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EPIDEMIOLOGY AND CONTROL OF BACTERIAL CANKER ON MONTMORENCY SOUR CHERRY CAUSED BY *PSEUDOMONAS SYRINGAE* PV. *MORSPRUNORUM*

By

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ABSTRACT

EPIDEMIOLOGY AND CONTROL OF BACTERIAL CANKER ON MONTMORENCY SOUR CHERRY CAUSED BY PSEUDOMONAS SYRINGAE PV. MORSPRUNORUM

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Rifampicin-resistant strains of Pseudomonas syringae pv. morsprunorum and P. syringae pv. syringae (PsmR and PssR, respectively) were used in epidemiological and chemical control studies on bacterial canker of Prunus cerasus L. cv. Montmorency (sour cherry) in East Lansing, Michigan. Rainwater was collected under a cherry tree with sequential rain samplers designed to study the dissemination of waterborne pathogens from trees. The apparatus collected rain from a 5,026-cm^2 area and saved 5- to 8-ml subsamples for each 0.5-mm rainfall. Two dispersal patterns of PsmR were observed from sampled rain periods. In the first pattern, populations of PsmR in rainwater increased and leveled off; while in the second, populations increased to the highest level in the first 2.5-mm rain, declined, and then leveled off. The first and second patterns were preceded by 5 days with and without rain, respectively. In 1980 and 1981, populations of PsmR in rainwater were higher in spring and autumn than in summer, whereas populations of PsmR associated with leaves were relatively constant (10^3-10^4 colony-forming units/cm^2 leaf). Ground cover plants in an orchard were inoculated with PsmR and PssR in November 1979 and 1980. Both strains were recovered from ground cover through the winter and in early spring but not in late spring and summer. In spring of 1980 and 1981, PsmR and PssR were

recovered from buds of fruit spurs following inoculation of leaf scars the preceding autumn. Recovery of PsmR and PssR from buds following leaf scar inoculations of fruit spurs in August 1980 were lower than for buds on spurs inoculated in October. Copper treatments were evaluated for reducing populations of PsmR on Montmorency sour cherry trees in spring and early summer 1980 and 1981. Populations of PsmR were reduced more by 636 and 949 mg/L tribasic copper sulfate (TBS) than by 200 mg/L Citcop 4E, but several applications were needed to reduce the populations to a low level. Citcop 4E at 200 mg/L was more phytotoxic to cherry foliage than 636 mg/L TBS. Phytotoxicity was related to the number of applications and was not reduced by adding hydrated lime to copper treatments. The decline of copper residues from leaves was related by multiple regression analyses with rainfall and initial level of copper on leaves.

To my father

Miles Beardsley Olson

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GENERAL INTRODUCTION

Bacterial canker on Prunus sp. is caused by Pseudomonas syringae pv. syringae (van Hall) Young et al. and P. syringae pv. morsprunorum (Wormald) Young et al. The disease is found in most regions of the world where Prunus sp. are grown, including California (51), Georgia (21), Michigan (36), Oregon (6), and Utah (2), USA; Nova Scotia (32) and Ontario (1) Canada; England (52); France (49); Germany (44); New Zealand (22); and Poland (39).

Pseudomonas syringae pv. syringae was first described by van Hall in 1902 as c using a leaf spotting on lilac (Syringae vulgaris L.) (48). Since 1902, P. syringae pv. syringae has been identified as a pathogen on more than 43 hosts, including such important crops as apple, cherry, Citrus sp., common bean, peach, and pear (7). In 1932, Wormald (52) identified P. syringae pv. morsprunorum as the cause of bacterial canker on plum. Pseudomonas syringae pv. morsprunorum has been identified as a pathogen on 10 hosts; i.e., almond, apricot, sweet and sour cherry, common plum, flowering plum, Myrobalan plum, peach, Prunus sibirica, and purple leaved plum (7). Pseudomonas syringae pv. morsprunorum and P. syringae pv. syringae are obligate aerobes, 0.7-1.2 by 1.5-3.0 um in size with polar multitrichous flagella, oxidase negative, and they produce a diffusible fluorescent pigment (20). Since Wormald described P. syringae pv. morsprunorum, there has been a controversy whether P. syringae pv. morsprunorum is

different enough from P. syringae pv. syringae to warrant a separate species name (33).

Many studies have focused on the taxonomic controversy of P. syringae pv. morsprunorum and P. syringae pv. syringae, fluorescent psuedomonads, and plant pathogenic bacteria in general (31, 33). In a taxonomic study, Garrett et al. (31) found P. syringae pv. syringae and P. syringae pv. morsprunorum gave positive and negative responses, respectively, to the following tests: liquefaction of gelatin; hydrolysis of casein, aesculin, and arbutin; L-leucine as sole carbon and nitrogen source; use of lactic acid; and metabolism of arbutin. Pseudomonas syringae pv. morsprunorum and P. syringae pv. syringae gave positive and negative responses, respectively, to the following tests: acid production on purple lactose agar, use of tartaric acid, and use of L-tyrosine as sole carbon and nitrogen source (31). Pseudomonas syringae pv. syringae and P. syringae pv. morsprunorum produced yellow and white pigment, respectively, in sucrose broth (31). Fruit inoculations with P. syringae pv. syringae produced black sunken lesions on pear and cherry fruitlets and lemons while P. syringae pv. morsprunorum produced no lesions on pear fruitlets and only small brown superfical lesions on cherry fruitlets and lemon (31). Garrett and Crosse (29) were able to distinguish strains of P. syringae pv. morsprunorum and P. syringae pv. syringae, indigenous to England, by phage typing. Presley and Crosse (42) also used phage typing to separate two races of P. syringae pv. morsprunorum, each pathogenic on different sweet cherry cultivars (Roundel and Napoleon).

Ice nucleation activity has been used to detect taxonomic differences between P. syringae pv. morsprunorum and P. syringae

pv. syringae. Hirano et al. (35) tested many fluorescent plant pathogenic pseudomonads for ice nucleation activity. Of 10 P. syringae pv. syringae isolates from Prunus species, six had ice nucleation activity at -5.5 C; while of 10 P. syringae pv. morsprunorum isolates, none had ice nucleation activity at -5.5 or -10.0 C (34). But, Latorre and Jones (37) found 48.6% of 105 isolates of P. syringae pv. syringae had ice nucleation activity between -4 and -6 C, while at the same temperatures only 24% of 96 P. syringae pv. morsprunorum isolates had ice nucleation activity.

Production of syringomycin has been used to separate P. syringae pv. morsprunorum from P. syringae pv. syringae. Seemuller and Arnold (46) found 34 of 40 isolates of P. syringae pv. syringae produced syringomycin in vitro and none of 36 P. syringae pv. morsprunorum isolates produced syringomycin. Similarly, Latorre and Jones (37) found 74.5% of 132 P. syringae pv. syringae isolates produced syringomycin and only three of 127 of P. syringae pv. morsprunorum isolates produced syringomycin.

By using the tests mentioned, P. syringae pv. morsprunorum can be separated from P. syringae pv. syringae. Often there were many strains of P. syringae pv. morsprunorum and P. syringae pv. syringae that did not respond typically to the tests mentioned. Therefore, some researchers prefer to identify species of P. syringae as physiotype 1 (P. syringae pv. syringae) or physiotype 2 (P. syringae pv. morsprunorum) from known hosts (17).

Crosse (17) suggested plant pathogenic bacteria should be classified according to characters of the bacteria that favored its natural selection in the field. Young et al. (53) proposed a change

in the nomenclature and classification of all plant pathogenic bacteria in 1978 that would lower many species to pathovars of one specie and the primary distinction between pathovars would be host range. This proposal was approved by the International Society for Plant Pathology in 1980 (24). Species names such as P. morsprunorum and P. syringae were changed to P. syringae pv. morsprunorum and P. syringae pv. syringae, pathovars of P. syringae. Pseudomonas syringae pv. morsprunorum was distinguished from P. syringae pv. syringae based on differences in host range; meanwhile both pathovars were still known pathogens of apricot, sweet and sour cherry, peach, and common plum (7). The new rules on classification stated that biochemical tests can aid in distingishing pathovars but host range must be the deciding factor for classification.

Disease symptoms of bacterial canker on Prunus species are most prevalent in spring. On sweet and sour cherry bacterial canker causes lesions on leaves and fruit, and cankers on limbs (36, 37). Lesions on leaves are angular, delimited by veins on the leaf, and often coalesce during severe infections (37). Chlorotic halo and water-soaked areas of leaf tissue commonly surround leaf lesions. During periods of hot dry weather, lesions may dry up and fall out of the leaf causing a tattered or shot-hole appearance. Lesions that develop on sweet and sour cherry fruit and pedicels begin as water-soaked areas that develop into dark, sunken regions (37). In Michigan, cankers caused by P. syringae morsprunorum on trunks and scaffold limbs are rare on sour cherry (37) but cankers on sweet cherry are commonly caused by P. syringae pv. syringae (36). Cankers on limbs

usually develop at the base of infected fruit spurs, and can girdle and destroy entire limbs of trees (9).

The disease cycle of bacterial canker on sweet cherry has been studied extensively in Oregon (7) and England (16) where bacterial canker is caused by P. syringae pv. syringae and P. syringae pv. morsprunorum, respectively. In England, P. syringae pv. morsprunorum overwinters in cankers that are active through the winter months in mild temperatures (9). In spring, P. syringae pv. morsprunorum are disseminated from cankers to swollen buds, flowers, and leaves by splashing rainwater (16). Only young cherry leaves are susceptible to leaf spotting (19). Schimdle and Zeller (44) determined from in vitro studies with sour cherry (Prunus cerasus) and an inoculum mixture of P. syringae pv. morsprunorum and P. syringae pv. syringae that the optimal temperature range for leaf spotting was 15 to 25 C with 100% relative humidity. Cold temperatures may predispose leaf tissue to leaf spotting because of the ice nucleation activity of some P. syringae pv. morsprunorum and P. syringae pv. syringae strains. Sour cherry trees inoculated with P. syringae pv. syringae and exposed to temperatures ranging from -2.0 to -0.5 C with 100% relative humidity developed more leaf spotting symptoms than trees exposed to 0 C (54). Similarly, terminal shoots of peach inoculated with P. syringae pv. syringae developed typical shoot lesions when exposed to -5 C for 15 days (50).

In spring, severe epidemics of bacterial canker are associated with cool moist weather conditions (10, 16). Crosse (10) reported in England that a severe outbreak of bacterial canker leaf spotting in May 1953 was associated with three consecutive days of rain

accompanied by heavy winds. Crosse (10) suggested that windblown rain containing P. syringae pv. morsprunorum inoculum was impacted into leaves via the stomata.

In summer, cankers are not active on sweet cherry trees and the bacteria in the cankers often die (9). But, Pseudomonas syringae pv. morsprunorum and P. syringae pv. syringae do survive as epiphytes associated with leaves and numerous studies have focused on this subject. Crosse (14) measured populations of P. syringae pv. morsprunorum on cherry leaves by determining the quantities of P. syringae pv. morsprunorum in leaf washings. Populations of P. syringae pv. morsprunorum on leaves of Napoleon, a bacterial canker-susceptible cultivar, were 1.2 to 3.3 times greater than those on Roundel, a bacterial canker-resistant cultivar (15). These differences correlated with leaf scar infections in autumn and were not associated with leaf spot infections in spring (15). In Poland, Burkowicz (5) measured inoculum potential of P. syringae pv. morsprunorum on sweet cherry (Czarna Pozna and Hedelfinger) leaves using Crosse's leaf washing procedure (14). For three years, populations of P. syringae pv. morsprunorum varied from 10^3 to 10^7 colony-forming units/leaf and populations were always highest in autumn (5). During the summer months in Michigan, Latorre and Jones (37) found that P. syringae pv. morsprunorum survived as an epiphyte associated with leaves of sour cherry. They also found P. syringae pv. morsprunorum was predominantly associated with leaf spotting on sour cherry in spring, but P. syringae pv. syringae and P. syringae pv. morsprunorum were found on symptomless leaves and the former was commonly found on ground cover plants in the orchard (37, 38). In

Georgia, pathogenic strains of P. syringae pv. syringae were also isolated from apparently healthy peach twigs through the growing season except from June to September (21). Because of the wide host range of P. syringae pv. syringae, it appears to be ubiquitous in many orchards. The significance of the simultaneous presence of P. syringae pv. morsprunorum and P. syringae pv. syringae in the orchard is not known.

The epiphytic stage of the life cycle permits P. syringae pv. morsprunorum and P. syringae pv. syringae to survive through the summer and infect woody plant tissue in autumn for overwintering. In England, P. syringae pv. morsprunorum infected sweet cherry leaf scars and when inoculum concentrations of P. syringae pv. morsprunorum were increased, the number of leaf scar infections increased (12). In autumn, leaf scars were most susceptible to infection in mid-October (12). Leaf scar infections on sweet cherry with a cherry strain of P. syringae pv. morsprunorum were 50% less when leaf scars were first inoculated with a P. syringae pv. morsprunorum strain from prune (19). During leaf scar infection, the bacteria were absorbed into the leaf scar tissue, eventually migrating to adjacent buds (11, 34). In England during mild winters, stem cankers developed from P. syringae pv. morsprunorum-infected leaf scars. In Oregon, buds on sweet cherry trees are infected directly by P. syringae pv. syringae from November to January rather than through leaf scars (6).

Throughout the growing season, rainwater is important for the dissemination of P. syringae pv. morsprunorum and P. syringae pv. syringae from cankers to leaves, from leaves to leaves, and from leaves to leaf scars (16). Fregoin and Crosse (26) have collected P.

syringae pv. morsprunorum isolates from rainwater but they did not determine the relationship between inoculum levels and disease incidence. Because many factors are involved for a severe epidemic of bacterial canker to occur, the disease is sporatic, but can unexpectedly cause extensive damage to cherry trees.

To reduce infection of cherry trees by rain-disseminated P. syringae pv. morsprunorum and P. syringae pv. syringae, chemical control practices have been evaluated. These studies have focused on reducing infection of leaves, blossoms, and fruit in spring, and leaf scars in autumn. In 1945, Montgomery and Moore (41) reported the incidence of bacterial canker on Bigarreau de Schrecken sweet cherry trees in England was less after 4 years of single-treatment applications of Bordeaux mixture in spring and autumn than on nonsprayed check trees. Bordeaux mixture applied after petal fall did cause severe copper phytotoxicity damage to leaves (41). In greenhouse studies, Dye (23) reported that 250 ug/ml streptomycin sulphate reduced wound-inoculated infections by P. syringae pv. syringae on peach seedlings by 84% compared to nonsprayed check seedlings. In the field Crosse (13) found three sprays of streptomycin hydrochloride applied at full bloom and at 75 and 100% petal fall reduced leaf spotting by 94%, while a single application of Bordeaux mixture at white bud had no effect on leaf spot infection. Copper sprays applied to sweet cherry trees in autumn reduced the level of leaf scar infection by P. syringae pv. morsprunorum that autumn as well as canker development the following spring (13). In Connecticut (43) and California (4), repeated applications of streptomycin and Bordeaux mixture before, during, and after bloom

significantly reduced the incidence of blossom blast on pear caused by P. syringae pv. syringae. In Oregon, chemical control practices are recomended for bacterial canker of sweet cherry (3). In Michigan, no control practices are recommended for bacterial canker on sweet and sour cherry.

