Plum Leaf Scald: Isolation, Culture, and Pathogenicity of the Causal Agent

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ABSTRACT

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A Gram-negative bacterium with rippled cell walls was consistently isolated on a cell-free medium from petiole, stem, and root tissues of plum showing leaf scald symptoms. Similar bacteria were also isolated from peach affected with phony disease, but not from healthy plants. Colonies developed in 8–16 days on BC-YE or BC-ZE medium and reached 0.1 to 0.9 mm in diameter after 3 wk. Bacterial cells measured 0.35 μm in diameter and 2–5 μm in length. One-year-old cultivar Myro plum seedlings inoculated with pure cultures of bacterial inoculum from plum or peach showed typical leaf scald symptoms in 5–6 mo. No symptoms were seen in plum controls. Bacteria isolated from all inoculated plums showing symptoms were morphologically, ultrastructurally, and serologically identical to original isolates from naturally diseased plum. Almond trees inoculated with plum bacteria showed no symptoms. Antiserum to the plum leaf scald bacterium reacted positively with extracts from diseased plum in ELISA and with tissue sections in immunofluorescence. No serological or pathological differences were observed among isolates of plum leaf scald bacteria from Georgia, Florida, and Argentina and the bacterium associated with phony peach disease. The plum leaf scald bacterium in pure culture or in diseased plants could be serologically distinguished from the Pierce's disease bacterium by ELISA and by its lack of pathogenicity on almond.

Additional key words: rickettsialike bacteria, phony peach disease, Pierce's disease of grape, ultrastructure.

Plum leaf scald (PLS) was first reported in 1935 in Argentina (4), and has since been causing severe losses in the Japanese plum (*Prunus saliciana* Lindl.) (17). PLS also affects other plum species (12,14,26). In the United States, PLS was reported in 1977 (6) and is a serious problem on plums in Alabama (14). PLS is also present in Brazil and Paraguay (5).

Xylem-limited rods with rippled cell walls, commonly termed rickettsialike bacteria, were associated first with Pierce's disease (PD) of grapevines (7,10) and later cultured in 1978 (2). Similar bacteria were associated with phony peach (PP) (11,18), periwinkle wilt (15), elm leaf scorch (9), and a ragweed disease (22). Bacteria resembling the PP organism in size and cell wall morphology were observed in xylem of diseased plum from Argentina (12), the United States (14), Brazil, and Paraguay (5). Bacteria have been isolated in vitro from peach and plum (1,25), elm (13), and ragweed (22). An etiological association between PLS and PP has been shown (6,26).

The leaves of Japanese plum affected by PLS first show chlorosis or browning at the tips and margins. The chlorotic areas later become brown and dry, and are separated from the unaffected areas by a light yellow margin. As PLS progresses, several necrotic bands on leaves usually appear. In early infection, only a few branches show symptoms, but later the whole plant is affected and fruit size, quality, and yield are reduced (12,14).

Koch's postulates have not yet been proven with the bacteria associated with PLS and PP disease. We previously reported a medium for the in vitro isolation of bacteria associated with PLS and PP (25). Here we report the culture of bacteria from diseased plum and peach, the reproduction of leaf scald symptoms in plums inoculated with the cultured organisms, the reisolation of the same organism from diseased plums, and the cultural and morphological characters of the bacterium.

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MATERIALS AND METHODS

Plant source. Healthy and diseased plum and peach trees were grown at the Southeastern Fruit and Tree Nut Research Laboratory, U.S. Department of Agriculture, Byron, GA. PLS, PP, and healthy trees were selected on the basis of symptoms, the occurrence of rod-shaped bacteria in root or xylem sap examined by phase-contrast microscopy (24), and by immunofluorescence (24,26). Bacteria were isolated from roots, stems, or petioles of 4-yr-old PP-affected Dixieland peach trees and from 5-yr-old Shiro (an interspecific *Prunus* hybrid) and cultivar Santa Rosa plum trees with PLS. Thereafter, bacteria were isolated from experimentally inoculated seedlings of plum cultivars Methley, Ozark Premier, Santa Rosa, and Shiro.

Isolation medium. The BC-YE agar medium was developed for the in vitro isolation and growth of bacteria from diseased peach and plum (25). After the initial isolation, the bacterial isolates were transferred and maintained on BC-ZE agar medium prepared as follows: 17.0 g Bacto-agar; 10.0 g yeast extract; 1.0 g K_2HPO_4: 1.0 g KH_2PO_4; 0.2 g $MgSO_4$; 2.0 g soluble starch; 4.0 g glucose; 0.25 g ferric pyrophosphate in 10 ml of solution, 0.50 g L-cysteine HCl in 10 ml of solution, and 980 ml distilled water. The pH was adjusted to 6.6 with 1N HCl or NaOH. The ferric pyrophosphate and cysteine stock solutions were prepared separately in 10 ml of distilled water, mixed, passed through a 0.20-μm membrane filter, and added to previously sterilized basal medium before it was poured. BC-ZE broth medium was prepared by omitting the agar.

