**FINAL REPORT**

**WTFRC Project #** **WSU Project # 13J-3661-5367**

**Project Title:** Epidemiology and Control of Bacterial Canker in Sweet Cherry

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**Objectives:**

1. Conduct field surveys to determine the occurrence of bacterial canker in eastern Washington State.

2. Collect bacterial strains from various locations and determine the copper sensitivity (or resistance) of *P. syringae* populations in sweet cherry orchards.

3. Evaluate chemical products for control of the disease.

The original proposal was not fully funded. The objectives listed above were in the modified proposal.

**Significant Findings:**

1. Bacterial canker occurs at low incidence among cherry blocks surveyed in the Yakima and Wenatchee areas, but severe epidemics have been observed at some specific sites. Bacterial canker is more noticeable in the northern fruit production areas from Pateros to Oroville.

2. Young trees (1 to 5 years old) are more prone to infection by *Pseudomonas syringae*. The disease was found in 2 of 24 young blocks in the Yakima area, 4 of 19 in the Wenatchee area, and 12 of 28 in the northern area.

3. The strains of *Pseudomonas syringae* isolated from cherry orchards exhibited varying levels of tolerance to copper, with 82% of strains having tolerance to copper at 0.25 mM $CuSO_4$ in vitro tests.

**Funding Request:**

No funding is requested. We are not continuing this project because of the current low level of the disease in most commercial orchards and lack of interest in the industry in funding a full program on this disease. However, as the acreage of new plantings increases, particularly those susceptible varieties, bacterial canker can be an important factor in the process of establishing profitable young orchards. We will key an eye on this disease.

**Methods:**

1. **Orchard survey.** The survey for bacterial canker was conducted in the Yakima Valley, Wenatchee area, and the northern fruit production region from Pateros to Oroville. Forty-two cherry orchards or blocks in the Yakima area, 20 in the Wenatchee area, and 40 in the northern

production region were randomly selected for this survey. The survey mainly focused on the young orchards. In each block, 60 trees were selected for examining bacterial canker symptoms.

2. **Isolation and copper sensitivity test** of ***P. syringae*.** In the spring, branches exhibiting canker symptoms and diseased buds were collected from nine orchards listed in Table 1.

Table 1. Sweet cherry orchards sampled for testing copper resistance of *Pseudomonas syringae.*

| Site | Variety | Age | History of copper sprays | Type of samples |
|---|---|---|---|---|
| 1 | Rainier | 5 years | NA[a] | Diseased and healthy buds |
| 2 | Lapins | 6 years | NA | Diseased buds |
| 3 | Rainier | 2 years | NA | Diseased buds |
| 4 | Chelan cherry on Mazzard | 3 years | 13.7 Kg/ha KOCIDE<br>10 % lime and sulfur solution<br>Once in spring | Diseased buds |
| 5 | 2009s on Mazzard | 3 years | 13.7 Kg/ha KOCIDE<br>1.18 L/ha NU-FILM 17<br>Once in both spring and fall | Diseased buds |
| 6 | 2525s and 4348s on Mazzard | 5 years | 13.7 Kg/ha KOCIDE<br>1.18 L/ha NU-FILM 17<br>Once in both spring and fall | Diseased buds |
| 7 | Sweetheart on Mazzard | 7 years | 13.7 Kg/ha NU-COP<br>1.18 L/ha NU-FILM 17<br>Once in both spring and fall | Diseased buds |
| 8 | Chelan cherry on Mazzard | 3 years | 13.7 Kg/ha KOCIDE<br>Twice in spring 2000<br>And<br>Once in spring 2001 | Diseased buds |
| 9 | Bing | 8 years | Copper product once in both spring and fall | Diseased buds |

[a] Detailed spray information not available but copper products were applied.

Samples were placed in zip lock bags, transported to the laboratory in a cooler and kept in a cold room until processed. Most bacterial strains were isolated from diseased buds. To isolate bacteria, outer bark tissues of selected buds were removed with a sterile scalpel, and small segments were excised from the buds. The excised segments were surface-disinfected for 30 seconds in 10% bleach and rinsed three times with sterile deionized water. Excised segments were then soaked in 5 mL of sterile potassium phosphate buffer (pH 7.0, 12.5 mM) with continuous rotary shaking (250 rpm) for 2 hours at 23°C. Serial dilutions (1/10, 1/100, 1/1000) in sterile water were made from the washings and plated onto King's medium B agar (KB). Plates were incubated at room temperature for four days and examined for fluorescent colonies under 366 nm (long wavelength) ultraviolet light. Representative fluorescent colonies were selected, purified on KB, and tested for the oxidase activity and the presence of arginine dihydrolase. Bacterial strains were stored in 0.01 M phosphate buffer at 4°C or in tryptic soy broth containing 15% glycerol at –80°C.

