Influence of Calcium, Nitrogen, and Indoleacetic Acid Applications on Phloem Tissue Susceptibility to *Pseudomonas syringae* pv. *syringae* Infection in Peach Trees Stressed with Ring Nematodes

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ABSTRACT

Two field experiments were conducted to study the effects of added nitrogen, calcium and indoleacetic acid, in the presence or absence of ring nematodes (*Mesocriconema xenoplax*), on susceptibility of peach to bacterial canker. When non-infested soil was inoculated with ring nematodes, peach tree susceptibility to bacterial canker infection caused by *Pseudomonas syringae* pv. *syringae* was dramatically increased after a period of two years. However, no evidence was found that ring nematode infestation increased tree water stress or in turn, altered plant calcium uptake. Soil fumigation with methyl bromide prior to planting in a commercial orchard significantly reduced both nematode populations and peach tree susceptibility to bacterial canker infection, when compared to nonfumigated treatments. In both experiments, tree susceptibility, as measured by canker length following inoculation of stems with *P. s.* pv. *syringae*, was negatively correlated with plant tissue nitrogen content and positively correlated with tissue calcium content. A principal components analysis showed that tissue nitrogen and calcium levels were negatively correlated, and that high nitrogen, low calcium tissues were less susceptible to bacterial canker than low nitrogen, high calcium tissues. These results indicate that the increased susceptibility of peach to *P. s.* pv. *syringae* under nematode infestation

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conditions is mediated by both nutritional effects (primarily nitrogen) and nutritional-independent effects, but do not support previous reports of beneficial effects of calcium for reducing bacterial canker.

INTRODUCTION

Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae*, is a devastating, widespread and economically important disease that can cause dieback of main scaffolds and death of stone fruit trees. Bacterial canker primarily occurs on trees that have been stressed by various biotic and abiotic factors (27). Probably the most important predisposing factor in California is high populations of ring nematodes (*Mesocriconema xenoplax*), which occur in many peach-growing districts, particularly on sandy soils. Lownsbery and coworkers (18,19) found that peach trees, if inoculated with ring nematodes, were more susceptible to *P. s.* pv. *syringae* infection as indicated by the length of cankers that developed after artificial inoculation with the bacterium. Increased susceptibility to bacterial canker was also observed in plum (24), peach (37,42), prune (8), and almond trees (21) stressed with the ring nematodes. However, the mechanism(s) by which ring nematodes induce stress(es) that cause bacterial canker in stone fruit is poorly understood.

Ring nematodes are ectoparasites that can feed on the root cortex cells and induce cellular modifications in host tissues that support sustained ingestion of nutrients by the nematode (11). Ring nematode infestations decreased the number of feeder roots and root volume, stunted growth, and reduced plant height, fresh and dry weight in peach (19,26,35), 'Myrobalan' plum (1), and 'French' prune (8). Sharpe and coworkers (35) reported that ring nematode infestation significantly decreased leaf concentrations of nitrogen, phosphorus, and potassium in peach. In addition, ring nematode infestation significantly increased water stress in plum leaves measured at midday (23,24). Decreased amounts of free amino acids due to high populations of ring

nematodes were also observed in peach roots and stems (25,28), which may suggest a reduction in nitrogen uptake by the damaged roots. These observations suggest that inadequacies or imbalances of mineral nutrients may be induced by high populations of ring nematodes. In addition, Carter (3) reported higher levels of indoleacetic acid in the vascular cambium of peach trees in nonfumigated soil containing ring nematodes compared to those in fumigated soil, and suggested that an imbalance of growth hormones could cause an early breaking of dormancy and in turn predispose trees to cold injury. Ogawa and English (27) speculated that IAA may interact with ice nucleation induced by *P. s.* pv. *syringae* and may be implicated in the bacterial canker syndrome when it is associated with freezing temperatures.

Information regarding mineral nutrition status and its relationship to bacterial canker of stone fruits is limited. English and coworkers (7) reported that more peach trees were killed by bacterial canker in low-nitrogen plots than in high-nitrogen plots located in a sandy orchard in California. Decreased incidence of bacterial canker has also been observed in 'French' prune trees when urea was applied via soil, compared to trees receiving low levels of nitrogen (36). A recent field experiment indicated that a compound fertilization with nitrogen, phosphorus, and potassium significantly decreased disease severity of bacterial canker in 'French' prune (33). A field survey of cherry trees in relation to bacterial canker in Michigan, indicated that leaf samples from most orchards with bacterial canker had insufficient levels of calcium and nitrogen (22). Vigouroux and Bussi inoculated peach growing in acidic soils with *P. s.* pv. *persicae* (40) and apricot with *P. s.* pv. *syringae* (41) during dormancy in France, and found that canker length in the stems was negatively correlated with calcium content in the bark. Calcium is absorbed by feeder roots (4), and water flow is the primary means of its translocation to different parts of the plant (15). Irrigation increases calcium content in bark and has been

reported to improve resistance to bacterial canker in peach (40). Field trials on peach tree short life (a disease in which bacterial canker is one of the major components) have demonstrated that most diseased peach trees had lower leaf calcium content compared to those growing in healthy orchards (10,34). However, the role of calcium in stone fruit tree resistance to bacterial canker should be further clarified.

In this study, we tested the following hypotheses: 1) Supplemental application of nitrogen may compensate for nitrogen that is not taken up as a result of nematode feeding, and would decrease the size of cambium lesions in nematode infested peach trees. 2) Nematode feeding may reduce water uptake, causing water stress and reducing calcium uptake. If this is true, supplemental applications of calcium may compensate for the loss of calcium uptake due to nematode feeding, and possibly decrease lesion length in nematode infested trees. 3) Supplemental applications of indoleacetic acid (IAA) might mimic nematode feeding effects, and possibly increase lesion length.

