Influence of rootstock, temperature and incubation duration on bacterial canker severity caused by *Pseudomonas syringae* pv. *syringae* in peach

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Influence of rootstock, temperature and incubation duration on bacterial canker severity caused by *Pseudomonas syringae* pv. *syringae* in peach.

Abstract – Introduction. Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae*, is a damaging disease of stone fruit worldwide. The effects of rootstock, temperature and incubation duration on bacterial canker in peach were assessed using both field and laboratory inoculation assays. **Materials and methods**. Both field and laboratory experiments were conducted to study the effects of rootstock, temperature and incubation duration on disease severity in peach. All inoculations were achieved with *P. syringae* pv. *syringae* strain B3A. Bacterial inoculations were applied to 1-year-old shoots of peach trees [*Prunus persica* (L.) Batsch]. After inoculation, the inoculated shoots were allowed to incubate either under field conditions or in a cold room at different temperatures [constantly at 0 °C, constantly at 14.4 °C, and in a fluctuating temperature regime of 12 h at 0 °C (night) and 12 h at 14.4 °C (day)] for excised shoots. The lesions were determined 1 to 6 weeks after inoculation to determine the effect of incubation duration. **Results and discussion**. The field experiment using peach grafted on three rootstocks (Nemaguard, K119-50 and P30-135) showed that shoots on Nemaguard developed the longest lesions and shoots on K119-50 the shortest among all three rootstocks. Shoots on Nemaguard had significantly lower bark calcium and higher nitrogen concentrations than those on K119-50 and P30-135. A negative correlation was found between lesion length and bark calcium concentration and the [calcium / nitrogen] ratio. Laboratory experiments with excised shoots on Nemaguard, K119-50, P30-135, Lovell and Guardian rootstocks growing in a second orchard showed inconsistent results. Shoots from Nemaguard developed significantly smaller lesions than those on K119-50 and P30-135. Shoots on Guardian and Lovell also developed significantly smaller lesions than those of shoots on K119-50 and P30-135. Temperature fluctuation during incubation (0 °C to 14.4 °C) had no effect on shoot lesion length compared with those incubated constantly at 14.4 °C, but produced significantly longer lesions than shoots incubated constantly at 0 °C. These inconsistent results suggest that, in the absence of major predisposing factors (*i.e.*, ring nematodes or low soil pH), rootstocks may play a minor role in peach susceptibility to bacterial canker even under favorable disease development conditions.

USA / *Prunus persica* / plant diseases / *Pseudomonas syringae* / experimental infection / rootstocks / temperature

Influence du porte-greffe, de la température et de la durée d'incubation sur la gravité du chancre bactérien provoqué par *Pseudomonas syringae* pv. *syringae* chez le pêcher.

Résumé – Introduction. Le chancre bactérien, causé par *Pseudomonas syringae* pv. *syringae,* est une maladie qui provoque des dommages aux fruits à noyau dans le monde entier. L'effet des porte-greffes, de la température et de la durée d'incubation sur le chancre bactérien ont été évalués chez le pêcher en utilisant à la fois des essais d'inoculation sur le terrain et en laboratoire. **Matériel et méthodes**. Des expériences en verger et en laboratoire ont été réalisées afin d'étudier les effets du porte-greffe, de la température et de la durée d'incubation sur la gravité de la maladie chez le pêcher. Toutes les inoculations ont été effectuées avec la souche B3A de *P. syringae* pv. *syringae*. Les inoculations bactériennes ont été appliquées sur des tiges de pêchers [*Prunus persica* (L.) Batsch] de 1 an. Après inoculation, les plantules inoculées ont été laissées en incubation soit en verger soit en chambre froide à différentes températures [constamment à 0 °C, constamment à 14,4 °C, ou avec une alternance de température de 0 °C pendant 12 h (nuit) et 14,4 °C pendant 12 h (jour)] pour des tiges excisées. Les lésions ont été déterminées 1 à 6 semaines après l'inoculation pour déterminer l'effet de la durée d'incubation. **Résultats et discussion**. L'expérience en verger utilisant des pêchers greffés sur trois porte-greffes (Nemaguard, K119-50 et P30-135) a montré que, parmi les trois porte-greffes, les tiges sur Nemaguard développaient les lésions les plus longues et les tiges sur K119-50 développaient les lésions les plus courtes. Les tiges greffées sur Nemaguard ont montré des teneurs en calcium de l'écorce significativement plus faibles et des concentrations en azote significativement supérieures à celles des tiges greffées sur K119-50 et P30-135. Une corrélation négative a été observée entre la longueur de la lésion et la teneur en calcium de l'écorce ainsi qu'entre la longueur de la lésion et le rapport [calcium / azote] de l'écorce. Les expérimentations en laboratoire avec des pousses excisées de plants sur porte-greffes Nemaguard, K119-50, P30-135, Lovell et Gardien issus d'un second verger ont donné des résultats contradictoires. Les tiges sur Nemaguard ont développé des lésions nettement plus petites que celles sur K119-50 et P30-135. Les tiges sur Guardian et Lovell ont également développé des lésions significativement plus petites que les pousses sur K119-50 et P30-135. La fluctuation de la température pendant l'incubation (0 °C à 14,4 °C) n'a eu aucun effet sur la longueur des lésions par rapport à celles continuellement incubées à 14,4 °C, mais elle a entrainé des lésions significativement plus longues que celles des pousses incubées continuellement à 0 °C. Ces résultats contradictoires suggèrent que, en l'absence des principaux facteurs prédisposants (par exemple, des nématodes en anneau ou un faible pH du sol), les porte-greffes peuvent jouer un rôle mineur dans la sensibilité du pêcher au chancre bactérien, même dans des conditions favorables au développement de la maladie.

