Symptomatology and Incidence of Prunus Necrotic Ringspot Virus in Peach Orchards in Georgia

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ABSTRACT

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Peach cultivars in central Georgia developed foliar chlorotic rings and necrosis during the first year after bud-inoculation with Prunus necrotic ringspot virus (PRSV). Twig dieback, bark necrosis, root sprouts, and trunk cankers developed during subsequent years. Symptoms on inoculated trees resembled those of the slow-decline disease prevalent in central Georgia orchards, particularly the extensive bark necrosis and longitudinal trunk cankers. The virus was detected in commercial orchards only in central Georgia by direct bioassay with Shirofugen flowering cherry or by enzyme-linked immunosorbent assay. In Peach County, 16% of apparently healthy (symptomless) trees and 25% of trees showing symptoms of slow decline were positive for PRSV. In Houston County, 42% of symptomless trees and 73% of trees in decline were positive. Prune dwarf virus was detected in only 7% of the PRSV-positive trees.

Various forms of peach decline have been recognized in peach orchards in Georgia and other peach-growing areas of the southeastern United States for more than 50 yr (16,18). Sudden, premature death of trees, the short-life syndrome, has been studied intensively and is recognized as the collapse of affected trees during spring or early summer (15). Prolonged or slow decline of peach trees, however, is not well understood. Slow decline, characterized by progressive bark necrosis and splitting, has contributed to mortality and low productivity of many orchards in Georgia. It is a condition variously attributed to root-knot nematodes, Clitocybe root rot, or bacterial canker acting independently of the short-life syndrome (1,14,17). Involvement of a virus such as Prunus necrotic ringspot virus (PRSV) has been suspected but not yet documented.

PRSV is commonly encountered in cultivated species of *Prunus* and is distributed worldwide. The virus is multiparticulate (8) and exists as a variety of biological forms or strains that cause a diversity of symptoms on many hosts (9,10). In the United States, it has been reported in peach-growing areas in the Northeast, West, and Midwest, where symptoms include foliar chlorotic rings, necrotic spots or deformation, and bark necrosis, pitting, splitting, or girdling (12). PRSV is transmitted by budding and is spread in nature by pollen (3,12). It is sometimes found in combination with other stone fruit viruses, particularly prune dwarf virus (PDV), that are difficult to distinguish by host reaction alone (11,13).

In 1953, a limited survey in Peach County, GA, the center of the peach industry, failed to detect PRSV in trees of the most common cultivars grown (7). In the 1970s, however, observations were frequently made of twig dieback and partial or complete trunk necrosis on peach trees undergoing a slow and progressive decline in Peach County and adjacent Houston County (H. C. Kirkpatrick, *unpublished*). Foliar symptoms were not common. An investigation was initiated to determine the characteristic symptomatology of strains of PRSV in Georgia and to evaluate the possible involvement of PRSV in slow decline of peach trees.

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MATERIALS AND METHODS

Virus sources. In 1972, budwood infected with PRSV was obtained from P. W. Cheney of the Fruit Research Lab, USDA-ARS, Wenatchee, WA, and by the second author from naturally infected trees in Peach and Houston counties in Georgia. Trees suspected to be natural sources were identified by symptoms and by reactions on a range of indicator hosts inoculated in the greeenhouse. Sources were tested for the presence of PDV by the cucumber-Butternut squash technique of Gilmer (4). Source buds were grafted onto and maintained on virus-free peach seedlings (*Prunus persica* (L.) Batsch 'Elberta'). Strains were severe PRSV WI161 and R19T11 from Houston and Peach counties, respectively; mild PRSV R19T8 from a tree in Houston County; and severe PRSV TR4T16 from the Fruit Research Lab, Wenatchee, WA.

