Incidence of Plum Leaf Scald

in Alabama

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Incidence of Plum Leaf Scald in Alabama

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INTRODUCTION

PLUMS ARE RELATIVELY EASY to grow and will produce well in Alabama. Yields in excess of 25,000 pounds per acre have been recorded from new, adapted cultivars and hybrids. Production of plums for the fresh market is increasing in Alabama and the Southeastern United States, with extensive plantings of A.U. Producer, Crimson, and Purple, and limited plantings of Homeside, selections released by the Auburn University Agricultural Experiment Station *(9, 10, 11)*. Also, there is interest in obtaining quantities of fruit suitable for commercial processing into jams and jellies.

Disease is a major factor limiting the production of plums, with leaf scald being one of the most serious problems. Leaf scald has been associated with a rickettsia-like organism, commonly designated as RLO *(6)*. The agent causing leaf scald *(3)* has shown relationship to the RLO that is considered to be the causal agent of peach phony disease *(4, 2)*. The effects of leaf scald are similar to those for phony disease of peach, i.e., reduction in new tree growth and in size, quality, and yield of fruit. In addition, leaf scald causes a die-back of terminal shoots followed by death of whole branches and finally the entire tree. Decline of trees may occur in one season or over 2 or more years.

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Description of Leaf Scald

Leaf scald has appeared on RLO infected Japanese plums (8) from mid-June until July. The first symptom is a slight chlorosis or bronzing along the margin or tip of a leaf. The discoloration intensifies, sometimes appearing water-soaked

