Interaction Between Nitrogen-Fertilized Peach Trees and Expression of *syrB*, a Gene Involved in Syringomycin Production in *Pseudomonas syringae* pv. *syringae*

Tiesen Cao, Roger A. Duncan, Michael V. McKenry, Kenneth A. Shackel, Theodore M. DeJong, and Bruce C. Kirkpatrick

First author: Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada; second author: University of California Cooperative Extension, Modesto 95358; third author: University of California Kearney Agricultural Center, Parlier 93648; fourth and fifth authors: Department of Pomology, and sixth author: Department of Plant Pathology, University of California, Davis 95616.

Accepted for publication 28 January 2005.

ABSTRACT

Cao, T., Duncan, R. A., McKenry, M. V., Shackel, K. A., DeJong, T. M., and Kirkpatrick, B. C. 2005. Interaction between nitrogen-fertilized peach trees and expression of *syrB*, a gene involved in syringomycin production in *Pseudomonas syringae* pv. *syringae.* Phytopathology 95:581-586.

The in vitro expression of the *syrB* gene that controls the synthesis of syringomycin, a non-host-specific phytotoxin produced by *Pseudomonas syringae* pv. *syringae* van Hall, was studied using aqueous extracts derived from bark tissues collected from nitrogen-fertilized and nonfertilized peach trees. Expression of the *syrB* gene was quantified as β-galactosidase activity expressed by *P. syringae* pv. *syringae* B3AR-132 containing a *syrB*::*lacZ* fusion. Gene expression was significantly less in three of four paired comparisons using extracts derived from fertilized versus nonfertilized trees; however, canker lengths were significantly different in only one of four comparisons. Expression was negatively correlated with plant tissue nitrogen content and positively correlated with a plant carbon/nitrogen ratio. Bark tissue from ring nematode-infested trees had significantly higher concentrations of total soluble phenolic compounds and carbon/nitrogen ratios than bark samples from trees without nematodes, and canker size was significantly greater in trees growing in ring nematode-infested soil compared with noninfested soil. Nitrogen fertilization significantly decreased the plant carbon/nitrogen ratio, which was positively correlated with the concentration of total soluble phenolic compounds. Canker size developing after bacterial inoculation was positively correlated with higher plant carbon/nitrogen ratios and total soluble phenolic compounds. These results support the hypothesis that one reason why nitrogen fertilization decreases host susceptibility to bacterial canker is by either reducing the amount of plant metabolites that can induce *syrB* gene expression, or producing or increasing the concentration of compounds that antagonize *syrB* inducing compounds.

The bacterium *Pseudomonas syringae* pv. *syringae* causes bacterial canker of stone fruit. *P. syringae* pv. *syringae* produces syringomycin (25), a cyclic lipodepsinonapeptide phytotoxin that kills host cells by disrupting cation exchange across the plasma membrane (11) and, subsequently, causes acidification of the cytoplasm (2). Inoculation with purified syringomycin caused peach stem necrosis that was morphologically indistinguishable from cankers produced by *P. syringae* pv. *syringae* (6). Syringomycin synthesis in *P. syringae* pv. *syringae* is controlled by the *syrB* gene (15) that is associated with the formation of two proteins, SyrB1 (68 kDa) and SyrB2 (35 kDa), of which SyrB1 protein is a peptide synthetase involved in syringomycin synthesis (2). Disease severity produced in cherry fruit following inoculation with *P. syringae* pv. *syringae* strain W4S770, a syringomycin-defective mutant containing a Tn5 insertion in the *syrB* gene, was approximately half that of the parental strain that contains an active *syrB* gene (28), which suggests that syringomycin significantly contributes to *P. syringae* pv. *syringae* virulence. A syringomycin synthesis indicator strain, B3AR-132, which contains a transcriptional fusion between the *syrB* and *lacZ* genes, can be used to assess the *syrB* promoter activity by quantifying β-galactosidase activity (16). Thus, this indicator strain is useful for quantifying *syrB* gene expression using plant-derived inducing compounds (12).

Several inducer compounds in plants can affect expression of syrB. Certain phenolic β-glucosides, such as arbutin, can induce the synthesis of syringomycin, and a few sugars, such as D-fructose, also can enhance the induction activity (15,21). The induction of the *syrB*::*lacZ* fusion by plant extracts correlates with the induction of the *syrB* gene during pathogenesis because factors that regulate expression of syringomycin production similarly control expression of the *syrB*::*lacZ* fusion (16).

Nitrogen fertilization has been proposed as an important factor that reduces peach (7) and 'French' prune (23,26) susceptibility to bacterial canker. However, the mechanism by which nitrogen fertilization reduces host susceptibility to bacterial canker is not well understood. One hypothesis that might explain why nitrogen fertilization affects plant susceptibility is called the carbon/nutrient balance theory (3). This theory suggests that concentrations of secondary plant compounds are related to the carbon/nitrogen ratio of a plant. When nitrogen availability is low, plant growth decreases more quickly than photosynthesis. Carbohydrates accumulate above levels required for growth under such conditions and high carbon/nitrogen ratios result. The accumulated carbohydrates then are redirected into the synthesis of carbon-based compounds such as phenolics (10). It may be possible that increased concentrations of phenolic compounds in response to nitrogen deficiency could play a role in predisposing stone fruit trees to bacterial canker disease.