Changes in cultural practices may also reduce the incidence of bacterial canker. In Oregon, it was reported (8) and recommended (3) that scions budded high on the rootstocks had lower incidence of bacterial canker than those budded at ground level.

Development of bacterial-resistant Prunus sp. cultivars is another means to control bacterial canker. Sour cherry scion cultivar Schattenmorello is highly resistant to leaf infection by P. syringae pv. syringae (45) and supports low epiphytic populations of P. syringae pv. morsprunorum compared with Nefris sour cherry (47). A drawback with resistant cultivars is that P. syringae pv. morsprunorum and P. syringae pv. syringae have many strains with different host ranges and levels of virulence; therefore, resistance to bacterial canker will probably be overcome by new strains of the pathogen.

For example, in England Garrett and Crosse (29) identified two strains of P. syringae pv. morsprunorum; one was a pathogen of prune and the other was a pathogen of cherry (race 1). Napoleon and Roundel sweet cherry cultivars were susceptible and resistant, respectively, to the cherry strain of P. syringae pv. morsprunorum (15). Later in 1974, race 2 of the cherry P. syringae pv. morsprunorum strain was identified using phage typing and was pathogenic on Roundel, the bacterial canker-tolerant sweet cherry cultivar (27). Garrett (28) reported F12/1 was tolerant to race 1 of P. syringae pv. morsprunorum

but was susceptible to race 2. Also, Allen and Dirks (1) screened many sweet cherry cultivars for resistance to P. syringae pv. morsprunorum and P. syringae pv. syringae with wound inoculations and found different pathogen strains were pathogenic on different cultivars. These examples of variability of P. syringae pv. morsprunorum and P. syringae pv. syringae indicate that a breeding program for bacterial canker-resistant cherry cultivars should be a continuous program. Evidence of an indigenous system of gene transfer has been found for P. syringae pv. morsprunorum (25), indicating the possibility P. syringae pv. morsprunorum may overcome host resistance by genetic recombination.

The objectives of this study were to: (i) develop a rain sampling device to monitor rain-disseminated pathogens from trees; (ii) investigate the dissemination of P. syringae pv. morsprunorum in rainwater and the overwintering of P. syringae pv. morsprunorum and P. syringae pv. syringae in leaf scars of fruit spurs and on ground cover in a Montmorency sour cherry orchard; (iii) investigate the effectiveness of fixed copper compounds for reducing populations of P. syringae pv. morsprunorum from early bud break through early summer on Montmorency sour cherry and to examine the retention of copper on the foliage; and (iv) to test the susceptibility of eight cherry rootstock cultivars to P. syringae pv. morsprunorum and P. syringae pv. syringae by wound inoculations.

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CHAPTER 1

A Sequential Sampler for Monitoring Water-Disseminated Pathogens from Trees

ABSTRACT

An apparatus for studying the dissemination of waterborne pathogens from trees is described and illustrated. It is used for monitoring a population of rifampicin-resistant Pseudomonas syringae pv. morsprunorum in rainwater collected under a Montmorency sour cherry tree. The apparatus collects rain from a 5,026-cm^2 area and saves 5- to 8-ml subsamples for each 0.5-mm rainfall. Simplicity of design and sequential sampling based solely on a mechanical mechanism are advantages of this apparatus for certain phytopathological studies.

INTRODUCTION

Many bacterial pathogens of fruit trees are waterborne during part of their life cycle; e.g., P. syringae pv. morsprunorum and Pseudomonas syringae pv. syringae, the cause of bacterial canker on cherry (2, 4). Splashing and windblown rain are considered important in the spread of these bacteria (2, 4). Bacterial canker is sporatic in occurrence; therefore, to comprehensively study the role of rain in removing and disseminating P. syringae pv. morsprunorum from leaves, we needed an apparatus that sequentially sampled rain dripping from

infected Prunus cerasus L. cv. Montmorency sour cherry trees. Sequential rain samplers are used for air pollution studies (1, 7, 8), but due to their large size, limited sampling capacity, and high cost, they are not practical for most epidemiological studies or for widespread monitoring of chemicals. This paper describes the design and use of a sequential rain sampler for monitoring runoff rainwater for a bacterial pathogen.

MATERIALS AND METHODS

Instrument design and operation. The self-advancing sequential rain sampler resembles a fraction collector and tipping bucket rain gauge combined (Figures 1.1 and 1.2). A large funnel collects the rain that fills and tips a divided bucket. On tipping, a subsample of water is siphoned into a 9-ml collection tube and the rack holding the tubes is automatically advanced.

The funnel is made of 8-mil polyethylene and has an 80-cm diam opening with a rim made from 1.9-cm diam rigid plastic tubing (Figure 1.1). The rim is supported 92 cm above the ground with four 51-cm lengths of 1.9-cm diam steel pipe attached to the sampler cover. A collar placed on the sampler cover is constructed from an inverted 20-cm diam plastic funnel, with a 10-cm diam orifice. The bottom of the polyethylene funnel is fitted into the collar and is held in place by inserting a 10-cm diam plastic funnel inside the polyethylene. The bottom of the 10-cm diam plastic funnel directs water into the tipping bucket and is held in place with a metal pin.

The sampler cover is made of a 25-cm diam x 31.7-cm high metal can (Figure 1.1). The sampler is mounted on two 1.9-cm thick plywood

boards spaced 10 cm apart with three 0.95x16-cm leveling bolts. The upper and lower boards are 55- and 77-cm equilateral triangles, respectively. The lower board is fastened to the ground with three 0.95x30-cm steel spikes.

The tipping bucket and sampling mechanism are constructed of 3.17- and 6.35-mm thick plexiglass (Figure 1.2). The tipping bucket has two compartments, each with a capacity of 280 ml. Tipping bucket volumes are adjusted by placing counterweights on a metal rod suspended below the tipping bucket. The axis for the tipping bucket is supported on both ends with removable bearing supports of Teflon (E.I. duPont de Nemours & Co., Wilmington, DE 19898).

The movement of the tipping bucket also advances the rack of collection tubes. A plexiglass arm, reinforced with a 2.17-mm steel shaft, is attached near the top center of the tipping bucket. A Teflon sliding bearing at the end of the steel shaft is inserted into a pivoting arm connected to the advancing mechanism located at the base of the sampler.

Each compartment of the tipping bucket empties into a separate drain. The floor of each drain slopes down 6.15 mm from the side walls to a center outlet. A 2.5-cm long x 2-cm wide x 2.5-cm high plexiglass box with an open top is placed on the floor of each drain directly above a row of collection tubes. Each box has a siphon made of 5-mm diam glass tubing for transferring water from the box to a collection tube each time the tipping bucket empties.

A turntable and rack of collection tubes are located underneath the floor of the drains. The turntable consists of three 21.6-cm diam plates made from 3.17-mm thick plexiglass. The top and bottom plates

are notched with 30 teeth pointing clockwise and counterclockwise, respectively, and are cemented to the center plate. The turntable revolves on a 10.16-cm diam Lazy Susan bearing (Triangle Manufacturing Co., Oshkosh, WI 54901). A removable rack for holding 13x100-mm test tubes is rotated by the turntable. The rack is made of three 20.5-cm diam plexiglass plates, two of which are 3.15-mm thick and drilled with holes in two concentric circles each to hold 30 tubes. Using this same pattern, the bottom plate, 6.35-mm thick, is drilled with wells 3.2-mm deep for stabilizing the respective tubes. Collection tubes in the outside circle are filled by the right siphon, those in the inside circle by the left siphon.

Two consecutive bucket tips fill a collection tube in each row of tubes and also advance the turntable one notch. The back and forth movement of the tipping bucket pivots the advancing arm and slides a Teflon advancing block back and forth in a partially enclosed box. An angled piece of flexible metal, protruding from the inside surface of the advancing block, catches a clockwise tooth on the turntable. When the left bucket tips, the advancing block moves from left to right, rotating the turntable counterclockwise one notch. As the advancing block moves from left to right, a second angled piece of flexible metal is exposed and catches a counterclockwise tooth, stopping the advancement of the turntable. The metal stop is pulled away from the turntable as the advancing block moves from right to left. Clockwise movement of the turntable is prevented by a stationary stop piece.

To record when rain samples are collected, an electronic switch (MICROswitch, Freeport, IL 61032) is attached to the pivoting arm just below the plexiglass arm coming from the tipping bucket. Each tipping

of the bucket causes the switch to complete an electronic circuit that sends a pulse to an event recorder (WEATHERtronics, Inc., West Sacramento, CA 95691).

Evaluation of instrument. To measure the volume of water needed to tip each bucket, known quantities of water were poured into the sampler until the bucket tipped. This was repeated 20 times per bucket for each of the three samplers. The volumes of water were multiplied by 1.99×10^{-3}, the millileters of rain necessary to collect 1 ml of water in the sampler, to obtain the quantity of rain needed to tip each bucket.

Redistribution of bacteria within each sampler was tested by pouring a bacterial suspension into the samplers, then removing bacteria that adhered to each sampler with simulated rain. A rifampicin-resistant strain of P. syringae pv. morsprunorum (PsmR) (6) grown on King's medium B (5) for 2 days at 22 C was suspended in 0.01-M phosphate buffer adjusted to pH 7.2. The sampler was washed thoroughly with phosphate buffer before pouring in enough bacterial suspension for two bucket tips. This was immediately followed by pouring in enough phosphate buffer for six bucket tips. Bacterial suspensions and phosphate buffer were poured around the perimeter of the funnel. From each of the eight collection tubes, duplicate 0.1-ml subsamples were pipetted with a Finnpipette (Finnpipette, Helsinki, Finland) onto a modified King's medium B amended with 50 µg/ml rifampicin (Calbiochem-Behring Corp., La Jolla, CA 92037) and 25 µg/ml cycloheximide (Sigma Chemical Co., St. Louis, MO 63178). Colonies were counted after 5-days incubation at 22 C and the colony-forming units (cfu)/ml sample were computed. The concentration of bacteria in

each collection tube was expressed as a percentage of the concentration of bacteria in the initial suspension (bacterial frequency). This experiment was replicated three times per sampler.

In the field a sequential rain sampler was positioned under the drip line of a Montmorency sour cherry tree in East Lansing, Michigan that was spray-inoculated with PsmR at sunset on 17, 29 April, 8, and 29 May 1980. Inoculum was prepared from 2-day-old cultures of PsmR grown on King's medium B incubated at 22 C. The cultures were suspended in phosphate buffer to give a final concentration of 10^8 cfu/ml. The Montmorency sour cherry leaves were lightly misted with 1.5 L of inoculum applied with a handgun sprayer operated at 28 kg/cm^2. Concentrations of PsmR in rainwater were determined for each collection tube by plating duplicate 0.1-ml subsamples onto a modified King's medium B amended with rifampicin and cycloheximide. Colonies were counted after 5-days incubation at 22 C and the cfu per milliliter of rainwater were computed. Rainwater was plated within 12 hr after the end of each rain period.

RESULTS AND DISCUSSION

The amount of rain needed to tip each bucket varied slightly among samplers and ranged from 0.533 $\pm$ 0.23 mm to 0.452 $\pm$ 0.023 mm.

When suspensions of bacteria were added to samplers that were still wet from being washed with phosphate buffer, there was a mean reduction of 16 and 9% in the concentration of bacteria in collection tubes 1 and 2, respectively, compared to the initial bacterial suspension (Figure 1.3A). When sterile phosphate buffer was added to the sampler (Figure 1.3A, samples 3 to 8), the concentration of

bacteria in successive collection tubes declined until no bacteria were detected.

The results fit a distinct pattern that was reconstructed with the following equation:

$$M_n = 0.9[0.9(I_n) + 0.1(I_{n-1})] + 0.1\ (M_{n-2}) \qquad \text{(Eq. 1)}$$

where M = concentration of bacteria in collection tube number n, I = concentration of bacteria in the solution entering the sampler, and n = collection tube number. This equation assumes that the funnel and sampling mechanism each retain 10% of the bacteria from the previous sample. Therefore, the quantities 0.1 (I_{n-1}) and 0.1 (M_{n-2}) represent the amount of bacteria remaining on the funnel and sampling mechanism, respectively. To reconstruct the experiment using equation 1, we assumed $I_{n-1} = 0$ and $M_{n-2} = 0$ for sample 1 and $M_{n-2} = 0$ for sample 2, since the sampler was cleaned with phosphate buffer prior to the experiment. When the equation was solved with known concentrations of bacteria entering the sampler, the predicted values were similar to the empirical data points (Figure 1.3B). A correlation coefficient of $r = 0.96$ was obtained when the empirical data were compared with the predicted data.

Equation 1 was used to estimate the concentration of bacteria in rain entering the sampler from the concentration of bacteria detected in the collection tubes. If the sampler was dry before a rain period, the concentration of bacteria in the first collection tube would be equal to the concentration of bacteria in water entering the sampler. The concentration of bacteria in the second collection tube would be

corrected for retention of bacteria on the funnel and the concentration of bacteria in the third to nth collection tubes would be corrected for retention of bacteria on the funnel and sampling mechanism. The prediction equations were:

1st tube $I_1 = M_1$ (Eq. 2)

2nd tube $I_1 = [M_2 - 0.1(I_1)]/0.9$ (Eq. 3)

3rd - nth tubes $I_n = [M_n - 0.09(I_{n-1}) - 0.1(M_{n-2})]/0.81$ (Eq. 4)

where, I = concentration of bacteria in the rain entering the sampler, M = measured concentration of bacteria in the collection tube, and n = collection tube number. Retention of bacteria on the funnel was accounted for by $0.1(I_1)$ and $0.09(I_{n-2})$ in equations 3 and 4, respectively, and retention of bacteria on the sampling mechanism was accounted for by $0.1(M_{n-2})$ in equation 4.

During a rain period on 3 October 1980, the sampler collected rainwater from a Montmorency sour cherry tree previously inoculated with PsmR. Actual concentrations of PsmR detected in the collection tubes and corrected concentrations were plotted over time (Figure 1.4). High concentrations of bacteria were detected in the first two samples collected after the onset of the rain. As the intensity of the rain increased, concentrations of bacteria in the samples decreased. But when the intensity of the rain decreased, concentrations of bacteria in subsequent samples increased with the maximum concentration being detected at 1630 hours. This may be explained if we consider that during a rain period the leaves are saturated with water and the bacteria in the leaves move out of the

leaves through the stomata at a constant rate (cfu per unit of time). When the rain intensity increases, the concentration per unit volume of water decreases and vice versa. After 1630 hours, there was a decline in the concentration of bacteria in the rain samples. Corrected concentrations of PsmR were similar to measured concentrations except in the last two samples. Corrected values were lower because many of the bacteria recovered were retained from the previous sample at 1630 hours.