États-Unis / *Prunus persica* / maladie des plantes / *Pseudomonas syringae* / infection expérimentale / porte greffe / température

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1. Introduction

Bacterial canker is a devastating and widespread disease in peach production in California. The causal organism of this disease, *Pseudomonas syringae* pv. *syringae*, causes serious canker damage only when the tree is in a dormant condition [1]. Research has shown that many factors can predispose stone fruit trees to bacterial canker [2, 3]. One important stress factor that is thought to predispose stone fruit trees to bacterial canker is the imbalance of mineral nutrition. Vigouroux and coworkers inoculated peach during the dormant season with *P. syringae* pv. *persicae* [4, 5], and apricot with *P. syringae* pv. *syringae* [6] and found that the lesion length that developed in the shoot was negatively correlated with the calcium concentration in the bark. Irrigation increased the bark calcium concentration of peach shoots and appeared to improve resistance to bacterial canker [5]. Moreover, nitrogen fertilization has been reported to reduce bacterial canker incidence in peach [7] and 'French' prune [8]. Calcium and nitrogen nutrition appear to be involved in the bacterial canker complex.

Rootstocks have been proposed as an important factor influencing the susceptibility of the scion to *P. syringae* pv. *syringae* infection. In California, apricots on peach rootstock are reported to be less susceptible to bacterial canker than apricots on Myrobalan plum rootstock, and plums on peach rootstock are more resistant to bacterial canker than plums on either Myrobalan or Marianna rootstocks [9]. Grower experience indicates that the industry shift from Lovell to Nemaguard rootstock resulted in greater susceptibility of peach to bacterial canker [10]. Nemaguard is frequently used as a peach rootstock because it is more resistant to root knot nematodes than Lovell rootstock [11]. Guardian has been more commonly used as a peach rootstock in the Southeast United States because of its resistance to both ring and root knot nematodes [12].

To evaluate the effect of rootstock on growth and bacterial canker susceptibility, rootstock experiments were established at the Kearney Agricultural Center of the University of California in Parlier and in a grower's orchard near Escalon, CA. A preliminary analysis indicated that more than a 50% increase in calcium concentration was observed in leaves of scions (cvs. Loadel and Flavorcrest) on K119-50 (*P. dulcis* × *P. salicina* hybrid) and P30-135 (*P. persica* × *P. salicina* hybrid) rootstocks, compared with trees on Nemaguard. Since bacterial lesion length has been reported to be negatively correlated with shoot calcium concentration [5, 6], it may be possible that this significant increase in leaf calcium might contribute to an increase in disease resistance to bacterial canker.

Bacterial canker development has been reported to be closely associated with temperature. Wilson compared the lesions developed at 18 °C to 21 °C, 10 °C and 2 °C, and found that trees held at 18 °C to 21 °C developed the largest lesions and trees held at 2 °C the least [13]. Dye reported an optimum temperature of 18.2 °C for peach shoot infection [14]. Wilson reported that cankers were initially larger at 21 °C to 23 °C than at lower temperatures, but a temperature of 15.5 °C was most favorable for canker extension after the plant had broken dormancy [15]. Weaver found that inoculated peach shoots had to be subjected to subfreezing temperature in order to develop typical bark cankers [16]. A higher incidence of cankers has been reported for the south compared to the north side of 'French' prune trunks [17]. In the dormant season in California daytime temperatures fluctuate considerably. However, the effect of temperature fluctuations during incubation on bacterial canker development has not been studied.

