Short Life of Peach Trees as Related to Tree Physiology, Environment, Pathogens, and Cultural Practices

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ABSTRACT

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Concentrations of prunasin, ninhydrin-positive material, and reducing sugars were determined in bark samples obtained from the north and south sides of trunks of healthy peach trees and those affected by peach tree short life (PTSL). Prunasin concentration was consistently decreased by greater than 90% on the south side of PTSL trees, where cold injury is commonly most severe, and by about 50% on the north side. Both the north and south sides of apparently healthy trees in PTSL orchards had about the same concentration of prunasin as trees in non-PTSL orchards. Concentrations of ninhydrin-positive materials did not change for PTSL trees. Reducing sugars decreased 50 and 30% on the south and north sides of PTSL trees, respectively, compared with apparently healthy trees in PTSL orchards. *Pseudomonas syringae* and Prunus necrotic ring spot virus were not determining factors in the PTSL syndrome. Populations of *Criconemella xenoplax* were significantly greater under PTSL trees than under apparently healthy trees in PTSL orchards. Soil pH of all PTSL orchards was within the range of 4.55–5.88.

Additional key words: bacterial canker, *Prunus persica*, replant disease

Peach tree short life (PTSL), defined as an unexpected and rapid death of peach trees (*Prunus persica* (L.) Batsch) in the spring and early summer, results from complex interactions of biotic and abiotic factors (14). PTSL is most prevalent where peaches have been grown previously, especially on sandy soils (14). Low soil pH, commonly ranging from 4.4 to 5.2, fall pruning, deep cultivation, and improper rootstock selection all have been implicated as poor orchard management practices that enhance the severity of PTSL (4,14).

The ring nematode (*Criconemella xenoplax* (Raski) Luc & Raski) appears to predispose the peach tree to cold injury and bacterial canker caused by *Pseudomonas syringae* pv. *syringae* van Hall (*P. s. syringae*) (9,10,14). Fluctuating winter temperatures, common in the Southeast, are apparently responsible for the cold injury that ultimately causes tree death (12). A major problem in PTSL research is that not all trees in an orchard are affected equally; therefore, uniform sampling before the onset of PTSL may be misleading. By comparing concentrations of prunasin, reducing sugars, ninhydrin-positive materials, and soluble protein, we investigated apparently healthy and dying peach trees in the same orchard and healthy trees in orchards with a low incidence of PTSL. Factors known to contribute to PTSL, such as pathogens, soil types, and cultural practices, were determined for each site and similarities and differences recorded.

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MATERIALS AND METHODS

Orchards. Orchards with greater than 10% tree loss caused by PTSL in 1984 were designated PTSL orchards (orchards 1–5). Orchards with no tree mortality (orchards 6–9) were then selected for similarity to PTSL orchards. Each orchard was at least 2 ha and consisted of a single cultivar/rootstock combination. Orchard age, scion/rootstock, cultural practices, and previous land use were recorded (Table 1). In two other 5-yr-old orchards (not listed in Table 1), trees affected by Clitocybe root rot (caused by *Clitocybe tabescens* (Scop.) Bres.) were compared with PTSL trees. All orchards

selected were within a 15-km radius of the Southeastern Fruit and Tree Nut Research Laboratory at Byron, GA. Sampling was done in April 1984.

Nematode and pH assays. The population of *Criconemella xenoplax* associated with each tree was estimated. Eight soil cores 2 cm in diameter were taken with a soil probe within the drip line around each tree to a depth of 25–30 cm. Ten dying and 10 apparently healthy trees were sampled from each PTSL orchard, and 10 healthy trees were sampled from each non-PTSL orchard. The same trees were used for all tests and determinations. After the soil was thoroughly mixed, the nematodes in 100 cm^3 of soil were extracted by centrifugal-flotation (5) and counted. Soil pH was determined with a glass electrode pH meter on a 1:1 soil:solution ratio.

Chemical assays. Duplicate trunk samples were taken with an arch punch from the north and south sides of each tree 20–30 cm above the ground line. Each sample was a 1.3-cm-diameter cylinder containing the bark and 5 mm of xylem. One sample was used for fresh- to dry-weight conversion and total protein. Fresh weight was determined and the sample wrapped in preweighed foil, placed in an oven at 80 C, and dried to constant weight. After dry weight was determined, the disk was then placed in 10 ml of 0.5 N NaOH, ground with a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY), and extracted at 60 C for 30 min. Protein in the NaOH extract was determined by the Bradford method (1). The second sample was processed to obtain extracts for measuring prunasin, ninhydrin-positive material, and reducing sugar. Prunasin was measured by the method of Reilly and Okie (13), free amino acids by the ninhdrin method (7), and reducing sugars by the method of Nelson (8).