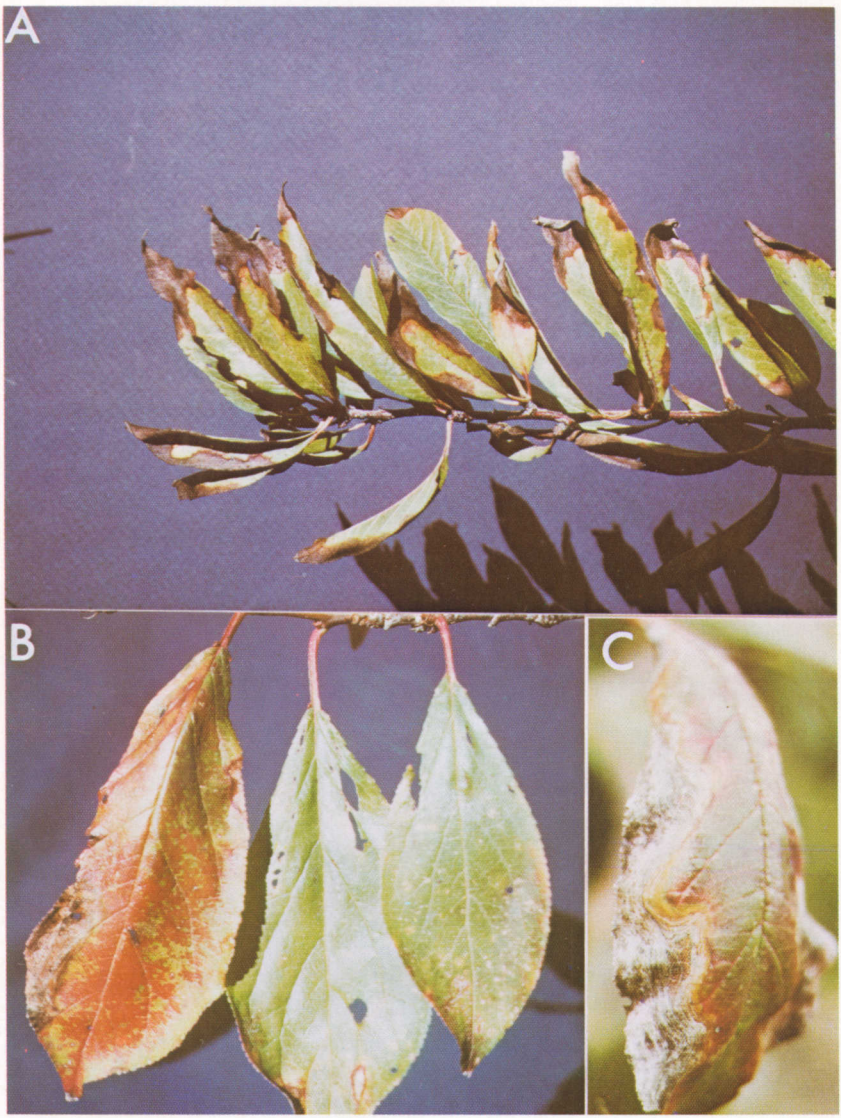

FIG. 1. Symptoms of leaf scald on plum: A, Bruce 12-4; B, Ozark Premier; C, Methley.

before turning brown and drying. The affected area becomes delineated from the unaffected area by a chlorotic band. As the die-back gradually progresses, several bands may appear in the necrotic tissue. Leaf scald may appear on one or more areas of an affected leaf and may involve as much as three-quarters of a leaf before abscission occurs.

In early phases of the disease, leaf scald may occur on only a few twigs or large branches, figure 1B; during the late phase, symptoms may appear on almost all of the foliage, figure 1A. The banded appearance of the necrotic tissue is especially evident during autumn, figure 1C. As a consequence of premature defoliation during September and October, diseased trees may develop new leaves that are malformed, leathery, and rolled; these leaves also may develop the scalded appearance.

This publication summarizes results of research on plum leaf scald by the Auburn University Agricultural Experiment Station.

MATERIALS AND METHODS

The plum cultivars and hybrids evaluated were planted at the Piedmont Substation, Camp Hill, in 1968, the Chilton Area Horticulture Substation, Clanton, in 1971, the Wiregrass Substation, Headland, in 1973, and the Main Station, Auburn, in 1974. All cultivars and hybrids were grown on Lovell peach rootstock. Root and twig samples were collected from four trees of each cultivar or hybrid at the Wiregrass Substation and from three trees at the Chilton Area Horticulture and Piedmont substations. Three root and nine twig samples were collected per tree and evaluated for disease.

Plant tissue examinations of disease incidence consisted of (1) staining root cross sections with acidified methanol according to the method of Hutchins (*5*); (2) vacuum infiltration of roots or twigs with 0.1 M potassium hydroxide and examination of extracts under a phase contrast microscope at 800X for presence of RLO; and (3) evaluation of RLO incidence from petioles of scalded leaves (*8*). Approximately 1 centimeter (0.39 inch) of petiole was crushed in 0.1 M potassium hydroxide on a microscope slide and the tissue extract was examined microscopically for RLO incidence in this latter method.

Estimates of leaf scald in cultivars or hybrids were made according to the following rating scale: 1 = 1-19 percent, 2 = 20-39 percent, 3 = 40-59 percent, 4 = 60-79 percent, and 5 = 80-100 percent of foliage showing scald symptoms.

Plum budwood of Bruce, Frontier, Methley, Ozark Premier, and Santa Rosa was obtained from Dr. P. R. Fridlund, Irrigation Agricultural Research and Extension Center, Prosser, Washington. To study RLO transmission and symptom development, buds from these cultivars were grafted on leaf scalded, 4-year-old Purple and Homeside plum trees on August 28, 1978, at the Main Station. Two buds of a given cultivar were grafted on a single twig; the other cultivars were budded onto different branches of the same tree. Two Homeside and two Purple trees were budded; one of the Homeside trees showed no leaf scald. On August 31, trees at the Wiregrass Substation were budded as described above using two trees of each of the following cultivars or hybrids: 5-year-old Ozark Premier, Ozark Premier F-2, and Purple trees with leaf scald disease symptoms. On September 1, leaf scalded, 10-year-old trees at the Piedmont Substation were budded as described above, using A.U. Producer. The roots and twigs of these trees had been examined for RLO using the French method *(2)* of vacuum infiltrating suspected roots and twigs with 0.1 M potassium hydroxide and examining the extracts under a phase contrast microscope *(7)*. Subsequently, a portion of each budwood stick from Prosser, Washington, was examined to determine possible RLO contamination using the French method *(2)*. Shoots that developed from the grafted buds were examined for symptoms of leaf scald at intervals throughout the 1979 growing season. During September, leaves were collected from each surviving shoot for examination in the laboratory. Petioles of four leaves from each bud were examined by the crushed-petiole microscopic examination of tissue extract method. Large twigs that developed from the grafted buds were collected and compared for RLO with other twigs on the same tree, using the French method *(2)*.

