Influence of mineral nutrients and freezing-thawing on peach susceptibility to bacterial canker caused by *Pseudomonas syringae* pv. *syringae*

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Influence of mineral nutrients and freezing-thawing on peach susceptibility to bacterial canker caused by *Pseudomonas syringae* pv. *syringae*.

Abstract — Introduction. Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae*, is a devastating disease of stone fruit worldwide. The effects of mineral nutrients and freezing-thawing on bacterial canker susceptibility were evaluated using potted peach trees in an attempt to understand predisposing factors in bacterial canker of stone fruit. **Materials and methods**. A split-plot experimental design with randomized complete block main plots (*i.e.,* inoculations associated with freezing-thawing or nonfrozen pretreatments) and subplots of trees with the seven treatments (*i.e.*, solutions deficient in N, P, K, Ca, Mg or Fe, respectively, and a full nutrient control) was adopted to study the effect of mineral deficiency and freezing-thawing on peach susceptibility to bacterial canker. **Results and discussion**. Phosphorus deficiency was the only treatment to significantly decrease lesion length that developed after inoculation with *P. syringae* pv. *syringae*, compared with the control trees that received full nutrients. Nitrogen and potassium deficiency treatments significantly decreased bark nitrogen and potassium concentrations accordingly, but had no clear effect on lesion sizes. Inoculation during freezing-thawing cycles significantly increased lesion length. In another independent experiment, nitrogen deficiency significantly increased the number of *P. syringae* pv. *syringae* leaf scar infections, but the subsequent infection was limited to a few millimeters. Nitrogen-deficient trees, which had higher [carbon / nitrogen] ratios, developed lesion sizes equivalent to trees provided with full nutrients. Collectively, these data suggest that, in the absence of other major predisposing factors (*i.e.*, low soil pH or ring nematodes), mineral nutrients may play a minor role in the susceptibility of peach to bacterial canker.

USA / *Prunus persica* / plant diseases / cankers / *Pseudomonas syringae* / nutrient deficiencies / lesions

Influence des éléments nutritifs et de la congélation-décongélation sur la sensibilité du pêcher au chancre bactérien provoqué par *Pseudomonas syringae* pv. *syringae*.

Résumé — Introduction. Le chancre bactérien, causé par *Pseudomonas syringae* pv. *syringae*, est une maladie dévastatrice des fruits à noyaux dans le monde entier. Les effets des éléments nutritifs et de la congélation-décongélation sur la sensibilité au chancre bactérien ont été évalués en utilisant des pêchers en pot afin d'essayer de comprendre les facteurs de prédisposition des fruits à noyau au chancre bactérien. **Matériel et méthodes**. Un dispositif expérimental en split-plot en blocs aléatoires complets avec des parcelles principales (inoculations associées à des prétraitements de congélation-décongélation ou sans congélation) et des parcelles secondaires d'arbres soumis à sept traitements (solutions déficientes en N, P, K, Ca, Mg ou Fe, respectivement, et un témoin disposant de tous les éléments nutritifs) a été adopté pour étudier l'effet de la carence en minéraux et de la congélation-décongélation sur la sensibilité du pêcher au chancre bactérien. **Résultats et discussion**. La carence en phosphore a été le seul traitement qui a diminué significativement la longueur des lésions développées après inoculation avec *P. syringae* pv. *syringae*, par rapport aux arbres témoins ayant reçu tous les éléments nutritifs. Les traitements de carence en azote et en potassium ont diminué de façon significative les concentrations en azote et potassium de l'écorce, mais ils n'ont eu aucun effet évident sur la taille des lésions. L'inoculation lors des cycles de congélation-décongélation a provoqué une augmentation considérable de la longueur de la lésion. Dans une autre expérience indépendante, la carence en azote a sensiblement augmenté le nombre de cicatrices foliaires infectées par *P. syringae* pv. *syringae*, mais l'infection qui en a résulté a été limitée à quelques millimètres. La carence en azote des arbres qui avaient eu des ratios [carbone / azote] plus élevés a développé des lésions de tailles équivalentes à celles des arbres ayant reçu tous les éléments nutritifs. Collectivement, ces données suggèrent que, en l'absence d'autres grands facteurs prédisposants (par exemple, faible pH du sol ou nématode *Criconemella xenoplax*), les éléments nutritifs pourraient jouer un rôle mineur vis-à-vis de la sensibilité du pêcher au chancre bactérien.