In our study, the effect of rootstock on peach susceptibility to bacterial canker in relation to calcium and nitrogen nutrition was evaluated. Laboratory inoculations with excised peach shoots from different rootstocks were performed in combination with incubation temperature treatments and incubation period to study the effects of rootstock, calcium and nitrogen nutrition, temperature fluctuation, and incubation duration on bacterial canker development.

2. Materials and methods

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

The plant materials used for all bacterial inoculations were 1-year-old shoots of peach trees [*Prunus persica* (L.) Batsch]. All treatments of plant materials are described subsequently in the following corresponding experiments. The bacterial strain used for all inoculations was *P. syringae* pv. *syringae* strain B3A [18], that was routinely maintained on King's medium B [19]. Bacterial strain *P. syringae* pv. *syringae* B3A (ice nucleation active), grown in King's medium B without agar for 2 days at 28 °C with shaking at 180 rpm, was harvested with centrifugation and adjusted to 10^8 CFU·mL^{-1} as estimated by measuring optical density at 600 nm with a spectrophotometer. Bacterial inoculations were accomplished as previously described [20]. After inoculation, the inoculated site was wrapped with a piece of Parafilm to keep the tissue moist. The inoculated shoots were allowed to incubate either in a cold room at different temperatures for excised shoots or under field conditions. After the completion of the incubation period, the length of lesions which were developed in the cambium and inner phloem was determined with a digital caliper after tangentially removing the bark with a razor blade.

2.2. Field inoculation experiment in Kearney Agricultural Center (KAC)

A field inoculation experiment was carried out in a peach orchard in KAC to evaluate the effect of rootstocks on scion susceptibility to bacterial canker. The peach trees, planted in a Hanford Fine Sandy Loam soil in January 1996, were flood-irrigated every two to three weeks during the growing season and fertilized with 504 kg·ha^{-1} calcium nitrate (equivalent to 78 kg·ha^{-1} nitrogen) on April 26, 2001. Two cultivars (Flavorcrest and Loadel) on three rootstocks [K119-50 (*P. dulcis* × *P. salicina* hybrid), P30-135 (*P. persica* × *P. salicina* hybrid) and Nemaguard (*P. persica* (L.) Batsch)] were arranged in a complete randomized split block design with 10 repetitions. Bacterial inoculations were carried out on 1-year-old shoots (one inoculation per tree) on March 10, 2002. A total of 60 trees were inoculated with *P. syringae* pv. *syringae*. The inoculations were allowed to incubate in the field for seven weeks. Prior to inoculation 1-year-old shoots were sampled for calcium and nitrogen analyses.

2.3. Peach rootstock bacterial canker trial in Escalon

The peach trees (cv. Ross) used in this experiment were planted in a sandy soil in February 2000. The trees were flood-irrigated 12 times each year and fertilized with UN-32 at a rate of 200 kg pure nitrogen per hectare per year. Five rootstocks (K119-50, Guardian [*P. persica* (L.) Batsch], Lovell [*P. persica* (L.) Batsch], Nemaguard and P30-135), with five trees per rootstock per plot, were arranged in a randomized complete block design with 8 repetitions. In January 2003, fifteen 100-cm-long 1-year-old shoots per rootstock per block were harvested for a total of 600 shoots and were brought into the laboratory in plastic bags. The shoots were kept in a cold room at −1 °C for three days before bacterial inoculation. No signs of freezing injury were observed during the period of cold storage at −1 °C. Three 100-cm-long shoots with similar diameter per rootstock per block were selected for bacterial inoculation. Six inoculations (10 cm to 14 cm apart) were accomplished in each shoot and the inoculation sites were wrapped with Parafilm to keep moist for infection. Then the three inoculated shoots were placed in three different long plastic bags, respectively. One bag was incubated in a cold room constantly at 0 °C, one bag in a room constantly at 14.4 °C, and the last bag was incubated in a fluctuating temperature regime of 12 h at 0 °C (night) and 12 h at 14.4 °C (day). The lesions were determined with a digital caliper 1, 2, 3, 4, 5 and 6 weeks after inoculation to determine the effect of incubation duration. No field inoculations were performed in this experiment.