Crosse (3) measured the inoculum potential of P. syringae pv. morsprunorum on sweet cherry leaves in vitro by washing detached leaves in water. The sequential sampler allows researchers to associate in vivo inoculum concentrations of pathogens in foliar-runoff rainwater with temporal environmental events.

One sampler functioned for two growing seasons and two samplers functioned for one growing season without problems. There were no clocks to wind or batteries to replace except on the event recorder. The samplers were easily transported, disassembled, and cleaned in the field. The sequential sampler may also be useful for monitoring fungal spores and pesticide runoff in rainwater from trees.

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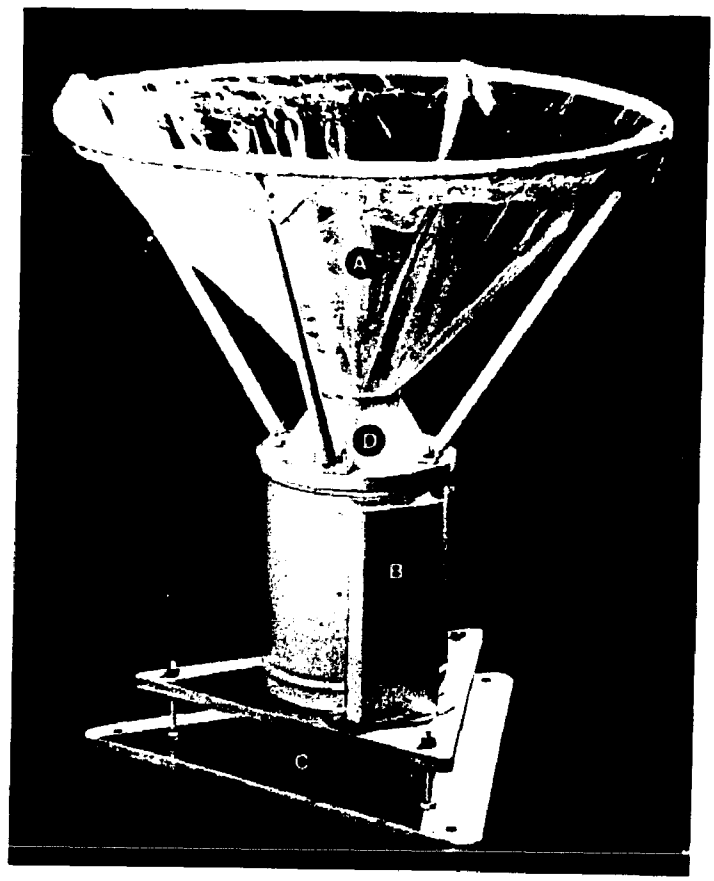

Figure 1.1. Sequential rain sampler, A, funnel; B, cover; C, platform with three leveling bolts; and D, collar.

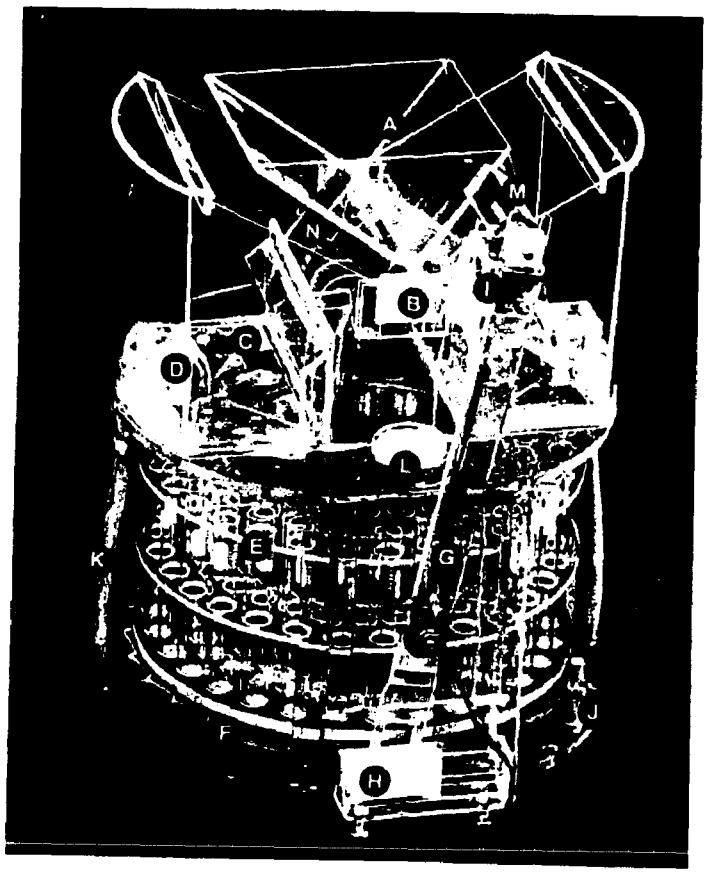

Figure 1.2. Mechanism for the sequential rain sampler. A, tipping bucket; B, bucket bearing; C, drain; D, plexiglass box and siphon; E, collection tube rack and tubes; F, turntable; G, pivoting arm; H, advancing block; I, electronic switch; J, stationary stop piece; K, drain tube; L, level; M, plexiglass arm; and N, counterweights.

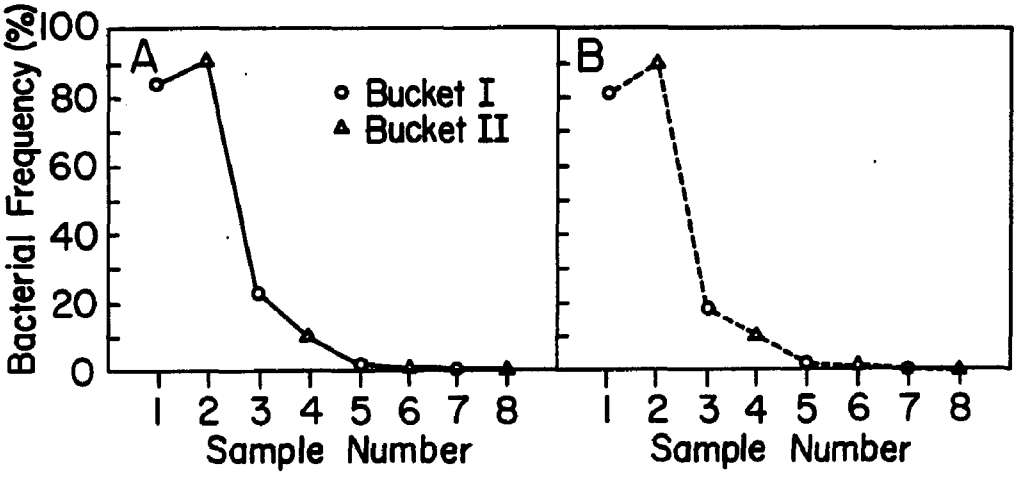

Figure 1.3. Bacterial frequency (%) in collection tubes where sufficient quantities of bacteria in suspension were added to the sampler for two bucket tips, followed by sufficient quantities of phosphate buffer for six bucket tips. Prior to sampling, the sequential rain samplers were thoroughly rinsed with phosphate buffer. A, Empirical data were the means of three replicate experiments from three samplers. B, Predicted values were from the sampler retention equation.

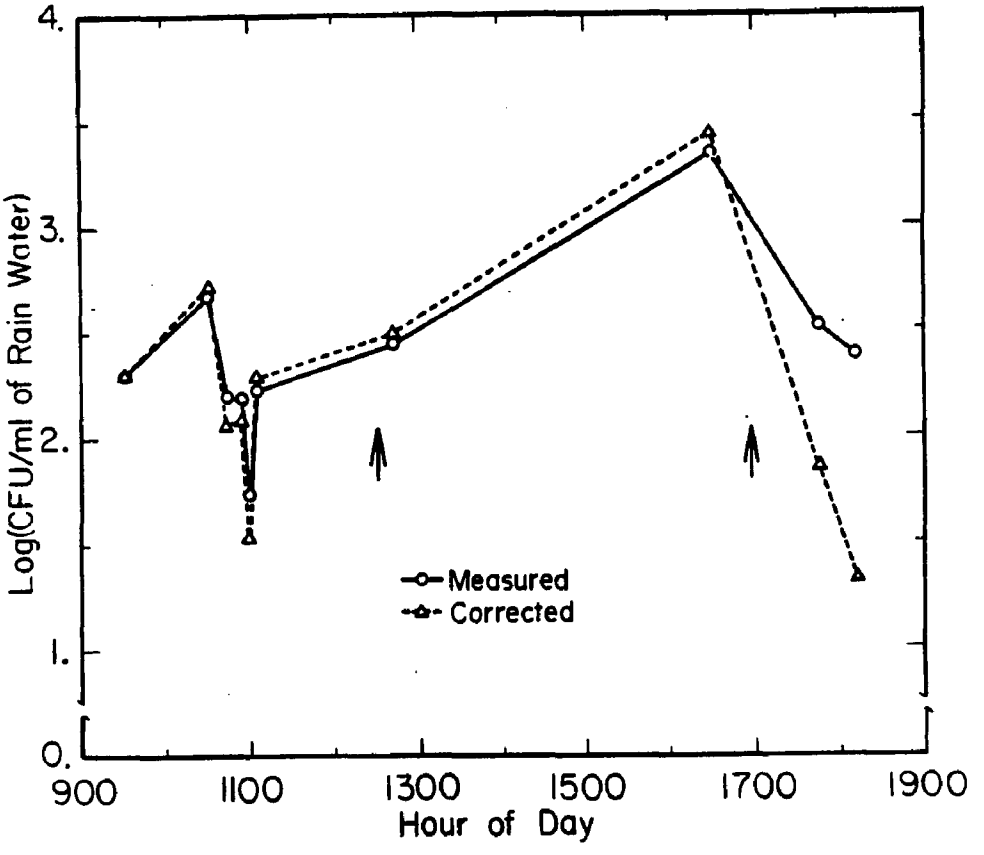

Figure 1.4. Concentrations of rifampicin-resistant Pseudomonas syringae pv. morsprunorum in samples of rainwater collected under a Montmorency sour cherry tree with a sequential sampler during a rain on 8 October 1980. Arrows indicate the beginning and end of a period of light mist.

CHAPTER 2

Dissemination in Rainwater and Overwintering of *Pseudomonas syringae* pv. *morsprunorum* and *P. syringae* pv. *syringae* in a Montmorency Sour Cherry Orchard

ABSTRACT

Rifampicin-resistant strains were used to study the survival and dissemination of *Pseudomonas syringae* pv. *morsprunorum* and *P. syringae* pv. *syringae* (PsmR and PssR, respectively) in a Montmorency sour cherry orchard. Orchard ground cover plants were inoculated with PsmR and PssR in November 1979 and 1980. Both strains were recovered from plants in the ground cover in winter and in early spring, but not in late spring and summer. In spring, PsmR and PssR were recovered from buds on fruiting spurs following the inoculation of leaf scars the preceding autumn. Recovery of PsmR and PssR from buds following leaf scar inoculations in August 1980 was lower than from buds inoculated in October 1980, but recovery of PsmR from buds inoculated in September and October 1979 were similar. In two growing seasons, populations of PsmR isolated from leaves were relatively constant (10^3-10^4 colony-forming units/cm^2 leaf), whereas populations of PsmR in rainwater were higher in spring and autumn than in summer. Two patterns of dispersal for PsmR were observed when rainwater was collected during rain periods with a sequential sampling apparatus. In the first pattern, populations of PsmR in rainwater increased

before leveling off; while in the second, initial populations in rainwater were higher but with time they declined and leveled off. The first and second patterns of dispersal were preceded by 5 days with and without rain, respectively.

INTRODUCTION

Bacterial canker on Prunus cerasus L. cv. Montmorency sour cherry in Michigan is caused by Pseudomonas syringae pv. morsprunorum (11). The disease is sporatic, but a severe epidemic occurred in 1976 (11). Leaf and fruit infections are the most common stages of bacterial canker noted on Montmorency sour cherry in Michigan.

The epiphytic nature of P. syringae pv. morsprunorum on stone fruits is well established (2, 11, 14), but research on the dissemination of P. syringae pv. morsprunorum in rainwater is limited mostly to field observations (6). Waterborne P. syringae pv. morsprunorum are considered the source of inoculum that infects leaves and flowers in spring (6) and leaf scars in autumn (5). On sweet cherry, P. syringae pv. morsprunorum infects fruit spurs through leaf scars from late September to mid-October, and the incidence of infection increases with increased inoculum concentrations (5). In England, P. syringae pv. morsprunorum overwinters in the vascular system of fruit spurs and in spring incites stem cankers and blossom blight (4, 8). In Oregon, P. syringae pv. syringae infects buds of sweet cherry directly rather than indirectly through leaf scars (7). Latorre and Jones (12) presented evidence that P. syringae pv. syringae overwinters on grasses and herbaceous plants under sour

cherry trees. The importance of this source of inoculum compared to other sources of inoculum in spring is not known.

The objectives of this study were to investigate the dissemination of P. syringae pv. morsprunorum in rainwater and the overwintering of P. syringae pv. morsprunorum and P. syringae pv. syringae in leaf scars of fruit spurs and on ground cover in a Montmorency sour cherry orchard.

MATERIALS AND METHODS

Rifampicin-resistant strains. The rifampicin-resistant strain of P. syringae pv. morsprunorum (PsmR) we used was selected by Latorre and Jones (12). Rifampicin-resistant strains of P. syringae pv. syringae (PssR) were selected by spreading 10^8 colony-forming units (cfu) of P. syringae pv. syringae onto King's medium B (10) containing 50 µg/ml rifampicin (Calbiochem-Behring Corp., La Jolla, CA 93037). Eleven isolates from sweet cherry leaves were screened for resistant strains. Colonies that developed after 4-days incubation at 22 C were considered rifampicin-resistant. Pathogenicity of parental and PssR strains were tested by inoculating tobacco leaves and Montmorency sour cherry fruit and leaves with inoculum concentrations of 10^6 cfu/ml 0.01-M phosphate buffer, pH 7.2. A strain of PssR similar in pathogenicity to the parental isolate was used in this study.

Inoculum of PsmR and PssR for each experiment was prepared from 2-day-old cultures grown on King's medium B incubated at 22 C. Petri plates were flooded with 10-ml phosphate buffer and the bacteria were suspended by stirring with a sterile glass rod. Inoculum concentrations of 10^8 cfu/ml phosphate buffer were determined

turbidimetrically and lower concentrations of inoculum were obtained by serial dilution in phosphate buffer.

