

AN ABSTRACT OF THE THESIS OF

Elzbieta Z. Krzesinska for the degree of Master of Science

in Horticulture presented on December 19, 1990.

Title: Assays to Determine Tolerance of Cherry Rootstock to Bacterial
Canker Caused by *Pseudomonas syringae* pv. *syringae*.

Abstract approved: _____

Anita N. Miller

Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae* is recognized as one of the greatest limiting factors in cherry production in Oregon. Disease incidence may be decreased when susceptible cultivars are high-grafted onto tolerant/resistant rootstocks. This research was begun to develop a rapid screening method which could be used to test cherry rootstocks for tolerance to *Pseudomonas syringae* pv. *syringae*. In 1988, one-year-old wood of 'Napoleon', 'Corum' and F/12-1 was collected at monthly intervals from November until January. 'Napoleon' and 'Corum' are found to be susceptible, and F/12-1 to be tolerant to *Pseudomonas syringae* pv. *syringae*. Twigs were inoculated with water, one avirulent, and three virulent strains of bacteria at 10^5 , 10^6 , and 10^7 cfu/ml. Browning, gummosis, and callus production were evaluated at the inoculation site after incubation for 4 weeks. Generally, browning and gummosis induced by concentrations of 10^6 and 10^7 cfu/ml across the virulent strains were not different. No gummosis and browning were observed on twigs inoculated with water or the avirulent strain. Callus production did not appear to be a usable

criterion of the disease in this assay. 'Napoleon' and 'Corum' had significantly higher browning and gummosis ratings than F/12-1.

In 1989, one-year-old twigs from 'Napoleon', 'Corum', and a number of cherry rootstocks were collected from October until December. The rootstocks included: F/12-1, M x M 2, M x M 39, M x M 60, GI 148-1, GI 148-8, GI 154-2, GI 154-5, GI 169-15, GI 172-9, and GI 173-9. Twigs were inoculated with water alone as a check, one avirulent and three virulent strains at 10^7 cfu/ml. Incision browning, gummosis and callus production were evaluated after incubation for 4 weeks. Based on incision browning and gummosis all the rootstocks tested were more tolerant than 'Napoleon' and 'Corum', and most did not differ from F/12-1. However, rootstocks GI 172-9 and GI 169-15 showed more browning than F/12-1 in a number of instances. On the last sampling date, rootstocks M x M 2, GI 173-9, GI 148-8, and GI 154-2 showed less browning than F/12-1.

The expression of virulence genes in *P. syringae* has been shown to be induced by plant signals extracted from cherry leaves. Crude aqueous extracts from 'Napoleon', 'Corum', and various cherry rootstock twigs were adjusted to a concentration of 0.2, 1.0, and 2.0 mg·ml⁻¹, and evaluated for the ability to induce virulence in *Pseudomonas syringae* pv. *syringae*. A decrease in activity was generally observed at the two highest concentrations of plant extracts. Reduced activity may have been due to inhibition of the assay or saturation of active sites of the enzyme produced by the bacteria. Compared to tolerant genotype F/12-1, 'Napoleon' and 'Corum' had the highest activity of all the genotypes tested. Rootstocks M x M 60, GI 148-1, GI 148-9, GI 154-5, and GI 169-15 had higher activity than F/12-1 in plant extracts of

0.2 mg·ml⁻¹. Activity induced in rootstocks M x M 2, M x M 39, GI 154-2, GI 172-9, and GI 173-9 was not different from that of F/12-1. The lowest activity observed was for 'Colt', however this activity was not statistically different from that induced by F/12-1.

Assays to Determine Tolerance of Cherry Rootstock to Bacterial
Canker Caused by Pseudomonas syringae pv. syringae.

BY

Elzbieta Z. Krzesinska

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Completed December 19, 1990

Commencement June 1991

APPROVED:

Professor of Horticulture in charge of major

Head of the Department of Horticulture

Dean of the Graduate School

Date thesis presented December 19, 1990.

Typed by Elzbieta Z. Krzesinska

TABLE OF CONTENTS

	<u>Page</u>
Chapter 1. INTRODUCTION.....	1
Chapter 2. LITERATURE REVIEW.....	4
Introduction.....	4
Dissemination.....	8
INA properties.....	9
Disease severity.....	10
Time of infection.....	11
Bud infection.....	13
Leaf infection.....	14
Flower infection.....	15
Fruit infection.....	15
Phytotoxin production.....	16
Control.....	17
Rootstocks.....	19
Chapter 3. EXCISED TWIG ASSAY TO SCREEN CHERRY ROOTSTOCKS FOR TOLERANCE TO <i>Pseudomonas syringae</i> pv. <i>syringae</i>	22
ABSTRACT.....	22
INTRODUCTION.....	24
MATERIALS AND METHODS.....	26
RESULTS AND DISCUSSION.....	28
Chapter 4. INDUCTION OF VIRULENCE GENES IN <i>Pseudomonas syringae</i> pv. <i>syringae</i> BY PLANT EXTRACTS FROM CHERRY ROOTSTOCKS.....	54
ABSTRACT.....	54
INTRODUCTION.....	56
MATERIALS AND METHODS.....	57
RESULTS AND DISCUSSION.....	59
Chapter 5. CONCLUSIONS.....	68
BIBLIOGRAPHY.....	71

LIST OF FIGURES

<u>Figure</u>	Chapter 4	<u>Page</u>
4.1	Comparison of β -galactosidase activity induced by plant extracts ($0.2 \text{ mg} \cdot \text{ml}^{-1}$) in <i>syrB::lacZ</i> fusion of <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain B3AR132 between F/12-1 and other cherry genotypes.....	65
4.2	Comparison of β -galactosidase activity induced by plant extracts ($1.0 \text{ mg} \cdot \text{ml}^{-1}$) in <i>syrB::lacZ</i> fusion of <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain B3AR132 between F/12-1 and other cherry genotypes.....	66
4.3	Comparison of β -galactosidase activity induced by plant extracts ($2.0 \text{ mg} \cdot \text{ml}^{-1}$) in <i>syrB::lacZ</i> fusion of <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain B3AR132 between F/12-1 and other cherry genotypes.....	67

LIST OF TABLES

<u>Table</u>	Chapter 3	<u>Page</u>
3.1	Source and pathogenicity of <i>Pseudomonas syringae</i> pv. <i>syringae</i> strains.....	31
3.2	Parentage of cherry rootstocks evaluated in the excised twig assay.....	32
3.3	The effect of inoculum concentration of three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> on browning at the incision made into twigs of three cherry genotypes, 4 weeks after inoculation.....	33
3.4	The effect of inoculum concentration of three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> on gummosis at the incision made into twigs of three cherry genotypes, 4 weeks after inoculation.....	34
3.5	The effect of inoculum concentration of three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> on callus production at the incision made into twigs of three cherry genotypes, 4 weeks after inoculation.....	35
3.6	The effect of genotype on browning at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10^5 cfu/ml, 4 weeks after inoculation.....	36
3.7	The effect of genotype on browning at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10^6 cfu/ml, 4 weeks after inoculation.....	37
3.8	The effect of genotype on browning at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10^7 cfu/ml, 4 weeks after inoculation.....	38
3.9	The effect of genotype on gummosis at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10^5 cfu/ml, 4 weeks after inoculation.....	39
3.10	The effect of genotype on gummosis at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10^6 cfu/ml, 4 weeks after inoculation.....	40
3.11	The effect of genotype on gummosis at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10^7 cfu/ml, 4 weeks after inoculation.....	41
3.12	The effect of genotype on callus production at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10^5 cfu/ml, 4 weeks after inoculation.....	42

3.13	The effect of genotype on callus production at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10 ⁶ cfu/ml, 4 weeks after inoculation.....	43
3.14	The effect of genotype on callus production at the incision after inoculation with three strains of <i>Pseudomonas syringae</i> pv. <i>syringae</i> at 10 ⁷ cfu/ml, 4 weeks after inoculation.....	44
3.15	Contrasts between F/12-1 and 12 cherry genotypes of lesion browning induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain W4N54.....	45
3.16	Contrasts between F/12-1 and 12 cherry genotypes of lesion browning induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain AP-1 in 1989.....	46
3.17	Contrasts between F/12-1 and 12 cherry genotypes of lesion browning induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain B-15 in 1989.....	47
3.18	Contrasts between F/12-1 and 12 cherry genotypes of gummosis induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain W4N54 in 1989.....	48
3.19	Contrasts between F/12-1 and 12 cherry genotypes of gummosis induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain AP-1 in 1989.....	49
3.20	Contrasts between F/12-1 and 12 cherry genotypes of gummosis induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain B-15 in 1989.....	50
3.21	Contrasts between F/12-1 and 12 cherry genotypes of callus production induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain W4N54 in 1989.....	51
3.22	Contrasts between F/12-1 and 12 cherry genotypes of callus production induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain AP-1 in 1989.....	52
3.23	Contrasts between F/12-1 and 12 cherry genotypes of callus production induced by inoculation with <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain B-15 in 1989.....	53

Chapter 4

4.1	Activity induced in a <i>syrB::lacZ</i> fusion of <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain B3AR132 by plant extracts obtained from extraction in different acetone concentrations.....	63
-----	--	----

4.2	Effect of plant extract concentration from cherry genotypes on β -galactosidase activity induced in <i>syrB::lacZ</i> fusion of <i>Pseudomonas syringae</i> pv. <i>syringae</i> strain B3AR132.....	64
-----	---	----

ASSAYS TO DETERMINE TOLERANCE OF CHERRY ROOTSTOCK TO BACTERIAL CANKER
CAUSED BY Pseudomonas syringae pv. syringae.

Chapter 1.

INTRODUCTION

Bacterial canker, caused by *Pseudomonas syringae*, is one of the greatest limiting factors to cherry production and the most serious disease of sweet cherries in the Pacific Northwest (Cameron, 1970). The disease occurs on the aboveground part of the tree and may result in localized cankers, and death of buds, limbs, or entire tree. The trees are seldom killed during one growing season, but severely infected trees may decline over several years.

Two pathovars of *P. syringae*, pv. *morsprunorum* and pv. *syringae*, incite similar symptoms on cherry trees throughout the world. However, the primary pathogen in Oregon is *P. syringae* pv. *syringae*. The typical symptoms, observed in the Northwest, are trunk and shoot cankers, dead buds and blossoms, and rarely leaf and fruit lesions (Cameron, 1962b).

Young trees seem more susceptible to the disease than older trees. Tree losses as high as 75% have been reported in young orchards in Oregon. In general, the disease occurrence appears to be greatest in one to six year old cherry orchards (Cameron, 1955, 1962b; Latorre et al., 1985; Ross and Hattingh, 1986b).

Some cultural practices provide a moderate level of control for this disease. Decreased incidence of the disease occurs when bactericides are applied in the fall and late winter and pruning is

delayed until the cool humid weather has passed in the spring. However, chemical control of bacterial canker is not very successful.

P. syringae can exist as an epiphyte on weeds, grasses, and apparently healthy plants (Baca and Moore, 1987b; Latorre and Jones, 1979b; Ross and Hattingh, 1986d). The pathogen was also isolated from internal tissues of apparently healthy cherry trees and from symptomless buds (Cameron, 1970; Ross and Hattingh, 1986a). Bacteria can enter the plant during the growing season and establish what is probably a very important source of inoculum inside symptomless tissue. This explains the low efficacy of the surface application of bactericides.

Grafting of susceptible genotypes high onto a tolerant rootstock, at approximately one meter above ground, also seems to decrease the incidence of disease (Cameron, 1971). It is critical to the industry to have rootstocks which are tolerant to *Pseudomonas syringae* pv. *syringae*. A clonally propagated selection of 'Mazzard' rootstock, F/12-1, is tolerant to the pathogen and is often used in the commercial orchards (Cameron, 1971; Webster, 1984).

Screening cherry genotypes for tolerance to bacterial canker is currently based on field observations. However, valid assessment of the disease can be made only after several years.

Recently, a number of new rootstocks from West Germany, the Giessen series (GI), has been introduced into the United States. They have only undergone field evaluation for tolerance to *P. syringae* pv. *morsprunorum*. Information about tolerance of M x M series, which was developed by Lyle Brooks in Oregon, to *P. syringae* pv. *syringae* is also lacking (Cummins, 1984).

Pseudomonas syringae produces a wide spectrum biocide, syringomycin (SR) (DeVay et al., 1968, Sinden et al., 1971). This toxin is not required for bacterial growth *in planta*, but significantly contributes to virulence (Xu and Gross, 1988). Several genes are required for syringomycin production, a few of them being components of the syringomycin synthetase complex (Xu and Gross, 1988). Expression of genes required for syringomycin production was promoted by plant signals present in cherry leaf extracts (Gross, personal communications).

In this study, an excised twig assay to screen for cherry rootstock tolerance to *Pseudomonas syringae* pv. *syringae* was developed, and cherry rootstocks from GI and M x M series were evaluated for their tolerance to bacterial canker. Crude extracts from these rootstocks were also evaluated for the ability to induce the *syrB* gene in *P. syringae* pv. *syringae*, and the activity of plant signals in the rootstocks was measured. A comparison of the results of the two assays was made.

