Production and Dispersal of Conidia of *Leucostoma cinctum* in Peach and Cherry Orchards Under Irrigation in Eastern Washington

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ABSTRACT

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The objectives of this study were to determine the seasonal production patterns of *Leucostoma cinctum* conidia in peach and cherry orchards and the effects of irrigation on dispersal of conidia. Conidia were trapped throughout the year and were present in highest numbers during spring and summer on both cherry and peach. The pathogen was dispersed 1.0 m by water drops that simulated irrigation drops that would impact cankers during over-the-canopy irrigation. Spore production and dispersal also resulted from the use of over-the-canopy and under-tree impact sprinklers. Although the two irrigation methods were not directly compared, the latter mode dispersed propagules of *L. cinctum* over horizontal distances of about 6 m, a distance sufficient to account for the movement of the pathogen between trees within and across orchard rows. It is concluded that disease management could be improved by alteration of horticultural and irrigation practices.

Additional keywords: *Leucostoma* canker, peach canker

Leucostoma canker (or Valsa canker), caused by *Leucostoma cinctum* (anamorph *Leucocytophthora cinctum*) and *Leucostoma persoonii* (anamorph *Leucocytophthora leucostoma*), is the primary canker disease of stone fruits in many regions of the world (4,5). Dead wood is the major source of *L. cinctum* and *L. persoonii* ascospores and conidia in the orchard. Conidia are considered to be the primary source of inoculum for new infections because it may take 2 to 3 years for perithecia to develop and dead wood is usually removed annually when trees are pruned. In Washington, *L. cinctum* is the primary wood pathogen on sweet cherry (*Prunus avium* L.) and peach (*P. persica* L.) Batsch. (1,9,16). Tree mortality in affected orchards can reach 3 to 6% per year (G. G. Grove, unpublished). Further economic losses result from the loss of bearing wood on surviving trees. Some growers estimate that this loss of bearing wood further reduces yield by up to 5% (G. G. Grove, unpublished). The

disease is particularly severe in orchards where over-the-canopy or under-tree impact sprinklers are used for irrigation and where intensive summer pruning is standard horticultural practice. The disease is sporadic and far less damaging in orchards where microsprinkler or drip irrigation is used.

Release and dispersal of conidia of *L. persoonii* was found to occur during rain events in all seasons on French prune in California (2). These authors determined that there was no evidence for any non-water-borne release of conidia. The primary means of dispersal was shown to be wind-blown rain. Also, increasing time and/or temperature between wetting events was correlated with increased numbers of conidia caught in funnel traps. In Colorado, *L. persoonii* conidia were found to be present in water-borne spore traps in all months from February through November (10). Over the course of the 1-year study, production of conidia appeared more responsive to relative humidity during the period February through June, whereas afterward and through November, production of conidia was more closely correlated with temperature. In Germany, *L. persoonii* on sweet cherry and *L. cinctum* on peach were observed to sporulate during the entire year, with maxima from November to March, and depending upon rainfall, also in June (16). The number of hours that temperatures were between 10 and 15°C, the duration of leaf wetness, and the length of time that relative humidity was above 90% all were correlated with numbers of conidia caught.

Commercial stone fruit production in Washington is limited primarily to irrigated areas east of the Cascade Mountains. There are no studies that have considered seasonal sporulation or addressed the role of irrigation in the sporulation and dispersal of conidia of *Leucostoma* spp. Furthermore, only one previous study has specifically addressed the production and dispersal of conidia of *L. cinctum* (17). The objectives of this study were to determine the seasonal production of conidia of *L. cinctum* on peach and cherry, and to assess conidia production and dispersal during irrigation events with under- and over-the-canopy impact sprinklers.

