Inducing the syrB Gene in 

**Pseudomonas syringae pv. syringae** in 

Twig Extracts from Cherry Genotypes

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Abstract. The syrB gene required by **Pseudomonas syringae pv. syringae** van Hall to produce the phytotoxin syringomycin is activated by plant signal molecules. Extracts from twigs of 12 cherry (**Prunus**) genotypes were tested for their ability to induce syrB::lacZ fusion in **P. syringae pv. syringae** strain B3AR132 to determine whether signal activity is correlated with susceptibility to bacterial canker. One-year-old twigs of ‘Napoleon’, ‘Corum’, and 12 cherry rootstocks (F12/1, ‘Colt’, MxM2, MxM39, MxM60, Gi 148-1, Gi 148-9, Gi 154-2, Gi 154-5, Gi 169-15, Gi 172-9, and Gi 173-9) were tested at concentrations of 0.2, 1.0, and 2.0 mg twig dry weight/ml solution for their ability to induce syrB::lacZ fusion, as measured by β-galactosidase activity. Extracts from all cherry genotypes induced syrB::lacZ fusion, but to varying degrees. The highest β-galactosidase activity was observed in ‘Napoleon’ and ‘Corum’, the most susceptible genotypes; activities were two to four times higher than that of F12/1, a disease-resistant genotype. Activities higher than that of F12/1 were induced at the lowest extract concentration by rootstocks MxM60, Gi 148-1, Gi 148-9, and Gi 154-5, whereas rootstocks MxM2, MxM39, Gi 154-2, Gi 172-9, Gi 173-9, and ‘Colt’ were not significantly different from F12/1. At the two highest extract concentrations tested, only ‘Napoleon’ and ‘Corum’ consistently had higher induc&betam;tion activity than F12/1. At high extract concentrations, interfering substances seemed to suppress or antagonize the induction of syrB::lacZ fusion. These results suggest that susceptible genotypes contain higher signal activities than resistant genotypes.

In the Pacific Northwest, bacterial canker caused by **Pseudomonas syringae pv. syringae** is a serious disease of sweet cherries (**Prunus avium** L.). Above-ground portions of trees are infected during the region’s cool, wet season from fall until early spring. Infection may produce localized cankers and kill buds, limbs, or the entire tree (Cameron, 1962a, 1962b). Leaf and fruit lesions are observed rarely.

**Pseudomonas syringae** pv. syringae produces syringomycin, an antibiotic-specific cyclic lipodepsipeptidolysin phytotoxin (Gross, 1991; Segre et al., 1989). This toxin disrupts ion transport across the plasmalemma of affected cells and leads to necrosis (Bidwai et al., 1987; Mott and Takemoto, 1988). Syringomycin production contributes significantly to virulence by increasing disease severity (Xu and Gross, 1988a, 1988b).

Five proteins are associated with syringomycin production and are believed to function as syringomycin synthetases (Gross, 1991; Morgan and Chatterjee, 1988). The formation of two large proteins, SR4 and SR5, is associated with the syrB gene (Xu and Gross, 1988b). SyrB either encodes a subunit of multimeric synthetase protein or is a positive regulatory protein that controls synthetase expression (Mo and Gross, 1991a). To study the expression of syringomycin production, Mo and Gross (1991a) mutagenized the syrB gene with TnHoH1 to generate fusions to a promotor-less lac operon. A transcriptional fusion of syrB with lacZ genes resulted in high β-galactosidase activity and was used to quantify syrB expression. The syrB::lacZ fusion was recombined into the genome of **P. syringae pv. syringae** strain B3A-R, which does not produce syringomycin in syringomycin minimal (SRM) medium (Mo and Gross, 1991a). The resulting recombinant B3AR132 also lacked β-galactosidase activity in SRM medium. However, adding cherry leaf extracts to SRM medium induced expression of syrB::lacZ fusion in strain B3AR132 (Mo and Gross, 1991a).

This virulence gene, which is partly responsible for syringomycin production by **P. syringae pv. syringae**, may be induced by compounds produced in the host plant’s leaves.