États-Unis / *Prunus persica* / maladie des plantes / chancre / *Pseudomonas syringae* / carence en substance nutritive / lésion

1. Introduction

Bacterial canker is a devastating, widespread and economically important disease of stone fruits. In California, bacterial canker is caused by *Pseudomonas syringae* pv. *syringae* van Hall, which normally resides as an epiphyte on healthy trees [1, 2]. Previous studies have demonstrated that bacterial canker occurs primarily on trees that have been predisposed by various stress factors such as nutrient deficiency [3–5], freezing temperatures [6, 7], high populations of ring nematodes [8–11] and low soil pH [4, 12]. Studies have shown that susceptibility to bacterial canker is reduced by nitrogen fertilization in peach [3, 10] and 'French' prune [5, 13]. Low available phosphate in the soil has been reported to reduce the susceptibility of plum to bacterial canker [14]. Daniell and Chandler found that bacterial canker severity in peach was reduced if iron was sufficient [15]. Vigouroux and Bussi reported that susceptibility to bacterial canker was reduced by calcium supplementation in peach and apricot [16, 17]. Canker length was also reduced in peaches supplemented with magnesium [16]. Supplemental fertilization with nitrogen, phosphorus, potassium and micronutrients significantly decreased bacterial canker severity in 'French' prune [13]. However, Beard and Wormald reported that plum susceptibility to bacterial canker was not decreased by nitrogen and potassium fertilization [14], and Wilson found no evidence that nitrogen fertilization reduced bacterial canker disease in stone fruit [18]. Vigouroux *et al.* reported an increased susceptibility in peaches that were supplemented with high levels of potassium [19]. Nitrogen and calcium fertilization failed to reduce the incidence of bacterial canker in young sweet cherry and peach trees [20, 21]. These variable results suggest that the influence of mineral nutrition on bacterial canker disease is debatable. It is difficult to separate the effects of individual elements that act together in the tree [16], but nutrient balance may play a more important role in host susceptibility than each element individually.

Previous studies indicated that freezing temperatures are an important predisposing factor in bacterial canker development. Cambial necrosis was more extensive when apricot [6, 22] and peach [7] stems were subjected to freezing temperatures following *P. syringae* pv. *syringae* inoculation compared with stems that were not subjected to freezing temperatures. Vigouroux reported that non-injurious freezing caused water-soaking in peach stems and suggested that freezing-induced water-soaking was important in promoting the ingress and spread of *P. syringae* pv. *syringae* in fruit tree stems [23, 24]. After freezing at –5 °C for (12 to 24) h, inoculations made during the thawing process produced significantly larger lesions than inoculations performed before freezing or after thawing [25]. However, the effect of the interaction between host mineral deficiency and exposure to freezing temperatures on bacterial canker has not been studied.

The objectives of our research were to evaluate the effect of tree mineral deficiencies on host susceptibility to bacterial canker. The effect of mineral deficiency on peach susceptibility to bacterial canker was studied in combination with freezing-thawing pretreatments. The effect of nitrogen deficiency on susceptibility to *P. syringae* pv. *syringae* infection through leaf scars and the subsequent development of cankers in the stems was also examined.

2. Materials and methods

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

Plant materials used for studying the effect of mineral deficiency and inoculation during freezing-thawing on lesion length were 1-year-old potted peach trees [*Prunus persica* (L.) Batsch]. Plant materials used for studying the effect of nitrogen deficiency on *P. syringae* pv. *syringae* infection through leaf scars and stem inoculations were 1-year-old potted peach trees produced from greenwood cuttings**.** The treatments of plant materials are described below for each individual experiment.

The bacterial strain used in our study, *P. syringae* pv. *syringae* strain B3A [26], was grown in liquid King's medium B [27] for two days at 28 °C with shaking at 180 rpm, and cells were harvested by centrifugation at 3000 × *g* for 7 min at 4 °C. Bacterial inoculum was prepared by suspending the bacterial pellet in sterile deionized water (sdH_2O) to a concentration of approximately 10^8 CFU·mL^{-1} as estimated by measuring the optical density at 600 nm. Bacterial inoculations were made with a pinprick inoculation procedure described previously [25]. The inoculated sites on each tree were wrapped with a piece of parafilm and the inoculated trees were placed either in a growth chamber or in a screenhouse for disease development. The extent of the canker that developed after incubation was measured with a digital caliper after cutting the stem tangentially with a razor blade to expose the brown necrotic area of infected cambium. Isolations from representative lesions were plated on King's medium B to confirm the recovery of the causal agent of *P. syringae* pv. *syringae* that typically fluoresces blue under UV light (354 nm), is oxidase-negative [28], and elicits a hypersensitive reaction in tobacco [29]. Negative controls were treated with sdH_2O instead of bacterial suspension after a wound in the bark was created with a pin-prick procedure [25].

