Incidence and Control of Cytospora Canker and Bacterial Canker in a Young Sweet Cherry Orchard in Oregon

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ABSTRACT

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A field study to evaluate control of Cytospora canker was initiated in 1981 by planting sweet cherry trees (cv. Bing). Treatments applied to the trees included white trunk paint, three levels of nitrogen, and application of benomyl (225 mg a.i. per liter) after dormant pruning or at popcorn, petal fall, and shuck split. Trees were evaluated annually from 1982 to 1986 for active trunk cankers, and isolations were made from margins of cankers. One to 5% of the trees were infected with *Cytospora cincta* each year, and 18% were infected by 1986. Bacterial canker, caused by *Pseudomonas syringae*, occurred in 13% of the trees in 1982 and in 25% of the trees by 1986. The death rates of trees infected with *C. cincta* and *P. syringae* were 16 and 17%, respectively. Disease incidence was highest in trees close to an old cherry orchard. Nitrogen or benomyl did not reduce the incidence of cankers. White trunk paint reduced the incidence of both Cytospora and bacterial trunk cankers.

Two of the most common canker diseases of sweet cherry (*Prunus avium* L.) in the Mid-Columbia region of Oregon are Cytospora canker, caused by *Cytospora cincta* Sacc. (teleomorph *Leucostoma cincta* (Fr.) Höhn.), and bacterial canker, caused by *Pseudomonas syringae* pv. *syringae* van Hall. In a survey conducted in 1980, 38% of 1,000 sweet cherry trees in 20 orchards had one or more cankers, with about equal numbers of Cytospora and bacterial canker (Spotts, *unpublished*).

Cytospora canker has been studied intensively since the 1930s, and most research has focused on peach as the host (35). *C. cincta* sporulates during the entire year (21,30), and infection occurs primarily in fall or spring (3,9,34) in wounded or dead tissue (35). Winter injury was considered the main contributing factor in an outbreak of Cytospora canker in sweet cherry in New York (16). Extensive canker development occurred during the second growing season after the winter injury. In a survey in Colorado, Cytospora canker of peach caused by *C. leucostoma* Sacc. (teleomorph *Leucostoma persoonii* Höhn.) was associated with winter injury or pruning wounds in 40 and 57% of the cankers, respectively (22). Wounds remained susceptible to invasion for 30–90 days or more (19,27). In Germany, field inoculation of cherry fruit scars with *C. cincta* was more successful than inoculation of leaf scars (29). Periderm formed in 4 and 8 days in leaf and fruit scars, respectively. *C. cincta* could not penetrate healed scars unless they were damaged by frost (12).

Cytospora spp. are considered weak pathogens (4) and often attack trees growing under stress. *C. leucostoma* caused larger cankers in nonvigorous prune trees than in vigorous trees in California (3). Cytospora canker was a more serious problem in peach trees grown in soil containing excessive or deficient nitrogen levels, rather than nitrogen levels considered adequate for tree growth (7).

Several researchers reported various degrees of control of Cytospora canker with fungicides. Benomyl at 300 μg/ml protected wounds in peach from infection by *C. leucostoma* but was ineffective as an eradicant (20). Application of benomyl at 500 μg/ml or captafol at 2,400 μg/ml at leaf drop gave good control of Cytospora canker of peach, but spring sprays were ineffective (25).

Serious outbreaks of bacterial canker of cherry occurred in Oregon as early as 1853 (1). Infection occurs mainly in young trees in tissue damaged by frost, pruning cuts, or insects (5,10). Infection occurs in fall or winter (5), and cankers are most active in cold, wet weather (11). Cankers are usually annual but can continue to develop for more than 1 yr (5). Tree mortality is generally greatest in the first 2 yr after planting (8), and trees are seldom killed after 8 yr (5). Current control measures for bacterial canker are inadequate. Bordeaux sprays gave some control, but effectiveness was erratic (5).

Cytospora canker and bacterial canker often occur together, and several researchers have studied the interaction between the two pathogens. In Hungary, a synergism was observed when *C. cincta* and *P. syringae* were inoculated simultaneously in apricot in winter but not in fall or spring (28). *Cytospora* spp. often invade tissues that were previously infected by *P. syringae* (6,26) and appear to actually prefer such tissue (11). Recently, it was shown that *C. cincta* dehy-

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drates and acidifies the inner bark in cankers, creating an unsuitable environment for *P. s.* pv. *syringae* (11). It was concluded that *P. s.* pv. *syringae* is incapable of long-term survival in the presence of *C. cincta*. In a study on peach tree short life involving both *Cytospora* sp. and *P. syringae*, 10 antibiotics and fungicides (including benomyl and streptomycin) gave no improvement in tree survival following postpruning applications (6).