MATERIALS AND METHODS

Plant materials, bacterial strain, inoculation, and disease evaluation. All bacterial inoculations were made on 1- or 2-year-old dormant stems of peach trees (*Prunus persica* (L.) Batsch). Field-grown trees were from either the University of California Kearney Agricultural Center (KAC), Parlier, CA, or from a commercial orchard in Modesto, CA. *P. s.* pv. *syringae* strain B3A (5), routinely maintained in King's medium B (14), was used in all of the inoculations. Bacterial inoculations were performed in the dormant seasons of 2000/2001, 2001/2002 and 2002/2003 (henceforth referred to as 2001, 2002 and 2003, respectively) using a procedure described previously (2). The inoculated sites on the stems were wrapped with parafilm to retain moisture. Inoculated stems were in the field as intact stems attached to trees during incubation. The incubation period for canker development was seven or eight weeks post inoculation. The extent

of canker development was evaluated by cutting the stem tangentially and measuring canker length with a digital caliper. In all cases, statistical separation of means was performed using log transformed values of lesion length (2). Extracts from some lesions were plated on King's medium B to confirm the presence of *P. s.* pv. *syringae* that was characterized with blue fluorescence under UV light (354 nm), negative oxidase reaction (16), and positive hypersensitivity reaction in tobacco (17).

Ring nematode inoculation at KAC. Eighteen plastic tanks (3.7 m × 2.4 m × 0.8 m deep) were arranged in 9 pairs (blocks), dug into the ground and filled with a locally-collected sandy soil (98% sand, 2% silt + clay). The pH in the tanks ranged from 6.8 along the surface 30-cm layer to 7.3 in the layer from 30 to 60 cm in depth. On March 20, 2000, the sandy soil was fumigated with methyl bromide (MB 0.68 kg/tank) to a depth of 45 cm and was covered with a plastic tarp for two weeks. On April 18, 2000, four 1-year-old peach trees (cv. Elegant Lady/Nemaguard) were planted in each tank, and on May 12, 2000, approximately 10,000 juvenile ring nematodes (*M. xenoplax*) were inoculated into the soil surrounding each tree in one randomly selected tank in each block.

Each of the trees in every tank was randomly assigned to one of four supplemental treatments, applied at 3- to 4-week intervals in June, July, August, and September of 2000 and 2001 respectively: 1) calcium trunk injection, 2) urea foliar application, 3) IAA foliar application, and 4) an untreated control. About 200 ml of 10 mM $CaSO_4$ were injected into the trunk each time (4 injections per year for a 2-year period) using the procedure described by Sánchez-Zamora and Fernández-Escobar (32), and approximately 1500 ml each of urea solution (1.1%) or IAA (10 ppm) were applied per tree as foliar sprays (4 sprays each per year for a 2-year period). Prior to application, 100 ppm stock solutions of IAA (Sigma) were freshly

prepared in 1 mM Na_2HPO_4-citrate buffer (pH 6.0) plus 0.2% Tween 20 (43), and a 10-fold dilution with water was used for spray. The experimental design was a split plot, randomized complete block, having with/without nematode tanks as main plots and trees as subplots.

Stem water potential (20) of lower canopy, shaded leaves was measured with a pressure chamber (Soil Moisture Equipment Corporation, Santa Barbara, CA), periodically through the season at midday, and on one occasion (October 4, 2001) over a diurnal cycle. Aluminized mylar bags were used to cover leaves for one hour prior to measurement, although preliminary experiments showed no change after 30 minutes of covering. Main stem (trunk) diameter was measured at a height of 5 cm above the soil line with a caliper at planting (April 18, 2000), and on January 9 in 2001 and 2002 respectively, and an equivalent circular circumference was calculated. Calcium and nitrogen analysis (see below) was performed on 10 to 15 mature leaves per tree harvested in September, 2000 and 2001 respectively, and on stem samples harvested prior to inoculation in January 2001 and 2002 respectively. Bacterial inoculations were performed in January, 2001 (one inoculation per tree) and 2002 (four inoculations per tree), and canker lesion length was recorded after about 60 days. Ring nematodes (*Mesocriconema xenoplax*) were extracted from soil samples collected in January 2002 using a centrifugal-flotation technique (12).

Soil fumigation, and supplemental nutrient application experiment in a commercial peach orchard in Modesto. Preplant soil fumigation treatments designed to reduce nematode populations were applied to a commercial peach orchard on sandy soil (88% sand, 8% silt, 4% clay) in Modesto, CA, which had successive generations of peach during the past 40 to 45 years. Fumigation was applied in October 2000 to 19.8-meter-wide strips perpendicular to the row direction and included methyl bromide (448 kg/ha) and an untreated control. Trees (cv. Loadel/Lovell) were planted on February 2, 2001 (918 trees/ha), and soil samples were collected on October 3, 2001

for extraction of both ring and root-lesion (*Pratylenchus vulnus*) nematodes. Main stem (trunk) circumference was measured at a height of 5 cm above the graft line with a steel tape on December 18, 2002.

Supplemental nitrogen (N) or calcium (Ca) treatments, beyond the grower's normal fertility program, were made to some trees in both MB fumigated and non-fumigated plots. The normal fertility practice (grower control) was application through the irrigation system of a total of 67.3 kg N/ha in the form of calcium nitrate and UAN-32 (32% nitrogen) in 2001 and 123.2 kg N/ha in the form of CAN-17 (17% nitrogen, 8.8% calcium, Chevron Co., Richmond, CA), and UN-32 in 2002. In 2001 there was no supplemental nitrogen applied, but trees in the supplemental Ca treatment were fertilized with oyster shell calcium flour (4.54 kg/tree), foliar micronutrients (5.6 kg/ha, RNA Microphos Response®, containing 15% P_2O_5, 30% potash, 7.7% Ca, 7.0% sulfur, 0.25% boron, and 10.0% zinc), and calcium chloride (1.1 kg Ca/ha). In 2002, in addition to 123.2 kg N/ha applied by the grower, the supplemental N treatment included applications through the irrigation system of 52.6 kg N/ha in the form of CAN-17 and UAN-32, and 56.0 kg N/ha in foliar applied low biuret urea; and 13.5 kg Ca/ha was applied to the supplemental Ca treatment in the form of calcium chloride through two irrigation injections and three foliar sprays.