2.2. Effect of mineral deficiency and inoculation during freezing-thawing on lesion length

Ninety-eight approximately 1-m-tall 1-year-old potted peach trees (cv. Fairtime / Nemaguard), which were kindly donated by Duarte Nurseries (Hughson, CA, USA), were transplanted into 7.6-liter pots filled with clean river sand on July 15, 1999. All potting soil attached to the root mass was removed and the roots were rinsed with deionized water prior to transplanting. After transplanting, the 98 potted trees were irrigated with deionized water for two weeks before the mineral-deficient treatments were initiated on July 28, 1999. Mineral-deficient treatments were essentially half-strength Hoagland's solution [30] that lacked nitrogen, phosphorus, potassium, calcium, magnesium or iron, respectively. Micronutrients such as boron, manganese, zinc, copper and molybdenum were all included in the solutions in the concentration as reported by Hoagland and Arnon [30]. Iron was added in the form of iron chelate micronutrient [Fe, 6%, technical sodium ferric ethylenediamine di-(O-hydroxy-phenylacetate), CIBA-GEIGY Co., Greensboro, NC] to a final concentration of 1.4 mg·L^{-1} to all treatments except for the iron deficiency treatment. The half-strength Hoagland's solution was adjusted to pH 7.0 before being applied to the potted trees. The trees were fertilized either with the deficient nutrient (treatment) or full nutrient (control) solutions twice a week. Three hundred mL were applied to each potted tree per application; any excess solution was allowed to drain from a hole in the bottom of the pot. The mineral-deficient treatments were imposed for 17 weeks and discontinued on November 29, 1999. After November 29, 1999, depending on weather conditions, the potted trees were irrigated only with an additional (1000 to 1500) mL of deionized water per week before they were inoculated on December 20, 1999. Bacterial inoculations were performed under two conditions, with and without a freezing-thawing pretreatment.

The experimental design was a split plot with randomized complete block main plots and subplots of trees with various mineral deficiency treatments. There were a total of seven blocks with two main plots (*i.e.,* inoculations associated with freezing-thawing or nonfrozen pretreatments) and seven subplots (*i.e.*, solutions deficient in N, P, K, Ca, Mg or Fe, respectively, and a full nutrient control) of each. The 98 potted trees were arranged on two benches according to the experimental design in a screenhouse in the field facility of the Department of Plant Pathology at the University of California Davis (USA).

On December 18, 1999, one-year-old stems (about 15 cm long) on the top portion of each tree were taken for mineral analysis to determine if the mineral deficiency treatments had significant effects on tree mineral concentrations. On December 20, 1999, when all trees were ready for bacterial inoculation, one group of 49 trees was

inoculated with *P. syringae* pv. *syringae* during the thawing process after trees had been exposed to freezing temperatures, and the other 49 trees were inoculated without the freeze-thaw pretreatment. The freezing pretreatment was accomplished by placing the potted trees in an insulation box in a cold room (–5 °C) for 24 h. During the freezing pretreatment, two belt heaters were placed underneath the pots to keep the pot soil temperature above 5 °C to avoid root freezing injury. Inoculations during freezing-thawing were performed during the thawing process immediately after the trees were moved out from the –5 °C cold room to room temperature (23 °C). Both inoculations during freezing-thawing and without freezing were performed at the same time using the same bacterial inoculum. Three inoculations, 15 cm apart, were made in the stem of each tree. The inoculated trees were placed in a walk-in growth chamber with a temperature of 15 °C (day) and 12 °C (night) for canker development. The growth chamber settings were as follows: 11 h of light (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR) with 50% relative humidity and 13 h of dark with 90% relative humidity. During incubation, each potted tree was irrigated with 500 mL of deionized water per week. The length of lesions that developed at the end of an incubation period of 3 weeks was determined with a digital caliper, and the average length of the three lesions was used for statistical analysis.