Isolation procedures. Stems, petioles, or roots with bark removed were cut into 3- to 5-mm pieces, surface sterilized in 1.5% sodium hypochlorite solution, and rinsed in sterile distilled water. Three isolation procedures were evaluated: sap was expressed by using sterile forceps and blotted directly onto the agar medium; plant tissue was aseptically transferred into a sterilized screw-capped centrifuge tube, 5–10 ml of filter-sterilized phosphate-buffered saline (PBS, 0.1 M, 0.85% NaCl, pH 6.6) was added, and contents were centrifuged at 20,000 *g* for 10 min, and 0.5–1.0 ml of buffer with the released bacteria was transferred onto each agar plate; or tissue was ground in 10 ml of filter-sterilized PBS in a

previously sterilized mortar and pestle, the supernatant was collected, and serial twofold dilutions (up to 1:4,096) were made in PBS and transferred to agar plates. Duplicate sets of plates were inoculated by each procedure: one set was incubated in complete darkness and the other in 12–14 hr of darkness per day. All inoculated plates were aerobically incubated at room temperature (25–26 C) and observed 8–14 days after inoculation.

After growth of bacteria was evident, single colonies were individually subcultured on agar media. All isolates were maintained on BC-ZE agar medium and subcultured weekly.

Pathogenicity studies. One-year-old Myro plum seedlings were inoculated with bacterial isolates from plum and peach grown for 8 days on BC-YE or BC-ZE agar medium. Bacterial inoculum was prepared from suspensions made by adding 5 ml of distilled water to each plate. The suspension (about 10^9 cells per milliliter) was mechanically vortexed gently and counted by phase-contrast microscopy. Xylem tissues of groups of four to 10 replicate plum seedlings were individually inoculated with four isolates from plum (PL-7, PL-20, PL-28, and PL-57) and two isolates from peach (PE-5 and PE-11) by needle injection (20). Each isolate originated from a different infected plant. The first injections were made at five different sites from the base of the stem to the tip and these were repeated 3 and 6 wk later. Ten control seedlings were similarly injected with distilled water.

Cultivar Mission almond (*Prunus amygdalus* Batsch) seedlings were similarly inoculated with a bacterial isolate from plum (PL-7) and with three isolates of PD bacteria isolated from diseased grape (*Vitis vinifera* L.), almond, and alfalfa (*Medicago sativa* L.). All inoculated plum and almond seedlings and water-injected control plants were kept in an insect-proof greenhouse and watered every 2–3 days.

Reisolations of bacteria were attempted from plum on BC-YE agar medium and from almond on RG-7 medium (19) 5–6 mo after inoculation. Colonial and cellular morphology of reisolated bacteria grown on plates in air at room temperature were compared with those of the original isolates.

Serology. Bacterial cultures PL-28 and PL-57 from plum and PE-11 from peach were grown in BC-ZE broth medium at 26 C on a gyratory shaker. Cells were harvested after 8–9 days and antigens were prepared as described before (20). Preimmune control serum was collected from female New Zealand white rabbits and immunization was by intravenous and intramuscular injections (20).

Antisera were also produced against Pierce's disease bacteria isolated from grape from California (PD-N5). Antisera titers were tested by slide agglutination test (20). The same test was also used to compare the PLS bacterial isolates from naturally diseased and inoculated Myro plums.

Enzyme-linked immunosorbent assay (ELISA). Gamma-globulin was purified (16) from antisera prepared against a plum isolate, PL-57, and a peach isolate, PE-11, and against a grape isolate of PD bacteria, PD-N5. Enzyme conjugation and test procedures were as previously described (16,19). Plates were read directly in a Titertek Multiskan ELISA reader (Flow Laboratories, Inc., Inglewood, CA 90302) at 405 nm.

Preparation of samples for ELISA. Root, stem, petiole, and leaf tissue samples were prepared by previously described methods (19) from peach and plum naturally infected with PP and PLS, respectively, and from plum inoculated with bacteria. Grape tissues with Pierce's disease, healthy checks, and plum tissue from plants with scorch caused by salt-damage were included.

Also tested were pure cultures of bacterial isolates from plum from Argentina (PL-01) (17) and from the United States (PL-57 and PL-04), one peach isolate (PE-11), two PD isolates (PD-N5 and PD-C8) and isolates of 21 other bacterial species used in previous comparative studies (16). Bacterial cultures were harvested by centrifugation after 8–9 days of growth, washed three times with PBS, and sonicated before use (20).

Immunofluorescence. Immunoglobulins were separated and conjugated from antisera to PL-57 and PD-N5 (23). Tests were conducted by methods described before (24) with field-collected diseased peach and plum, with inoculated Myro plum, and with PD-affected grape. Healthy plant samples and cells from pure cultures of *Agrobacterium tumefaciens, Pseudomonas savastanoi, Pseudomonas syringae,* and *Bacillus subtilis* were included as controls. Treated materials were viewed with a Zeiss GFL microscope equipped with an HB-200W mercury lamp and filters.

