

Effect of leaf scar age, chilling and freezing-thawing on infection of *Pseudomonas syringae* pv. *syringae* through leaf scars and lenticels in stone fruits

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Effect of leaf scar age, chilling and freezing-thawing on infection of *Pseudomonas syringae* pv. *syringae* through leaf scars and lenticels in stone fruits.

Abstract – Introduction. Bacterial canker, caused by *P. syringae* pv. *syringae*, is an important disease of stone fruit worldwide. The possibility of *P. syringae* pv. *syringae* infection through leaf scars and lenticels was evaluated in cherry, peach and prune. **Materials and methods.** Laboratory and field inoculations were performed using cherry, peach and prune stems to evaluate leaf scar age, chilling and freezing-thawing on bacterial infection through leaf scars and lenticels. **Results and discussion.** Increasing leaf scar age was associated with significant decreases in disease incidence and length of lesions resulting from leaf scar inoculation with *Pseudomonas syringae* pv. *syringae* in cherry, peach and prune. A significant reduction in incidence and lesion length was observed after 4 h of air exposure, and both measures of infection were reduced to essentially 0 by 2 days of exposure. Prolonged chilling temperature (2.2 °C) prior to leaf removal had no clear effect on disease incidence of leaf scar infection, but significantly decreased lesion length due to leaf scar infection. Cherry was more susceptible to *P. syringae* pv. *syringae* infection through leaf scars than peach and 'French' prune. The leaf scar inoculation results were consistent with the previous studies. The disease incidence of lenticel infection caused by bacterial inoculation in 'French' prune was very low, but significantly higher than the water control. Freezing-thawing significantly increased both the disease incidence and the lesion size via lenticel infection. The lenticel inoculation data suggest that *P. syringae* pv. *syringae* infection through lenticels is possible under field conditions.

USA / *Prunus persica* / *Prunus avium* / *Prunus domestica* / plant diseases / cankers / *Pseudomonas syringae* / experimental infection / lenticels / lesions

Effet de l'âge de la cicatrice foliaire, de la réfrigération et de la congélation-décongélation sur l'infection des cicatrices foliaires et des lenticelles par *Pseudomonas syringae* pv. *syringae* chez les arbres fruitiers à noyau.

Résumé – Introduction. Dans le monde entier, le chancre bactérien causé par *P. syringae* pv. *syringae* est une maladie importante des fruitiers à noyaux. La possibilité de l'infection des cicatrices foliaires et des lenticelles par *P. syringae* pv. *syringae* a été évaluée chez le cerisier, le pêcher et le prunier. **Matériel et méthodes.** Des inoculations ont été effectuées en laboratoire et sur le terrain en utilisant des tiges de cerisier, pêcher et prunier pour tester l'effet de l'âge de la cicatrice foliaire, de la réfrigération et de la congélation-décongélation sur l'infection bactérienne au travers des cicatrices foliaires et des lenticelles. **Résultats et discussion.** L'âge de la cicatrice foliaire a été associée à une diminution significative de l'incidence de la maladie et de la longueur des lésions résultant de l'inoculation de cicatrices foliaires par *P. syringae* pv. *syringae* chez le cerisier, le pêcher et le prunier. Une réduction significative de l'incidence et de la longueur des lésions a été observée après 4 h d'exposition à l'air, et ces deux mesures de l'infection ont été réduites à pratiquement zéro après 2 jours d'exposition. Une réfrigération prolongée (2,2 °C) avant l'effeuillage n'a pas eu d'effet clair sur l'incidence de la maladie sur les cicatrices foliaires, mais elle a significativement diminué la longueur des lésions dues à l'infection. Le cerisier a été plus sensible à l'infection des cicatrices foliaires par *P. syringae* pv. *syringae* que le pêcher et le prunier 'French'. Les résultats de l'inoculation des cicatrices foliaires ont été cohérents avec ceux d'études antérieures. Sur le prunier 'French', l'incidence de la maladie sur l'infection des lenticelles a été très faible, mais elle a été nettement supérieure à celle mesurée sur les plantes du traitement témoin inoculées avec de l'eau. La congélation-décongélation a considérablement augmenté à la fois l'incidence de la maladie et la taille des lésions dues à l'infection des lenticelles. Les données sur l'inoculation des lenticelles suggèrent que l'infection des lenticelles par *P. syringae* pv. *syringae* est possible en vergers.