Corresponding author: B. C. Kirkpatrick; E-mail address: bckirkpatrick@ucdavis.edu

DOI: 94.1094/PHYTO-95-0581

In this research, expression of the *syrB* gene was quantified in vitro by determining β-galactosidase activity expressed by *P. syringae* pv. *syringae* B3AR-132 in response to exposure to aqueous bark extracts from nitrogen-fertilized, low-nitrogen, and non-fertilized peach trees growing in soils with or without ring nematodes. The effects of nitrogen fertilization and nematode infestation on the quantities of plant carbon, nitrogen, and soluble phenolic compounds also were examined and correlated with the size of cankers that developed after inoculation with *P. syringae* pv. *syringae*.

MATERIALS AND METHODS

Plant materials, treatments, and field bacterial inoculation. Two field experiments were the source of peach tissues used in this study. In April 2000, 1-year-old peach trees (*Prunus persica* (L.) Batsch, cv. Elegant Lady grafted on Nemaguard rootstocks) were planted in 18 tanks (tank dimension: 3.7 by 2.4 by 0.8 m) filled with methyl bromide-fumigated sandy soil (100% sand) at the University of California, Kearney Agricultural Center (KAC), Parlier. In May 2000, approximately 10,000 juvenile ring nematodes (*Mesocriconema xenoplax*) were inoculated into the soil around the four trees growing in each tank. Nine tanks were inoculated with ring nematodes and nine served as uninoculated controls. The nitrogen fertilization treatment included four foliar sprays of 1.1% urea per year from June to September for a period of 2 years. Within each tank, one tree was treated with foliar urea and one tree was an untreated control. The experimental design was a split plot with randomized complete block main plots of tanks (nine blocks) with ring nematode inoculations (nematode inoculation versus nematode free) and subplots of trees (urea foliar spray versus untreated control). One-year-old stems were inoculated with *Pseudomonas syringae* pv. *syringae* strain B3A (6) in January 2001 and 2002 using a pinprick inoculation procedure described previously (5). The inoculated plants were grown under field conditions for 2 months. The lesions beneath each inoculation site were measured by tangentially cutting off the outer bark. Leaf samples (10 to 15 leaves per tree) were taken in September 2000 and 2001. Stem tissue samples were taken in January 2001 and 2002 just prior to bacterial inoculation. After a period of two growing seasons, the average ring nematode population in the nematode-inoculated tanks was 896 per liter of soil versus 0 per liter soil in the uninoculated control tanks.

A second trial was performed in a soil fumigation plot that had been continuously planted with peach (cv. Loadel grafted on Lovell rootstocks) during the past 40 to 45 years, located near Modesto, CA. In February 2001, 1-year-old peach trees were planted in sandy soil (88% sand, 8% silt, and 4% clay) with or without preplant soil fumigation with methyl bromide (MB) at a rate of 448 kg/ha. Average ring nematode population in the non-fumigated soil was 523 per liter of soil compared with 4 per liter of soil in the MB-treated soil. The nitrogen fertilization treatment included applications of nitrogen through irrigation at a rate of 16.8 kg/ha on 26 April 2002 and 22.4 kg/ha on 30 May 2002 in the form of CAN-17 (17% nitrogen, 8.8% calcium; Chevron Co., Richmond, CA), 13.4 kg/ha in the form of UN32 on 9 August 2002, and a foliar application of nitrogen at 56 kg/ha on 29 October 2002 in the form of low-biuret urea (biuret is a nitrogenous impurity formed in the manufacture of urea which may cause leaf burn during foliar application if high-biuret urea is used). In addition, the grower applied CAN-17 and UN32 at 123.2 kg/ha to all trees in the orchard throughout the growing season of 2002; thus, the plants in the low-nitrogen control received CAN-17 and UN32 at 123.2 kg/ha. The entire experimental design resulted in a combination of preplant (MB fumigation versus no fumigation) and postplant treatments (high-nitrogen application versus low-nitrogen control), with four 20-tree replications of each combination in two adjacent rows. One-year-old stems were inoculated as previously described with *P. syringae* pv. *syringae* strain B3A in December 2002. The inoculated plants were grown under field conditions for 7 weeks prior to sampling. Lesion lengths were determined as previously described after completion of the incubation period. Stem tissue samples used for elemental analyses were collected in December 2002 just prior to bacterial inoculation.

Preparation of bark extracts. Bark extracts essentially were prepared following the procedure described by Mo et al. (15). One-year-old dormant stem samples collected from the KAC and Modesto experimental plots were washed with deionized water and air dried for 3 to 5 min at room temperature. Fresh bark tissue was removed from the woody stem and ground in liquid nitrogen, and an aqueous extract was obtained by homogenizing 3 g of frozen bark tissue with 9 ml of deionized water. The homogenate was centrifuged (10,000 × *g*, 10 min) to precipitate insoluble plant material. The supernatant was filter sterilized and adjusted to a final concentration of 75% (vol/vol) acetone, stirred vigorously for 10 min, and centrifuged (10,000 × *g*, 10 min) to pellet insoluble material that was discarded. The acetone was allowed to evaporate in a fume hood overnight and the remaining aqueous extract was stored at –20°C until used. A 10-fold dilution of the aqueous phase was used for the β-galactosidase assay.