2.3. Effect of nitrogen deficiency on *P. syringae* pv. *syringae* infection through leaf scars and stem inoculation

Because the mineral deficiency experiment was unable to demonstrate that nitrogen deficiency could increase bacterial canker, which was inconsistent with the previous observations [3, 5, 10, 11, 13], a second nitrogen deficiency experiment was initiated in 2001. Thirty 1-year-old peach trees (cv. Angelus) obtained from greenwood cuttings were grown in 3.8-liter pots and fertilized weekly with full-strength Hoagland's solution either lacking nitrogen or with complete nutrients (control) in a greenhouse from May to September, 2001. In October 2001, the trees were moved into a walk-in growth chamber for two weeks to induce premature dormancy using the following conditions: 11 h of daytime at 20 °C with light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR, and 13 h of nighttime at 15 °C with relative humidity of 90%. Bacterial inoculation on leaf scars was accomplished by forcibly removing the leaves and immediately placing a drop (5 µL) of bacterial suspension onto the fresh leaf scars. Five leaf scars were inoculated per tree and a total of 15 trees were inoculated for each treatment. The inoculated trees were incubated for two months for canker development in the same growth chamber and under the same conditions as described above except the temperature was adjusted to 15 °C (daytime) and 10 °C (nighttime). The inoculated trees were fully irrigated only with deionized water twice a week during this incubation period. Bacterial infection of leaf scars was evaluated at the end of the incubation period. The number of infected and total inoculated leaf scars was recorded, and the length of the lesions beneath the infected leaf scars (if any) was determined. Because leaf scar infections were only limited to a few millimeters, *P. syringae* pv. *syringae* was also inoculated into the stems on January 04, 2001 after recording the leaf scar infection results. The inoculated trees were then allowed to incubate in a screenhouse under ambient conditions from January 04 to March 01, 2002. During this incubation period, only water was provided by a drip system for 15 min every other day. Lesions were evaluated at the end of the incubation period. Prior to inoculation of the stems, pieces of 1-year-old stems of the top portion of each tree were taken for nitrogen analysis to determine if there was any decrease in nitrogen concentration due to the nitrogen deficiency treatment.

2.4. Tree nitrogen, carbon, [carbon/nitrogen] ratio, and mineral element determination

Stem samples were briefly rinsed with deionized water and dried with paper towels. About 20 g of fresh bark was collected

Table I.
Effect of nutrient deficiency treatments on calcium, nitrogen, phosphorus, potassium, magnesium and iron concentrations in peach bark[1].

Treatment	N	P	K	Ca	Mg	Fe ($mg \cdot L^{-1}$)
			(%)			
Full nutrient	3.07 ± 0.11 a	0.242 ± 0.01 b	0.50 ± 0.08 b	3.12 ± 0.23 cd	0.26 ± 0.02 a	177 ± 20 a
Ca-deficient	2.35 ± 0.05 b	0.236 ± 0.02 b	0.55 ± 0.05 b	3.27 ± 0.26 bcd	0.27 ± 0.02 a	171 ± 16 a
N-deficient	2.15 ± 0.09 b	0.246 ± 0.01 b	0.63 ± 0.08 b	4.29 ± 0.34 a	0.30 ± 0.01 a	230 ± 31 a
P-deficient	2.99 ± 0.11 a	0.241 ± 0.01 b	0.68 ± 0.11 b	3.73 ± 0.25 abc	0.30 ± 0.01 a	171 ± 28 a
K-deficient	2.90 ± 0.11 a	0.246 ± 0.01 b	0.24 ± 0.03 c	3.41 ± 0.16 bcd	0.29 ± 0.01 a	191 ± 19 a
Mg-deficient	2.97 ± 0.07 a	0.251 ± 0.01 ab	0.95 ± 0.11 a	2.98 ± 0.20 d	0.26 ± 0.02 a	160 ± 24 a
Fe-deficient	3.19 ± 0.09 a	0.285 ± 0.01 a	0.65 ± 0.12 b	3.93 ± 0.20 ab	0.30 ± 0.01 a	158 ± 38 a

[1] Mean of 14 replicates ± standard error.
Means followed by the same letter are not significantly different at $P < 0.05$ based on Duncan's Multiple Range Test.

from each sample by tangentially removing all tissues down to the cambium with a razor blade, and the scrapings were dried in an oven for three days at 70 °C. The dried samples were then ground in a grinder-mill (Arthur H. Thomas Co., Laboratory Apparatus, Philadelphia, USA) to pass through a 40-mesh sieve. Total bark nitrogen and carbon were determined by a combustion gas analysis method [31, 32] in which (2 to 3) mg of plant sample wrapped in tin foil was combusted in an element analyzer (NA 1500, Fisons Instruments, Italy). [Carbon/nitrogen] ratios were calculated from carbon and nitrogen concentrations in the bark.