États-Unis / *Prunus persica* / *Prunus avium* / *Prunus domestica* / maladie des plantes / chancre / *Pseudomonas syringae* / infection expérimentale / lenticelle / lésion

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RESUMEN ESPAÑOL, p. 169

1. Introduction

Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae*, is a devastating disease of stone fruit due to its impact on branch dieback and tree mortality. The disease has a distinct blast phase on leaves and blossoms in some areas with cool moist weather in spring and a canker phase on the stem and branches in the dormant season. The blast infection is thought to occur mainly through stomata, wounds or injuries caused by freezing temperatures [1, 2]. In England, Crosse demonstrated that the main avenue of fall infection for the canker phase on stems and branches caused by *Pseudomonas morsprunorum* in cherry trees is through leaf scars, with 90% to 100% of branch cankers being found at the base of a dead spur [3, 4]. This association, together with the frequent occurrence of dead spurs with incipient cankers at the base and dead spurs without basal cankers, suggested leaf scars provided the wounds necessary to initiate infection of spurs in sweet cherry [5]. Montgomery and Moore achieved some control of the canker phase by fall applications of copper Bordeaux sprays, which further suggested that the spurs became infected through the leaf scars during the fall leaf-fall period [6]. During rainy periods, inoculum is washed onto scars and moved into the vessels of the leaf traces. Wind-driven rain appears to provide the ideal conditions for infection, by simultaneously removing leaves and distributing inoculum [3]. In attempts to reproduce cankers that were comparable with naturally occurring ones, the cankers that formed following artificial inoculations of leaf scars of spurs with *P. morsprunorum* were indistinguishable in form and severity from those occurring naturally [5]. Leaf scars as the principal infection sites for the canker phase were also reported on peach in California [7] and in France [8]. However, branch cankers of peach did not occur following leaf scar inoculation in North Carolina [9]. In Oregon, Cameron reported that infections with *P. syringae* in cherry occurred through bud scales rather than leaf scars, between November and February [10]. In Australia, infections of apricot and cherry were reported not to occur

through leaf scars, but only stem and bud inoculations consistently led to the establishment of cankers [11]. No cankers resulted from inoculation of leaf scars with *P. syringae* pv. *syringae* at leaf drop in pear trees [12]. Crosse pointed out that leaf scars are not a major avenue of infection in areas where bacterial canker of stone fruit is due to *P. syringae* [1]. The variable results obtained from these studies indicate that the role of leaf scars in initiating infections by *P. syringae* in stone fruits is debatable.

Lenticels are the only permanent natural openings to the internal tissues of stems, since the susceptible cortical and phloem tissues are covered with a thick periderm. Wormald drew attention to the lenticels as possible points of entry [13], but later research showed that cankers could not be produced by spraying lenticels of plum and cherry with *P. syringae* pv. *syringae* inoculum under pressure [1, 14]. Thus, the role of lenticel infection in bacterial canker of stone fruits is not conclusive.

Temperature has also been reported to influence bacterial canker development [15]. The optimum temperature for stem infection in peach seedlings was found to be 18.2 °C in the greenhouse [16]. Dormant peach trees held at 15 °C developed larger cankers than trees held at 6 °C [7]. Bacterial canker severity increased when peach branches or shoots were exposed to freezing temperatures a few days after inoculation with *P. syringae* pv. *persicae* [17]. Noninjurious, freezing-induced water-soaking has been reported to be important in promoting the ingress and spread of *P. syringae* pv. *syringae* in fruit tree stems [18, 19]. Research has shown that peach seedlings subjected to chilling injury conditions (25 to 30 days at 6 °C) are more susceptible to *P. syringae* pv. *syringae* infection than unchilled seedlings [20]. However, the effect of chilling temperature on leaf scar infection and the influence of freezing-thawing on lenticel infection have not been studied. In our study, the potential of leaf scars and lenticels as the avenues of *P. syringae* pv. *syringae* ingress and the effects of leaf scar age, chilling and freezing-thawing on bacterial infection through leaf scars and lenticels were investigated.

2. Materials and methods

2.1. Plant materials, bacterial strain, inoculation and disease evaluation

The plant materials used in the leaf scar inoculation experiment were the current season growing shoots with 8 to 20 leaf scars of 5- to 8-year-old cherry [*Prunus avium* L. cv. Bing], peach [*Prunus persica* (L.) Batsch, cv. Fantasia] and prune [*Prunus domestica* L., cv. French] trees that were growing in the experimental orchards of the Department of Plant Pathology of the University of California (UC) at Davis, USA.

The plant materials used for the lenticel inoculation experiment were (15- to 20)-cm-long 3-year-old dormant stems of 'French' prune. *Pseudomonas syringae* pv. *syringae* strain B3A [21], an ice nucleation-active strain [22] used in all inoculation experiments, was grown in King's medium B liquid as described previously [19].