Cherry trees grafted high (100 cm) on a tolerant rootstock, such as F12/1, sometimes have fewer cankers in the scion than lower grafts (Cameron, 1971). High grafting currently is recommended to reduce disease incidence. Several interspecific hybridrootstocks, the MxM and Gisela clones, are being evaluated for horticultural acceptability in the United States. In an earlier study, the susceptibility of many of these rootstocks to **P. syringae pv. syringae** was determined in a twig assay (Krzesinska and Miller-Azarenko, 1992). In the study we report here, our objective was to test whether extracts of 1-year-old wood of various cherry genotypes with different susceptibilities to **P. syringae pv. syringae** (Krzesinska and Miller-Azarenko, 1992) could induce the expression of syrB::lacZ gene fusion in strain B3AR132.

Ten ‘Napoleon’ sweet cherry twigs were inoculated in Mar, 1990 with 10 colony forming units (cfu)/ml of strain B3AR132 to determine if this strain could induce the same symptoms as the four virulent strains used in an earlier twig assay (Krzesinska and Miller-Azarenko, 1992). A 20-µl droplet of inoculum was placed onto a 0.5 × 2-cm flaps cut into the twig to expose the cambium. Twigs (1-year-old wood, 15 cm long) were placed into sterile test tubes on water-saturated cotton, covered, and incubated for 4 weeks at 15C in darkness. Incision browning (1 = yellow pith, 4 = dark brown) was assessed after incubation and used to score strain virulence. After 4 weeks of incubation, browning and gummosis were observed on ‘Napoleon’ twigs inoculated with strain B3AR132; thus, B3AR132 was pathogenic and retained at least some virulence. Therefore, it was used to evaluate cherry rootstock genotypes with different levels of disease resistance for relative plant signal activities.

One-year-old wood was collected from trees of ‘Napoleon’, ‘Corum’, F12/1, ‘Colt’, MxM2, MxM39, MxM60, Gi 148-1, Gi 148-9, Gi 154-2, Gi 154-5, Gi 169-15, Gi 172-9, and Gi 173-9 genotypes in Mar. 1990. ‘Napoleon’, ‘Corum’, and F12/1 are *P. avium* selections. ‘Napoleon’ and ‘Corum’ were susceptible controls and F12/1 was resistant control. The MxM rootstocks are interspecific *P. avium* × *P. mahaleb* L. hybrids. The Gi series was selected from interspecific crosses between *P. avium*, *P. cerasus* L., *P. canescens* Bois., and *P. fruticosa* Pall. (Cummins, 1984). Twigs were washed in water, air-dried, cut in 1.0-cm pieces, frozen in liquid nitrogen, and lyophilized. The dried samples then were ground in a Wiley mill, passed through a 40-mesh screen, and stored at -20C.

Water extracts were prepared from lyophilized ground twig samples of the various *Prunus* genotypes. Tissue weights of 20,100, and 200 mg were homogenized in a Waring blender with 100 ml of double-distilled water. For each genotype and concentration, 2 ml of aqueous extract was evaporated to dryness and
resuspended in 2 ml of 75% acetone to remove proteins. After 30 min, the samples in 75% acetone were evaporated to dryness, resuspended in 2 ml of distilled water, filter-sterilized, and stored at -20°C. Each genotype was assayed three times, with two replications each time.

Twig aqueous extracts were assayed for their effects on the expression of \( \beta \)-galactosidase activity by \( \text{syrB}:\text{lacZ} \) fusion in B3A132 as described by Mo and Gross (1991a). Plant extracts (100 µl) containing 1, 5, or 10mg (dry weight) of sample were added to 4.9 ml of SRM medium containing 10^7 cfu/ml of \( P. \text{syringae} \) pv. \( \text{syringae} \) mutant strain B3A132. Duplicate SRM cultures also were prepared with the parental strain B3A-R or without bacteria; these cultures were the controls and were used to measure background activity in the assay. After being shaken and incubated for 3 days at 25°C, cells from 0.5 ml of culture were pelleted in microcentrifuge tubes, suspended in Z-buffer (1.4 ml), repelleted, and resuspended in 1.4 ml Z-buffer (Miller, 1972). A portion of the washed cells (1 ml) was measured for bacterial cell density at 600 nm. Twenty microliters of 0.05% sodium dodecyl sulfate and 20 µl of chloroform were added to 0.5 ml of the washed cells. The cells were lysed by vortexing them for 10 sec and then incubating them for 10 min at 28°C. The assay reaction was started by adding 100 µl of \( o \)-nitrophenyl- \( \beta \)-d-galactopyranoside (4 mg ml\(^{-1} \)) to the culture and then incubating it for 10 min at 28°C. The reaction was terminated by adding 250 µl of 1mNa,CO\(_3\). Cellular debris was removed by pelleting the culture in a microcentrifuge; the absorbency of the solution was determined at 420 nm. Miller units of \( \beta \)-galactosidase activity were calculated using the formula given by Stachel et al. (1985).

