Project Title: Etiology of Cherry Cankers and Dieback in the Pacific Northwest

Report Type: FINAL Project Report (NCE 2024)

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Project Duration: 1-Year

Total Project Request for Year 1 Funding: $ 9960 (**NCE**)

Other related/associated funding sources: None

WTFRC Collaborative Costs: None

Budget 1

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Wages[1]	$591.00
Benefits	$59.00
RCA Room Rental	
Shipping	
Supplies[2]	$3,720.00
Travel[3]	$3,590.00
Plot Fees	
Miscellaneous[4]	$1,000.00
Total	$8,960.00

Footnotes: [1] = timeslip labor for media preparation; [2] = petri plates, growth media (2 types), antibiotics, and orchard tools; [3] overnight (Grove) and local travel (Grove and DuPont); [4] = autumn canker/ dieback workshop

Budget 2
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Salaries	
Benefits	
Wages	
Benefits	
RCA Room Rental	
Shipping	
Supplies[1]	$500.00
Travel[2]	$500.00
Plot Fees	
Miscellaneous	
Total	$1,000.00

Footnotes: [1] = lab, field, outreach costs; [2] = local travel

Objectives
A. Sample symptomatic cherry trees in multiple orchards in Eastern WA and OR, where dieback has previously documented and include additional orchards in other regions in the PNW (no more than 5 per orchards per county).
B. Remove bark on and adjacent to diseased tissue and (using magnification) search for fungal fruiting bodies that could enable field identification.
C. Isolate, purify, morphologically characterize and store fungal and bacterial isolates from symptomatic tissue using standard sterile microbiological techniques. Photographically document canker morphology and isolate growth habit/color on growth medium.
D. Summarize known information about cherry dieback in the PNW and distribute the information to industry.

Methods
Over 70 orchards were sampled in 2023 while 16 additional orchard were sampled in 2024. Samples were collected in Adams, Benton, Grant, Hood River, Okanogan, Chelan, Yakima, Franklin, Walla Walla, Klickitat, Douglas, and Wasco Counties (Table 1). Cankers and dieback were found in most locations. Bark on and adjacent to diseased tissue was removed and (using magnification) observed for fungal fruiting bodies that in some cases facilitated identification (Figures 6 and 7). Six to eight fragments (~8 mm; 0.31") from the margin between healthy and necrotic tissue (Figures 2 and 3) were be taken from each sample. Fungal isolation was performed by surface sterilization using 95% ethanol or 0.055 NaOCL followed by immediate flaming and subsequent placement onto Potato Dextrose Agar (PDA) and Malt agar (MA) amended with 100 ppm (0.1g/L) tetracycline and 2.5 ml / l lactic acid. Pure cultures of each fungal isolate were obtained using single hyphal tip sub-culturing methods on PDA and MA (Figure 4). Cultures were incubated at ambient temperature conditions in darkness and then photographed to document colony color and morphology.

Hyphal transfers from pure cultures were made to glass microscope slides and observed for conidia and conidiophore morphology at 40 - 250 X. If fruiting bodies were observed in pure cultures, representative samples were transferred to glass slides observed at 100 X to determine fruiting body and ascospore or conidia morphology.

Significant findings:
- Canker / dieback issues are widespread and quite severe
- Bacterial, Leucostoma (=Cytospora), Eutypa, Calosphaeria, and Botryosphaeria cankers were documented in the region
- Bacterial canker and Leucostoma cankers were the most frequently encountered
- Calosphaeria canker was documented in 12 orchards (10 of those 12 were in Oregon).
- Multiple instances of mixed infections were documented
- > 30 fungi distinct from those listed above were collected from diseased tissue and stored in long-term culture. These await positive identification and proof of pathogenicity.
- Fungal fruiting bodies were easier to detect during late summer /fall *in situ*

Results and Discussion

Leucostoma (=Cytospora) and bacterial cankers were documented in the late 20th century as the primary canker issues facing cherry growers in the Pacific Northwest (PNW). However, cankers of dieback of

cherry have become more prevalent over the last decade and in some cases appear to different from known diseases. A canker survey was conducted during the 2023 and 2024 growing seasons when 86 orchards were sampled (Table 1). Many of these sites had disease symptoms and signs different from those of a typical bacterial canker infection. Plant material was observed for disease signs (fungal reproductive structures; Figures 6 and 7) and then tissue was removed from the edges of cankers (Figures 1 and 2) and cultured (Figure 2) on potato dextrose agar (PDA) and malt agar (MA). Mycelium from the edges of colonies were transferred to PDA and MA and incubated 14-21 days in darkness at 22 C (71.6 F) to purify cultures (Figure 4).

