FINAL PROJECT REPORT

Project Title: Prevalence, biology and management of bull's eye rot in apple

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Total Project Request: $50,000 **Year 1:** $25, 000 **Year 2:** $25,000 **Year 3: $0**

Other funding sources: Technical Assistance for Specialty Crop **(**TASC) $1000 per annum (2016-17) [funding dedicated only for information transfer from previous and future research on management of bull's eye rot].

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Item	2016	2017
Salaries[1]	$18,000	$18,000
Benefits	$2,000	$2,000
Wages		
Benefits		
Equipment		
Supplies	$3000	$3000
Travel[2]	$1000	$1000
Miscellaneous		
Plot Fees	$1,000	$1,000
Total	$25,000	$25,000

Footnotes: [1]Funding is requested to support a Research Assistant; [2]Travel to field sites, packing sheds, etc.

Objective:

1. Conduct a survey of packing houses and orchards to assess the prevalence of bull's eye rot and perennial canker in Washington State.
2. Prepare a collection of *Neofabraea* spp. isolates from different geographic regions in Washington State and assess fungicide sensitivity/resistance of the pathogen population.
3. Test and compare the efficacy of fungicide treatments and orchard management for the control of bull's eye rot in commercial orchards.
4. Develop methodology for control of bull's eye rot in storage and in orchards both post-harvest and pre-harvest.

Significant Finding:

1. *Neofabraea* spp. were isolated from samples collected from major apple production areas of Washington State, including Okanogan, Chelan, Wenatchee, Brewster and Yakima.
2. A total of 85 isolates of *Neofabraea* spp. was evaluated for sensitivity to four preharvest and two postharvest fungicides. The isolates were most sensitive to the preharvest fungicide Luna Sensation and the postharvest fungicide Penbotec.
3. Trials were conducted over two years to assess the efficacy of selected fungicides for the control of postharvest decay incited by *Neofabraea* spp. when applied in either the preharvest or postharvest setting. Findings to date indicate that the preharvest fungicide Luna Sensation is most effective in controlling postharvest bull's eye rot.

Methods:

Objective 1: Conduct a survey of packing houses and orchards to assess the prevalence of bull's eye rot and perennial canker in Washington State.

Symptomatic fruit were collected from various packing houses and information was collected relative to orchard location from which fruit were sourced, packing house location, and preharvest and/or postharvest fungicide applications that were implemented. The symptoms on each fruit were assessed visually and were recorded photographically. The fruit were then surface disinfected using 70% Ethanol, and fruit tissue was aseptically excised from the margin of diseased and healthy areas on the symptomatic fruit. This tissue section was transferred aseptically onto Potato Dextrose Agar (PDA) media and incubated at 70°F (20°C) in the dark for 2-3 weeks. The morphology of the fungi that emerged from the symptomatic tissues was recorded and was identified using taxonomic descriptions previously published. DNA was extracted from pure cultures of isolates that were identified as *Neofabraea* based upon morphological characteristics. This DNA was then used for molecular identification to species level through PCR amplification using species-specific primers.

Objective 2: Prepare a collection of Neofabraea spp. isolates from different geographic regions in Washington State and assess fungicide sensitivity/resistance of the pathogen population.

A total of 110 isolates of *Neofabraea* spp. were tested for sensitivity to the 6 selected fungicides listed below.

- Penbotec (Pyrimethanil)
- Mertect (Thiabendazole)
- Topsin (Thiophanate methyl)
- Luna Sensation (Fluopyram + Trifloxystrobin)
- Inspire Super (Difenoconazole+ Cyprodinil)
- Aprovia (Solatenol®)

Sensitivity of the fungal isolates to the selected fungicides was examined *in vitro* using agar-based assays that included media amended with a specific individual fungicide chemistry. For each test fungicide, the active chemistry was incorporated into media at five different (10-fold dilution series) concentrations, with each trial including a no fungicide control growth medium. At each fungicide concentration, each isolate was represented by three replicate test agar plates. Fungal growth and development traits that were measured in these studies included spore germination and mycelial growth across the agar surface. Assessments on fungicide amended media were compared relative to fungal growth and sporulation recorded on media without fungicide amendment. Fungicide sensitivity was expressed in terms of minimum inhibitory concentration (MIC; the minimum concentration that inhibits visible growth of the fungus) and EC_{50}, which is the concentration that reduces fungal growth by 50% compared with growth attained on media in the absence of the fungicide.