Chapter 2

LITERATURE REVIEW

Introduction

Bacterial canker, caused by *Pseudomonas syringae*, is recognized as a serious disease of stone fruit trees. *P. syringae* occurs as a resident epiphyte on these tree species throughout the world. The bacterium has been reported in Chile (Latorre *et al.*, 1985), England (Grosse, 1963, 1966), France (Gardan *et al.*, 1972), Poland (Sobiczewski, 1978), South Africa (Ross and Hattingh, 1986), and United States (Cameron, 1960, 1962, 1970, 1971; English and Davis, 1960; Jones, 1971; Weaver, 1978).

Investigations as to the causal agent of bacterial canker began in the second half of the 19th century. At this time, the term "canker" was first used in the disease description. It was thought that canker was caused by a variety of factors, including inorganic, such as frost, poor orchard management, occurrence of excessive amount of sap in the trees, insect damage, and also by the saprophytic fungus *Nectria ditissin*. The first formal description of the disease was made by Sorauer (1881). Work on the disease was continued by Bos (1899), and finally Van Hall (1902). In 1902, Van Hall's report provided evidence that a bacterium was the cause of cankers and gummosis in fruit trees. Brzezinski (1902) in Poland showed that blight and cankers occurring on stone fruit trees, apples, pears, and hazelnuts were mainly of bacterial

origin. In Germany, Aderhold and Ruhland (1907) published a report about a bacterial caused gum-flow in fruit trees.

Presence of the disease in Oregon was first reported by Griffin (1911), who reported a bacterial bud blight in dormant sweet cherry trees. Barrs (1913) later demonstrated that some of the bacteria isolated by Griffin could also cause cankers on limbs. Moreover, Barrs mentioned several outbreaks of bacterial canker which occurred as early as 1853 on dormant sweet cherry trees in Oregon. Later, comparative studies, conducted in the United States, revealed that the aforementioned bacterial pathogen was the same organism which caused bacterial blight of lilac, *Pseudomonas syringae*. More detailed studies of bacterial canker of sweet cherries were done by Cameron (1960, 1962, 1970, 1971). Numerous common names have been used to describe the disease caused by *P. syringae*: 'blossom blast', 'gummosis', 'bacterial canker', 'die-back', 'shoot blight', 'bacterial sour-sap', 'cherry gummosis', 'spur blight', 'blast of stone fruits', 'sour-sap', and 'wither tip'. These names originated from the different symptoms caused by *P. syringae*. Presently, the common name 'bacterial canker' is used in most countries to describe the disease.

Two pathovars of *P. syringae* cause similar symptoms on sweet cherries. Pathovar *morsprunorum* is reported to induce symptoms on cherries in England (Crosse, 1963), Poland (Sobiczewski, 1978), South Africa (Ross and Hattingh, 1986), the eastern states of the United States, and Michigan (Cameron, 1962; Jones, 1971). In Oregon, only the pathovar *syringae* caused symptoms on sweet cherry trees (Cameron, 1962).

Several potential sources of inoculum in the field have been

identified by various researchers. Buds are regarded as major overwintering sites of *P. syringae* (Leben, 1981; Mansveld and Hattingh, 1987; Ross and Hattingh, 1986a). Bacteria were detected inside apparently healthy apple and pear buds during growing and dormant seasons over a 2-year period in South Africa. The pathogen appears to be sheltered inside the bud during the hot, extremely dry summer months. During the fall, *P. syringae* was detected on each of the individual bud scales and also on the primordium. Pathogenic bacteria were also isolated from many apparently healthy buds of stone fruit trees (Ross and Hattingh, 1986a). Healthy buds can harbor the inoculum in the absence of disease. When buds develop and leaves unfold, the bacteria are probably already present on the developing leaves and are distributed by splashing water to other parts of the plant. Active, expanding buds contained a higher level of bacteria than did dormant buds (Ross and Hattingh, 1986a). Mansvelt and Hattingh (1987) showed that population levels of bacteria on the bud scales decreased during winter but rose sharply in spring. The bacteria were also present on newly formed buds.

The epiphytic population of these bacteria may play an important role in their survival. Epiphytic bacteria survive on the surface of the plant tissue and may cause infection when suitable environmental conditions develop. Several studies have demonstrated that *P. syringae* can exist as an epiphyte on weeds, grasses, and apparently healthy plants (Baca and Moore, 1987b; Latorre and Jones, 1979b; Ross and Hattingh, 1986d). *P. syringae* strains have been isolated from numerous non-symptomatic plants, showing that the bacterium survives as an

epiphyte without causing disease (Lindow, 1978a). Workers from different parts of the world have reported that *P. syringae* could be isolated during the growing season from weeds and grasses (Baca and Moore, 1987b; Latorre and Jones, 1979b; Lyskanowska, 1976; Ross and Hattingh, 1986c). *P. syringae* was the dominant organism isolated from the broadleaved herbaceous plants grown in stone fruit orchards in South Africa. An epiphytic phase of *P. syringae* on weeds might have important implications on the epidemiology of stone fruit trees (Ross and Hattingh, 1986d). Pathogenic *Pseudomonas* may spread from weeds by splashing rain and become established on stone fruit trees as a resident population (Crosse, 1963; Ross and Hattingh, 1986c). Latorre and Jones (1979a) speculated that these bacteria overwintered on weeds in Michigan.

P. syringae also survived throughout the growing season on the surface of symptomless leaves of host trees in South Africa. The resident phase of the bacterium was probably initiated in the spring by inoculum present in active cankers and buds of infected trees (Ross and Hattingh, 1986a). Epiphytic populations of *P. syringae* were also found on symptomless buds or other aerial parts of cherry trees, and it was suggested that they may serve as a very important source of primary inoculum (Crosse, 1959, 1966; Gross *et al.*, 1984a; Latorre and Jones, 1979b). However, Malvic and Moore (1988) suggested that epiphytic *P. syringae* populations may be important for survival and secondary spread during the growing season, but may not be important relative to survival and overwintering of the primary inoculum.

P. syringae was also isolated from the internal tissues of apparently healthy cherry trees (Cameron, 1970). In addition, the

systemic spread of the bacteria was demonstrated by isolation from symptomless leaves and petioles. Bacteria can enter the plant during the growing season and establish a very important source of inoculum inside symptomless tissue (Ross and Hattingh, 1987). Signs of the infection may not be seen until the following growing season. Cameron (1970) pointed out the significance of this type of silent infection to chemical disease control. This may explain the low efficacy of protective bactericides applied to the tree surface.

Dissemination

Knowledge about the ways by which an organism is disseminated is important in disease control. *P. syringae* can be moved from place to place by wind or rain (Crosse, 1966; Malvic and Moore, 1988). These factors are also reported to spread the inoculum from weeds (Crosse, 1963) and winter cankers (Cameron, 1962a) to the trees and new foliage.

Dispersion of the disease by infected nursery stock has been suggested (Crosse, 1955; Wormland, 1942). It was shown that a significant portion of apparently healthy budwood from stone fruit trees carries pathogenic *Pseudomonas* (Ross and Hattingh, 1986a). The organism can be spread by budwood to nursery trees, which will not show symptoms, until predisposing field conditions favor disease development. In some nurseries in Poland only 10% of cherry buds inserted into rootstocks survived. The high failure rate was ascribed to bud infection by *P. syringae* (Lyskanowska, 1976). Since cankers do not always appear in the first years, the trees could be planted in the orchard where disease outbreak would occur and infection could be spread to other healthy

trees. This type of spread has important implications for plant breeders and producers. The demand for bacteria-free budwood is evident. Cameron (1962a) pointed out that some nursery practices may promote outbreak of the disease. Of considerable concern is the practice of tying trees in tight bundles, and then heeling in the bundles under several inches of soil or sawdust. Open wounds made during the tying and cool, wet spring weather offer ideal conditions for infection. All these occur together when the trees are planted out in the nursery beds.

Wounding seems to play a significant role in most of these infections. Whether mechanically induced, or caused by frost injury, wounds predispose trees to blossom blight and bacterial canker. Pruning wounds also aid infection (Cameron, 1962a and b; Ross and Hattingh, 1986b).

INA properties

Numerous workers have reported that bacterial canker in the field is related to cold temperatures (Panagopoulos and Crosse, 1964; Weaver, 1978). Ice nucleation active (INA) bacteria *P. syringae* and *Erwinia herbicola* are associated with the surface of most plant species. They are the most well known biogenic sources which can cause ice nucleation at about -1.5 °C (Gross *et al.*, 1983). In spring, the first symptoms of bacterial canker often appear after a late frost. This phase of the disease is the most dangerous because a large portion of expanding leaves and flowers may be damaged (Gross *et al.*, 1984b). Bacterial canker can develop in naturally infected and artificially inoculated

trees and on excised limbs in the laboratory within a few days after a freeze (Weaver, 1978). The pathogen enters the plant through frost-induced wounds, and contents of ruptured cells are used as a nutrient source for the pathogen (Sule and Seemuller, 1987). Potential ability to induce frost injury to leaves and flowers of fruit trees may facilitate infection and amplify multiplication of the INA pseudomonad component of the bacterial microflora on fruit tree surfaces (Gross *et al.*, 1984b). Many *P. syringae* strains isolated from woody plants in Oregon and Washington have ice nucleation activity but differ in their INA characteristics (Baca *et al.*, 1987a).

Disease severity

The severity of bacterial canker symptoms depends on climatic conditions and cultivar variety (Cameron, 1962b). Plant age may also play an important role in disease severity. Several researchers stated that disease incidence appears to be greatest in one to six year-old cherry orchards (Cameron 1955, 1962b; Latorre *et al.*, 1985; Ross and Hattingh, 1986b). Tree losses as high as 75% have been reported in young orchards in Oregon. However, trees were seldom killed after they were in the orchard for eight years (Cameron, 1962b). In general, young succulent tissues are most susceptible to infection. Once the bacteria become established in the young orchard, the tops of the trees may be killed. Older trees, eight years and more, are usually not as susceptible as younger ones and thus are less severely infected. Cankers that occur on older trees are often located on smaller limbs where they do not kill the tree. However, bacteria from these infections later

could move down the limbs, as rain spreads the bacteria around the tree. New cankers may form on the scaffold branches and trunk. This is often the cause of the dead shoots and branches that appear in many sweet cherry orchards during spring and early summer in Oregon (Cameron, 1970). Infection of spurs and buds by *P. syringae* is usually more severe on mature trees, but cankers are more important on young trees (Cameron, 1962b).

Cankers on the trunk and scaffold limbs probably cause the greatest damage to cherry trees by affecting the vascular cambium. That portion of the tree is effectively girdled and the area above the girdle will eventually die. If the canker is on the trunk below the scaffold limbs, the entire top of the tree may be killed.

Disease outbreak is frequently found in areas characterized by cool, wet springs, and is associated with periods of high winds and continued moisture (Cameron, 1962b; Crosse, 1956a). Variations in available moisture and temperature may influence fluctuations in bacterial distribution and frequency during the seasons. Highest bacteria populations occur in early spring, with a moderate increase after the first fall rains. Lowest populations are in midsummer and during the coldest weeks of winter (Cameron, 1970).

Time of infection

P. syringae usually infects limbs during fall and winter (Garrett, 1981). Bacteria enter the plant through the bases of infected buds and spurs, as well as through leaf scars, pruning cuts, and injuries caused by various agents. After entering the plant, bacteria migrate

intercellularly and progress into the bark and the medullary rays of xylem and phloem. In advanced stages of infection, the bacteria assail and break down parenchyma cells, which results in the formation of lysogenic cavities filled with bacteria. Infected areas are slightly sunken and may have a slightly darker brown color than the rest of the bark. When the canker area is cut, the bark may be any shade from bright orange to brown. The cambium may or may not be affected. At both the upper and lower margins of the canker, narrow brown streaks extend into the healthy tissue.

As the trees break dormancy in the spring, gum may be formed by the tissue surrounding the canker and may exert enough pressure to break through the bark and run down the outside of the limb. Cankers that do not produce gum are similar, but usually are moister, sunken, and may have a sour odor. Development of the cankers is relatively rapid in fall, after the trees have gone into dormancy, but before low winter temperatures occur. During cold winter periods, canker development is slow, but then increases rapidly between the end of the cold weather and the beginning of rapid tree growth in the spring. Infected areas increase in size during winter and cankers become visible in early spring. Infections during the active growing season are not very important, as they are very quickly isolated by callus tissue. The ability to wall-off the infection seems to be correlated with varietal resistance but is also affected by the age and succulence of the plant, the temperature and rainfall during a season, and the type of rootstock on which the tree is growing. In general, *P. syringae* is a rather weak

pathogen and will cause serious canker damage only when the tree is in a dormant condition (Cameron, 1962b).

Bud infection

Two hypotheses explain how bacteria enter the bud. The first states that bacteria enter the spur via the vascular traces of the leaf scar. The organism is sucked into the vessels during rain and high winds, and the pathogen then spreads into the living tissue (Crosse, 1954, 1955). Cameron (1962a) reported that infection of the buds in the Pacific Coast states does not occur through the leaf scars. Bacteria are washed into cracks at the base of the slightly open bud scales and infection occurs at the base of the outside bud scales. The organism is then spread throughout the base of the bud, killing the tissues across the base, and separating the growing point from the rest of the plant. The bacteria sometimes spread downward and kill the stem tissues around the base of the bud. The seriousness of this infection is greatly increased in years when bud swell begins earlier in the year. First symptoms may be observed by sectioning buds in late February and early March. Brown discolored areas may be observed at the base of the bud scales of infected buds. The brown area extends across the base of the bud and the entire bud eventually dies (Cameron, 1962b). Both flower and leaf buds are equally affected. The severity of bud infection may be observed during spring by comparison of the heavy bloom on healthy trees with the black skeletons of diseased trees.