Leaf scar inoculations were carried out by placing a drop (about 5 μ L) of bacterial suspension (10^8 CFU·mL⁻¹) on the surface of the scars. The stems with inoculated leaf scars were then covered with plastic bags and placed in an incubator for two weeks at 12 °C for disease development.

Lenticel inoculations were conducted by spraying bacterial suspension onto the surface of 3-year-old stems of 'French' prune with a plastic squeeze bottle sprayer. Prior to lenticel inoculation, a 0.05% solution of Breakthru[®] (polyether-polymethylsiloxane copolymer) surfactant (Plant Healthy Technologies, Lathrop, CA) was added to the bacterial suspension to increase penetration of the bacteria into the lenticels. Preliminary experiments indicated that the surfactant did not significantly influence bacterial viability (data not shown). The stems with inoculated lenticels were covered with plastic bags and kept in an incubator for three weeks at 12 °C for disease development.

The length of the lesion that developed during the incubation period and the incidence of leaf scar and lenticel infections were examined by tangential dissection of

the stem after completion of the incubation period. A subset of suspected lesions was analyzed by dissecting the margin of the lesions, aseptically chopping the tissue in sterile deionized water (sdH₂O) and plating a portion of the tissue macerate on King's medium B as previously described [23]. In most cases, fluorescent *P. syringae* pv. *syringae* colonies, characterized by a negative oxidase reaction and a positive hypersensitive response in tobacco leaves [24, 25], were recovered from the brown-colored lesions following inoculation with *P. syringae* pv. *syringae*. Only in such a case when *P. syringae* pv. *syringae* colonies were confirmed was regarded as a positive bacterial infection.

2.2. Effect of leaf scar age on infection

In November 1998, leaves with petioles on current season growing shoots of 8-year-old cherry, peach and prune trees growing in the field at UC Davis were forcibly removed 5, 2 and 1 days, and 12, 4, 2 and 0 hours prior to inoculation, respectively. Three stems of each leaf scar age of each species were inoculated with bacterial suspension and an additional stem of each leaf scar age of each species was inoculated with sdH₂O. Inoculations were carried out by placing a drop (5 μ L) of bacterial suspension on scars from a hypodermic syringe with a 25-gauge needle. The 0-h treatment was performed by placing a drop of bacterial suspension on the surface of the fresh leaf scar immediately after removing the petioles from the stems in the laboratory. The inoculated stems were then covered with plastic bags and placed in an incubator for two weeks at 12 °C for disease development. After the completion of incubation, the length of the resulting brown lesion beneath the leaf scar was then measured after removing a thin layer of the bark with a razor blade. The number of infected and total inoculated leaf scars for each stem was recorded. At the end of October 2002, the same experiment was repeated but with four stems per treatment of each species being included.

2.3. Effect of chilling temperature on leaf scar infection

Four-month-old, self-rooted peach seedlings were produced from greenwood cuttings using the method developed by Couvillon and coworkers [26]. After root formation, the rooted peach cuttings (cv. Angelus) were planted in 3.8-liter pots filled with sand and vermiculite (sand:vermiculite = 3:1). The plants were fertilized with half-strength Hoagland's solution [27] for about one month in the greenhouse of UC Davis. Prior to inoculation, plants with 2 to 6 leaves were exposed to a chilling temperature (2.2 °C) in a growth chamber for 0 (control), 1, 2, 4 and 7 days, respectively. After the completion of the chilling treatment, leaves were forcibly detached and fresh leaf scars were immediately inoculated with *P. syringae* pv. *syringae* by placing 1 drop (5 µL) of bacterial suspension on the surface of the scar. Two to six leaf scars were inoculated with *P. syringae* pv. *syringae* on each plant, that was then covered with a plastic bag, kept moist for two days, and incubated in one of the two growth chambers for three weeks for disease development. The settings of the two growth chambers were as follows: growth chamber 1, 11 h of light at 11.6 °C to 12.5 °C, and 13 h of dark at 10.9 °C to 11.9 °C; growth chamber 2, 11 h of light at 11.3 °C to 15.0 °C, and 13 h of dark at 6.8 °C to 8.4 °C. Each chilling treatment was replicated with 10 plants and an additional two plants of each treatment were inoculated with sdH₂O as a negative control. After a 3-week incubation period, the lesion length beneath each leaf scar was measured and the number of both infected and total leaf scars was recorded.