For the analysis of Ca, P, K, Mg and Fe, 100 mg of the dried sample was baked at 500 °C overnight, and the ash was dissolved in 10 mL 1N HNO_3 on a hot plate at 80 °C for 20 min. The acid extract was filtered through Whatman No. 1 filter paper and the volume of the acidic solution was increased to 50 mL by adding deionized water. A ten-fold dilution of the acid extract was analyzed for Ca, P, K, Mg and Fe with an ICP spectrometer (Thermo Jarrell Ash Co.) in the Division of Agriculture and Natural Resources Analytical Laboratory, University of California Davis, CA, USA.

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, log transformation was applied to the raw data to establish a normal distribution and homogeneity of variance before subjecting it to statistical comparison.

3. Results

3.1. Effect of mineral deficiency treatments on tree mineral concentration and trunk growth

Analysis of variance of tree mineral concentrations indicated significant differences in nitrogen, phosphorus, potassium and calcium concentrations in the bark across the seven different mineral fertilization levels, and nitrogen- and potassium-depleted treatments significantly decreased bark nitrogen and potassium levels, respectively (*table I*). The calcium-deficient treatment also significantly decreased bark nitrogen concentration (*table I*). Compared with the trees provided with full nutrients, nitrogen-deficient treatment significantly increased bark calcium concentration; magnesium-deficient treatment significantly increased bark potassium concentration; and iron-deficient treatment significantly increased bark phosphorus and calcium concentrations (*table I*).

Table II.
Effect of nutrient deficiency treatments on peach trunk growth[1].

Treatment	Trunk diameter (mm)		
	Initial	Final	Increase
Full nutrient	7.0 ± 0.3 a	9.4 ± 0.2 a	2.4 ± 0.1 abc
Ca-deficient	7.1 ± 0.3 a	9.0 ± 0.2 ab	1.9 ± 0.1 d
N-deficient	6.5 ± 0.3 ab	8.5 ± 0.3 b	2.0 ± 0.2 cd
P-deficient	6.8 ± 0.2 ab	8.9 ± 0.2 ab	2.1 ± 0.2 bcd
K-deficient	6.7 ± 0.3 ab	9.0 ± 0.3 ab	2.3 ± 0.1 abcd
Mg-deficient	6.4 ± 0.2 ab	9.1 ± 0.2 ab	2.7 ± 0.2 a
Fe-deficient	6.2 ± 0.2 b	8.7 ± 0.2 ab	2.5 ± 0.2 ab

[1] Mean of 14 replicates ± standard error. Trunk diameter was measured at 5 cm above the graft union on July 24 and November 28, 1999, respectively. Means followed by the same letter are not significantly different at $P < 0.05$ based on Duncan's Multiple Range Test.

Table III.
Effect of mineral nutrient treatments on lesion size in peach stems that were developed after bacterial inoculations (14 replicates)[1].

Treatment	Log mean[2]	Lesion length[3] (mm)
Full nutrient (control)	1.369 ab	33.8 ± 7.2
Mg-deficient	1.396 a	39.9 ± 9.0
Ca-deficient	1.378 ab	37.0 ± 8.2
K-deficient	1.360 ab	36.8 ± 8.3
N-deficient	1.332 abc	34.0 ± 7.9
Fe-deficient	1.319 bc	30.6 ± 6.4
P-deficient	1.264 c	27.7 ± 6.2

[1] Replication number includes *P. syringae* pv. *syringae* inoculations made during freezing-thawing and inoculations without freezing pretreatments.

[2] Log mean = $\log_{10}$ (lesion length). Means followed by the same letter are not significantly different at $P < 0.05$ based on Duncan's Multiple Range Test.

[3] Mean of lesion length ± standard error.

In general, the seven levels of mineral fertilization generated a group of trees with different bark mineral concentrations. Trunk growth as indicated by trunk diameter increase did not significantly differ from those in the control in response to mineral depletion treatments except the calcium deficiency treatment, which had significantly less trunk growth compared with the control (*table II*).