Table I.
Analysis of variance for lesion length due to bacterial canker in peach (cvs. Flavorcrest, Loadel; Rootstocks, K119-50, Nemaguard, P30-135) in Kearney Agricultural Center of the University of California in Parlier, USA.

Source	Degrees of freedom	Mean square	Prob > *F*
Block	9	46.71	< 0.0489
Rootstock[1]	2	663.90	< 0.0001
Block × rootstock (error A)	18	29.85	–
Cultivar[2]	1	361.62	< 0.0041
Block × cultivar (error B)	9	24.80	–
Rootstock × cultivar	2	179.03	< 0.0015
Error	18	18.90	–

[1] Rootstock: testing for the effect of rootstocks on bacterial infection.
[2] Cultivar: testing for the effect of cultivars on bacterial infection.

2.4. Nitrogen and calcium determination

One-year-old shoots were harvested before inoculation, washed with deionized water, and dried with paper towels. About 20 g of fresh bark were collected using a razor blade, removing the phloem down to the cambium layer, and were dried in an oven for 3 days at 70 °C. The dried bark samples were ground in a grinder-mill (Arthur H. Thomas Co., Laboratory Apparatus, Philadelphia, USA) to pass a 40-mesh sieve. Total bark nitrogen was determined by a combustion gas analysis method [21, 22] in which 2 mg to 3 mg of bark sample wrapped in tin foil was combusted in an element analyzer (NA 1500, Fisons Instruments, Italy). For calcium analysis, 20 mg to 30 mg of the dried sample was incinerated at 500 °C for 4 h, and the ash was dissolved in 5 mL 1N HNO_3 on a hot plate at 80 °C for 10 min. The acid extract was filtered through Whatman No. 1 filter paper and was brought to 50 mL by adding deionized water, and analyzed for calcium by atomic absorption spectrometer (Analyst 800, Perkin Elmer Instruments, 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, USA).

3. Results

3.1. Effect of rootstock on susceptibility to bacterial infection in the Kearney Agricultural Center experiment

Analysis of variance of lesion length obtained in the spring of 2002 showed significant differences among rootstocks, cultivars and the interactions between rootstock and cultivar (*table I*). Shoots on Nemaguard rootstock developed the longest lesions among the three rootstocks for both cultivars, and shoots on K119-50 developed the shortest lesions among all three rootstocks for 'Flavorcrest' but not for 'Loadel' (*table II*). In both cultivars, shoots on Nemaguard rootstock had the lowest calcium and the highest nitrogen concentrations in the bark among all three rootstocks (*table II*). In contrast, shoots on K119-50 rootstock had the highest calcium and lowest nitrogen concentrations in the bark among all three rootstocks for both cultivars (*table II*). Of the two cultivars, 'Flavorcrest', which had significantly lower bark nitrogen than 'Loadel' but a similar level of bark calcium, developed significantly longer lesions than 'Loadel' (data not shown). In addition, the lesion length of the two cultivars was negatively correlated with the bark calcium concentration (R^2 = 0.191, r = –0.437, P < 0.0005, n = 60) and [calcium / nitrogen] ratio (R^2 = 0.2016, r = –0.449, P < 0.0003, n = 60) (*figure 1*).

Table II.
Effect of rootstock on lesion length due to bacterial canker, bark calcium and nitrogen concentrations in peach (cvs. Flavorcrest, Loadel; Rootstocks, K119-50, Nemaguard, P30-135) in Kearney Agricultural Center of the University of California in Parlier, USA.

Cultivar	Rootstock	Lesion length[1] (mm)	Bark calcium[1] (%)	Bark nitrogen[1] (%)
Flavorcrest	Nemaguard	26.3 ± 2.8 a	1.89 ± 0.13 c	1.53 ± 0.06 a
	P30-135	21.2 ± 1.6 a	2.78 ± 0.13 b	1.37 ± 0.04 b
	K119-50	10.2 ± 1.7 b	3.26 ± 0.15 a	1.21 ± 0.03 c
Loadel	Nemaguard	19.0 ± 1.0 a	2.02 ± 0.08 c	1.59 ± 0.05 a
	P30-135	11.9 ± 1.3 b	3.03 ± 0.20 b	1.43 ± 0.04 b
	K119-50	12.1 ± 1.1 b	3.56 ± 0.16 a	1.36 ± 0.06 b

[1] Mean of 10 replicates ± standard error. Means followed by the same letter are not significantly different at $P < 0.05$ based on Duncan's Multiple Range Test.