For this study the experimental design consisted of preplant soil treatments as main plots and postplant nutrient supplements as subplots. Each of 4 replicated subplots consisted of 10 consecutive trees in two adjacent rows, with centrally located six trees in each row used for study. Bacterial inoculations were made into 1-year-old dormant stems (one inoculation per tree) on January 24, 2002 and December 18, 2002 and lesion length was recorded after about 50 days of incubation under ambient field conditions. Prior to inoculation in December 2002, 1-

year-old stems were sampled for calcium and nitrogen analysis. For canker length, measurements were made on the centrally located six trees from each row with the mean of each row considered as a subsample, but the mean of the two subsamples per replicate was used in the statistical analysis. For nutrient analyses, subsample measurements were performed on pooled 1-year-old stems that were sampled from the centrally located six trees of each row, and the mean of the two subsamples per replicate was used in the statistical analysis.

Nitrogen, carbon, and calcium analysis. Leaf and bark samples collected from the KAC and Modesto experiment sites were washed, oven-dried, and ground based on a standard procedure (13). Total leaf and bark nitrogen was determined by a combustion gas analysis method (30, 31) in which 2 to 3 mg of plant sample wrapped in a tin foil was combusted in an element analyzer (NA 1500, Fisons Instruments, Italy). Leaf and bark calcium concentrations were determined using an atomic absorption spectrometer (Analyst 800, Perkin Elmer Instruments) (38).

Data analysis. Data were analyzed for statistical significance using the general linear model (GLM) procedure (Statistical Analysis System; SAS Institute, Cary, NC). For canker length data, log transformed values were used when appropriate (9), but in some cases the means of both the transformed and the original values will be presented for clarity.

RESULTS

Ring nematode inoculation at KAC. In 2001, after a single season of growth in nematode infested soil, there was no difference in the development of bacterial canker associated with the presence of nematodes or with any of the additional nutrient treatments (Fig. 1, ANOVA not shown). In 2002, the lesion length that developed in non-infested soil was somewhat larger than that which developed in 2001 (Fig. 1), but a very large increase in lesion length was clearly associated with nematode infestation across all supplemental treatments (Fig. 1). In 2002, some of the lesions were long enough to coalesce and extended from the four inoculated branches to the central

trunk, so that expressing the canker length per inoculation, as was done in 2001, can be regarded as a conservative estimate of the extent of canker development. Analysis of variance of lesion length in 2002 indicated a highly significant nematode effect, but the block, treatments (calcium injection, IAA and urea foliar sprays), and the interaction between nematode and treatment were not significant (Table 1). Ring nematode counts for soil samples taken on January 10, 2002, averaged 896 nematodes per liter soil in the nematode infested tanks and 0 in the control tanks.

Nematode infestation had no detectible effect on overall tree growth, as measured by the increase in trunk diameter (Fig. 2), and no effect on midday stem water potential (SWP) was observed in either experimental year at KAC (Fig 3). In both years, SWP was substantially below (more stressed than) the value expected for a fully irrigated prune or almond tree in June/July, but gradually increased to this value over the growing season (Fig. 3). Stem water potential clearly responded to the normal diurnal changes in evaporative demand, but at no time in the diurnal cycle was there any evidence of a nematode infestation effect (Fig. 4).

Both bark and leaf nitrogen content responded positively to foliar applied urea, and negatively to the presence of nematodes, although the separation in treatment means was less clear for bark than for leaf nitrogen (Table 2). Foliar applied urea raised leaf N content under nematode infested conditions to significantly above that of control, non-infested conditions, more than compensating for the nematode effect (Table 2). For bark N content there were no statistically significant treatment effects, but a compensatory effect of foliar applied urea for nematode infestation also occurred. Calcium injection treatment did not significantly increase calcium content in leaves or bark (data not shown). To quantify the movement of calcium after trunk injection, 5-cm trunk sections were analyzed at the point of injection and 15 cm and 30

cm above the injection site, respectively. The calcium content at the injected site was significantly higher in the treated trees than in the control trees (0.35% versus 0.28%, respectively), and at the site 15 cm above the injected site (0.32% versus 0.21%, respectively), but was not different in the section 30 cm above the injected site (data not shown), presumably reflecting a relatively low mobility of the applied calcium.

Soil fumigation, and supplemental nutrient application in Modesto. As in the KAC experiment, after the first year there was no significant difference in lesion length (ANOVA not shown) between trees growing in fumigated versus non-fumigated soil (38.2 mm versus 21.7mm average lesion length, respectively, in 2002). These lesions were also of a similar length to those developed in the first year of the KAC study (Fig. 1). Analysis of variance of lesion length induced by bacterial inoculation after two years however, showed significant differences for MB fumigation, supplemental nutrients (calcium and urea fertilizations), and the interaction between soil fumigation and supplemental nutrients (Table 3). In all cases, both soil fumigation and supplemental nutrients numerically reduced lesion length compared to their respective controls (Table 4), with the significant interaction term (Table 3) due to a larger reduction in lesion length by nutrients under non-fumigated conditions than under fumigated conditions (Table 4). Fumigation had significantly reduced the levels of ring nematodes in the soil (0 to 1.5 m in depth) at the end of the first season of tree growth, from 523 nematodes per liter soil in the untreated control to 4 ring nematodes per liter soil in the MB fumigated treatment. MB fumigation also significantly reduced root-lesion nematode populations (data not shown)

In contrast to the KAC experiment, tree growth as measured by trunk diameter was significantly ($p = 0.017$) greater in the fumigated (7.8 cm), compared to the non-fumigated (5.0 cm) treatment, although growth was not affected by any of the supplemental nutrient treatments

(data not shown). Supplemental nitrogen significantly increased bark nitrogen content compared to the control trees under both fumigated and nonfumigated conditions (Table 4). However, bark calcium content did not significantly increase in response to supplemental calcium under either condition (Table 4).