Symptomatology and host range. Ten to 15 2-yr-old peach trees (cultivars June Gold, Mayflower, and Maygold budded on Lovell rootstocks) were inoculated from PRSV source trees in August and September 1974 by inserting three T-buds per tree at the bases of scaffold limbs. Symptoms were recorded during the subsequent 5 yr. Ten trees of two of these cultivars were tested simultaneously under controlled greenhouse environment (24 C during the day and 13 C at night) for 2 yr with 6- to 8-wk periods of dormancy. Uninoculated (unbudded) checks were included in each test.

Native and other cultivated species of *Prunus* were also tested by bud and root-graft inoculations in the greenhouse. Species included Chickasaw plum (*P. angustifolia* Marsh), Mariana plum (interspecific hybrid *P. domestica* × *P. cerasifera* × *P. munsoniana*), wild black cherry (*P. serotina* Ehrh.), Mahaleb cherry (*P. mahaleb* L.), Shirofugen flowering cherry (*P. serrulata* Lindl. 'Shirofugen'), Kwanzan cherry (*P. serrulata* Lindl. 'Kwanzan'), Nanking cherry (*P. tomentosa* Thumb), Jordanola almond (*P. dulcis* (Mill.) D. A. Webb), and peach cultivars Elberta, Lovell, and Redskin. Five to 10 2-yr-old trees were budded from PRSV-infected peach sources between July and September 1974 during active growth. Roots were also wedge-grafted to seedling taproots of half of the plants. Plants were transferred to 3 C for 6–8 wk for winter dormancy. Five additional 2- to 3-yr-old plants were budded in the field for confirmatory observations. Before inoculations, plants were indexed on 3- to 4-yr-old Shirofugen trees in the field to verify freedom from PRSV and PDV (6,13). Symptoms were observed for 3 yr in the greenhouse and for 5 yr in the field.

County surveys and classification of diseased trees. Surveys for PRSV were conducted between June and August 1981 in 14 selected orchards in the

principal peach-growing areas in Georgia. Orchards with trees in decline were selected when possible. Cultivar data were not taken because of the prevalence of mixed plantings. Twig samples were taken from 40–60 symptomless trees per orchard and, when possible, from an equal number of trees with one or more of the symptoms of slow decline. Symptomatic trees were placed in one of four categories: 1) dieback of terminal twigs only; 2) pronounced dieback extending to scaffold limbs with bark, and sometimes, trunk necrosis (cankers); 3) severe dieback, severe necrotic splitting of the bark and trunk, and vigorously growing basal root sprouts; and 4) root sprouts dominant and cultivar severely weakened with necrosis, stunting, and bark splitting. Trees with root sprouts only, without signs of terminal dieback or cankers, were not considered diseased. Trees in sudden collapse from the short-life syndrome were not sampled. Three twigs from each tree sampled were cut and placed in a plastic bag for transportation to the laboratory. One bud from each twig was indexed on Shirofugen flowering cherry. Twigs were then shipped to Wenatchee, WA, where serological tests and additional Shirofugen bioassays were conducted.

Indexing and serology. Buds from survey or test twigs were indexed on Shirofugen flowering cherry at the Southeastern Fruit and Tree Nut Laboratory, Byron, GA, within 6 hr of collection and at the Fruit Research Laboratory, Wenatchee, within 5 days of collection. Three buds were tested from each survey tree. Positive checks were buds from known PRSV source trees, and negative checks were buds from known healthy trees. Three weeks after budding, the cambium was cut above bud insertions and the tissue examined for necrosis.