Studies of colony morphology. The presence of bacteria isolated from diseased plum on BC-YE agar was confirmed by phase-contrast microscopy, and single-colony subcultures were made one or more times on the same medium or on BC-ZE agar. One isolate from plum (ATCC 33490) and the other from peach (ATCC 33489) were deposited at the American Type Culture Collection.

Colony morphology was studied by light microscopy and scanning electron microscopy (SEM). Bacterial colonies for SEM examination were prepared from 7-day-old cultures grown on BC-ZE agar medium and transferred with a #1 cork borer to 3% glutaraldehyde in 0.06 M phosphate buffer, pH 6.8, for 12 hr. Samples were washed three times with phosphate buffer, dehydrated in a graded acetone series to 90% in 80 min, then transferred twice each to 100% acetone for 20 min, to a 2:1, then a 1:2 acetone:Freon TF (Dupont Chemicals) mixture for 20 min, and finally in 100% Freon TF for 20 min at 0 C. After critical-point drying, specimens were mounted and coated with SEM stubs. Colony morphology was examined with a JEOL JSM35 SEM, then some colonies were ruptured and individual cells were examined.

Morphology and ultrastructure. Leaves from inoculated plums showing symptoms and from controls were collected, fixed, and processed as described before (26). Ultrathin sections were observed with a transmission electron microscope (model AEI EM6B at 60 kV).

A drop of BC-ZE medium containing plum bacteria (PL-06) from a 4-day-old subculture was mixed with a drop of 2% potassium phosphotungstate in phosphate buffer (pH 7.0) on a Parafilm® sheet and a collodion-coated grid was placed in contact with the mixture for a few seconds. Excess fluid was removed with a piece of filter paper and grids were examined immediately after drying.

Intact colonies of plum bacteria isolated from inoculated Myro plum and grown on BC-ZE agar medium were embedded in 1% sterilized Noble agar (Difco) and processed (25). Ultrathin sections were cut, stained with uranyl acetate and lead citrate, then examined (25).

RESULTS

Isolation in culture. Rod-shaped aflagellate and nonspore-forming bacteria 0.35 μm in diameter and of variable length (2–5 μm) were consistently isolated on BC-YE agar medium from diseased plum and peach, but not from healthy tissue (Table 1). Colonies, whether from peach or plum, were small, white, blue, or greenish white and were observed at ×25 with a dissecting

TABLE 1. Isolation of bacteria on BC-YE medium from leaf scald-affected plum (*Prunus saliciana*) and phony-affected peach (*Prunus persica*)

Plant material	Trees tested (no.)	Type of tissue	Attempts (no.)	Isolations (no.)	Successful isolations (%)
Plum					
Healthy	5	Root	33	0	0
		Stem	28	0	0
		Petiole	25	0	0
Diseased	12	Root	42	32	76
		Stem	60	51	84
		Petiole	48	46	96
Peach					
Healthy	5	Root	26	0	0
		Stem	21	0	0
		Petiole	18	0	0
Diseased	7	Root	42	30	71
		Stem	41	8	20
		Petiole	16	0	0