MATERIALS AND METHODS

Seasonal production of conidia. Spore samplings were made in both cherry and peach orchards. Cherry studies were conducted from January 2001 until December 2002 in a 5.3-ha orchard located 4 km north of Prosser, WA. The orchard was comprised of 30-year-old 'Bing' trees planted on Mazzard rootstock on a 2.7 × 5.5 m (tree × row) spacing. The orchard was irrigated using under-the-canopy impact sprinklers (Model F33S, Nelson Irrigation, Darra, Queensland, Australia). Peach studies were conducted during 1993 to 1995 in a 12.4-ha orchard located 5 km north of Wapato, WA, and comprised of 12-year-old cv. Redhaven peaches. Trees were planted on a 4.3 × 4.9 m spacing and irrigated using over-the-canopy model 30H impact sprinklers (Rainbird, Inc., Glendora, CA) positioned about 2 m above tree canopies. Canker incidence was nearly 100% in both orchards. Twenty cankers on each host were used to study conidia production. To accommodate water-borne spore traps identical to those described previously (8), cankers were chosen that were located within 1.5 m of the proximal end of main scaffold branches and positioned 1 to 2 m above the orchard floor. To facilitate the collection of rain- and wash-water, triangular funnels were prepared from 0.025-mm aluminum sheeting. A semicircular notch was cut in the broad end of the funnel so that the funnel would fit snugly against the cankered branches. The aluminum was creased longitudinally to form a path for water flow. The notched end of the funnel was placed on the branch bottom immediately beneath the canker and secured with pieces of aluminum wire.

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The opposite end of the funnel was then tilted downward at a 45-degree angle, terminating about 2.5 cm inside the spore trap opening. Cankers were washed at monthly intervals by applying about 100 ml of sterile distilled water to canker surfaces with a small wash bottle. If rainwater was present in the traps, cankers were washed as described above and the total volume of water in the spore trap determined with a graduated cylinder. Effluent was transferred to 0.95-liter Mason jars and transported to the laboratory. The number of conidia per milliliter of effluent was determined using a hemacytometer. The numbers of conidia per mm² of canker surface area was determined by dividing the estimated total number per volume of wash water by canker surface area. Cankers were either circular or elliptical in shape, and their surface areas were estimated by substituting the measured canker radii in formulae for determining the surface area of a circle (πr^2) or ellipse ($\pi r_1 r_2$). A Campbell Scientific CR-10 Datalogger located 0.5 km from the orchard provided continuous measurements of temperature, precipitation, relative humidity, leaf wetness, wind speed, wind direction, and solar radiation. To verify the presence of *L. cinctum*

in canker wash samples, effluent from some cankers was placed directly onto Leucostoma selective medium (LSM; G. G. Grove *unpublished*) containing 12.5 g of malt extract and 20.0 g of Difco agar per liter. The base medium was amended with imazilil at 25 µg/ml, terrazole at 100 µg/ml, tetracycline at 100 µg/ml, streptomycin sulfate at 100 µg/ml, and chloramphenicol at 50 µg/ml after autoclaving. Cultures were incubated 10 to 15 days at 20°C, and putative colonies of *L. cinctum* were confirmed by microscopic observation at ×20. In the peach orchard, the effluent collected in traps was removed immediately prior to each over-the-canopy irrigation set and the concentration of spores of *L. cinctum* determined as described above. Traps were reattached after trees had dried following the irrigation set.

Production of conidia during over-the-canopy irrigation. The water-borne spore traps were also used to study the production and dispersal of conidia during over-the-canopy irrigation events in the peach orchard. Samples were taken during four irrigation events in 1994 and seven events in 1995. Traps were emptied of any natural precipitation prior to irrigation studies and the water processed as de-

scribed above. Irrigation water was then applied beginning at 0615 PDT and canker effluent collected in the aforementioned traps for about 6 h. The concentration of conidia of *L. cinctum* was determined as described above.

Dispersal of *L. cinctum* conidia. Laboratory experiments. Active cankers about 6 cm long were collected from peach branches and used in laboratory experiments. Cankers were secured horizontally to a ring stand. The canker was moistened with 50 ml of sterile distilled water 5 min before experiments commenced. Petri dishes containing 20 ml LSM were placed at 20-cm intervals to a distance of 100 cm from the canker. Four respective rows of dishes were positioned at 0, 90, 180, and 270 degrees azimuth from the canker. In order to mimic the impaction of droplets from over-the-canopy sprinklers and the dripping of irrigation from foliage and limbs in the tree canopy, 10 ml of sterile distilled water was released from a burette positioned 2 m above the canker and allowed to impact the canker surface. Two dishes were placed at each position: the cover of one dish was removed during the release of water while the other was left covered at all times. At the conclusion of

Fig. 1. Effect of time of year on sporulation of *Leucostoma cinctum* on cankers on 'Redhaven' peach trees in Wapato, WA, during 1993 to 1995. Presented are A, average monthly temperature and total monthly precipitation and B, number of conidia per mm² of canker surface area collected from water-borne spore traps. Values represent the mean number of conidia per mm² recovered from 20 cankers.

the experiment, cultures were as described above. The number of colonies of *L. cinctum* was determined by microscopic observation at $\times 20$. The experiment was conducted twice.