All trees in the test plots were fertilized each year according to recommended management practices.

Attempts were made to culture RLO from plant tissues on JD-1 and JD-3 media *(1)*. Leaf petioles were surface-sterilized in 0.5 percent sodium hypochlorite for 3 minutes and washed in sterile, distilled water. Plant sap was forcibly expressed

from the petioles with tweezers and blotted directly onto the medium. Surface-sterilized, mid-rib segments were also plated onto nutrient agar and then incubated.

Preparation of tissue extracts for electron microscopy was as follows: a drop of RLO suspension was placed on a carbon-stabilized Formvar film on a copper specimen grid; after 1 minute the excess extract was removed with a filter paper and a drop of 2.0 percent potassium phosphotungstate (pH 6.5) was added and then removed with filter paper after 30 seconds. The grid was dried for 10 minutes and examined with a Philips EM-300 electron microscope.

Tissue samples for electron microscopy were from leaves exhibiting leaf scald. Small pieces (1 x 2 mm) of petiole and leaf mid-ribs were fixed in a 3 percent solution of glutaraldehyde. The tissues were washed in cold 0.05 M cacodylate buffer (pH 6.8) and postfixed overnight at 4°C (40 °F) in 2 percent OsO_4. Subsequently, the tissues were dehydrated in an ethanol series graduating into propylene oxide and embedded in Epon. Thin sections were stained with 70 percent alcoholic uranyl acetate and lead citrate and examined with the electron microscope.

RESULTS AND DISCUSSION

Histochemical Diagnosis of Plum Leaf Scald

Since the plum trees were on Lovell peach rootstock, it was expected that Hutchins' histochemical test *(5)* would provide a rapid diagnosis of disease as used for detection of phony dis-

TABLE 1. COMPARISON OF HUTCHINS' HISTOCHEMICAL TEST AND FRENCH'S MICROSCOPIC TEST FOR PRESENCE OF RLO IN PLUMS FROM THE CHILTON AREA HORTICULTURE SUBSTATION

Plum cultivar or hybrid	Root reaction tests					
	Histochemical[1]			Microscopic[2]		
Bruce 12-4	0	0	0[3]	T	2	2
Burbank D-1	3	2	3	T	1	1
Crimson	0	0	1	1	2	2
Early Gold	1	3	2	T	2	1
Homeside	0	3	1	T	2	1
Methley A-21	2	1	0	T	3	1
Ozark Premier	2	2	3	2	3	2
Santa Rosa	3	0	3	1	3	2

[1]Root cross section (CS) immersed in acidified methanol 5 minutes. Reaction key: 0 = none; 1 = faint purple CS; 2 = purple blotch in CS; 3 = distinct purple spots.

[2]Roots infiltrated with 0.1 M potassium hydroxide, extracted under vacuum; extracts examined for RLO. Reaction key: T = trace of RLO; 1 = 5-10 RLO; 2 = < 50 RLO; 3 = > 50 RLO.

[3]Average reaction of three roots from each of three different trees.

ease of peach. However, consistent results were not apparent in initial examinations when the root-staining procedure was compared with the microscopic examination of root extracts, table 1. Some stained roots showed distinct purple spots, others showed purple blotches, and a third group showed a faint purple coloration throughout the cross section, figure 2.