Bacterial strains, culture media, and inoculum preparation. *P. syringae* pv. *syringae* strain B3AR-132 containing the *syrB*::*lacZ* fusion (16) was used for all *syrB* promoter induction assays. B3AR-132 was grown in syringomycin minimal medium (SRM) (9), which contains 1.0% D-glucose, 0.4% L-histidine, 0.8 mM $MgSO_4 \cdot 7H_2O$, 0.8 mM potassium phosphate, and 10 μM $FeCl_3$, which was added immediately before use to ensure adequate Fe^{3+} for maximum *syrB* expression. Following the procedure of Sayler (22), cells of B3AR-132 were streaked onto solid SRM medium, and single colonies that grew after 24 h were transferred to 25 ml of SRM medium. The cultures were incubated overnight at 28°C with rotary shaking at 150 rpm, and then incubated at room temperature without shaking for 3 days. Cells of B3AR-132 were harvested by centrifugation and resuspended in SRM medium containing 15% glycerol (vol/vol) to a concentration of 5×10^8 CFU/ml and stored at –80°C until used.

Assay for β-galactosidase. Bark extracts (100 μl) were added to 4.9 ml of SRM medium containing *P. syringae* pv. *syringae* B3AR-132 at 10^6 CFU/ml. Duplicate SRM cultures were made for each bark extract sample. Sterile deionized water (sdH_2O) (100 μl) added to 4.9 ml of SRM medium containing B3AR-132 at 10^6 CFU/ml was used as a negative control. The positive control was prepared by adding 50 μl of 10 mM arbutin in sdH_2O and 50 μl of 10% D-fructose in sdH_2O to 4.9 ml of SRM medium containing B3AR-132 at 10^6 CFU/ml. After briefly shaking, the cultures were allowed to incubate at room temperature for 3 days without shaking. Following inoculation, cells from 500 μl of the culture were harvested by centrifugation and suspended in 1 ml of Z-buffer, which contains $Na_2HPO_4 \cdot 7H_2O$ at 16.1 g/liter, $NaH_2PO_4 \cdot H_2O$ at 5.5 g/liter, KCl at 0.75 g/liter, $MgSO_4 \cdot 7H_2O$ at 0.246 g/liter, and β-mercaptoethanol, pH 7.0, at 2.7 ml/liter (14). Cell densities were measured with a spectrophotometer at 600 nm (A_{600}). The cells were lysed by adding 20 μl each of chloroform and 0.05% sodium dodecyl sulfate (12). After vortexing for 10 s, the cell extracts were incubated at 28°C for 10 min, then assayed for β-galactosidase by adding 100 μl of freshly prepared o-nitrophenyl-β-D-galactopyranoside (4 mg/ml). The reaction was allowed to proceed for 10 min at room temperature, and then terminated by adding 250 μl of 1 M Na_2CO_3. Cell debris was pelleted in a microcentrifuge, and the absorbency of the supernatant was determined at 420 nm (A_{420}). β-Galactosidase units were calculated using the formula $(A_{420} \times 1{,}000)/(A_{600} \times 10 \text{ min})$ (27).

Total soluble phenolic compounds. Soluble phenolic compounds were determined following the procedure of Omokolo et

al. (18), with some modifications. Fresh bark tissue (20 to 30 mg) from 1-year-old stems, collected in the dormant season prior to field bacterial inoculation, was washed with deionized water, ground in liquid nitrogen, and extracted with 5 ml of 80% ethanol on a shaker for 20 min at 200 rpm. The homogenate was centrifuged at 1,000 × *g* for 10 min and the supernatant was transferred to a clean test tube. The plant debris pellet was re-extracted with 5 ml of 80% ethanol and centrifuged. The two ethanol bark extracts were combined and mixed. Total soluble phenolic compounds were determined colorimetrically by adding 100 µl of bark extract and 350 µl of Folin and Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis) to 4.9 ml of deionized water. The color reaction was initiated by adding 700 µl of saturated Na_2CO_3 to the solution, which then was incubated at room temperature for 1 h. The optical density of the solution was determined at 725 nm and the concentration of total soluble phenolic compounds was calculated from a standard curve made from a dilution series of phenol.

Carbon and nitrogen analysis. Leaf samples were collected from KAC in September 2000 and 2001 for carbon and nitrogen analysis. In all, 10 to 15 leaves from the middle part of the current season's shoots were harvested as a representative sample and put into plastic bags. The leaf samples were washed with deionized water, dried in an oven for 72 h at 70°C, ground in a grinder mill (Arthur H. Thomas Co., Laboratory Apparatus, Philadelphia), and passed through a 40-mesh sieve. One-year-old stem samples collected in the dormant season at the KAC and Modesto sites, were washed with deionized water and ≈20 g of fresh bark were collected using a razor blade to remove the phloem down to the cambium layer. The bark samples were dried and ground as described above. Total leaf and bark carbon and nitrogen was determined by a combustion gas analysis method (19,20) in which 2 to 3 mg of plant sample was wrapped in aluminum foil and combusted in an element analyzer (NA 1500; Fisons Instruments, Italy).