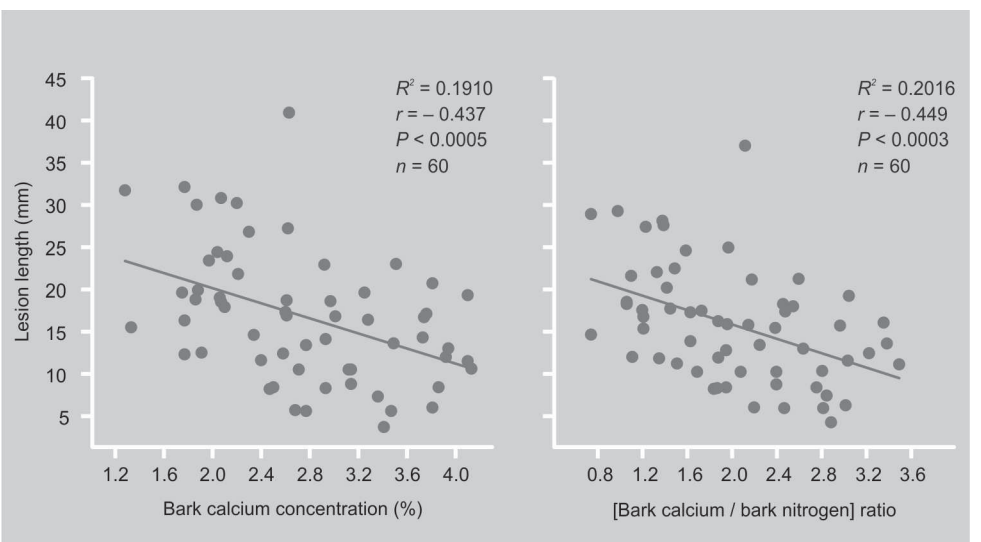

Figure 1.
Correlations between lesion length due to bacterial canker and bark calcium concentration, and lesion length and the [calcium / nitrogen] ratio in peach in Kearney Agricultural Center of the University of California in Parlier, USA.

3.2. Effect of rootstock, incubation temperature and duration of incubation on bacterial lesion length

Analysis of variance of the lesion length data obtained from inoculating excised shoots sampled from Escalon showed significant effects of block, rootstock, incubation temperature, and an interaction between rootstock and incubation temperature (*table III*). In this experiment, peach shoots on K119-50 and P30-135 developed significantly longer lesions than those on Guardian, Lovell and Nemaguard (*table IV*). The bark calcium concentration of the shoots on K119-50 rootstock was significantly higher than those on Guardian, Lovell and P30-135 rootstocks (*table IV*). The bark calcium concentration of the shoots on Nemaguard rootstock was significantly higher than those on Guardian rootstock (*table IV*). However, the bark nitrogen concentration of the shoots did not significantly differ from each other across all five rootstocks and was limited within a very narrow range (*table IV*). Accordingly, there

Table III.
Analysis of variance for lesion length due to bacterial canker in excised peach shoots (cv. Ross; Rootstocks, Guardian, K119-50, Lovell, Nemaguard, P30-135) in Escalon (CA), USA.

Source	Degrees of freedom	Mean square	Prob > *F*
Block	7	219.84	< 0.0001
Rootstock (R)[1]	4	677.54	< 0.0060
Block × rootstock (error A)	28	149.71	–
Incubation temperature (T)[2]	2	1672.10	< 0.0006
Block × temperature (error B)	14	125.63	–
Incubation duration (I)[3]	5	87.96	> 0.0830
Block × incubation duration (error C)	35	41.02	–
R × T	8	163.67	< 0.0010
R × I	20	61.09	> 0.2180
T × I	10	46.66	> 0.4919
R × T × I	40	46.71	> 0.5690
Error	546	49.42	–

[1] Rootstock: testing for the effect of rootstocks on lesion length.

[2] Incubation temperature: testing for the effect of temperature on lesion length.

[3] Incubation duration: testing for the effect of incubation period on lesion length.

Table IV.
Effect of rootstocks on lesion length due to bacterial canker in excised peach shoots, and bark calcium and nitrogen concentrations in Escalon (CA), USA (2003).