Objective 3: Test and compare the efficacy of fungicide treatments and orchard management for the control of bull's eye rot in commercial orchards.

Preliminary in vitro studies indicated that the pre-harvest fungicides Luna Sensation and Topsin and postharvest fungicides Penbotec and Mertect are effective in suppressing spore germination and mycelial growth. Hence, Luna Sensation and Topsin were selected for use as pre-harvest fungicide treatment and Mertect and Penbotec were utilized as post-harvest fungicide treatments in studies to examine fungicide efficacy in orchard trilas for the control of postharvest bull's eye rot in storage. One commercial orchard located at Orondo was included in this study because of the high incidence of bull's eye rot and perennial canker previously observed in fruit and trees at this site. The cultivars Golden Delicious, Red Delicious and Fuji were included in this study.

In pre-harvest trials, each selected fungicide was represented by 4 replicates, and each replicate consisted of 5 trees in a completely randomized design. A no fungicide treatment was included as the control. Fungicide treatments were applied to fruit 2 weeks prior to harvest and fifty fruit per replicate were harvested on commercial harvest dates. Golden Delicious fruit were harvested on September 6th, 2016, Red Delicious fruit harvested on

September 14th 2016 and Fuji fruit were harvested on September 30th 2016. In 2017 Golden Delicious was harvested on 20th September, Red Delicious on 2nd October and Fuji on 20th October. Fruit was stored in boxes lined with plastic liners and on fiber trays in a cold room at regular atmosphere and 32° F (0° C) for up to 8 months post-harvest (mph). Fruit will be monitored monthly for any bull's eye rot development starting at 3 mph and continuing for the duration of the storage period. Incidence of bull's eye rot in control fruit versus fungicide treated fruit will be compared to assess fungicide efficacy.

In the study of post-harvest fungicide treatments, for each variety and on each harvest date as stated above, 50 fruits were harvested per replicate with a total of 4 replicates per treatment. Again fruits were harvested from the same orchard and same block as the pre-harvest fungicide trial. A buffer zone was employed that consisted of two rows between the tree rows treated with preharvest fungicide and those tree rows from which fruit were harvested for application of postharvest drench and no fungicide control. Fruit were harvested on the same dates as noted above and received a drench of the representative fungicide treatment. Fruit were stored in boxes in the same manner as described above and in regular atmosphere at 32°F in cold rooms and inspected monthly for the incidence of bull's eye rot from 3 mph to 8 mph. Fungicide efficacy was expressed as relative incidence of bull's eye rot in treated fruit compared to the no treatment control. The experiment was conducted for two years.

Objective 4: Develop methodology for control of bull's eye rot in storage and in orchards both post-harvest and pre-harvest.

This research is directed towards controlling bull's eye rot in commercial orchards. Information from fungicide trials and orchard management trials will be incorporated towards development of grower bulletin / handouts that will be circulated online or in print.

Results:

Objective: 1 *survey of packing houses and orchards to assess the prevalence of bull's eye rot and perennial canker in Washington State.*

Isolates that were collected from 2011-2017 and also some from 2005 were included in this study as the initial information regarding the distribution of the pathogens across Washington State (Table1). *Neofabraea perennans* was the primary species of the causal fungus isolated from the samples collected in the scope of this study. However, *N. kienholzii* was recovered from samples collected in a previous survey from 2005. *N. perennans* was recovered from samples collected from all major apple producing areas in the state including the north, central and southern regions.

Table 1: Distribution of *Neofabraea* spp. complex in Washington State, with information on application of pre-harvest or postharvest treatments, variety of apple, location of orchard and identity of species.