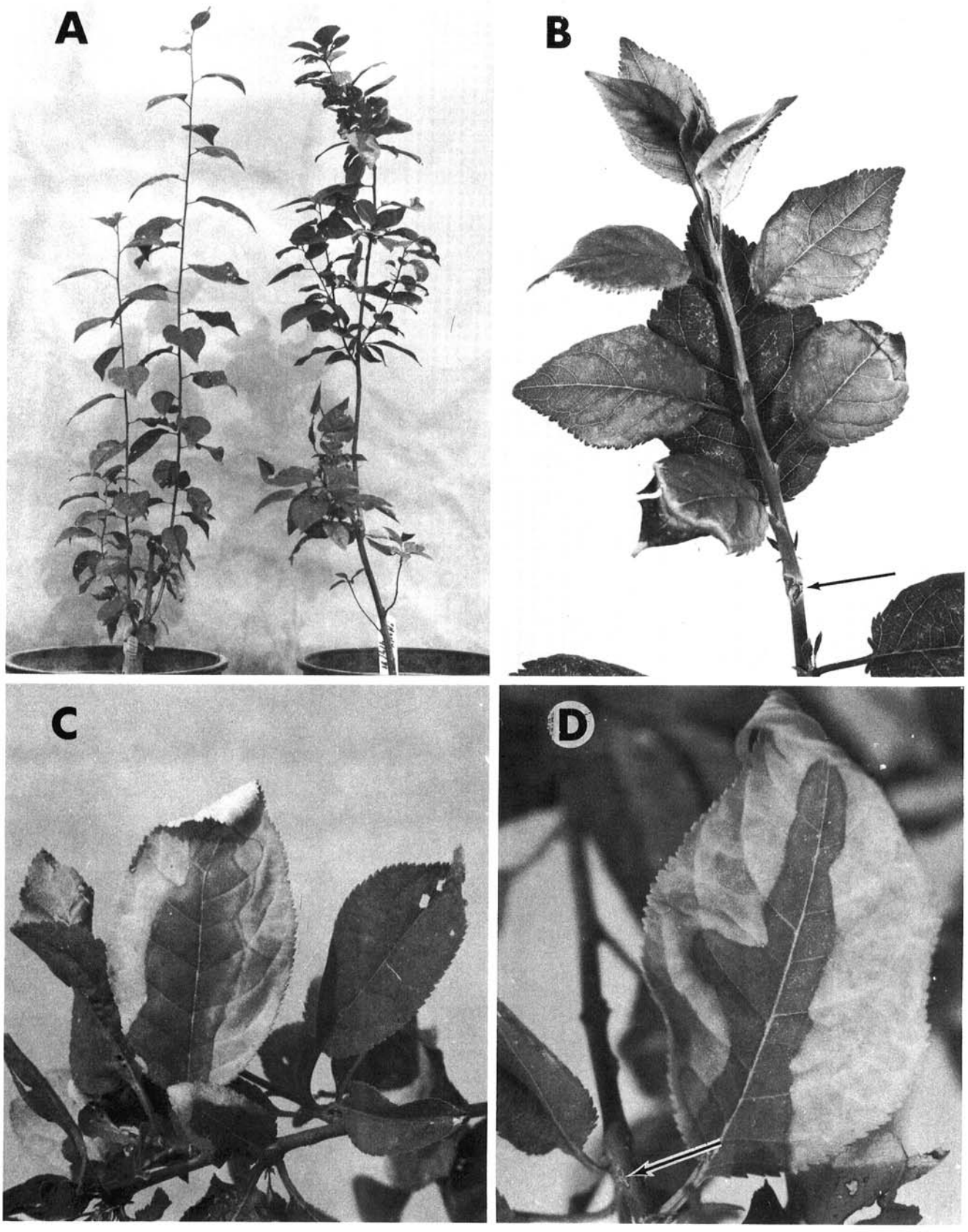

Fig. 1. Symptoms of plum leaf scald (PLS) on cultivar Myro plum seedlings 6 mo after inoculation by needle injection with a pure culture of bacteria isolated from naturally diseased plum. **A,** Plant (left) inoculated with water (check) and plant (right) showing early symptoms of rosetting. **B,** Marginal and tip necrosis just above the inoculation point (arrow) on a young shoot. **C** and **D,** Leaf scalding typical of PLS disease. Arrow indicates the point of inoculation in D.

microscope 8–10 days postinoculation. Bacteria were isolated from roots, stems, and petioles of naturally diseased plum trees but only from roots and stems of peach (Table 1). None were isolated from healthy checks or from tissues scalded by drought or salt injury.

Bacteria were successfully isolated from diseased plum and peach by each of the three isolation procedures tested. Isolations from expressed sap were most useful with petioles, small stems, and roots, whereas extraction by centrifugation was useful with larger stems and roots. We were able to obtain bacterial lawns in the primary isolation with the centrifugation procedure. The average colony diameter in plates incubated in darkness after 10 days was 0.6 mm compared to 0.4 mm in plates incubated in light. On some occasions no bacterial growth was seen in plates incubated in light, whereas colonies developed from the same material in darkness. We were unable to isolate bacteria from roots or stems of cherry graft-inoculated with tissue from PP-affected peach despite high concentrations of bacteria in the tissues.

Pathogenicity studies and reisolation. Twenty of the 24 Myro plums inoculated by needle injection showed PLS symptoms (Fig. 1) and stunting in 5–6 mo (Table 2). Eight of 10 plums inoculated with peach isolates showed leaf scald symptoms in about 6 mo (Table 2). None of the controls developed leaf scald. Leaf scald symptoms observed on inoculated plums were identical to those on naturally infected plants, starting at leaf tips and progressing downward. Average height of control plants was 68 cm, and that of inoculated plants was 46 cm. Bacteria were observed in tissues of inoculated plum by immunofluorescence 4 mo after the initial inoculation, or 1–2 mo prior to symptom appearance.

Bacteria were readily reisolated on BC-YE agar medium from plum showing leaf-scald symptoms (Table 2), but not from control plants. Colonies indistinguishable from the original isolates were visible 10–12 days after plate inoculation. Rod-shaped bacteria of variable lengths were observed in water-mounted specimens by phase-contrast microscopy. Bacteria were Gram-negative, catalase-positive, and identical in shape, size, and serological properties to those originally isolated from naturally infected plum.

Almonds inoculated with PLS bacteria developed no symptoms; however, 12 of the 18 almonds inoculated with PD bacteria showed typical symptoms of almond leaf scorch. Rods identical to the PD bacterium were isolated from symptomatic almonds, but none from checks or from asymptomatic almonds.

Serology. Antisera prepared against PLS and PD bacteria had homologous titers of 2,048–4,096 in slide agglutination tests. Bacteria isolated from naturally infected plums (isolates PL-7, PL-20, PL-28, and PL-57) and from inoculated Myro plums (isolates PL-06, PL-07, and PL-09) reacted identically against PLS bacterial antiserum.