Data analysis. Data were analyzed for statistical significance using the general linear model (GLM) procedure (Statistical Analysis System; SAS Institute, Cary, NC). Log transformations (8), when appropriate, were applied to the data before subjecting it to statistical analysis.

RESULTS

Effect of nitrogen fertilization on *syrB* expression as indicated by β-galactosidase activity and bacterial lesion length. Compounds present in peach bark extracts induced the expression of the *syrB*::*lacZ* gene fusion in *P. syringae* pv. *syringae* B3AR-132 (Fig. 1). β-Galactosidase activity induced by extracts from trees with less nitrogen was significantly higher than extracts from trees with nitrogen fertilization at the Modesto site. This response occurred in the presence or absence of ring nematodes (Fig. 1A). In the KAC experiment, nitrogen fertilization significantly decreased β-galactosidase activity in the absence of ring nematodes, but not in the presence of ring nematodes (Fig. 1B). β-Galactosidase activity was negatively correlated with leaf nitrogen content ($R^2 = 0.124$, $P < 0.035$, $n = 36$) at the KAC site and bark nitrogen content ($R^2 = 0.455$, $P < 0.0041$, $n = 16$) in the Modesto plot. Regardless of site, the presence or absence of ring nematodes did not have a significant effect on the ability of bark extracts to induce expression of the *syrB*::*lacZ* reporter. The high β-galactosidase activity induced by the positive control and the low activity of the negative control validated the assay (Fig. 1).

The lesions resulting from *P. syringae* pv. *syringae* inoculation were significantly smaller in nitrogen-fertilized trees than in the control trees in the presence of nematodes in the Modesto experiment plot (Fig. 2A), but not in the KAC plot (Fig. 2B). Nitrogen fertilization did not have a significant effect on lesion size in the absence of nematodes in either experimental plot (Fig. 2). The presence of nematodes significantly predisposed the host plants to develop larger lesions at both experimental sites, except the nitrogen supplement treatment in the Modesto experimental plot (Fig. 2).

Effect of nematode infestation and nitrogen fertilization on the carbon/nitrogen ratio in peach trees. Nematode infestation resulted in a significant increase in the carbon/nitrogen ratio in the leaves; however, the numerically higher carbon/nitrogen ratio in the bark was not significant in the KAC experiment (Table 1). The bark carbon/nitrogen ratio was slightly higher in trees growing in the nonfumigated soil compared with the MB-fumigated soil in the Modesto experiment, but the difference was not significant (Table 1). Nitrogen fertilization resulted in a significant decrease in the carbon/nitrogen ratio in leaves, but the decrease in the bark was not significant in the KAC experiment (Table 2). In the Modesto experiment, the carbon/nitrogen ratio in bark tissues was significantly lower in nitrogen-fertilized plants than in the control (Table 2).

Correlation analysis showed that β-galactosidase activity was positively correlated with leaf carbon/nitrogen ratio ($R^2 = 0.131$, $P < 0.030$, $n = 36$) in the KAC experiment and bark carbon/nitrogen ratio ($R^2 = 0.344$, $P < 0.017$, $n = 16$) in the Modesto experiment.

Effect of nematode infestation and nitrogen fertilization on the concentration of total soluble phenolic compounds in peach tissues. Total soluble phenolic compounds in the bark significantly increased in response to nematode infestation in both locations (Table 3). Nitrogen treatments did not significantly decrease total soluble phenolic compounds in the bark in either