Enzyme-linked immunosorbent assays (ELISA) were conducted at the Fruit Research Laboratory, Wenatchee, on leaf tissues combined from three twig samples per tree. One-half gram of pooled tissue was homogenized in 5 ml of ELISA grinding buffer (2) and filtered into test tubes coated with silicone (Sigmacote; Sigma, Inc., St. Louis, MO). ELISA wells were each coated with antigen by procedures previously described (2) and then with 200 μl of tissue extract. Antisera was against the G strain of PRSV (obtained from R. W. Fulton, University of Wisconsin, Madison) and against Fulton's B strain of PDV (PVAS 33, American Type Culture Collection, Rockville, MD). Gamma globulin was purified from antisera by ammonium sulfate precipitation, dialysis, and elution on a DEAE column; the peroxidase was conjugated and tests were conducted as described by Clark and Adams (2). Coating and conjugate gamma globulins were used at 1:1,000 and 1:800 dilutions, respectively, with two wells per sample, one for PRSV and the other for PDV. Enzyme reactions were measured at 540 nm by spectrophotometer (Varian 635 D, Varian Assoc., Los Altos, CA). Optical density readings greater than pooled negative (buffer) controls plus two standard deviations were considered positive. Presumptive evidence for PRSV in sampled trees was based on positive Shirofugen reactions with negative ELISA for PDV or on any positive ELISA reaction for PRSV. Evidence for PDV in sampled trees was based on positive ELISA reactions.

RESULTS

Symptoms and host range. All PRSV source trees were negative for PDV as determined by cucumber-Butternut squash bioassay or by ELISA (based on a single well per sample). Symptoms induced on peach and other *Prunus* hosts were generally characteristic of those

Table 1. Occurrence of specific symptoms of slow decline of peach on cultivars inoculated with severe and mild strains of Prunus necrotic ringspot virus (PRSV)[a]

	Peach cultivar and inoculated PRSV strain					
	June Gold		Mayflower		Maygold	
Symptoms[b]	Severe	Mild	Severe	Mild	Severe	Mild
No symptoms	0/28[c]	24/28	2/30	25/30	2/10	10/10
Foliar chlorotic rings[d]	28/28	4/28	16/30	0/30	5/10	0/10
Foliar necrotic rings[d]	28/28	2/28	14/30	0/30	5/10	0/10
Twig dieback	28/28	1/28	12/30	1/30	6/10	0/10
Basal sprouts	18/28	1/28	23/30	5/30	4/10	0/10
Bark necrosis	5/28	2/28	23/30	1/30	2/10	0/10
Bark splitting	8/28	2/28	23/30	1/30	2/10	0/10
Stunting	10/28	0/28	8/30	0/30	0/10	0/10

[a] Combined data for severe strains TR4T16 and W1161 and mild strain R19T8 in bud or root-graft inoculations (August–September 1974); symptom data recorded for 5 yr.
[b] No symptoms developed in uninoculated checks (10 trees for June Gold and Mayflower, five trees for Maygold).
[c] Number trees positive/total tested.
[d] Foliar symptoms transitory, appearing first year only.

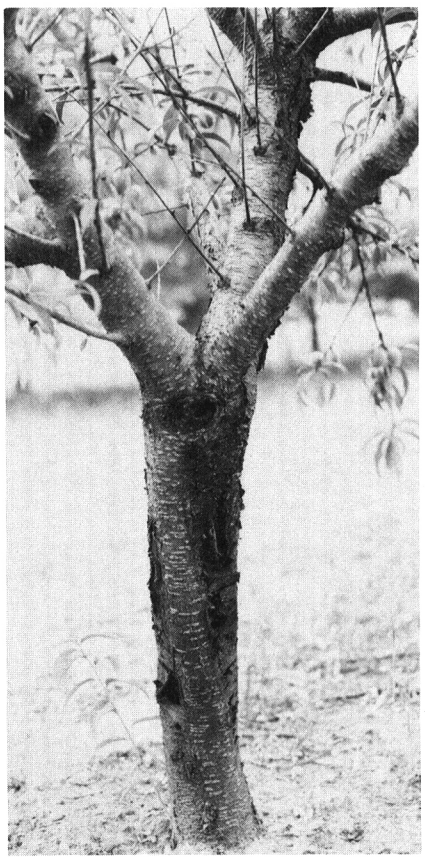

Fig. 1. Bark necrosis and longitudinal splitting on a 4-yr-old Junegold peach tree 20 mo after inoculation by bud and root grafts from a source of severe Prunus necrotic ringspot virus.