Bacteria cultured from scorch-diseased tissue reacted positively with homologous antiserum in ELISA using 10^{10} cells per milliliter, 2.0 mg of purified coating gamma-globulin per milliliter, and a 1/1,000 dilution of conjugate. Root, stem, and petiole extracts of naturally infected plum reacted positively with PLS and PD bacterial antisera (Table 3). The $A_{405\ nm}$ values ranged from 0.092 to 1.240 and were always higher than those obtained with PD bacterial antiserum or with healthy plant material (Table 3). When concentrations of bacteria (and $A_{405\ nm}$ values) in plums were low, positive reactions were obtained only with PLS bacterial antiserum.

Positive ELISA reactions were also observed with inoculated and symptomatic Myro plums, and with root and stem extracts of phony-affected peach using antisera prepared against PLS and PD bacteria (Table 3). Negative tests were obtained with extracts from petioles of diseased peach, with healthy plum and peach control, and with plums showing leaf scorch symptoms due to salt damage.

Stem extracts of diseased grape stem reacted positively in ELISA with antisera prepared against PD and PLS bacteria. The $A_{405\ nm}$ values of homologous antiserum were always higher than the values obtained with heterologous antiserum (Table 3). Healthy grape tissues produced no color reaction.

Positive reactions in ELISA were obtained with three isolates of PLS bacteria in culture from Georgia, Florida, and Argentina by using antisera prepared against PLS and PD bacteria (Table 4). No major difference in $A_{405\ nm}$ values were observed among the PLS

TABLE 2. Reisolation of bacteria from 1-yr-old cultivar Myro plums inoculated with pure cultures of bacteria originally isolated from diseased peach and plum trees

Isolate[a]	Inoculum source[b]	Plants inoculated (no.)	Plants with symptoms[c] (no.)	Plants with positive reisolation[d] (no.)
PL-7	Plum	5	5	5
PL-20	Plum	4	4	4
PL-28	Plum	5	3	3
PL-57	Plum	10	8	8
PE-5	Peach	5	3	3
PE-11	Peach	5	5	5

[a] Each isolate used as inoculum was subcultured five or six times from a single colony.
[b] Plum and peach were naturally affected by leaf scald and phony disease, respectively.
[c] Symptoms consisted of leaf scalding and stunting.
[d] Bacteria were isolated on BC-YE agar medium using petioles or stems.

TABLE 3. Absorbance ($A_{405\ nm}$) from enzyme-linked immunosorbent assays on extracts from diseased and healthy plum, peach, and grape with antiserum to plum leaf scald and Pierce's disease bacteria

Plant material	Plants tested (no.)	Type of tissue	Average $A_{405\ nm}$[a] PLSB[b]	PDB[c]
Healthy plum	5	Root	0.010	0.014
		Stem	0.006	0.009
Naturally diseased plum	12	Root	0.744	0.280
		Stem	0.504	0.201
Experimentally diseased plum	14	Stem	0.560	0.207
Healthy peach	5	Root	0.013	0.016
		Stem	0.011	0.014
Naturally diseased peach	6	Root	0.602	0.247
		Stem	0.301	0.099
Healthy grape	7	Stem	0.008	0.015
Naturally diseased grape	10	Stem	0.246	0.804
Phosphate-buffered saline check	–	–	0.012	0.010

[a] Values represent three replicated, composite samples per test. Values for diseased tissues are significantly different ($P = 0.01$) from those for healthy tissues.
[b] Antiserum prepared against plum leaf scald bacteria.
[c] Antiserum prepared against Pierce's disease bacteria.

TABLE 4. Serological cross reactions of various isolates of plum leaf scald bacteria (PLSB) with other xylem-limited bacteria from various sources

Strain[a]	Source Host[b]	Area	Average $A_{405\ nm}$[c] PLSB[d]	PDB[e]
PL-57	Plum	Georgia	1.148	0.412
PL-04	Plum	Florida	1.156	0.400
PL-01	Plum	Argentina	1.150	0.429
PL-06	Plum	Georgia	1.140	0.401
PE-11	Peach	Georgia	1.164	0.414
PD-N-5	Grape	California	0.364	1.586
PD-C-8	Almond	California	0.389	1.570
Phosphate-buffered saline check	–	–	0.004	0.007

[a] Cell preparations adjusted to 10^{10} cells per milliliter. Cells were washed three times in buffer before use.
[b] All naturally infected hosts except for the plum source of PL-06, which was artificially inoculated.
[c] Averages of three composite replicates per test. Values among replicates not significantly different ($P = 0.01$).
[d] Antiserum prepared against plum leaf scald bacterium.
[e] Antiserum prepared against Pierce's disease bacterium.

bacterial isolates. PLS bacteria isolated from inoculated Myro plum (isolate PL-06) reacted the same as the isolates from naturally infected plum (Table 4). Positive reactions were also observed with PP-associated and PD bacteria from grape and almond. The $A_{405\ nm}$ values in homologous tests with PLS bacteria were higher than those obtained with PD bacterial antiserum (Table 4). Similar results were obtained with the peach isolate.

No color reaction in ELISA was observed with any of the 21 other test bacteria in assays utilizing antiserum prepared against the PLS bacterium.