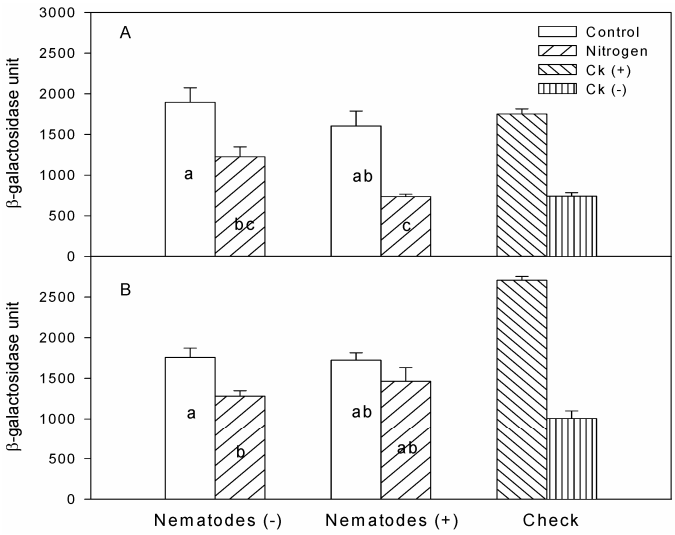

Fig. 1. Effect of nitrogen fertilization on the induction of *syrB* gene expression, indicated by a β-galactosidase reporter construct, using aqueous bark extracts from peach. **A,** Induction activity of extracts from cv. Loadel grafted on Lovell rootstocks growing near Modesto. Samples were collected from peach trees growing in soils naturally infested with ring nematodes (Nematodes +) or from trees growing in soil that was treated with methyl bromide (Nematodes –). **B,** Induction activity of extracts from cv. Elegant Lady grafted on Nemaguard rootstocks growing at the Kearney Agricultural Center (KAC). Samples were collected from peach trees growing in tanks that were experimentally inoculated with ring nematodes (Nematodes +) or from trees growing in tanks without ring nematodes (Nematodes –). Controls were a low level of nitrogen applied by the grower in the Modesto experimental site (**A**) and no nitrogen applied at KAC (**B**). Bark extracts were added to syringomycin minimal medium (SRM) containing *Pseudomonas syringae* pv. *syringae* strain B3AR-132 at 10^6 CFU/ml and β-galactosidase activity was measured spectrophotometrically. The positive check (Ck+) was arbutin and D-fructose and the negative check (Ck–) was sterile deionized water added to SRM medium containing *P. syringae* pv. *syringae* strain B3AR-132 at 10^6 CFU/ml. Data shown are means of **A,** eight or **B,** nine samples ± one standard error. Bars with the same letter within each experimental site are not different at $P < 0.05$ based on Tukey's studentized range test.

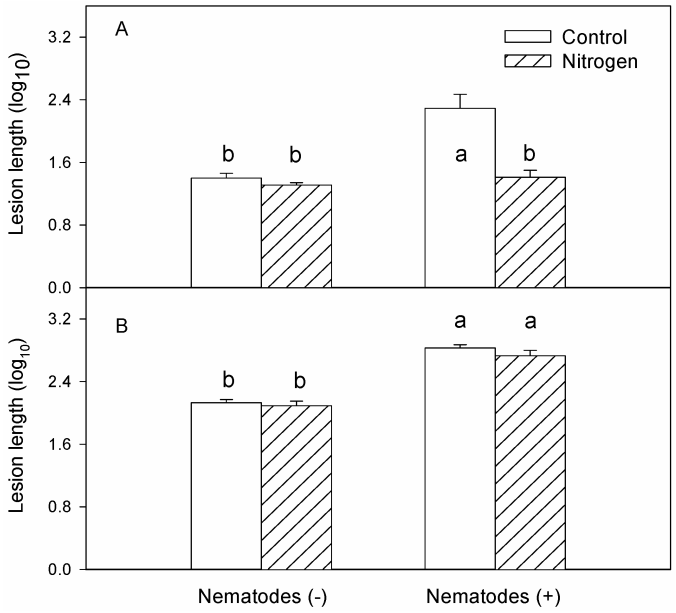

Fig. 2. Effect of nitrogen fertilization and nematodes on the size of bacterial cankers that developed following inoculation with *Pseudomonas. syringae* pv. *syringae* strain B3A. **A,** Samples were collected from peach trees growing in soils naturally infested with ring nematodes (Nematodes +) or from trees growing in soil that was treated with methyl bromide (Nematodes –). Lesion length produced in stems of cv. Loadel grafted on Lovell rootstock trees growing near Modesto. **B,** Samples were collected from peach trees growing in tanks that were experimentally inoculated with ring nematodes (Nematodes +) or from trees growing in tanks without ring nematodes (Nematodes –). Lesion length produced in stems of cv. Elegant Lady grafted on Nemaguard trees growing at Kearney Agricultural Center. Data shown are means of **A,** eight or **B,** nine samples ± one standard error. Bars with the same letter within each experimental site are not different at $P < 0.05$ based on Tukey's studentized range test.

TABLE 1. Effect of nematode infestation on the carbon/nitrogen (C/N) ratios of peach tissues

Location[x]	Soil treatment[y]	No. of samples	Tissue	C/N ratio[z]
KAC	Nematode-inoculated	18	Leaf	25.8 ± 1.1 a
KAC	MB-fumigated	18	Leaf	22.1 ± 1.3 b
KAC	Nematode-inoculated	18	Bark	40.5 ± 2.3 a
KAC	MB-fumigated	18	Bark	35.5 ± 1.5 a
Modesto	Nonfumigated	8	Bark	25.9 ± 0.8 a
Modesto	MB-fumigated	8	Bark	24.5 ± 1.0 a

[x] KAC = University of California Kearney Agricultural Center.
[y] MB = methyl bromide.
[z] Mean ± one standard error. Means followed by the same letter within each tissue and location combination are not different at $P < 0.05$ based on Student's *t* test.

experiment, although comparatively low levels of total phenolic compounds were found in the nitrogen-treated trees (data not shown).

