# Incidence of Prunus Necrotic Ringspot Virus in Some Pennsylvania Peach Orchards and Nurseries

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**ABSTRACT**

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Sixteen percent of 324 trees sampled in 12 commercial orchards (23–30 trees per orchard) were positive for Prunus necrotic ringspot virus (PNRSV) as determined by enzyme-linked immunosorbent assay. Among orchards, the median and range of PNRSV-infected trees were 6 and 0–83%, respectively. If data from all orchards were combined, there appeared to be a statistical correlation between incidence of virus and canker of the trunk or scaffolds. However, there was no apparent statistical relationship between the presence of virus and cankers if the two orchards with the highest incidence of PNRSV were removed from the data set. PNRSV incidence in three nurseries ranged from 0 to 10% and appeared to be related to the source of budwood and seed.

In many peach orchards in Pennsylvania and the mid-Atlantic fruit-growing region, a significant number of trees decline by the 10th year after planting (5). The reason for the decline is not always clear. Affected trees often have cankers on the trunks or main scaffolds that appear to be caused by winter injury followed by *Cytospora* spp. infections. Several reports (1,2,15,16,18,21,22) indicated an association between Prunus necrotic ringspot virus (PNRSV) and Cytospora-like canker, winter injury, and other decline symptoms in peach. A recent survey showed that PNRSV is prevalent in West Virginia (1,2). Therefore, a survey was conducted in Pennsylvania to obtain additional information on the incidence of PNRSV in peach orchards and its possible correlation with cankers of the trunk and scaffolds.

## MATERIALS AND METHODS

In April 1984 and 1985, 324 peach trees in 12 commercial orchards (9–17 yr old, four orchards each of cultivars Loring, Redhaven, and Garnet Beauty) in Adams and Franklin counties of Pennsylvania were rated for severity of cankers on the trunks or main scaffolds and assayed for PNRSV. Canker ratings were none, moderate, or severe. Trees rated moderate or severe usually displayed other decline symptoms (poor growth and dieback), whereas trees without canker were not rated or sampled unless they appeared healthy and vigorous. Where possible, 10 trees in each canker category were sampled per orchard. During bloom, one terminal shoot (20–30 cm long) from each of four sides of each tree were selected unless the tree was rated moderate; shoots from these trees were collected from the affected side of the tree. Samples from a known PNRSV-infected tree and a noninfected tree were included as controls. Shoots were incubated in beakers (four shoots from one tree per beaker) containing tap water for 2–5 days at 22 ± 2 C. Leaf tissue (a 0.2-g composite sample from the young leaves of four shoots) was triturated in 5 ml of 0.02 M potassium phosphate buffer, pH 7.4, containing 0.15 M sodium chloride, 0.05% Tween 20, and 20% polyvinylpyrrolidone, mol wt 40,000 (PBS-Tween-PVP).

Double-sandwich enzyme-linked immunosorbent assay (ELISA) was performed as described by Clark and Adams (4). γ-Globulin was partially purified as described by Powell and Derr (17). Plates were coated at 4 C for 16 hr with anti-PNRSV-G γ-globulin (10 μg/ml, ATCC PVAS 22) in 0.05 M sodium carbonate buffer, pH 9.6. Crude sap prepared in PBS-Tween-PVP was incubated in duplicate microtiter plate wells at 4 C for 16 hr. The anti-PNRSV-G enzyme conjugate (1/1,500 dilution in PBS-Tween-PVP) was incubated at 37 C for 4 hr. A sample was scored positive if the $A_{405\,nm}$ was twice that of the healthy controls and had a minimum absorption of 0.1 after 30 min. Data were subjected to chi-square analysis.

In May and June 1985, Loring and/or Garnet Beauty peach trees in the nursery row, budded in July 1984, were sampled in three Pennsylvania nurseries. Two leaves were collected from each tree (50 or 100 trees per cultivar per nursery), sealed in plastic bags, stored overnight at 5 C, and processed by ELISA (0.1 g of tissue per 5 ml of buffer) as described. Trees were selected according to a systematic sampling plan, and estimates of the standard errors of the percentages were determined (9).

## RESULTS

Sixteen percent of the 324 trees sampled in the 12 commercial orchards were positive for PNRSV (Table 1). The median and range of PNRSV-infected trees were 6 and 0–83%, respectively. Most (69%) of the infected trees were located in two of the Garnet Beauty orchards. Chi-square analysis of combined data from all sites or from only Garnet Beauty sites suggested an apparent statistical relationship between the presence of virus and the occurrence of canker (Table 1). However, if the two Garnet Beauty sites with a high incidence

**Table 1.** Incidence of Prunus necrotic ringspot virus in relation to canker and cultivar[a]

| | Percent PNRSV infection | | | |
|---|---|---|---|---|
| | Cultivar | | | Mean of all cultivars |
| Canker | Garnet Beauty | Redhaven | Loring | |
| None | 12 (41)[b] | 7 (55) | 3 (32) | 8 (128) |
| Moderate | 38 (37) | 7 (30) | 8 (40) | 18 (107) |
| Severe | 67 (33) | 0 (19) | 3 (37) | 26 (89) |
| All classes | 37 (111) | 6 (104) | 5 (109) | 16 (324) |
| $P$[c] | 0.001 | 0.488 | 0.540 | 0.002 |

[a] Twenty-three to 30 trees in each of 12 orchards (four orchards each of Garnet Beauty, Redhaven, and Loring) were rated for canker on the trunks and main scaffolds and assayed for virus by ELISA.