A strong fluorescence reaction was observed with PLS bacterial antiserum and diseased plum, peach, and grape root, and with pure cultures of PLS, PD, and PP-associated bacteria. Similar reactions were observed with PD bacterial antiserum. There was no difference in the fluorescence reaction between original PLS bacterial isolates and those isolated from inoculated Myro plum. No reactions were observed with any other bacteria or healthy controls.

Colony morphology. Colonies were generally circular, domed, or raised, and others appressed to the agar surface. Circular colonies with convoluted margins, and tubular or ovoid colonies with smooth margins were observed by scanning electron microscopy (Fig. 2). Individual bacteria could be observed on the surface and interior of colonies (Figs. 2B and D).

Cellular morphology and ultrastructure. Bacteria with rippled cell walls were present in the xylem tissues of Myro plum trees inoculated with pure cultures of bacteria and showing leaf scald symptoms, but not in healthy plants. Bacteria were identical to

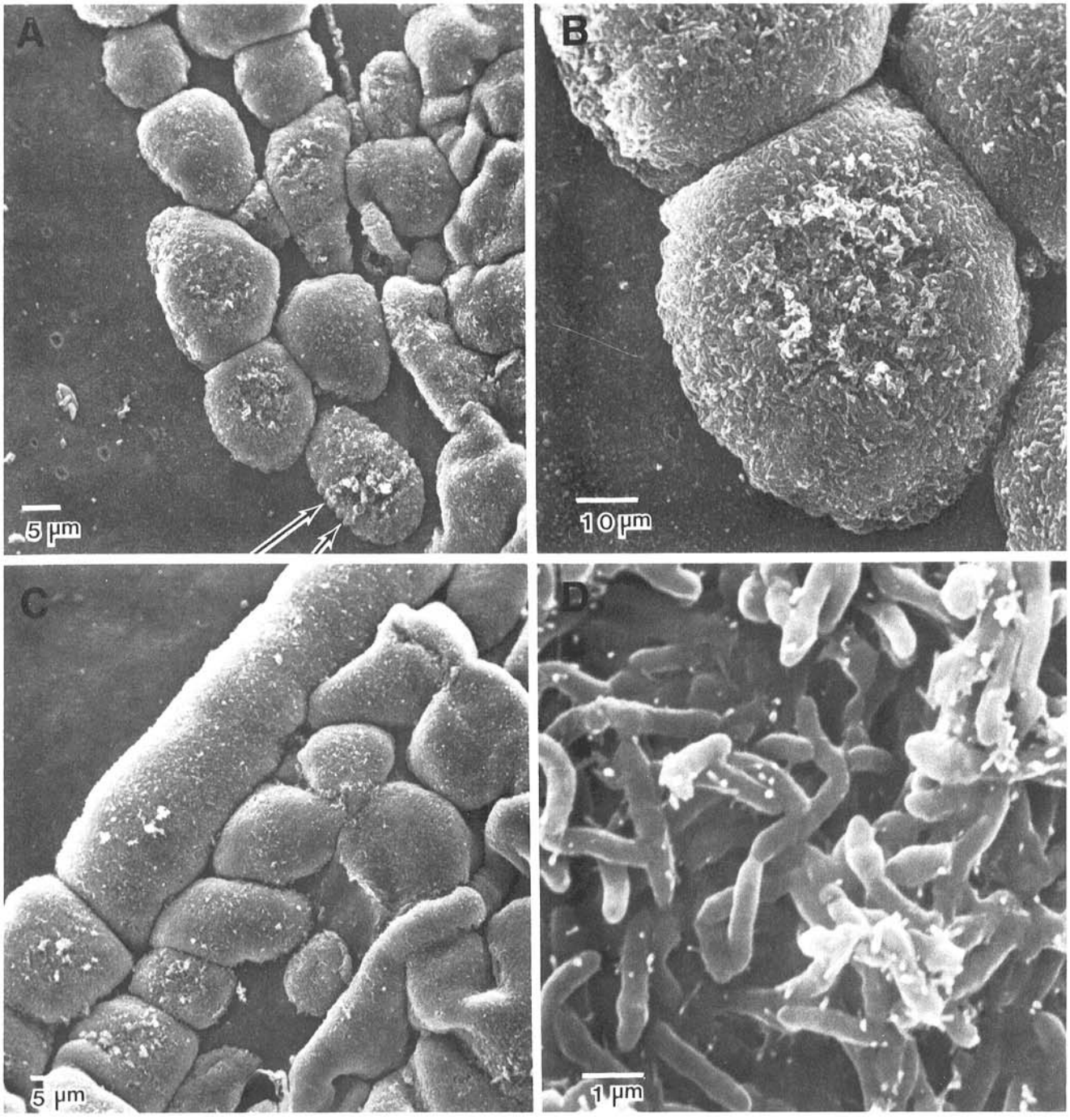

Fig. 2. Scanning electron microscopy of the plum leaf scald bacterium grown on BC-ZE agar medium. **A,** Round to ovoid colonies showing rough edges (arrows) (×300). **B,** High magnification of some colonies from A (×1,000). **C,** Longer, slender, smooth-edged colony mixed with rough-edged round colonies (×300). **D,** Presence of bacteria in colonies (×10,000).

those previously reported in diseased plum (26).

