

Incidence of Prunus Necrotic Ringspot Virus in Selected Peach Orchards of South Carolina

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ABSTRACT

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A survey was conducted in 1987 to determine the prevalence of Prunus necrotic ringspot virus (PNRSV) in peach orchards of South Carolina and to investigate variation in the incidence of the virus among the major cultivars and production areas. Virus detection was by ELISA of floral or juvenile tissues. The major peach cultivars grown in the state were sampled, with the design of the survey being based on the counts reported in a triennial statewide census of peach trees last completed in 1985. Samples were collected from trees 6-10 yr old. A bud stick was collected from each quadrant of a tree, and tissue from the four sticks was combined to form the sample extracted for ELISA. A total of 5,833 trees was sampled from seven cultivars in 114 orchards in the three peach-growing regions of the state. Observed incidence of PNRSV in individual orchards ranged from 0 to 100%. After adjustment for effects of regions, growers, and orchard age, the estimated incidences of PNRSV in the cultivars were 6.9% in Harvester, 10.6% in Junegold, 15.0% in Redglobe, 39.5% in Loring, 44.1% in Blake, 52.9% in Coronet, and 74.5% in Redhaven. Some young orchards were apparently 100% infected; these were inferred to have been established with trees propagated from sources of scionwood infected with PNRSV. At these levels of infection the potential yield losses caused by this virus are immense. As the virus was detected by ELISA, however, certain constraints on estimating losses based upon such data exist.

South Carolina ranks second in peach production in the United States. Although much is known about occurrence and effects of fungal and nematode problems that affect peach orchards in this state, little is known about viruses. At least 30 viruses infect peach trees, and their consequences range from latent infection, with little apparent effect on the host, to plant death resulting either directly or indirectly from infection (19). There is little or no information about prevalence of any of these viruses in South Carolina or elsewhere in the southeastern United States.

Prunus necrotic ringspot virus (PNRSV) occurs worldwide, infects the majority of *Prunus* species, and exists as different strains that can cause distinctly different diseases in the same host (11). Most studies of this virus have worked with strains isolated from cherry, e.g., the causal agents of sour cherry Stecklenberg disease and rugose mosaic disease. Effects of PNRSV on peach are not documented extensively. Losses in fruit yield (20-22), reduced tree growth (21,24), changes in fruit color (24), and

delayed fruit maturity (20) have all been associated with infection by PNRSV, although these effects may be strain-specific.

The virus is believed to be transmitted mainly through pollen (12,28) and seed (6,9,10,17,27) and possibly by a nematode vector (13). Asexual propagation of peach using infected sources of scionwood produces virtually 100% virus-infected trees. The only method of controlling spread of infection from this source is by planting virus-free material.

The constraints and criteria that should be used in designing surveys for virus diseases were discussed by Barnett (3). The "finite" population of peach orchards in South Carolina appears to be an ideal situation in which to apply these concepts. Census information on peach trees in South Carolina was used in planning this large-scale survey as an aid to accurate population definition.

The survey conducted in 1987 was intended to establish the general prevalence of PNRSV in commercial peach orchards of South Carolina and to investigate variation in virus incidence among the major cultivars and production regions.

MATERIALS AND METHODS

Census information was used to define the peach tree population to be surveyed and to design a sampling scheme. All population counts and proportions

quoted here are derived from the 1985 South Carolina Fruit Tree Survey (Document 440, May 1986, published by the South Carolina Agricultural Statistics Service, P.O. Box 1911, Columbia 29202).

Survey design. The critical role of population definition in survey design is emphasized by Barnett (3). The availability of a statewide census, cross-classified by cultivars and age groups in regions, together with listings for individual growers, proved invaluable in defining the population of peach trees to be sampled in this survey. We chose to exclude orchards in the more advanced age groups (>8 yr old), reluctantly, in view of their potential status as reservoirs of virus, but their decreasing contributions to future production did not justify a proportionate allocation of our limited sampling resources. Recent plantings were also excluded; unless they had been established from an infected source of budwood, their time at risk was too short to warrant proportionate sampling. Moreover, in the absence of significant changes in peach crop ecology, virus prevalence in the recent plantings (as they mature) will probably attain the general levels found in peach crops at the ages that were sampled. As a consequence of these considerations, only the 4- to 8-yr-old census group was included, and these trees were 6-10 yr old when the survey was conducted, at which time ages of individual plantings (to the nearest year) were obtained from growers.