Leaf infection

Leaf infection appears on young succulent leaves. It occurs most frequently in areas with cool, wet springs and during periods of high winds and continued moisture. As leaves mature, they become less susceptible. Leaf infection is rare late in the season. Infection takes place through stomata. From there, the pathogen spreads intercellularly through the mesophyll, resulting in small angular leaf spots, due to collapse and death of the cells. Bacteria then progress intercellularly from the mesophyll, through the parenchyma bundle sheath into the vascular system of the minor vein, and from there to the main vein. As the invasion continues, bacteria may be found in the xylem (Ross and Hattihgh, 1987). The foliage becomes pale green to yellowish-green, leaf margins are rolled and the leaves appear wilted. These symptoms are visible at any time from bud break into the middle of summer. Infected areas appear as angular or circular spots, 1-2 mm in diameter, dark green, water soaked, with a yellow halo. Old infections become dry and brittle and fall out of the leaf. Affected leaves may have either a shot-hole appearance, that may be quite irregular, or the entire leaf tip and margin may drop. Leaf petioles may also show similar symptoms. The affected branches usually wilt and die before fall. During wet weather, bacteria ooze out of the spots and are spread to other leaves by direct contact, insects, and rain. Leaf infection is rather uncommon in Oregon (Cameron, 1962b).

Flower infection

Flower infection by *P. syringae* is rare but it may be very severe under favorable conditions. Bacteria enter the flowers through natural openings and through wounds made by insects, hail, or wind-blown rain. Under very humid conditions, the bacteria spread through the floral parts quickly and may advance into spurs and twigs, where they can initiate canker formation. Infected blossoms appear water soaked, turn brown and hang down. The common disease name, 'blast', originated from these symptom descriptions (Cameron, 1962b).

Fruit infection

Fruit infection appears as flat, irregular, dark brown to black lesions, 2-3 mm in diameter. Spots may be depressed and have underlying gum pockets. The infection may sometime reach the stone (Cameron, 1962b; Dye, 1953; Jones, 1971).

The majority of the described symptoms can occur to varying degrees wherever the bacteria are found. However, certain areas are more predisposed to a particular phase of the disease. Cankers on trunks and limbs are very common on susceptible cultivars in England (Crosse, 1954) and New Zealand (Dye, 1953), moderate in western Oregon and California, and mild in eastern Oregon (Cameron, 1962b). Death of dormant buds is very severe in western Oregon, but is less significant in California and other parts of the world (Cameron, 1962b). Shoot and spur withering is common in England, but rarely occurs along the Pacific coast (Anderson, 1956). Leaf spotting is especially severe in England (Crosse, 1956b),

almost nonexistent in California (Anderson, 1956), and mild in Oregon. Blossom and fruit symptoms occur in New Zealand (Dye, 1953), Great Britain (Wormland, 1937), California (Anderson, 1956), and occasionally in Oregon (Cameron, 1962 b). Whether this is a result of strain differences of the pathogens, or of different climatic conditions, has not been determined.

Phytotoxin production

Plant pathogenic strains of *P. syringae* produce a broad spectrum biocide, syringomycin (SR) (DeVay *et al.*, 1968; Sinden *et al.*, 1971). The phytotoxicity of SR and its production by all pathogenic isolates tested suggests its involvement in bacterial canker of stone fruit trees (DeVay *et al.*, 1968). Symptoms produced by *P. syringae* on inoculated peach shoots were similar to symptoms produced by SR (Sinden *et al.*, 1971). Syringomycin is a peptide-containing phytotoxin that is not host-specific but biocidal to a wide spectrum of organisms (DeVay *et al.*, 1968). It disrupts physiological functions within the plasma membrane of host cells, thus producing necrosis which resembles at least part of the natural disease syndrome (Gross, 1985). Syringomycin enhances disease development during pathogenesis by killing a large number of host cells, which results in more necrosis and larger lesion size. It was shown that toxin is not required for bacterial growth *in planta* and pathogenicity, but contributes significantly to virulence (Xu and Gross, 1988).

Production of syringomycin is associated with four proteins, which are believed to function as syringomycin synthetases (Morgan and

Chatterjee, 1988). Several genes are required for syringomycin production, however, few of them may be directly responsible for its biosynthesis, e.g. *syrA* and *syrB* genes are required for the formation of two proteins, which are believed to be components of the syringomycin synthetase complex (Xu and Gross, 1988).

Current research in Dr. Gross's laboratory at Washington State University (Gross, personal communication) has identified that signal molecules present in cherry leaf tissue are partially responsible for the induction of syringomycin production in *Pseudomonas syringae* pv. *syringae*. Gross recombined the *syrB::lacZ* fusion into the genome of *P. s. syringae* strain B3A, which, in comparison to some other *P. syringae* pv. *syringae* strains, does not produce syringomycin in SR-minimal (SRM) medium. The resulting mutant B3AR132 also lacks activity in SRM medium. In comparison, both strains expressed activity in potato-dextrose broth (PDB), which suggests that a component of potato was responsible for induction of *syrB* gene. Addition of cherry leaf extracts to SRM medium induced expression of *syrB::lacZ* fusion in strain B3AR132, suggesting that the virulence genes which are responsible for syringomycin production in *P. syringae* pv. *syringae* may be induced by endogenous plant substrates (Gross, personal communication).

Control

Chemical control for bacterial canker is very limited. In general, copper compounds and streptomycin are used. However, repeated applications of one bactericide may lead to the selection of copper- or

streptomycin-resistant mutants (De Boer, 1980; Young, 1977). Cameron (1962b) stated that leaf spot and killing of buds have been successfully controlled by spray applications of bactericides, but control of the canker phase has generally been erratic (Cameron, 1962b).

Cultural practices play a role in decreasing disease severity. Summer pruning of old cankers may help reduce the amount of inoculum (Wilson, 1953). Another method, widely used in New Zealand, is based on burning *P. syringae* cankers with a propane burner. With this method further spread of bacteria was limited. Cauterization seems to be an easy method, and most cankers are controlled after one treatment (Hawkins, 1976).

Time of pruning is also a very important factor in disease control. Pruning wounds provide entrances for bacteria, especially during wet periods (Crosse, 1954). However, no evidence of increasing canker development has been noted between fall, winter, and summer pruning in the Pacific Northwest (Cameron, 1962b).

As early as 1913, Barss suggested the use of resistant or tolerant trunk stocks. He reported that Mazzard as a trunk stock is a "thoroughly practical way of protecting cherry from the disease". The top of the tree seemed to be less susceptible to the disease if the trunk and the scaffold limbs were resistant stocks. Grubb (1944) observed that rootstocks grafted high were less susceptible than those grafted low. Several experiments carried out by Cameron (1960, 1971) lead to similar conclusions. Cultivars grafted on scaffold limbs of Mazzard had fewer cankers than when budded low on the rootstock. This

justified the practice of high working trees on a tolerant trunk stock. Reduced canker development may be due to a degree of resistance imparted from the rootstock to the scion. An influence of rootstock on the susceptibility of scion cultivars of plum (*Prunus domestica*) to bacterial canker has been demonstrated (Shanmuganatham and Crosse, 1963). The decrease in disease incidence in cherry might also be a result of tolerance of the rootstock. Nevertheless, the nature of the rootstock-scion combination are both of great importance (Cameron, 1962b).

Rootstocks

Research in several countries aims to develop new, good quality, commercially desirable cherry rootstocks and cultivars. One of the attributes will be a high degree of resistance to bacterial canker. Primary cherry rootstocks in use in the world are seedlings or clonal selections of *Prunus avium* L. known as 'Mazzard' and *Prunus mahaleb* L. known as 'St. Lucie' or 'Mahaleb'.

Cherry rootstocks most commonly used in Willamette Valley are F/12-1, 'Mazzard' and minor acreage on 'Colt'. F/12-1 is a clonal selection of *P. avium* released by East Malling Experimental Station in England and is recognized as being canker-resistant (Webster, 1984). In Oregon, the rootstock F/12-1 is reported to be quite tolerant to *P. syringae* pv. *syringae*. 'Colt', a hybrid of *Prunus avium* x *Prunus pseudocerasus*, was also released by East Malling and is resistant to *P. syringae* pv. *morsprunorum* (Webster, 1984). The introduction of this 'canker resistant', semi-dwarfing rootstock 'Colt' (Webster, 1980), to

replace the older, moderately canker-tolerant but very vigorous F/12-1 rootstock, has facilitated disease control (Garrett, 1986). However, 'Colt' was tested for tolerance to the bacterial canker caused by pathovar *morsprunorum* but not to pv. *syringae* which is the casual agent of bacterial canker in Oregon.

Resistance to bacterial canker in *P. avium* is very low (De Vries, 1965). However, rootstock F/12-1 which is clonal selection of *P. avium* is known to be tolerant to the disease, as a result of field selection for tolerance to *Pseudomonas syringae*.

Recently, new interspecific hybrid rootstocks were introduced in the United States. They include M x M clones, selected by Lyle Brooks in Oregon, which are hybrids of *P. mahaleb* x *P. avium*. The Giessen clones selected and evaluated by Gruppe at the Justus Liebig University in Giessen, West Germany, became available 4-5 years ago. The Giessen stocks are interspecific hybrids, crosses between *P. avium*, *P. canescens*, *P. cerasus*, *P. fruticosa*, *P. mahaleb* and *P. pseudocerasus* (Cummins, 1984).

The absence of effective bactericides to control bacterial canker caused by *P. syringae* encourages plant breeders to develop resistant genotypes. Screening cherry rootstocks for resistance to bacterial canker is currently based mainly on field observations. However, several years of observations are required for valid assessments. Also, variation in environmental conditions from year to year can influence results of field screening tests. Differences in environment, soil, and strains of the pathogen between location may account for contradictory results in screening tests for a particular genotype.

Inoculation of branches and leaf scars of trees in the field was used in England to screen for tolerance to *Pseudomonas syringae* pvs. *morsprunorum* and *syringae* (Crosse and Garrett, 1966; Garrett, 1981)). However, branch inoculations required 3 months, and leaf scar inoculations even longer for symptoms to develop. The latter method does not find practical use in screening for tolerance to pathovar *syringae* because the leaf scar is not the primary route of entry for this pathogen (Garrett, 1981). Garrett (1981) mentioned the use of potted trees in controlled environment greenhouse for inoculation, but 4 to 6 months would still be required for symptom expression.

Chapter 3

EXCISED TWIG ASSAY TO SCREEN CHERRY ROOTSTOCKS FOR TOLERANCE TO
Pseudomonas syringae pv. syringae

ABSTRACT

An excised twig assay was developed to evaluate cherry rootstocks for tolerance to *Pseudomonas syringae* pv. *syringae*. In 1988, one-year-old twigs of 'Napoleon', 'Corum', and F/12-1 were collected at monthly intervals from November until January. Twigs were inoculated with water alone, one avirulent (K-4), and three virulent (W4N54, AP-1, and B-15) strains of bacteria at 10^5 , 10^6 , and 10^7 cfu/ml. Incision browning, gummosis, and callus production were evaluated after 4 weeks of incubation at 15 °C and high relative humidity.

No gummosis or browning were observed on twigs inoculated with water alone or the avirulent strain. Callus production does not appear to be a usable criterion for genotype tolerance in this assay. In general, there was no significant inoculum concentration effect on browning, gummosis, or callus development. However, in some cases concentrations of 10^6 and 10^7 induced more browning and gummosis than 10^5 cfu/ml. The susceptible genotypes, i.e. 'Napoleon' and 'Corum' had higher incision browning and gummosis ratings than the tolerant genotype, F/12-1.

In 1989, 'Napoleon', 'Corum' and a number of cherry rootstocks, including: F/12-1, M x M 2, M x M 39, M x M 60, GI 148-1, GI 148-8,

GI 154-2, GI 154-5, GI 169-15, GI 172-9, and GI 173-9 were evaluated. One-year-old wood was collected at monthly intervals from October until December. Twigs were inoculated with water and the same strains of *P. syringae* pv. *syringae* used in the previous year at 10^7 cfu/ml. Ratings of incision browning, gummosis, and callus production were made after 4 weeks of incubation. Again, 'Napoleon' and 'Corum' had the highest browning and gummosis ratings. In general, there were no differences in browning and gummosis ratings between F/12-1 and any other the rootstocks tested. However, on some occasions, rootstock GI 172-9 and GI 169-15 had higher browning ratings than F/12-1. On the last sampling date, rootstocks M x M 2, GI 173-9, GI 148-8, and 154-2 showed less browning than F/12-1.

INTRODUCTION

Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae*, is a serious disease of sweet cherries (*Prunus avium* L.) in the Pacific Northwest. The infection occurs on the aboveground part of the tree. The typical symptoms observed in the Northwest are: trunk and shoot cankers, dead buds and blossoms, and occasionally leaf and fruit lesions (Cameron, 1962a and b). Infection may result in localized cankers, death of buds, limbs, or the entire tree.

Chemical control for bacterial canker is not very successful. The most commonly used bactericides are copper compounds and sometimes streptomycin. Cameron (1962a) stated that leaf-spot and the killing of buds have been successfully controlled by spray applications, but chemical control of the canker phase has generally been erratic. Repeated use of one bactericide has resulted in the selection of copper- or streptomycin-resistant mutants (De Boer, 1980; Young, 1977).