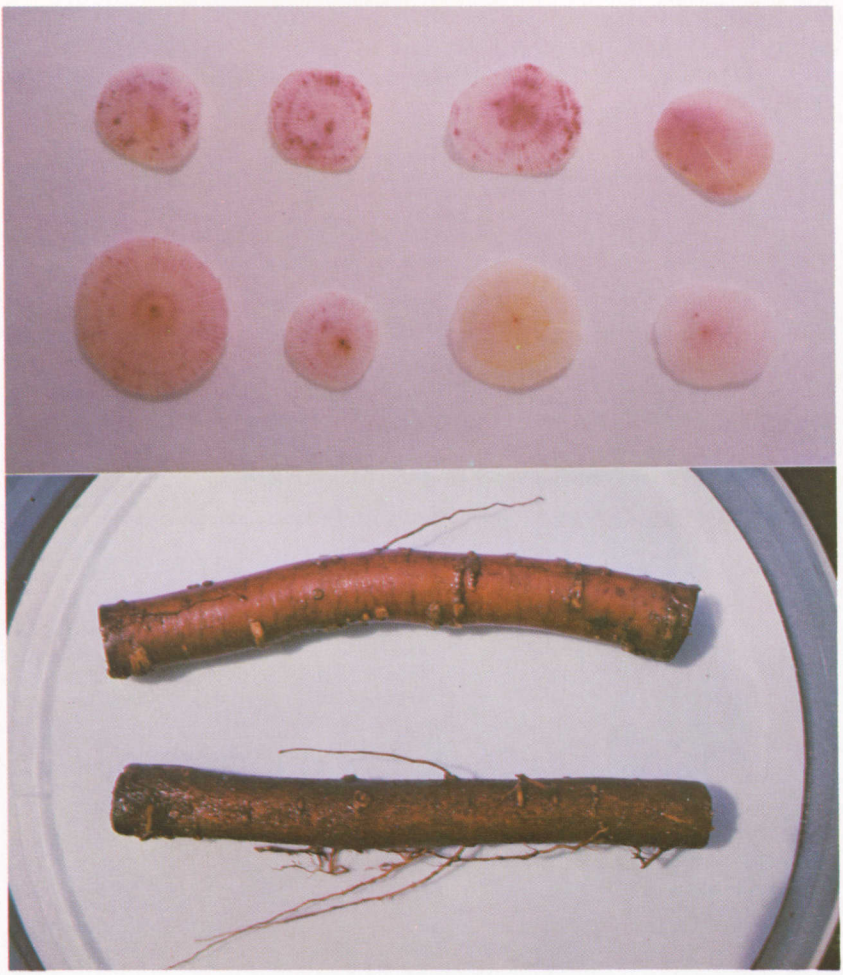

FIG. 2. Peach and plum roots and cross sections (CS). Top: Sections with acidified methyl alcohol—upper row, left to right, Giant Cherry, Burbank D-1, Ozark Premier, Methley A-39; lower row, left to right, CS 1 & 2 = Bruce 12-4 (from pink roots), CS 3 = Bruce 12-4 (from dark brown roots), CS 4 = peach (check). Bottom: Root types recovered from plum trees on Lovell rootstocks—upper root, pink, smooth root = peach; lower root, dark brown = plum.

As the work progressed, two types of roots were evident: the first type was dark brown, had a coarse, rough epidermis, and grew just under the soil surface; the second type was pink, had a smooth root surface interrupted by irregular spaced rings, and grew deep in the soil. An examination of uprooted trees indicated that dark brown roots grew from the scion or were plum roots, whereas pink roots were peach understock roots, figure 2. Subsequently, examination of acidified methanol stained roots from three plum trees was made in conjunction with microscopic examination of potassium hydroxide extracts from different portions of the same roots. Dark brown (plum) roots yielded a uniform purpling throughout the cross section with some faint spots. The pink (peach) roots yielded distinct spots with some blotches. Microscopic examinations of extracts revealed the presence of RLO from plum roots, thus confirming Hutchins' *(5)* report that *Prunus* spp. other than peach gave variable reactions in the histochemical test. Consequently, Hutchins' method is not recommended due to variable results.