We excluded more than 40 cultivars with low census counts and no anticipation of becoming fashionable in future plantings. Another consideration was to include cultivars that occurred abundantly in more than one region. With the exception of Jefferson (which is grown predominantly in only one region), we included all cultivars (seven) with census counts in excess of 60,000 trees in the 4- to 8-yr age group. Altogether they accounted for 43% of the 1.467 million trees contributed by more than 50 cultivars to that age group, which itself contained 40% of the statewide census. This initial definition of the population of interest was modified by practical limitations.

Available resources permitted

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processing samples from approximately 6,000 trees and, in accordance with our second objective, were deployed equally to the 17 cultivar-region combinations shown in Table 1 (353 trees each). The census count of a particular cultivar in the specified age group in a particular region was divided by 353 to establish a sampling fraction for that combination. For example, the census count for 4- to 8-yr-old trees of the cultivar Blake in the Ridge region was 54,931, yielding an intended average sampling rate of one of every 156 trees. Intended average sampling rates for other region-cultivar combinations were: Coastal Plain—Coronet (36), Harvester (71), Junegold (114), Loring (39), and Redglobe (50); Ridge—Coronet (126), Harvester (82), Junegold (52), Loring (91), Redglobe (115), and Redhaven (137); Upstate—Blake (153), Coronet (78), Loring (70), Redglobe (158), and Redhaven (177). In practice, these intentions had to be modified; there were too many growers with small orchards (<1,000 trees) of the specified cultivars and ages for all to be visited and sampled. Many small orchards were therefore excluded and the 353 samples allocated to the remaining orchards in the final sampling plan. In fact, the smallest number of trees sampled in any one orchard was eight. The outcome of this modified sampling plan was a redefinition of the population actually sampled to an average of 64% of that identified originally (39% in the Upstate region, 73% in the Ridge region, and 91% in the Coastal Plain).

The resultant population, stratified by 17 combinations of cultivar and region, is shown in Table 1. All growers visited had orchards of more than one cultivar so that even though 114 orchards are listed, only 37 growers are represented.

Sampling. The longest diagonal of trees in an orchard was selected for sampling. The number of trees in that diagonal was estimated (from counts of numbers of trees per row and of rows) and divided by the number of samples to be collected to derive an interval between sample trees (e.g., a sample was collected from every fifth tree along the diagonal). Single bud sticks approximately 30 cm long were collected from each quadrant of a sampled tree, wrapped in moist paper towels, and

enclosed in plastic bags. The original intention was to store samples in a cool room at 4 C until assays could be performed, at which time the material would be removed from storage, placed in buckets of water, and transferred to a greenhouse at 20 C where buds would be "forced." These intentions were modified, as described later. Samples of flower or fresh leaf tissue were taken from each of the four bud sticks sampled from an individual tree, combined, and prepared for ELISA by use of a leaf squeezer and 0.03 M sodium phosphate buffer, pH 7.0, containing 0.01 M sodium diethyldithiocarbamate + 0.5% Tween 20. Tissue and buffer were ground in an approximate ratio of 1:5 (w/v).

Assay technique. Direct, double-antibody sandwich ELISA was used with conjugates and coating antibodies prepared from American Type Culture Collection antiserum PVAS-22 raised against Fulton's strain G of PNRSV. All solutions used in ELISA, as well as the antibodies and conjugates, were prepared as described by McLaughlin et al (15). Both conjugated and coating antibodies were used at dilutions of 1:800. Plates were coated for 1 hr at room temperature, then washed, the sample extract was added, and the plates were incubated overnight at 4 C. After a second wash, conjugate was added and the plates were incubated overnight again at 4 C. After a final rinse, substrate was added and the plates were incubated for 1 hr at room temperature.

All ELISA plates contained controls (of corresponding peach tissues) known to produce positive and negative reactions. Positive reactions of tree samples were recorded after visual examination of color development in wells.