Weather conditions during incubation. Temperature conditions, as measured by both hours below 0^{o}C and average daily minimum, were colder during the second year of the KAC experiment compared to the first, whereas the opposite was the case at the Modesto site (Table 5). At both sites, increased rainfall was associated with a decreased temperature range experienced, with the first year being wetter than the second for the KAC site, and drier than the second at the Modesto site (Table 5).

Correlations between lesion length and plant tissue nitrogen and calcium content. Lesion length was negatively correlated with bark nitrogen content in the Modesto experiment and also negatively correlated with bark and leaf nitrogen content in the KAC experiment (Table 6). The correlations between lesion length and bark nitrogen content were consistent for the two experiments in Modesto and KAC in that smaller lesion lengths were associated with higher bark nitrogen contents. Lesion length was positively correlated to leaf and bark calcium in both locations, although only significantly so at KAC (Table 6).

A principal components (PC) analysis was performed for the control (non-sprayed or injected with supplemental nitrogen, calcium or IAA) trees with and without nematodes at the KAC site, for which leaf and bark nitrogen and calcium levels had all been determined in 2001 and 2002. The matrix of correlation coefficients showed that for the same element, leaf and bark levels were always positively correlated, but that the two elements were always negatively correlated (increases in nitrogen associated with decreases in calcium) independent of tissue

type (Table 7). Because of the correlations within and between elements, the first PC explained most (72%) of the systematic variance in nitrogen and calcium levels (Table 8). Based on this first PC, the loadings (correlation coefficients) for nitrogen were always positive and for calcium were always negative, with the first PC capturing between 53 and 85% of the variance, depending on the element and tissue type (Table 9). A bi-variate plot showed that the two years of data mainly differed in terms of the first PC, with generally high nitrogen in bark and leaf tissues and low leaf calcium in 2001, and the opposite tissue condition in 2002 (Fig. 5). For these data, a strong negative correlation was found between lesion length and the first PC (Fig. 6), consistent with the negative and positive correlations that were found for nitrogen and calcium, respectively, across all treatments and at both sites (Table 6). The first PC (Fig. 6) was used as a covariate to compare the lesion length of nematode infested and nematode free trees, essentially adjusting them to the same mineral nutrition levels. The slope of the relation between $\log_{10}$(lesion length) and the first PC was not influenced by the presence of nematodes (no heterogeneity of slope), but the adjusted $\log_{10}$(lesion length) was significantly greater for nematode infested trees (2.20) than for nematode free trees (1.78, $p<0.0008$, ANCOVA not shown). These results indicate that the increased susceptibility to *P. s.* pv. *syringae* under nematode infestation conditions is mediated by both nutritional effects and nutritional-independent effects.

DISCUSSION

Ring nematode infestation played a significant role in predisposing peach trees to *P. s.* pv. *syringae* infection. A clear increase in susceptibility to *P. s.* pv. *syringae* infection, as measured by lesion length, was associated with nematode presence at KAC (Fig. 1, Table 1),

and a clear reduction in susceptibility was associated with soil fumigation that reduced nematode numbers in Modesto (Table 3). These results are in agreement with the results of previous studies on peach (18,19), plum (24), and prune trees (8). Significant increases in susceptibility to bacterial canker were observed in peach trees within a 1-year-period in an experiment using potted plants when the plants were inoculated with 20,000 ring nematodes per plant (19), thus it is possible to achieve nematode predisposing effects on bacterial canker within a short period if plants are exposed to very high densities of ring nematodes. Our results indicate that a 2-year-period was necessary to predispose trees to severe bacterial canker when inoculated with 10,000 juvenile ring nematodes per tree. A similar duration to achieve the nematode predisposing effect was also observed in the Modesto experiment site.

The predisposing effect of ring nematode infestation has been recognized for many decades (24), and is often attributed to the occurrence of plant stress (27), but the mechanism of predisposition and the specific stress or stresses involved are unclear and many alternatives have been suggested. Overall tree growth should integrate the effects of both biotic and abiotic stresses, and treatment effects on tree growth were found in Modesto but not in KAC, even though susceptibility differences due to nematodes were observed at both locations. Hence, our data do not support the hypothesis that overall tree stress is required for predisposition to bacterial canker disease. Mojtahedi and coworkers (24) found that nematode infestation was associated with increased levels of water stress in plum trees at midday, but not at night, consistent with the expectation that damage to roots caused by nematode feeding might be most apparent under conditions of transpiration. It is also reasonable to assume that tree calcium nutrition may be influenced directly by nematode feeding or indirectly by plant water relations effects (41). However, the data in our study indicated that the clear predisposing effects of

nematode feeding on tree susceptibility to bacterial canker occurred in the absence of either a water stress effect or reduced tissue levels of calcium.

Temperature is important in bacterial canker development (29), with a reported optimum temperature of 18.2^{o}C in the greenhouse (6), very close to the average daily maximum temperatures recorded at both experimental sites (Table 6). Severity of bacterial canker increased when peach branches or shoots were exposed to freezing temperatures a few days after inoculation with *P. s.* pv. *persicae* (39), and Ogawa and English (27) suggested that IAA induced cambial activity may predispose this tissue to ice nucleation at freezing temperatures, and hence promote the spread of *P. s.* pv. *syringae* and subsequent disease development. We found no evidence that foliar applied IAA increased cambial activity (trunk growth, data not shown) or susceptibility of trees to bacterial canker (Table 1). In one-year-old potted peach seedlings, we found that IAA application did significantly increase vegetative growth, but did not influence the development of bacterial canker, even when inoculations were performed during freezing/thawing (data not shown). In the KAC experiment, no conspicuous cold or freezing injury was found in the IAA treated trees compared to the control plants either year, although freezing/thawing temperatures were recorded one week post bacterial inoculations (Table 6). Collectively, these results do not support the hypothesis (27) that plant tissues are predisposed to cold injury by the interaction of IAA and the ice nucleation properties of *P. s.* pv. *syringae,* increasing the severity of bacterial canker.