In 1981 we initiated a field study to evaluate several cultural and chemical methods for control of Cytospora canker of sweet cherry caused by *C. cincta*. Treatments were begun in a new planting during the first season, and incidence of cankers was determined annually for 5 yr. It became obvious early in the study that considerable bacterial canker was also developing. This provided an opportunity to study the long-term progress of both diseases and the interaction between *C. cincta* and *P. s.* pv. *syringae*. An abstract of this study has been published (31).

MATERIALS AND METHODS

Orchard design. Sweet cherry trees (cv. Bing on Mazzard rootstock) were planted in March 1981. Trees were spaced 3.3 m apart in the row and with 6.7 m between rows. Trees were headed to 90 cm height after planting. A 0.3-m length of cherry limb infected with *C. cincta* and containing abundant pycnidia was placed at the base of each tree to provide inoculum throughout the planting. Commercial orchard management practices for weed and insect control and irrigation were applied uniformly to all trees.

Treatments. A factorial experiment was designed with factors (treatments) consisting of three rates of nitrogen fertilization, two applications of benomyl (Benlate 50W), and application of white paint to trunks. The experiment contained 384 trees, with 192 trees treated with each benomyl and paint level and 128 trees with each nitrogen level. Nitrogen was applied at 0, 113, or 453 g per tree in February 1982 and 1983. Ammonium nitrate was used in 1982 and urea in 1983. Leaf tissue was analyzed annually for nitrogen from 1981 to 1986 by the Oregon State University Plant Analysis Laboratory, Corvallis. Benomyl was applied to runoff at 225 mg a.i. per liter. Dormant applications were made immediately after pruning in January through March, 1981 to 1985. Spring applications were made at popcorn bloom stage, petal fall, and shuck split (separation of floral tissue from enlarging fruit) in early April, late April, and early May, respectively, 1981–1985. White latex paint was applied to the trunks of half of the trees in each of the nitrogen and benomyl treatments. Tree trunks were painted in fall 1981 and repainted in 1983. The experiment was arranged as a randomized complete block design with eight blocks oriented perpendicularly to the slope. A planting of mature cherry trees bordered the experimental orchard at the top of the slope.

Canker sampling and pathogen identification. Beginning in spring of 1982, the trunks of all trees were examined for cankers. Pieces of tissue at canker margins were removed with a knife sterilized with 95% ethanol. In the laboratory, the outer bark was removed and the tissue cut into 5- × 5-mm pieces. Tissue pieces were surface-sterilized for 15 sec in 0.525% NaOCl and rinsed in sterile distilled water; four pieces were plated on potato dextrose agar (Difco) acidified with 1.5 ml of 85% lactic acid per liter (APDA) and on *Pseudomonas* agar F (Difco). Plates were incubated at 25 C for 5 days. If no pathogen was isolated from canker tissue, the canker was sampled one to three additional times during the growing season.

Growth and morphology of *Cytospora* isolates on APDA at 25 and 33 C were used to distinguish between *C. cincta* and *C. leucostoma* (3,9,34). *Pseudomonas* agar F plates were examined for colonies producing fluorescent pigment. Fluorescent colonies were purified on nutrient agar, then tested for oxidase activity (32) and pathogenicity to green cherry fruitlets (14). In addition, 36 isolates were tested using the GATTa tests (G = gelatin liquefaction, A = aesculin hydrolysis, T = tyrosinase activity, Ta = tartarate utilization) to distinguish *P. s.* pv. *syringae* from *P. s.* pv. *morsprunorum* (18).

RESULTS

Twenty-seven isolates of *Cytospora* sp. were tested for growth at 25 and 33 C. Growth of all isolates after 5 days was much greater at 25 C (60-mm average colony diameter) than at 33 C (17-mm average colony diameter). Based on criteria of Willison (34) and growth at 25 and 33 C (3,9), all isolates were considered *C. cincta*.

Thirty-two of the 36 *Pseudomonas* isolates from canker tissue that were fluorescent, oxidase negative, and pathogenic to cherry fruitlets also conformed to at least three of the four GATTa tests and were considered *P. s.* pv. *syringae*. The remaining four isolates differed from *P. s.* pv. *syringae* in two or three of the GATTa tests. However, none conformed to *P. s.* pv. *morsprunorum*, nor was *P. s.* pv. *morsprunorum* identified from a group of 82 *Pseudomonas* isolates from fruit trees in the Pacific Northwest (13,14). On this basis, we limited testing of further isolates of bacteria from cherry canker tissue to fluorescence, oxidase, and pathogenicity to cherry fruitlets to determine whether or not they were *P. syringae*.