Overwintering. Survival of PsmR and PssR on ground cover plants was studied in a 5-yr-old Montmorency sour cherry orchard in 1979 and 1980 in East Lansing, Michigan. The predominant plant in the ground cover was Poa pratensis L. Other plants in decreasing frequency were Medicago sativa L., Festuca elatior var. arundinacea (Shreb.), Panicum dichotomiflorum Michx., Stellaria media (L.) Cyrill., Medicago lupulina L., Taraxacum officinale Weber., Rumex acetosella L., Plantago lanceolata L., Plantago major L., and Lychnis alba Mill. Suspensions of PsmR and PssR (10^8 cfu/ml phosphate buffer) were sprayed onto these plants with a backpack sprayer charged with CO_2 gas and operated at 2.8 kg/cm^2. Four separate plots were inoculated with each isolate. A plot consisted of a row of three cherry trees with a strip of ground cover in the tree row killed with herbicide. A 9-m long x 30-cm wide area on each side of the herbicide strip was spray-inoculated with 1 L of inoculum.

From each plot, two bulk samples of plant material were collected, one on each side of the herbicide strip. Plants were sampled at 1-m intervals along the strips. Samples were weighed and a 20-g subsample from each sample was homogenized for 2 min in a blender (Waring Products Inc., New Hartford, CT 06057) with 300-ml phosphate buffer. The homogenate was serially diluted in phosphate buffer and duplicate 0.1-ml subsamples were plated on a modified King's medium B (KBrc) amended with 50 µg/ml rifampicin and 25 µg/ml cycloheximide (Sigma Chemical Co., St. Louis, MO 63178). Colonies were counted

after 5-days incubation at 22 C and the cfu per gram of plant material were calculated.

In summer, leaves were collected from the three trees in each plot to determine if PsmR or PssR had moved up into the trees from the ground cover. Each tree was divided into quadrants and 10 leaves were randomly collected from each quadrant. The 40 leaves were measured with an area meter (Model LI-3000, LAMBDA Instrument Comp., Lincoln, NB 68504) before homogenizing them for 2 min in a blender with 300-ml phosphate buffer. The homogenate was serially diluted in phosphate buffer and duplicate 0.1-ml subsamples were plated on KBrc. Colonies were counted after 5-days incubation at 22 C and the cfu of PsmR per cm^2 of leaf were determined.

Leaf scars on fruiting spurs were inoculated with PsmR and PssR on 21 September, 5 October 1979; 19 August, 11 September, and 8 October 1980 in East Lansing. Three Montmorency sour cherry trees were inoculated with each isolate. A leaf was removed from each spur and 0.05 ml of inoculum was placed on the exposed leaf scar. Inoculum concentrations were 10^4, 10^6, and 10^8 cfu/ml phosphate buffer in 1979 and 10^2, 10^4, and 10^6 cfu/ml phosphate buffer in 1980. Each concentration was applied to 18 leaf scars per tree. Inoculum was kept in an ice bath during the inoculation period. Inoculated spurs were collected during bloom on 15 May 1980 and 14 May 1981, and individually frozen at -20 C. Isolations were made by dicing the buds of each spur with a sterile razor blade and placing the pieces in 10-ml phosphate buffer. After 1 hr, the suspension was mixed and 0.1 ml was plated on KBrc. The presence of rifampicin-resistant strains was

recorded after 5-days incubation at 22 C and the percentage of infected leaf scars was calculated for each replication.

Monitoring bacterial populations. Populations of PsmR associated with Montmorency sour cherry leaves were monitored in East Lansing. A single 12-yr-old Montmorency sour cherry tree was spray-inoculated on 17, 29 April, 8, 29 May 1980; 15, 22, 29 April, and 7 June 1981 with 1.5-L phosphate buffer containing 10^8 cfu PsmR/ml using a handgun sprayer operated at 28 kg/cm^2. All spray inoculations were applied at sunset. Leaves were collected eight and 18 times from 4 June to 23 September 1980 and from 23 May to 10 October 1981, respectively (Figure 2.1). The tree was divided into quadrants and 10 leaves were collected at random from each quadrant. The 40 leaves were measured with an area meter before they were homogenized for 2 min in a blender with 300-ml phosphate buffer. The homogenate was serially diluted in phosphate buffer and duplicate 0.1-ml subsamples were plated on KBrc. Colonies were counted after 5-days incubation at 22 C and the cfu of PsmR per cm^2 of leaf were calculated.

Populations of PsmR from individual leaves on terminal shoots were determined in 1981. Two 13-yr-old Montmorency sour cherry trees were spray-inoculated for the first time in 1981 as described for the single tree. Populations associated with leaves were determined on 26 May, 2, 17, 24 June, and 2 July. Leaves on six to 10 terminal shoots were harvested, measured with an area meter, and homogenized individually for 2 min in a blender with 50-ml phosphate buffer. The homogenate was serially diluted in phosphate buffer and duplicate 0.1-ml subsamples plated on KBrc. Colonies were counted after 5-days

incubation at 22 C and the cfu of PsmR per cm^2 of leaf were calculated.

In 1980 and 1981, one and three sequential rain samplers (see Chapter 1), respectively, were placed under the drip line of the inoculated tree used for monitoring bacterial populations on leaves. From each 5- to 8-ml sample of rainwater, duplicate 0.1-ml subsamples were plated on KBrc within 12 hr after each rain ended. Colonies were counted after 5-days incubation at 22 C and the cfu of PsmR per milliliter of rainwater were determined.

Environmental monitoring. Air temperature was recorded with a 7-day recording hygrothermograph (Bendix Co. Inc., Baltimore, MD 21204) placed in a weather shelter 2 m above the ground in the orchard. Calibration of the hygrothermograph was checked weekly with a sling psychrometer. Rainfall was measured with a tipping bucket rain gauge (WEATHERtronics, Inc., West Sacramento, CA 95691) and recorded with an event recorder or microcomputer (9). Average populations of PsmR in rainwater for each rain period and populations of PsmR associated with leaves were separately correlated with daily average air temperatures for the preceding 10 days.

Data analyses. All populations of PsmR in rainwater and associated with leaves were transformed by adding 1 to each value before calculating the $\log_{10}$. All data were analyzed with the Stat 4 statistical program (1) and Cyber 750 computer (Control Data Corp., Minneapolis, MN 55440).

RESULTS

Overwintering on ground cover. Following inoculation of the orchard ground cover on 15 November 1979, populations of PssR and PsmR were high (4.21 to 4.47 $\log_{10}$ cfu/g ground cover) on 18 January (Table 2.1). Fewer bacteria were recovered on 11 March and 17 April, and no bacteria were recovered on 16 May, 23 June, and 24 September 1980. Following a second inoculation on 2 November 1980, populations of PssR were high while populations of PsmR were quite low on 13 November. Both strains were recovered from the ground cover on 16 December 1980, only PssR was recovered on 8 March, and neither PssR or PsmR were recovered on 28 May 1981. Neither PsmR or PssR were recovered from Montmorency sour cherry leaves collected on 23 June 1980 and 8 June 1981.

Overwintering in leaf scars. In the spring of 1980 and 1981, PsmR and PssR were recovered from buds of spurs inoculated in the autumn of 1979 and 1980, respectively (Table 2.2). In both years, recovery of PsmR and PssR from buds increased as inoculum concentrations were increased. Recovery of PssR from spurs inoculated 5 October 1979 was higher than from spurs inoculated 21 September 1979, but the recovery of PsmR did not differ significantly between inoculation dates. In 1980, the recovery of PsmR and PssR was significantly higher for spurs inoculated 8 October than for those inoculated 19 August.

Populations associated with leaves. Through the 1980 and 1981 growing seasons, populations of PsmR associated with symptomless leaves were about 4 $\log_{10}$ cfu/cm^2 leaf (Figure 2.1). In 1980 and 1981, populations above 5 $\log_{10}$ cfu/cm^2 leaf were recorded only once

(24 June 1980); populations below 3 $\log_{10}$ cfu/cm^2 leaf were recorded twice (27 June and 13 August 1981). Fluctuations in air temperature did not appear to significantly alter populations of PsmR associated with leaves.

Populations of PsmR associated with individual leaves of terminal shoots were determined in 1981. On 2, 17, 24 June, and 2 July newly emerged leaves had significantly ($P = 0.05$) lower populations of PsmR than older leaves (Table 2.3). Usually, no PsmR were isolated from the youngest leaves, but populations of 2 to nearly 3 $\log_{10}$ cfu/cm^2 leaf were recovered from the older basal leaves.

Populations in rainwater. In 1980, populations of PsmR in rainwater collected from 29 May to mid-June were 1.3 to 3.7 $\log_{10}$ cfu/ml rainwater and from 1 July to mid-August were 0.0 to 0.7 $\log_{10}$ cfu/ml rainwater (Figure 2.1A). Populations of PsmR increased to 3.1 $\log_{10}$ cfu/ml rainwater on 31 August, but on the following day populations were much lower (0.5 $\log_{10}$ cfu/ml rainwater). Populations of PsmR increased from 0.5 $\log_{10}$ cfu/ml rainwater on 1 September to 3.0 $\log_{10}$ cfu/ml rainwater on 13 and 17 October. Prior to leaf fall on 10 November, populations of PsmR dropped to 1.9 $\log_{10}$ cfu/ml rainwater on 3 and 8 November.

In 1981, the highest populations of PsmR in rainwater, 3.5 $\log_{10}$ cfu/ml rainwater, were recorded on 10 May, 2 days before full bloom (Figure 2.1B). From 24 May to 13 June, populations of PsmR were 1.6 to 2.9 $\log_{10}$ cfu/ml rainwater. On 8 June, populations of PsmR may have been higher than normal because the tree was inoculated on 7 June. From 22 June to 22 September, populations of PsmR in rainwater

were 0.0 to 1.9 $\log_{10}$ cfu/ml; on 25 September they increased to 2.6 $\log_{10}$ cfu/ml and in autumn, populations peaked at 3.0 $\log_{10}$ cfu/ml on 21 October. Populations of PsmR decreased to 0.3 $\log_{10}$ cfu/ml rainwater on 5 November, 3 days before leaf fall. Correlation coefficients between populations of PsmR in rainwater and average daily temperatures for the preceding 10 days were -0.5436 and -0.5096 ($\underline{P}$ = 0.01) for 1980 and 1981, respectively, indicating that as air temperature increased, populations of PsmR in rainwater decreased and vice versa (Figure 2.1).

Dispersal patterns of bacteria in rainwater. Populations of PsmR in serially collected rain fractions revealed two types of dispersal patterns (Figure 2.2). In a type 1 pattern, populations of PsmR increased during the early stages of a rain period, then leveled off. Rains resulting in a type 1 dispersal pattern were preceded within 5 days by rain. In a type 2 pattern, populations of PsmR were high in the first fractions, declined and leveled off in the later fractions. Rains resulting in type 2 patterns were not preceded within 5 days by rain. When five or more fractions were collected and PsmR were isolated, the dispersal pattern was classified. Of 52 rain periods in 1980 and 1981, 24 were not classified because no bacteria were isolated (six periods) or less than five fractions were collected (18 periods). Of 21 rain periods classified as type 1, 20 were preceded within 5 days by rain. None of the seven rain periods classified as type 2 were preceded within 5 days by rain. In 1981, dispersal patterns did not differ between the three samplers.

DISCUSSION

Both PsmR and PssR overwintered in Montmorency sour cherry buds of fruit spurs following leaf scar inoculations in autumn. As reported in England (5), infection through leaf scars with PsmR was greater in late autumn than in early autumn. In this study, the incidence of infection through leaf scars by P. syringae pv. syringae and P. syringae pv. morsprunorum were similar, while Crosse and Garrett (7) found the incidence of infection with a strain of P. syringae pv. syringae from Oregon, USA was less than with a strain of P. syringae pv. morsprunorum from England. These differences in efficiency of leaf scar infection between P. syringae pv. syringae and P. syringae pv. morsprunorum may be caused by differences in the way infection was determined. Crosse and Garrett (7) determined infection by visual observation of symptoms, while we isolated the pathogen.

Through the winter and early spring, PsmR and PssR were recovered from plants in the ground cover but not in late spring or summer, and were not disseminated up into the Montmorency sour cherry tree. These results indicate PsmR and PssR failed to become established on new ground cover growth in spring, probably because the strains were not pathogenic to any of the available plant species. Latorre and Jones (12) suggested P. syringae pv. morsprunorum did not overwinter in the ground cover because its host range was more limited than that of P. syringae pv. syringae.

In this study, populations of PsmR associated with Montmorency leaves were relatively constant through the growing season; while in Poland (2, 14), populations of P. syringae pv. morsprunorum fluctuated during the growing season on Nefris, North Star, and Schattenmorele

sour cherry as well as on sweet cherry. These differences may be caused by sampling technique; in other studies (2, 14), populations were determined from leaf washings and expressed as cfu per leaf, while in this study total populations asssociated with leaves were expressed as cfu per cm^2 of leaf.

Recovery of PsmR from newly emerged Montmorency sour cherry leaves was infrequent, while PsmR was always recovered from older fully expanded leaves. Therefore, PsmR was either washed by rainwater from older infected leaves to newly emerged leaves or newly emerged leaves were infected but the bacteria were not detectable with our isolation procedure.

Populations of PsmR in rainwater collected under the drip line of a Montmorency sour cherry tree correlated negatively with air temperature. Cool air temperatures and high populations of PsmR in rainwater correspond with infection of leaves and leaf scars in spring and autumn, respectively. Schimdle and Zeller (13) demonstrated that at 100% relative humidity, air temperatures between 15 and 20 C were optimal for infection and disease development of P. syringae pv. morsprunorum on sour cherry. In this study, populations of PsmR associated with leaves were not altered by air temperature; but air temperature may have affected the survival of PsmR in rainwater or release of PsmR from the leaves.

The two dispersal patterns of PsmR in rainwater were dependent on the release of PsmR from Montmorency sour cherry leaves. When rains were more than 5 days apart, PsmR probably built up on or near the leaf surface and were released in high numbers with the onset of rain. Dew periods may have contributed to this buildup by providing

conditions suitable for egress of bacteria through stomata onto leaves and for growth of bacteria on the leaf surface.

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Table 2.1. Recovery of rifampicin-resistant Pseudomonas syringae pv. morsprunorum (PsmR) and P. syringae pv. syringae (PssR) from plants in the orchard ground cover following inoculation in the autumns of 1979 and 1980.