2.4. Lenticel inoculation

Healthy 3-year-old dormant stem pieces of 15 to 20 cm in length and about 5 cm in diameter were cut off from 'French' prune trees growing in the orchard of UC Davis with a handsaw in the morning and brought into the laboratory. The two ends and any fresh wounds of each stem piece were sealed with hot wax before inoculation. One group of stems (30 stems) was

exposed to -5 °C overnight as a freezing pretreatment prior to inoculation, while another group (61 stems) was stored at 4 °C. Inoculations were carried out by spraying a bacterial suspension supplemented with 0.05% Breakthru[®] (polyether-polymethylsiloxane copolymer) surfactant (Plant Healthy Technologies, Lathrop, CA) on the entire surface of the stems either during the transition period when the frozen stems were thawing or on stems that were being stored at 4 °C. The inoculated stems were then covered with plastic bags and placed in an incubator for three weeks at 12 °C. After the completion of incubation, the lesion length under the inoculated lenticels (if any) was measured and both the number of infected and total inoculated lenticels of each stem piece was recorded. Only lesions from which *P. syringae* pv. *syringae* could be isolated were considered as lenticel infections.

2.5. Data analysis

Data were analyzed for statistical significance using the general linear model (GLM) procedure (Statistical Analysis System; SAS Institute, Cary, NC). When appropriate, arcsine and square root transformations [28, 29] were applied to the data in order to establish a normal distribution and homogeneity of variance before subjecting it to statistical comparison. The means of both the transformed and untransformed data are presented.

3. Results

3.1. Effect of leaf scar age on infection

An analysis of variance of disease incidence of leaf scar infection indicated highly significant main effects of leaf scar age, species and year, and only one significant interaction term (year × species, *table D*). Since leaf scars inoculated with sdH₂O developed no infections (data not shown), only those inoculated with *P. syringae* pv. *syringae*

Table I.

Analysis of variance of arcsine-transformed disease incidence of leaf scar infection in cherry, peach and prune stems tested for the effects of leaf scar age, species, year, and interaction between treatment and species.

Source	Degree of freedom	Mean square	Prob > F
Leaf scar age	6	0.643	< 0.0001
Species	2	0.170	< 0.0067
Year	1	0.895	< 0.0001
Year × species	2	0.105	< 0.029
Year × treatment	6	0.063	> 0.055
Species × treatment	12	0.017	> 0.672
Error	12	0.022	–

Table II.

Effect of leaf scar age on the average disease incidence of leaf scar infection in cherry, peach and prune (21 stems tested for each leaf scar age).

Leaf scar age	Arcsine mean ¹	Disease incidence ² (%)
0 h	0.985 a	69.1 ± 4.8
2 h	0.920 a	65.4 ± 6.0
4 h	0.682 b	46.5 ± 6.5
12 h	0.488 c	26.6 ± 5.5
1 day	0.451 c	22.7 ± 4.2
2 days	0.200 d	3.0 ± 1.8
5 days	0.147 d	0

¹ Arcsine-transformed value = $(1/2) \times \{\arcsin[(X / (n + 1))^{0.5}] + \arcsin[((X + 1) / (n + 1))^{0.5}]\}$, where X = number of infected leaf scars per stem, n = total number of inoculated leaf scars per stem.

Means followed by the same letter are not different at $P < 0.05$ according to Duncan's multiple range test.

² Mean of disease incidence ± standard error.

were used for statistical analysis. Leaf scars were more susceptible to bacterial infection if they were exposed to the air for less than 2 h, compared with leaf scars that were exposed to the air for 4 h to 1 day (*table II*). Leaf scars were almost immune to bacterial infection if they were exposed to the air for two or more days (*table II*). Cherry leaf scars appeared to be significantly more susceptible to bacterial infection than peach and prune (*table III*). The significant interaction between species and year was due to the fact that, in 1998, disease incidence was significantly higher in cherry than in prune

and peach in descending order, but, in 2002, no significant differences were detected, although cherry still ranked the highest, peach the next and prune ranked the lowest (data not shown). The significant year effect was the result of a much higher disease incidence in 2002 than in 1998 (data not shown).

An analysis of variance of the length of the lesions caused by leaf scar infection also showed significant main effects of leaf scar age and a significant interaction term (year × species), but the year effect was not significant (data not shown). Infections on

Table III.

Disease incidence resulting from leaf scar infection in cherry, peach and prune (49 stems treated for each species).

Species	Arcsine mean ¹	Disease incidence ² (%)
Cherry	0.679 a	42.8 ± 4.6
Peach	0.507 b	30.1 ± 5.2
Prune	0.473 b	27.0 ± 4.3

¹ Arcsine-transformed value = $(1/2) \times [\arcsin\{(X / (n + 1))^{0.5}\} + \arcsin\{((X + 1) / (n + 1))^{0.5}\}]$, where X = number of infected leaf scars per stem, n = total number of inoculated leaf scars per stem.

Means followed by the same letter are not different at $P < 0.05$ according to Duncan's multiple range test.