Fig. 2. Bark necrosis and development of vigorous root sprouts on a 3-yr-old cultivar Mayflower peach tree inoculated with a severe strain of Prunus necrotic ringspot virus.

previously reported for PRSV (12).

Peach trees generally developed foliar chlorotic rings typical of "shock" symptoms within 2 wk of inoculation. "Shothole" necrosis on leaves and twig dieback were evident during the first and second years after inoculation. Cultivar differences in susceptibility were evident. June Gold was most susceptible. All plants inoculated with the severe strains of PRSV developed foliar symptoms (Table 1). Mayflower was intermediate in susceptibility; Maygold was least susceptible, with only 50% of inoculated plants developing foliar symptoms. On the average, foliar symptoms developed on about 70% of trees inoculated with severe strains and on 3–6% of trees inoculated with mild strains.

Terminal dieback, bark necrosis and splitting, and basal sprouts were chronic symptoms developing in the later stages of disease. Some trees inoculated with severe strains of PRSV developed basal sprouts and bark necrosis within 6 mo. Foliar symptoms were seldom seen on trees 3 yr after inoculation. Typical symptoms during the third and fifth years of infection included severe bark necrosis with longitudinal splitting of trunk and scaffold limbs (Fig. 1). Strong, vigorous basal sprouts were also typical on trees infected with severe PRSV (Fig. 2). In this stage of decline, cultivar differences in susceptibility were also evident. Twig dieback, for example, developed in all June Gold trees inoculated with severe strains but only in about 50% of inoculated Mayflower and Maygold (Table 1). Bark necrosis and splitting, however, was most prevalent (76%) in Mayflower trees inoculated with severe strains compared with less than 28% in trees of the other cultivars.

In the final stages of decline of affected trees, sprouts became dominant and wood of the original cultivar weakened and died. However, most trees that were kept under standard cultivation practices in the orchard persisted in a stunted and weakened condition because of loss of wood from necrosis.

The host range of PRSV isolates from Georgia was also typical of that reported for the virus. Bark necrosis developed within 2 wk around bud insertions on Shirofugen. Symptoms on *P. dulcis*, *P. tomentosa*, *P. serotina*, and Mariana plum included foliar chlorotic rings, some mottling, and leaf dehiscence (especially with *P. serotina*) within 2–7 mo of inoculation. On *P. mahaleb*, foliar chlorotic rings did not appear until 11–12 mo after inoculation. No symptoms appeared on *P. angustifolia*, on *P. serrulata* 'Kwanzan,' or on the uninoculated checks. At least 50% of inoculated trees of each species were positive when indexed on Shirofugen during the first year. No differences were noted in symptom expression between greenhouse- and field-grown plants and between root- and bud-grafted plants during a 3-yr observation period.

Orchard surveys and indexing. Orchards with trees showing symptoms of slow decline were found in Peach and Houston counties in central Georgia. In Brooks County (south) and to a limited extent in Talbot County (west), trees were found with some terminal dieback but none of the associated symptoms of slow decline, particularly bark necrosis, trunk splitting, and root sprouts (Table 2). In the northern and northeastern growing areas (Morgan and McDuffee counties), no decline symptoms were found.

PRSV was commonly detected in trees in Peach and Houston counties. Incidence was high in symptomless as well as symptomatic trees (Table 2). On the average, 42% of symptomless and 73% of symptomatic trees in Houston County were positive for PRSV on the basis of bioassay or ELISA. Four of the 61 samples with positive ELISA reactions (7%) in Houston County were also positive for PDV. In Peach County, index reactions were positive for 16% of symptomless and 25% of symptomatic trees. One of the seven samples with positive ELISA reactions in Peach County was also positive for PDV. No PRSV or PDV was detected in samples from the other counties surveyed. Trees in Brooks and Talbot counties that had terminal dieback on twigs but no other symptoms were also negative.

