Ilarviruses: Evidence for Rapid Spread and Effects on Vegetative Growth and Fruit Yields of Peach Trees

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ABSTRACT

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Over a 4-yr period, the incidence of trees infected by prune dwarf virus (PDV) and Prunus necrotic ringspot virus (NRSV) in two young peach (*Prunus persica*) orchards progressed from 27 to 94% in one subplot and from zero to 72% in the other. For trees infected with peach stunt disease (PSD = infections by PDV and NRSV), fruit production, trunk diameter, and tree height were reduced by an average of 30, 23, and 12%, respectively, over 3 yr. Single virus infections by NRSV, but not PDV, also significantly affected tree growth and fruit yield. Honeybees tested positive for both viruses and are likely involved in transporting virus-contaminated pollen to healthy trees.

Two viruses, prune dwarf (PDV) and Prunus necrotic ringspot (NRSV), were recently detected in a large number of young cling peach orchards (*Prunus persica* (L.) Batsch) in California (13). Both viruses are transmitted during bloom when pollen from diseased trees is available (2–4,6,11). When these viruses occur in combination, the infected peach trees often lack obvious foliage symptoms but are stunted and exhibit shortened internodal shoot growth. This condition is known as peach stunt disease (PSD) (5). These symptoms are most pronounced in the early spring, but as the mean daily temperatures rise, the affected trees become less conspicious.

Several reports have appeared describing the relative spread rates of PDV and NRSV and the effects of these viruses alone or in combination on trunk growth and fruit yield of peach trees. Although these viruses occasionally spread relatively slowly (14), incidences of newly infected trees approaching 80% or higher occurred after 10 or more cropping seasons (9,12), and yields from PSD trees were compared with trees infected with NRSV (9,10) or healthy trees (8).

In 1987, two adjacent, first-leaf orchards planted with a common peach cultivar were found where one orchard contained 25% diseased trees and the other had none. During the next 3 yr, we conducted annual virus assays to determine the occurrence of new infections and annually measured tree heights, trunk diameters, and peach yields to investigate the effects of single and double virus infections compared with uninfected trees. Our results are being reported here.

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MATERIALS AND METHODS

In 1987, two different sources of Carson peach trees were used to establish two adjacent orchards located near Hughson, CA. The distance between orchards was approximately 15 m. The west orchard contained 1,205 trees that originated from one nursery. Trees were arranged in a square block (35 rows × 35 trees). The east orchard had 559 peach trees obtained from a second nursery. These were planted in a rectangular block (37 rows × 17 trees). Tree density was 331 per hectare in both orchards. The remaining half of the east site had been planted in 1982 to nectarine trees (*P. persica* var. *nectarina* (Aiton) Maxim. 'Summer Grand').

Indirect enzyme-linked immunosorbent assay (ELISA) was used to detect and identify both PDV and NRSV in tissue extracts as described (13), except that alkaline phosphatase was substituted for peroxidase on the enzyme-conjugated antibody. Bioassays on field-grown indicator trees of Shirofugen flowering cherry (*P. serrulata* Lindl.) were performed by T-bud grafts. Host response was determined after 4–6 wk of incubation (13).

Initial ELISA tests were made in April 1987 when 20 trees located in the southeast corner of the west orchard and 10 from the southwest corner of the east orchard were sampled. The same trees were indexed on Shirofugen in June. In the following year, a more intensive assay was done with ELISA. Prior to budbreak in February 1988, dormant buds from trees in the west (12 rows × 12 trees) and east orchards (10 rows × 17 trees) were collected and assayed by ELISA. During April of each year from 1988 to 1990, succulent shoots and leaves from trees in the west (nine rows × nine trees) and east orchards (10 rows × 17 trees) were similarly assayed. In April 1989, 10 nectarine trees immediately bordering the north edge of the east orchard were assayed for PDV and NRSV. In mid-June during 1988–1990, 64 trees (eight rows × eight trees representing yield plot trees) in the west orchard were bud-grafted on Shirofugen. The yield plot trees from the east orchard were similarly graft-indexed in 1989 and 1990.