Data analysis. Estimates of prevalence (\hat{p}) in the defined population of each cultivar in each region were calculated from observed sample incidences and census counts as follows: $\hat{p} = (\sum_i t_i r_i / n_i) / (\sum_i t_i)$, $\text{var}(\hat{p}) = p(1 - p)(\sum_i t_i^2 / n_i) / (\sum_i t_i)^2$, where t_i is the census count of trees, and r_i is the observed count of infections among the n_i sampled trees, in the i th orchard of the specified cultivar in the specified region. Given a fixed sampling resource, $N = \sum_i n_i$, for a given cultivar in a particular region, $\text{var}(\hat{p})$ is

minimized by using a uniform sampling fraction: $(n_i / t_i) = N / (\sum_i t_i)$. This condition was intended in the design of our survey and was realized with only two exceptions, but the sampling fraction varied considerably from cultivar to cultivar.

For the purpose of making comparisons among cultivars and regions free of effects of age and individual grower practices (assumed common to all orchards owned by a grower), logit-linear analysis (7) was applied to all observations in the cross-classification of cultivars and regions, with growers nested in regions, and allowing for yearly age effects. Thus, the least squares estimates reported for cultivar incidences are adjusted for region, grower, and age and those for regional incidences are adjusted for cultivar and age.

RESULTS

Sampling began on 9 February in the Coastal Plain and ended on 23 April in the Upstate region. Our intent to collect only dormant budwood, store it, and perform ELISA on tissues from forced blooms at a later date had to be modified because the onset of flowering was earlier and the rate of blossom development faster than had been anticipated. Early samples were stored as planned initially, but samples collected between 15 March and 7 April were beginning to bloom; therefore, fresh blossoms were assayed immediately. Moreover, samples collected after 7 April were in full bloom accompanied by early leaf development. These blossoms were fragile and handled poorly, so ELISA was performed on newly developed leaf tissue as soon as possible after sampling. Thus, a mixture of tissues was used for ELISA: forced blossoms (mostly in the Coastal Plain), fresh blossoms (mostly in the Ridge region), and fresh leaves (mostly in the Upstate region).

In the Ridge region, the number of samples assayed was reduced from the planned 2,471 to 2,439 by errors in sample collection and labeling. In the Upstate region, the numbers of trees sampled from cultivars Redglobe and Loring were reduced from the intended 353 to 331 and 240, respectively, because of labeling errors and erroneous attribution of cultivars to growers in the 1985 census report. In all, assays were performed on tissues from 5,833 trees sampled from the 382,492 trees in the defined population.

Observed incidence of PNRSV infection in individual orchards spanned the full range from 0 to 100%. No virus was detected in four orchards of Redglobe, three of Junegold, two of Harvester, and one each of Loring and Redhaven. In five orchards of Redhaven and in one each of Blake and Coronet, however, all sampled trees were infected.

Table 1. Number of orchards sampled in three peach-growing regions of South Carolina and number of 4- to 8-yr-old trees in those orchards

Cultivar	Number of trees (number of orchards)			
	Coastal Plain	Ridge region	Upstate region	Total
Blake	0 (0)	41,154 (11)	23,750 (7)	64,904 (18)
Coronet	12,500 (2)	35,013 (12)	15,200 (6)	62,713 (20)
Harvester	18,880 (4)	22,620 (10)	0 (0)	41,500 (14)
Junegold	39,577 (5)	14,142 (8)	0 (0)	53,719 (13)
Loring	12,400 (3)	24,972 (7)	10,400 (4)	47,772 (14)
Redglobe	16,620 (4)	28,054 (8)	13,750 (5)	58,424 (17)
Redhaven	0 (0)	28,607 (10)	24,853 (8)	53,460 (18)
Total	99,977 (18)	194,562 (66)	87,953 (30)	382,492 (114)

Estimates of prevalence for each of the 17 cultivar-region combinations, weighted by orchard census numbers and reported as percentages, are shown in Table 2. Results from the logit-linear analysis of infected and noninfected tree counts, back-transformed to percentages, are given in Table 3. There was no evidence of cultivar \times region interaction in logit-incidence, so that marginal incidences only are listed for separate cultivars and regions; those for cultivars are from least squares means adjusted for individual grower practices, assumed uniform in average effect across all cultivars used by a grower, as well as for regional and age effects.

DISCUSSION

The availability of a statewide census, with its listings for individual growers, simplified the tasks of defining the population precisely and of designing and executing this survey in accordance with its objectives. The general prevalence is reported in Table 2 with confidence that it is an unbiased and accurate representation of a population of almost 382,500 trees in 114 orchards.