DISCUSSION

PRSV was found in peach orchards in central Georgia. In 7% of the cases detected by serology, PDV was also found. Incidence of virus in trees in slow decline showing symptoms of bark necrosis, splitting, and basal root sprouts was about twice that in symptomless trees in the same orchards. Outside of the central Georgia area, typical symptoms of slow decline were not found, neither was there evidence of PRSV or PDV. In inoculation tests with strains of PRSV free of PDV, symptoms of slow decline were reproduced in the greenhouse and orchard. We conclude, therefore, that PRSV is a contributing factor to tree decline in central Georgia.

Variability in cultivar susceptibility to PRSV was evident in our replicated tests. This factor may explain some of the distribution of PRSV and decline in orchards in Georgia. Cultivars of peaches commonly planted in the southeast should be tested for resistance. Screening for disease resistance in advanced breeding selections should include a test for susceptibility to PRSV.

Bark necrosis, cankering, and necrotic

Table 2. Incidence of Prunus necrotic ringspot virus (PRSV) and prune dwarf virus (PDV) in selected peach orchards in Georgia (1980–1981)

Orchard		Total trees	Symptomless	Symptomatic trees by disease category[a]				Percent infection	
County	Age (yr)	indexed	trees	1	2	3	4	Symptomless trees	Symptomatic trees
Houston	8	40	15/19	2/2[b]	5/6	11/11	2/2	79	95
	7	33	7/19	0/0	3/4	6/6	1/4	37	71
	8	40	5/20	1/2	3/5	7/9	2/4	25	65
	6	39	4/20	1/2	2/5	6/12[c]	0/0	20	47
	5	40	10/20	3/4	6/6[c]	8/8	0/2	50	85
Peach	4	40	3/20	0/0	2/6[d]	2/8	3/6	15	35
	5	41	0/20	0/3	0/4	0/4	2/10	0	9
	6	41	1/21	0/1	0/3	0/6	3/10	5	16
	4	41	7/21	1/2	2/3	4/9	3/6	33	50
	4	40	5/20	2/4	4/5	4/8	2/3	25	30
Morgan	5	40	0/40	0/0	0/0	0/0	0/0	0	0
McDuffee	6	40	0/40	0/0	0/0	0/0	0/0	0	0
Talbot	5	50	0/40	0/10	0/0	0/0	0/0	0	0
Brooks	8	120[e]	0/60	0/60	0/0	0/0	0/0	0	0

[a] All infections PRSV unless footnoted. 1 = Dieback of terminal twigs only; 2 = pronounced dieback extending to scaffold limbs with bark, and sometimes, trunk necrosis (cankers); 3 = severe dieback, severe necrotic splitting of the bark and trunk, and vigorously growing basal root sprouts; and 4 = root sprouts dominant and cultivar severely weakened with necrosis, stunting, and bark splitting.

[b] Number positive/total trees indexed. Indexing by Shirofugen bioassay and by ELISA. Positive reactions to either assay considered a sign of infection.

[c] Two trees ELISA-positive for PDV.

[d] One tree ELISA-positive for PDV.

[e] Shirofugen bioassay only.

longitudinal splitting of the trunk and scaffold limbs are prominent symptoms of PRSV on some cultivars of peach in Georgia. Although these specific symptoms of PRSV have been described previously on peach (12), they are not frequently encountered in orchards in the northwestern or eastern United States. Canker symptoms on peach infected with PRSV are not typical in the mid-Atlantic seaboard and in the northeastern United States (J. M. Wells, H. C. Kirkpatrick, and C. L. Parish, *personal observations and communications*). It is possible that a combination of factors involving susceptible cultivars, climatic conditions, and perhaps biological variants (strains) of PRSV are responsible for the characteristic cankering on peach in central Georgia.

ELISA was rapid and efficient for detecting PRSV and PDV in vitro. However, 8% of the tests were ELISA-positive but Shirofugen-negative, and 15% were ELISA-negative and Shirofugen-positive. Reasons for discrepancies between the serological assay and the bioassay are not known. Possible explanations are 1) leaves were used in ELISA, whereas buds were used in the bioassay; 2) not all strains of PRSV from survey trees reacted with ELISA antiserum, whereas most all should have reacted with Shirofugen; or 3) the virus is not totally systemic in the trees. Variability may also have come from the use of only one ELISA well per sample.