Orchard studies using over-the-canopy sprinklers. The peach orchard was used for these dispersal studies with water applied in 12-h sets at approximately 2.46 kg/cm². The dispersal of *L. cinctum* was studied by placing 100-mm-diameter petri dishes containing LSM medium on steps of 1.8-m aluminum ladders placed about 1 m from infected trees. Dishes were positioned at heights of 0.5 to 1.8 m. Ladders were positioned so that they would be within the tree drip lines. Totals of 72 and 84 petri dishes were used in the two runs of the experiment (four plates each were also positioned in the crotches of three trees during the second experiment). The covers of one-half of the plates were removed immediately prior to the beginning of the irrigation set. Sets commenced at 0615 PDT, and the plates were uncovered for 15 min. At the conclusion of the experiment, dishes were covered and transported to the laboratory and incubated as described above. The presence of putative colonies of *L. cinctum* was determined by microscopic observation at $\times 20$.

Orchard studies using under-the-canopy impact sprinklers. A second orchard (20 ha) located 5 km south of Yakima, WA, was used for these studies. The orchard was comprised of 12-year-old 'Redhaven' planted on a spacing of 4.3 m between trees and 4.9 m between rows and irrigated using under-the-canopy sprinklers (model L20H Rainbird). A mature tree with a large canker in the main scaffold/trunk area was used as an inoculum source in dispersal experiments. Petri dishes 150 mm in diameter containing 40 ml of LSM were placed 0.5, 0.9, 1.4, 1.8, 2.3, 2.7, 3.2, 3.6, 4.1, 4.5, 5, 5.4, and 5.9 m from the canker and arranged 110 degrees azimuth diagonal to the canker. Two dishes were placed atop a 10-cm-high cinder block at each location; the cover of one was removed during watering while the second remained covered to serve as a control. The angle of the water stream of an impact sprinkler 2.1 m from the canker was adjusted so that the water stream impacted directly on the canker. About 10 ml of sterile distilled water was applied to the canker 1 h prior to the experiment. Water was applied at 2.46 kg/cm². The water stream was allowed to impact on the canker 10 times, after which dishes were covered. At the conclusion of the experiment, dishes were transported to the laboratory, incubated as described above, and the number of colonies of *L. cinctum* was determined. The experiment was repeated 12 days later.

Data analyses. *Seasonal spore production.* The relationship between monthly spore production, temperature, and precipitation was examined using the correlation

analysis procedure of JMP Statistical Discovery Software (SAS Institute, Cary, NC). In order to stabilize variances, the $\log_{10}(\text{base } 10)$ of monthly conidia concentrations + 1 were used in all correlation analyses. The significance of correlation coefficients was determined with a standard *t* test at $P < 0.05$. The relationships between spore production during irrigation sets and temperature were determined similarly.

Dispersal studies. Regression analysis was used to examine the relationship between the $\log_{10}(\text{base } 10)$ of colony numbers + 1 (*Y*) and horizontal distance (*D*) from the canker, degrees azimuth (*A*) to the canker, and distance $\times$ degrees azimuth (*AD*).

RESULTS

Seasonal production of conidia. All sporulation values presented are the number of conidia per mm² canker surface area $\times 10^5$. In general, sporulation was highest during the spring and summer months, but conidia were produced throughout the year. For example, in the peach orchard in

1994, sporulation ranged from a minimum of 0.41 in November to a maximum of 20.0 in May (Fig. 1). In 1995, values ranged from 1.2 in December to 26.4 in May. A similar pattern of spore production was observed on cherry (Fig. 2). Values ranged from 0.3 in February to 8.7 in June 2002. In 2003, values ranged from 0.5 in January to 9.0 in June. Numbers of conidia were positively correlated ($P < 0.05$) with temperature on cherry during both years of the study and on peach in 1994 and 1995, but not in 1993 (Table 1). Significant ($P < 0.05$) correlations between sporulation and precipitation were not detected for either host.