Year Collected	Location of Orchard	Variety	Treatment	Species identified
2017	Othello	Fuji	_	*N. perennans*
2017	Othello	Pink Lady	_	*N. perennans*
2016	Orondo	Golden Delicious	_	*N. perennans*
2015	Brewster	Granny Smith	_[a]	*N. perennans*
2015	Chelan	Gala (twig)	_	*N. perennans*
2015	Orondo	Golden Delicious	Scholar Max (MCP)	*N. perennans*
2015	Wenatchee	Gala	Pyrimethanil	*N. perennans*
2015	East Wenatchee	Golden Delicious	Scholar Max (MCP)	*N. perennans*
2015	Wenatchee	Cripps Pink	Organic	*N. perennans*
2013	Manson	Golden Delicious	no PH treatment	*N. perennans*
2013	Manson	Red Delicious	no PH treatment	*N. perennans*
2011	Yakima	Granny Smith	_	*N. perennans*
2005	Wapato	Golden Delicious	_	*N. perennans*
2005	Mattawa	Fuji	DPA [c] drenched	*N. kienholzii*
2005	Tonasket	Red Delicious	drenched	*N. kienholzii*

[a] '-' represents no information available regarding treatments applied
[b] PH meaning postharvest
[c] DPA diphenylamine

Objective 2: Prepare a collection of Neofabraea spp. isolates from different geographic regions in Washington State and assess fungicide sensitivity/resistance of the pathogen population.

Among 110 isolates tested, a total of 85 isolates produced conidia that germinated or showed growth when transferred onto PDA plates that contained no fungicide. Hence, a final analysis of EC 50 and MIC was analyzed using these 85 isolates. Below is a summary of results obtained from the analysis of EC_{50} and MIC data:

- All 85 isolates examined were sensitive to the pre-harvest fungicide containing fluopyram and trifloxystrobin (Luna Sensation). EC_{50} ranged from less than 0.1 -1 ppm and MIC 0.1 -100 ppm. Most isolates were highly sensitive to this chemistry (79 out of 85) having an EC_{50} of less than 0.1 ppm.

- EC_{50} for preharvest fungicide containing thiophanate methyl (Topsin) ranged from 1ppm to greater than 1000 ppm. The MIC was greater than 1000 ppm for 40 of the 85 isolates examined. This finding indicates that a high percentage of the isolates comprising the *Neofabraea* population examined possesses resistance to this fungicide chemistry.

- When tested against the fungicide Solatenol (Aprovia), EC_{50} ranged from 0.1ppm- 100 ppm. MIC ranged from 10ppm-1000ppm, and one isolate possessed and MIC greater than 1000ppm.

- In assays conducted using fungicide containing difenoconazole and cyprodinil (Inspire Super), *Neofabraea* spp. isolates exhibited EC_{50} values ranging from 0.1ppm-140ppm. MIC ranged from 0.1ppm to greater than 1000ppm.

- Isolates screened against the postharvest fungicide pyrimethanil (Penbotec) had EC_{50} values ranging from less than 0.1 to 482.7 ppm. MIC ranged from 1ppm to 150ppm.

- When screened against the postharvest fungicide containing thiabendazole, EC_{50} ranged from less than 0.1 to 4775 ppm. MIC ranged from 10 ppm to greater than 1000 ppm. 14 out of 85 isolates tested had MIC greater than 1000 ppm indicating the presence of resistant strains in the population.

Objective 3: Test and compare the efficacy of fungicide treatments and orchard management for the control of bull's eye rot in commercial orchards.

In 2016-17 season, preharvest application of fungicide containing fluopyram and trifloxystrobin (Luna Sensation) was most effective in controlling the postharvest incidence of bull's eye rot in Golden Delicious, Red Delicious and Fuji apple. However, postharvest fungicide containing pyrimethanil (Penbotec) was equally as effective in controlling bull's eye rot in Golden Delicious and Fuji apple. Initial screening of fruit collected during the 2017-18 season for symptom development was initiated in December 2017.