PRSV has been calculated to cause significant losses in peach orchards in California. In a recent analysis, an initial infection level of 4% of trees in a fourth-year orchard results in losses of more than $400 per hectare by the eighth to ninth year and linearly increasing losses every additional year (5). Although these estimates may not be fully applicable to growing conditions and cultural practices in Georgia and in the southeastern United States, it is clear that PRSV is a disease that should be controlled. Screening of budwood sources and nursery stock against PRSV and PDV should be practiced rigorously.

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LITERATURE CITED

1. Barker, K. R., and Clayton, C. N. 1973. Nematodes attacking cultivars of peach in North Carolina. J. Nematol. 5:265-271.
2. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-484.
3. Cole, A., Mink, G. I., and Regev, S. 1982. Location of Prunus necrotic ringspot virus on pollen grains from infected almond and cherry trees. Phytopathology 72:1542-1545.
4. Gilmer, R. M. 1961. The rapid determination of necrotic ring spot and sour cherry yellows viruses of stone fruits. Plant Dis. Rep. 45:608-611.
5. Heaton, C. R., Ogawa, J. M., and Nyland, G. 1981. Evaluating economic losses caused by pathogens of fruit and nut crops. Plant Dis. 65:886-888.
6. Helton, A. W. 1962. Relative merits of Shirofugen and peach trees as indicators for Prunus ringspot virus in prune trees. Phytopathology 52:846-849.
7. KenKnight, G., and Jones, J. F. 1953. Apparent absence of the ring spot virus in the peach variety collection at the U.S. Horticultural Station, Fort Valley, Georgia. Plant Dis. Rep. 37:346.
8. Loesch, L. S., and Fulton, R. W. 1975. Prunus necrotic ringspot virus as a multicomponent system. Virology 68:71-78.
9. Mink, G. I. 1980. Identification of rugose mosaic-diseased cherry trees by enzyme-linked immunosorbent assay. Plant Dis. 64:691-694.
10. Mink, G. I., and Aichele, M. D. 1984. Use of enzyme-linked immunosorbent assay results in efforts to control orchard spread of cherry rugose mosaic disease in Washington. Plant Dis. 68:207-210.
11. Mink, G. I., and Aichele, M. D. 1984. Detection of Prunus necrotic ringspot and prune dwarf viruses in *Prunus* seed and seedlings by enzyme-linked immunosorbent assay. Plant Dis. 68:378-381.
12. Nyland, G., Gilmer, R. M., and Moore, J. D. 1974. Prunus ring spot group. Pages 104-132 in: Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. U.S. Dep. Agric. Agric. Handb. 437. 433 pp.
13. Parker, K. G., and Cochran, L. C. 1951. Similarities of symptoms produced by the viruses causing ring spot of peach and necrotic ring spot of sour and sweet cherry. Phytopathology 41:942.
14. Petersen, D. H., and Dowler, W. M. 1965. Bacterial canker of stone fruits in southeastern states. Plant Dis. Rep. 49:701-702.
15. Ritchie, D. F., and Clayton, C. N. 1981. Peach tree short life: A complex of interacting factors. Plant Dis. 65:462-469.
16. Savage, E. F., and Cowart, F. F. 1942. Factors affecting peach tree longevity in Georgia. Ga. Agric. Exp. Stn. Bull. 219. 7 pp.
17. Savage, E. F., Weinberger, J. N., Lutrell, E. S., and Rhoads, A. S. 1953. Clitocybe root rot - a disease of economic importance in Georgia. Plant Dis. Rep. 37:269-270.
18. Taylor, J., Biesbrock, J. A., Hendrix, F. F., Jr., Powell, W. M., Daniell, J. W., and Crosby, F. L. 1970. Peach tree decline in Georgia. Ga. Agric. Exp. Stn. Res. Bull. 77. 45 pp.