Distribution of Rickettsia-like Bacteria in Peach, and Their Occurrence in Plum, Cherry, and some Perennial Weeds

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ABSTRACT

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Rickettsia-like bacteria (RLB) were approximately × 10 more concentrated in 0.1 M KOH extracts of root sections of peach trees with symptoms of phony disease than in twig sections. RLB also were present in symptomless trees in orchards with 7 and 52% disease incidence. Positive symptoms of phony disease followed within 3–12 mo of detection of RLB in eight of 10 initially symptomless trees examined. RLB counts in roots and twigs of peach trees in phony-infected orchards were significantly higher in May than in February, August, or November. RLB also were found in several species of cherry and plum, and in perennial weeds surrounding peach orchards. Positive immunofluorescent tests for RLB were obtained with extracts from Mazzard cherry (*P. avium*), Shiro plum (interspecific *Prunus* hybrid), and Johnson grass (*Sorghum halapense*).

Rickettsia-like bacteria (RLB) are a class of small, Gram-negative bacteria now recognized as being causal agents of several plant diseases (7). These organisms were recently associated with phony peach disease when found in infected peach trees, *Prunus persica* (L.) Batsch, and in the wild (Chickasaw) plum, *P. angustifolia* Marsh (2,6).

Phony disease is a serious problem in peach orchards in the southeastern United States. Affected peach trees have a flattened or umbrella-like canopy due to shortening of terminal internodes. Foliage is generally denser and darker green than in normal trees. Tree longevity is not affected. However, phony trees produce progressively smaller fruit until eventually, 3–5 yr after symptom appearance, the fruit are too small to market (8).

Phony peach disease was first observed on peaches in Marshallville, GA in 1890 and was epidemic by 1915 (8). The disease can occur in other members of the genus *Prunus* such as apricots (*P. hortulana*, L.), Mexican plums (*P. domestica*, L.) (9), and in the Chickasaw plum which is an important natural reservoir of the disease organism (1). Incidence has been suppressed by roguing of wild plum and diseased peach trees. Periods of outbreak (such as a current epidemic) have been attributed in part to a relaxation of the program.

Since the development of a vacuum infiltration technique to recover RLB from peach tissues (3), relatively large surveys of occurrence and distribution may now be made rapidly.

Quantifying the presence and concentration of RLB in tissues depends on a knowledge of the natural variability of occurrence, the distribution of the organism within the host, and any other influencing environmental factors. This report summarizes research on the variability of RLB in peach tissues, on the relationship between appearance and symptom expression, and on the presence of RLB in other *Prunus* species and some perennial weeds.

MATERIALS AND METHODS

Peach trees in experimental and commercial orchards in Peach County and Houston County, GA were selected for study and categorized by symptomatology as either infected with phony disease, symptomless, or "questionable." Growth habits of phony trees were typically more compact and flattened than normal trees and foliage tended to be a darker green. Symptomless trees had normal growth habits and normally pigmented foliage. Trees of questionable symptomatology had growth habits and foliage color intermediate to the phony and symptomless trees. Questionable trees could not positively be categorized as phony, but possessed characteristics suggesting early stages of disease.

Tissues were examined for RLB by the method of French et al (3) with slight modifications. Root and twig sections approximately 0.5 cm in diameter were trimmed to 6 cm in length, and 1 ml 0.1 M KOH was drawn through by vacuum infiltration within 4 hr of sampling. To reduce variability and to expedite sampling, 1 ml of KOH extracts from three root or twig samples per tree were combined and examined as a composite sample for that tree. Two drops from the KOH extract were placed on a microscopic slide and dried for subsequent examination.

Slides were examined at ×400 with a microscope (Universal Microscope, Carl Zeiss Inc., New York, NY) equipped with phase contrast optics and an objective lens with a 20-mm field of vision. Three representative fields per slide were examined, counts made of the numbers of RLB, and averages were calculated and rounded off to the nearest whole number for that sample. Only particles with light transmittance characteristics and rodlike dimensions of typical RLB were counted. In samples with high numbers of RLB, one quadrant per field was counted and the result was multiplied by four.