Sampling dates	Recovery on KBrc[x] ($\log_{10}$[colony-forming units + 1/g ground cover]) PsmR	PssR
(Inoculated 15 Nov 1979)[y]		
18 Jan 1980	4.21 ± 0.27[z]	4.47 ± 0.34
11 Mar 1980	3.96 ± 0.54	4.27 ± 0.55
17 Apr 1980	1.38 ± 0.17	2.52 ± 1.49
16 May 1980	0.00 ± 0.00	0.00 ± 0.00
23 Jun 1980	0.00 ± 0.00	0.00 ± 0.00
24 Sep 1980	0.00 ± 0.00	0.00 ± 0.00
(Inoculated 2 Nov 1980)[y]		
13 Nov 1980	0.22 ± 0.38	3.64 ± 0.25
16 Dec 1980	1.62 ± 0.76	3.74 ± 0.29
8 Mar 1981	0.00 ± 0.00	1.29 ± 0.85
28 May 1981	0.00 ± 0.00	0.00 ± 0.00

[x]KBrc = A modified King's medium B amended with 50 µg/ml rifampicin and 15 µg/ml cycloheximide.

[y]For each pathovar a plot was inoculated with 2 L of 10^8 colony-forming units/ml phosphate buffer and each plot contained two 30-cm wide by 9-m long areas separated by a row of three Montmorency sour cherry trees.

[z]Mean of four plots followed by the standard error of the mean.

Table 2.2. Percent recovery of rifampicin-resistant *Pseudomonas syringae* pv. *morsprunorum* (PsmR) and *P. syringae* pv. *syringae* (PssR) from buds on fruit spurs of Montmorency sour cherry in spring following leaf scar inoculations the previous autumn.

Inoculation date	Inoculum concentrations (cfu/ml buffer)[y] PsmR 10^2	10^4	10^6	10^8	Means[z]	PssR 10^2	10^4	10^6	10^8	Means
21 Sep 1979	-	25	56	61	47±19	-	0	7	14	7±11
5 Oct 1979	-	5	60	78	48±28	-	12	24	54	30±22
Means	-	15±13	58±16	70±19	-	-	6±10	15±10	34±26	-
19 Aug 1980	2	24	62	-	29±25	2	9	55	-	22±26
11 Sep 1980	2	34	64	-	34±26	2	25	65	-	31±28
8 Oct 1980	2	61	80	-	48±35	2	16	71	-	30±32
Means	2±3	40±19	69±11	-	-	2±3	16±15	64±15	-	-

[y]cfu = Colony-forming units. Each value is the mean of three replications each consisting of 12 to 18 leaf scar inoculations. Buds were collected on 15 May 1980 and 14 May 1981, and infection was established by isolating on King's medium B amended with 50 µg/ml rifampicin and 25 µg/ml cycloheximide.

[z]Values are the mean and standard error of the mean.

Table 2.3. Populations of rifampicin-resistant Pseudomonas syringae pv. morsprunorum on individual leaves taken from terminal shoots of Montmorency sour cherry trees in 1981.

	Sampling date				
Leaf[x]	26 May	2 June	17 June	24 June	2 July
2	2.03(6)[y]a[z]	2.24(6) a	2.16(10) a	2.86(10) a	2.93(10) a
4	1.19(6) a	1.30(6) a	1.67(10) a	2.54(10) a	2.82(10) a
6	1.62(5) a	0.78(6) a	1.00(10) ab	2.01(10) ab	2.18(10) ab
8	-	0.00(3) b	1.51(10) a	1.42(10) b	1.93(10) ab
10	-	-	0.00(9) b	0.37(10) c	1.24(9) b
12	-	-	-	0.17(8) c	2.04(5) ab

[x]Leaves are numbered starting from the base of the shoot.

[y]Mean of the $\log_{10}$ (colony-forming units + 1 per cm^2 leaf) followed by the number (in parentheses) of leaves sampled. Bacteria were recovered on a modified King's medium B amended with 50 µg/ml rifampicin and 25 µg/ml cycloheximide.

[z]Values in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

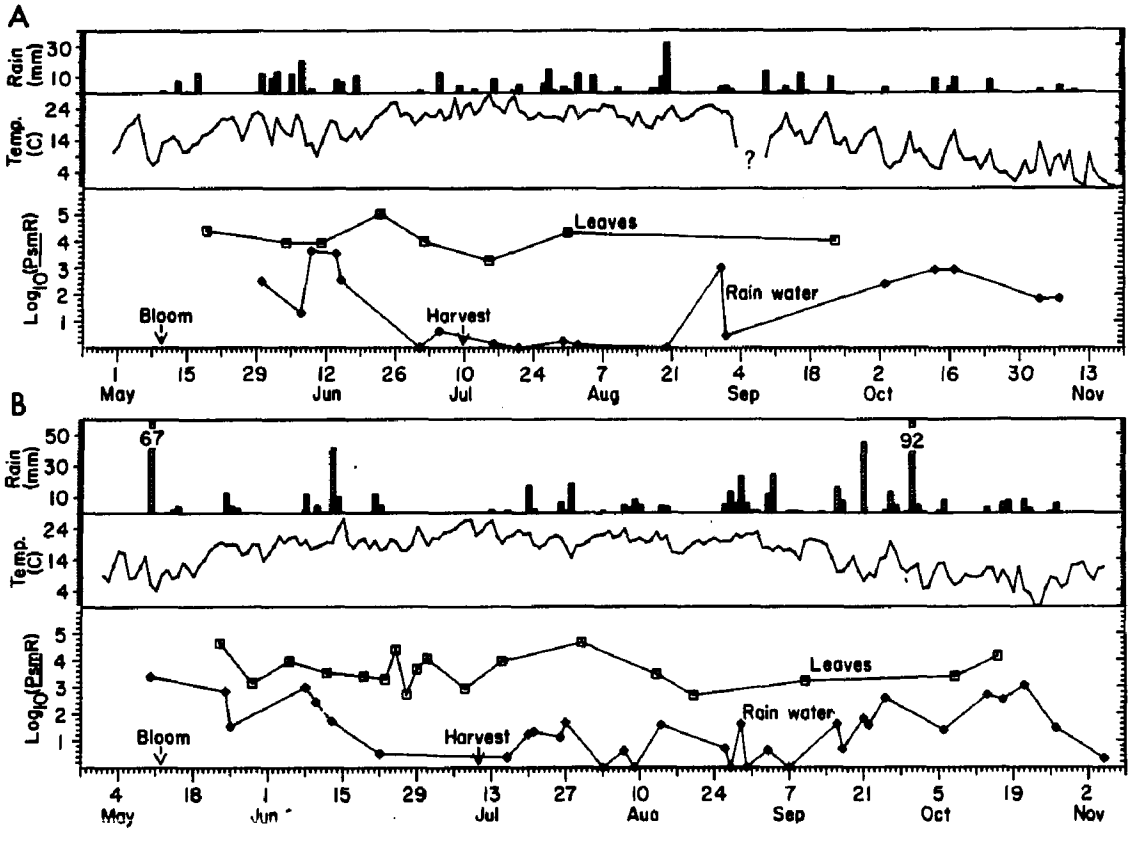

Figure 2.1. Recovery of rifampicin-resistant Pseudomonas syringae pv. morsprunorum (PsmR) from leaves and runoff rainwater from a Montmorency sour cherry tree in relation to mean daily air temperatures and rainfall. Populations were expressed as $\log_{10}$ (colony-forming units of PsmR + 1 per milliliter rainwater) or per cm^2 leaf. A, 1980; B, 1981.

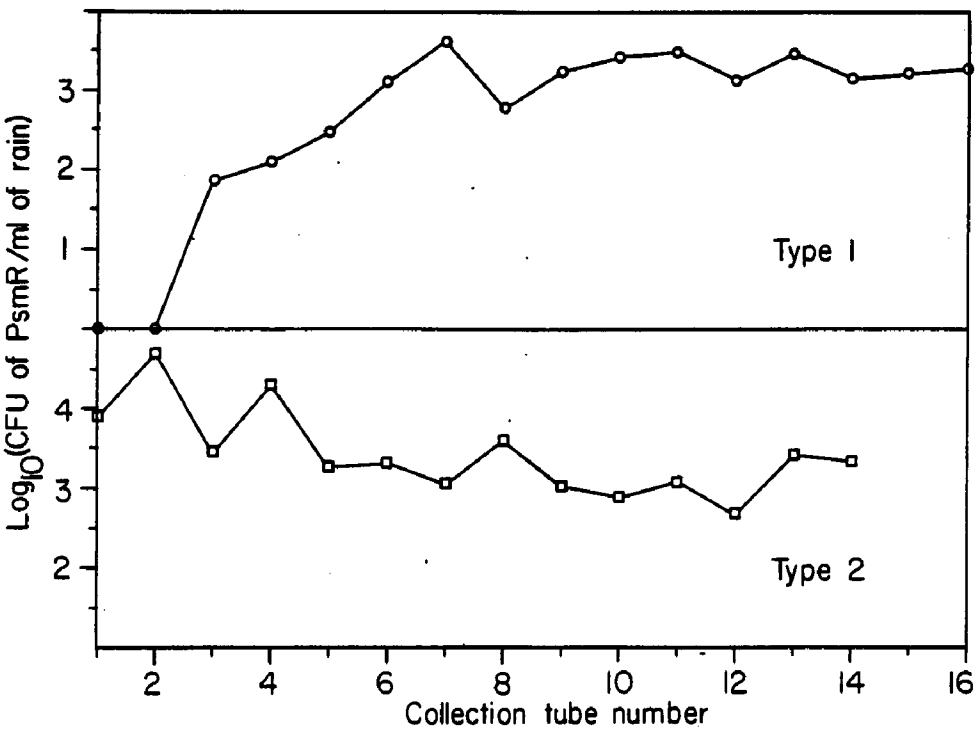

Figure 2.2. Dispersal patterns for rifampicin-resistant Pseudomonas syringae pv. morsprunorum (PsmR) in runoff rainwater collected serially under a Montmorency sour cherry tree during rains on 14 (type 2) and 15 (type 1) June 1980.

CHAPTER 3

Reduction of Pseudomonas syringae pv. morsprunorum on Montmorency Sour Cherry with Copper and Dynamics of the Copper Residues

ABSTRACT

A rifampicin-resistant strain of P. syringae pv. morsprunorum (PsmR) was used to study the effectiveness of tribasic copper sulfate (TBS) and Citcop 4E for reducing populations of PsmR on Montmorency sour cherry trees in spring and early summer. Populations of PsmR were reduced more by 636 and 949 mg/L TBS than by 200 mg/L Citcop 4E, but several applications were needed to reduce the populations to a low level. Citcop 4E at 200 mg/L was more phytotoxic to cherry foliage than TBS at 636 mg/L. Phytotoxicity was related to the number of applications and was not reduced by adding hydrated lime to the copper treatments. The decline of copper residues from leaves was related by multiple regression analyses with rainfall and initial level of copper on leaves.

INTRODUCTION

Bacterial canker, caused by Pseudomonas syringae pv. morsprunorum, was first observed on Prunus cerasus L. cv. Montmorency sour cherry in Michigan in 1976 (13) and no effective control measures have been tested. In Oregon (2), application of copper compounds have been recommended in autumn and just before bud break to control

infection of dormant buds on sweet cherry. In England (15), Bordeaux mixture applied to sweet cherry trees at white bud stage and again at petal fall reduced the leaf spot phase of the disease, but the petal fall spray was highly phytotoxic to the leaves. A single spray of Bordeaux mixture at white bud had no significant effect on the leaf spot phase (8, 15), but sprays of streptomycin at full bloom and at 75 and 100% petal fall reduced leaf spot symptoms by 93 to 96% (6). In California (4) and Connecticut (17), blossom blast of pears, caused by P. syringae pv. syringae, was reduced by a series of streptomycin or fixed copper treatments before, during, and after bloom. These studies suggest that repeated spray treatments in spring may control leaf, blossom, and fruit infections by P. syringae pv. morsprunorum in Michigan.

The objectives of this study were to investigate the effectiveness of fixed copper compounds for reducing populations of P. syringae pv. morsprunorum from early bud break through early summer on Montmorency sour cherry and to examine the retention of copper on the foliage. Copper compounds were evaluated rather than streptomycin because copper compounds have activity against some important fungal pathogens of cherry, and registration of these compounds by regulatory agencies was more likely than registration of streptomycin.

MATERIALS AND METHODS

Field inoculations. The rifampicin-resistant strain of P. syringae pv. morsprunorum (PsmR) selected by Latorre and Jones (14) was used in all experiments. Inoculum grown on King's medium B (12) for 2 days at 22 C was suspended in 10 ml of 0.01-M phosphate buffer

(pH 7.2) by stirring with a sterile glass rod. Inoculum concentrations of 10^8 colony-forming units (cfu)/ml phosphate buffer were obtained by adjusting the turbidity of suspensions to 0.04 absorbance with a spectrophotometer (Spectronic 20, Bausch and Lomb, Rochester, NY 14625) set at 625 nm. Montmorency sour cherry trees in East Lansing, Michigan were spray-inoculated on 17, 29 April, 8, 29 May 1980; 15, 22, 29 April, and 7 June 1981. All trees were inoculated at sunset with a handgun sprayer (28 kg/cm^2) until leaves were lightly wet. The amount of inoculum applied to each tree was 0.2 to 1.5 L depending on tree size.

Spray trials. On inoculated trees, tribasic copper sulfate 53W (TBS) and Citcop 4E (48% copper salts of fatty and rosin acids; Cities Service Co., Atlanta, GA 30302) were evaluated along with streptomycin sulfate 21.2W (Pfizer Inc., New York, NY 10017). Concentrations of spray solutions containing copper were based on the amount of copper salt per liter. Hydrated lime [$Ca(OH)_2$] was evaluated for reducing phytotoxicity from copper sprays. Because of captafol's (Difolatan 4F, Chevron Chemical Co., Richmond, CA 94804) persistence when applied to apple trees (16), the possibility it may improve the retention of copper on cherry leaves was evaluated. All treatments (Tables 3.1 and 3.2) were applied to runoff with a handgun sprayer operated at 28 kg/cm^2. Application dates were 21 April, 2, 27 May, 12 June, 1, 14, 29 July 1980; 17, 27 April, 13, 21, 27 May, 3, 11, 17, and 25 June 1981. Treatments were applied to single-tree plots arranged in a randomized complete block design according to tree size. Each treatment was replicated five times.

In 1981, defoliation was rated by visually estimating the percentage of fallen leaves per replication on 6 and 8 July, 11 and 13 days, respectively, after the final copper treatment on 25 June. Concentrations of copper on leaves were measured on 8 July, 3 days after the onset of defoliation. Five terminal shoots, each with 12 intact leaves, were selected at random from each replication. Starting from the base of each shoot, leaves at nodes 1, 3, 6, 8, and 11 were harvested and pooled by node number. The leaves were measured with an area meter (Model LI-3000, LAMBDA Instruments Corp., Lincoln, NB 68504) and copper concentrations were measured with a plasma emission spectrometer described later. Leaf age was estimated using a degree-day leaf emergence model developed for Montmorency sour cherry (10). Temperatures were recorded with a hygrothermograph (Bendix Co. Inc., Baltimore, MD 21204) placed in a weather shelter 1.5 m above the orchard floor. The number of copper sprays applied to each leaf was estimated based on the approximate date of leaf emergence.