Plant substances present in cherry twigs induced expression of \( \text{syrB}:\text{lacZ} \) fusion in B3A132 (Fig. 1). Activity varied with rootstock. In general, as the concentration of cherry extracts increased, the \( \beta \)-galactosidase activity decreased. This result was attributed to the presence of substances in crude extracts, which, at higher concentrations, inhibited \( \text{syrB} \) induction or antagonized the effect of the plant signals. Twig extracts containing no bacteria in the SRM medium had background \( \beta \)-galactosidase activity similar to that of the extracts with the nonsyringomycin-producing parental strain B3A-R (data not shown).

Compared to F12/1, ‘Napoleon’ and ‘Corum’ had the highest activity, regardless of plant extract concentration (Fig. 1). When inoculated with the lowest concentration of plant extract, rootstocks MxM2, MxM39, Gi 154-2, Gi 172-9, Gi 173-9, and ‘Colt’ were not different from F12/1. The \( \beta \)-galactosidase activity was higher for extracts from rootstocks MxM60, Gi 148-1, Gi 148-9, Gi 154-5, and Gi 169-15 than those from F12/1.

In many cases, degree of incision browning in an excised twig assay, as observed in an earlier study with some of the same rootstocks (Krzesinska and Miller, 1992), paralleled the degree of \( \text{syrB} \) induction. In both assays, ‘Napoleon’ and ‘Corum’ had the highest browning and \( \beta \)-galactosidase activity. Rootstock Gi 169-15 at the lowest extract concentration induced high \( \beta \)-galactosidase activity and also was more susceptible in the twig assay. Differences were observed with rootstocks MxM60, Gi 148-1, Gi 148-9, and Gi 154-5, which were similar to F12/1 in the earlier twig assay but had high activity at low extract concentrations in the present bioassay. The opposite situation was observed for rootstock Gi 172-9, which seemed to be susceptible to \( P. \text{syringae} \) pv. \( \text{syringae} \) in the twig assay (Krzesinska and Miller-Azarenko, 1992) but not different from F12/1 in \( \beta \)-galactosidase activity. In both assays, the other rootstocks did not differ from F12/1 in susceptibility. One explanation for these differences in the assays may be the physiological stage of the twigs. For the bioassay, twigs were collected only once, in Mar. 1990, whereas samples were collected earlier in the winter for disease susceptibility tests.

The cherry substances serving as \( \text{syrB} \)-inducing plant signals remain to be identified.
Nevertheless, Mo and Gross (1991b) identified some phenolic glycosides that function as signal molecules in syrB induction in P. syringae pv. syringae. The structural requirement for maximum syrB induction is a glucosylated benzene ring. Phenolic compounds generally are stored as glycosides to increase water solubility and decrease reactivity (Hösel, 1981). Many phenolic glucosides are found in plant species within the host range of P. syringae pv. syringae (Miller, 1973; Paris, 1963). Additionally, sugars such as sucrose and fructose enhance the response of P. syringae pv. syringae to the phenolic signal (Mo and Gross, 1991a). Sugars seem to be an integral part of the signal transduction process.

The results may vary with sampling time, since there may be seasonal fluctuation in the concentration of plant signals or substances that antagonize induction by plant signals. Identifying the signal molecules that induce phytoxin production by P. syringae pv. syringae and determining seasonal fluctuation in their concentrations may lead to a better understanding of the host-pathogen interaction and provide a basis for more effective disease-control practices.

**Literature Cited**


Cummins, J.N. 1984. Fruit tree rootstocks recently introduced and soon to be introduced. Compact Fruit Tree 17:57-63.