Microscopic Examination of Tissue Extracts

Although all the plum cultivars were on Lovell peach rootstock, great variability in numbers of RLO was found in

TABLE 2. NUMBER OF RICKETTSIA-LIKE ORGANISMS AND LEAF SCALD RATINGS FOR PLUM CULTIVARS AND HYBRIDS, WIREGRASS SUBSTATION, OCTOBER 1977

Cultivar or hybrid	Bacterial counts		Leaf scald rating[3]
	Roots[1]	Twigs[2]	
Burbank D-1	45	36	5
Crimson	20	11	5
Frontier	69	47	5
Giant Cherry	44	6	3
Homeside	3	8	1
Mariposa	19	2	1
Methley	21	12	5
Methley A-21	14	1	1
Methley E-1	9	30	5
Morris	45	2	1
Ozark Premier	25	9	[4]
Ozark Premier F-1	52	117	5
Ozark Premier F-2	44	46	5
Purple	38	110	5
Santa Rosa	89	75	5

[1]Average number of bacteria from roots in six microscope fields at 800X.
[2]Average number of bacteria from twigs in 180 microscope fields at 800X.
[3]Scale of 1-5, based on percent leaf scald per tree, with four replications totaled and averaged.
[4]Trees completely defoliated.

TABLE 3. NUMBER OF RICKETTSIA-LIKE ORGANISMS FROM PLUM CULTIVARS AND HYBRIDS, CHILTON AREA HORTICULTURE SUBSTATION, DECEMBER 1977

Cultivar or hybrid	Bacterial counts	
	Roots[1]	Twigs[2]
Abundance G-47	83	1.1
A.U. Producer	134	1.1
Bruce 12-4	21	1.3
Burbank D-1	88	4.2
Crimson	90	1.2
Early Gold	72	1.3
Homeside	18	.4
Methley A-21	32	1.0
Ozark Premier	16	5.0
Santa Rosa	117	23.2

[1]Average number of bacteria from roots in 45 microscope fields at 800X.
[2]Average number of bacteria from twigs in 135 microscope fields at 800X.

the roots. At the Wiregrass Substation, the highest RLO number (89) was found in the roots of Santa Rosa. The lowest average RLO count (3) occurred with Homeside, table 2. Both scald and leaf reddening were evident on Methley A-21 in October. Trees that appeared green and relatively disease-free were Homeside, Mariposa, and Morris. Except for Morris, these cultivars had low RLO counts from roots and low leaf scald ratings.

At the Chilton Area Horticulture Substation, RLO numbers were higher in A.U. Producer than in Santa Rosa. Homeside was very low in RLO counts, with only Ozark Premier showing a lower count in the same orchard. Trends in high and low RLO counts appear similar between the stations except for Ozark Premier, tables 2 and 3.

Counts of RLO in twigs of Methley A-21 at both substations and Mariposa and Morris at the Wiregrass Substation were only 0 to 2 for each microscope field. With such negligible bacterial counts, it is possible that these cultivars possess resistance factors that might be utilized by plum breeders. These cultivars may provide material that could be tolerant to plum leaf scald.

The technique of French *(2)* for potassium hydroxide vacuum infiltration and phase contrast microscopic evaluation of extracts for presence of RLO in stone fruits makes it possible for plum breeders to evaluate their plants for presence of RLO. Extractions made during summer, fall, and winter indicated that RLO counts were highest when trees were in full leaf. Bacterial counts dropped drastically in twig samples made during the winter, with the extraction process requiring more

time with dormant twigs. The method provides a better procedure for evaluation and confirmation of plum leaf scald than Hutchins' (5) acidified methanol test. However, the method of crushing petioles from scalded leaves in potassium hydroxide and examining the extracts by phase contrast microscopy appears to be a more rapid spot check for the disease, since bacteria occur in high numbers in leaf petioles.

