V. albo-atrum was reisolated from inoculated plants with symptoms.

Inoculated plants that did not show either foliar or vascular browning symptoms (less than 1% of those inoculated) were reinoculated by root dip, and survivors were reselected. Seed from random cross-pollination by hand was collected, replanted and inoculated by root dip. A high percentage (over 50%) of the progeny were symptomless compared with 0% symptomless for the unselected CUF101 control.

Cowpea (California Blackeye 5) was highly susceptible following inoculation of soil with spores (table 3). About 10 to 14 days after inoculation, lower leaves became flaccid and growth was suppressed. The effect of the high degree of susceptibility of cowpea to Verticillium wilt is not known, since the disease has not been seen in the field. We isolated *V. albo-atrum*, alfalfa strain, in routine samples from cowpea plants at the UC Riverside experimental farm; the plants were growing near a plot of naturally infected alfalfa plants but did not show active symptoms. In selection of rotation crops for control of Verticillium wilt, cowpea would not be an advisable choice. Cotton (SJ-2) was susceptible following root drench inoculation, but the expression of disease was not as severe as that caused by inoculation with a defoliating isolate of *V. dahliae*.

Conclusions

Since Verticillium wilt appears to be established in two areas of southern California, high temperature will not limit its spread in California alfalfa-growing areas as had been earlier believed.

The most effective control of Verticillium wilt in other areas where dormant and semidormant alfalfa is grown is the use of resistant cultivars, some of which have been selected from existing cultivars. Unfortunately, resistant nondormant alfalfa cultivars are not yet available. Since we were able to select plants that were resistant to *V. albo-atrum*, and seed progeny from random crosses was also resistant, it appears that obtaining resistance will be the most effective approach to control of the disease in nondormant alfalfa.

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Survey detects viruses in almond, prune, and sweet cherry orchards

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Prunus necrotic ringspot and/or prune dwarf viruses were found in young California orchards, averaging 20% infection in almond and prune and 4% in sweet cherry. Nursery stock was implicated as the primary source, and efforts are now under way to propagate disease-free trees.

Prune dwarf and Prunus necrotic ringspot viruses have plagued California's tree fruit and nut orchards ever since these crops were established. Their effect on orchard trees was uncertain, however, until research in the 1940s and 1950s demonstrated that virus infections were responsible for reduced tree growth and fruit yield. During this same period, rapid diagnostic and virus elimination procedures were also being developed. The accumulated findings were put into commercial practice from mid-1960 to late 1970, resulting in the production and planting of virus-free fruit and nut orchards.

In 1987, however, we found that one in four cling peach (*Prunus persica*) orchards contained a high incidence of prune dwarf virus, Prunus necrotic ringspot virus, or both. The cling peach industry was back in a disease situation similar to that of earlier times. Economic difficulties had apparently led to increased demand for less expensive, noncertified cling peach trees in late 1970 to early 1980. The relatively high disease incidence in cling peach orchards included first-leaf trees, suggesting the use of infected budwood and/or seed or seedling rootstocks in propagation. During 1988, we expanded the surveys to determine virus incidence in almond (*P. dulcis*), sweet cherry (*P. avium*), and prune (*P. domestica*).

Economic importance

Prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV) are serologically unrelated but share similar biologic properties. For example, they are pollen- and seed-borne, and they are spread to healthy trees through pollination with virus-infected pollen and by grafting.

In the nursery, grafting diseased scions onto healthy understocks or healthy scions onto diseased stocks can result in a low percentage of bud take and retarded growth of the budling shoot.

In orchards, virus-infected trees may show leaf symptoms ranging from scattered or clustered chlorotic spots (caused, for example, by almond calico, a strain of PNRSV) to an initial chlorosis that turns into brown (necrotic) rings or areas that fall out (caused by strains of PDV and PNRSV). The latter results in a shredded-leaf appearance. The calico and rugose mosaic strains of

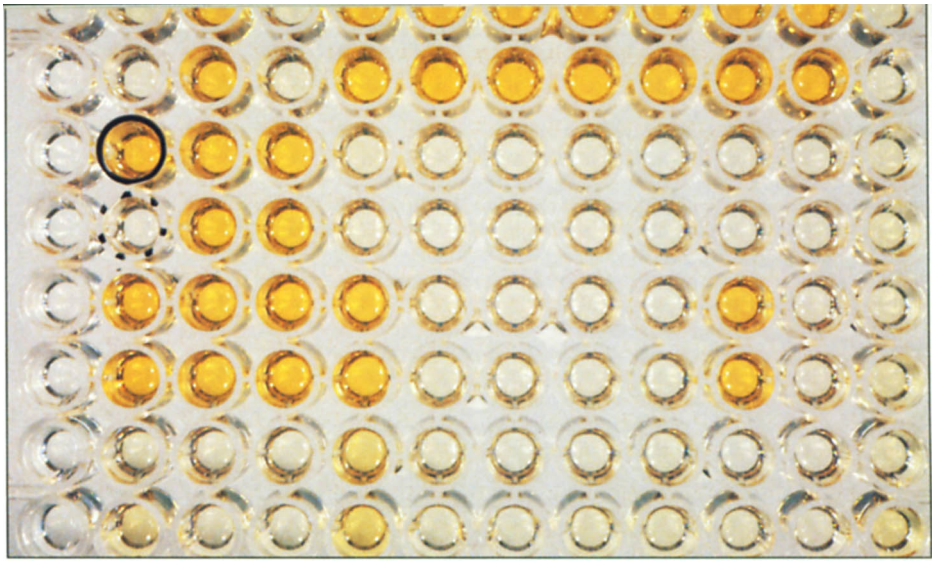
PNRSV also induce the infectious form of bud failure in almond, and the rugose mosaic strain causes fruit deformity and tree decline in sweet cherry. In peach, dual virus infections cause severe tree stunting (hence the disease name "peach stunt") along with reduced fruit yield.