² Mean of disease incidence ± standard error.

Table IV.

Effect of leaf scar age on the average lesion length of leaf scar infection in cherry, peach and prune (21 stems treated for each leaf scar age).

Leaf scar age	Square root mean ¹	Lesion length ² (mm)
0 h	1.955 a	3.5 ± 0.3
2 h	1.813 ab	2.8 ± 0.2
4 h	1.643 b	2.3 ± 0.2
12 h	1.272 c	1.2 ± 0.2
1 day	1.323 c	1.6 ± 0.3
2 days	0.838 d	0.3 ± 0.2
5 days	0.707 d	0

¹ Square root-transformed value = $(\text{lesion length} + 0.5)^{0.5}$. Means followed by the same letter are not different at $P < 0.05$ according to Duncan's multiple range test.

² Mean of original disease severity ± standard error.

leaf scars exposed to the air for less than 4 h developed significantly longer lesions compared with those exposed to the air for 12 h and 1 day (*table IV*). Infections on leaf scars exposed to the air for more than 1 day developed the smallest lesions among all the leaf scar ages (*table IV*). The significant interaction between year and species in lesion length was similar to that of disease incidence in that, in 1998, lesion length was significantly higher in cherry than in peach and prune in descending order while, in 2002, the lesion length of all

three species was the same but in this case cherry did not rank the highest (data not shown).

3.2. Effect of chilling on leaf scar infection

The statistical analysis of disease incidence data indicated that no significant effect of duration of chilling (2.2 °C) could be detected, although there was a trend for lower disease incidence with increasing

Table V.

Analysis of variance of arcsine-transformed lesion length of leaf scar infection in 'Angelus' peach tested for the effects of duration of chilling temperature (0, 1, 2, 4 and 7 days at 2.2 °C), chamber, and interaction between duration of chilling temperature and chamber.

Source	Degree of freedom	Mean square	Prob > F
Treatment (duration at 2.2 °C)	4	0.434	< 0.016
Chamber (chamber 1 & chamber 2)	1	0.004	> 0.739
Treatment × chamber (error)	4	0.035	–

Square root-transformed value = (lesion length + 1)^{0.5}.

Table VI.

Effect of duration of chilling on the average lesion length of leaf scar infection in 'Angelus' peach (10 stems tested for each chilling duration).

Duration of chilling	Square root mean ¹	Lesion length ² (mm)
No chilling	2.333 a	4.49 ± 0.38
1 day	2.062 b	3.27 ± 0.19
2 days	2.024 b	3.12 ± 0.18
4 days	1.779 c	2.24 ± 0.27
7 days	1.900 bc	2.61 ± 0.18

¹ Square root-transformed value = (lesion length + 1)^{0.5}.

Means followed by the same letter are not different at $P < 0.05$ according to Duncan's multiple range test.

² Mean of original disease severity ± standard error.

chilling (data not shown). The duration of chilling did have a significant effect on lesion length (table V). Significantly longer lesions were observed in *P. syringae* pv. *syringae*-infected leaf scars without chilling, compared with those with chilling treatment (table VI). Both the disease incidence and lesion length results suggest that leaf scar resistance to bacterial infection is increased as the plants are acclimatized to the chilling temperature, although 4 to 7 days of chilling were required to reduce lesion length by about 50% (table VI) which was comparable with that achieved by only 4 to 12 h of leaf scar aging (table IV).

3.3. Effect of freezing-thawing on lenticel infection

The incidence of lenticel infection on 'French' prune stems was very low, but statistical analysis on pooled data indicated that the disease incidence of lenticel infection due to bacterial inoculation was significantly higher than those inoculated with sdH₂O (table VII). Freezing-thawing had significant effects on both increasing disease incidence of lenticel infection and increasing the lesion length beneath the infected lenticels (table VII).

Table VII.
Effect of freezing-thawing on lenticel infection in 'French' prune.

Treatment	Inoculation	% of lenticel infection ¹ (%)	Number of infected lenticels	Total lenticels inoculated	Lesion length ² (mm)
Freezing/thawing	Bacteria	1.3 a	28	2101	31.1 ± 2.9 a
Nonfrozen	Bacteria	0.8 b	29	3752	16.8 ± 1.2 b
Control	sdH ₂ O	0.0 c	0	1241	0

¹ Data followed by the same letter are not significantly different at $P < 0.05$ based on the χ^2 test.

² Means (\pm standard error) followed by the same letter are not significantly different at $P < 0.05$ based on the t test.