[b] Numbers within parentheses indicate sample size.

[c] $P$ values were obtained by chi-square analysis.

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**Table 2.** Incidence of Prunus necrotic ringspot virus in relation to canker in two Garnet Beauty orchards and in 10 other orchards[a]

| Canker | Percent PNRSV infection | |
|---|---|---|
| | Garnet Beauty orchards[b] | Other orchards |
| None | 31 (13)[c] | 5 (115) |
| Moderate | 70 (20) | 6 (87) |
| Severe | 90 (20) | 7 (69) |
| $P$[d] | 0.002 | 0.850 |

[a] Twenty-three to 30 trees in each of 12 orchards (four orchards each of Garnet Beauty, Redhaven, and Loring) were rated for canker on the trunks and major scaffolds and assayed for virus by ELISA.
[b] These orchards contained 69% of the infected orchard trees.
[c] Numbers within parentheses indicate sample size.
[d] $P$ values were obtained by chi-square analysis.

**Table 3.** Incidence of Prunus necrotic ringspot virus (PNRSV) in 1-yr-old peach trees in three Pennsylvania nurseries[a]

| Nursery | Cultivar | Budwood source[b] | Seed source[c] | Trees sampled (no.) | PNRSV-positive (% ± SE)[d] |
|---|---|---|---|---|---|
| 1 | Garnet Beauty | NI | NI | 100 | 8 ± 2.6 |
| 2 | Garnet Beauty | NI | NI | 50 | 10 ± 3.8 |
| 1 | Loring | $I_1$ | NI-I | 100 | 2 ± 1.4 |
| 2 | Loring | NI | NI | 50 | 8 ± 3.8 |
| 3 | Loring | $I_2$ | NI | 100 | 0 |

[a] Leaf tissue was assayed for virus by ELISA.
[b] Budwood sources: NI = budwood collected from nonindexed trees that in turn were propagated using seed and budwood from nonindexed trees; $I_1$ = budwood collected from 5-yr-old, nonindexed trees propagated using seed and budwood from trees that indexed negative for PNRSV; $I_2$ = budwood collected from 12-yr-old, nonindexed trees propagated using seed from nonindexed trees and budwood from trees that indexed negative for PNRSV.
[c] Seed sources: NI = from nonindexed trees and NI-I = about 50% of seeds were from trees that indexed negative for PNRSV.
[d] Standard errors of the percentages were calculated according to Mendenhall et al (9).

of virus were removed from the data set, no such relationship was observed (Table 2). In the three nurseries, 0–10% of the trees sampled were positive for PNRSV (Table 3). Canker or foliar symptoms were not observed in these nurseries. Source of budwood and seed appeared to affect the incidence of virus. Although budwood sources were not virus-indexed, the sources had been propagated with indexed budwood in two instances; in these two instances, PNRSV incidence was low (Table 3).

## DISCUSSION

Before virus-indexing programs were available, PNRSV was considered a common virus in stone fruit orchards (3,7,14,15). Our data and those of Barrat (1,2) show that this virus is still prevalent in the mid-Atlantic region. Results from our nursery survey demonstrate that at least some cultivars from some nurseries are a significant source of virus. Nursery infection may originate from infected seed (13) and/or infected budwood (3,7,15). PNRSV is pollen-transmitted in cherry (8,11), and field spread was reported in cling peaches in California (18).

Several papers (16,18,21), including a recent study in Georgia (22), indicate that PNRSV can induce serious disease(s) in peach. Our data indicate that PNRSV was not a major factor contributing to canker in 10 of the 12 orchards surveyed. However, in two Garnet Beauty orchards with high incidences of canker and virus, the possibility exists that the virus significantly contributed to tree canker and tree decline. Disease severity of PNRSV-infected cherry varied with cultivar (12), and it is possible that Garnet Beauty is more susceptible than Redhaven and Loring. Disease severity may also be affected by strain of PNRSV (10,12,15,22), and it is possible that trees in two of the Garnet Beauty orchards were infected with a severe strain. Symptomless strains of PNRSV in cherry were reported (6); such strains might explain the presence of PNRSV in apparently healthy peach trees sampled in this and other studies (22). Other factors affecting symptom expression of PNRSV-infected trees include host status, environment, and dual infections with other viruses (15,19,20).

Correlative data such as those provided in this study cannot prove the involvement or lack of involvement of PNRSV in peach tree decline. The virus is prevalent in the mid-Atlantic region, and controlled experiments are required to determine the effects of different strains of PNRSV on different cultivars of peach under mid-Atlantic environmental conditions. To reduce incidence of this virus, growers should purchase nursery trees that were propagated from virus-free seeds and buds.


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