Reductions of plant nitrogen concentrations in response to ring nematode infestation have been reported in peach (35) and plum (23,24). In this study, ring nematode infestation was always associated with lower nitrogen levels in both tissues that were evaluated (leaves and bark), although in many cases, particularly in bark tissue, the differences were small and not

statistically significant. Supplemental nitrogen fertilization has reduced bacterial canker disease in peach (7) and 'French' prune (36), and of the two factors investigated in this study (nitrogen and calcium), only nitrogen showed a reduction in lesion length associated with an increase in nutrient concentration (Table 6). Presumably, the positive correlation of lesion length to calcium concentration (Table 6) was only the result of the overall negative correlation between calcium and nitrogen (Table 7), and together with the principal components analysis (Figs. 5 and 6) reinforces the hypothesis that increased tissue nitrogen decreases host susceptibility to *P. s.* pv. *syringae*. Hence, we may conclude that supplemental nitrogen is a reasonable cultural practice to reduce peach tree susceptibility to bacterial canker, but it is also clear that there are additional physiological factors which predispose peach trees to this disease and which change as a result of nematode feeding, independently of tissue nitrogen status.

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TABLE 1. Analysis of variance for log-transformed lesion length observed in the dormant season of 2002 in peach (cv. Elegant Lady/Nemaguard) at KAC. Lesions were evaluated two months post inoculation with *P. syringae* pv. *syringae*.

Source	Degrees of freedom	Mean square	Prob>F
Nematode[x]	1	8.420	<0.0001
Block	8	0.023	>0.2414
Nematode × Block (Error A)	8	0.025	
Treatment[y]	3	0.020	>0.3283
Nematode × Treatment	3	0.013	>0.5046
Error B	48	0.017	

[x]Nematode: Testing for the effect of nematode inoculation.

[y]Treatment: Testing for the effect of calcium injection, IAA and urea foliar application.

TABLE 2. Effect of urea foliar spray on tissue nitrogen content in peach trees growing in 2001 at KAC (cv. Elegant Lady/Nemaguard)

Supplemental treatment	Soil treatment	No. of replicates	Bark N (%)[z]	Leaf N (%)[z]
Urea spray	Nematode inoculation	9	1.26 ± 0.05 a	2.10 ± 0.11 b
Control	Nematode inoculation	9	1.08 ± 0.07 a	1.46 ± 0.03 c
Urea spray	MB fumigation	9	1.35 ± 0.10 a	2.68 ± 0.14 a
Control	MB fumigation	9	1.32 ± 0.09 a	1.69 ± 0.09 c

[z] Mean ± 1 standard error. Means followed by the same letter are not different at $P<0.05$ based on Tukey's Studentized Range Test.

TABLE 3. Analysis of variance for log-transformed lesion length observed in the dormant season of 2003 in peach in Modesto (cv. Loadel/Lovell). Lesions were evaluated seven weeks post inoculation with *P. syringae* pv. *syringae*.

Source	Degrees of freedom	Mean square	Prob>F
Methyl Bromide (MB)[x]	1	1.217	<0.0281
Block	3	0.116	>0.1436
MB × Block (Error A)	3	0.076	
Treatment[y]	2	0.482	<0.0039
MB × Treatment	2	0.326	<0.0145
Error B	12	0.053	

[x]Nematode: Testing for the effect of methyl bromide fumigation.

[y]Treatment: Testing for the effect of calcium and urea fertilization.

TABLE 4. Effect of preplant soil fumigation and supplemental nitrogen (N) and calcium (Ca) on bark N and Ca levels, and lesion length in Modesto (cv. Loadel/Lovell). Inoculations with *P. syringae* pv. *syringae* were made on December 18 2002, and lesions were evaluated on Feb. 7 2003. Stem samples for N and Ca determination were taken on December 18 2002.

Treatment combination	Original canker lesion length (mm)[y]	Log- mean lesion length[z]	Bark N (%)[y]	Bark Ca (%)[y]
Control/Nonfumigated	283.1 ± 111.4	2.292 a	1.74 ± 0.03 c	2.12 ± 0.15 ab
Calcium/Nonfumigated	100.1 ± 65.0	1.740 ab	1.83 ± 0.08 bc	2.66 ± 0.31 a
Nitrogen/Nonfumigated	27.9 ± 6.5	1.407 b	2.01 ± 0.05 ab	1.77 ± 0.13 b
Control/Fumigated	26.9 ± 4.5	1.400 b	1.75 ± 0.03 c	2.22 ± 0.07 ab
Calcium/Fumigated	24.7 ± 3.0	1.381 b	1.91 ± 0.03 bc	2.06 ± 0.20 ab
Nitrogen/Fumigated	20.5 ± 1.4	1.307 b	2.15 ± 0.04 a	2.08 ± 0.22 ab

[y] Mean ± 1 standard error. Means are followed by the same letter are not different at $P<0.05$ based on Tukey's Studentized Range Test.

[z] Log-transformed data = $\log_{10}$(original canker length).

TABLE 5. Air temperature and precipitation recorded at the nearest weather stations to the experiment sites during the period of canker development.

Location	Incubation period	Average air temperature[z] (°C)		Daily air temperature range (°C)	Hours below 0 °C	Precipitation (mm)
		Daily minimum	Daily maximum			
KAC[x]	Jan. 10 2001 to Mar. 13 2001	3.7 ± 3.0bc	14.1 ± 3.1ab	-1.9 – 20.5	34	108.7
KAC	Jan. 09 2002 to Mar. 09 2002	2.8 ± 3.3ab	15.0 ± 4.9b	-2.9 – 24.9	85	25.9
Modesto[y]	Jan. 24 2002 to Mar. 11 2002	1.9 ± 3.6a	15.3 ± 3.5b	-4.3 – 20.9	101	29.0
Modesto	Dec. 18 2002 to Feb. 07 2003	4.5 ± 3.1bc	12.5 ± 2.8a	-4.0 – 17.3	19	56.8

[x] Data obtained from a local weather station (CIMIS #39, Parlier).

[y] Data were obtained from a local weather station (CIMIS #71, Modesto)

[z] Means ± 1 standard deviation. Means followed by the same letter are not different at $P<0.05$ based on Student's t-test.