The influence of rootstock on the susceptibility of scion cultivars of plum (*Prunus domestica*) to bacterial canker has been demonstrated (Shanmuganathan and Crosse, 1963). Cameron (1962) showed that cherry cultivars grafted high on a tolerant rootstock sometimes showed reduced occurrence of canker on the scion.

The primary cherry rootstocks planted around the world are seedlings or clonal selections of *Prunus avium* L. known as 'Mazzard' and *Prunus mahaleb* L. known as 'Mahaleb' or 'St. Lucie'. Cherry rootstocks most commonly used in the Willamette Valley are F/12-1, 'Mazzard' seedlings and minor acreage on 'Colt'. F/12-1, a clonal selection of *P. avium*, is

reported to be tolerant to *P. syringae* pv. *syringae* in Oregon. 'Colt', a hybrid of *Prunus avium* x *P. pseudocerasus* was tested for tolerance to the bacterial canker caused by *P. syringae* pv. *morsprunorum*, but not to *P. syringae* pv. *syringae* (Webster, 1980). Recently, new interspecific hybrid rootstocks were introduced in the United States. They include the M x M clones, which are hybrids of *P. mahaleb* x *P. avium*, and the Giessen (GI) clones, which are interspecific crosses between *P. avium*, *P. cerasus*, *P. canescens*, *P. fruticosa*, *P. mahaleb* and *P. pseudocerasus* (Cummins, 1984). Little is known about the tolerance of these rootstocks to *P. syringae* pv. *syringae*.

Screening cherry rootstocks for resistance to bacterial canker is primarily based on field observations. However, valid assessment of resistance can be made only after several years. In field testing, at a single location, the variation in environmental conditions from year to year can influence the result of screening tests.

An excised twig assay was used previously with peach to determine interaction between *P. syringae* and freezing (Weaver, 1978). Swell and Wilson (1959) suggested that dormant twig assays in the laboratory could substitute for field inoculated screening trials.

The purpose of this study was to develop an excised-twig assay for *P. syringae* pv. *syringae* tolerance and to compare the results based on the rapid assay with known disease reaction in the field, and to use the developed method to screen newly introduced cherry rootstocks for resistance to bacterial canker caused by *P. syringae* pv. *syringae*.

MATERIALS AND METHODS

ASSAY DEVELOPMENT

Strains and dilutions

Three virulent (W4N54, AP-1, B-15) and one avirulent (K-4) strains of *P. syringae* pv. *syringae* were used. The sources and pathogenicity of the strains are summarized in Table 3.1. Strains were cultured on King's medium B (KB) agar (King, 1954) for 3 days at 26 °C and suspended in sterile deionized water to a final cell density of 10^5 , 10^6 , and 10^7 cfu/ml as determined spectrophotometrically and confirmed by a standard dilution plate assay.

Experimental procedure

One-year old twigs of 'Napoleon', 'Corum', and 'F/12-1' were excised from trees growing at the Lewis-Brown Horticulture Research Farm, Oregon State University, Corvallis, Oregon. Sampling dates were 22 November 1988, 23 December 1988, and 26 January 1989. All twigs were placed in polyethylene bags and brought to the laboratory. Twenty cm sections were excised from the apical end of each shoot. Twig sections were surface sterilized by dipping for 30 seconds in 75% ethanol, then for 10 minutes in an 0.05% solution of sodium hypochlorite. Twigs were then rinsed in distilled water three times, and blotted dry. A 2-cm segment was cut from basal ends of twigs to remove portions that may have absorbed sodium hypochlorite. A sterile razor blade was used to make a longitudinal incision, 0.5 x 2 cm flap, on each twig to expose the

cambium. A 20 μ l drop of bacterial suspension was placed in each incision with a sterile pipette. Ten twigs per genotype were inoculated with each strain and concentration, and with water which served as a control. After inoculation, twigs were placed on water saturated cotton in the bottom of sterile glass test tubes (2.5 x 25 cm). Tubes were covered with plastic caps and incubated at 15 °C in the dark. After 4 weeks, twigs were evaluated for the amount of gummosis (0-2, 0 = no gummosis), callus production (0-1, 0 = no callus), and browning of the incision (1-4, 1 = yellow pith).

ROOTSTOCK EVALUATION

'Napoleon', 'Corum', and cherry rootstocks from the M x M and Giessen (GI) series were tested for tolerance to bacterial canker. The rootstocks that were evaluated and their parentage are listed in table 3.2. Twigs were collected on 20 October, 25 November, and 20 December 1989. Plant material for rootstock testing (M x M and GI series) was collected from Meadow Lake Nursery Co, McMinnville, Oregon.

Twigs were assayed as above using the same strains of *P. syringae* pv. *syringae* at a concentration of 10^7 cfu/ml. Water was used as a control.

RESULTS AND DISCUSSION

ASSAY DEVELOPMENT

Concentration effect.

Twigs inoculated with the avirulent strain or water did not cause incision browning or induce gummosis. The means were 1.0 and 0.0, respectively. Since inoculation with water and the avirulent strain did not cause browning or gummosis in any case, the browning and gummosis of an incision were assumed to be an indicator of susceptibility.

In general, there was no concentration effect on incision browning or gummosis (Tables 3.3 and 3.4). If there was an effect, concentrations of 10^6 and 10^7 induced more browning and gummosis than 10^5 cfu/ml, with one exception when concentrations 10^5 and 10^7 induced the highest browning response on 'Corum' inoculated with W4N54 on 23 December. Also, a greater gummosis rating was observed on 'Napoleon' after inoculation with a bacterial suspension of W4N54 on 26 January at concentrations 10^5 and 10^6 cfu/ml.

In general, no significant differences in callus production were observed among genotypes tested regardless of concentration (Table 3.5). This apparent lack of response may have resulted from the rating scale, which was based on presence or absence of callus, and did not account for the amount of callus that developed.

Genotype effect

'Napoleon' and 'Corum' had the highest browning and gummosis ratings 4 weeks after inoculation (Tables 3.6 - 3.11). However, in a few cases, 'Napoleon' had a higher browning and gummosis rating than 'Corum', and in one case 'Corum' responded with the highest gum production. Both cultivars appear to be highly susceptible to the pathogen in our excised twig assay, which agrees with results from field observations.

F/12-1 consistently had the lowest browning and gummosis rating, however, some gummosis and browning were observed in almost all cases. This suggests that F/12-1 is tolerant, rather than resistant to *P. syringae* pv. *syringae*, which confirms the field observation that some disease occurs on this rootstock in Oregon.

Generally, there are no differences among genotypes relative to the presence of callus on the incision (Table 3.12 - 3.14).

ROOTSTOCK EVALUATION

'Napoleon' and 'Corum' usually had the highest browning rating compared to all other genotypes (Table 3.15 - 3.17). Rootstocks GI 172-9 and GI 169-15 had higher browning rating than F/12-1 on two out of three dates, when inoculated with strain W4N54 (Table 3.15). The other rootstocks, in general, were not significantly different from F/12-1. After inoculation with strain AP-1, only the rootstock GI 172-9 had a higher browning rating on two out of three dates, in addition to 'Napoleon' and 'Corum' (Table 3.16). A browning rating lower than F/12-1, was observed on the last date on rootstocks M x M 2, GI 173-9, GI 148-8 and GI 154-2 after inoculation with all the virulent strains.

This might be a result of the physiological stage of this rootstocks. Rootstocks from the last inoculation date were evaluated in mid January, and at this time some buds were breaking on inoculated twigs, which suggests that rest was satisfied and dormancy broken.

The gummosis ratings were generally higher for 'Napoleon' and 'Corum' in than for F/12-1, when inoculated with strain W4N54 (Table 3.18). Inoculation with strain AP-1 and B-15 on the last two dates also resulted in higher gummosis rating of 'Napoleon' than this for F/12-1 (Table 3.19 and 3.20). Only on the last date did 'Corum' have a higher gummosis rating than F/12-1 when inoculated with strain AP-1 and B-15. GI 169-15 was the only rootstock evaluated which had a more gummosis in comparison to F/12-1. This occurred when twigs were inoculated with strain AP-1 on the last date (Table 3.19). The degree of gummosis appeared to increase as samples were collected later in the winter months.

Callus production was again not a usable evaluation criterion for rootstock tolerance. The susceptible genotypes 'Napoleon' and 'Corum' did not differ from F/12-1 in their callus ratings (Table 3.21- 3.23).

Results based on previously reported field observations and those from the excised twig assay in this assay show high susceptibility of 'Napoleon' and 'Corum' to the virulent strains of *P. syringae* pv. *syringae*. Lesion browning and gummosis were not observed on twigs inoculated with water and the avirulent strain in the excised twig assay. These data confirm the reliability of the excised twig assay.

Table 3.1. Source and pathogenicity of *Pseudomonas syringae* pv. *syringae* strains.

Strain	Source	Pathogenicity
W4N54	Anjou Pear	+ ^x
B-15	Peach	+
AP-1	Asian Pear	+
K-4	Anjou Pear	-

^xCherry fruitlet pathogenicity test.

Table 3.2. Parentage of cherry rootstocks evaluated in the excised twig assay.

Rootstock	Species or Hybrid
F/12-1	<i>P. avium</i>
Colt	<i>P. avium</i> x <i>P. pseudocerasus</i>
MxM 2	<i>P. mahaleb</i> x <i>P. avium</i>
MxM 39	" "
MxM 60	" "
GI 148/1	<i>P. cerasus</i> Schattenmorelle x
GI 148/8	<i>P. canescens</i> EMRS
GI 154/2	<i>P. cerasus</i> x <i>P. canescens</i>
GI 154/5	" "
GI 169/15	<i>P. cerasus</i> x <i>P. avium</i>
GI 172/9	<i>P. fruticosa</i> 64 x <i>P. avium</i>
GI 173/9	<i>P. fruticosa</i> 64 x <i>P. cerasus</i> Schattenmorelle

(Cummins, 1984)

Table 3.3. The effect of inoculum concentration of three strains of *Pseudomonas syringae* pv. *syringae* on browning at the incision made into twigs of three cherry genotypes, 4 weeks after inoculation.²

Genotype	Conc. (cfu/ml)	Strain								
		W4N54			B-15			AP-1		
		22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.
Napoleon	10 ⁵	2.4a ^y	2.2a	3.0	1.7a	2.7	1.6a	2.4	2.9	2.8
	10 ⁶	3.1b	2.8ab	3.4	2.3b	2.6	2.6b	2.6	3.2	2.9
	10 ⁷	3.5b	3.6b	3.5	2.6b	2.8	3.5c	2.5	3.0	3.0
Corum	10 ⁵	2.9	3.1a	3.1	1.8a	2.7	2.3a	2.6	2.6	2.4a
	10 ⁶	3.1	2.3b	3.5	2.3ab	2.7	2.5a	2.8	2.9	3.3b
	10 ⁷	3.4	3.4a	3.5	2.9b	3.0	3.0b	3.0	3.0	3.7b
F/12-1	10 ⁵	1.7	1.1a	1.1a	1.1	1.6	1.0	1.5	2.2	1.6
	10 ⁶	1.7	1.6b	2.1b	1.4	1.5	1.1	1.1	2.4	1.9
	10 ⁷	1.7	1.5b	2.3b	1.4	1.8	1.1	1.4	2.5	2.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^yBrowning rating: 1-4 (1-yellow, 4-dark brown pith).

Table 3.4. The effect of inoculum concentration of three strains of *Pseudomonas syringae* pv. *syringae* on gummosis at the incision made into twigs of three cherry genotypes, 4 weeks after inoculation.²

Genotype	Conc. (cfu/ml)	Strain								
		W4N54			B-15			AP-1		
		22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.
Napoleon	10 ⁵	1.1 ^Y	0.6a	1.6a	0.4	1.7	1.4	1.4	1.8	1.9
	10 ⁶	1.0	2.0b	1.8a	0.6	2.0	1.6	1.3	2.0	1.6
	10 ⁷	1.2	1.8b	0.9b	0.8	2.1	1.9	1.5	1.9	1.9
Corum	10 ⁵	1.4	1.6	1.2	0.8	2.0	0.4a	1.1	1.4	1.6
	10 ⁶	1.1	1.8	1.3	0.9	1.8	0.8a	1.1	1.8	1.6
	10 ⁷	1.5	1.8	1.4	0.8	2.0	1.8b	1.1	1.7	1.8
F/12-1	10 ⁵	0.5	0.1a	0.4a	0.1	0.9	0.6	0.0a	1.5	0.8
	10 ⁶	0.2	0.5ab	1.2b	0.3	0.6	0.6	0.6b	1.5	0.8
	10 ⁷	0.3	0.9b	1.2b	0.2	0.9	1.0	0.1a	1.6	1.1

²Each value represents the mean for ten twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^YGummosis rating: 0-2 (0-no gummosis, 2-severe gummosis).