In mid-July of each year during 1988–1990, all trees in the yield plots (eight rows × eight trees of both

orchards) were picked twice, about 1 wk apart because of uneven fruit ripening. The harvests from the two orchards were kept separate. Weights of No. 1 fruits (unblemished fruit with a minimum diameter of 6 cm) from both harvests were combined. All other fruits were discarded. In the first harvest year (1988), yields from the 17 PSD trees were compared with yields from 111 uninfected trees. Thereafter, as new infections (i.e., trees with PDV, NRSV, or PSD) occurred, the kind and number of diseased and healthy trees changed each year, and yields and growth measurements from those trees were compared accordingly. Measurements of tree heights and trunk diameters (the latter at 0.5 m above the soil line) were made annually during August.

All yields and growth measurements were analyzed by analysis of variance and Fisher's least significant difference test at the 5% level of probability.

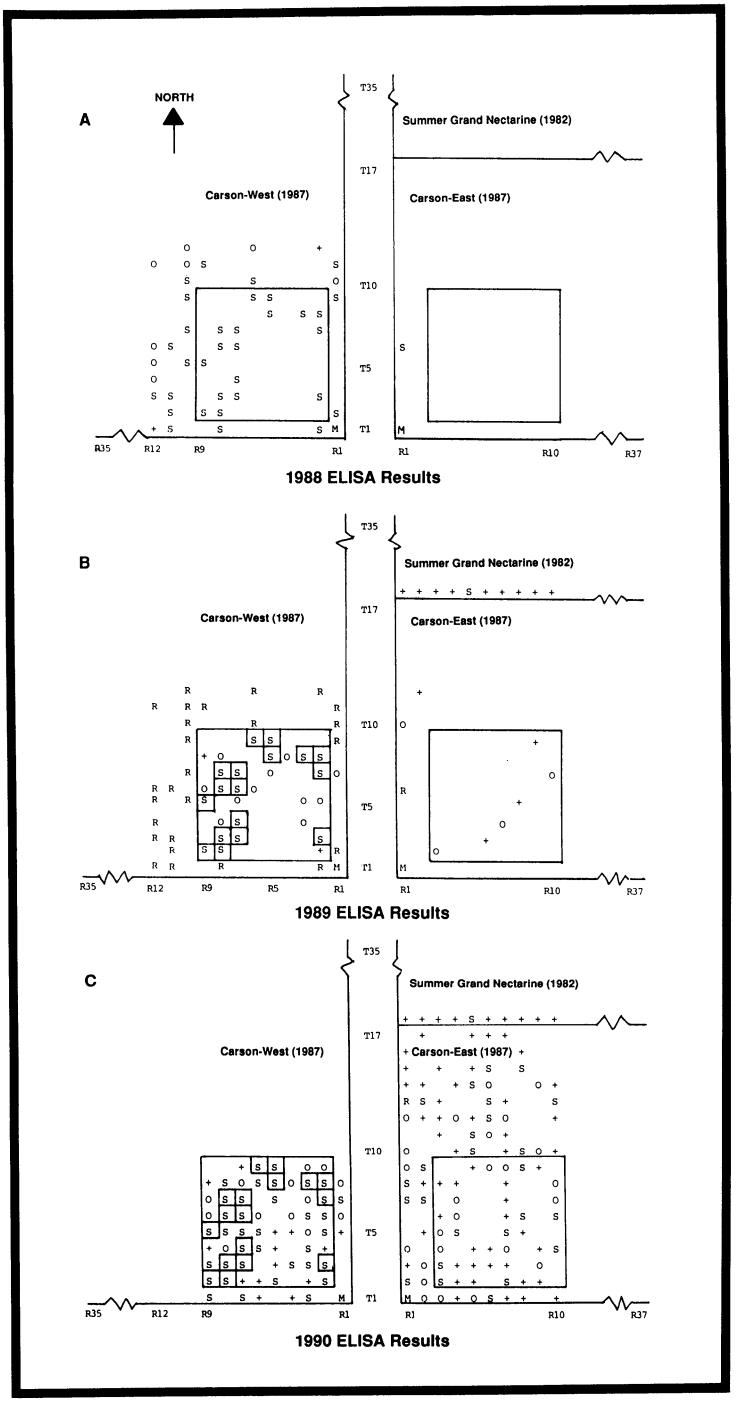

Fig. 1. Two adjacent peach (*Prunus persica* 'Carson') orchards were established in 1987; trees in the west and east orchards were from different nursery sources. An older block of nectarine trees, located immediately north of the Carson east orchard, was planted in 1982. The square areas are composed of eight rows × eight trees used to determine virus spread rates, fruit yields, and tree growths. **(A)** ELISA results completed in 1988. Blocks of 12 rows × 12 trees (west) and 10 rows × 17 trees (east) were sampled for the 1988 ELISA assays. M = missing tree, S = PSD, O = PDV, and + = NRSV. **(B)** Results of ELISA done in April 1989. Collections were made in the west (nine rows × nine trees) and east orchards (10 rows × 17 trees). Also, 10 nectarine trees bordering the east orchard were assayed. The symbol R within the orchard boundaries indicates trees removed in February 1988 and replaced 1 yr later; all PSD-1987 trees left standing in the west yield plot are highlighted by smaller squares, other symbols as indicated above. **(C)** Results of ELISA in April 1990. All symbols as previously noted.

RESULTS

Virus assays. Visual observations and ELISA data revealed that the west orchard had been planted with PSD trees. The initial incidence was 25%, i.e., five PSD trees were detected among 20 trees. The 10 east orchard trees tested negative. On Shirofugen, infective buds were detected only from the ELISA-positive trees. All ELISA-negative trees yielded Shirofugen-negative buds. Assays of buds collected in February 1988 indicated that 43 of 143 trees (27%) were infected in the west orchard (Fig. 1A). Thirty-three trees were infected with both viruses (of which 17 trees were located within the yield plot area), and 10 trees were infected by PDV or NRSV alone. A single PSD tree was detected in the east orchard. Prior to budbreak in 1988, all infected trees in the rows that surrounded the eight row × eight tree yield plot in the west were removed. This was done to partially reduce the inoculum potential immediately surrounding the west yield plot trees. See Figure 1B for tree positions marked R where infected trees were removed. The single PSD tree in the east orchard was also removed.