Canker and dieback was widespread and in some sites *quite* severe, far more than what was apparent in similar surveys conducted in late 20th century. Both bacterial and Leucostoma cankers were quite common but cankers caused by Eutypa (Figures 1 and 2), Calosphaeria (Figures 6 and 7), and Botryosphaeria were also documented in the region. Mixed infections (bacterial canker + Leucostoma, Leucostoma+ Eutypa, and Leucostoma + Calospshaeria) were also discovered (Figures 4-5) in multiple regions. However, over 30 fungi distinct from *L. cinctum*, *E. lata*, *C. pulchella,* and *Botrysphaeria* were isolated from diseased tissues. The pathogenicity of these isolates is unclear as are their respective roles in the cherry canker complex. Aside from the notable prevalence of *C. pulchella* in Oregon, no discernable geographic patterns were observed for the distribution of the other fungal pathogens. Canker/dieback causation varied significantly within production regions (Figure 4).

Table 1. Number (out of 86 orchards sampled) and percentage of orchards affected by Leucostoma, Eutypa, Calosphaeria, Botryosphaeria, Pseudomonas, and mixed infections.

	Leucostoma (Cytospora)	**Eutypa**	**Calosphaeria**	**Botryosphaeria**	**Pseudomonas (bacterial canker)**	**Mixed Infections[3]**
Number of orchards affected[1]	41	8	10[2]	2	12	14
Percent of orchards sampled	47.7	9.3	11.6	2.4	14	16.3

Footnotes: [1] of 86 orchards sampled; [2]80% of these orchards were in Oregon; [3]Leucostoma: Eutypa, Leucostoma:Calofsphaeria, Leucostoma: Pseudomonas

Figure 1. Tissue was taken from the margins of diseased tissue and plate on potato dextrose (PDA) and malt (agar) amended with oxytetracycline and lactic acid.

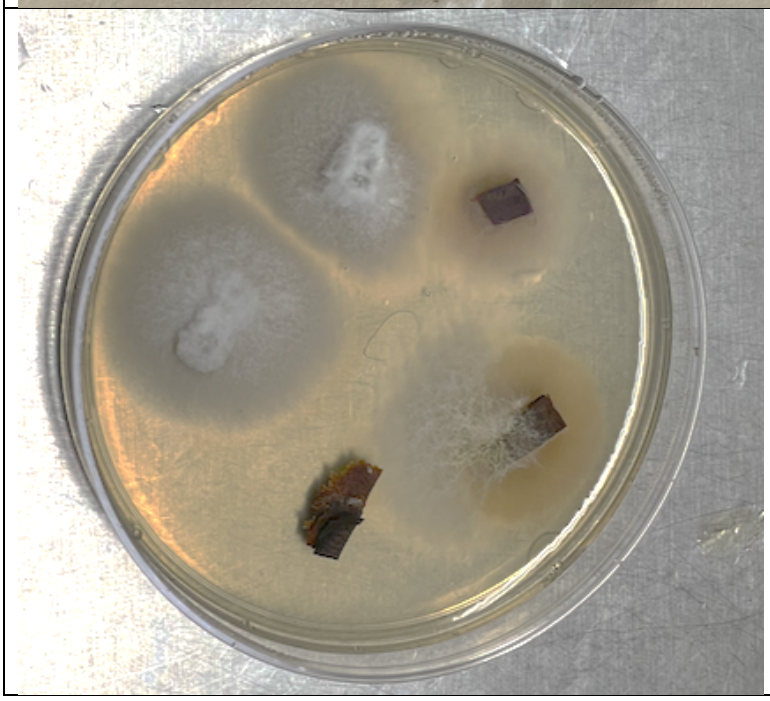

Figure 2. *Eutypa lata* growing from diseased cherry wood. Tissue was taken from the margins of diseased tissue and plate on potato dextrose (PDA) and malt (agar) amended with oxytetracycline and lactic acid.