Sampling and diagnosis

We collected leaf tissues from trees in almond and prune orchards in the Sacramento and San Joaquin valleys and sweet cherry orchards in San Joaquin County during March and April 1988. The sample size per orchard varied according to the crop. Five almond trees per cultivar per site were sampled, because almond orchards contain two or more cultivars planted in alternate rows to ensure adequate pollination. Sweet cherry also requires cross-pollination, but the ratio of fruit cultivar trees to pollenizers is large (8-15:1). Prune is largely self-fertile, and California orchards are planted extensively to a single cultivar, the Improved French. In cherry and prune orchards, we sampled 10 trees per site. Test orchards ranged in age from first- to sixth-leaf stages of growth.

To detect and identify virus-infected trees, we used a serological test called ELISA (enzyme-linked immunosorbent assay). The test involved the extraction of leaf tissues in carbonate buffer, the product of which was spotted in duplicate wells in an ELISA microtiter plate. (Microtiter plates for PNRSV were precoated with virus-specific trapping antibodies; such trapping antibodies were not required for PDV.) Next, and in their proper sequence, unlabeled and peroxidase enzyme-conjugated antibodies were added to the wells, allowed to react, and then rinsed. Finally a substrate solution was placed in the wells, which in the presence of the enzyme is hydrolyzed to produce a yellow color. Wells with healthy leaf extracts remain clear. For control standards, each plate contained extracts prepared from known virus-infected and healthy tissue.

To confirm the ELISA results, we resampled selected orchard trees in June, when budsticks were collected, and grafted three budchips per source tree onto Shirofugen flowering cherry (*P. serrulata*), a sensitive indicator for both PDV and PNRSV.



ELISA microtiter plate used in testing for virus. Colored wells indicate a positive reaction. Well outlined in solid black contains a known virus sample; dash-lined well contains a healthy sample.

After 4 to 6 weeks of incubation, the underlying bark tissue surrounding the inserted budchips was examined for necrosis.

Results and discussion

Virus infections in almond and prune averaged 20% each, and in sweet cherry, 4% (table 1). We detected PNRSV in all test species, whereas PDV occurred in cherry and prune but not almond. The bioassays on Shirofugen supported the ELISA results.

Based on virus incidences in individual orchards of almond and prune, it was clear that diseased scions, rather than diseased understocks, were used to produce budling trees. For example, PNRSV was detected in 2 almond orchards (in their first-leaf stage of growth) planted to 6 different cultivars. On these sites, all 20 sampled trees (representing 4 cultivars) tested positive by ELISA. The 2 remaining cultivars were ELISA-negative. In 3 other almond orchards planted to a given cultivar of the same age, the incidence of PNRSV was 0%, 0%, and 100%. Similarly, 6 prune orchards contained virus levels ranging from 50% to 100%, while another 16 orchards showed no diseased trees. These findings indicate that certain source trees used for budwood were infected, while other source trees were healthy.

In contrast, lower percentages of infection in some almond and cherry orchards, where the incidence was 10% or less, suggested that infected understocks were probably involved. Seed-to-seedling transmission of PDV and PNRSV is reported to vary from a trace to 12%, and cultivars of almond and cherry are commonly propagated on seedling roots: Lovell or Nemaguard peach understock for almond, and mahaleb or mazzard understock for sweet cherry.

Control

The widespread occurrence of PNRSV and/or PDV in young orchards, as detected in 1988 in almond and prune as well as our previous findings in cling peach, suggested that nursery stock was the primary source of diseased trees. When we completed the 1987 cling peach surveys, we informed the nursery industry of our findings. They moved immediately to obtain assistance from the California Department of Food and Agriculture (CDFA) and provided CDFA with the necessary funds to establish a self-supporting ELISA facility early in 1988. Financial support was also allocated to the nursery certification program of CDFA to update their virus indicator tree planting.

During the first year of operation, some 7,000 potential source trees for scion wood and seed were tested by ELISA for PDV and PNRSV, and materials from clean trees were used to propagate nursery stock beginning in 1988. Currently the outlook on the availability of virus-tested trees is excellent. However, continuance of this program depends on grower demand for certified nursery stock to help offset the costs involved in the virus indexing program.

TABLE 1. Incidence of prune dwarf (PDV) and Prunus necrotic ringspot viruses (PNRSV) in young orchard trees

Crop	Trees tested*	No. trees infected			Percent infection
		PDV	PNRSV	PDV+PNRSV	
Almond	360	0	73	0	20
Cherry	90	2	2	0	4
Prune	260	1	45	6	20

NOTE: Almond, first- to sixth-leaf trees sampled; cherry and prune, second- and fourth-leaf trees.
* Number of trees tested by ELISA.

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