3.2. Effect of mineral nutrient deficiency and freeze-thawing on lesion length

Regression analysis revealed significant positive correlations between lesion lengths of the three inoculations on each tree [$P < 0.0001$, $R^2 = (0.676, 0.704, 0.794)$, $n = 98$, for each paired correlation analysis, respectively], suggesting that similar stems had similar responses to the bacterial infection. This result validated the bacterial inoculation procedure. Analysis of variance of lesion lengths from the inoculations with *P. syringae* pv. *syringae* indicated significant differences among treatments (withholding specific minerals, $P < 0.0047$), inoculation subsequent to freeze-thaw treatments ($P < 0.0001$), and blocks ($P < 0.0282$) (data not shown). Multiple mean comparisons indicated that the phosphorus deficiency treatment significantly decreased lesion length compared with the control (*table III*). No other mineral deficiency treatments significantly differed from the control, although the magnesium deficiency treatment had the longest lesion lengths (*table III*). Inoculation subsequent to the freeze-thaw treatment yielded an average lesion length (± standard error) of (59.9 ± 2.2) mm ($n = 49$) which was significantly ($P < 0.0001$) longer than the (8.6 ± 0.2) mm ($n = 49$) obtained without freeze-thaw pre-inoculation treatment (data not shown). When the data were pooled together according to pre-inoculation treatments (*i.e.*, freeze-thaw *vs.* not frozen), a regression analysis indicated that only phosphorus was positively correlated with lesion length ($P < 0.027$, $n = 49$) when infection occurred without freeze-thaw pretreatment, but it only accounted for 10% variability of the lesion length. A multiple regression analysis indicated that only 21% of the variability of the lesion length was accounted for by nitrogen, phosphorus, potassium, calcium, magnesium and iron ($P > 0.1042$, $n = 49$).

Table IV.
Effect of nitrogen deficiency on peach leaf scar susceptibility to bacterial canker.

Treatment	Leaf scar infection[1] (%)	No. of leaf scars infected	No. of leaf scars inoculated	Lesion length[2] (mm)
Nitrogen-deficient	77.3 a	58	75	1.5 ± 0.1 a
Full nutrient	61.3 b	46	75	2.8 ± 0.7 a

[1] Percentiles followed by the same letter are not significantly different at $P < 0.05$ based on Student's *t*-test.

[2] Mean of 15 replicates ± standard error. Means followed by the same letter are not significantly different at $P < 0.05$ based on Student's *t*-test.

Table V.
Effect of nitrogen deficiency on bark nitrogen and carbon concentrations and lesions in peach stems that were developed after bacterial inoculations[1].

Treatment	Log mean[2]	Lesion length (mm)	Bark nitrogen concentration (%)	Bark carbon concentration (%)	Bark [C / N] ratio
Nitrogen-deficient	1.39 a	34.0 ± 8.3	1.44 ± 0.05 b	48.77 ± 0.26 a	34.4 ±1.3 a
Full nutrient	1.20 a	16.2 ± 1.2	3.01 ± 0.10 a	47.66 ± 0.23 b	16.1 ± 0.6 b

[1] Mean of 15 replicates ± standard error. Means followed by the same letter are not significantly different at $P < 0.01$ based on Student's *t*-test.

[2] Log mean = $\log_{10}$ (lesion length). Means followed by the same letter are not significantly different at $P < 0.05$ based on Student's *t*-test.

3.3. Effect of nitrogen deficiency on the incidence of *P. syringae* pv. *syringae* infection through leaf scars, size of stem lesion lengths and bark [carbon/nitrogen] ratios

Nitrogen-deficient trees had a significantly higher incidence of infections through leaf scars than those that received full nutrients. However, the lesion length due to infection through leaf scars was limited to just a few millimeters and was not significantly influenced by nitrogen deficiency (*table IV*). The subsequent bacterial inoculation in the stems, however, resulted in equivalent lesion lengths irrespective of how different the bark nitrogen concentrations were (*table V*). Nitrogen-deficient plants had significantly lower bark nitrogen and higher bark carbon concentrations, and therefore a significantly higher [carbon/nitrogen] ratio than those provided with full nutrients (*table V*). Comparing the bark nitrogen concentration of trees in the nitrogen deficit treatment to trees in the mineral nutrient deficit experiment (*table I*), the nitrogen concentration of trees in the nitrogen deficiency experiment was much lower (*table V*). This was probably due to the differences in growth conditions of the experimental trees (*i.e.*, greenhouse *vs.* outdoor screenhouse).

4. Discussion

Nitrogen and potassium deficiency treatments significantly decreased tree nitrogen

and potassium concentrations, respectively, but did not have clear effects on the size of lesions that developed after inoculation with *P. syringae* pv. *syringae* (*tables I, III*). Although the phosphorus deficiency treatment did not significantly decrease plant phosphorus concentration, the lesions that developed after inoculation were significantly smaller in the phosphorus deficiency treatment trees than in trees receiving full nutrients (*table III*). This is consistent with previous observations made by Beard and Wormald [14] that low availability of soil phosphate reduced plum susceptibility to bacterial canker.