Production of conidia during over-the-canopy irrigation. Conidia were detected in all 1994 and 1995 irrigation experiments (Fig. 3). In 1994, values ranged from 1.5 on 2 June to 29.0 on 23 June. During 1995, values ranged from 7.0 on 13 July to 34.0 on 5 June. The number of conidia produced per mm² of canker surface area was not significantly ($P < 0.05$) correlated with temperature during the irrigation.

Fig. 2. Effect of time of year on sporulation of *Leucostoma cinctum* on cankers on 'Bing' cherry trees in Prosser, WA, during 2001 to 2002. Presented are **A**, average monthly temperature and total monthly precipitation and **B**, number of conidia per mm² of canker surface area collected from water-borne spore traps. Values represent the mean number of conidia per mm² recovered from 20 cankers.

Dispersal of *L. cinctum* conidia. Laboratory experiments. The impaction of water drops on canker surfaces dispersed conidia up to 100 cm from the canker (Fig. 4). Colony numbers were highest in dishes placed 20 and 40 cm from the canker. In general, the number of colonies decreased with increasing distance from the canker, e.g., at 180 degrees azimuth 95, 60, 1, 0, and 0 colonies were recorded in dishes 20, 40, 50, 80, and 100 cm from the canker in experiment 2, respectively. Colonies of *L. cinctum* were not present in any dishes left covered during the experiments.

An *F* test (15) indicated that the regression results from experiments 1 and 2 were not significantly different. The data from the two experiments were combined, and the equation

$$\text{Log}(Y + 1) = 5.6 + -0.06 * D$$

(where *Y* = number of *L. cinctum* colonies per petri dish and *D* = horizontal distance from canker) described the relationship between colony number and horizontal distance from the canker with a coefficient of determination (*R*²) of 0.81 at *P* < 0.01. Degrees azimuth and interactions between distance and degrees azimuth were not significant (*P* < 0.05).

Orchard studies using over-the-canopy irrigation. The volume of water in each petri dish precluded determining the number of *L. cinctum* colonies. Seventy-six and 93% of dishes contained *Leucostoma* colonies in the first and second experiments, respectively (data not shown). Colonies were detected at heights of up to 1.52 and 1.83 m in the first and second experiments, respectively. All dishes positioned in tree crotches contained *L. cinctum*. Colonies of *L. cinctum* did not develop in dishes left covered during irrigation.

Orchard studies using under-the-canopy irrigation. Colonies of *L. cinctum* were observed in petri dishes situated from 0.5 to 5.9 m from cankers during the application of water. There was no apparent pat-

Table 1. Coefficients of correlation (*r*) between average monthly temperature, precipitation, and production of conidia by *Leucostoma cinctum* on peach and cherry cankers in the Yakima Valley during 1993 to 1995 and 2001 to 2002, respectively

Host	Year	<i>r</i> ^{a,b}	
		Temp. ^b	Precip. ^b
Peach	1993 ^c	-0.5346NS ^d	-0.5585NS
	1994	0.6582* ^c	-0.1951NS
	1995	0.6435*	-0.4111NS
Cherry	2001	0.6375*	-0.1437NS
	2002	0.5947*	-0.1709NS

^a The significance of correlation coefficients was determined with a standard *t* test at *P* < 0.05.

^b Analyses for temperature and precipitation were conducted on log transformed data.

^c Data collected from July to December.

^d NS = not significant.

Fig. 3. Sporulation of *Leucostoma cinctum* on cankers on 'Redhaven' peaches during over-the-canopy irrigation in Wapato, WA, during 1994 to 1995. Conidia were collected in water-borne spore traps positioned beneath cankers for 6 h during irrigation sets. Values represent the mean number of conidia per mm² of canker surface area recovered from 20 cankers.

Fig. 4. Number of colonies of *Leucostoma cinctum* splash dispersed to petri dishes positioned at different horizontal distances from a canker. Water drops were released onto canker surfaces from a butte positioned 2 m above the canker.

tern to dispersal using this irrigation method, and the number of colonies in dishes was not a function of distance from the inoculum source at the distances tested. Colonies of *L. cinctum* did not develop in dishes left covered during the application of water.