Rootstock	Lesion length[1] (mm)	Bark calcium concentration[2] (%)	Bark nitrogen concentration[2] (%)
K119-50	17.2 ± 0.8 a	1.88 ± 0.07 a	1.63 ± 0.05 a
P30-135	17.2 ± 1.0 a	1.58 ± 0.10 bc	1.68 ± 0.04 a
Guardian	14.2 ± 0.7 b	1.43 ± 0.08 c	1.65 ± 0.04 a
Lovell	13.1 ± 0.3 b	1.50 ± 0.06 bc	1.65 ± 0.04 a
Nemaguard	12.7 ± 0.3 b	1.72 ± 0.07 ab	1.63 ± 0.04 a

[1] Mean of lesion length of 144 replicates ± standard error. Means followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

[2] Mean of 8 replicates ± standard error.

were no apparent correlations between lesion length and bark calcium and nitrogen concentrations.

The effect of incubation temperatures indicated that lesions developed at fluctuating temperatures of 0 °C (12 h) to 14.4 °C (12 h) did not significantly differ from those developed at a constant temperature of 14.4 °C, but were significantly larger than those developed at 0 °C (*table V*). The significant interaction between rootstock and incubation temperature was due to the fact that lesions developed at a constant temperature of 14.4 °C and fluctuating temperature of 0 °C (12 h) to 14.4 °C (12 h) were significantly larger than those at 0 °C for shoots on P30-135, K119-50 and Guardian rootstocks, but the lesions were not significantly

Table V.
Influence of incubation temperature on lesion length due to bacterial canker in excised peach shoots in Escalon (CA), USA (2003).

Incubation temperature	Lesion length[1] (mm)
14.4 °C	16.5 ± 0.6 a
0 °C (12 h)–14.4 °C (12 h)	16.4 ± 0.6 a
0 °C	11.9 ± 0.2 b

[1] Mean of lesion length of 240 replicates ± standard error. Means followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

Table VI.
Influence of incubation temperature on lesion length due to bacterial canker in excised peach shoots in Escalon (CA), USA (2003).

Rootstock	Incubation temperature	Lesion length[1] (mm)
P30-135	14.4 °C	19.1 ± 2.1 a
	0 °C (12 h)–14.4 °C (12 h)	20.0 ± 1.8 a
	0 °C	12.5 ± 0.4 cd
K119-50	14.4 °C	19.2 ± 1.3 a
	0 °C (12 h)–14.4 °C (12 h)	20.1 ± 1.8 a
	0 °C	12.3 ± 0.5 cd
Guardian	14.4 °C	17.5 ± 1.4 ab
	0 °C (12 h)–14.4 °C (12 h)	14.6 ± 1.2 bc
	0 °C	10.7 ± 0.3 d
Lovell	14.4 °C	13.5 ± 0.5 cd
	0 °C (12 h)–14.4 °C (12 h)	13.7 ± 0.4 cd
	0 °C	12.2 ± 0.4 cd
Nemaguard	14.4 °C	13.1 ± 0.5 cd
	0 °C (12 h)–14.4 °C (12 h)	13.5 ± 0.6 cd
	0 °C	11.6 ± 0.3 cd

[1] Mean of lesion length of 48 replicates ± standard error. Means followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's Multiple Range Test.

different across the three incubation temperature regimes for peach shoots on Lovell and Nemaguard (*table VI*).

In addition, no significant effect of incubation duration on lesion length was determined in this experiment (*table III*). When the lesion length was plotted over the incubation days, there was only a slight increase in lesion length over time for shoots incubated at 0 °C and fluctuating temperatures of 0 °C (12 h) to 14.4 °C (12 h), but no increase in lesion length over

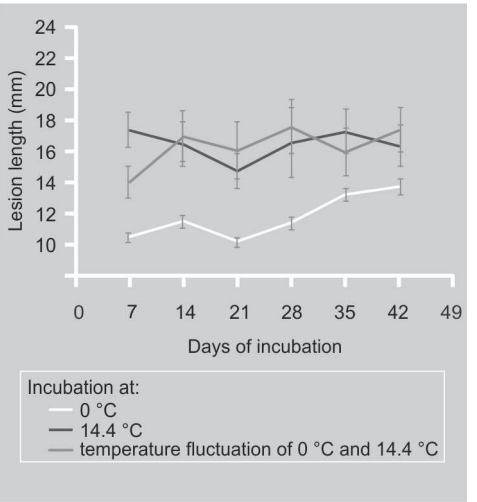

Figure 2.
Lesion length due to bacterial canker developed in excised peach shoots at different incubation temperature regimes. Each point represents a mean of 8 replicates of 5 rootstocks ± standard error.

time for shoots incubated at a constant temperature of 14.4 °C (*figure 2*). In addition, the lesion sizes were more variable for shoots incubated at a constant temperature of 14.4 °C or fluctuating temperatures of 0 °C (12 h) to 14.4 °C (12 h) than at a constant temperature of 0 °C (*figure 2*).