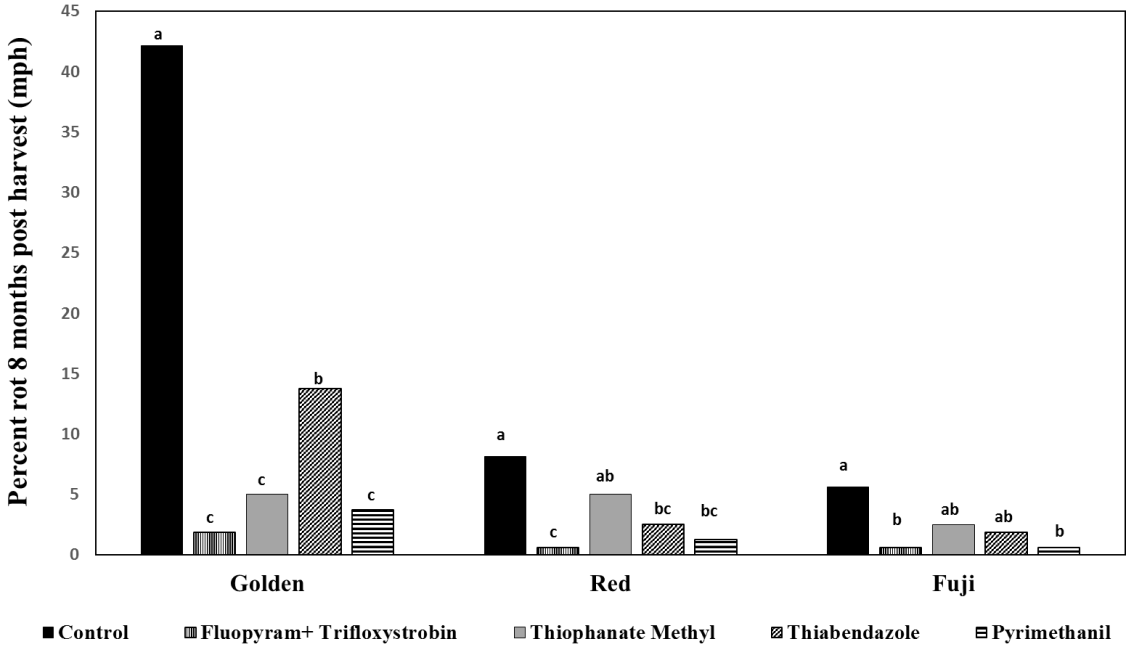

Fig 1: Efficacy of selected fungicide treatments for the control of postharvest decay (Bull's eye rot) in apple incited by *Neofabraea* spp. during the 2016-2017 harvest/storage season. Three apple varieties were tested i.e. Golden Delicious, Red Delicious and Fuji. For a given apple cultivar, means (bars) designated with the same letter are not significantly ($P < 0.05$) different.

Objective 4: Develop methodology for control of bull's eye rot in storage and in orchards both post-harvest and pre-harvest.

The findings from these studies were presented at American Phytopathological Society annual meeting in 2016 and at the Pacific Division meeting of American Phytopathological Society in 2017. All results will summarized and prepared for submission to peer reviewed journal by 2018.

Executive Summary

Neofabraea perennans and N. kienholzii are major causal pathogens of bull's eye rot of apple in Eastern WA. These fungi cause significant economic loss to the Washington State apple industry and have been listed as quarantine pathogens. Previous experiments indicate that fungicide treatments containing thiabendazole and thiophanate methyl provide effective bull's eye rot control, however use of this treatment is not recommended due to the potential for resistance development in populations of *Penicillium* spp. Hence, the objective of this research was to screen newer commercially available fungicides, possessing multiple active chemistries, for bull's eye rot control. Fungal sensitivity and disease control efficacy of the selected fungicides were evaluated *in vitro* and in orchard trials, respectively. Results from these trials with *Neofabraea* spp. isolates suggest that fungicide containing fluopyram and trifloxystrobin may inhibit *Neofabraea* spp. *in vitro* at a level equivalent to that of thiabendazole. *Neofabraea* spp. isolates resistant to preharvest fungicide difenoconazole and cyprodinil, and postharvest fungicide pyrimethanil were identified in the trial. Field trials utilizing preharvest sprays and postharvest drenches indicate that preharvest applications two weeks before harvest with fungicide containing fluopyram and trifloxystrobin is most effective in controlling postharvest bull's eye rot in storage.