Virus and microorganism assays. Young leaves were randomly collected from each sample tree and stored for no more than 3 days in polyethylene bags in a refrigerator until assayed for Prunus necrotic ring spot virus (PNRSV) with the enzyme-linked immunosorbent assay (ELISA) procedure described by Clark and Adams (2). Antiserum prepared by R. W. Fulton against isolate G of PNRSV was obtained from the American Type Culture Collection, Rockville, MD. Samples were ground in 0.1 M sodium phosphate buffer, pH 7.0, at 1:20 (w/v). Immunoglobulin and conjugated immunoglobulin were used at 1 μg/ml. Incubation periods were 4 hr for coating and conjugate fixation and 12 hr at 6 C for the sap-filled plates. All ELISA readings were at 410 nm with a Dynatech MR 600 Microplate Reader (Dynatech Instruments, Inc., Torrence, CA). A positive test was defined as one in which the absorbance at 410 nm of the sample wells was >5× the absorbance of the virus-free control wells (plant of the same species and growth stage).

P. s. syringae was isolated from bark of PTSL and healthy trees in PTSL orchards 2 and 3 and from trees in healthy orchard 9. Five pieces of bark were obtained from the interface of healthy and necrotic areas on the trunk or scaffold branches of each sample tree. Portions about 5 cm in diameter from each sample of a tree were combined, placed in a sterilized mortar, and ground in 5 ml of distilled water. The bark slurry was streaked onto *Pseudomonas* agar F (Difco), incubated at 25 C for 3 days, then examined with ultraviolet light for fluorescence. Fluorescing colonies were tested for pathogenicity on peach seedlings by puncturing the stem and placing one drop of bacterial suspension on the puncture. Cankers developed within 7 days.

Orchard 3 was removed in June 1984 and roots and trunks visually assessed for root-knot nematode (*Meloidogyne* sp.), Clitocybe root rot, and crown gall (caused by *Agrobacterium tumefaciens* (E. F. Smith & Town.) Conn).

RESULTS

Orchard conditions. PTSL orchards had 11–30% tree loss, whereas healthy orchards had only 0.3–1.5% tree loss to PTSL. Of the five short-life orchards selected for this study, four were on land previously planted to peaches. Spring or summer pruning was conducted in three of the orchards. PTSL developed even in a summer-pruned orchard with no known history of peaches planted on the site (Table 1). Of the healthy orchards selected, one was summer-pruned, two were fall-pruned, and one was spring-pruned. *C. xenoplax* populations in these orchards were relatively low compared with PTSL trees in the PTSL orchards and about equal to those in the apparently healthy trees from those orchards. The *C. xenoplax* population of the one orchard not previously planted to peaches was the highest for any of the PTSL orchards (Table 2). The soils of all orchards were sandy loam. With the exception of healthy orchard 6 (pH 6.1), the pH of topsoil and subsoil of healthy and PTSL orchards did not differ greatly and were all within a range of 4.6–5.9.

Chemical analysis. Protein concentration and dry matter of samples from PTSL trees and apparently healthy trees of an orchard were equal on both south and north sides. This indicated that major components of the bark appeared equal; however, specific chemical components changed. The prunasin concentrations of trees from the healthy orchards ranged from 0.92 to 1.82 mg/cm^2 of bark, which was similar to the range for healthy trees in PTSL orchards (Table 3). A striking decrease in prunasin concentration was detected in PTSL trees, particularly on the south side, where levels ranged from 0 to 0.09 mg/cm^2 of bark. On the north side, prunasin levels ranged from 0 to 0.95

Table 1. History of orchards selected for high incidence of peach tree short life (PTSL) and for absence or low incidence of PTSL

Orchard and type	Age (yr)	Scion/ rootstock	Previous peach land/last orchard[a]	Time of pruning
1 (PTSL)	9	Harvester/Lovell	Yes/5 yr	Aug. 1983
2 (PTSL)	6	Loring/Lovell	Yes/2 yr	Nov. 1983
3 (PTSL)	3	Brighton/Lovell	Yes/1 yr	Nov. 1983
4 (PTSL)	7	Redskin/Lovell	Yes/16 yr	Mar. 1984
5 (PTSL)	12	Coronet/Lovell	No	Aug. 1983
6 (Healthy)	9	Harvester/Lovell	No	Nov. 1983
7 (Healthy)	6	Loring/Nemaguard	Yes/15 yr	Dec. 1983
8 (Healthy)	7	Redglobe/Lovell	No	Mar. 1984
9 (Healthy)	9	Harvester/Lovell	No	Aug. 1983

[a] Years between previous orchard and current planting.