4. Discussion

Leaf scars that were exposed to the air for less than 2 h had the highest disease incidence and lesion length, and those exposed to the air for 2 days or more were almost immune to bacterial infection. There was a clear progressive reduction in both disease incidence and lesion length with time after removal of the leaf petiole and bacterial inoculation. Similar results were also observed on sweet cherry [3, 5] and sour cherry [30]. The decreased disease incidence and severity due to the increase in leaf scar age was believed to be related to the quantity of inoculum penetrating the scars [3]. In a field experiment using cherry, the bacterial inoculum applied directly after removal of petioles was drawn rapidly into the scar within a few seconds, which resulted in higher disease incidence and severity the following year compared with those inoculations on prolonged leaf scars [3]. Crosse reported that, as the leaf scar ages, the amount of bacterial inoculum drawn into the xylem vessel of the leaf trace decreases, which decreases the disease incidence and severity [3]. The data obtained from our research support this previous conclusion. In addition, Crosse inoculated two types of fresh cherry leaf scars, those resulting from natural leaf abscission and those following artificial leaf removal, and the resulting disease due to inoculation was more severe in leaf scars produced by artificial leaf removal than in scars produced by natural abscission [3]. An experiment using acid fuchsin as a marker also demonstrated that visible penetration of the dye decreased as the leaf scar

aged in cherry [3]. These and our studies suggest that wound healing, which results in polyphenolic compounds accumulating in the wound tissues and the formation of tyloses or gums in the vessels, may mediate a defensive mechanism to reduce leaf scar infection [3].

In previous experiments, inoculum entered leaf scars via the xylem vessels of the leaf trace and frequently penetrated the main vascular system of the fruit spur, invaded the medullary rays and spread into the cortex and pith. Seven months after the leaf scar inoculation, cankers up to 30 cm long had developed [3]. Our data indicated that the lesion length due to leaf scar infection was only a few millimeters within an incubation period of two weeks. Compared with the lesion length (typically, 2 or 3 cm) that developed after a similar incubation period following wound inoculation with *P. syringae* pv. *syringae* in peach stems [19], leaf scar inoculation resulted in a very limited infection in our experiments. However, it may be possible that a more extensive leaf scar infection would occur over a longer incubation period.

The data also demonstrated a significant difference between the two years of the experiment. Both the disease incidence and lesion length were much greater in 2002 than in 1998 (data not shown). Crosse demonstrated that, in cherry, leaf scars were more susceptible in the period from the beginning of September to the latter part of October than in November [4]. This seasonal variation of susceptibility may provide an explanation for the significant year effect in

this study because the experiment conducted in 2002 was in October compared with November in 1998.

Duration of chilling did not have a statistically significant effect on disease incidence, but significantly decreased the lesion length. English and Davis hypodermically inoculated *P. syringae* pv. *syringae* into 3-month-old Lovell seedlings that were subjected to chilling-injury conditions and they found that seedlings with chilling treatment (25 to 30 days at 6 °C) were more susceptible to canker development than those without chilling treatment [20], which is contrary to the trend that we observed. Crosse found a dramatic decrease in susceptibility of leaf scars to bacterial infection in fall, and attributed this to the decreased vascular tension in trees resulting from reduced transpiration and increased soil moisture [4], which limited the amount of inoculum drawn into the xylem vessels of the leaf scars [1]. In California, defoliation in late fall always coincides with a drop in temperature. The low temperature during defoliation may be a factor contributing to the resistance of leaf scars to *P. syringae* pv. *syringae* infection. Collectively, the leaf scar inoculation data were consistent with the previous studies [1, 4]. This suggests that it may be beneficial to time bactericidal sprays during defoliation in the fall in order to reduce the inoculum for leaf scar infection.

Lenticels have been suggested to serve as an avenue for bacterial infection [13], but cankers could not be produced by spraying lenticels with inoculum under pressure [1, 14]. The data of lenticel inoculation in this study demonstrated that disease incidence through lenticel infection was very low. Freezing-thawing significantly increased both disease incidence and lesion length due to lenticel infection. The low incidences of lenticel infections suggest that *P. syringae* pv. *syringae* infection through lenticels is possible under field conditions since there are millions of lenticels on each plant. It may be possible that lenticel infection is significant even with such a low infection rate.

In conclusion, our results support the hypotheses that leaf scars and lenticels could serve as an avenue for *P. syringae* pv.

syringae to initiate infection in stone fruit trees. However, the facts that only a few millimeters in lesion length were developed after infection through leaf scars and lenticels suggest that the stem materials used for inoculation were not fully susceptible to *P. syringae* pv. *syringae*, compared with the major bacterial canker predisposing factors such as ring nematode infestation [31, 32]. The role of leaf scars and lenticels as infection avenues could be further validated if ring nematode predisposed stems were inoculated with *P. syringae* pv. *syringae*.