The overall infection rate of cherry trees in all treatments by *C. cincta* varied from 4.1 to 5.4% each year, except for 0.3% in 1985 (Table 1). Incidence of infection of trees by *P. syringae* was greatest in 1982 at 13.5%, then declined to 2.3–4.4% per year from 1983–1986 (Table 1). At the end of the study in 1986, 18% of the original 384 trees were infected with *C. cincta* and 25% with *P. syringae*. Trees that died were deleted from calculations in following years. The mortality rates of trees infected with *C. cincta* and *P. syringae* were 16 and 17%, respectively, at the end of the 6-yr study. Both *C. cincta* and *P. syringae* were isolated from the same canker in 38 trees. *C. cincta* was isolated before *P. syringae* in four of these trees but followed a primary infection of *P. syringae* in 21 trees.

The location of trees in the orchard affected the incidence of Cytospora canker, bacterial canker, and tree mortality. Disease and mortality were greatest in trees nearest to the old orchard (Table 2). Regressions of mortality and disease on distance were significant at $P = 0.05$ and $P = 0.01$, respectively.

In 1982, the amount of nitrogen in leaf tissue from trees fertilized with 0, 113, and 453 g of nitrogen per tree was 2.0, 2.2, and 2.5%, respectively. Levels decreased during the study, and the 5-yr average nitrogen concentrations in leaf tissue of trees receiving 0, 113, and 453 g were 1.8, 2.1, and 2.4%, respectively. Levels less than 2.3% are considered deficient (33). The rate of the nitrogen fertilization did not affect the incidence of Cytospora canker, bacterial canker,

Table 1. Incidence of Cytospora canker and bacterial canker in sweet cherry trees from 1982 to 1986

	Trees infected with			
	Cytospora cincta		*Pseudomonas syringae*	
Year	Number	Percent[a]	Number	Percent[a]
1982	16	4.2	52	13.5
1983	15	4.1	16	4.4
1984	17	4.8	10	2.8
1985	1	0.3	10	2.9
1986	19	5.4	8	2.3

[a] Values adjusted for dead trees and based on 384, 367, 357, 346, and 346 trees in 1982, 1983, 1984, and 1986, respectively. Numbers indicate new infections for each year and are not cumulative values.

or tree mortality (Table 3).

Dormant benomyl applied immediately after pruning did not affect the incidence of Cytospora canker or bacterial canker (Table 3). However, a significant ($P = 0.05$) paint × dormant benomyl interaction was found, in which mortality of unpainted trees decreased from 26 to 15% when trees were treated with benomyl. The spring application of benomyl did not affect disease or mortality (Table 3), but a significant ($P = 0.01$) paint × spring benomyl interaction occurred, in which incidence of Cytospora canker of unpainted trees increased from 19 to 29% when trees were treated with benomyl. The incidence of Cytospora canker in painted trees decreased from 16 to 7% when trees were treated with benomyl, but the decrease was not significant ($P = 0.05$).

The incidence of Cytospora canker and bacterial canker was significantly ($P = 0.01$) less in trees that received white paint on the trunks than in unpainted trees (Table 3). Painted trees also had significantly ($P = 0.01$) less mortality than unpainted trees.

DISCUSSION

Although most field research on Cytospora canker and bacterial canker is done in established orchards, we initiated treatments during the first year that trees were planted and monitored infection annually for 5 yr. The study was designed to evaluate the methods of control of Cytospora canker, but the development of bacterial canker provided an opportunity to study the progress of both diseases and their interactions. Whereas infection with *C. cincta* was relatively constant at 4–5% a year, most infections with *P. syringae* occurred during the first year after planting. This agrees with previous observations (5,8) and we emphasize the importance of control of bacterial canker in young orchards. Cytospora canker, however, is a continuing problem that probably requires control for the life of the orchard.

Mortality of trees infected with bacterial canker (17%) was similar to mortality of those with Cytospora canker (16%). However, the majority of trees infected with either pathogen remained alive for the duration of the study. In 1988, 2 yr after the study ended, we sampled 100 cankers in the same orchard and recovered *C. cincta* and *P. syringae* from only 18 and 6%, respectively, of the cankers (Spotts, *unpublished*). Thus, many cankers were inactive, and trees appeared healthy and vigorous. *P. syringae* usually preceded *C. cincta* in dual infections, and this agrees with previous observations (6,11,26).