In retrospect, it is clear that without census information this survey would have been conducted very differently, with the consequences of uncertainty about the population being sampled and about the reliability of the results obtained. Even given the census information, there are obviously many different approaches to designing a survey in relation to different objectives, largely by different choices of variables with which to stratify the population. In this first extensive survey, the primary objectives were to establish a foundation of general prevalence in commercial orchards (>1,000 trees of the same cultivar) dominating production now and in the foreseeable future and to identify potential cultivar problems in distinct peach-growing regions of the state. If the primary objective had been to investigate more intimate epidemiological features of potential importance, then an entirely different stratification would have been appropriate, e.g., stratification of cultivars by years since their release and by an ordering of their seasonal flowering dates.

Prior to the survey, much work had been completed in calibrating the ELISA system, with presumptive detections of the virus being verified by bioassay using *Prunus serrulata* Lindley 'Shiro-fugen.' Antiserum to PNRSV-G was used, as this isolate virtually has the status of type strain for this virus. Sera against this strain have been used by a number of other workers (2,18,25), giving indications of activity to a broad range of strains of PNRSV and providing us with the opportunity to make limited comparisons with other published work. In our hands, PVAS-22 has been useful

Table 2. General incidence^a of Prunus necrotic ringspot virus in peach orchards of South Carolina

Region	Cultivar						
	Harvester	June-gold	Loring	Coronet	Redglobe	Blake	Redhaven
Coastal							
Plain	1.7 \pm 0.7	6.5 \pm 1.3	56.3 \pm 2.6	59.4 \pm 2.6	8.5 \pm 1.5
Ridge	6.3 \pm 1.3	7.6 \pm 1.4	21.1 \pm 2.3	50.4 \pm 2.8	15.2 \pm 1.9	52.8 \pm 2.7	64.7 \pm 2.6
Upstate	28.2 \pm 2.9	38.4 \pm 2.7	22.6 \pm 2.4	39.0 \pm 2.6	85.8 \pm 1.9

^aEstimated percentage of trees infected followed by standard error, weighted by census tree counts but otherwise unadjusted.

in detecting PNRSV both in samples of peach from diverse sources and in other species.

Floral and juvenile leaf tissues are recognized as being the optimal tissues in fruit trees in which to detect PNRSV by ELISA. They contain high concentrations of the virus and have low background readings (1,25). In our system, optical densities recorded for these tissues from healthy peach ranged from 0.09 to 0.2. Later in the season, the background reading from leaf tissue can increase up to 0.6, and discrimination between healthy and infected trees on the basis of ELISA alone is not possible. Our visual inspection of ELISA plates detects as unequivocally positive only those wells with OD readings of above 0.4. Bioassays of bud sticks collected in the spring for which ELISA readings were 0.4 or less gave no indications of the presence of PNRSV.

Some authors (5,16,25) report that infection by PNRSV is systemic in *Prunus* species, including peach. Peach trees in South Carolina have been found in which the virus is restricted in its distribution (23). Repeated exhaustive testing of these trees has in some instances shown no spread of the virus from season to season. In "blind" tests, however, it was always possible to detect infection of these trees by using a composite sample of tissue from the four quadrants of the tree. A sample from each quadrant corresponds approximately to a sample from each scaffold limb of the tree, as peach trees in South Carolina are pruned to have four or five scaffold limbs. The use of samples taken from around the tree to ensure detection of recent infections, which may be localized, was previously suggested by Torrance and Dolby (25).

The general incidence of PNRSV reported here is much higher in some cultivar-region combinations than had been anticipated. In a 1982 survey of six South Carolina orchards, R. W. Miller, T. Watson, M. Zimmerman, and J. Golden (*unpublished*) found that infection per orchard averaged 20.8%. Barrat and Otto (4) reported that 29.5% of 1,264 trees in West Virginia were infected with PNRSV. In this survey, there are large populations (50,000+ trees) of Redhaven and Coronet cultivars in which PNRSV incidence is estimated to be 50–75%.