In East Lansing, I studied the loss of copper from leaves on Montmorency sour cherry trees treated once with 6 g/L TBS + 12 g/L hydrated lime and from trees treated four times with 2.4 g/L TBS with and without 4.8 g/L hydrated lime. Treatments were applied to 30-yr-old trees and each treatment was replicated four times in a randomized complete block design. Treatments were applied as described earlier. Each replicate contained two or three trees. The high rate of TBS was applied on 18 September 1981 and the lower rate was applied on 18, 28 September; 8 and 19 October 1981.

Monitoring bacterial populations. Recovery of bacteria from plant material sprayed with copper compounds can be difficult when

using standard isolation procedures. In 1978, Young (20) reported that the bactericidal activity of copper was inactivated with phosphate buffer. To verify this report, we determined the survival of PsmR in distilled water and phosphate buffer with and without TBS + hydrated lime. Treatment solutions of phosphate buffer (13.6 mg/ml KH_2PO_4 + 14.2 mg/ml Na_2HPO_4) and distilled water were amended with 18.7 mg/ml TBS + 39.6 mg/ml hydrated lime and then diluted 10- and 100-fold with phosphate buffer and distilled water, respectively. A stock solution containing approximately 10^4 cfu PsmR/ml distilled water was divided into 100-ml aliquots. Aliquots of stock solution were mixed with 10-ml aliquots of treatment solutions to give the treatments listed in Table 3.3. After 10 min, duplicate 0.1-ml subsamples from each replication were plated onto King's medium B. Colonies were counted after 5-days incubation at 22 C. Each treatment was replicated five times.

Populations of PsmR were monitored on each inoculated single-tree replicate. Each tree was divided into quadrants and 10 fully expanded leaves were randomly chosen from each quadrant. The leaves were measured with an area meter before each 40-leaf sample was homogenized for 2 min with 300-ml phosphate buffer in a blender (Waring Products Inc., New Hartford, CT 06057). The homogenate was serially diluted in phosphate buffer and duplicate 0.1-ml subsamples were pipetted onto a modified King's medium B amended with 50 µg/ml rifampicin (Calbiochem-Behring Corp., La Jolla, CA 92037) and 25 µg/ml cycloheximide (Sigma Chemical Co., St. Louis, MO 63178). Colonies were counted after 5-days incubation at 22 C and the cfu of PsmR per cm^2 of leaf were determined.

Monitoring copper deposits on leaves. To determine the rate of copper loss from Montmorency leaves, leaf samples were collected after treatments were applied. Samples were taken on 2, 10 June, 1, 7, 14, 29 July, 6, 12 August 1980; 2 June, 8 July, and 10 September 1981. In autumn of 1981, samples were collected on 18, 23, 28 September, 3, 8, 13, 19, 24, and 29 October.

To determine concentration of copper on leaves, each replicate was divided into quadrants and 10 leaves were randomly collected per quadrant. The quantity of copper per cm^2 of leaf area was determined for each replication by cutting 1.5-cm^2 leaf discs from the center of all 40 leaves with a cork borer. The discs were dried at 72 C for at least 18 hr, weighed, and ground to a fine powder with a mortar and pestle. For each replication, 100 mg of leaf powder was digested for 18 hr at 72 C with 2 ml of "Baker Instra-Analyzed" nitric acid (J. T. Baker Chemical Co., Philipsburg, NJ 08865) in a 10-ml screw-cap Teflon vial. Using a volumetric flask, each digested replication was increased to 10 ml with double-glass-distilled water filtered through a Milli-Q water purification system (Millipore Corp., Bedford, MA 01730). Each diluted sample was poured into a 16x125-mm culture tube with a screw cap and held at room temperature for 24 hr until precipitates formed. Each sample was centrifuged at 150 g for 30 min and the supernatant was decanted into a clean culture tube.

Digested samples were analyzed for copper using a plasma emission spectrometer (Jarrel-Ash Model 955 Plasma Atom Comp.; Fisher Scientific, Waltham, MA 02254) with the inductively coupled plasma operated at 1.1-kw forward power, 1.6-mm flame height, 0.84 to 0.98 kg/cm^2 nebulizer pressure, and a sample flow rate of 1.4 ml/min. The

spectrometer was standardized with a 10 µg/ml stock solution of copper in 20% "Baker Instra-Analyzed" nitric acid prepared by serially diluting a 1,000 µg/ml copper atomic spectral standard solution (J. T. Baker Chemical Co., Philipsburg, NJ 08865). The results from the spectrometer were used to determine the micrograms of copper per cm^2 of leaf. All vials, glassware, and utensils used in the copper analyses were washed in 24% nitric acid (commercial grade) for 30 min before rinsing with distilled and double-glass-distilled Milli-Q filtered water. Blank samples were processed repeatedly to monitor for copper in reagents and on glassware.

The relationship between copper loss from the leaves and rainfall was established using stepwise regression (9). The amount of rainfall and the difference in the level of copper on leaves between two sampling periods were computed. Rainfall was measured with a tipping bucket rain gauge (WEATHERtronics Inc., West Sacramento, CA 95691) and recorded with an event recorder or a microcomputer-based instrument (11). Citcop 4E and TBS treatments were analyzed separately using a second degree polynomial of the general form:

$$CL = b_0 + b_1C + b_2R + b_3RC + b_4C^2 + b_5R^2 + b_6(RC)^2 + e$$

where CL = the difference in the level of copper (µg Cu^{++} per cm^2 leaf) on leaves between two sampling periods, R = rainfall in milliliter between sampling periods, C = the level of copper (µg per cm^2 leaf) on leaves for the first of the two sampling periods, b's = partial regression coefficients, and e is a normally distributed

random variable with mean zero and variance σ^2. Factors included in the final equation were significant at $P = 0.01$. Only the regression models having the best combination of high coefficients of determination and residuals supporting the assumptions that errors were independent and normally distributed (see Appendix 2) were retained (9). The data were plotted using a computer program designed to plot randomly selected three-dimensional data points (19).

General data analyses. Data collected sequentially during the growing season were analyzed as a split-plot with time (18). Treatment differences in most experiments were analyzed using planned paired comparisons. All data were analyzed with the Stat 4 statistical program (3) and Cyber 750 computer (Control Data Corp., Minneapolis, MN 55440). Unless stated otherwise, differences were significant at the $P = 0.01$ level.

RESULTS

Copper phytotoxicity. In 1980, leaf chlorosis and defoliation were not observed in any copper treatment. In 1981, copper-treated trees had significantly greater defoliation than nonsprayed, streptomycin-, and captafol-sprayed trees (Table 3.4). Trees sprayed with Citcop 4E had significantly more defoliation than trees sprayed with TBS. Hydrated lime and captafol did not reduce the level of defoliation in the TBS and Citcop 4E treatments.

Leaves at the base of terminal and lateral shoots were the first to show injury from copper sprays. Trees sprayed with Citcop 4E had more leaves showing injury than trees sprayed with TBS (Table 3.5).

On 8 July, the amount of copper on leaves sprayed with TBS or Citcop 4E was significantly ($P = 0.05$) higher on leaves that emerged on 11 May than emerged on 26 June (Table 3.5). The approximate threshold concentration of copper that resulted in phytotoxicity was 8.3 and 1.4 $\mu g\ Cu^{++}/cm^2$ of leaf for TBS and Citcop 4E, respectively. These leaves received seven and four applications of TBS and Citcop 4E, respectively.

Recovery of bacteria from buffered copper solutions. High counts of PsmR (278 to 372 cfu/ml) were recovered from all phosphate buffer solutions containing TBS (Table 3.3). Only a few PsmR (0 to 3 cfu/ml) were recovered from distilled water amended with 0.017 mg/ml TBS + 0.036 mg/ml hydrated lime. Recovery of PsmR from the phosphate buffer + TBS treatments and from phosphate buffer alone were similar and significantly greater than recovery of PsmR from distilled water alone.

Effect of copper on bacterial populations. In 1980, trees treated with copper had significantly lower populations of PsmR than trees treated with captafol (Table 3.1). Among the copper treatments, trees treated with TBS had significantly fewer PsmR than trees treated with Citcop 4E, and trees treated with 200 mg/L Citcop 4E had significantly fewer PsmR than trees treated with 100 mg/L. Following the last inoculation on 29 May, populations of PsmR declined steadily as four applications of copper were applied (Figure 3.1A). By 31 July, populations of PsmR on trees treated with TBS and Citcop 4E (200 mg/ml) were 98.9 and 95.0% less, respectively, than those on captafol-treated trees. The decline in populations of PsmR from 4 June to 10 June in the TBS treatment occurred despite the

development of leaf symptoms of bacterial canker starting 10 June. However, leaf symptoms were not severe enough to detect differences between treatments.

In 1981, trees treated with copper and streptomycin had significantly fewer PsmR than nontreated and captafol-treated trees (Table 3.2). Streptomycin-treated trees had populations of PsmR similar to trees treated with TBS or Citcop 4E. Trees treated with TBS had significantly fewer PsmR than trees treated with Citcop 4E. Except on 20 June, populations of PsmR were lower on TBS-treated trees than on nontreated or captafol-treated trees (Figure 3.1B). On 29 June and 10 September, populations of PsmR on the TBS-treated trees were 94 and >99% less than their respective check treatments. Populations of PsmR on trees treated with 200 mg/ml Citcop 4E were below populations on nonsprayed trees except on 14 July (data not shown). Populations of PsmR were not affected by adding hydrated lime or captafol to the copper treatments in 1980 and 1981. No symptoms of bacterial canker developed in 1981.

Dynamics of copper deposition. In autumn of 1981, the amount of copper loss for trees treated with 2.4 g/L TBS with and without hydrated lime were 28 and 27%, respectively; therefore, data from these two treatments were combined. Leaves from trees sprayed with 6 g/L TBS had 9.5 and 6.0 µg more copper per cm^2 leaf on 18 and 23 September, respectively, than trees sprayed with 2.4 g/L TBS (Figure 3.2). On 28 September, the concentrations of copper on leaves retreated with 2.4 g/L TBS increased from 3 to 10 $\mu g/cm^2$ leaf, and was 3 µg higher than the concentration remaining in the 6 g/L TBS treatment. Copper concentrations dropped dramatically between 18 and

23 September and between 28 September and 3 October because of 40- and 89-mm rain on 21 and 30 September, respectively. On 3 October, copper concentrations for the 2.4 and 6.0 g/L TBS treatments were 3 $\mu g/cm^2$ leaf and concentrations in the latter treatment remained relatively constant. On 8 October, the 2.4 g/L TBS treatment was sprayed a third time and the concentration of copper increased to 10.2 $\mu g/cm^2$ leaf, remaining at this concentration through 13 October due to lack of rain. But after 15 mm of rain, the concentration of copper dropped to 4.8 $\mu g/cm^2$ leaf by 19 October. On this date, the 2.4 g/L TBS treatments were sprayed a fourth time, and concentrations of copper increased to 9.5 $\mu g/cm^2$ leaf but dropped to 6.1 $\mu g/cm^2$ leaf on 24 October, following a 8-mm rain. On 27 October, 5-mm rain had no effect on the concentration of copper.

Copper residue data for Citcop 4E in 1980 and 1981 were analyzed using stepwise regression to determine if the loss of copper (CL) from leaves was a function of rainfall (R) and initial concentration of copper (C) on leaves. The data included 225 points and the resulting model was:

$$CL = -7.6827 \times 10^{-3} + 1.6186 \times 10^{-1}C + 1.9529 \times 10^{-2}RC - 3.080 \times 10^{-4}R^2$$

The R^2 value was 0.823. A nomogram of the original data shows how copper loss ($\mu g\ Cu^{++}$ per cm^2 leaf) increased as rainfall and concentration of the initial deposits of copper on leaves increased (Figure 3.3A).

A similar analysis of residue data for TCS in 1980 and 1981 included 194 points and the resulting model was:

$$CL = -1.6584 + 2.8058 \times 10^{-1}C + 1.2271 \times 10^{-1}R + 5.5985 \times 10^{-3}RC - 1.167 \times 10^{-3}R^2$$

The R^2 value was 0.9073. As rainfall amounts and the initial concentrations of copper on leaves increased, greater quantities of copper were lost from leaves (Figure 3.3B).

There were no differences in copper loss between copper treatments with and without captafol or hydrated lime.

DISCUSSION

In the early 1950's, organic fungicides began to replace copper compounds for the control of cherry leaf spot and brown rot on Montmorency sour cherry in Michigan. By 1970, copper compounds were eliminated from most cherry disease control programs. Bacterial canker developed for the first time on Montmorency sour cherry in Michigan in 1976 (13) and has continued to be a significant but sporatic problem. In this study, repeated application of copper compounds reduced populations of *P. syringae* pv. *morsprunorum*, the cause of bacterial canker in Michigan (13). This suggests that, prior to 1970, the frequent use of copper to control other diseases also controlled bacterial canker. Now that copper compounds are not used on cherry, the pathogen is no longer being suppressed.

Tribasic copper sulfate has several advantages over Citcop 4E for use on Montmorency sour cherry. Tribasic copper sulfate was more effective in reducing populations of PsmR than Citcop 4E, was less phytotoxic than Citcop 4E, and resulted in higher concentrations of copper on leaves than Citcop 4E. Unlike Bordeaux mixture, the addition of hydrated lime to TBS and Citcop 4E treatments did not reduce the phytotoxic effects of copper.

This results indicate the following strategy should be used for reducing populations of P. syringae pv. morsprunorum on Montmorency sour cherry. Tribasic copper sulfate should be applied every 7 to 10 days starting at green tip bud stage to prevent buildup of populations of P. syringae pv. morsprunorum on emerging leaves and flower parts. Populations of PsmR were probably higher in the experimental orchard than populations of P. syringae pv. morsprunorum in commercial orchards because we made several artifical inoculations. Therefore, populations of P. syringae pv. morsprunorum in commercial orchards should be reduced to levels below those recorded in this study. As reported by other workers (6, 15), repeated applications were more effective than single applications for bacterial canker disease control. However, applications should be discontinued after shuck-fall bud stage to avoid phytotoxicity.

In England (5), leaf scars of sweet cherry infected with P. syringae pv. morsprunorum in autumn developed cankers the following spring, and application of Bordeaux mixtures to sweet cherry trees in autumn decreased the development of cankers by 75 to 85% (6). In Michigan, Montmorency sour cherry was susceptible to leaf scar infection by P. syringae pv. morsprunorum and P. syringae pv. syringae

in September and October (see Chapter 2). Therefore, repeated application of coppers in autumn should help control leaf scar infections, particularly during periods of frequent rainfall. Early defoliation from autumn treatments of Bordeaux mixture was a problem on sweet cherry (1); but in this study, defoliation by TBS applied in autumn was not observed on Montmorency sour cherry. This suggests autumn treatments with TBS may be safer than with Bordeaux mixture.