Disease Transmission

An examination of 1978 budgrafts during March 1979 showed 58 percent survival. Bud survival had dropped to 28 percent on May 15 and to 17 percent on July 27, table 4. During July, symptoms of scald were observed on leaves of the shoots of each of the five different plum cultivars. Leaf scald symptoms appeared identical to those on the rest of the tree.

TABLE 4. SHOOT GROWTH FROM DISEASE-FREE BUDS GRAFTED ON TREES INFECTED WITH RICKETTSIA-LIKE ORGANISMS, 1978[1]

Bud/stock	Bud survival			
	Mar. 15	May 15	July 27	Sept. 12
Piedmont Substation	*No.*	*No.*	*No.*	*No.*
Bruce/A.U. Producer	2	2	2	1
Frontier/A.U. Producer	2	2	2	1
Methley/A.U. Producer	0	0	0	0
Ozark Premier/A.U. Producer	0	0	0	0
Santa Rosa/A.U. Producer	0	0	0	0
Main Station				
Bruce/Homeside	2	2	1	1
Frontier/Homeside	2	2	0	0
Methley/Homeside	2	2	1	1
Ozark Premier/Homeside	2	2	1	1
Santa Rosa/Homeside	2	2	1	1
Wiregrass Substation				
Bruce/Ozark Premier	4	2	1	1
Frontier/Ozark Premier	4	2	1	1
Methley/Ozark Premier	4	0	0	0
Ozark Premier/Ozark Premier	4	0	0	0
Santa Rosa/Ozark Premier	2	0	0	0
Bruce/Ozark Premier F-2	4	0	0	0
Frontier/Ozark Premier F-2	4	0	0	0
Methley/Ozark Premier F-2	4	4	3	2
Ozark Premier/Ozark Premier F-2	4	0	0	0
Santa Rosa/Ozark Premier F-2	2	0	0	0
Bruce/Purple	4	2	1	1
Frontier/Purple	6	4	3	1
Methley/Purple	2	2	1	1
Ozark Premier/Purple	6	4	2	1
Santa Rosa/Purple	2	0	0	0
Pct. survival	*58*	*28*	*17*	*12*

[1]Buds grafted August 28 through September 1, 1978.