TABLE 6. Correlation analysis between canker lesion length (transformed[y]) and nitrogen or calcium content (cv. Loadel/Lovell, Modesto; Elegant Lady/Nemaguard, KAC)

Location	Independent variable	No. of sample[z]	Slope	R^2	Prob>F
Modesto	Bark nitrogen	24	-1.314	0.286	<0.0071
KAC	Bark nitrogen	72	-0.924	0.338	<0.0001
KAC	Leaf nitrogen	72	-0.611	0.439	<0.0001
Modesto	Bark calcium	24	+0.151	0.025	0.463
KAC	Bark calcium	72	+0.409	0.241	<0.0001
KAC	Leaf calcium	72	+0.605	0.536	<0.0001

[y] Transformed canker lesion length = $\log_{10}$(original canker lesion length).

[z] In Modesto, the sample number included all 4 replications in nitrogen and calcium treatments and the control in both MB fumigated and untreated areas. In KAC, the number of samples were the pooled data for 2001 and 2002.

Table 7. Correlation coefficient matrix for bark and leaf N and Ca levels in control trees with and without nematodes at KAC for 2001 and 2002.

	Bark Ca	Bark N	Leaf Ca	Leaf N
Bark Ca	1	-0.47	+0.50	-0.57
Bark N		1	-0.66	+0.71
Leaf Ca			1	-0.80
Leaf N				1

Table 8. Eigenvalues of the correlation matrix shown in Table 7, and the relative proportion of variance attributable to that eigenvalue (sum of proportions = 1).

Order	Eigenvalue	Proportion
1	2.877	0.72
2	0.584	0.15
3	0.352	0.09
4	0.187	0.04

Table 9. Loadings (correlation coefficients) and communality estimates (% variance) for a single PC analysis of the correlation matrix shown in Table 7.

Measure	First PC loading	Communality estimate (% variance)
Bark Ca	-0.73	53%
Bark N	+0.84	71%
Leaf Ca	-0.89	79%
Leaf N	+0.92	85%

Figure legends:

Figure 1. Mean (N = 9) canker lesion length (non-transformed) ± the approximate 95% confidence interval for ring nematode infestation and post planting treatments over 2 years in the KAC experiment. In 2001 there were no significant differences (based on log-transformed values) between trees with and without nematodes for any treatment combinations. In 2002, lesion lengths were significantly greater in trees stressed with ring nematodes than in trees free of nematodes. Canker lesions were also significantly longer in 2002 than in 2001for trees stressed with ring nematodes.

Figure 2. Tree size, as measured by trunk diameter, from planting until the end of the experiment, under nematode free and nematode infested conditions at KAC. Values shown are the mean ± 2SE (N = 36).

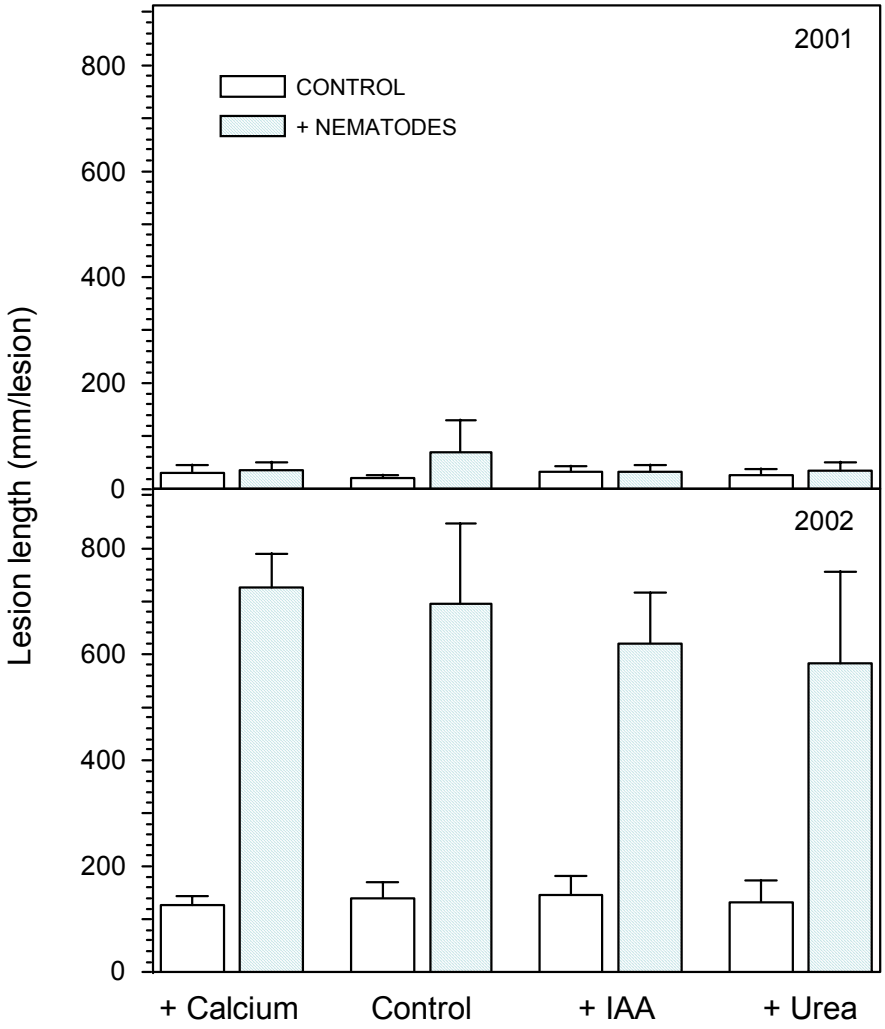
Figure 3. Seasonal patterns in midday stem water potential (SWP) in 2000 and 2001 for trees under nematode free and nematode infested conditions at KAC. Values shown are the mean ± 2SE (N = 9). Also shown for reference is the midday SWP expected for a fully irrigated (baseline) prune or almond tree under the same midday conditions of temperature and humidity.