Of the three peach-growing regions

Table 3. Adjusted incidences^a of Prunus necrotic ringspot virus in peach orchards of South Carolina

Variable	Incidence
Cultivar^b	
Harvester	6.9 \pm 3.6
June-gold	10.6 \pm 4.8
Redglobe	15.0 \pm 4.7
Loring	39.5 \pm 8.0
Blake	44.1 \pm 9.5
Coronet	52.9 \pm 8.3
Redhaven	74.5 \pm 8.7
Region^c	
Coastal Plain	28.0 \pm 8.7
Ridge	31.8 \pm 6.7
Upstate	30.6 \pm 8.4
Age (yr)^d	
6	10.6 \pm 7.4
7	36.3 \pm 9.6
8	24.5 \pm 5.8
9	28.9 \pm 11.1
10	61.6 \pm 19.7

^aLogit-linear analysis of infected and non-infected tree counts; least squares means of logit-incidence back-transformed to percentage, followed by standard error.

^bIncidence adjusted for region, grower, and age.

^cIncidence adjusted for cultivar and age.

^dIncidence adjusted for cultivar, region, and grower.

surveyed, the Upstate (a piedmont region) experiences fewer than 220 frost-free days per year, and so cultivars with relatively high chilling-hour requirements (850–950 hr) are common. In contrast, the Coastal Plain experiences more than 240 frost-free days per year, and cultivars with low chilling-hour requirements (450 hr) may be grown. The Ridge region is intermediate in these respects. After adjustment for the effects of cultivar and age, no differences in prevalence of PNRSV among regions were detected in this survey.

There is an indication of a possible increasing trend in prevalence with age (over the range of 6–10 yr, Table 3). This agrees with the reports of Schmitt et al (22) and Barrat and Otto (4) but is complicated by another factor contributing to prevalence levels. The observation that several young orchards are apparently 100% infected suggests they had been established from infected sources of scionwood. Investigation of a possible age trend in incidence therefore requires populations from which such orchards are excluded.

PNRSV frequently causes visible

symptoms on peaches during the early, relatively short acute stage of infection, but in the subsequent, much longer chronic phase, the virus is latent (19). Thus, in the absence of symptoms it is difficult to present a convincing argument to a grower that infection by the virus causes a yield loss. Saunier (21) examined trees propagated from healthy and PNRSV-infected clones of the cultivars Springtime and Robin and observed an average annual reduction in yield over 4 yr of 55 and 31%, respectively. Yield reductions were particularly severe (82%) for infected trees of Springtime in a year when severe winter cold damage was recorded. These trees had been infected throughout their lives and correspond to the situation in young orchards where we found all trees were infected. If similar losses per tree occurred with the cultivars we surveyed, then even though levels of infection in most plantings did not reach 100% (mean 30%, range 7-74%), the potential yield losses caused by this virus on a statewide basis are immense.

When applied to estimation of yield losses, however, these results must be interpreted remembering that ELISA was used in detection. Double-antibody sandwich direct ELISA is extremely strain-specific (26). Even though we used antiserum against PNRSV-G, we may not have detected either all strains of the virus present in peach or infections by other viruses closely related serologically. Thus our results may be an underestimation of PNRSV sensu Francki et al (8) in these orchards. In addition, detection of a virus is no indication that the virus is causing a disease. Howell and Mink (14) have detected strains of PNRSV in cherry that give high ELISA readings but do not cause symptoms (HENS strains). If strains similar to those in cherry exist in peach, then our results might cause an overestimate of the effects of PNRSV, particularly as the disease syndromes associated with the effects of PNRSV in peach are not as unequivocally defined as are those caused by different strains of PNRSV in cherry and other *Prunus* species.

In addition to any losses directly attributable to viral infection, the stress that infection places on a tree must be considered. In general, viral infection debilitates the tree, making it more susceptible to other forms of stress. As shown by Saunier (21), yield losses are greater when extremely cold winter

conditions are recorded. In the extreme situation, a combination of infection by PNRSV and extremely low winter temperatures leads to increased tree mortality.

The infrastructure of the peach-growing industry in the southeastern United States and the nurseries that supply planting material to that industry is such that the results of a survey in South Carolina are applicable over a much greater region of the country. Nurseries that supply the southeastern states also supply trees to many of the eastern, southern, and southwestern states. The industry is based in Tennessee, where many of the nurseries do not use virus-free seed to establish rootstocks and collect much of their scionwood from plantings of peach cultivars (some in South Carolina) that are not routinely indexed for the presence of viruses. Considerable potential for the propagation of trees from infected scionwood exists. As spread of PNRSV in the southeastern United States appears to be primarily by infected nursery stock (2), it is highly likely that the situation we observed for PNRSV in South Carolina may also be true in other peach-growing areas of the United States.

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