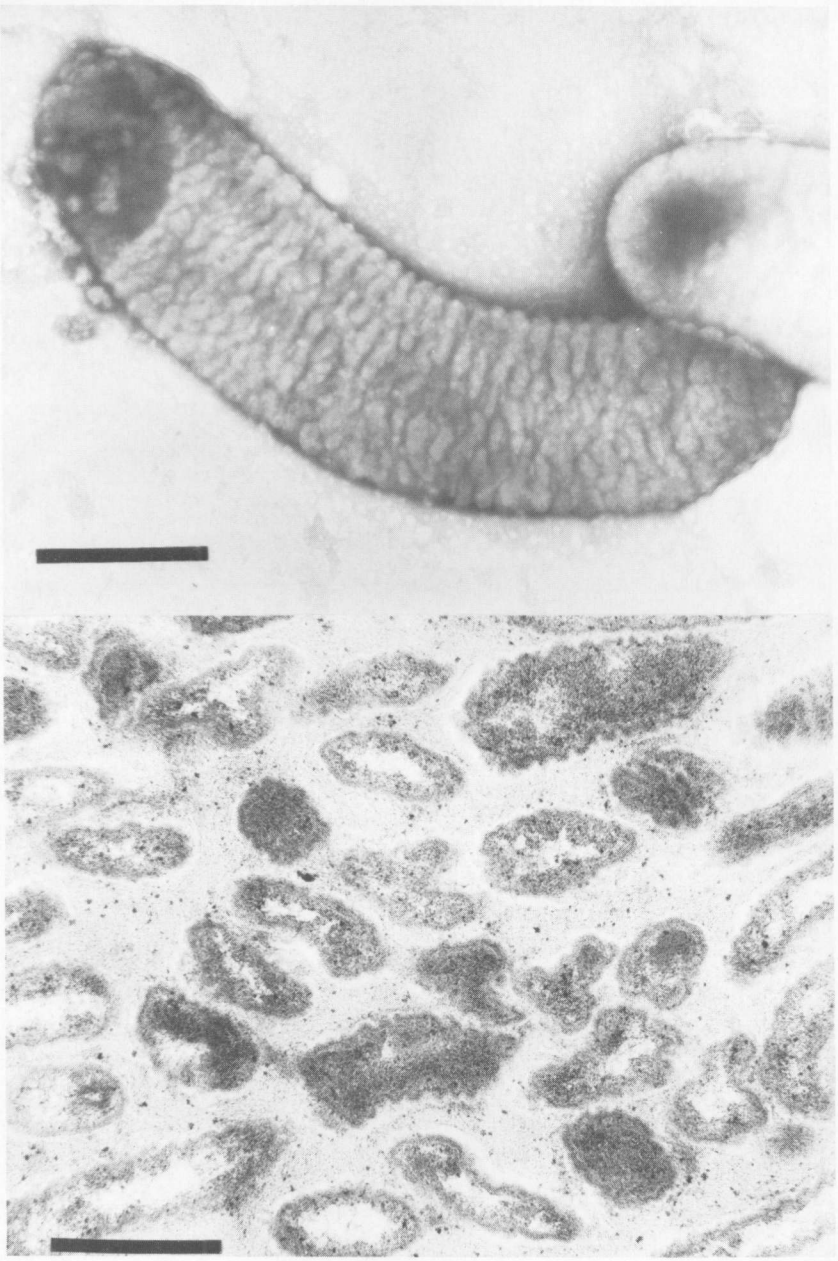

FIG. 3. Micrograph of rickettsia-like organisms from trees with plum leaf scald. Top: Negatively stained bacteria extracted from plum twigs exhibit typical rippled cell walls. Bottom: Cross section through xylem vessel showing bacteria in mid-rib tissue. Scale bar = 0.5 μm.

A special study of the Main Station Homeside tree which exhibited no leaf scald symptoms during 1978 gave a count of 5 RLO from only one of the four roots sampled. One branch on this tree exhibited leaf scald symptoms during 1979. Shoots from buds of Bruce and Methley were free of RLO and leaves showed no symptoms of scald. Santa Rosa developed only as a rosette of leaves and the Frontier buds died, table 4. The surviving Ozark Premier bud grew well, with approximately 10 percent of the leaves on the shoot exhibiting leaf scald symptoms. No RLO could be extracted from the twigs of Ozark Premier, but the leaves with scald were positive for RLO. Non-scalded leaves from the same branch did not show presence of RLO.

Attempted Culture of Pathogen

Culture of RLO on the specialized JD- 1 and JD-3 media was negative. Scalded leaf, mid-rib tissue placed on nutrient agar produced species of *Alternaria, Cephalosporium, Fusarium,* and *Gloeosporium*, all apparently saprophytes. A yellow bacterial culture developed from 40 percent (40 of 98 isolations) and a white bacterial culture developed from 11 percent (11 of 98 isolations) of the cultures. The bacterial cultures were identified as *Erwinia herbicola, Pseudomonas marginata,* and *Xanthomonas* sp., all secondary saprophytes. Some of these smooth-walled saprophytic bacteria were observed among the epidermal cells in sections examined with the electron microscope.

Electron Microscopy

Electron microscopic examinations conducted on potassium hydroxide extracts of plum tissue confirmed the presence of rippled cell walls characteristic of RLO, figure 3. Furthermore, evaluations of petiole and mid-rib xylem cross sections with the electron microscope revealed the characteristic thick, rippled cell walls attributed to RLO in plum afflicted with leaf scald, figure 3. No virus or mycoplasma-like organisms were observed in the plum tissue sections.