Table 3.5. The effect of inoculum concentration of three strains of *Pseudomonas syringae* pv. *syringae* on callus production at the incision made into twigs of three cherry genotypes, 4 weeks after inoculation.²

Genotype	Conc. (cfu/ml)	Strains								
		W4N54			B-15			AP-1		
		22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.
Napoleon	10 ⁵	0.4 ^y	0.3a	1.0	0.3	1.0	0.6ab	0.9ab	0.8	1.0
	10 ⁶	0.6	0.8ab	1.0	0.1	1.0	1.0a	1.0a	1.0	1.0
	10 ⁷	0.7	1.0b	1.0	0.1	1.0	0.3b	0.5b	1.0	0.9
Corum	10 ⁵	0.9	0.8ab	1.0	0.8	1.0	0.7	0.7	0.9	0.8
	10 ⁶	0.5	0.5ab	1.0	0.5	0.9	0.6	0.5	1.0	1.0
	10 ⁷	0.9	1.0a	0.8	0.5	1.0	1.0	0.8	0.8	0.9
F/12-1	10 ⁵	0.0	1.0	1.0	0.8	0.8	1.0	0.0	1.0	1.0
	10 ⁶	0.1	0.9	1.0	0.6	1.0	1.0	0.3	1.0	1.0
	10 ⁷	0.3	1.0	1.0	0.7	1.0	1.0	0.1	0.8	1.0

²Each value represents the mean for 10 twigs. Mean separation by genotype within columns was by Waller-Duncan k-ratio t-test, k=100.

^yCallus rating: 0-1 (0-no callus, 1-callus present).

Table 3.6. The effect of genotype on browning at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^5 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov. 23	Dec. 26	Jan.	22 Nov. 23	Dec. 24	Jan.	22 Nov. 23	Dec. 24	Jan.	-	-
Napoleon	2.2a ^y	2.2a	3.0a	1.7	2.4a	1.6a	2.4a	2.9	2.8	1.0	1.0
Corum	2.9a	3.1b	3.1a	1.8	2.7a	2.3b	2.4a	2.6	2.4	1.0	1.0
F/12-1	1.7b	1.1c	1.1b	1.1	1.6b	1.0c	1.5b	2.2	2.6	1.0	1.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by Waller-Duncan k-ratio t-test, k=100.

^yBrowning rating: 1-4 (1-yellow, 4-dark brown pith).

Table 3.7. The effect of genotype on browning at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^6 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	24 Jan.	22 Nov.	23 Dec.	24 Jan.	-	-
Napoleon	3.1a ^Y	2.8a	3.4a	2.3a	2.6a	2.6a	2.6a	3.2a	2.9a	1.0	1.0
Corum	3.1a	2.3ab	3.5a	2.3a	2.7a	2.5a	2.8a	2.9ab	3.3a	1.0	1.0
F/12-1	1.7b	1.6b	2.1b	1.4b	1.5b	1.1b	1.1b	2.4b	1.9b	1.0	1.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^YBrowning rating: 1-4 (1-yellow, 4-dark brown pith).

Table 3.8. The effect of genotype on browning at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^7 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	24 Jan.	22 Nov.	23 Dec.	24 Jan.	-	-
Napoleon	3.5a ^Y	3.6a	3.5a	2.6a	2.8a	3.5a	2.5a	3.0a	3.0b	1.0	1.0
Corum	3.4a	3.4a	3.5a	2.9a	3.0a	3.0b	3.0a	3.0a	3.7a	1.0	1.0
F/12-1	1.7b	1.5b	2.3b	1.4b	1.8b	1.1c	1.4b	2.5b	2.0c	1.0	1.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^YBrowning rating: 1-4 (1-yellow, 4-dark brown pith).

Table 3.9. The effect of genotype on gummosis at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^5 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	-	-
Napoleon	1.1a ^y	0.6a	1.6a	0.4ab	1.7a	1.4	1.4	1.8	1.9a	0.0	0.0
Corum	1.4a	1.6b	1.2a	0.8a	2.0a	0.4	1.1	1.4	1.6a	0.0	0.0
F/12-1	0.5b	0.1a	0.4b	0.1b	0.4	0.6	0.0	1.5	0.8b	0.0	0.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^yGummosis rating: 0-2 (0-no gummosis, 2-severe gummosis).

Table 3.10. The effect of genotype on gummosis at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^6 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	-	-
Napoleon	1.0a ^Y	2.0a	1.8	0.6	2.0a	1.1	1.3a	2.0	1.6a	0.0	0.0
Corum	1.1a	1.8a	1.3	0.9	1.8a	0.8	1.1ab	1.8	1.6a	0.0	0.0
F/12-1	0.2b	0.5b	1.2	0.3	0.6b	0.6	0.6b	1.5	0.8b	0.0	0.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^YGummosis rating 0-2: (0-no gummosis, 2-severe gummosis).

Table 3.11. The effect of genotype on gummosis at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^7 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	-	-
Napoleon	1.2a ^y	1.8a	0.9	0.8a	2.0a	1.6a	1.5a	1.9	1.9a	0.0	0.0
Corum	1.5a	1.8a	1.4	0.8a	2.0a	1.8a	1.1b	1.7	1.8a	0.0	0.0
F/12-1	0.3b	0.9b	1.2	0.2b	0.9b	1.0b	0.1c	1.6	1.1b	0.0	0.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^yGummosis rating: 0-2 (0-no gummosis, 2-severe gummosis).

Table 3.12. The effect of genotype on callus production at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^5 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	-	-
Napoleon	0.4a ^Y	0.3a	1.0	0.3	1.0	0.6	0.9a	0.8	1.0	1.0	1.0
Corum	0.9b	0.8b	1.0	0.8	1.0	0.7	0.7a	0.9	0.8	1.0	1.0
F/12-1	0.0c	1.0b	1.0	0.8	0.8	1.0	0.0b	1.0	1.0	1.0	1.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^YCallus rating: 0-1 (0-no callus, 1-callus present).

Table 3.13. The effect of genotype on callus production at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^6 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	-	-
Napoleon	0.6 ^Y	0.8	1.0	0.1	1.0	1.0	1.0a	1.0	1.0	1.0	1.0
Corum	0.5	0.5	1.0	0.5	0.9	0.6	0.5ab	1.0	1.0	1.0	1.0
F/12-1	0.1	0.9	1.0	0.6	1.0	1.0	0.3b	1.0	1.0	1.0	1.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^YCallus rating: 0-1 (0-no callus, 1-callus present).

Table 3.14. The effect of genotype on callus production at the incision after inoculation with three strains of *Pseudomonas syringae* pv. *syringae* at 10^7 cfu/ml, 4 weeks after inoculation.²

Genotype	Strains										
	W4N54			B-15			AP-1			K-4	Water
	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	22 Nov.	23 Dec.	26 Jan.	-	-
Napoleon	0.7ab ^Y	1.0	1.0	0.1a	1.0	0.3a	0.5ab	1.0	0.9	1.0	1.0
Corum	0.9a	1.0	0.8	0.5ab	1.0	1.0b	0.8a	0.8	0.9	1.0	1.0
F/12-1	0.3b	1.0	1.0	0.7b	1.0	1.0b	0.1b	0.8	1.0	1.0	1.0

²Each value represents the mean of 10 twigs. Mean separation by genotype within columns was by the Waller-Duncan k-ratio t-test, k=100.

^YCallus rating: 0-1 (0-no callus, 1-callus present).

Table 3.15. Contrasts between F/12-1 and 12 cherry genotypes of lesion browning induced by inoculation with *Pseudomonas syringae* pv. *syringae* strain W4N54.²

Genotype	Browning rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	1.1	1.2	1.5
Napoleon	3.3 ***	2.9 ***	3.2 ***
Corum	2.3 ***	1.2 NS	3.2 ***
MxM 2	1.0 NS	1.3 NS	1.0 *
MxM 39	1.6 *	1.2 NS	1.4 NS
MxM 60	1.2 NS	1.2 NS	1.2 NS
GI 148-1	1.6 *	1.3 NS	1.4 NS
GI 148-8	1.5 NS	1.3 NS	1.0 *
GI 154-2	1.1 NS	1.2 NS	1.0 *
GI 154-5	1.5 NS	2.6 ***	1.1 NS
GI 169-15	1.8 **	1.0 NS	2.0 *
GI 172-9	1.6 *	1.8 **	2.1 **
GI 173-9	1.2 NS	1.6 *	1.0 *

²Each value represents the mean of 10 twigs.

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

^yBrowning rating: 1-4 (1-yellow, 4-dark brown pith).

Table 3.16. Contrasts between F/12-1 and 12 cherry genotypes of lesion browning induced by inoculation with *Pseudomonas syringae* pv. *syringae* strain AP-1 in 1989.^z

Genotype	Browning rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	1.0	1.1	1.4
Napoleon	2.3 ***	2.7 ***	3.9 ***
Corum	2.1 ***	2.9 ***	3.4 ***
MxM 2	1.0 NS	1.0 NS	1.0 *
MxM 39	1.0 NS	1.1 NS	1.5 NS
MxM 60	1.2 NS	1.0 NS	1.0 *
GI 148-1	1.0 NS	1.0 NS	1.2 NS
GI 148-8	1.0 NS	1.0 NS	1.0 *
GI 154-2	1.0 NS	1.1 NS	1.0 *
GI 154-5	1.1 NS	1.7 NS	1.3 NS
GI 169-15	1.0 NS	1.0 NS	1.3 NS
GI 172-9	1.3 *	1.7 ***	1.7 NS
GI 173-9	1.2 NS	1.4 *	1.0 *

^zEach value represents the mean of 10 twigs.

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

^yBrowning rating: 1-4 (1-yellow, 4-dark brown pith).

Table 3.17. Contrasts between F/12-1 and 12 cherry genotypes of lesion browning induced by inoculation with *Pseudomonas syringae* pv. *syringae* strain B-15 in 1989.^z

Genotype	Browning rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	1.4	1.2	1.6
Napoleon	2.7 ***	3.1 ***	3.6 ***
Corum	2.8 ***	3.0 ***	2.9 ***
MxM 2	1.0 NS	1.0 NS	1.1 *
MxM 39	1.1 NS	1.5 NS	1.4 NS
MxM 60	1.0 NS	1.0 NS	1.0 **
GI 148-1	1.0 NS	1.1 NS	1.2 *
GI 148-8	1.7 NS	1.4 NS	1.0 **
GI 154-2	1.1 NS	2.3 ***	1.0 **
GI 154-5	1.2 NS	1.6 NS	1.2 *
GI 169-15	1.9 *	1.3 NS	1.6 NS
GI 172-9	1.5 NS	1.6 NS	1.5 NS
GI 173-9	1.9 *	1.3 NS	1.1 *

^zEach value represents the mean of 10 twigs.

NS, *, **, *** Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively.

^yBrowning rating: 1-4 (1-yellow, 4-dark brown pith).

Table 3.18. Contrasts between F/12-1 and 12 cherry genotypes of gummosis induced by inoculation with *Pseudomonas syringae* pv. *syringae* strain W4N54 in 1989.²

Genotype	Gummosis rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	0.0	0.2	0.8
Napoleon	1.2 ***	0.5 ***	1.8 ***
Corum	0.5 ***	0.2 NS	1.4 ***
MxM 2	0.0 NS	0.0 NS	0.0 ***
MxM 39	0.0 NS	0.0 NS	0.1 ***
MxM 60	0.0 NS	0.0 NS	0.0 ***
GI 148-1	0.0 NS	0.0 NS	0.0 ***
GI 148-8	0.0 NS	0.0 NS	0.0 ***
GI 154-2	0.0 NS	0.0 NS	0.0 ***
GI 154-5	0.0 NS	0.0 NS	0.2 ***
GI 169-15	0.0 NS	0.0 NS	0.3 **
GI 172-9	0.0 NS	0.0 NS	0.6 NS
GI 173-9	0.0 NS	0.0 NS	0.1 **

²Each value represents the value of 10 twigs.

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

^yGummosis rating 0-2 (0-no gummosis, 2-severe gummosis).

Table 3.19. Contrasts between F/12-1 and 12 cherry genotypes of gummosis induced by inoculation with *Pseudomonas syringae* pv. *syringae* strain AP-1 in 1989.²

Genotype	Gummosis rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	0.0	0.0	0.5
Napoleon	0.0 NS	0.6 ***	2.0 ***
Corum	0.0 NS	0.0 NS	1.9 ***
MxM 2	0.0 NS	0.0 NS	0.0 ***
MxM 39	0.0 NS	0.0 NS	0.2 *
MxM 60	0.0 NS	0.0 NS	0.0 ***
GI 148-1	0.0 NS	0.0 NS	0.0 ***
GI 148-8	0.0 NS	0.0 NS	0.0 ***
GI 154-2	0.0 NS	0.0 NS	0.0 ***
GI 154-5	0.0 NS	0.0 NS	0.2 *
GI 169-15	0.0 NS	0.0 NS	0.9 **
GI 172-9	0.0 NS	0.0 NS	0.3 NS
GI 173-9	0.0 NS	0.0 NS	0.5 NS

²Each value represents the mean of 10 twigs.

NS,*,**,*** Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively.

^yGummosis rating: 0-2 (0-no gummosis, 2-severe gummosis).

Table 3.20. Contrasts between F/12-1 and 12 cherry genotypes of gummosis induced by inoculation with *Pseudomonas syringae* pv. *syringae* strain B-15 in 1989.^z

Genotype	Gum rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	0.0	0.5	1.1
Napoleon	0.0 NS	1.2 ***	1.9 ***
Corum	0.0 NS	0.4 NS	1.7 ***
MxM 2	0.0 NS	0.0 *	0.0 ***
MxM 39	0.0 NS	0.0 *	0.0 ***
MxM 60	0.0 NS	0.0 *	0.0 ***
GI 148-1	0.0 NS	0.2 NS	0.0 ***
GI 148-8	0.0 NS	0.0 *	0.0 ***
GI 154-2	0.0 NS	0.1 *	0.0 ***
GI 154-5	0.0 NS	0.4 NS	0.0 ***
GI 169-15	0.0 NS	0.5 NS	0.4 ***
GI 172-9	0.0 NS	0.0 *	0.2 ***
GI 173-9	0.0 NS	0.0 *	0.0 ***

^zEach value represents the mean of 10 twigs.