DISCUSSION

The results demonstrate that conidia of *L. cinctum* are produced in cherry and peach orchards in Eastern Washington throughout the year and the inoculum concentration is highest during spring and summer. The spring-summer sporulation peak documented in our studies is similar to patterns documented in other irrigated orchards (2,11). Bertrand and English (2) demonstrated wind-blown rain was a primary means of dispersal for conidia in French Prune orchards. Our results show *L. cinctum* appears to be dispersed in stone fruit orchards by natural precipitation and irrigation. In addition, both over-the-canopy and under-tree sprinkler irrigation can promote sporulation and disperse conidia during the growing season in Eastern Washington. This may be particularly important in regions where measurable precipitation is very sporadic during the growing season. These findings offer growers in Eastern Washington a means to reduce disease losses by altering pruning and irrigation practices that contribute to the development of *Leucostoma* canker.

The recognition that irrigation practice can be an important factor in the dispersal of conidia of *L. cinctum* has implications for disease management in the region. Producers of stone fruit in Eastern Washington have considered *L. cinctum* to be a pathogen most active during the autumn and winter months (9), when most annual precipitation is received, and they have adjusted their dormant pruning practices to avoid tree wounding during these periods. Our results on peach and cherry suggest pruning periods may be coincident with the early periods of the sporulation peak, and therefore growers should consider pruning trees during mid- to late-winter when inoculum concentrations are lower. Inoculum production and dispersal by irrigation practices commonly used in Eastern Washington should be considered when developing a pruning plan. "Summer" pruning, which consists of the removal of excess vegetative growth during early summer, is a practice commonly used to promote the development of anthocyanins in fruit to enhance marketability (12,13). The resulting wounds may become infected if irrigation water impacts cankers and splashes inoculum to wounds prior to healing. Delaying irrigation until wounds have healed could perhaps reduce infection of summer pruning in orchards

with canker-infested trees. Complete healing of peach wounds requires 390 (base = 0°C) accumulated degree-days (3). Based on temperatures recorded in the Wapato, WA, orchard during 2000 to 2004, healing would have required 14 to 21 days. Irrigation delays of this magnitude would be acceptable to Washington growers provided that compensatory water was applied during previous irrigation sets.

The sporulation and dispersal of *L. cinctum* during irrigation sets also raises interesting issues about the types of irrigation utilized in stone fruit orchards and their role in the prevalence and severity of the disease in the region. It is possible that the conversion to irrigation systems less likely to wet canker surfaces and distribute the causal organism to infection courts might over the long term reduce the incidence and severity of the disease. The two options available to growers appear to be to convert impact sprinkler systems to underground drip or microsprinkler irrigation. Both methods would be less likely to wet canker surfaces and provide the water drops necessary for dispersal. Alteration of irrigation types and/or practices has been recommended to reduce the incidence and severity of several orchard diseases (6,7,14). Michailides and Morgan (14) recommended lowering sprinkler angles to facilitate the orchard management of panicle blight of pistachio. Grove and Boal (7) recommended shortening the duration of over-the-canopy irrigation sets to reduce the incidence of fruit rots of apple and pear caused by *Phytophthora cactorum*. However, neither of these recommendations involved the expenses incurred during conversion of an impact sprinkler irrigation system to drip or microsprinkler systems. Therefore, producers should carefully weigh the costs versus benefits of switching irrigation methods. The cost of a microsprinkler irrigation system in a new orchard is estimated at US\$5,375 per hectare (10). The costs could be higher in an established orchard. Therefore, the decision to convert irrigation systems depends in large part on the profitability of the crop. For example, gross returns from a mature sweet cherry orchard in Washington are about US\$33,700 per hectare (10). At loss levels due to *Leucostoma* canker of 5% per year, the costs required for the installation of an irrigation system less conducive to the spread of *L. cinctum* would be recouped in 3 to 4 years. At current prices, recovering irrigation conversion costs in peach and nectarine orchards would require 6 to 7 years (G. G. Grove, unpublished). Because of high irrigation conversion costs and economic losses related to *Leucostoma* canker, growers should therefore consider the effects of irrigation system on disease development

when planning and designing new orchards.

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