The concentration of total soluble phenolic compounds in the bark was positively correlated with the carbon/nitrogen ratios of leaves and bark and negatively correlated with nitrogen content in the bark and leaf tissues in the KAC experiment (Table 4). The correlation between total soluble phenolic compounds and carbon/nitrogen ratios in the bark was not significant at the Modesto site (data not shown). The lesion length (log-transformed value) caused by *P. syringae* pv. *syringae* inoculation was positively correlated with both bark and leaf carbon/nitrogen ratios and total bark phenolic compounds in the KAC experiment using the pooled data collected from 2001 to 2002 (Table 5). A positive correlation also was found between the lesion length (log-transformed value) and bark carbon/nitrogen ratio in the Modesto

TABLE 2. Effect of nitrogen treatment on carbon/nitrogen (C/N) ratios of peach tissues

Location[y]	Treatment	No. of samples	Tissue	C/N ratio[z]
KAC	Urea foliar spray	18	Leaf	19.8 ± 0.9 b
KAC	Control	18	Leaf	28.1 ± 0.8 a
KAC	Urea foliar spray	18	Bark	36.2 ± 1.4 a
KAC	Control	18	Bark	39.7 ± 2.5 a
Modesto	Nitrogen	8	Bark	23.1 ± 0.6 b
Modesto	Control	8	Bark	27.3 ± 0.4 a

[y] KAC = University of California Kearney Agricultural Center.
[z] Mean ± one standard error. Means followed by the same letter within each tissue and location combination are not different at $P < 0.05$ based on Student's *t* test.

TABLE 3. Effect of nematode infestation on concentration of total soluble phenolic compounds in bark tissues of peach stems

Location[x]	Soil treatment[y]	No. of samples	Compounds (mg/g)[z]
KAC	Nematode-inoculated	18	40.5 ± 2.0 a
KAC	MB-fumigated	18	34.4 ± 1.7 b
Modesto	Nonfumigated	8	115.4 ± 3.3 a
Modesto	MB-fumigated	8	97.8 ± 4.0 b

[x] KAC = University of California Kearney Agricultural Center.
[y] MB = methyl bromide.
[z] Total soluble phenolic compounds (mg) in phenol equivalents per gram fresh tissue. Mean ± one standard error. Means followed by the same letter within each location are not different at $P < 0.05$ based on Student's *t* test.

TABLE 4. Correlation between total soluble bark phenolic compounds and carbon/nitrogen (C/N) ratios or nitrogen content in peach tissues at the University of California Kearney Agricultural Center

Independent variable	No. of samples	Slope	R^2	$P > F$
Bark C/N	36	0.347	0.1240	<0.0352
Leaf C/N	36	0.628	0.1676	<0.0132
Bark nitrogen (%)	72	–12.708	0.2730	<0.0001
Leaf nitrogen (%)	72	–8.154	0.3333	<0.0001

experiment (Table 5). There was a trend toward longer lesions associated with higher total phenolic compounds in the Modesto experiment, and the correlation was almost significant (Table 5). Obviously longer cankers were associated with higher carbon/nitrogen ratios and higher phenolic compounds in the bark.

DISCUSSION

Bark extracts derived from nitrogen-fertilized peach trees significantly reduced the expression of the *syrB*::*lacZ* gene fusion in three of the four paired comparisons compared with extracts from nonfertilized or less-fertilized trees. The negative correlations between *syrB* induction and tissue nitrogen content suggest that nitrogen fertilization may reduce plant *syrB* inducer compounds or increase plant compounds that reduce the activity of plant signal molecules, which in turn decreases the expression of the *syrB* gene in *P. syringae* pv. *syringae.* The effect of nitrogen application on reducing *syrB* gene expression is in agreement with the effect that increased nitrogen applications had on decreasing bacterial canker severity in 'French' prune (23,26) and in experimentally inoculated peach trees stressed with ring nematodes in the Modesto experiment (4). Surprisingly, however, ring nematode infestation did not have a significant effect on the ability of bark extracts from infested trees to induce *syrB* gene expression at either experimental site. Krzesinska et al. (12) studied the ability of different cherry genotypes to induce expression of the *syrB*::*lacZ* gene fusion using twig extracts. They found that extracts of susceptible cherry genotypes induced higher levels of *syrB* expression than did extracts of resistant cherry genotypes.

TABLE 5. Correlation between lesion length (log-transformed) due to bacterial canker and carbon/nitrogen (C/N) ratios or total soluble phenolic compounds in the bark of peach[y]

Location[z]	Independent variable	No. of samples	Slope	R^2	$P > F$
KAC	Bark C/N	72	<0.040	0.3436	<0.0001
KAC	Leaf C/N	72	0.070	0.4372	<0.0001
KAC	Bark phenolics	72	0.037	0.3263	<0.0001
Modesto	Bark C/N	16	0.115	0.4427	<0.0049
Modesto	Bark phenolics	16	0.017	0.2472	>0.0501

[y] Log-transformed value = $\log_{10}$(lesion length).
[z] KAC = University of California Kearney Agricultural Center.

However, Sayler (22) found no differences in *syrB* expression using twig extracts derived from nitrogen-stressed or fertilized prunes, and concluded that nitrogen fertilization of prune trees had little effect on the induction of syringomycin synthesis by *P. syringae* pv. *syringae*.