After budbreak in 1988, a second ELISA test confirmed the locations of the PSD trees in the west yield plot and indicated that the remaining east orchard trees were virus negative. In June 1988, a total of 18 trees in the west yield plot indexed positive on Shirofugen. These included all 17 of the PSD-ELISA positive trees. The extra Shirofugen-positive tree had been negative in two previous ELISA tests that year.

In 1989, ELISA results indicated that in addition to the original PSD-infected trees (hereafter referred to as PSD-1987 trees), there were other infected trees, e.g. 10 with PDV and two with NRSV, present in the west yield plot (Fig. 1B). These included the single new infection detected by Shirofugen in 1988. Collections from the east orchard showed that it now contained five trees each with PDV or NRSV. All ELISA-positive trees in each yield plot were confirmed by bud grafting to Shirofugen in June 1989. Moreover, these bud grafts identified additional infective bud sources among the ELISA-negative trees. There were nine and 11 newly infected trees in the west and east yield plots, respectively (Table 1). All 10 nectarine trees were ELISA-positive for NRSV and one was

also infected with PDV.

To validate the reliability and sensitivity of each assay, we tested tissue extracts by ELISA and inserted buds from the same shoots into Shirofugen for trees sampled in April 1990. By that time, 55 and 39 infected trees were found in the west and east yield plots, respectively. Both assays detected the same virus-infected trees, indicating that ELISA was as accurate as the bud graft indexing method for samples tested in the spring. However, Shirofugen results with budwood collected in June 1990 indicated the presence of more infected trees (five trees in the west and seven in the east yield plots), all of which had tested negative in both April assays (Table 1). Soon after these determinations were made and before harvest, we collected three to five undersized fruits (i.e., one fruit per scaffold) from the trees that had assayed negative in April and indexed Shirofugen-positive in June. A composite sample of fruit tissues (skin-flesh) per tree was extracted and assayed by ELISA. PDV only was detected in nine fruit extracts and NRSV only in three others. All of the NRSV-infected fruit collections were from the trees in the east yield plot. By combining results of all assays done in 1990, the incidence of infected trees was 94 (60/64) and 72% (46/64), respectively, in the west and east yield plots. The interpretation of these results indicated that other healthy trees had become infected during the most recent pollination period. For the remainder of the east orchard collection, there were 63 ELISA-positive trees, for a total of 109 (64.5%) (Fig. 1C).

Based on the ELISA results in 1989 and 1990, the frequency of new infections caused by either virus in the yield plot trees was similar—59 trees with PDV and 62 trees with NRSV. Of these, 25 trees were infected with both viruses, 34 by PDV alone, and 37 by NRSV alone.