The calcium deficiency treatment resulted in significantly decreased tree nitrogen concentration, but not in decreased bark calcium, probably due to its low mobility within the tree. However, the calcium deficiency treatment did not have a clear effect on lesion size following inoculation with *P. syringae* pv. *syringae*. The magnesium deficiency treatment did not decrease tree magnesium concentration, but the trees in this treatment had significantly higher bark potassium concentration than trees that received complete nutrients. Considering the relatively wide range of calcium concentrations (2.98% to 4.29%) in the bark, there was no correlation between lesion length and bark calcium concentration, which is not consistent with the results of Vigouroux and Bussi [16, 17]. However, the pH of soils in the studies of Vigouroux and Bussi [16, 17] were considerably lower than the pH of the soils in our experiments (pH 7.0). Low pH can have significant effects on the availability of macro- and micronutrients, and low pH has also been shown to increase the susceptibility of bacterial canker in cherry [4] and peach trees [12].

Our data showed a significant effect of the freeze-thaw pretreatment on increasing lesion size after inoculation with *P. syringae* pv. *syringae*. This is consistent with our previous observations made with excised peach stems [25] and the results of Vigouroux [23, 24]. Our preliminary observations indicated that bacterial suspension of *P. syringae* pv. *syringae* strain B3A nucleated ice when it was exposed to –1 °C for only a few hours in contrast to the unchanged liquid form of sdH_2O that was treated under the same conditions (data not shown), suggesting that *P. syringae* pv. *syringae* strain B3A is an ice nucleation active bacterium. However, it remains controversial to regard ice nucleation activity in *P. syringae* pv. *syringae* as a virulence factor in the infection process in stone fruit [2]. The role of ice nucleation activity in *P. syringae* pv. *syringae* during infection in peach stems may be of minor importance, given the fact that the lesion size due to bacterial infection was very comparable with the extent of dye ingress and spread in the cortical parenchyma in peach stems [33].

The mineral deficiency treatments did not have a significant influence on tree nutrient concentration except for nitrogen and potassium. This is probably due to the relatively low mobility of some elements such as calcium and iron within the tree and the relatively short duration of these treatments.

Because of inconsistencies in the effect of nitrogen on canker severity in previous experiments [3, 5, 10, 11, 13], the nitrogen deficiency experiment was repeated in 2001. In this experiment the nitrogen-depleted nutrient treatment significantly decreased bark nitrogen concentration (*table V*) and increased the number of leaf scars infected with *P. syringae* pv. *syringae* (*table IV*). Thus, nitrogen status appears to be a potential factor that influences bacterial infection through leaf scars, in addition to leaf scar age, as reported by Crosse [34]. However, nitrogen-deficient trees failed to develop significantly longer lesions than those provided with full nutrients after inoculation with *P. syringae* pv. *syringae*, which is consistent with our nutrient deficiency experiment, but not in agreement with previous observations in peach [3, 10, 11] and 'French' prune [5, 13]. However, the beneficial effects of nitrogen fertilization on reducing bacterial canker severity have mostly been observed in peach trees growing in sandy soils [3, 10, 11] containing high populations of ring nematodes, that are an important factor in California for predisposing *Prunus* trees to bacterial canker [8–11]. In the presence of high ring nematode populations, high-nitrogen and low-calcium tissues have been found to be less susceptible to

bacterial canker than low-nitrogen and high-calcium tissues [11]. In this study, the effects of mineral nutrients (particularly nitrogen and calcium) on susceptibility to bacterial canker were not pronounced in the absence of predisposing factors such as ring nematode infestation or low soil pH.

When nitrogen availability is reduced, plant growth declines more rapidly than photosynthesis, carbohydrates can accumulate and compounds such as phenolics begin to accumulate [35]. In this study, longer lesions appeared to be associated with higher carbon and lower nitrogen, and therefore higher [carbon/nitrogen] ratios. Our previous work also demonstrated higher [carbon/nitrogen] ratios associated with significantly larger lesions in peach stems that were inoculated with *P. syringae* pv. *syringae* [10]. It may be possible that increased phenolics in response to nitrogen deficiency are involved in altering host susceptibility to bacterial canker because of a positive correlation between the [carbon/nitrogen] ratio and soluble phenolic compounds [10].

