

Canker Development on Plum Shoots Following Systemic Movement of *Xanthomonas campestris* pv. *pruni* from Inoculated Leaves

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ABSTRACT

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Leaves on Golden King plum trees in a greenhouse were inoculated with *Xanthomonas campestris* pv. *pruni* to determine if systemic movement of the pathogen leads to the development of summer and spring cankers. Summer cankers occurred only if the pathogen had been introduced into petioles and main veins within 5 wk of shoot emergence. Application of inoculum by spraying into intercellular spaces of unwounded leaf surfaces resulted in spring cankers during budbreak on trees previously chilled to dormancy at 4 C in the dark. Both types of cankers are apparently associated with systemic movement of the pathogen from infected leaf and shoot tissues.

Additional key words: bacterial spot, *Prunus salicina*

Spring and summer canker symptoms of bacterial spot disease caused by *Xanthomonas campestris* pv. *pruni* (Smith) Dye on peach (*Prunus persica* (L.) Batsch) trees are distinctly different (12). Spring cankers develop after budbreak on shoots of the previous season's growth, whereas summer

cankers appear on new green shoots, usually as a result of infection of foliage. Both types of cankers occur on plum (*P. salicina* L.), apricot (*P. armeniaca* L.), and peach trees in South Africa (H. J. du Plessis, *personal observations*). Fresh leaf scars associated with storm damage to trees in the fall provide sites for the infection courts that lead to spring canker development (4,5,7). Wind-driven rainfall appears to disperse bacteria from lesions on leaves and summer cankers to the leaf scars; however, the infection courts

and origin of inoculum leading to summer cankers seem to be unknown.

In a scanning electron microscopic study, *X. c.* pv. *pruni* entered plum shoots systemically from lesions on leaves (2). Further systemic movement was confirmed by the recovery of a rifampicin-resistant strain of the pathogen from symptomless petiole and shoot tissue as far as 13 cm from the site of inoculated leaves (3). Moreover, disease symptoms were observed on main and secondary veins of uninoculated leaves. However, cankers failed to develop on trees grown for 3 mo in a greenhouse, despite the high numbers of *X. c.* pv. *pruni* in shoots.

The purpose of this study was to determine if cankers would develop on Golden King plum trees after systemic movement of *X. c.* pv. *pruni* from inoculated leaves.

MATERIALS AND METHODS

Trees. Two-year-old plum trees (Golden King budded on Marianna rootstocks) in a nursery were pruned, removed from the soil, stored in plastic

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bags at 4 C for 3 mo, and then planted in 20-cm-diameter pots containing a mixture of equal volumes peat, loam, and sand. The potted trees were grown in a greenhouse at 27 ± 3 C and were given weekly applications of balanced nutrient solution (6). Each tree was allowed to develop six new shoots. Other shoots were removed.

Inoculum. *X. c. pv. pruni* strain 6 (2) was grown on Difco nutrient agar plates at 27 C for 48 hr. Suspensions of the culture, about 1×10^8 , 1×10^7 , or 5×10^4 colony-forming units (cfu) per milliliter, were prepared in sterile distilled water (SDW). The suspensions were used within 1 hr after preparation.

Inoculation of petioles and veins. Leaves on trees were inoculated in the greenhouse at 27 C. A complete randomized design with a fractional $3 \times 2 \times 3$ factorial arrangement of treatments was used. The factors were inoculation sites (petioles, main veins, or secondary veins), shoot age (4–5 or 7–8 wk after shoot emergence), and inoculum suspensions (1×10^8 , 1×10^7 , or 5×10^4 cfu/ml), respectively. However, main veins received only one inoculum concentration (1×10^7 cfu/ml). The first 14 fully expanded leaves from the tips of three of the shoots chosen at random on each of 10 trees were used per inoculation site or inoculum suspension. A 5- μ l sample of inoculum was applied to the abaxial side of the leaves at a point halfway along the length of a petiole, main vein, or secondary vein. A 26-gauge needle was used to puncture through the droplet into the vascular system. Control leaves on the remaining three shoots on each tree were treated with SDW. Trees were grown in the greenhouse at 27 C and inspected regularly for canker symptoms 2–8 wk after inoculation.