As peach trees are largely self-pollinated and the pollen grains are borne in a sticky matrix, pollen dispersal to neighboring or distant trees would require an insect such as the honeybee (*Apis mellifica* Linnaeus). On 20 March 1990, when the trees were at nearly 90% petal fall (west orchard), a sampling of 52 honeybees was made and extracts of the pollen sacs were assayed by ELISA. Viral antigens were detected in 41 extracts, i.e., 37 with PDV alone and four with PDV and NRSV, with the A_{405nm} values averaging 1.02 (PDV pollen sac extracts), 0.23 (NRSV extracts), and 0.08 (apparently healthy pollen sac extracts). Controls of infected and healthy peach tissues produced A_{405nm} values of 1.39 and 0.11 (for PDV ELISA) and 0.66 and 0.02 (for NRSV ELISA), respectively.

Fruit yields and tree growth measurements. Fruit yields and growth measurements of similarly infected (i.e., trees with PDV alone, NRSV alone, or PSD-1987 and -1990) and uninfected trees in the west and east plots were compared and found to be statistically similar (LSD, $P = 0.05$). Therefore, irrespective of the year infections were detected and, except for PSD-1987 and -1990 trees, data from the PDV- or NRSV-infected or uninfected trees were combined and compared against each other and the other treatments.

Diseased trees generally produced fewer fruit than healthy trees (Table 2). For all harvests, the yield loss from PSD-1987 trees was 29% when compared with uninfected trees, which was significant statistically (LSD, $P = 0.05$). Likewise, the yield reduction of 31% from new PSD trees detected in 1990 (PSD-1990) were statistically similar to those of PSD-1987 trees. Trees infected by NRSV, but not PDV, produced significantly less fruit (i.e., 18%) than uninfected trees during 1990.

Virus infections also influenced the vegetative growth of the trees. When compared with measurements of healthy trees, the largest differences in trunk diameter occurred with PSD-1987 (with 22.5% less growth) and -1990 (10% less) trees, followed by NRSV-infected trees (7% less) (Table 3). These differences were statistically significant (LSD, $P = 0.05$). All infected trees were significantly shorter than the control trees. The shortest were PSD-1987 and -1990 trees with a 12% reduction in height (Table 3). This was followed by reductions of 5 or 3%, respectively, for NRSV- and PDV-infected trees (Table 3). All were significant (LSD, $P = 0.05$) when compared with uninfected trees.

DISCUSSION

The results of repeated virus assays clearly indicated that during each flowering season, additional peach trees had become infected. Because peach pollen is not effectively windborne, it appears that honeybees are involved in transporting diseased pollen grains to open blossoms on uninfected trees. From there, the viruses are evidently transmitted to uninfected plants through the fertilization process (1-4,6,11). More honeybee virus assays, where the bees

Table 1. Evidence of peach stunt disease (PSD), prune dwarf virus (PDV), and Prunus necrotic ringspot virus (NRSV) spread in orchards of the peach cv. Carson[t]

Test block	Year	Number of trees with[u] PSD	PDV	NRSV	Number of Shirofugen positives of ELISA-negative trees[v]
West	1988	17	0	0	1[w]
	1989	17	10	2	9[x]
	1990	33	11 (+5)[y]	11	5[y]
East	1988	0	0	0	NT
	1989	0	4	3	11[z]
	1990	9	10 (+4)[y]	20 (+3)[y]	7[y]

[t] Both 64-tree test blocks were established in 1987.
[u] Based on results of ELISA done in April.
[v] All ELISA-positive trees were infective on Shirofugen and not included in the totals; bioassays done each June. NT = not tested.
[w] Identified as PDV in April 1989 ELISA.
[x] Five trees as PSD, one PDV, and three NRSV in April 1990 ELISA.
[y] Number in parentheses indicates infected trees identified by July 1990 ELISA using fruit extracts of positive trees listed in the right-hand column.
[z] Two trees each as PSD or PDV and seven NRSV in April 1990 ELISA.

Table 2. Effect of peach stunt disease (PSD), prune dwarf virus (PDV) and Prunus necrotic ringspot virus (NRSV) on peach yield

Tree status	No. of trees harvested	Yield (t/ha)[y]
1988		
Healthy	111	3.1 a
PSD-1987[z]	17	1.3 b
1989		
Healthy	92	23.0 a
PDV-1989	14	22.6 a
NRSV-1989	5	21.7 a
PSD-1987	17	11.4 b
1990		
Healthy	34	29.5 a
PDV-1989 and 1990	21	29.4 a
NRSV-1989 and 1990	31	24.2 b
PSD-1987	17	21.2 bc
PSD-1990	25	20.5 c

[y] For each harvest year, means followed by the same letter are not significantly different, LSD, $P = 0.05$.
[z] The year when the original PSD trees were planted; others indicate the year new infections were detected.