4. Discussion

Peach rootstocks appear to have a significant influence on mineral nutrition in the scion bark in some circumstances. In the Kearney Agricultural Center experiment, peach shoots on K119-50 and P30-135 rootstocks had correspondingly 72% and 47% higher bark calcium concentration than shoots on Nemaguard rootstock, but also had correspondingly 21% and 10% lower bark nitrogen concentration than those on Nemaguard rootstock for 'Flavorcrest' (*table II*). For 'Loadel', shoots on K119-50 and P30-135 rootstocks had correspondingly 76% and 50% higher bark calcium and correspondingly 14% and 10% lower bark nitrogen than those on Nemaguard rootstock (*table II*). Shoots on Nemaguard and P30-135 rootstocks developed significantly longer lesions than those on K119-50 rootstock for 'Flavorcrest'. However, for 'Loadel', shoots on P30-135 and K119-50 rootstocks developed significantly smaller lesions than those on Nemaguard rootstock. Lesion length was also found to be negatively correlated with the bark calcium concentration, which is consistent with previous studies in France [4–6] but not consistent with the results observed by Cao *et al.* [23, 24]. Vigouroux *et al.* found quite high negative correlations (correlation coefficient ranged from 0.591 to 0.604) between lesion length and bark calcium concentration of stone fruit trees growing in acidic soil in France [4, 5]. In the current study, we found that the correlation coefficient (r = –0.437, P < 0.0005, n = 60) of lesion length versus bark calcium concentration was lower than those obtained in France [4, 5]. The low correlation coefficient between lesion length and bark calcium found in our experiment suggests the presence of calcium-independent factors such as sandy soil, ring nematodes, soil pH, etc. Interestingly, the lesion length was not influenced by the significant changes in bark nitrogen concentration due to rootstock, which may be attributed to a relatively narrower range of bark nitrogen observed in this experiment compared with other experiments [23–25].

In contrast, in the Escalon experiment, the bark calcium concentration in shoots on K119-50 and P30-135 rootstocks did not significantly differ from those on Nemaguard rootstock and the lesions obtained from the laboratory inoculation in excised shoots on K119-50 and P30-135 rootstocks were significantly longer than those on Nemaguard rootstock. Shoots on Guardian, Lovell and Nemaguard did not differ in their ability to promote lesion development after inoculations, although shoots on Guardian rootstock had significantly lower bark calcium than those on Nemaguard rootstock. Generally, the bark calcium concentrations of the shoots on all the five rootstocks in the Escalon experiment were limited within a much narrower range than the calcium concentrations in shoots on Nemaguard, P30-135 and K119-50 rootstocks in the Kearney Agricultural Center (KAC) experiment. Therefore, it is not surprising that correlations between lesion length and bark calcium concentration and the [calcium / nitrogen] ratio were not significant in the Escalon experiment. In addition, the soil in the Escalon orchard was much sandier than

that in the KAC experiment, and the fertilization rate of pure nitrogen was much higher in the Escalon plot than in the KAC experiment. However, the calcium fertilization rate in the KAC experiment was much higher than in the Escalon plot because no calcium fertilizer was applied to Escalon trees. The differences in these field operations and soil conditions between the two orchard sites would make the trees quite different in nutrient status.

Incubation temperatures played a significant role in bacterial canker development. Incubation at 0 °C significantly decreased the lesion length compared with those at 14.4 °C and fluctuating temperatures of 0 °C (12 h) to 14.4 °C (12 h), which is consistent with previous studies [3, 13]. Incubation at fluctuating temperatures of 0 °C (12 h) to 14.4 °C (12 h) did not significantly affect the lesions compared with those incubated at a constant temperature of 14.4 °C. Wilson reported that the most favorable temperature for bacterial canker extension was 15.5 °C [15], which is very close to the 14.4 °C used in this study. These results suggest that temperature fluctuations (0 °C to 14.4 °C) may be equally conducive to bacterial canker development as an optimal temperature and more similar to environmental conditions that exist in the field.