Inoculation of unwounded leaves. Ninety trees with six 4- to 5-wk-old shoots (25–30 cm long) were transferred to a controlled environment (27 C, relative humidity 95–100%) 6 hr before inoculation. Three well-developed leaves located near the tip, midway, and base of three randomly chosen shoots on each tree were tagged for inoculation. Leaves were inoculated by spraying inoculum (containing 5×10^4 cfu/ml) onto one-third (from the tip) of the abaxial surface with a spray gun connected to a compressed air supply (1.7 kg/cm²) until the underlying tissue became uniformly water-soaked. Corresponding control leaves on the other three shoots were not treated. Trees were returned to the greenhouse 1 hr after inoculation. Two weeks after inoculation, samples of 30 trees in a complete randomized design were subjected to one of the following environmental conditions: 27 ± 3 C for 5 mo in the greenhouse, 15 C for 4 mo in a growth room timed to a 16-hr photoperiod followed by 4 C for 3 mo in the dark, and 4 C for 3 mo in the dark. Trees were

examined monthly for symptom expression. Trees held at 4 C were returned to the greenhouse (27 C) and kept for 6 wk before final inspection.

RESULTS

Lesions typical of bacterial spot disease developed after 2–4 wk on veins of leaves inoculated by puncture. No symptoms appeared on leaves of control shoots. Cankers were observed on younger shoots (4–5 wk old) on trees grown at 27 C within 4 wk. These symptoms were observed at adjacent nodes of leaves on trees that had been inoculated in petioles or main veins. Water-soaked areas appeared immediately below the junction of petioles with shoots. Three weeks later, these lesions had the same appearance as summer cankers on green shoots in an infected plum orchard.

Regression analyses of mean percentages of summer cankers recorded at each of 14 successive nodes for each inoculum concentration (1×10^8 , 1×10^7 , or 5×10^4 cfu/ml) or inoculation site (i.e., petiole or main vein) are shown in Figure 1. In all instances, canker development declined significantly from the first (near the tip) to the last node (regression coefficients $\neq 0$; $P < 0.05$); however, slopes of regression lines did not differ significantly from each other ($F_{3,48} = 1.149$, $P > 0.10$). The different inoculum concentrations and inoculation sites had a highly significant effect (F intercepts_{3,51} = 183.016, $P < 0.001$) on the incidence of cankers. Cankers were not detected on control shoots inoculated with SDW.

Cankers failed to develop after introduction of inoculum in secondary veins. Furthermore, on older shoots (7–8 wk old), cankers did not appear, even if inoculum had been introduced into petioles or veins.

Spray-inoculated, unwounded leaves developed lesions of bacterial spot disease after 7–14 days. The number of lesions per leaf ranged between 150 and 500. After 2 mo at 27 C, lesions were occasionally seen on veins of uninoculated leaves, apparently because of systemic movement of the pathogen (3); however, summer canker symptoms were not observed on trees held at 27, 15, or 4 C. Trees became dormant at 4 C, and all leaves were shed within 3 mo.

Cankers, typical of spring canker symptoms of bacterial spot disease, developed within 4 wk after trees had been transferred from cold storage to the greenhouse at 27 C. Percentages of cankers recorded at distal ends, midway, and proximal ends of shoots were 0, 2.2, and 6.7% ($\chi^2_{2df} = 7.214$; $P < 0.05$), respectively, for trees treated at 15 and 4 C, and 9.5, 19.1, and 13.1% ($\chi^2_{2df} = 3.52$; $P > 0.05$), respectively, for trees chilled at 4 C. Most (78%) of the cankers seen developed at nodes of inoculated leaves. The remaining cankers (22%) developed one to two node positions away from inoculated leaf positions in both directions. No cankers were observed on control shoots.