Through the study, RLB counts were made of tissues sampled from *Prunus* spp. growing in experimental plots around orchards with phony peach disease, and a search was made for RLB in perennial weeds in the vicinity.

Samples of RLB from hosts other than peach were studied for serological relationships to phony peach RLB by immunofluorescence. Antiserum prepared against the phony peach bacterium was obtained from N. W. Schaad, Department of Plant Pathology, University of Georgia, Experiment. Immunoglobins were prepared and plant tissues stained by methods described in a companion report (10). Some confirmatory immunofluorescence work was done by the courtesy of W. J. French, IFAS, University of Florida, Monticello by methods previously described (5).

Statistical treatment of data included a transformation of averages to square root of (x + 0.5), an analysis of variance, and a Duncan's multiple range test. Differences were considered significant, $P = 0.05$.

TABLE 1. Distribution of rickettsia-like bacteria (RLB) in each quadrant of a peach tree with symptoms of phony disease

Tree quadrant[y]	Average RLB counts[z]		
	Roots	Twigs	Root/twig ratio
NW	150.2	12.4	12.1
NE	33.0	3.4	9.7
SE	36.2	9.8	3.7
SW	74.0	4.2	17.6
Average	70.85 a	7.45 b	10.8

[y]Samples collected July 1977.
[z]RLB counts in 1 ml 0.1 M KOH extract of 6 cm twig or root sections. Each value represents an average of three samples collected; those not followed by the same letter are significantly different, $P = 0.05$.

TABLE 2. Bacterial counts in roots and twigs from peach trees in orchards with different incidences of phony disease symptoms

Diseased trees in orchard (%)	Bacterial counts from trees with indicated symptoms[z]						Combined (orchard) totals
	Negative		Questionable		Positive		
	Roots	Twigs	Roots	Twigs	Roots	Twigs	
0.1	0	0	0	2	136	71	209 a
7	31	22	23	99	536	117	828 b
52	56	29	53	96	407	47	688 b
Average root/twig ratios	1.9		2.5		4.5		
Combined (symptom) totals	138 a		278 a		1,314 b		

[z]Counts taken on samples collected May 1977, and based on averages of three samples per tree, five trees per plot. Averages not followed by the same letter are significantly different, $P = 0.05$.

TABLE 3. Progression of bacterial counts in roots of initially healthy trees and development of phony peach disease symptoms during a 3-yr period

Tree number	Bacterial counts in roots at indicated dates[a]								
	1977			1978			1979		
	Apr	Jul	Oct	Apr	Jul	Oct	Apr	Jul	Oct
1	0	1	1	30	*16*[b]	x[b]	x	x	x
2	0	0	0	3	*10*	x	x	x	x
3	0	0	0	0	5	26	4	8	3
4	0	0	1	0	0	0	20	*36*	x
5	0	0	0	48	*102*	x	x	x	x
6	0	0	25	*80*	*23*	x	x	x	x
7	0	0	3	*50*	7	x	x	x	x
8	0	0	0	0	0	4	48	*14*	x
9	0	0	0	0	0	0	11	0	0
10	0	0	0	2	*18*	x	x	x	x

[a]Based on averages of three samples per tree at each examination.
[b]Italicized numbers indicate tree with positive symptoms of disease, "x" indicates tree removed.

RESULTS

Considerable variability occurred in counts of RLB within some trees. In one extensive sampling, average RLB counts of terminal twigs from tree quadrants ranged from 36.2 to 150.2 (Table 1), with individual numbers ranging from 1 to 284 per microscopic field. However, with data based on averages of three root or twig samples, there were no statistical differences in counts among the tree quadrants.

Roots from trees with positive symptoms of phony peach disease generally contained greater numbers of RLB than did twigs. Although root/twig ratios for individual trees ranged widely, ratios of 4.5 to 10.8 were typical for multiple samples taken from one tree (Table 1), or for averages of several trees (Table 2) during the late spring and early summer months.