SUMMARY

Although the plum cultivars were grown on peach rootstock, root cross sections stained with acidified methanol gave vari-

able or inconsistent results. The confusion was resolved with the discovery that plum scions sometimes produced roots and these roots did not stain with the expected reaction known for peaches.

A wide range in leaf coloration associated with symptom patterns and intensity of leaf scald was found on leaves of the cultivars and hybrids involving the species *Prunus americana, P. cerasifera, P. munsoniana, P. salicina, P. simoni,* and *P. triflora.* Rickettsia-like organisms were found in 0.1 M potassium hydroxide extracts from all cultivars and seedlings 4 years of age or older. Bacteria were extracted from Lovell peach rootstocks when sufficient samples were made, while twig samples of the several cultivars and seedlings varied greatly in RLO counts. Fewest RLO and mildest leaf scald symptoms were found in Homeside, Mariposa, Morris, and the seedling Methley A-21. Leaf scald symptoms generally correlated with bacterial counts in roots or twigs.

Transmission of RLO from infected trees through disease-free buds and into their shoots occurred in all trees showing symptoms of leaf scald. The technique of phase contrast microscopic examination of crushed petiole extracts for RLO incidence is a rapid technique used to establish the presence of the bacteria.

Electron microscopy of xylem extracts and xylem cross sections revealed RLO with characteristic rippled cell walls and dimensions.

ACKNOWLEDGMENT

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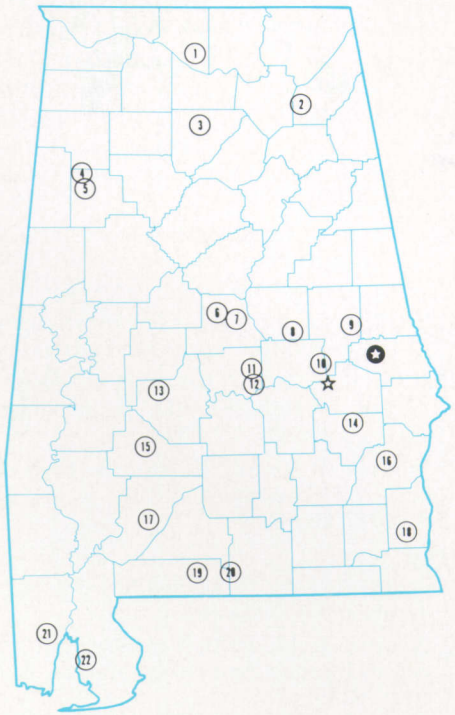

Research Unit Identification

★ Main Agricultural Experiment Station, Auburn.
☆ E. V. Smith Research Center, Shorter.

1. Tennessee Valley Substation, Belle Mina.
2. Sand Mountain Substation, Crossville.
3. North Alabama Horticulture Substation, Cullman.
4. Upper Coastal Plain Substation, Winfield.
5. Forestry Unit, Fayette County.
6. Foundation Seed Stocks Farm, Thorsby.
7. Chilton Area Horticulture Substation, Clanton.
8. Forestry Unit, Coosa County.
9. Piedmont Substation, Camp Hill.
10. Plant Breeding Unit, Tallassee.
11. Forestry Unit, Autauga County.
12. Prattville Experiment Field, Prattville.
13. Black Belt Substation, Marion Junction.
14. The Turnipseed-Ikenberry Place, Union Springs.
15. Lower Coastal Plain Substation, Camden.
16. Forestry Unit, Barbour County.
17. Monroeville Experiment Field, Monroeville.
18. Wiregrass Substation, Headland.
19. Brewton Experiment Field, Brewton.
20. Solon Dixon Forestry Education Center, Covington and Escambia counties.
21. Ornamental Horticulture Field Station, Spring Hill.
22. Gulf Coast Substation, Fairhope.