Table 2. Number of *Criconemella xenoplax* under peach tree short life (PTSL) trees and healthy trees in PTSL or non-PTSL orchards in Georgia in April 1984

Orchard and type	No. trees/ orchard	Percent PTSL	No. *C. xenoplax* /100 cm^3 of soil	
			PTSL trees[a]	Healthy trees[a]
1 (PTSL)	638	11.0	346	237
2 (PTSL)	3,248	28.0	356	139
3 (PTSL)	780	30.4	293	170
4 (PTSL)	921	27.5	158	134
5 (PTSL)	664	17.3	810	67
Mean		22.8	392**[b]	149**
6 (Healthy)	496	0.6	···	127
7 (Healthy)	935	0.5	···	3
8 (Healthy)	780	1.5	···	362
9 (Healthy)	700	0.3	···	109
Mean		0.7	···	150

[a] Mean of 10 trees (PTSL-type orchard) or six trees (healthy-type orchard).
[b] ** = Means of PTSL trees and healthy trees in PTSL orchards are significantly different ($P = 0.01$).

mg/cm^2 of bark (Table 3). The south side of the trunk was exposed to direct sunlight throughout the winter, which would elevate temperatures of the tissue even during the coldest days, and this may have accelerated prunasin breakdown.

Trees killed by *C. tabescens* appeared similar to PTSL-affected trees in early spring, but trees with root rot had a different pattern of prunasin distribution. These trees were easily distinguished from PTSL trees by the mycelial fans clearly visible under the root bark, which was a dark brown, and the appearance of trunk bark 20–30 cm above the ground that still retained a yellow-green color although the trees were dead. Analysis of 15 trees with Clitocybe root rot, from a site not included in Table 1, showed the bark prunasin levels were 0.82 ± 0.73 mg/cm^2 of bark on the south side and 0.57 ± 0.69 mg/cm^2 of bark on the north side of the trunk. Five trees from a second site had the same pattern, although the levels of prunasin were lower (0.11 ± 0.22 and 0.14 ± 0.28 mg/cm^2 of bark for the south and north sides, respectively).

Ninhydrin-positive materials (a measure of free amino acids) were similar on both sides of PTSL trees (0.05–0.10 mM/g fresh weight), apparently healthy trees from the same orchard (0.04–0.16 mM/g fresh weight), or healthy trees (0.05–0.08 mM/g fresh weight). The range of values for ninhydrin-positive materials found in the bark of trees with Clitocybe root rot was similar to those in other categories of trees.

Reducing sugars from trees in healthy orchards were somewhat lower than those found in healthy trees in the PTSL orchards and were the same on both sides of the tree (Table 4). Reducing sugars decreased by an average of 50% on the south side of the PTSL trees compared with the south side of apparently healthy trees from the PTSL orchards, whereas the north side had an average decrease of 30% (Table 4). Reducing sugars of the *Clitocybe*-affected trees were equal on both sides of the trunk and were within the same range as those of healthy orchard trees.

Viruses and microorganisms. On the basis of ELISA results, none of the trees assayed in orchards 1, 3, or 4 were infected with PNRSV; however, PNRSV was detected in one PTSL tree in orchard 2, in two PTSL trees in orchard 5, and in three healthy trees in orchard 5. Bacterial canker caused by *P. s. syringae* did not appear to be associated with PTSL in this study. Of the 60 samples from healthy and PTSL trees from three orchards, *P. s. syringae* was isolated from only eight, and these were randomly distributed throughout the samples.

Of the 780 trees in PTSL orchard 3, 187 were 1-yr-old replants (24%) and were not included in the visual assessment after the trees of that orchard had been uprooted in preparation for removal. Root-knot nematode was detected on the root systems of 53 trees not affected by PTSL (14.9%) but only on those of 25 PTSL trees (10.5%). Clitocybe root rot was present on seven PTSL trees (2.9%) but not on others. Crown gall appeared on the root systems or trunks of nine short-life trees (3.8%) and of 12 unaffected trees (3.4%).

Table 3. Mean prunasin content of peach trees affected by peach tree short life (PTSL), from apparently healthy trees in the same orchard, and from trees in non-PTSL orchards

Orchard and type	Prunasin content (mg/cm^2 of bark)[a] (±SD)			
	PTSL trees		Healthy trees	
	South	North	South	North
1 (PTSL)	0.09 ± 0.20	0.95 ± 0.31	1.03 ± 0.50	1.40 ± 0.48
2 (PTSL)	0.09 ± 0.27	0.22 ± 0.44	0.93 ± 0.31	0.99 ± 0.36
3 (PTSL)	0.00	0.00	1.28 ± 0.27	1.33 ± 0.56
4 (PTSL)	0.03 ± 0.09	0.58 ± 0.71	1.66 ± 0.45	1.45 ± 0.51
5 (PTSL)	0.00	0.44 ± 0.66	1.04 ± 0.36	1.64 ± 0.46
6 (Healthy)	...	...	1.30 ± 0.60	1.16 ± 0.54
7 (Healthy)	...	...	1.82 ± 0.54	1.66 ± 0.34
8 (Healthy)	...	...	1.16 ± 0.56	1.32 ± 0.44
9 (Healthy)	...	...	1.46 ± 0.72	0.92 ± 0.14

[a] Samples of bark tissue were taken from the north and south sides of each tree 20–30 cm from ground level. Ten trees of each type were sampled in PTSL orchards and six trees in healthy orchards.