Cytospora canker and bacterial canker were worse in trees closer to an old cherry orchard than in trees farther away. Many old trees were infected with *C. cincta* and *P. syringae* and were in various stages of decline. Infected tissue in these trees was probably a source of inoculum for infection of experimental trees.

Nitrogen level did not affect incidence of Cytospora or bacterial canker. The nitrogen level of leaves from trees receiving the two lowest rates was less than 2.3 and is considered deficient (33). Cytospora canker of peach was worse when trees were grown in nitrogen-deficient soil than when levels were adequate (7). Nutritional effects on disease may result from the interaction of more than one nutrient, such as nitrogen and potassium (7), and these interactions are difficult to control and study under field conditions.

Benomyl had no overall effect on Cytospora canker but in one interaction appeared to cause an increase in the incidence of Cytospora canker. Eighteen isolates of *C. cincta* were tested for resistance to benomyl, and all were sensitive to 1 μg/ml (Spotts, *unpublished*). Control of Cytospora canker has been obtained with benomyl in other studies (20,25). Benomyl applications were designed to protect pruning wounds and other susceptible tissue for a few weeks during late winter and spring. Many trunk cankers appeared to originate in winter-injured tissue on the west and southwest sides of the trees. The single postpruning benomyl application provided little protection of this tissue. Control of infection of leaf scars in the fall was not studied herein but may be of importance in the Mid-Columbia region of Oregon. Rain is the primary mechanism of dispersal for conidia of *C. cincta* and *C. leucostoma* (2,30). Total rain during leaf drop in October and November in Hood River, Oregon, exceeds 150 mm and is similar to rain amounts in eastern Canada, where infection of leaf scars of peach by *C. cincta* is considered important (25). In contrast, infection of leaf scars with *C. leucostoma*

Table 2. Effect of location of cherry trees in orchard on incidence of Cytospora canker, bacterial canker, and mortality

Distance from edge of orchard[a] (m)	Percent trees infected with		Dead (%)
	C. cincta	*P. syringae*	
6	21	42	23
18	27	35	10
30	25	25	17
42	25	29	8
54	19	21	15
66	8	23	6
78	6	10	8
90	6	15	8
Intercept[b]	29.8	40.6	18.6
slope[b]	−0.26	−0.33	−0.14
r[c]	−0.86**	−0.93**	−0.71*

[a] Distances are from border of orchard to the center of each block. Orchard of mature trees is located adjacent to first block at top of slope.
[b] Linear regression based on the formula $Y = b + mx$, where Y = cumulative percent disease or mortality from 1982 to 1986, x = meters from edge of orchard to center of block, b = Y intercept, and m = slope.
[c] r = Correlation coefficient. ** and * indicate significance at $P = 0.01$ and $P = 0.05$, respectively.

Table 3. Effect of nitrogen, benomyl, and white trunk paint treatments on Cytospora canker, bacterial canker, and mortality of sweet cherry trees from 1982 to 1986

Treatment	Level	Percent trees infected with		Dead (%)
		C. cincta	*P. syringae*	
Nitrogen[a]	0	18[b]	23	14
	113 g/tree	20	30	12
	453 g/tree	16	24	11
Dormant benomyl[c,d]	+	21	29	11
	−	15	22	13
Spring benomyl[c,d]	+	18	26	11
	−	18	25	13
White paint[c]	+	12**	20**	4**
	−	24	31	20

[a] Each value represents 128 trees.
[b] Values followed by ** indicate effect of factor is significant at $P = 0.01$. Numbers indicate cumulative values from 1982 to 1986.
[c] Each value represents 192 trees.
[d] Dormant benomyl applied immediately after pruning; spring benomyl applied at popcorn, petal fall, and shuck split.

is of no importance in Colorado, where rainfall in October and November is less than 40 mm (22).

Incidence of Cytospora canker and bacterial canker was significantly less ($P = 0.01$) in trees with trunks painted with white paint than in unpainted trees. Painting the trunks of fruit trees white has been recommended to reduce winter freeze damage (24). White paint on tree trunks can maintain the temperature of cambium 8–16 C lower in painted than in unpainted trees (15,17,24) and can reduce the sudden drop in temperature following a sunny winter day (23). Winter-injured tissue can be an important site of infection and is readily colonized by *C. cincta*, *C. leucostoma* (16,22), and, apparently, *P. syringae*.

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