One important group of plant signal molecules that can induce the expression of *syrB* gene in *P. syringae* pv. *syringae* is phenolic β-glucosides, such as arbutin (15,21). In our experiments, ring nematode infestation had a significant effect on increasing total soluble bark phenolic compounds (Table 3) and carbon/nitrogen ratios (Table 1). Our experiments also showed that nitrogen fertilization significantly decreased plant carbon/nitrogen ratios (Table 2), which is in agreement with the carbon/nutrient balance theory (3). Ring nematode infestation also significantly decreased bark nitrogen content in the KAC experiment (4). Previous studies also showed that ring nematode infestation decreased plant nitrogen (17,24). The data obtained in this research indicate that higher concentrations of total soluble bark phenolic compounds were associated with higher plant carbon/nitrogen ratios, lower plant nitrogen concentrations (Table 4), and larger lesions (Table 5). The ability to increase *syrB* gene expression increases when plant tissues have higher carbon/nitrogen ratios and *P. syringae* pv. *syringae* inoculation of stems with comparatively high carbon/nitrogen ratios produced larger lesions (Table 5). Further investigation to identify specific plant phenolic β-glucosides in trees that have been stressed by ring nematodes may help to further understand the specific mechanism, compound, or compounds that predispose stone fruit trees to bacterial canker. Determining whether changes occur in plant sugars such as D-fructose in response to ring nematode infestation also could be informative, because D-fructose enhances *syrB* gene expression in the presence of plant phenolic β-glucosides (15).

It should be noted that the supplemental nitrogen treatment had a significant effect in decreasing peach susceptibility to *P. syringae* pv. *syringae* infection as indicated by lesion length in the presence of nematodes in the Modesto plot. However, a similar effect was not found using foliar applications of urea in trees stressed with ring nematodes at the KAC site. This may be due to the smaller amount of nitrogen that was applied to trees at KAC compared with the Modesto plot. This also is supported by the fact that the bark nitrogen content did not significantly increase in response to the foliar nitrogen treatment at the KAC site (data not shown). It also should be noted that the scion cultivars and rootstocks were different at the two experimental sites. However, no scion cultivars of peach are known to possess resistance to bacterial canker (13,29), and both rootstocks are good hosts for the ring nematode (1,13). Although Lovell rootstock provided better control of peach tree short life (PTSL) than Nemaguard rootstock in South Carolina (30), winter cold injury is a significant component of the PTSL syndrome and Nemaguard is more susceptible to cold injury than Lovell (13). Winter injury is not an important problem in California and no difference was found in the susceptibility to bacterial canker of Lovell and Nemaguard grafted peach trees growing (in containers) in California (13); however, recent field trials have shown Nemaguard to be more susceptible than Lovell to bacterial canker (R. Duncan, *unpublished data*). In addition, the higher ring nematode population at the KAC site (896 ring nematodes/liter of soil) compared with the Modesto site (523 ring nematodes/liter of soil) also may have contributed to the difference of the host plants in response to *P. syringae* pv. *syringae* infection. Other factors, such as soil composition, which varied somewhat at the two experimental sites, soil microflora, or some other cultural factor also could have influenced the susceptibility of the trees at the two experimental plots.

In conclusion, the data obtained from this study support the hypothesis that one effect nitrogen fertilization has on decreasing tree susceptibility to bacterial canker in peach is by either reducing the concentration of *syrB* gene inducer compounds or increasing the concentration of plant metabolites that reduce the activity of *syrB* gene inducer compounds. Although this research focused on the relationship between nitrogen fertilization and ring nematode infestation of peach trees and the ability of polyphenolic extracts to induce *syrB* gene expression, other factors besides syringomycin synthesis also mediate the ability of *P. syringae* pv. *syringae* to cause bacterial canker disease.

ACKNOWLEDGMENTS

This study was supported by the California Prune, Cling Peach, and Almond Boards. We thank D. Gross for providing *P. syringae* pv. *syringae* strain B3AR-132 used in this study, R. Sayler for initial instruction in use of the β-galactosidase assay, T. Buzo for assistance in field operations, N. Kline for providing peach trees for field inoculations, and H. Hu for assistance in polyphenolic extraction.

LITERATURE CITED

1. Barker, K. R., and Clayton, C. N. 1973. Nematodes attacking cultivars of peach in North Carolina. J. Nematol. 5:265-271.
2. Bender, C. L., Alarcón-Chaidez, F., and Gross, D. C. 1999. *Pseudomonas syringae* phytotoxins: Mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiol. Mol. Biol. Rev. 63:266-292.
3. Bryant, J. P., Chapin, F. S., and Klein, D. R. 1983. Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357-368.
4. Cao, T. 2004. Host susceptibility factors in bacterial canker of stone fruit caused by *Pseudomonas syringae* pv. *syringae*. Ph.D. diss. University of California, Davis.
5. Cao, T., Sayler, R. J., DeJong, T. M., Kirkpatrick, B. C., Bostock, R. M., and Shackel, K. A. 1999. Influence of stem diameter, water content and freezing-thawing on bacterial canker development in excised stems of dormant stone fruit. Phytopathology 89:962-966.
6. DeVay, J. E., Lukezic, F. L., Sinden, S. L., English, H., and Coplin, D. L. 1968. A biocide produced by pathogenic isolates of *Pseudomonas syringae* and its possible role in the bacterial canker disease of peach trees. Phytopathology 58:95-101.
7. English, H., DeVay, J. E., Lilleland, O., and Davis, J. R. 1961. Effect of certain soil treatments on the development of bacterial canker in peach trees. (Abstr.) Phytopathology 51:65.
8. Fernandez, G. C. J. 1992. Residual analysis and data transformations: Important tools in statistical analysis. HortScience 27:297-300.
9. Gross, D. C. 1985. Regulation of syringomycin synthesis in *Pseudomonas syringae* pv. *syringae* and defined conditions for its production. J. Appl. Bacteriol. 58:167-174.
10. Hoffland, E., van Beusichem, M. L., and Jeger, M. J. 1999. Nitrogen availability and susceptibility of tomato leaves to *Botrytis cinerea*. Plant Soil 210:263-272.