Table 4. Mean reducing sugar content of bark samples from peach trees affected by peach tree short life (PTSL), from apparently healthy trees in the same orchard, and from trees in non-PTSL orchards

Orchard and type	Reducing sugar (mg/g fresh weight)[a] (±SD)			
	PTSL trees		Healthy trees	
	South[b]	North	South	North
1 (PTSL)	40.4 ± 11.0	75.1 ± 10.4	76.6 ± 7.2	61.0 ± 5.8
2 (PTSL)	16.5 ± 11.8	20.4 ± 9.6	44.4 ± 3.4	43.5 ± 6.3
3 (PTSL)	13.6 ± 5.6	12.9 ± 4.9	38.2 ± 4.6	31.9 ± 5.5
4 (PTSL)	43.0 ± 16.7	61.7 ± 27.6	88.9 ± 13.6	79.5 ± 11.8
5 (PTSL)	62.1 ± 14.8	66.0 ± 11.7	93.8 ± 11.8	91.3 ± 11.9
6 (Healthy)	...	...	41.5 ± 5.8	37.4 ± 4.7
7 (Healthy)	...	...	35.6 ± 3.6	35.7 ± 4.4
8 (Healthy)	...	...	36.2 ± 4.1	36.5 ± 4.0
9 (Healthy)	...	...	61.2 ± 5.9	61.4 ± 5.7

[a] Reducing sugars expressed as milligrams of glucose equivalents.
[b] Samples of bark tissue were taken from the north and south sides of each tree 20–30 cm from ground level. Ten trees of each type were sampled in PTSL orchards and six trees in healthy orchards.

DISCUSSION

Predisposing factors such as *C. xenoplax*, site factors (yet unknown), low soil pH, rootstocks, and incorrect pruning times reduce the cold-hardiness of the tree trunk in late winter (14), mainly on the south side, where solar radiation elevates temperatures higher than on the north side. During subsequent periods of subfreezing weather, this susceptible area of trunk tissue is thought to suffer damage that may result in the loss of cell membrane integrity. Our data do not allow us to determine if the loss of prunasin, a cyanogenic glucoside, is the result of cold injury and tissue death or if in fact prunasin breakdown contributes to tissue death. Prunasin and certain enzymes must come in contact for the degradation of prunasin and the release of cyanide to occur (3).

The concentration of prunasin was about 1 mg/cm^2 of bark on both the north and south side of the healthy trees, but averaged only 0.5 mg/cm^2 on the north side and less than 0.1 mg/cm^2 on the south side of the PTSL trees. The release of cyanide (88 μg/mg of prunasin) could produce considerable tissue damage. Metabolic pathways have been elucidated by which cyanide is detoxified in cyanogenic plants (3). Cyanide is incorporated into amino acid metabolism with the end product being asparagine or arginine. Recently, we presented evidence that amino acid metabolism in roots and shoots of peach seedlings was drastically altered by the feeding of *C. xenoplax*, resulting in decreases of aspartic acid and arginine in the roots (11). Reducing sugars decreased on both the north and south sides of PTSL trees. The decrease in reducing sugar concentration of PTSL trees (Table 4) may be a result of fermentation by organisms that invade dead and dying tissue.

Under field conditions, it appears that a blend of factors including site, ring nematode populations, soil pH, and pruning practices interact to predispose the trees to PTSL. The orchards in this study were all well below the recommended soil pH of 6.5 (6) except orchard 6. This

orchard, pruned in the fall, had a pH greater than 6.0 and a population of 127 *C. xenoplax* per 100 cm^3 of soil but only 0.6% PTSL. Orchard 7, also pruned in the late fall, on Nemaguard rootstock and planted on land previously in peaches, had only three *C. xenoplax* per 100 cm^3 of soil and 0.5% PTSL. In contrast, PTSL orchards 1–5 all had soil pH of less than 6.0 and high nematode populations (average 392 *C. xenoplax* per 100 cm^3 of soil). In this study, PTSL was related to high *C. xenoplax* populations and low soil pH more than to pruning times. The primary injury to PTSL trees appeared to be from cold damage that occurred most severely on the south side of the trunk.

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