In 1980, PsmR was recovered from Montmorency leaves even after repeated application of coppers in spring and summer. In laboratory studies, survival of PsmR in distilled water was dramatically reduced when amended with TBS + hydrated lime at concentrations similar to those applied to Montmorency sour cherry trees in 1980. This indicates PsmR may survive in or on leaf tissues in areas protected from copper being deposited on the leaf surface. It may also be possible that the mucoid substances referred to by Crosse (7) protect the bacteria from copper residues.

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Table 3.1. Effect of copper treatments on populations of rifampicin-resistant Pseudomonas syringae pv. morsprunorum (PsmR) on Montmorency sour cherry leaves and the analysis of variance for a set of planned paired comparisons in 1980.

Treatments and rates per liter	PsmR recovered on KBrc[v] ($\log_{10}$[cfu+1/cm^2 leaf])	Reduction in bacteria (%)[w]
Captafol, 986 mg	3.05 ± 1.26	0
Tribasic copper sulfate, 949 mg + lime[x], 3.59 g	1.84 ± 1.54	94
Citcop 4E, 100 mg	2.69 ± 1.23	56
Citcop 4E, 100 mg + lime, 1.19 g	2.83 ± 1.05	40
Citcop 4E, 100 mg + captafol, 986 mg	2.77 ± 0.98	48
Citcop 4E, 200 mg	2.06 ± 1.39	90
Citcop 4E, 200 mg + lime, 1.19 g	2.02 ± 1.22	91
Citcop 4E, 200 mg + captafol, 986 mg	2.46 ± 1.29	74

Planned paired comparisons between treatments[y]	df	ms	F[z]
Captafol vs copper	1	15.6	10.6**
Tribasic copper sulfate vs Citcop 4E	1	13.6	9.4**
Citcop 4E, 100 mg vs Citcop 4E, 200 mg (I)	1	20.0	13.8**
Citcop 4E alone vs Citcop 4E + lime and Citcop 4E + captafol (II)	1	1.1	0.8
Citcop 4E + lime vs Citcop 4E + captafol (III)	1	1.4	1.0
Interaction between comparisons I and II	1	0.1	0.0
Interaction between comparisons I and III	1	2.5	1.7
Blocks	4	7.2	5.2*
Error	28	1.5	

[v]KBrc = A modified King's medium B amended with 50 µg/ml rifampicin and 25 µg/ml cycloheximide. Each value is the mean of five replications measured on eight sampling dates followed by the standard error of the mean.

[w]Percent reduction in bacteria is the difference in recovery of PsmR between the captafol (alone) treatment and each copper treatment divided by the captafol treatment and multiplied by 100.

[x]Lime = hydrated lime, $Ca(OH)_2$.

[y]Planned paired comparisons between treatments were determined for PsmR recovered on KBrc.

[z]* = significant ($P = 0.05$), ** = significant ($P = 0.01$).

Table 3.2. Effect of copper treatments on populations of rifampicin-resistant Pseudomonas syringae pv. morsprunorum (PsmR) on Montmorency sour cherry leaves and the analysis of variance for a set of planned paired comparisons in 1981.

Treatments and rates per liter	PsmR recovered on KBrc[v] (log_{10}[cfu+1/cm^2 leaf])	Reduction in bacteria (%)[w]
Check (no spray)	2.76 ± 0.72	0
Captafol, 491 mg	2.79 ± 0.93	0
Tribasic copper sulfate, 636 mg	2.16 ± 1.04	76
Tribasic copper sulfate, 636 mg + lime[x], 3.59 g	1.91 ± 1.20	86
Tribasic copper sulfate, 636 mg + captafol, 491 mg	2.05 ± 1.02	81
Citcop 4E, 200 mg	2.40 ± 0.74	58
Citcop 4E, 200 mg + lime, 3.59 g	2.52 ± 0.91	44
Citcop 4E, 200 mg + captafol, 491 mg	2.27 ± 1.12	69
Streptomycin sulfate, 121 mg	2.03 ± 1.08	82

Planned paired comparisons between treatments[y]	df	ms	F[z]
Check vs captafol alone	1	0.0	0.0
Check and captafol vs copper and streptomycin	1	37.2	28.3**
Copper vs streptomycin	1	2.1	1.6
Tribasic copper sulfate vs Citcop 4E (I)	1	13.1	10.0**
Copper alone vs copper + lime and copper + captafol (II)	1	0.8	0.2
Copper + lime vs copper + captafol (III)	1	0.2	0.2
Interaction between comparisons I and II	1	0.8	0.6
Interaction between comparisons I and III	1	2.5	1.9
Blocks	4	12.5	9.5**
Error	32	1.3	

[v]KBrc = A modified King's medium B amended with 50 µg/ml rifampicin and 25 µg/ml cycloheximide. Each value is the mean of five replications measured on 15 sampling dates followed by the standard error of the mean.

[w]Percent reduction in bacteria is the difference in recovery of PsmR between check treatments (mean of check and captafol treatments) and each copper treatment divided by check treatments and multiplied by 100.

[x]Lime = hydrated lime, $Ca(OH)_2$.

[y]Planned paired comparisons between treatments were determined for PsmR recovered on KBrc.

[z]** = significant ($P = 0.01$).

Table 3.3. Recovery of rifampicin-resistant Pseudomonas syringae pv. morsprunorum (PsmR) from distilled water and phosphate buffer each containing tribasic copper sulfate (TBS) + hydrated lime (lime).

Suspending solution	Concentration of amendments: TBS (mg/ml)	Concentration of amendments: Lime (mg/ml)	PsmR recovered on KBrc[x] (colony-forming units/ml)
Distilled water	0.000	0.000	187 ± 83[z]
Distilled water	0.017	0.036	3 ± 1
Distilled water	0.170	0.360	0 ± 0
Distilled water	1.700	3.600	0 ± 0
Phosphate buffer[y]	0.000	0.000	294 ± 80
Phosphate buffer	0.017	0.036	372 ± 72
Phosphate buffer	0.170	0.360	278 ± 56
Phosphate buffer	1.700	3.600	371 ± 71

[x]KBrc = A modified King's medium B amended with 50 µg/ml rifampicin and 25 µg/ml cycloheximide.

[y]Phosphate buffer at 0.01 M (pH = 7.2).

[z]Mean of five replications followed by the standard error of the mean. Using a set of planned paired comparisons between treatments, distilled water without TBS + hydrated lime vs all phosphate buffer treatments were significantly ($P = 0.01$) different (ms = 80712, F = 13.5, error df = 16, and error ms = 5968).

Table 3.4. Defoliation of Montmorency sour cherry trees from various copper treatments and the analysis of variance for a set of planned paired comparisons in 1981.

Treatments and rates per liter	Defoliation (%)[x]
Check (no spray)	0.1 ± 0.3
Captafol, 491 mg	0.3 ± 0.5
Tribasic copper sulfate, 636 mg	6.5 ± 6.5
Tribasic copper sulfate, 636 mg + lime[y], 3.59 g	7.1 ± 8.7
Tribasic copper sulfate, 636 mg + captafol, 491 mg	11.7 ± 9.2
Citcop 4E, 200 mg	43.7 ± 30.8
Citcop 4E, 200 mg + lime, 3.59 g	45.4 ± 32.6
Citcop 4E, 200 mg + captafol, 491 mg	37.1 ± 26.6
Streptomycin sulfate, 121 mg	0.1 ± 0.3

Planned paired comparisons between treatments	df	ms	F[z]
Copper vs no copper	1	12575	38**
Tribasic copper sulfate vs Citcop 4E (I)	1	16951	51**
Copper alone vs copper + lime and copper + captafol (II)	1	0	0
Copper + lime vs copper + captafol (III)	1	35	0
Interaction between comparisons I and II	1	96	0
Interaction between comparisons I and III	1	419	1
Check vs captafol alone	1	0	0
Captafol vs streptomycin sulfate	1	0	0
Block	4	243	1
Error	32	331	

[x]Each value is the mean of five replications followed by the standard error of the mean. Data were recorded on 6 and 8 July.

[y]Lime = hydrated lime, $Ca(OH)_2$.

[z]** = significant ($P = 0.01$).

Table 3.5. The relationship of the number of copper sprays applied to Montmorency sour cherry leaves and residues of copper on the leaves to phytotoxicity.

Leaf emergence (date)[s]	Sprays (no.)[t]	µg Cu^{++}/cm^2 leaf[u]			Phytotoxicity[y]		
		Check[v]	TBS[w]	Citcop 4E[x]	Check	TBS	Citcop 4E
11 May	7	0.4 a[z]	8.3 a	2.3 a	-	+	+
22 May	5	0.5 a	7.2 b	1.5 b	-	-	+
2 June	4	0.5 a	5.5 c	1.4 b	-	-	+
11 June	3	0.4 a	4.6 d	0.9 bc	-	-	-
26 June	1	0.3 a	2.6 e	0.6 c	-	-	-

[s]Knowing the position of the leaf on the terminal, the date of leaf emergence was calculated with a Montmorency sour cherry leaf emergence degree-day model.

[t]Estimated number of sprays applied to each leaf.

[u]Each value is the mean of five replications sampled on 8 July 1981.

[v]Nonsprayed.

[w]TBS = Tribasic copper sulfate at 636 mg/L.

[x]Citcop 4E at 200 mg/L.

[y]+ = chlorotic leaves observed, - = no chlorotic leaves observed.

[z]Values within a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

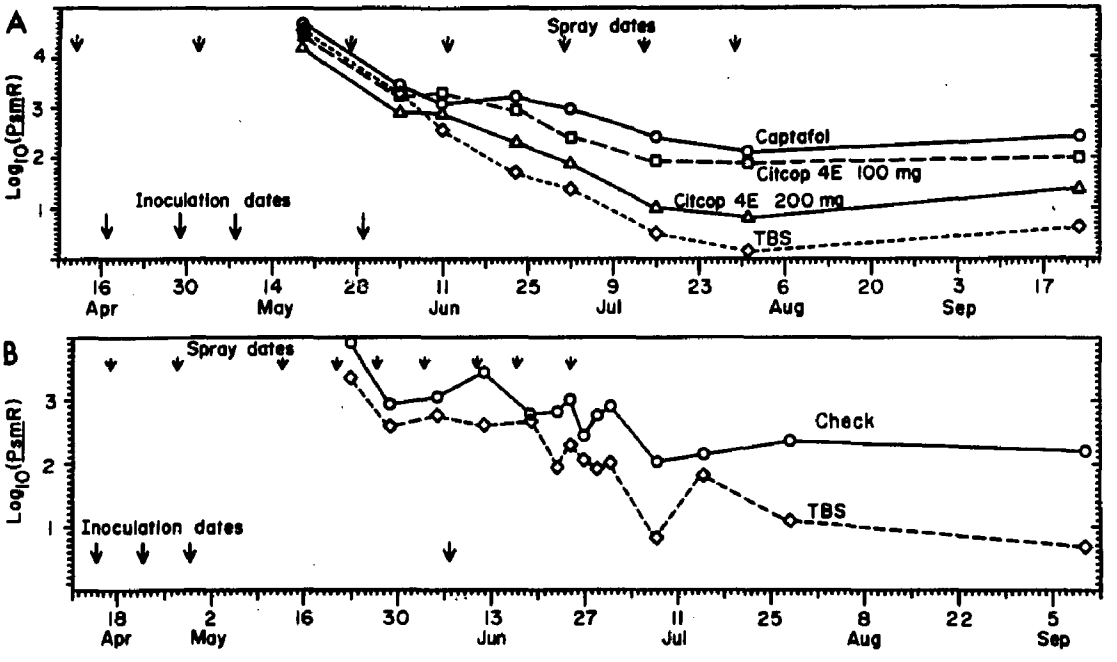

Figure 3.1. Populations of rifampicin-resistant Pseudomonas syringae pv. morsprunorum (PsmR) on Montmorency sour cherry leaves sprayed with copper treatments. A, In 1980, treatments were 986 mg/L captafol, 949 mg/L tribasic copper sulfate (TBS), and 100 and 200 mg/L Citcop 4E. B, In 1981, treatments were 636 mg/L TBS and check treatments (nonsprayed and 491 mg/L captafol combined).

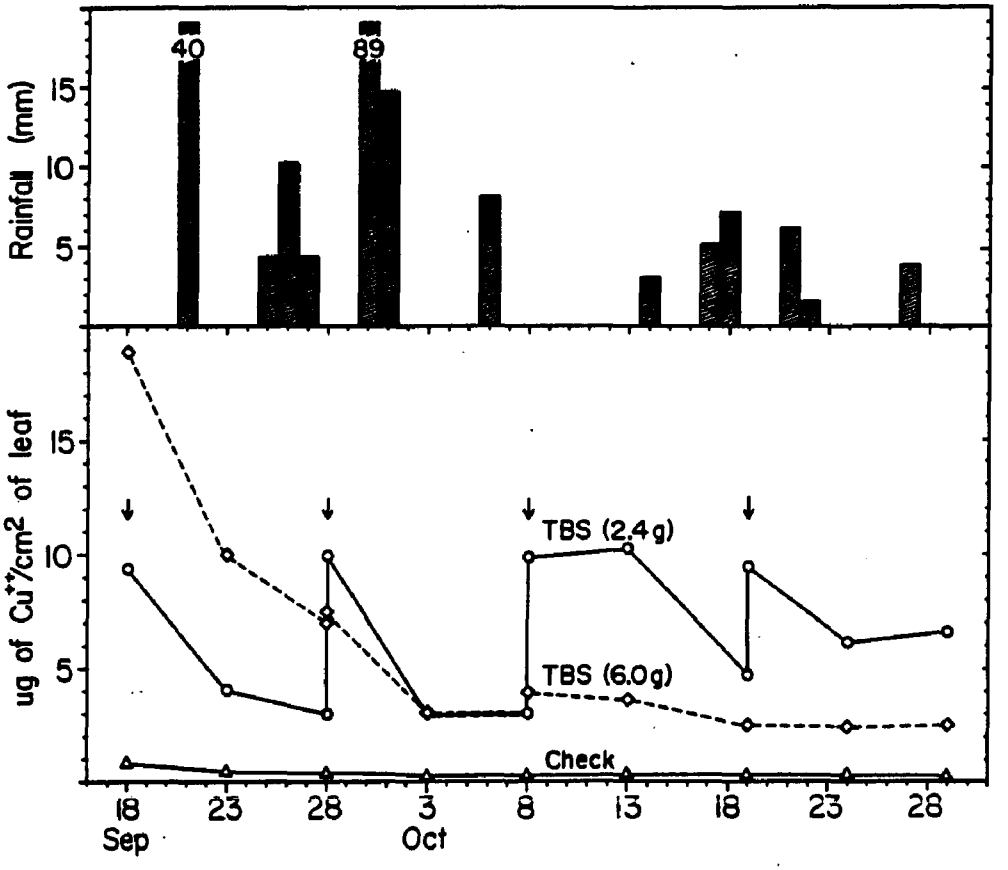

Figure 3.2. The relationship between rainfall and the loss of copper from Montmorency sour cherry leaves. Trees were sprayed on 18 September 1981 with 6 g/L tribasic copper sulfate (TBS) + 12 g/L hydrated lime and on 18, 28 September, 8, and 19 October with 2.4 g/L TBS with or without 4.8 g/L hydrated lime. Arrows indicate the dates sprays were applied. Check trees were not sprayed.