To assay copper tolerance in culture, modified low-complexing mineral salts medium, casitone-yeast extract-glycerol medium (CYE) was used for these assays. Copper from a stock solution of $CuSO_4$ was added to autoclaved CYE medium cooled to 50°C immediately before pouring to achieve the desired copper concentrations of 0, 0.25, 0.5, 1 and 2 mM of $CuSO_4$. Copper gradient media was prepared by dispensing 15 ml of CYE amended with cupric sulfate in 100 X 15-mm Petri dish plates. After the medium solidified, the dishes were placed in a horizontal position. Gradient plates were stored at 4°C for at least 8 hours before use.

Bacteria were grown in test tubes each containing 4 ml of tryptic soy broth (Difco, Detroit) on a rotary shake incubator at 250 rpm at 23˚C. Preliminary tests showed that after 4 hours in soy broth, a 1000-fold diluted bacterial suspension resulted in approximately $10^6$ cfu/ml. One hundred µl of such bacterial suspensions of each test strain was spread on a plate containing the copper-amended CYE medium. Three replicates of each copper concentration were included in the experiment for each isolate. Plates were incubated at 21°C for 72 hr in an inverted position, after which visible confluent growth of a bacterial strain on copper-amended CYE medium was considered copper-resistant at the corresponding concentration of $CuSO_4$.

3. **Chemical control of bacterial canker.** Lime sulfur and copper products are commonly used on cherry trees in the fall or early spring. Mixture of copper products and mancozeb has been reported to enhance the bactericidal activity of copper products for control of bacterial diseases on other crops. In the fall of 2000, we set up a field trial in a commercial orchard to evaluate effects of lime sulfur, copper hydroxide, copper sulfate, mancozeb, and the mixture of copper hydroxide and mancozeb on control of bacterial canker. Seven treatments were included in this trial: (1) lime sulfur in the fall and spring; (2) lime sulfur in the fall and copper hydroxide in the spring; (3) copper hydroxide in both fall and spring; (4) copper sulfate in both fall and spring; (5) mancozeb in both fall and spring; (6) mixture of copper hydroxide and mancozeb in both fall and spring; and (7) unsprayed control. Treatments were arranged in a randomized complete design, with 3 replicates (two trees for each treatment within each replicate).

**Results and Discussion:**

1. A total of 42 cherry blocks were surveyed in Yakima area. Bacterial canker was found in four blocks with different ages. Of 24 blocks at age of l to 5, two had bacterial canker, with mean incidence (percentage of trees with the disease) of 3%; of the 11orchards at age of 6 to 10, one had bacterial canker with a disease incidence of 2%; and among 7 blocks at age of more than 10 years, one block under the cover had bacterial canker with the incidence of 2% (Table 2).

In the Wenatchee area, of the 19 blocks at age of 1 to 5, four had bacterial canker, with mean disease incidence of 3% (Table 2).

In the northern fruit production area, 40 blocks were surveyed and bacterial canker was found in 18 blocks. Bacterial canker was more noticeable in the region. Among the 28 blocks at age of 1 to 5, 12 had bacterial canker (43% of the blocks), and the incidence of disease in these blocks ranged from 2% to 77% with mean incidence of 14%. Of the eight blocks at age of 6 to 10, five had bacterial canker, with mean disease incidence of 6% (Table 2).

Table 2. Summary of orchard survey of bacterial canker on cherry trees in eastern Washington.

| **Production [a] Region** | **Age of Block** | **Number of orchard surveyed** | **Number of orchard with the disease** | **Incidence of disease (%) in the orchards with the disease [b]** | |
|---|---|---|---|---|---|
| | | | | **Range** | **Mean** |
| Yakima | 1 – 5 | 24 | 2 | 3 – 3 | 3 |
| | 6 – 10 | 11 | 1 | 2 | 2 |
| | > 10 | 7 | 1 | 2 | 2 |
| | | | | | |
| Wenatchee | 1 – 5 | 19 | 4 | 2 – 5 | 3 |
| | 6 – 10 | 1 | 0 | | |
| | > 10 | | | | |
| | | | | | |
| Northern Area | 1 – 5 | 28 | 12 | 2 – 77 | 14 |
| | 6 – 10 | 8 | 5 | 3 – 10 | 6 |
| | > 10 | 4 | 1 | | 5 |

[a] The Yakima survey included the following areas: Moxee, Union Gap, Parker, Buena, and Zillah. The Wenatchee survey included Wenatchee, East Wenatchee, Rock Island, and Rocky Reach Dam. The northern area included Pateros, Brewster, Bridgeport, Okanogan, Omak, Tonasket, and Oroville.