11. Hutchison, M. L., Tester, M. A., and Gross, D. C. 1995. Role of biosurfactant and ion channel-forming activities of syringomycin in transmembrane ion flux: A model for the mechanism of action in the plant-pathogen interaction. Mol. Plant-Microbe Interact. 8:610-620.
12. Krzesinska, E. Z., Azarenko, A. N., and Gross, D. C. 1993. Inducing the *syrB* gene in *Pseudomonas syringae* pv. *syringae* in twig extracts from cherry genotypes. HortScience 28:335-337.
13. Lownsbery, B. F., English, H., Noel, G. R., and Schick, F. J. 1977. Influence of Nemaguard and Lovell rootstocks and *Macroposthonia xenoplax* on bacterial canker of peach. J. Nematol. 9:221-224.
14. Miller, J. H. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
15. Mo, Y.-Y., Geibel, M., Bonsall, R. R., and Gross, D. C. 1995. Analysis of sweet cherry (*Prunus avium* L.) leaves for plant signal molecules that activate the *syrB* gene required for synthesis of the phytotoxin, syringomycin, by *Pseudomonas syringae* pv. *syringae*. Plant Physiol. 107:603-612.
16. Mo, Y.-Y., and Gross, D. C. 1991. Expression *in vitro* and during plant pathogenesis of the *syrB* gene required for syringomycin production by *Pseudomonas syringae* pv. *syringae*. Mol. Plant-Microbe Interact. 4:28-36.
17. Mojtahedi, H., and Lownsbery, B. F. 1975. Pathogenicity of *Criconemoides xenoplax* to prune and plum rootstocks. J. Nematol. 72:114-119.
18. Omokolo, N. D., Tsala, N. G., and Djocgoue, P. F. 1996. Changes in carbohydrate, amino acid and phenolic contents in cocoa pods from three clones after infection with *Phytophthora megakarya* (Brasier and Griffin). Ann. Bot. 77:153-158.
19. Pella, E. 1990. Elemental organic analysis. Part 1. Historical developments. Am. Lab. 2:116-125.
20. Pella, E. 1990. Elemental organic analysis. Part 2. State of the art. Am. Lab. 8:28-32.
21. Quigley, N. B., and Gross, D. C. 1994. Syringomycin production among strains of *Pseudomonas syringae* pv. *syringae*: Conservation of the *syrB* and *syrD* genes and activation of phytotoxin production by plant signal molecules. Mol. Plant-Microbe Interact. 7:78-90.
22. Sayler, R. J. 2001. Disease mechanisms and evaluation of cultural and chemical controls for bacterial canker of 'French' prune trees. Ph.D. diss. University of California, Davis.
23. Sayler, R. J., and Kirkpatrick, B. C. 2004. The effect of copper sprays and fertilization on bacterial canker in French prune. Can. J. Plant Pathol. 25:406-410.
24. Sharpe, R. R., Nyczepir, A. P., and Reilly, C. C. 1986. Effect of nematodes on nutrient uptake in Lovell and Nemaguard peach seedlings. Stone Fruit Tree Decline, Third Workshop Proceedings. Clemson University, Clemson, SC.
25. Sinden, S. L. 1966. The nature of the wide spectrum antibiotic produced by pathogenic *Pseudomonas syringae* and its role in the bacterial canker disease of *Prunus persicae*. Ph.D. thesis. University of California, Davis.
26. Southwick, S. M., Yeager, J. T., Weis, K. G., Kirkpatrick, B. C., Little, E. L., and Westerdahl, B. B. 1997. Relationship between nitrogen fertilization and bacterial canker in 'French' prune. (Abstr.) HortScience 32:520.
27. Stachel, S. E., An, G., Flores, C., and Nester, E. W. 1985. A Tn3 *lacZ* transposon for the random generation of β-galactosidase gene fusion: Application to the analysis of gene expression in *Agrobacterium*. Eur. Mol. Biol. Organ. J. 4:891-898.
28. Xu, G.-W., and Gross, D. C. 1988. Evaluation of the role of syringomycin in plant pathogenesis by using Tn5 mutants of *Pseudomonas syringae* pv. *syringae* defective in syringomycin production. Appl. Environ. Microbiol. 54:1345-1353.
29. Yadava, U. L., and Doud, S. L. 1989. Rootstock and scion influence growth, productivity, survival, and short life-related performance of peach trees. J. Am. Soc. Hortic. Sci. 114:875-880.
30. Zehr, E. I., Miller, R. W., and Smith, F. H. 1976. Soil fumigation and peach rootstocks for protection against peach tree short life. Phytopathology 66:689-694.