NS,*,**,*** Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

^yGummosis rating: 0-2 (0-no gummosis, 2-severe gummosis).

Table 3.21. Contrasts between F/12-1 and 12 cherry genotypes of callus production induced by inoculation with *Pseudomonas syringae* pv. *syringae* strain W4N54 in 1989.^z

	Callus rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	1.0	1.0	0.9
Napoleon	1.0 NS	0.6 **	1.0 NS
Corum	1.0 NS	1.0 NS	1.0 NS
MxM 2	1.0 NS	1.0 NS	1.0 NS
MxM 39	1.0 NS	0.2 ***	1.0 NS
MxM 60	1.0 NS	0.3 ***	1.0 NS
GI 148-1	0.3 ***	0.0 ***	0.7 NS
GI 148-8	0.0 ***	0.2 ***	1.0 NS
GI 154-2	1.0 NS	1.0 NS	1.0 NS
GI 154-5	0.8 NS	0.0 ***	1.0 NS
GI 169-15	0.9 NS	0.3 ***	0.5 ***
GI 172-9	0.8 NS	0.0 ***	0.9 NS
GI 173-9	1.0 NS	1.0 NS	0.8 NS

^zEach value represents the mean of 10 twigs.

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

^yCallus rating: 0-1 (0-no callus, 1-callus present).

Table 3.22. Contrasts between F/12-1 and 12 cherry genotypes of callus production induced after inoculation with *Pseudomonas syringae* pv. *syringae* strain AP-1 in 1989.²

Genotype	Callus rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	1.0	1.0	1.0
Napoleon	1.0 NS	1.0 NS	1.0 NS
Corum	1.0 NS	0.6 *	1.0 NS
MxM 2	1.0 NS	1.0 NS	1.0 NS
MxM 39	1.0 NS	0.8 NS	0.9 NS
MxM 60	1.0 NS	1.0 NS	1.0 NS
GI 148-1	1.0 NS	0.8 NS	1.0 NS
GI 148-8	1.0 NS	0.3 ***	1.0 NS
GI 154-2	1.0 NS	1.0 NS	1.0 NS
GI 154-5	0.8 ***	0.2 ***	1.0 NS
GI 169-15	1.0 NS	0.9 NS	0.5 ***
GI 172-9	1.0 NS	0.3 ***	1.0 NS
GI 173-9	1.0 NS	0.7 NS	1.0 NS

²Each value represents the mean of 10 twigs.

NS,*,**,***Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively.

^yCallus rating 0-1: (0-no callus, 1-callus present).

Table 3.23. Contrasts between F/12-1 and 12 cherry genotypes of callus production induced after inoculation with *Pseudomonas syringae* pv. *syringae* strain B-15 in 1989.^z

Genotype	Callus rating ^y		
	29 Oct.	25 Nov.	20 Dec.
F/12-1 vs.	1.0	1.0	1.0
Napoleon	1.0 NS	1.0 NS	1.0 NS
Corum	0.9 NS	1.0 NS	1.0 NS
MxM 2	1.0 NS	1.0 NS	1.0 NS
MxM 39	1.0 NS	1.0 NS	1.0 NS
MxM 60	1.0 NS	1.0 NS	1.0 NS
GI 148-1	1.0 NS	1.0 NS	1.0 NS
GI 148-8	0.8 NS	0.6 ***	1.0 NS
GI 154-2	0.7 NS	0.9 NS	1.0 NS
GI 154-5	0.8 NS	0.9 NS	1.0 NS
GI 169-15	1.0 NS	1.0 NS	0.7 ***
GI 172-9	1.0 NS	0.3 NS	1.0 NS
GI 173-9	1.0 NS	0.8 NS	1.0 NS

^zEach value represents the mean of 10 twigs.

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01 , or 0.001 , respectively.

^yCallus rating 0-1: (0-no callus, 1-callus present).

Chapter 4.

INDUCTION OF VIRULENCE GENES IN *Pseudomonas syringae* pv. *syringae*
BY PLANT EXTRACTS FROM CHERRY ROOTSTOCKS

ABSTRACT

Specific chemical signals in cherry leaf tissue reportedly induce the expression of genes for syringomycin production in *Pseudomonas syringae* pv. *syringae*. Therefore, extracts from twigs of cherry rootstock were tested to see if they would induce the expression of one of the genes for toxin production. One-year-old twigs of 'Napoleon', 'Corum', and cherry rootstocks including: F/12-1, 'Colt', M x M 2, M x M 39, M x M 60, GI 148-1, GI 148-9, GI 154-2, GI 154-5, GI 169-15, GI 172-9 and GI 173-9 were collected in March 1990, freeze-dried, extracted in water and then in 75% acetone. Extracts were diluted to concentrations equivalent to 0.2, 1.0 and 2.0 mg·ml⁻¹ (dry weight), and assayed for effects on the expression of the *syrB::lacZ* fusion in *Pseudomonas syringae* pv. *syringae* strain B3AR132. Expression of the gene was measured by the level of β-galactosidase activity.

A decrease in enzyme activity was generally observed at the two highest concentrations of plant extracts. This may be the result of inhibition or saturation of the active sites of the enzyme produced by the bacteria. The plant extract concentration of 0.2 mg·ml⁻¹ appeared to be the best description of activity in this assay.

The highest activity was observed on 'Napoleon' and 'Corum', as compared to F/12-1. Activity higher than F/12-1 was also induced by rootstocks M x M 60, GI 148-1, GI 148-9, GI 154-5, and 169-15. Rootstocks M x M 2, M x M 39, GI 154-2, GI 172-9, and GI 173-9 were not significantly different from F/12-1. The lowest activity was observed for 'Colt', however, the activity induced by this rootstock was not statistically different from F/12-1.

INTRODUCTION

Pseudomonas syringae pv. *syringae*, which causes diseases in numerous plant species including monocots and dicots, produces syringomycin, a non-host specific phytotoxin (Bradbury, 1986; Gross, 1977). A rapid detergent-like lysis of plant and fungal cellular membranes is induced by this toxin (Backmann, 1971). Syringomycin production contributes significantly to virulence by increasing severity and frequency of disease incidence.

Production of syringomycin is associated with four proteins, which are believed to function as syringomycin synthetases (Morgan, 1988). Also, several genes are required for syringomycin production, but only a few may be directly responsible for its biosynthesis. The *syrB* gene is associated with the formation of two proteins which are believed to function as syringomycin synthetases (Xu and Gross, 1986).

P. syringae pv. *syringae* strain B3A did not produce syringomycin in syringomycin minimal medium (SRM). The *syrB::lacZ* fusion was recombined into the genome of this strain and the resulting mutant B3AR132 also lacked activity in SRM medium. Addition of extracts from cherry leaves to SRM medium induced expression of *syrB::lacZ* fusion in strain B3AR132. The data suggested that the virulence genes, which are responsible for syringomycin production in *P. syringae* pv. *syringae*, may be induced by endogenous plant substrates (Gross, personal communication).

The objectives of this study were to test whether cherry rootstocks with different susceptibility to *P. syringae* can induce the expression of different level of *syrB::lacZ* gene fusion in strain B3AR132.

Secondly, the amount of activity induced in this strain by crude plant extracts was quantified.

MATERIALS AND METHODS

STRAINS AND MEDIA

Two strains of *P. syringae* pv. *syringae*: B3AR132, the mutant strain containing the *syrB::lacZ* fusion marker exchanged into its genome, and the parental strain, B3A were used in this study (Gross, 1990). Strains were cultured on King's medium B (KB) agar (King, 1954), for 3 days at 27 °C. Ten ml of SR-minimal (SRM) medium (Gross, 1985) were inoculated with a single bacterial colony, shaken, and incubated overnight at 25 °C, and incubated for 2 more days without shaking. The cultures were suspended in SRM medium to a final concentration of 10^8 cfu/ml as determined spectrophotometrically, and preserved in glycerol for short-term storage (Gross, 1983).

VIRULENCE TEST

'Napoleon' twig samples were inoculated with 10^7 cfu/ml of strain B3AR132 as described in chapter 3. Twigs were examined for lesion browning after 4 weeks of incubation.

PLANT MATERIAL PREPARATION

One-year-old wood was collected from 'Napoleon', 'Corum', 'Colt' and several other cherry rootstocks in March 1990 (Table 2.2). Twigs were washed in water, air-dried, cut in 1 cm pieces, frozen in liquid

nitrogen, and freeze-dried. The dried samples were then ground in a Wiley mill, passed through a 40 mesh screen and stored at -20 °C.

EXTRACTION PROCEDURE

Ten grams (dry weight) of ground twig samples of 'Napoleon' by homogenizing the twigs in a blender with 100 ml of 0, 20, 40, 60, and 80% acetone for 10 minutes. The homogenate was centrifuged at 16,000 x g for 10 minutes and the supernatant was filter-sterilized. The extracts were evaporated to dryness, resuspended in 10 ml of distilled water, and centrifuged at 16,000 x g. The supernatant was then filter-sterilized, frozen, and stored at -20 °C.

Extracts were assayed for effects on the expression of the *syrB::lacZ* fusion in B3A-R132 (β-galactoside activity) by the method described by Miller (1972) as modified by Stachel *et al.* (1985). Diluted plant extracts (100 μl) containing 1, 5 and 10 mg (D.W.) of sample were added to 4.9 ml of SRM medium containing 10⁶ cfu/ml of *Pseudomonas syringae* pv. *syringae* mutant strain B3A132 prepared as described above. Duplicate SRM cultures were also prepared with the parental strain B3AR as a measure of background activity in the assay.

After incubation 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 again in Z-buffer (1.4 ml). A portion of the washed cells (1 ml) was measured for bacterial cell density at 600 nm. Twenty μl of 0.05% sodium dodecyl sulfate (SDS) and 20 μl of chloroform were added to 0.5 ml of the washed cells. The cells were

lysed by vortexing for 10 seconds followed by 10 minutes of incubation at 28 °C. The assay reaction was started by adding 100 µl of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (4mg/ml), followed by incubation at 28 °C for 10 min. The reaction was terminated by adding 250 µl of 1 M Na₂CO₃. Cellular debris was removed by pelleting in a microcentrifuge, and the absorbance spectrum of the solution determined at 420 nm. Miller units of β-galactosidase activity were calculated using the formula given by Stachel *et al.* (1985a).

EVALUATION OF CHERRY GENOTYPES

Plant extracts in water were prepared by the method described above. For each genotype, 2 ml of extract was evaporated to dryness and resuspended in 2 ml of 75% acetone to precipitate the proteins. After 30 minutes, samples were evaporated to dryness, resuspended in 2 ml of distilled water, filter-sterilized, and stored at -20 °C.

Cherry twig extracts were assayed for the effects on expression of the *syxB::lacZ* fusion in B3AR132. Cultures were incubated and assayed for β-galactosidase activity, as described above. Each genotype was assayed 3 times with 2 replications at a time.

RESULTS AND DISCUSSION

VIRULENCE TEST.

After three weeks of incubation, browning and gummosis were observed on twigs of 'Napoleon' inoculated with the test strain. These

observations suggest that this strain could induce trunk symptoms on cherries and, therefore, could be used in this bioassay.

EVALUATION OF EXTRACTION PROCEDURE

Extraction of plant material from 'Napoleon' twigs in water gave the highest β -galactosidase activity, regardless of the concentration of plant extracts used in the assay (Table 4.1). As the concentration of acetone increased, the activity decreased. It appears that the compounds responsible for induction of the virulence genes are insoluble in acetone.

EVALUATION OF CHERRY GENOTYPES

In general, as the concentration of cherry extracts increased, the β -galactosidase activity decreased when expressed on a dry weight basis (Table 4.2). An exception was observed for 'Napoleon', where concentrations of 0.2 and 1.0 mg/ml induced the highest activity, and for 'Corum', where all three concentrations induced significantly different activity. The general decrease in activity with increasing plant extract concentration could be explained by the presence of substances in crude extracts, which at higher concentrations act as reaction inhibitors. This decrease of activity at higher concentrations of plant extracts may also be a result of saturation of the active sites of the enzyme produced by the bacteria.

No difference in activity was recorded on M x M 2 regardless of concentration. The increase in activity with increasing concentration of plant extracts, which was observed on GI 172-9, may be explained by

an insufficient amount of plant signal at low concentrations, or relatively low levels of inhibitory substances present in this rootstock. For rootstock F/12-1, concentration 1.0 mg/ml induced the highest β -galactosidase activity, but the 0.2 and 2.0 mg/ml were not significantly different.

In comparison to F/12-1, 'Napoleon' and 'Corum' had the highest activity regardless of concentration of plant extract (Figure 4.1-4.3). When inoculated with the lowest concentration of plant extract, rootstocks M x M 2, M x M 39, GI 154-2, GI 172-9, GI 173-9, and 'Colt' were not different from F/12-1. A β -galactosidase activity higher than for F/12-1 was recorded for rootstocks M x M 60, GI 148-1, GI 148-9, GI 154-5, and 169-15.