The length of incubation did not affect the lesions in these experiments. The average lesion lengths obtained from both field and laboratory inoculations were about 20 mm and were very comparable with each other. The lesions were also quite comparable with the lesions obtained from excised fresh shoots after *P. syrinage* pv. *syringae* was inoculated without freezing-thawing in our previous study [20]. However, compared with lesions that developed on ring nematode-stressed peach trees (usually longer than 100 mm within a seven-week incubation period [23, 25]), the lesions developed in shoots on different rootstocks were very limited. These data suggest that the predisposing effect of rootstock on peach susceptibility to bacterial canker may be very limited compared with the predisposing effect by ring nematodes. The overall lesion lengths developed within a six-week period after inoculations were not dependent on the length of incubation, suggesting that the shoot materials were not especially susceptible to *P. syrinage* pv. *syringae* infection and the influence of rootstock on peach scion susceptibility to the bacterial infection may be of minor importance.

In summary, our data indicate that shoots on Nemaguard rootstock tend to develop larger lesions following bacterial inoculations than those on K119-50 and P30-135 rootstocks in the field under circumstances where shoot calcium concentrations differ substantially. Calcium or the [calcium /nitrogen] ratio appear to be involved in shoot susceptibility to *P. syrinage* pv. *syringae* infection in peach on different rootstocks. Compared with the ring nematode infestation predisposing effect, the rootstock effect on host susceptibility to *P. syrinage* pv. *syringae* infection may play a minor role.

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Influencia del porta injerto, de la temperatura y de la duración de incubación en la gravedad del chancro bacteriano, provocado por *Pseudomonas syringae* pv. *syringae* en el melocotonero.

Introducción. El chancro bacteriano, causado por *Pseudomonas syringae* pv. *syringae* es una enfermedad que provoca daños en los frutos de hueso en el mundo entero. Se evaluó el efecto de los porta injertos, de la temperatura y de la duración de incubación del chancro bacteriano en el melocotonero, empleando a la vez pruebas de inoculación sobre el terreno y en laboratorio. **Material y métodos**. Se realizaron experimentos en vergel y en laboratorio con el fin de estudiar los efectos del porta injertos, de la temperatura y de la duración de incubación en la gravedad de la enfermedad en el melocotonero. Todas las inoculaciones se llevaron a cabo con la cepa B3A de *P. syringae* pv. *syringae*. Las inoculaciones bacterianas se aplicaron en los troncos de los melocotoneros [Prunus persica (L.) Batsch] de 1 año. Tras inoculación, las plántulas inoculadas se dejaron en incubación en vergel o en cámara fría bajo diferentes temperaturas [constantemente a 0 °C, constantemente a 14,4 °C, o con una alternancia de temperatura de 0 °C durante 12 h (noche) y 14,4 °C durante 12 h (día)] para los troncos extirpados. Las lesiones se determinaron entre 1 y 6 semanas tras inoculación de modo a precisar el efecto de la duración de incubación. **Resultados y discusión**. El experimento en vergel mediante el uso de melocotoneros injertados en tres porta injertos (Nemaguard, K119-50 y P30-135) mostró que, entre los tres porta injertos, los troncos en Nemaguard desarrollaban las lesiones más largas y los troncos en K119-50 desarrollaban las lesiones más cortas. Los troncos injertados en Nemaguard mostraron contenidos de calcio en la corteza significativamente más reducidos y las concentraciones de nitrógeno significativamente superiores a las de los troncos injertados en K119-50 y P30-135. Se observó una correlación negativa entre la longitud de la lesión y el contenido de calcio de la corteza, así como entre la longitud de la lesión y la relación [calcio / nitrógeno] de la corteza. Los experimentos en laboratorio con vástagos extirpados de plantones en porta injertos Nemaguard, K119-50, P30-135, Lovell y Gardien, pertenecientes a un segundo vergel, dieron resultados contradictorios. Los troncos en Nemaguard desarrollaron lesiones bastante más pequeñas que las de K119-50 y P30-135. Los troncos en Guardian y Lovell desarrollaron igualmente lesiones bastante más pequeñas que los vástagos en K119-50 et P30-135. La fluctuación de la temperatura durante la incubación (0 °C a 14,4 °C) no tuvo ningún efecto en la longitud de las lesiones, en relación con aquéllas que se incubaban continuamente a 14,4 °C, pero dio lugar a lesiones significativamente más largas que las de los vástagos incubados continuamente a 0 °C. Dichos resultados contradictorios sugieren que, en ausencia de los principales factores predisponentes (por ejemplo, los nematodos redondos o un reducido pH del suelo), los porta injertos pueden desempeñar un pequeño papel en la sensibilidad del melocotonero frente al chancro bacteriano, incluso en condiciones favorables para el desarrollo de la enfermedad.

EUA / *Prunus persica* / enfermedades de las plantas / *Pseudomonas syringae* / infección experimental / portainjertos / temperatura