DISCUSSION

Higher pathogen populations introduced in petioles rather than main veins

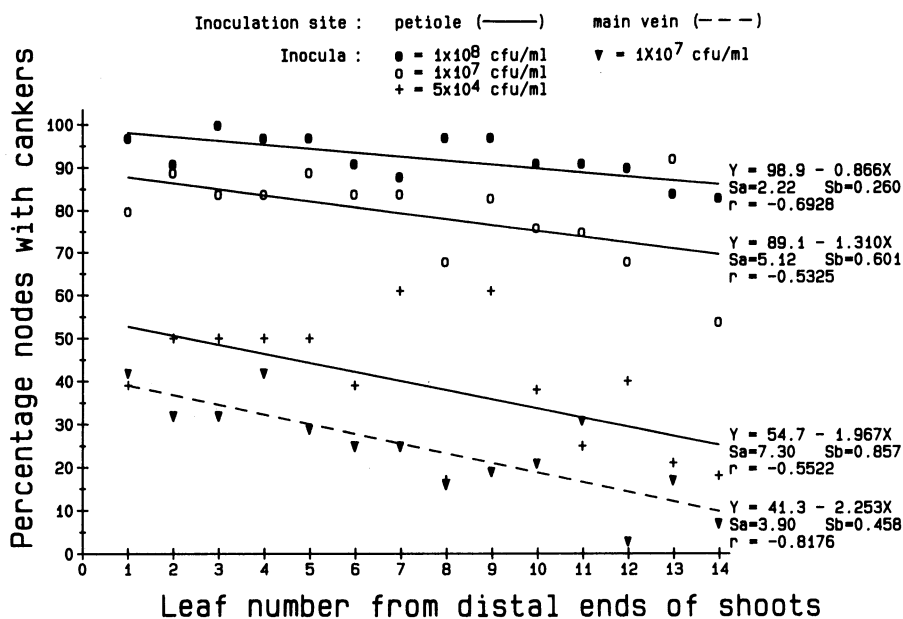


Fig. 1. Regression analyses of summer canker development at nodes of Golden King plum inoculated with *Xanthomonas campestris* pv. *pruni*. Inoculum (5- μ l sample) was applied on the abaxial side of a leaf at a point halfway along the length of a petiole or main vein. A 26-gauge needle was used to puncture the droplet into the vascular system. Correlation coefficients (r) of all four lines were significantly different at $P < 0.05$. Standard deviations of intercepts (Sa) and slopes (Sb) are indicated.

of young shoots (4–5 wk old) obviously favored effective entry and subsequent summer canker development in shoots. Even low pathogen populations (about 250 cfu) applied to petioles, caused reasonably high percentages (29–54%) of cankers at nodes. In contrast, older shoots (7–8 wk old) were immune to summer canker development. Progressive resistance of nodes away from distal ends of shoots (Fig. 1) demonstrates the increased restraining effect of older tissue on summer canker development. Resistance of rice and citrus leaf tissue to *Xanthomonas* also increases as leaves mature (8,11).

If wounding of plum petioles or main veins during wind-driven rainstorms exposes vessel elements (2), the chances of summer canker development might increase. Leaf injury is also an important predisposing factor favoring shoot infection by other bacterial plant pathogens of deciduous fruit trees including plum (1,9,10). In infected orchards, however, summer cankers are frequently detected at nodes and internodes adjacent to visually unwounded leaves (H. J. du Plessis, *personal observations*). I suspect that *X. c. pv. pruni* may move systemically from old tissues into new shoots.

Budbreak evidently predisposes plum

shoot tissues colonized by *X. c. pv. pruni* to develop spring cankers. Development of 22% of the spring cankers one to two nodes away from inoculated leaf positions in both directions confirmed the systemic movement of the pathogen in shoots (3). Cankers developed equally well at the tip or base of shoots on trees chilled only at 4 C. Similar results were reported by Gasperini et al (5). In contrast, peach leaf scars inoculated at the distal end of shoots develop many more spring cankers than those proximal (4).

Both summer and spring cankers on plum are due to systemic movement of *X. c. pv. pruni* from infected leaf and shoot tissues. The importance of leaf scar infection of plum (5) in South Africa remains in doubt. Wind-driven rains that dislodge leaves are rare in the local deciduous fruit-growing areas.

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