At the two highest concentrations, rootstocks M x M 39, GI 148-9, GI 154-2, GI 154-5, GI 169-15, GI 173-9, and 'Colt' had lower activity than F/12-1. In comparison to F/12-1, rootstocks M x M 2, GI 148-1, and GI 172-9 were not significantly different at a concentration of 1.0 mg/ml, but the activity was higher at a concentration of 2.0 mg/ml. Regardless of concentration of the plant extracts, the lowest activity was induced by the rootstock 'Colt', when compared to all other genotypes tested.

The lowest concentration (0.2 mg/ml dry weight) of tissue sample appears to be better suited for a description of the plant signals activity in this assay than the higher ones. It may be due to active sites in the enzyme being unsaturated, or possibly less inhibitory substances present at this concentration. However, for some rootstocks, e.g., GI 172-9, this concentration of extract may be too low, to supply

sufficient amounts of plant signals for full expression of *syrB::lacZ* fusion in bacteria.

Results from the bioassay showed that plant substances present in cherry twigs were able to induce the genes for syringomycin production. Activity of these signals varied from rootstock to rootstock.

Table 4.1. Activity induced in a *syrB::lacZ* fusion of *Pseudomonas syringae* pv. *syringae* strain B3AR132 by plant extracts obtained from extraction in different acetone concentrations.²

Acetone conc.	Activity (Miller units)		
	Concentration (mg·ml ⁻¹)		
	0.2	1.0	2.0
0%	61a	269a	352a
20%	24b	86b	173b
40%	18c	60b	149b
60%	14c	49b	141b
80%	10d	23b	69c

²Mean separation by concentration within columns was by Waller-Duncan k-ratio t-test, k=100.

Table 4.2. Effect of plant extract concentration from cherry genotypes on β -galactosidase activity induced in a *syxB::lacZ* fusion of *Pseudomonas syringae* pv. *syringae* strain B3AR132.²

Genotype	Activity (Miller units·mg ⁻¹)		
	Concentration (mg·ml ⁻¹)		
	0.2	1.0	2.0
Napoleon	611a	590a	380b
Corum	592a	564b	341c
F/12-1	169b	232a	160b
MxM 2	192	196	260
MxM 39	252a	138b	73b
MxM 60	439a	194b	181b
GI 148-1	427a	218b	257b
GI 148-9	378a	94b	86b
GI 154-2	181a	55b	93b
GI 154-5	540a	77b	58b
GI 169-15	580a	153b	102b
GI 172-9	43c	199b	255a
GI 173-9	298a	75b	35b
Colt	166a	12b	8b

²Mean separation by concentration across the row was by Waller-Duncan k-ratio t-test, k=100.

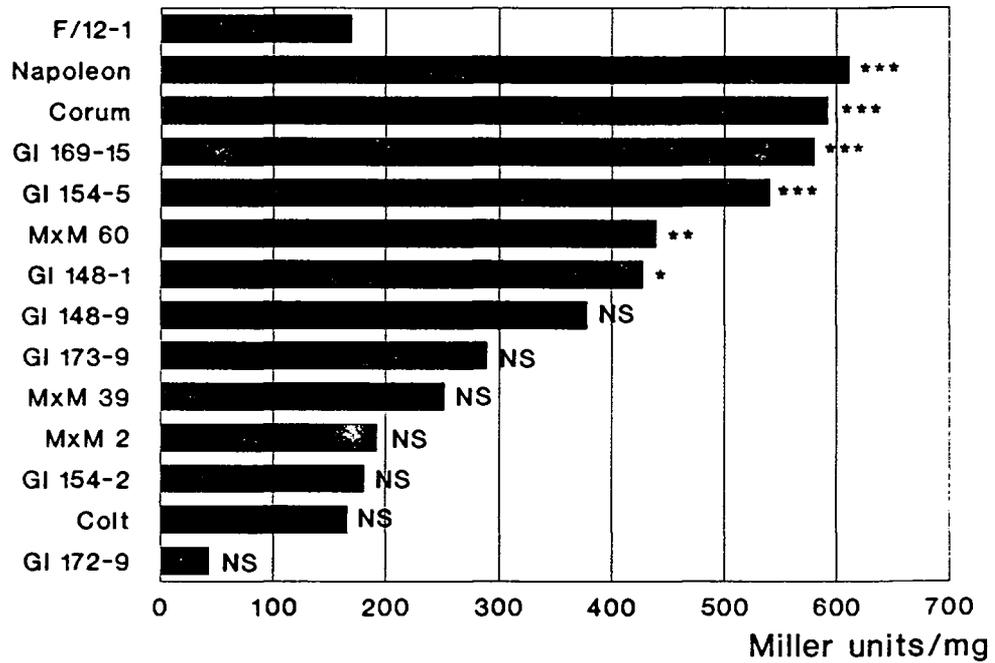


Figure 4.1. Comparison of the β -galactosidase activity induced by plant extracts ($0.2 \text{ mg} \cdot \text{ml}^{-1}$) in a *syrB::lacZ* fusion of *P. syringae* pv. *syringae* strain B3A-R132 between F/12-1 and other cherry genotypes (NS, *, **, *** Nonsignificant or significant at $P=0.05$, 0.01 , or 0.001 , respectively).

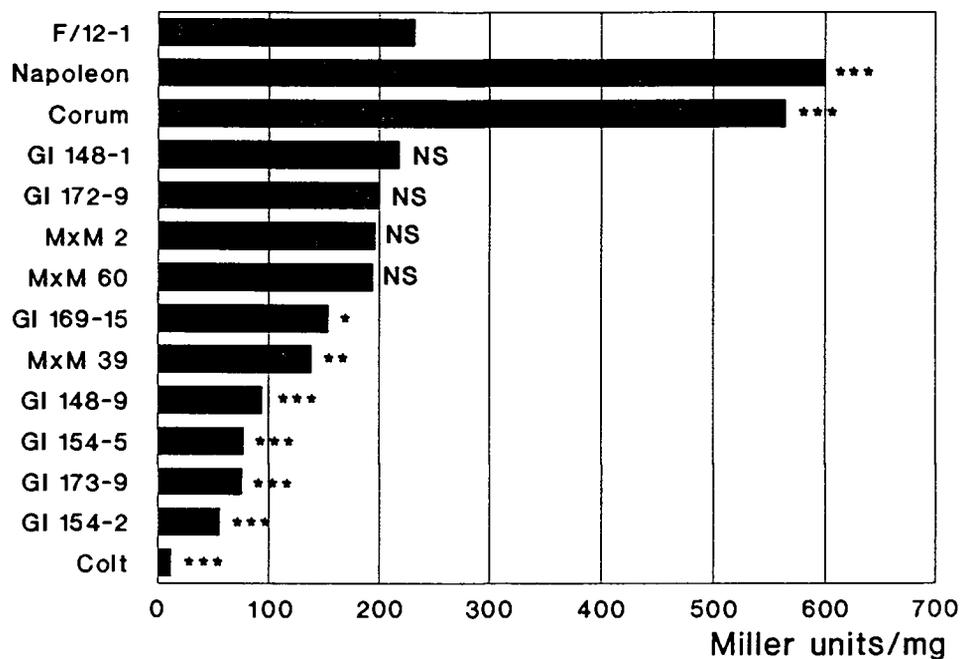


Figure 4.2. Comparison of β -galactosidase activity induced by plant extracts ($1.0 \text{ mg} \cdot \text{ml}^{-1}$) in a *syrB::lacZ* fusion of *P. syringae* pv. *syringae* strain B3A-R132 between F/12-1 and other cherry genotypes (NS, *, **, *** Nonsignificant or significant at $P=0.05$, 0.01 , or 0.001 , respectively).

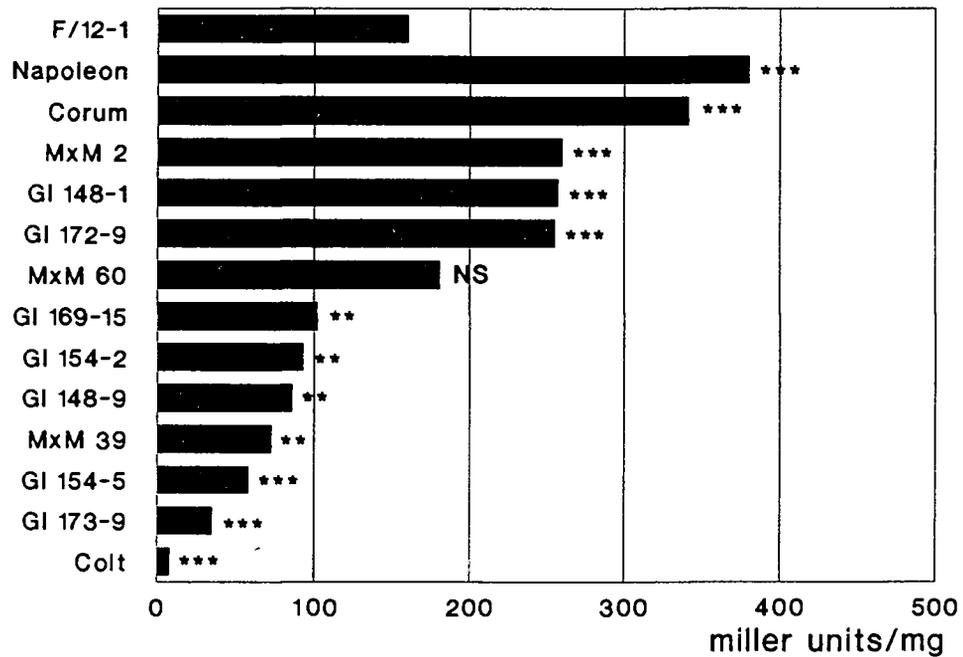


Figure 4.3. Comparison of β -galactosidase activity induced by plant extracts ($2.0 \text{ mg} \cdot \text{ml}^{-1}$) in a *syrB::lacZ* fusion of *P. syringae* pv. *syringae* strain B3A-R132 between F/12-1 and other cherry genotypes (NS, *, **, *** Nonsignificant or significant at $P=0.05$, 0.01 , or 0.001 , respectively).

Chapter 5

CONCLUSIONS

'Napoleon' and 'Corum', two cultivars which, based on field observations, are highly susceptible to the bacteria, had the highest browning and gummosis ratings in our assay. Lack of lesion browning and gummosis on twigs inoculated with water and an avirulent strain, in comparison to the results from inoculations with virulent strains, suggests that the excised twig assay is reliable under these conditions. Based on the twig assay, almost all the rootstocks tested appear to be as tolerant to the pathogen as F/12-1. Two rootstocks GI 172-9 and GI 169-15 seemed to be less tolerant than F/12-1 to *P. syringae* pv. *syringae*.

The time of the inoculation may be important in screening cherry genotypes. The results from the assay suggest that testing should probably be done before the chilling requirement of the tree is satisfied. The twigs were less susceptible to the disease when samples were taken at the end of the dormancy period. Some of the rootstocks from the last sampling date broke buds in the test tubes; also, less severe disease symptoms were observed than for the first two sampling dates.

Results from the bioassay using the mutant strain B3AR132 showed that plant substances present within cherry twigs were able to induce the *syrB* gene for syringomycin production. Activity of these signals varied from rootstock to rootstock. Further purification of the crude

extracts could perhaps improve the accuracy of the assay by removing substances which might interfere with the assay.

Browning from the excised twig assay were somewhat correlated with the the syringomycin production in bioassay with the mutant strain. In both assays, 'Napoleon' and 'Corum' had either highest browning or β -galactosidase activity. Rootstock GI 169-15 appears to have higher β -galactosidase activity and to be more susceptible than others. But differences could be observed with rootstocks M x M 60, GI 148-1, GI 148-9, and GI 154-5, which are similar to F/12-1 in twig assay, but have high activity in bioassay. The opposite situation can be observed for rootstock GI 172-9 which appears to be susceptible to *P. syringae* in the twig assay, but not different in activity from F/12-1. The other rootstocks, in both assays, were not different from F/12-1. One of the explanations for these differences in the assays may be relate to variable physiological stages of the twigs. For the bioassay, they were collected only once, in March 1990. At this time of the year, the trees had already satisfied rest, and changes in their chemical composition may have occurred. Dr. Gross (personal communication) pointed out there are some substrates in the plant tissue, when in combinaton with other plant signals, are enhancers for the induction of the *syrB* gene. The fluctuation in the amount of plant signals, enhancers, or even inhibitors in the plant tissue, not only during the growing season, but also during dormancy, may influence the results of the test. Determination of the seasonal pattern of the signals in the plant could play an important role in disease control. Also identification of the signal molecules, as well as the enhancers, would provide a better

understanding of host-pathogen interactions.