In negatively stained preparations and in thin sections, the PLS bacteria isolated from inoculated Myro plum were rod-shaped cells with rounded ends or with one tapered end (Fig. 3). Average cellular dimensions were 0.35 μm in diameter and 2 μm in length. Some cells were as long as 5 μm.

Negatively stained PLS bacteria revealed numerous irregular ridges or folds on the surface of the cell wall. Fimbrialike fibrils occurred peritrichously, but were more prominent at the polar regions (Fig. 3A and B). Cross-sectional views of PLS bacteria were spherical or ovoid (Fig. 3C). Wall profiles consisted of an outer rippled membrane, an intermediate electron-dense peptidoglycan (or R) layer, and an inner, smooth cytoplasmic membrane (CM) (Fig. 3D). Ribosomes, vesicles, mesosomes, filaments, and DNA-like granules were evident in the cytoplasm enclosed by the CM.

DISCUSSION

Bacteria were consistently isolated in culture from plums with symptoms of PLS from Georgia, Florida, and Argentina. These bacteria were morphologically, ultrastructurally, and serologically identical to those present in the xylem of diseased plants. Healthy plums inoculated with the cultured organisms developed typical leaf scald symptoms. Bacteria indistinguishable by the same criteria from the original isolates were reisolated from these plants, thus establishing their pathogenicity.

The PLS bacterium could not be isolated or subcultured on media suitable for the PD bacterium (2). The PD bacterium, however, grew well on BC-YE and BC-ZE media, as did bacteria isolated from elm leaves affected with scorch (13), from wilt-affected periwinkle (*unpublished*), and from ragweed (22). Of the xylem-limited bacteria recently isolated, the PD bacterium appears to be the least fastidious since the bacteria from periwinkle and ragweed cannot be isolated or grown on PD bacterial media. The nutritional requirements, therefore, of PLS and PD bacteria are different even though both are xylem-limited pathogens transmitted by sharpshooter leafhoppers.

The PD, but not the PLS, bacterium produced leaf scorch in inoculated almond, and could be reisolated from symptomatic almond. The PLS bacteria are thus physiologically and pathologically different from the PD bacteria. Further studies are in progress on the pathological relationships of the bacteria of PD, PLS, and those associated with ragweed, periwinkle, and elm.

In the southeastern United States, PD, PLS, and PP are prevalent, whereas in California only PD in grape, almond, and alfalfa is present. Sharpshooter leafhoppers, however, are present in both areas. In the southern United States, both PLS and PP are present and cause considerable damage in plum and peach (4,14,26), but no PP has been reported from South America although peach orchards are adjacent to diseased plum orchards and the PP bacterium is capable of causing leaf scald in plum (6,26). Similar observations have been made with PD and almond leaf scorch. The PD bacterium causes PD in grape and leaf scorch in almond (3), but in some field situations in California, no PD occurs in grapes growing next to severely diseased almond and vice versa. Vector preferences may account for this situation.

One-year-old Myro plum seedlings kept under water stress showed excellent leaf scald symptoms in 5–6 mo. Two inoculated plants not subjected to water stress showed only mild symptoms after 8 mo, suggesting that water stress enhances leaf scald symptoms in plum.

A bacterium isolated from phony-diseased peach produced leaf scalding in plum, indicating that PLS and the bacterium associated with PP are pathogenically similar (6,26) in addition to being

