Leaf Scald on Plum Shoots Growing from Disease-free Buds

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ABSTRACT

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Buds free of leaf scald symptoms were grafted on plum trees infected with rickettsialike organisms. Typical scald symptoms developed on plum shoots from surviving buds. Rickettsialike organisms with characteristic rippled cell walls were seen by electron microscopy in xylem extracts and xylem cross sections of petioles from leaf scald-affected leaves. Crushing petioles in 0.1 M KOH and examining the extract under a phase contrast microscope was an effective method for evaluating leaves when a vacuum extraction method could not be used.

The association of plum leaf scald with rickettsialike organisms (RLO) was first reported by Kitajima et al (5) and later by French et al (3), who showed that RLO from cultivated plum trees were homologous, according to indirect immunofluorescence, to RLO associated with phony disease of peach. Twenty-six cultivars or hybrids of plums in Alabama were reported by Kitajima et al (5) and were evaluated for RLO by French's method (2). Leaf scald symptoms were evident on all plants 4 yr or older (6) but not in younger plants. These preliminary reports (2,6) contain the only data on the occurrence of plum leaf scald in the southeastern United States.

Our investigations were made to determine whether shoots arising from disease-free buds would produce scalded leaves, to study symptom development, and to establish the presence of RLO in scalded leaf tissues.

MATERIALS AND METHODS

Plum budwood of 'Bruce' [(*Prunus salicina* × *P. angustifolia*) × (*P. salicina* × *P. munsoniana*)], 'Frontier' (*P. salicina*), 'Methley' (*P. salicina* × *P. cerasifera*), 'Ozark Premier' [(*P. salicina* × (*P. salicina* × *P. cerasifera*)], and 'Santa Rosa' (*P. salicina*) were obtained from P. R. Fridlund, Irrigation Agricultural Research and Extension Center, Prosser, WA.

By a "T" budding procedure, buds from these cultivars were grafted on leaf-scalded 4-yr-old cv. Purple and Homeside plum trees at the Horticulture Farm, Main Station, Auburn, AL, on 28 August 1978. Two buds of a cultivar were grafted on one twig; the other cultivars were budded onto different branches of the same tree. Two Homeside and two Purple trees were budded; one Homeside tree showed no leaf scald.

On 31 August trees at the Wiregrass Substation, Headland, AL, were budded on two trees with leaf scald of each of the following cultivars or hybrids: 5-yr-old Ozark Premier, Ozark Premier F-2, and Purple.

On 1 September leaf-scalded 10-yr-old trees at the Piedmont Substation, Camp Hill, AL, were budded on cv. A.U. Producer. The roots and twigs of these trees had been examined for RLO by French's method (2) for vacuum infiltrating suspected roots and twigs with 0.1 M KOH and examining the extracts under a phase contrast microscope (6). A 2.5-cm portion of each budwood stick from Prosser, WA, also was examined to determine possible RLO contamination. No disease-free trees were available for inclusion in this study.

Shoots that developed from the grafted buds were examined for symptoms at intervals throughout the 1979 growing season. During September, leaves with plum leaf scald were collected from each surviving shoot and examined. Approximately 1.0-cm of leaf petiole was crushed in 0.1 M KOH on a microscope slide and the tissue extract was examined for RLO under phase contrast microscopy (×800). Petioles of four leaves from each bud were examined. Large twigs that developed from the grafted buds were collected and compared with other twigs on the same tree.

Attempts were made to culture RLO on nutrient agar and on JD-1 and JD-3 media (1). Leaf petioles were surface-sterilized in 0.5% sodium hypochlorite for 3 min and washed 10 times in sterile, distilled water. Plant sap was squeezed with sterile forceps from the petioles and blotted onto the medium.

Tissue extracts were prepared for electron microscopy as follows: a drop of RLO suspension was placed on a carbon-stabilized Formvar film on a copper specimen grid; after 1 min the excess extract was removed with a filter paper and a drop of 2.0% potassium phosphotungstate (pH 6.5) was added and removed with filter paper after 30 sec. The grid was dried for 10 min and examined with a Philip's EM-300 electron microscope.

Tissue samples for electron microscopy were from leaves with leaf scald. Small pieces (1 × 2 mm) of petiole and leaf midribs were fixed in a 3% solution of glutaraldehyde. The tissues were washed in cold 0.05 M cacodylate buffer (pH 6.8) and postfixed overnight at 4 C in 2% OsO_4. Subsequently, the tissues were dehydrated in an ethanol series graduating into propylene oxide and embedded in

Fig. 1. Leaf of plum with symptoms of leaf scald. The necrotic bands form as a result of gradual dieback of leaf tissue.