BIBLIOGRAPHY

- Aderhold, R.W. and W. Ruhland. 1907. Der Bakterienbrand der Kirschbaume. Arb. Kaiserl. Biol. Anst. f. Land und Fortwirtschaft 5:293-340.
- Anderson, H.W. 1956. Diseases of fruit crops. McGraw-Hill Book Co., New York, pp.501.
- Baca, S., M.L. Canfield and L.W. Moore. 1987a. Variability in ice nucleation strains of *Pseudomonas syringae* isolated from diseased woody plants in Pacific Northwest nurseries. Plant Dis. 71:412-415.
- Baca, S. and L.W. Moore. 1987b. Colonization of grass species and cross infectivity to woody nursery plants. Plant Dis. 71:724-726.
- Backman, P.A. and DeVay, J.E. 1971. Studies on the mode of action and biogenesis of the phytotoxin syringomycin. Physiol. Plant Path. 1:215-234.
- Barss, H.P. 1913. Cherry gumosis: A preliminary report. Oreg. Crop. Pest. and Hort. Rpt. for 1911-1912, pp.199-217.
- Bidwai, A.P., L. Zhang, R.S. Bachmann, and J.Y. Takemoto. 1987. Mechanism of action of *Pseudomonas syringae* phytotoxin, syringomycin: stimulation of red beet plasma membrane ATPase activity. Plant Physiol. 83:39-43.
- Bos, R.J. 1899. Eene bacterienziekte der syringen. Tijdschr. Plantenziekten 5:177-183.
- Bradbury, J.F. 1986. Guide to plant pathogenic bacteria, p.175-177. CAB International Mycological Institute, Kew, England.
- Brzezinski, J. 1902. Etiologia raka bakteryjnego na drzewach owocowych. Akademia Nauk pp. 134.
- Cameron, H.R. 1955. Bacterial canker and dead bud in sweet cherries in Oregon. 47th Ann. Rpt of Oregon State Hort. Soc. pp. 103-104.
- Cameron, R.H. 1960. Death of dormant buds in sweet cherry. Plant Dis. Rptr. 44(2):139-143.
- Cameron, H.R. 1962a. Mode of infection of sweet cherry by *Pseudomonas syringae*. Phytopath. 52(9):917-921.
- Cameron, H.R. 1962b. Diseases of deciduous fruit trees incited by *Pseudomonas syringae* van Hall. Oregon Exp. St. Tech. Bull. 66. pp. 3-64.

- Cameron, H.R. 1970. *Pseudomonas* content of cherry trees. *Phytopath.* 60:1343-1346.
- Cameron, H.R. 1971. Effect of root or trunk stock on susceptibility of orchard trees to *Pseudomonas syringae*. *Plant Dis. Rptr.* 55(5):421-423.
- Crosse, J.E. 1954. Bacterial canker, leaf spot, and shoot wilt of cherry and plum. *Ann. Rept. of East Malling Res. Sta. for 1953, Sec. IV*, pp. 202-207.
- Crosse, J.E. 1955. Bacterial canker of stone-fruits. I. Field observations on the avenues of autumnal infection of cherry. *J. Hort. Sci.* 30:131-142.
- Crosse, J.E. 1956a. Bacterial canker of stone-fruits. II. Leaf scar infection of cherry. *J. Hort. Sci.* 31:212-224.
- Crosse, J.E. 1956b. An epidemic leaf spot and spur wilt of cherries caused by *Pseudomonas morsprunorum*. *Ann. Rept. East Malling Res. Sta. for 1955*:121-125.
- Crosse, J.E. 1959. Bacterial Canker of Stone-fruits. IV. Investigation of method for measuring the inoculum potential of cherry trees. *Ann. of Applied Biol.* 47:306-317.
- Crosse, J.E. 1963. Bacterial canker of stone-fruits. V. A comparison of leaf surface populations of *Pseudomonas morsprunorum* in autumn on two cherry varieties. *Ann. of Applied Biol.* 52:97-104.
- Crosse, J.E. 1966. Epidemiological relations of the pseudomonad pathogens of deciduous fruit trees. *Ann. Rev. of Phytopath.* 4:291-310.
- Crosse, J.E. and C.M.E. Garrett. (1966). Bacterial canker of stone fruits. VII. Infection experiments with *Pseudomonas morsprunorum* and *P. syringae*. *Ann. of Applied Biol.* 58:31-34.
- Cummins, J.N. 1984. Fruit tree rootstocks recently introduced and soon to be introduced. *Compact Fruit Tree* 17:57-63
- De Boer, S.H. 1980. Leaf spot of cherry laurel caused by *Pseudomonas syringae*. *Can. J. Plant Pathol.* 2:235-238.
- DeVay, J.E., F.L. Lukezic, S.L. Sinden, H. English, and D.L. Coplin. 1968. A biocide produced by pathogenic isolates of *Pseudomonas syringae* and its possible role in the bacterial canker disease of peach trees. *Phytopath.* 58:95-101.
- De Vries, D.P. 1965. Field resistance to bacterial canker in some cherry seedling populations. *Euphytica* 14:78-82.
- Dye, D.W. 1953. Blast of stone fruit in New Zealand. *N. Z. J. of Sci.*

- and Tech., Sect. A 35:451-461.
- English, H. and J.R. Davis. 1960. The source of inoculum for bacterial canker and blast of stone fruit trees. *Phytopath.* 50:634.
- Gardan, L., J. Luisetti, and J.P. Prunier. 1972. Variation of inoculum level of *Pseudomonas morsprunorum persicae* on leaf surface of peach trees. Pages 87-94 in: H.P. Maas-Geesteranus. Proc. of the Third Intl. Conf. on Plant Pathogenic Bacteria, Wageningen.
- Garrett, C.M.E. 1981. Screening for resistance in *Prunus* to bacterial canker. Proc. Fifth Int. Conf. Plant Path. Bact. Cali.
- Garrett, C.M.E. 1986. Influence of rootstock on the susceptibility of sweet cherry scions to bacterial canker caused by pvs. *morsprunorum* and *syringae*. *Plant Pathol.* 35:114-119.
- Griffin, F.L. 1911. A bacterial gumosis of cherries. *Science* 34:615-616.
- Gross, D.C. 1985. Regulation of syringomycin synthesis in *Pseudomonas syringae* pv. *syringae* and defined conditions for its production. *J. of Applied Bact.* 58:167-174.
- Gross, D.C. 1985. Mechanisms of plant pathogenesis by *Pseudomonas* species. *Can. J. Microbiol.* 31:403-410.
- Gross, D.C., Y.S. Cody, E.L. Proebsting, Jr., G.K. Rademaker, and R.A. Spotts. 1983. Distribution, population dynamic and characteristic of INA bacteria in deciduous fruit tree orchards. *Applied Env. Microb.* 46(6):1370-1379.
- Gross, D.C., Y.S. Cody, E.L. Proebsting, Jr, G.K. Rademaker, and R.A. Spotts. 1984a. Ecotypes and pathogenicity of INA *P. syringae* isolated from deciduous fruit tree orchards. *Phytopath.* 74:241-248.
- Gross, D.C. and J.E. DeVay. 1977. Population dynamics and pathogenesis of *Pseudomonas syringae* in maize and cowpea in relation to the *in vitro* production of syringomycin. *Phytopath.* 67:475-483.
- Gross, D.C., E.L. Proebsting, Jr., and P.K. Andrews. 1984b. The effect of INA bacteria on temperatures of ice nucleation and free injury of *Prunus* flower buds at various stages of development. *J. Amer. Soc. Hort. Sci.* 109(3):375-380.
- Grubb, N.H. 1944. The comparative susceptibility of high and low worked cherry trees in the nursery to bacterial canker. Ann. Rept. of East Malling Res. Sta. for 1943. Sect. III, pp. 43-44.
- Hawkins J.E. 1976. A cauterization method for the control of cankers caused by *Pseudomonas syringae* in stone fruit trees. *Plant Dis. Rptr.* 60(1):60.

- Jones, A.L. 1971. Bacterial canker of sweet cherry in Michigan. *Plant Dis. Rptr.* 55(11):961-965.
- King E.O., M.K. Ward, and D.E. Raney. 1954. Two simple media for demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44:301-307.
- Latorre, B.A., J.A. Gonzales, J.E. Coxs, and F. Vial. 1985. Isolation of *Pseudomonas syringae* pv. *syringae* from cankers and effect of free moisture on its epiphytic populations on sweet cherry trees. *Plant Dis.* 69:409-412.
- Latorre, B.A., and A.L. Jones. 1979a. Evaluation of weeds and plant refuse as potential sources of inoculum of *Pseudomonas syringae* in Bacterial canker of cherry. *Phytopath.* 69:1122-1125.
- Latorre, B.A. and A.L. Jones. 1979b. *Pseudomonas morsprunorum*, the cause of bacterial canker of sour cherry in Michigan, and its epiphytic association with *P. syringae*. *Phytopath.* 69:335-339.
- Leben, C. 1981. How plant pathogenic bacteria survive. *Plant Dis.* 5:633-637.
- Lindow, S. E. 1978a. Population dynamics of epiphytic INA bacteria on frost sensitive plants and frost control by means of antagonistic bacteria. In P.H.Li and A.Sakai (eds). *Plant cold hardiness and freezing stress, Vol II.* Academic Press, New York.
- Lindow, S.E. 1983. Methods of preventing frost injury caused by epiphytic INA bacteria. *Plant Dis.* 67:327-333.
- Lindow, S. E., D. C. Arny, C. D. Upper 1978b. Distribution of INA bacteria on plants in nature. *Applied Env. Microbiol.* 36:831-838.
- Lyskanowska, M.K. 1976. Bacterial canker of sweet cherry (*Prunus avium*) in Poland. I. Symptoms, disease development and economic importance. *Plant Dis. Rptr.* 60:465-469.
- Malvic, D.K. and L.W. Moore. 1988. Survival and dispersal of a marked strain of *Pseudomonas syringae* in a maple nursery. *Plant Pathol.* 37:573-580.
- Mansvelt, E.L. and M.J. Hattingh. 1987. *Pseudomonas syringae* pv. *syringae* associated with apple and pear buds in South Africa. *Plant Dis.* 71:789-792.
- Miller J.H. 1972. *Experiments in molecular genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
- Morgan, M. K. and A. K. Chatterjee. 1988. Genetic characterization and regulation of proteins associated with production of syringotoxin by *Pseudomonas syringae* pv. *syringae*. *J. Bacteriol.* 170:5689-5697.

- Panagopoulos, C.G. and J.E. Crosse. 1964. Frost injury as a predisposing factor in blossom blight of Pear caused by *P. syringae* van Hall. *Nature* (London) 202:1352.
- Ross, I.M.M, and M.J. Hattingh. 1986a. Pathogenic *Pseudomonas* spp. in stone fruit buds. *Phytophylactica* 18:7-9.
- Ross, I.M.M. and M.J. Hattingh. 1986b. Bacterial canker of sweet cherry in South Africa. *Phytophylactica* 18:1-4.
- Ross, I.M.M. and M.J. Hattingh. 1986c. Weeds in orchards as potential source of inoculum for bacterial canker of stone fruit. *Phytophylactica* 18:5-6.
- Ross, I.M.M. and M.J. Hattingh. 1986d. Resident populations of *Pseudomonas syringae* on stone fruit tree leaves in South Africa. *Phytophylactica* 18:55-58.
- Ross, I.M.M. and Hattingh, M.J. 1987. Systemic invasion of cherry leaves and petioles by *Pseudomonas syringae* pv. *morsprunorum*. *Phytopath.* 77:1246-1252.
- Shanmuganathan, N. and J.E. Crosse. 1963. Experiments to test the resistance of plum rootstocks to bacterial canker. Report of East Malling Research Stat. for 1962, pp.101-104.
- Sinden, S., DeVay, J., and P. Backman. 1971. Properties of syringomycin, a wide spectrum antibiotic and phytotoxin produced by *Pseudomonas syringae*, and its role in the bacterial canker disease of peach trees. *Physiol. Plant Pathol.* 1:199-213.
- Sobiczewski, P. 1978. Epiphytic populations of pathogenic *Pseudomonas* on sour cherry leaves. Pages 753-762 in: Proc. of the Fourth Intl. Conf. on Plant Pathogenic Bacteria. Vol. II. NRA, Angers.
- Sorauer, P. 1891. Neue Krankheitseracheinung bei Syringa. *Zeitschr. Pflanzenkr.* 1:186-188.
- Stachel S.E., An G., Flores C., and Nester E.W. 1985. A Tn3 *lacZ* transposon for the random generation of β -galactosidase gene fusions: application to the analysis of gene expression in *Agrobacterium*. *EMBO J.* 4:891-898.
- Sule, S. and E. Seemuller. 1987. The role of ice formation in sour cherry leaves by *P. syringae*. *Phytopath.* 77:173-177.
- Van Hall, C.J.J. 1902. Bijlragen tot de kennis der bacterielle plantenziekten. Ph.D. thesis, University of Amsterdam.
- Weaver, D.J. 1978. Interaction of *P. syringae* and freezing in bacterial canker on excised peach twigs. *Phytopath.* 68:1460-1463.

- Webster, T. 1980. Dwarfing rootstocks for plums and cherries. *Acta Horticulturae* 114:201-207.
- Webster, T. 1984. Practical experiences with old and new plum and cherry rootstocks. *Compact Fruit Tree* 17:103-117.
- Wilson, E.E. 1953. Bacterial canker of stone fruits. *Year Book of Agriculture*, U.S.D.A., Washington D. C., pp.722-729.
- Wormland, H. 1932. Bacterial diseases of stone fruit trees in Britain. IV. The organism causing bacterial canker of plum trees. *Trans. Brit. Mycol. Soc.* 17:157-169.
- Wormland, H. 1942. Bacterial disease of stone fruit trees in Britain. VIII. Bacterial canker of peach. *Trans. Brit. Mycol. Soc.*, 25:246-249.
- Xu, G. and D.C. Gross. 1988. Physical and functional analyses of the *syrA* and *syrB* genes involved in syringomycin production by *Pseudomonas syringae* pv. *syringae*. *J. of Bacteriology*. pp.5680-5688.
- Young, J.M. 1977. Resistance to streptomycin in *Pseudomonas syringae* from apricot. *N.Z.J. Agric. Res.* 20:249-251.