Phony peach RLB were frequently detected in root and twig samples from trees showing no symptoms of the disease. In an orchard with only 0.1% phony trees, RLB generally were found only on those trees positive for disease symptoms (Table 2). However, in orchards with 7 or 52% phony trees, RLB were found in the questionable as well as any symptomless trees selected at random. Total RLB counts were significantly higher in trees with symptoms than in symptomless or questionable trees and root/twig ratios tended to be higher. Average RLB counts were significantly less in the orchard with 0.1% phony disease symptoms than in the 7 or 52% infected orchards. However, there was no significant difference in RLB levels between the trees of the 7 or 52% infected orchards.

Generally the presence of RLB in symptomless peach trees was followed by symptom development within as little as 3 mo. In a study in which 10 symptomless trees were monitored for RLB in the roots over a 30-mo period, nine trees eventually and consistently showed the presence of RLB, and eight of those developed disease symptoms (Table 3). RLB, above the level of one per field, were detected in trees from 3–12 mo prior to symptom expression. Twig counts were not as reliable indicators of the disease status of the trees since only four of the eight diseased trees yielded RLB from twig samples during the span of this study.

The level of RLB in root and twig extracts from trees in phony orchards varied with the season of the year. The tendency for RLB counts to be higher in May as compared to February, August, or November was evident in symptomless, questionable, and symptom-positive trees (Table 4). The peak of RLB counts in the spring quarter was consistent and statistically demonstrable with aggregate data from roots or twigs from all trees sampled. Levels of RLB were lowest during the autumn (November) and winter quarters (February).

Rod-shaped particles of morphology identical to that of phony peach RLB were found in many species of *Prunus* (Table 5). RLB counts were particularly high in mature trees of Mazzard cherry (*P. avium* L.), Shiro plum (interspecific *Prunus* hybrid), wild plum (*P. angustifolia*), and Montmorency cherry (*P. cerasus* L. 'Montmorency'). RLB were occasionally found in such perennial weeds as Johnson grass (*Sorghum halepense* [L.] Pers.) and Sickle pod (*Cassia tora* L.), but in numbers of less than five per field.

Generally the presence of RLB in domestic plum (*P. domestica* L.) was associated with marginal scorching of the leaves, confirming an observation recently made by French et al (4). Compact growth habit or darker leaf color were either not present or could not be consistently correlated with the presence of RLB in plum trees. Symptoms suggestive of phony disease were also absent in the other *Prunus* species examined.

Extracts of host tissues containing RLB were tested by immunofluorescent cross reaction to phony peach RLB antisera. Root tests were positive for the RLB from Mazzard cherry, Shiro plum, wild plum, domestic plum, and peach. Twig and stem tests were positive for Mazzard cherry, Shiro plum, peach, and Johnson grass (Table 5).

DISCUSSION

Root tissues were a rich source of RLB in peach trees affected by phony disease. Terminal twig sections from the current year's growth contained fewer bacteria than did roots, but because of ease of sampling, twigs were useful in diagnostic work. Bacterial counts were generally higher during the late spring months than at any other time of the year. Diagnosis of phony peach disease based on

TABLE 4. Bacterial counts at quarterly intervals from 1977 to 1979 on trees in an orchard with an initial 7% incidence of phony peach disease

Sampling date	Bacterial counts from trees with indicated symptoms[y]							
	Healthy		Questionable		Diseased		Combined symptom totals and average[z]	
	Roots	Twigs	Roots	Twigs	Roots	Twigs	Roots	Twigs
Feb 1977	5	0	6	0	73	3	84	3
1978	19	1	1	0	58	1	78	2
1979	4	0	4	0	230	0	238	0
Average	9.3	0.3	3.7	0	120.3	1.3	133.3 a	1.7 a
May 1977	31	32	23	99	536	117	590	248
1978	6	2	8	2	207	9	221	13
1979	25	11	13	2	512	15	550	28
Average	62.0	45.0	14.7	34.3	418.3	47.0	453.7 b	96.3 b
Aug 1977	4	13	1	40	90	4	95	57
1978	1	0	2	0	21	1	24	1
1979	9	1	8	2	176	2	193	5
Average	4.7	4.7	3.7	14.0	95.7	2.3	104.0 a	21.0 ab
Nov 1977	4	0	5	0	59	0	68	0
1978	3	0	1	0	4	0	8	0
1979	1	0	1	0	97	0	99	0
Average	2.7	0	2.3	0	50	0	58.3 a	0 a

[y]Counts based on averages of three samples per tree, five trees per plot.
[z]Averages in each column not followed by same letter are significantly different, $P = 0.05$.