The nitrogen effects on bacterial canker severity were consistent for the two independent experiments included in our research. The nitrogen-depleted trees in the nitrogen deficiency experiment had much lower bark nitrogen concentration (*i.e.*, 1.44%, *table V*) than the nitrogen-deficient trees in the mineral-deficient experiment (*i.e.*, 2.15%, *table I*), but this decrease in nitrogen concentration did not increase lesion size after inoculation with *P. syringae* pv. *syringae* as trees in both experiments developed similar-sized lesions (*i.e.*, 34.0 mm, respectively, *tables III, V*). Independent field experiments indicated that higher nitrogen concentration in the bark had an effect of decreasing susceptibility to bacterial canker in peaches that were predisposed by ring nematodes [10, 11]. The inconsistency of the nitrogen effect on bacterial canker susceptibility found in our current study suggests the presence of nitrogen-independent factors involved in peach susceptibility to bacterial canker.

Previously, Crosse demonstrated that the main avenue of fall infection of bacterial canker on stems and branches caused by *Pseudomonas morsprunorum* in cherry trees is through leaf scars [34, 36, 37]. Leaf scars as principal infection sites for canker development were also reported on peach in California [38] and in France [39]. In our current study, nitrogen deficiency appeared to increase leaf scar infection rate but had no clear effect on promoting further canker development in peach. Earlier, Davis and English reported severe canker development through leaf scar inoculation in orchard conditions, but they were unable to induce canker development in the growth chamber where the temperature was controlled to a constant 15 °C, which led them to speculate that other factors such as rain and freezing temperatures were involved in the disease development [38]. With reference to the previous studies on leaf scar infection, the inconsistency of bacterial canker establishment through leaf scar infection observed in the present study indicates that other nitrogen-independent factors are involved in predisposing peach trees to bacterial canker.

Our current results combined with the literature regarding ring nematode populations [8–11] and soil pH [4, 12] on the predisposition to bacterial canker suggest that tree mineral nutrients may play a secondary role in altering host susceptibility to bacterial canker. In other words, tree mineral nutrients (*i.e.*, nitrogen and calcium) may have an effect on host susceptibility to bacterial canker after the tree host has been predisposed to infection by more significant factors such as ring nematodes or low soil pH.

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Influencia de los elementos nutritivos y de la congelación-descongelación en la sensibilidad del melocotonero al chancro bacteriano provocado por *Pseudomonas syringae* pv. *syringae*.

Resumen — Introducción. El chancro bacteriano, causado por *Pseudomonas syringae* pv. *syringae*, es una enfermedad devastadora que afecta los frutos de hueso del mundo entero. Se evaluaron, por medio de melocotoneros en maceta, los efectos que representan los elementos nutritivos y la congelación-descongelación para la sensibilidad al chancro bacteriano, con el fin de intentar entender los factores que hacen que los frutos de hueso estén predispuestos al chancro bacteriano. **Material y métodos**. Para poder estudiar el efecto que tiene la falta de minerales y la congelación-descongelación en la sensibilidad del melocotonero al chancro bacteriano, se adoptó un dispositivo experimental en parcelas subdivididas en bloques aleatorios completos con parcelas principales (inoculaciones asociadas a pre-tratamientos de congelación-descongelación o sin congelación) y con parcelas secundarias de árboles sometidos a siete tratamientos (soluciones con insuficiente N, P, K, Ca, Mg o Fe, respectivamente, y un testigo que dispusiera de todos los elementos nutritivos). **Resultados y discusión**. El único tratamiento que disminuyó significativamente la longitud de las lesiones desarrolladas tras inoculación con *P. syringae* pv. *syringae* fue el tratamiento que carecía de fósforo, de acuerdo con los árboles testigo que recibieron todos los elementos nutritivos. Los tratamientos faltos de nitrógeno y de potasio hicieron disminuir de modo significativo las concentraciones de nitrógeno y potasio de la corteza, pero no tuvieron ningún efecto evidente en el tamaño de las lesiones. La inoculación en el momento de los ciclos de congelación-descongelación provocó un aumento considerable de la longitud de la lesión. En otro experimento independiente, la falta de nitrógeno hizo que aumentara sensiblemente el número de cicatrices foliares infectadas por *P. syringae* pv. *syringae*, pero la infección resultante se limitó a algunos milímetros. La falta de nitrógeno en árboles con ratios [carbono / nitrógeno] más elevados desarrolló lesiones de tamaños equivalentes a los de los árboles que recibieron todos los elementos nutritivos. En conjunto, estos datos sugieren que, en ausencia de otros grandes factores predisponentes (por ejemplo, reducido pH del suelo o nematodo *Criconemella xenoplax*), los elementos nutritivos podrían desempeñar un papel minoritario, en relación con la sensibilidad del melocotonero al chancro bacteriano.

EUA / *Prunus persica* / enfermedades de las plantas / necrosis cancerosa / *Pseudomonas syringae* / deficiencias nutritivas / lesiones