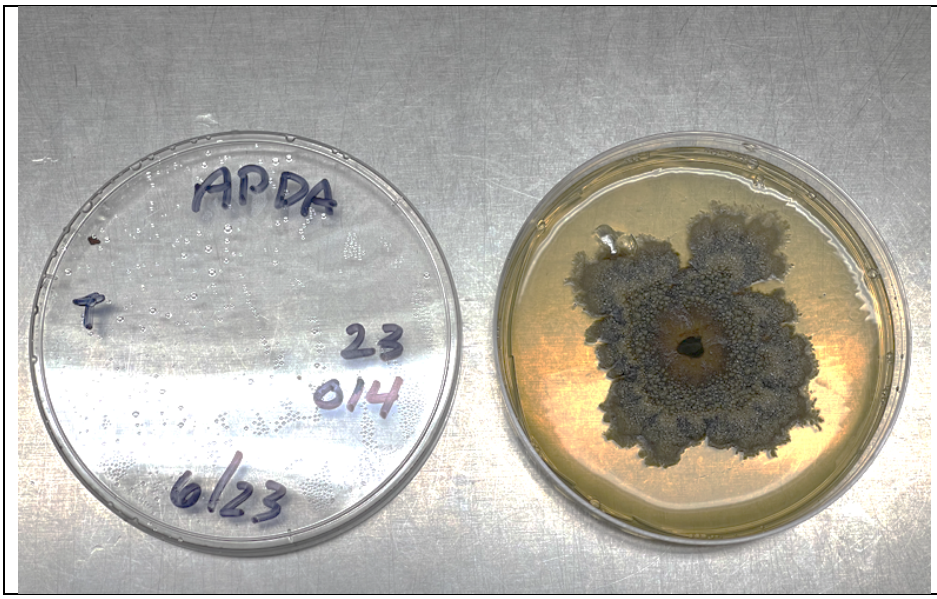

Figure 3. Leucostoma (=Cytospora) spp. Isolated from symptomatic "Chelan" trees near Wallula, WA. Isolate was obtained from a tree infected by both *Calosphaeria pulchella* and this isolate. See Figs. 6 and 7 below.

Figure 4. Results from various orchard sites in Franklin and parts of Adams and Walla Walla counties. Note that variation in causal agents and mixed infections.

	**Figure 5 (mixed infection).** Diseased Chelan tree near Wallula, WA. Tree is infected by both *Calosphaeria pulchella, Leucostoma* (=Cytospora) *cinctum,* and a second undetermined Leucostoma species (see Figure 4).
	**Figure 6**. Signs of *Calosphaeria pulchella*. Black areas are clusters of the sexual state (perithecia) of this pathogen.

Figure 7. Signs of *Calosphaeria pulchella.* Black-gray area is a clustes of the sexual state (perithecia) of this pathogen at higher magnification than in Figure 7. Each club-like structure is a perithecium (source of ascospores).

Extension. Current information on cankers of cherry was distributed via oral presentations at The Columbia Basin Tree Fruit Club (April 26, 2023), OSU Wasco County Preharvest Cherry Tour (June 6, 2023), Cherry Day in The Dalles (February 27, 2024), NCW Stone Fruit Day (January 17, 2027) Okanogan Horticultural Association Summer Field Day (August 3, 2023), Legacy Fruit Annual Meeting (March 14, 2024), G.S. Long Hood River Grower Meeting (January 5, 2024), and publications on the Tree Fruit Web Site / Fruit Matters:

Dupont, T., and Grove, G., and Thompson, A. 2023. Fungal Canker and Dieback Pathogens of Stone Fruit. *Fruit Matters*, August 2023. https://treefruit.wsu.edu/fungal-canker-and-dieback-pathogens-of-stone-fruit/.

Dupont, T., and Grove, G., and Thompson, A. 2024. Fungal Canker and Dieback Pathogens of Stone Fruit. *Fruit Matters*, August 2023. https://treefruit.wsu.edu/fungal-canker-and-dieback-pathogens-of-stone-fruit/.

Grove, G.G., and Sallato, B. 2023. The Fungi Among Us. *Fruit Matters*, August 2023. https://treefruit.wsu.edu/article/the-fungi-among-us/.

Executive Summary

A total of 86 orchards were sampled (70 in 2023, 16 in 2024) for wood dieback/cankers of *unknown (bacterial canker was not identified as the primary pathogen prior to the orchard visit)* etiology. Tissue samples were taken from the edges of cankers using a sterile razor blade, surface-disinfested using either sodium hypochlorite or ethanol / brief exposure to flame and placed on potato dextrose agar amened with tetracycline and lactic acid. Pure cultures of putative isolates were obtained by taking hyphae from the edges of fungal cultures and transferred to fresh PDA or malt agar plates. Colony morphology and the presence/morphology of fungal fruiting bodies and spore size/morphology were used to identify isolates. Leucostoma (=Cytospora) was isolated from samples taken from 47.7% of orchards sampled. Eutypa, Calorphaeria, and Botryosphaeria were isolated from samples taken from 9.3%, 11.6%, and 2.4% of orchards, respectively. Calosphaeria was the primary fungal pathogen isolated from Oregon orchards (9.3% of the aforementioned 11.6% of total orchards). Mixed infections were detected in 16.3% of the orchards sampled. Leucostoma and bacterial cankers were the most common. Numerous other fungi were isolated during the course of the study but their pathogenicity to cherry remains unclear; they are most likely saprophytic organisms common in diseased wood tissue. The epidemiology of Eutypa and Calosphaeria in the context of our regional climate and cultural practices needs further study.