[b] The incidence of disease was expressed as percentage of trees exhibiting bacterial canker symptoms in the orchards. Sixty trees were examined for the presence of the disease in each block.

2. Nine orchards with a history of bacterial canker disease were selected to be monitored for copper resistance of *Pseudomonas syringae* populations. A total of 110 *Pseudomonas syringae* strains were isolated and tested for resistance to copper sulfate. The strains isolated from cherry trees in selected orchards exhibited varying levels of tolerance to copper ions added to CYE medium in the vitro test (Table 3).

   Bacterial strains that were able to grow at 0.25 mM $CuSO_4$ in the medium were considered resistant to copper sulfate. Of the 110 *Pseudomonas syringae* strains tested, 82% (90 strains) were tolerant to copper at 0.25 mM $CuSO_4$ in the medium. Copper resistant strains of *P. syringae* were found in seven orchards. The strains from orchards 7 and 9 were sensitive to copper, but only a few from those orchards were tested for copper sensitivity. All strains tested from orchards 3, 4, 5, 6, and 8 were resistant to copper sulfate at 0.25 mM $CuSO_4$ in the medium while 26% and 83% of strains tested from orchards 1 and 2, respectively, were resistant. Among the strains resistant to copper, 39% of the bacterial strains also were resistant to 0.5 mM $CuSO_4$. Strains from orchards 1 and 2 were also resistant to copper sulfate at 1 mM $CuSO_4$ in the medium, indicating highly resistant populations of the pathogen in these orchards, but these strains were at a low frequency of 4% and 11% for orchard 1 and orchard 2, respectively.

Table 3. Resistance to copper sulfate of *Pseudomonas syringae* strains collected from sweet cherry orchards in Washington State from 2000 to 2001.

| Orchard | Number of strains tested | % of strains growing | | | | |
|---|---|---|---|---|---|---|
| | | Copper sulfate in CYE medium (mM) | | | | |
| | | 0 | 0.25 | 0.5 | 1 | 2 |
| 1 | 23 | 100 | 26 | 26 | 4 | 0 |
| 2 | 18 | 100 | 83 | 78 | 11 | 0 |
| 3 | 14 | 100 | 100 | 93 | 0 | 0 |
| 4 | 15 | 100 | 100 | 0 | 0 | 0 |
| 5 | 4 | 100 | 100 | 50 | 0 | 0 |
| 6 | 15 | 100 | 100 | 100 | 0 | 0 |
| 7 | 2 | 100 | 0 | 0 | 0 | 0 |
| 8 | 15 | 100 | 100 | 0 | 0 | 0 |
| 9 | 4 | 100 | 0 | 0 | 0 | 0 |

3. The chemical control trial was conducted in a 2-year-old Lapins block in a commercial orchard in Omak. This block was adjacent to a 7-year-old Lapins block with severe bacterial canker. However, in our test block no canker lesion developed in the spring, and the grower also trimmed old canker tissues from the tree. Thus, no disease rating data was generated from this trial.

In summary, bacterial canker occurs with relatively low incidence in the Yakima and Wenatchee area. The disease was more noticeable in the northern fruit production region from Pateros to Oroville. Young trees were particularly prone to infection by *Pseudomonas syringae*. The strains of *Pseudomonas syringae* isolated from cherry orchards exhibited varying levels of tolerance to copper in the in vitro test.

Information on the occurrence of the disease was from large field surveys from which cherry blocks were randomly selected. Although the survey indicates a relatively low incidence of bacterial canker, we have also been invited to some young commercial cherry blocks at some specific sites and have seen severe epidemics of bacterial canker in the blocks, particularly on varieties Lapins and Sweetheart. As the acreage of new plantings increases, bacterial canker can be an important factor in the process of establishing profitable young orchards. Dr. Gary Grove and I will keep eyes on this disease.

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