TABLE 5. Occurrence of rickettsia-like bacteria in *Prunus* spp. and in some perennial weeds commonly found near peach orchards, and immunofluorescent (IMF) reaction of root extracts to phony peach antiserum

Host plant		Bacterial counts[a]									
		1977		1978		1979		3-yr average		IMF reactions[c]	
Common name	Scientific nomenclature	Roots	Twigs	Roots	Twigs	Roots	Twigs	Roots	Twigs	Roots	Twigs
Mazzard cherry	*P. avium*, L.	2,450	7	819	2	583	2	1,284	3	+++	++
Mahaleb cherry	*P. mahaleb*, L.	21	16	7	5	1	0	10	7	–	–
Montmorency cherry	*P. cerasus* L. 'Montmorency'	174	33	52	6	3	2	76	14	–	–
Shirofugen cherry	*P. cerasus* Spaeth 'Shirofugen'	15	7	8	3	10	1	13	4	–	–
Wild black cherry	*P. serotina* Ehrh.	32	5	13	0	3	0	16	2	–	–
Peach cv. 'Babygold'	*P. persica* (L.) Batsch	126	16	40	9	80	2	82	9	+	+
P. davidiana	*P. davidiana*, French	0	1	0	0	0	0	0	0	–	–
Shiro plum	Interspecific *Prunus* hybrid[b]	107	40	642	475	283	75	344	193	+++	+
Myra plum	*P. cerasifera* Ehrh.	685	241	75	12	766	87	509	114	+	–
Wild plum	*P. angustifolia*, Marsh	243	106	76	52	153	21	157	60	+	–
Domestic plum	*P. domestica*, L.	36	6	11	4	70	5	39	5	+	–
Johnson grass	*Sorghum halepense* (L.) Pers.	5	2	6	2	11	3	7	2	(d)	+
Sickle pod	*Cassia tora* L.	2	3	2	1	4	1	3	2	(d)	–

[a]Based on averages of three samples from three plants per host collected between June and August of each year.
[b]*P. simonii* × *P. solicina* × *P. cerasifera* × *P. munsoniana* hybrid.
[c]Tested on 1979 extracts.
[d]Only stem samples tested for perennial weeds.

the presence of RLB, therefore, was most accurate with root tissues sampled in May and June. Because of the variability in the distribution of RLB in the tree, examinations of at least three root or twig samples were considered minimal for reliable diagnosis.

Based on published data by Hutchins (9) and by French (2), and on data presented in this paper, wild native plums and certain other species of *Prunus* are or may be alternate hosts of the phony peach RLB. This paper extends the probable list of alternate hosts to wild and several species of domestic cherry, and to the perennial weed, Johnson grass. In a companion report (10), other lines of evidence including electron microscope studies confirm the involvement of Johnson grass as a possible alternate host of phony peach disease.

The sampling method developed and utilized in this study is useful in early detection and roguing of trees infected with phony peach disease. Detection of RLB in root or twig tissues indicates good probability of symptom development in 3 to 18 mo. It may be possible, with successful infiltration of antibiotics in peach tissues negative for phony symptoms but positive for RLB to arrest the development of the disease, and to provide a practical control measure without loss of the tree. To the author's knowledge, this is the first report of seasonal variability in RLB in a host plant. Temperature sensitivity of the organism may be the probable cause of reduced concentrations of RLB in roots and twigs during the hot months of the late summer and cold months of the winter. Greenhouse tests are now under way to test this hypothesis.

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