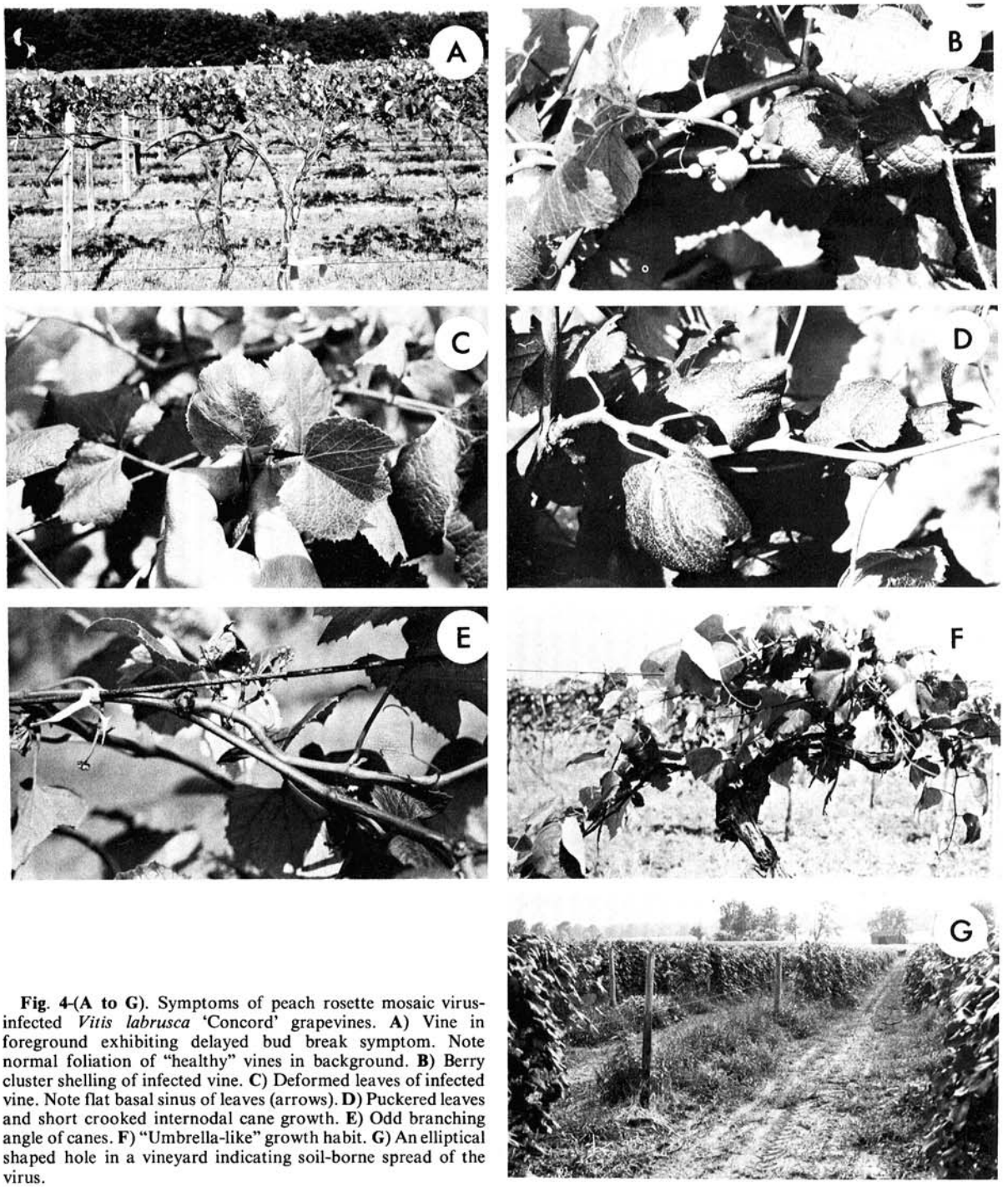

Fig. 4-(A to G). Symptoms of peach rosette mosaic virus-infected *Vitis labrusca* 'Concord' grapevines. **A)** Vine in foreground exhibiting delayed bud break symptom. Note normal foliation of "healthy" vines in background. **B)** Berry cluster shelling of infected vine. **C)** Deformed leaves of infected vine. Note flat basal sinus of leaves (arrows). **D)** Puckered leaves and short crooked internodal cane growth. **E)** Odd branching angle of canes. **F)** "Umbrella-like" growth habit. **G)** An elliptical shaped hole in a vineyard indicating soil-borne spread of the virus.

Trichodorus sp. were also present in low populations at some of the locations.

DISCUSSION.—PRMV was present in 23.8% of the 'Concord' vines surveyed exhibiting grapevine 'degeneration' symptoms. One PRMV-positive vineyard was formerly planted to peaches and the other was planted adjacent to an old peach orchard.

X. americanum, which was present beneath PRMV-positive vines at location B, has been shown by Dias and Cation (4) to transmit PRMV erratically and with low frequency from herbaceous host to herbaceous host, but not to grape, after the nematodes artificially were made viruliferous. *C. xenoplax,* associated with PRMV-positive vines at location A, was previously reported by Klos et al. to be a vector of PRMV in peach (9).

It is possible that there is immunity from PRMV already present in *V. labrusca* 'Delaware' grape. Apparently healthy, high-yielding Delaware vines are growing in the exact location at vineyard A and at a second location not in this survey, where Concord vines previously died or were removed because of PRMV infection (authors, *unpublished*).

Kirkpatrick et al. (8) have shown that *Xiphinema index,* added to potted *Vitis vinifera* 'Carignane' grape at the rate of 500 nemas per 6,000 cc soil, resulted in delayed bud break, marked reduction in extension growth, berry shatter, and fruit size reduction by the end of the second year as compared to noninoculated controls. These symptoms are similar to the symptoms observed on PRMV-free 'degeneration' vines in this survey. Cohn (3) has demonstrated that *X. index* and *Longidorus africanus* caused blackening and cortical disintegration of *V. vinifera* seedling roots. Raski and Radewald (12) found that *C. xenoplax* multiplied when on *V. vinifera* 'Thompson Seedless' grape roots, but that the nematodes did not cause any marked pathogenic symptoms, even after feeding for the 142-147 days. Lownsbery et al. (10) recently showed that *C. xenoplax* is a primary pathogen of peach when allowed to feed on the test host for 2.3 yr.

Nonsap-transmissible virus(es) not yet detected and/or high populations of *X. americanum* or *C. xenoplax* under the nonPRMV 'degeneration' vines compared to those under apparently healthy vines at locations C through F could account for the 'degeneration' symptoms exhibited by those vines. Pathogenicity tests are necessary to determine the direct pathogenic effects of *X. americanum* and *C. xenoplax* to Concord grapevines.

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