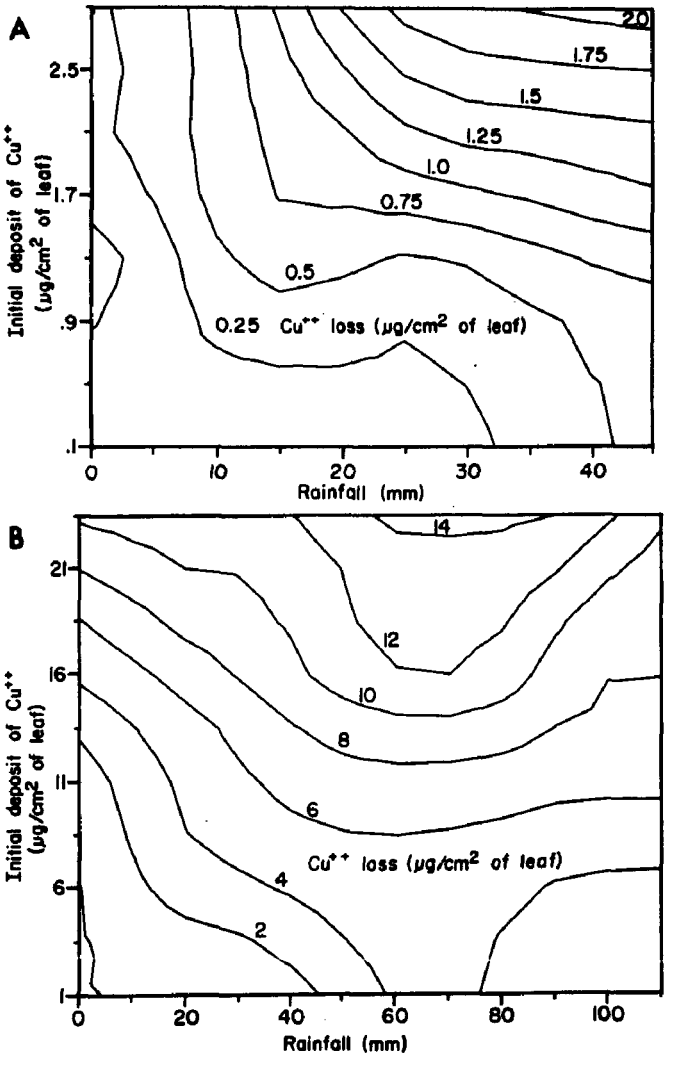

Figure 3.3. Nomogram relating the amount of copper lost from Montmorency sour cherry leaves to initial deposits of copper on the leaves and to the amount of rainfall. A, Citcop 4E; B, Tribasic copper sulfate.

APPENDICES

APPENDIX 1

Susceptibility of *Prunus* Rootstock Cultivars to *Pseudomonas syringae* pv. *morsprunorum* and *P. syrinage* pv. *syringae*

ABSTRACT

Terminal shoots of eight cherry rootstock cultivars were wound-inoculated with *Pseudomonas syringae* pv. *morsprunorum* and *P. syringae* pv. *syringae*. The shortest cankers developed on Colt and Mahaleb rootstocks and the longest cankers developed on MxM 2. Cankers on F12/1, MxM 14, MxM 39, MxM 97 rootstocks were intermediate in length.

INTRODUCTION

Bacterial canker on *Prunus* sp. caused by *Pseudomonas syringae* pv. *morsprunorum* (5, 11) and *P. syringae* pv. *syringae* (3, 7) can cause severe injury to susceptible cultivars. Resistance to bacterial canker has been measured by length of cankers after inoculation of leaf scars (6) and wound-inoculation of stems (1, 6, 10), and by severity of leaf spotting after natural infection of leaves (13). In Canada, two distinct patterns of host susceptibility were recorded with *P. syringae* pv. *morsprunorum* and *P. syringae* pv. *syringae* on wound-inoculated sweet cherry cultivars (1). On sweet cherry in England, cankers were 50% longer after wound inoculation with *P. syrinage* pv. *syringae* from Oregon, USA than with *P. syringae* pv.

morsprunorum from England (6). Using the same isolates, cankers were 30% longer after inoculation of leaf scars with P. syringae pv. morsprunorum than with P. syringae pv. syringae (6).

The purpose of this research was to test the susceptibility of eight cherry rootstock cultivars to P. syringae pv. morsprunorum and P. syringae pv. syringae by wound inoculations.

MATERIALS AND METHODS

One of strain P. syringae pv. morsprunorum and two strains of P. syringae pv. syringae were used to wound-inoculate the following rootstocks: Colt (P. avium x P. pseudocerasus), F12/1, Mahaleb (P. mahaleb L.), MxM 2 (F12/1 x Mahaleb), MxM 14, MxM 39, MxM 60, and MxM 97. The P. syringae pv. morsprunorum isolate was a rifampicin-resistant strain (PsmR) initially isolated from Montmorency sour cherry (11). One P. syringae pv. syringae strain was isolated from chokecherry (P. virginiana L.) leaves (PssC) and the other was a rifampicin-resistant strain (PssR) initially isolated from sweet cherry leaves (see Chapter 2). Inoculum was grown on King's medium B (8) for 2-days incubation at 22 C and suspended in 0.01-M phosphate buffer (pH 7.2) by stirring with a sterile glass rod. Inoculum concentrations of 10^8 colony-forming units/ml phosphate buffer were obtained by adjusting the turbidity of the suspension to 0.04 absorbance with a spectrophotometer (Spectronic 20; Bausch & Lomb, Rochester, NY 14625) set at 625nm. Inoculum was kept in an ice bath during the inoculation period.

Twenty to 30 1-yr-old trees of each rootstock cultivar were planted in East Lansing, Michigan in June 1980, and were inoculated in

the summer of 1981. The rootstocks were pruned twice in 1981 to maintain four actively growing terminal shoots per tree. Each terminal was inoculated three leaf nodes below the meristem by inserting a 25-gauge needle longitudinally a few millimeters into the terminal and placing a 0.05 ml of inoculum at the point of insertion. Rootstocks were inoculated on 16 June with a mixture of PsmR and PssR, 13 July with PssC, and 31 August with PssC, and the cankers were measured on 11 July, 20 August, and 15 November, respectively. Three terminals per tree were inoculated with bacteria. A fourth terminal on each tree was inoculated with phosphate buffer without bacteria to serve as a control.

Differences in length of cankers between rootstock cultivars for each inoculation date were analyzed using Duncan's multiple range test. The error mean square was pooled from all three inoculation dates. The error mean square for each comparison was recalculated according to the number of replications in each treatment (14).

RESULTS AND DISCUSSION

Terminals inoculated only with phosphate buffer formed callus tissue around the point of inoculation. The area of callusing was raised and was 0- to 1-mm long. Mean length of cankers varied between inoculation dates; therefore, length of cankers were included for each inoculation date.

The greater the length of time cankers were allowed to develop, the larger the cankers were that developed (Figure 4.1). The longest cankers developed after the inoculation on 31 August with PssC and data were taken 76 days later, while the smallest cankers developed

after the inoculation on 13 July with PssC and data were taken 38 days later. Cankers from the latter were smaller than cankers that developed after the inoculation on 16 June with PssR + PsmR and data were taken 25 days later. The small cankers, developed after the 13 July inoculation, may be caused by canker inactivity in summer or differences in virulence between PssC and PsmR + PssR. In England (5) and Oregon (3), cankers on sweet cherry were inactive in summer and the bacteria in the cankers frequently died. In this study, the activity of cankers, or rate of canker growth, from inoculations on 13 July (summer) and 31 August (autumn) were similar. Differences in length of cankers formed from inoculations on 16 June and 13 July were probably caused by PssC being less virulent than PsmR + PssR.

Although the time of year inoculations were performed may have affected the rate of canker growth, growth rate of cankers was greatest for inoculations made on 16 June (0.41 mm/day) compared to 0.20 and 0.24 mm/day for inoculations on 13 July and 31 August, respectively. Crosse (5) reported canker growth was most active in spring and was associated with actively growing plant material. In this study, rootstocks were deliberately pruned twice in one growing season to maintain actively growing shoots. Therefore, differences in growth rate of cankers may be associated again with differences in virulence between PssC and PsmR + PssR.

Aside from the differences in virulence and length of time for canker development, the rootstocks can be divided into three groups based on differences in length of cankers (Table 4.1). Generally, cankers on MxM 2 were longest; on F12/1, Mahaleb, MxM 14, MxM 39, MxM 60, and MxM 97 were intermediate; on and Colt were shortest. MxM 2

had the longest cankers on 16 June with PsmR + PssR and on 13 July with PssC. On 31 August, cankers on MxM 2 were 20-cm long, second to the 26-mm cankers on MxM 97. On all three inoculation dates, Colt had the shortest cankers. Cankers on MxM 39 inoculated on 16 June were in the shortest size group, while on 13 July and 31 August the cankers were intermediate in length. Opposite to this were lengths of cankers on MxM 14. These differences coincide with the use of different pathogen strains. Similar differences between strains of P. syringae pv. morsprunorum and P. syringae pv. syringae and cultivars of sweet cherry were reported by Allen and Dirks (1). Similarly, Garrett (9) reported that F12/1 was moderately susceptible to race 1 of P. syringae pv. morsprunorum, but was highly susceptible to the newly described race 2 of P. syringae pv. morsprunorum.

Rootstocks resistant to bacterial canker, such as Colt, may be useful to growers if they are budded high as reported and recommended in Oregon (2, 4). If rootstock cultivars resistant to bacterial canker are widely used, new virulent strains of P. syringae pv. morsprunorum and P. syringae pv. syringae may appear or develop because of host selection pressure. Therefore, all indigenous strains of the pathogen should be used for the development of bacterial canker resistance in an ongoing Prunus species breeding program.

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Table 4.1. Canker lengths on Prunus rootstock cultivars following wound inoculations with Pseudomonas syringae pv. morsprunorum P. syringae pv. syringae.

	Date of inoculation[w]		
Rootstock	16 June	13 July	31 August
Colt	4.6[x] a	3.7 a	13.1 a
Mahaleb	6.0 a	11.4 cd	13.6 a
F12/1	10.8 b	8.1 b	13.4 a
MxM 14	12.9 bc	4.9 a	14.8 a
MxM 39	5.4 a	8.5 bc	18.7 b
MxM 60	13.6 bc	6.2 ab	19.4 b
MxM 97	10.9 b	5.1 a	26.2 c
MxM 2	14.9 c	12.2 d	20.0 b
Check[y]	0.5	0.4	0.8
Mean	10.3	7.5	18.1
Rate of growth[z]	0.41	0.20	0.24

[w]Actively growing terminal shoots were inoculated with 0.05 ml of 10^8 colony-forming units/ml phosphate buffer in 1981. On 16 June, terminals were inoculated with a 1:1 mixture of rifampicin-resistant strains of P. syringae pv. syringae recovered from sweet cherry and P. syringae pv. morsprunorum recovered from sour cherry, and data were taken on 11 July. On 13 July and 31 August, a strain of P. syringae pv. syringae from chokecherry was used to inoculate terminal shoots, and data were taken on 20 August and 15 November, respectively.

[x]Each value is a mean of 19 to 54 cankers and values in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

[y]Mean length of cankers for all rootstocks inoculated with phosphate buffer alone.

[z]The mean length of all cankers on a given inoculation date was divided by the number of days between the inoculation date and date data were taken to give the mm of canker growth per day.

APPENDIX 2

RESIDUAL PLOTS FOR REGRESSION EQUATIONS OF CITCOP 4E AND TRIBASIC COPPER SULFATE

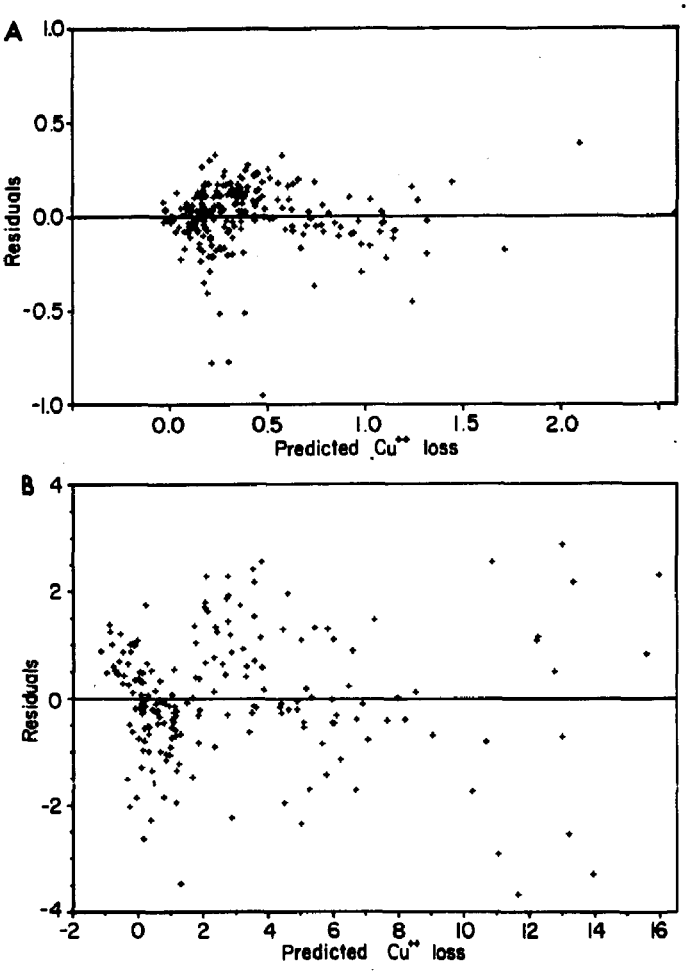

Figure 5.1. Plot of residuals (observed minus predicted values) against predicted amounts of copper loss indicating that the errors are independent, have zero mean with a constant variance and follow a normal distribution. A, Citcop 4E; B, Tribasic copper sulfate.