Figure 4. Diurnal pattern in SWP for trees under nematode free and nematode infested conditions at KAC on October 4, 2001. Values shown are the mean ± 2SE (N = 9).

Figure 5. Bivariate plot of the first two principal components (PC1, PC2) for each of the 9 replicate control trees at KAC with and without nematodes in 2001 and 2002. Also shown are

the Eigenvectors for the measurements of bark calcium (BC) bark nitrogen (BN), leaf calcium (LC) and leaf nitrogen (LN).

Figure 6. Correlation of $\log_{10}$(lesion length) to the first principal component (PC1) for each of the 9 replicate control trees at KAC with and without nematodes in 2001 and 2002. Line shown is a linear regression ($R^2 = 0.60$, $p<0.0001$).



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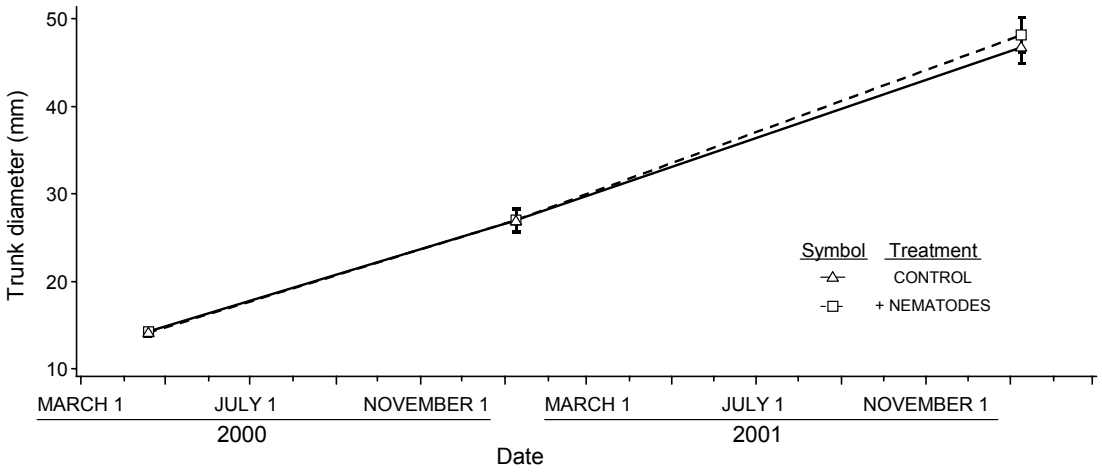

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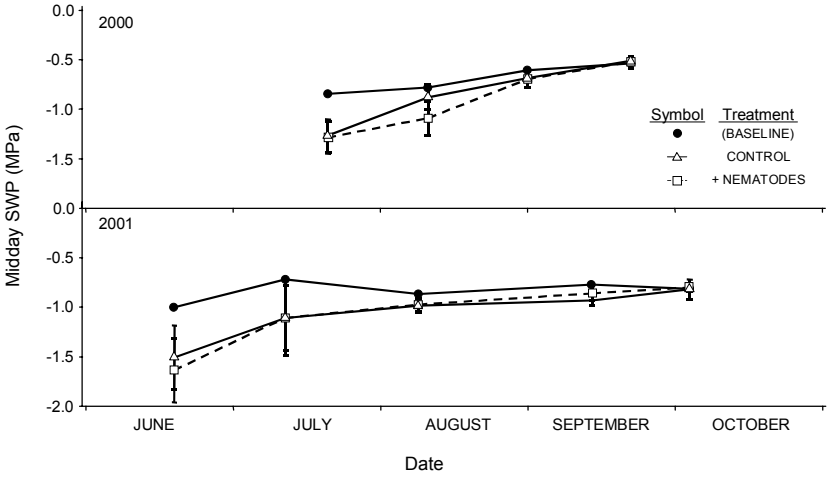

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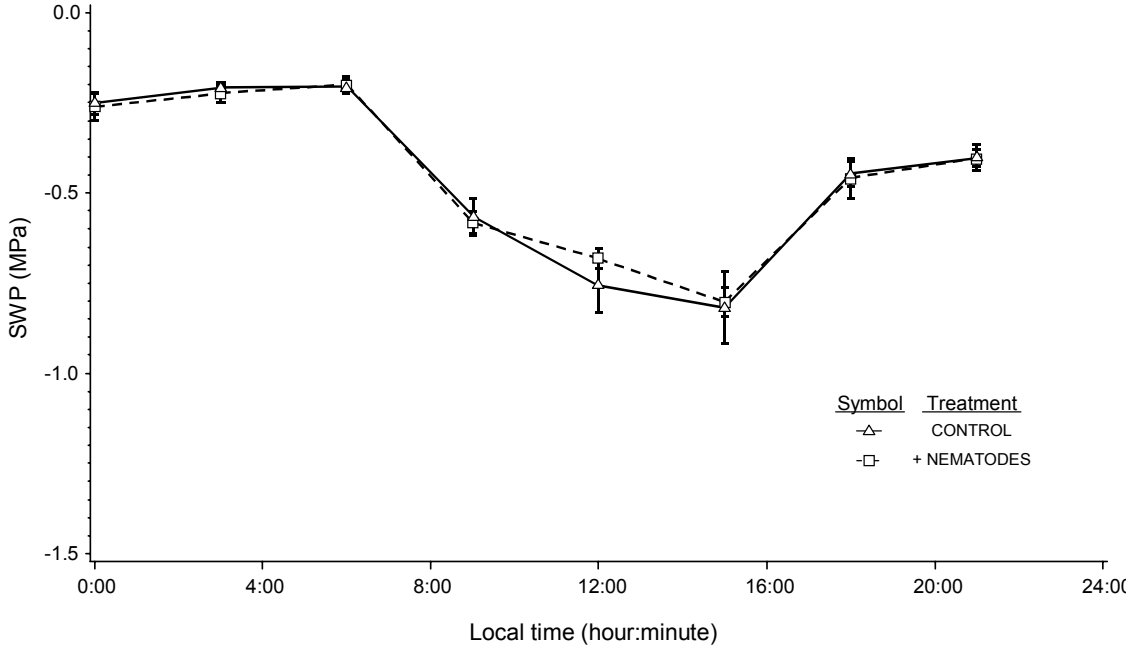