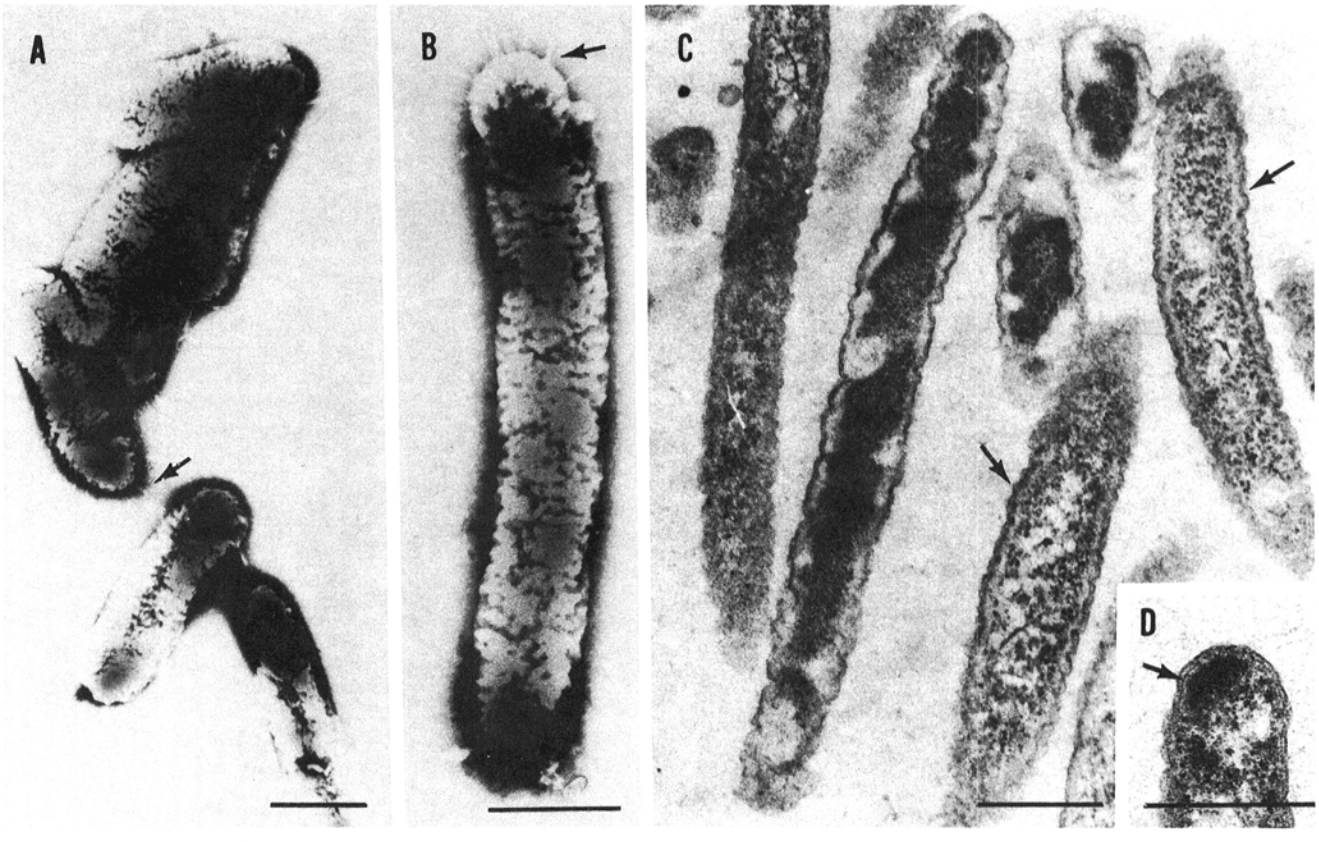

Fig. 3. Electron micrographs of plum leaf scald (PLS) bacteria obtained from BC-ZE agar medium. **A,** Negatively stained preparation with rod-shaped cells possessing numerous minute, irregular ridges or folds and fimbriae (arrow), mostly polar, on the surface of the cell wall. Bar = 0.5 μm. **B,** A typical organism with a dimension of 0.35 × 2 μm. Bar = 0.5 μm. **C,** A group of organisms in longitudinal view of thin section display the characteristic rippled cell wall (arrows). The rippled appearance on the wall is produced by the surface ridges when sectioned. Note also the DNA-like fibrils interspersed in the ribosome-rich cytoplasm of some cells. **D,** A portion of a bacterial cell in the polar region reveals a cell wall profile in which the peptidoglycan or R layer is seen as electron-dense layer (arrow) between the outer membrane and the cytoplasm membrane.

serologically similar (Table 4). However, confirmation of the reciprocal pathogenicity awaits the results of cross-infection tests now in progress. PP symptoms can be seen in peach 2 yr after inoculation, but the PP organism produces no leaf scalding symptoms in peach in nature.

Immunofluorescence was not useful in making distinctions among the PLS, PD, and PP bacteria. However, by using homologous and heterologous antisera with ELISA, we were able to separate PLS bacteria from PD bacteria. Among themselves, the PLS bacterial isolates were indistinguishable in ELISA. Pathogenicity studies showed the same relationships, indicating that PLS in South America and the United States is caused by the same organism. The PLS bacterial antiserum in ELISA was highly specific and reacted with the xylem-limited bacteria, but not with other bacteria. By using ELISA, it was also possible to distinguish PLS-affected plant materials from PD-affected tissues (Table 3). Our preliminary data (*unpublished*) indicate that ELISA also can be used to screen sharpshooter populations for the presence of PLS bacteria. This test also is useful for finding alternate or reservoir hosts of PLS and PP. With ELISA, we were able to discover several important wild hosts of PD in California (16,21), to detect PD bacteria in insects, and to study the relative sensitivity of various grape cultivars (8,21).

The rippled wall of PLS bacteria is a common characteristic among the xylem-limited, leafhopper-transmitted bacteria and is attributable to the presence of surface ridges on the cell wall of the organism when sectioned. The ridges or folds reported here also have been previously reported in PD bacteria (7,10), in PP-associated bacteria (11,18,26), and in the PLS pathogen (12,14). The fimbrialike fibriles (Fig. 3B) in the PLS bacteria were similar to those previously reported (5).

Our serological studies, cellular fatty acid profiles, and data on GC composition of the DNA (*unpublished*) indicate no similarities between either the PD or PLS bacteria and the true rickettsia. Therefore, we propose adoption of the descriptive term "xylem-limited bacteria (XLB)" instead of the term "rickettsialike" to describe these bacteria until their taxonomy is further clarified.

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