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References

- [1] Crosse J.E., Epidemiological relations of the pseudomonad pathogens of deciduous fruit trees, *Ann. Rev. Phytopathol.* 4 (1966) 291–310.
- [2] Süle S., Seemüller E., The role of ice formation in the infection of sour cherry leaves by *Pseudomonas syringae* pv. *syringae*, *Phytopathology* 77 (1987) 173–177.
- [3] Crosse J.E., Bacterial canker of stone-fruits. II. Leaf scar infection of cherry, *J. Hortic. Sci.* 31 (1956) 212–224.
- [4] Crosse J.E., Bacterial canker of stone-fruits. III. Inoculum concentration and time of inoculation in relation to leaf-scar infection of cherry, *Ann. Appl. Biol.* 45 (1957) 19–35.
- [5] Crosse J.E., The leaf scar as an avenue of infection for the cherry bacterial canker organism, *Pseudomonas morsprunorum* Wormald, *Nature* 168 (1951) 560–561.
- [6] Montgomery H.B.S., Moore M.H., The control of bacterial canker and leaf spot in sweet cherry, *J. Pomol.* 21 (1945) 155.
- [7] Davis J.R., English H., Factors related to the development of bacterial canker in peach, *Phytopathology* 59 (1969) 588–595.

- [8] Vigouroux A., Observations de contaminations tardives des plaies pétiolaires du pêcher par *Pseudomonas mors-prunorum* f.sp. *persicae* Prunier, Luisetti, Gardan, Ann. Phytopathol. 8 (1976) 111–115.
- [9] Endert E., Ritchie D.F., Overwintering and survival of *Pseudomonas syringae* pv. *syringae* and symptom development in peach trees, Plant Dis. 68 (1984) 468–470.
- [10] Cameron H.R., Mode of infection of sweet cherry by *Pseudomonas syringae*, Phytopathology 52 (1962) 917–921.
- [11] Wimalajeewa D.L.S., Seasonal variation in susceptibility of apricot to *Pseudomonas syringae* pathovar *syringae* (bacterial canker), and sites of infection in apricot and cherry, Aust. J. Exp. Agric. 27 (1987) 475–479.
- [12] Spotts R.A., Cervantes L.A., Factors affecting the severity of bacterial canker of pear caused by *Pseudomonas syringae* pv. *syringae*, Plant Pathol. 44 (1995) 325–331.
- [13] Wormald H., Bacterial canker of stone fruit trees in Britain. III. The symptoms of bacterial canker in plum trees, J. Hortic. Sci. 9 (1931) 239–256.
- [14] Shanmuganathan N., Studies on bacterial canker of plum and cherry (*Pseudomonas morsprunorum* Wormald), Univ. Lond., Thesis, U.K., 1962.
- [15] Otta J.D., English H., Epidemiology of the bacterial canker disease of ‘French’ prune, Plant Dis. Rep. 54 (1970) 332–336.
- [16] Dye D.W., The effect of temperature on infection by *Pseudomonas syringae* van Hall, N.Z. J. Sci. Tech. ser. A 38 (1957) 500–505.
- [17] Vigouroux A., Obtention de symptômes de bacteriose du pêcher (*Pseudomonas mors-prunorum* f. sp. *persicae*) sur rameaux de pêcher détachés et conservés en survie. Effet du froid, Ann. Phytopathol. 6 (1974) 95–98.
- [18] Vigouroux A., Ingress and spread of *Pseudomonas* in stems of peach and apricot promoted by frost-related water-soaking of tissues, Plant Dis. 73 (1989) 854–855.
- [19] Cao T., Saylor R.J., DeJong T.M., Kirkpatrick B.C., Bostock R.M., Shackel K.A., Influence of stem diameter, water content, and freezing-thawing on bacterial canker development in excised stems of dormant stone fruit, Phytopathology 89 (1999) 962–966.
- [20] English H., Davis J.R., Effect of temperature and growth state on the susceptibility of *Prunus* spp. to *Pseudomonas syringae*, Phytopathology 59 (1969) 1025.
- [21] DeVay J.E., Lukezic F.L., Sinden S.L., English H., Coplin D.L., A biocide produced by pathogenic isolates of *Pseudomonas syringae* and its possible role in the bacterial canker disease of peach trees, Phytopathology 58 (1968) 95–101.
- [22] Cao T., Kirkpatrick B.C., Shackel K.A., DeJong T.M., Influence of mineral nutrients and freezing-thawing on peach susceptibility to bacterial canker caused by *Pseudomonas syringae* pv. *syringae*, Fruits 66 (2011) 441–452.
- [23] Spotts R.A., Cervantes L.A., *Pseudomonas* canker of pear trees in Oregon, cultivar resistance, and effect of trunk guards on canker incidence and bacteria survival on bark, Plant Dis. 78 (1994) 907–910.
- [24] Chandler W.A., Daniell J.W., Effect of leachates from peach soil and roots on bacterial canker and growth of peach seedlings, Phytopathology 64 (1974) 1281–1284.
- [25] Weaver D.J., Interaction of *Pseudomonas syringae* and freezing in bacterial canker on excised peach twigs, Phytopathology 68 (1978) 1460–1463.
- [26] Couvillon G.A., King G.A., Moore C.M., Bush P., Obtaining small peach plants containing all bud types for “rest” and dormancy studies, HortScience 10 (1975) 78–79.
- [27] Hoagland D.R., Arnon D.I., The water culture method for growing plants without soil, Calif. Agric. Exp. Stn. Circ. 347 (1938) 39 p.
- [28] Zar J.H., Biostatistical analysis, 2nd edition, Prentice-Hall, Inc., Englewood Cliffs, N.J., U.S.A., 1984.
- [29] Fernandez G.C.J., Residual analysis and data transformations: Important tools in statistical analysis, HortScience 27 (1992) 297–300.
- [30] Bech K., Susceptibility to bacterial canker in sour cherry varieties: Orchard observations and inoculation trials, J. Phytopathol. 134 (1992) 238–246.
- [31] Cao T., Duncan R.A., McKenry M.V., Shackel K.A., DeJong T.M., Kirkpatrick B.C., The interaction between nitrogen fertilized peach trees and the expression of *syrB*, a gene involved in syringomycin production in *Pseudomonas syringae* pv. *syringae*, Phytopathology 95 (2005) 581–586.
- [32] Cao T., McKenry M.V., Duncan R.A., DeJong T.M., Kirkpatrick B.C., Shackel K.A., Influence of ring nematode infestation and calcium, nitrogen, and indoleacetic acid applications on peach susceptibility to *Pseudomonas syringae* pv. *syringae*, Phytopathology 96 (2006) 608–615.