Table 3. Cumulative effects of peach stunt disease (PSD), prune dwarf virus (PDV), and Prunus necrotic ringspot virus (NRSV) on growth of fourth-leaf peach trees

Tree status	Trunk diameter (cm)[z]	Tree height (m)
Healthy	12.45 a	4.73 a
PDV-1989 and 1990	12.09 ab	4.58 b
NRSV-1989 and 1990	11.60 bc	4.51 b
PSD-1990	11.18 c	4.18 c
PSD-1987	9.65 d	4.18 c

[z] All measurements taken in August 1990. Within each column, means followed by the same letters are not significantly different, LSD, $P = 0.05$.

were collected in other peach and prune orchards, were also ELISA-positive for PDV and NRSV (J. K. Uyemoto, *unpublished*). Previously, honeybees have been implicated in the long-distance spread of NRSV, i.e., with the movement of commercial hives from California to Washington (7). Also, Smith et al (12) discussed their potential role in the spread of both viruses in peach orchards.

Both assay procedures used were comparable in detecting virus-infected trees in an April collection. However, with ELISA, caution must be exercised when shoot-leaf tissues are taken during periods of wide fluctuation in ambient temperatures. For example, we had previously reported erratic ELISA results for shoot-leaf collections made in early May 1987 when day temperatures exceeded 38 C over a 12-day period (13). Presently, if trees are sampled for ELISA during the summer months in California, extracts of skin-flesh of fruits are preferred over other tissues (J. K. Uyemoto, *unpublished*). For graft-indexings on Shirofugen, budwood may be sampled throughout the year.

Our data further indicated that depending upon when tissues were collected, there were new ELISA- and Shirofugen-positives among trees that had assayed negative previously. This situation presents a dilemma for the California clean stock program. The program, under the auspices of the California Department of Food and Agriculture, involves the annual testing for PDV and NRSV by ELISA or Shirofugen in all *Prunus* sources used to produce nursery trees. Usually, the tests are done once each year (e.g., graft-indexing on Shirofugen during the winter or summer months or ELISA tests of extracts prepared from dormant buds or recently developed shoot-leaf tissues in winter and spring months, respectively) and budwood from virus-negative trees is harvested as needed. It is conceivable that an ELISA-negative peach tree when tested during March–April may later test positive (infected during the recent bloom period) during May–June when current season shoots are usually harvested for nursery tree production. To partially guard against unknowingly propagating from such trees, it is suggested that a subsample of each budwood collection be tested for PDV and NRSV. Hence, assay results would be valid for a particular collection and not necessarily the source tree(s). Thereafter, when more budwood is removed from the same (or different) source tree later in the growing season, the new collection must be retested.

Our yield results showed that PSD, regardless of whether trees were infected for 1 yr or longer, reduced fruit production by nearly a third when compared with yields from uninfected trees. Also, trees infected with NRSV, but not those infected with PDV, caused significant yield losses (nearly one-fifth) in 1990. In other yield trials, Pine (8) observed losses of 6 and 33% for trees infected with NRSV and PSD, respectively, compared with healthy tree yields. Schmitt et al (9) reported yield reductions of 16% for NRSV-infected trees compared with healthy trees and 31% for trees infected with PSD compared with NRSV-infected trees. Smith and Challen (10) also compared fruit yields of trees infected with PSD and NRSV-infected trees (healthy trees not involved) and found that the latter trees yielded three times as much crop. These reports, combined with our results, suggest that NRSV-infected and PSD-infected trees are disadvantaged economically. In contrast, yields from PDV-infected trees were unaffected as fruit yields were statistically similar to uninfected ones (LSD, $P = 0.05$).

Pine (8) reported decreases in trunk girth of 12–13% for NRSV-infected trees and 34 or 51% for PSD-infected trees. Trunk measurements of our diseased and healthy trees showed considerably less differences, e.g., 7, 10, and 23% for NRSV-infected, PSD-1990, and -1987 trees, respectively. The discrepancy is presumably attributable to different virus isolates and peach cultivars used in the respective trials.

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