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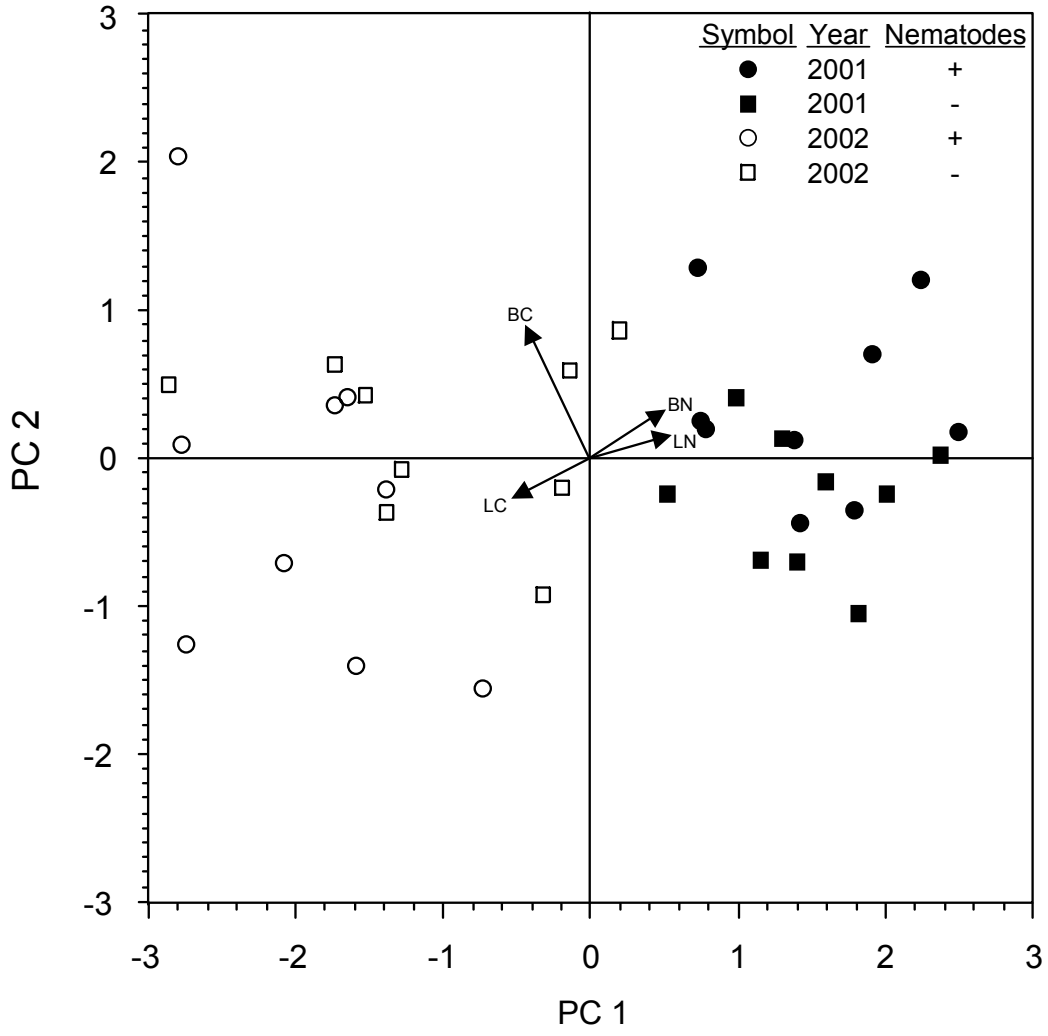

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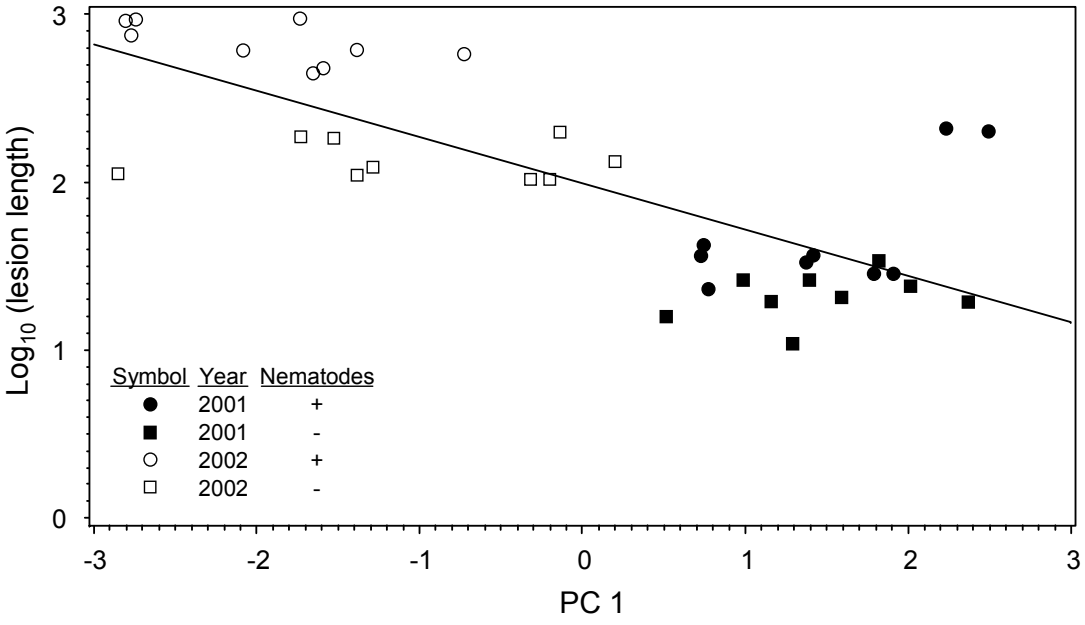

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