Efecto de la edad de la cicatriz foliar, de la refrigeración y de la congelación-descongelación en la infección de cicatrices foliares y de las lenticelas por *Pseudomonas syringae* pv. *syringae* en los frutales de hueso.

Resumen – Introducción. En el mundo entero, el chancro bacteriano causado por *P. syringae* pv. *syringae* es una enfermedad importante de los frutales de hueso. Se evaluó la posibilidad de infección de las cicatrices foliares y de las lenticelas por *P. syringae* pv. *syringae* en el cerezo, el melocotonero y el ciruelo. **Material y métodos.** Se efectuaron inoculaciones en laboratorio y sobre el terreno, empleando ramas de cerezo, melocotonero y ciruelo para testear el efecto de la edad de la cicatriz foliar, de la refrigeración y de la congelación-descongelación en la infección bacteriana, a través de las cicatrices foliares y de las lenticelas. **Resultados y discusión.** La edad de la cicatriz foliar se asoció a una significativa disminución de la incidencia de la enfermedad y de la longitud de las lesiones, que resultaron de la inoculación de cicatrices foliares por *P. syringae* pv. *syringae* en el cerezo, melocotonero y ciruelo. Se observó una reducción significativa de la incidencia y de la longitud de las lesiones tras 4 h de exposición en el aire, y se redujeron a casi cero estas dos medidas de la infección tras 2 días de exposición. La refrigeración prolongada (2,2 °C) antes de la deshojadura no tuvo ningún efecto claro en la incidencia de la enfermedad en las cicatrices foliares, pero disminuyó significativamente la longitud de lesiones causadas por la infección. El cerezo fue más sensible a la infección de las cicatrices foliares por *P. syringae* pv. *syringae* que el melocotonero y el ciruelo 'French'. Los resultados de la inoculación de las cicatrices foliares fueron coherentes con los de los estudios anteriores. En el ciruelo 'French', la incidencia de la enfermedad en la infección de las lenticelas fue muy escasa, pero fue considerablemente superior a la que se midió en las plantas del tratamiento testigo inoculadas con agua. La congelación-descongelación aumentó considerablemente tanto la incidencia de la enfermedad como el tamaño de las lesiones causadas por la infección de las lenticelas. Los datos sobre la inoculación de las lenticelas sugieren que es posible la infección de las lenticelas por *P. syringae* pv. *syringae* en vergeles.

EUA / *Prunus persica* / *Prunus avium* / *Prunus domestica* / enfermedades de las plantas / necrosis cancerosa / *Pseudomonas syringae* / infección experimental / lenticel / lesiones