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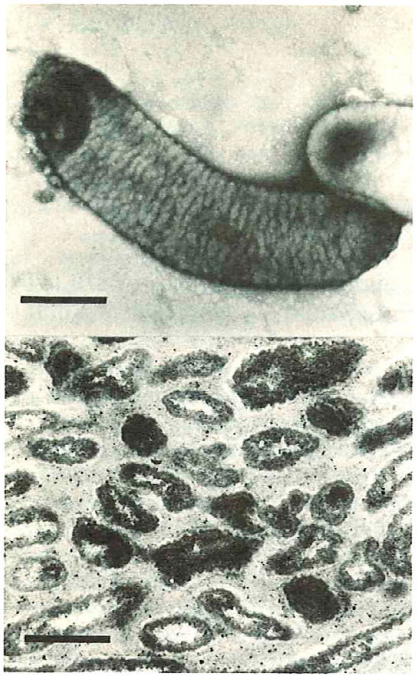

Fig. 2. Electron micrographs of rickettsialike organisms: **(Top)** Negatively stained bacteria extracted from plum twig with 0.1 M KOH. Note rippled cell walls. **(Bottom)** Bacteria in cross section through xylem vessel. Scale bar = 0.5 μm.

Epon. Ultrathin sections were stained with 70% alcoholic uranyl acetate and lead citrate and examined with the electron microscope.

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

RESULTS

Leaf scald has appeared on RLO-infected Japanese plums (6) in mid-June to July. The first symptom is slight chlorosis or bronzing along the margin or tip of a leaf. The discoloration intensifies, sometimes appearing water-soaked before turning brown and drying. The affected area becomes delineated by a chlorotic band. As the die back gradually progresses, several bands may appear in the necrotic tissue (Fig. 1). 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. The banded appearance of the necrotic tissue is especially evident during autumn. In early phases of the disease, leaf scald may occur only on a few twigs or large branches; during the late phase, symptoms may appear on almost all of the foliage. As a consequence of premature defoliation, diseased trees may develop new leaves during September and October; leaf scald also may develop on these malformed, leathery, and rolled leaves.

No RLO were associated with the budwood from Washington State. In March 1979, 58% of the buds of the five cultivars were surviving. Bud survival was 28% on 15 May, 17% on 27 July, and 12% on 12 September. During July, symptoms of scald occurred on leaves arising from the shoots of each of the five plum cultivars at the Wiregrass and Piedmont substations and on some cultivars at the Main Station. Leaf scald symptoms were identical to those on the rest of the tree. Mature scalded leaves showed 50–100 RLO per microscope field in the petiole extracts.

Five RLO were observed in one of four roots from the Homeside tree without symptoms of plum leaf scald. During 1979 typical leaf scald symptoms were evident on one branch on this tree. Counts of 2, 14, 4, and 0 RLO were seen in sections taken at four 15-cm intervals from the base of a branch with symptoms. Approximately 100 RLO per microscope field were seen in 0.1 M KOH extracts of petioles from the leaves with symptoms of plum leaf scald on this branch. No RLO were observed in sections from twigs selected randomly from the rest of the tree.

The amounts of shoot growth produced from Washington State buds on the Homeside tree, by cultivar, were: Bruce, 76.7 cm; Methley, 133.9 cm; Ozark Premier, 162.6 cm; and Santa Rosa, only a rosette of leaves. Symptoms did not occur on shoots of the cultivars Bruce, Methley, or Santa Rosa, and extracts from shoots or petioles showed no RLO. However, approximately 10% of the leaves on the Ozark Premier shoots had leaf scald symptoms; laboratory examination of these shoots showed no RLO in extracts from the twigs, but RLO were found in petiole extracts made from scalded leaves of this cultivar. Leaves with no symptoms of leaf scald taken from the same shoot were negative for RLO.

No bacteria except contaminants were cultured on artificial media.

Rod-shaped organisms extracted from twigs and examined with the electron microscope had a distinctive rippled cell wall (Fig. 2). Electron micrographs of petiole sections showed the characteristic thick, rippled cell walls attributed to RLO in plum tissues (5). No virus or mycoplasmalike organisms were observed in the petiole and midrib cross sections.

DISCUSSION

Microscopic examination of petioles crushed in 0.1 M KOH appears to be the most rapid procedure to determine RLO. This technique may serve as a rapid spot check of scalded leaves to determine RLO incidence and to make certain that the leaf symptoms are not related to root problems or nutritional deficiencies. The crushed tissue debris on the slides may complicate observations unless RLO occur in high numbers. Debris may be less a problem in vacuum extraction of roots and twigs for determining RLO incidence, but it is a slower procedure.

RLO associated with plum leaf scald may be identified by use of the immunofluorescence test (3,4).

The incidence of RLO in the Homeside tree at the Main Station at Auburn apparently was low because RLO were not transmitted to the new shoots that arose from buds of the grafted cultivars Bruce, Methley, and Santa Rosa. French et al (2) indicated that RLO may occur in pockets in peaches affected with phony disease. This situation also may prevail for RLO in plum, thus explaining why some branches may have scalded leaves when others do not.

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