FINAL PROJECT REPORT

Project Title: Fungicide evaluation for the control of bull's eye rot of apple

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Cooperators:

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Total Project Funding: $39,700

Budget History:

Item	Year 1: 2014	Year 2: 2015
Salaries*	$14,000	$15,000
Benefits	$2,000	$2,200
Wages		
Benefits		
Equipment		
Supplies	$2,000	$2,500
Travel	$500	$500
Plot Fees	$1,000	$1,000
Miscellaneous		
Total	$19,500	$21,200

Footnotes: *Funding is requested to support a Research Assistant

OBJECTIVES

(1) Evaluate the efficacy of select pre-harvest fungicides and post-harvest fungicide drenches for the control of bull's-eye rot of apple incited by *Neofabraea perennans* and *Cryptosporiopsis kienholzii*.

(2) Determine the effectiveness of fungicide applications in the control of early versus late season apple fruit infection by *N. perennans* and *C. kienholzii* occurring in the field.

SIGNIFICANT FINDINGS

During both annual trials of this experiment, the only pre-harvest fungicide treatment providing statistically significant pre-harvest control of bull's eye rot caused by *Neofabraea perennans* or *Cryptosporiopsis kieholzii* was thiophanate-methyl (Topsin-M).

During both annual trials of this experiment, the only postharvest fungicide treatments providing statistically significant control of bull's eye rot caused by *N. perennans* or *C. kienholzii* were thiabendazole (Mertect) and pyrimethanil (Penbotec).

Inoculations conducted later in the growing season (at two weeks before harvest) resulted in higher incidences of bull's eye rot compared to early and mid-season inoculation periods (eighteen and five weeks before harvest, respectively). Regardless of inoculation timing, the pre-harvest fungicide thiophanate-methyl, and postharvest fungicides thiabendazole and pyrimethanil were most effective for control of *N. perennans* and *C. kienholzii* infections.

RESULTS

Efficacy of pre-harvest fungicide applications

Only two fungal inoculation time-points were explored during the first year of this study, (five and two weeks prior to harvest). Infection was significantly greater when inoculations were conducted at two weeks prior to harvest compared to inoculations at five weeks before harvest ($P < 0.0001$). Overall the proportion of apples with bull's eye rot decay due to *N. perennans* infection was not significantly different from infections attributed to *C. kienholzii* (P=0.0960). Average bull's eye rot recovery for fruit treated with zinc (Ziram), and pyraclostrobin plus boscalid (Pristine) was not significantly different from average disease incidence recorded in the no fungicide control treatment. Only fruit treated with thiophanate-methyl (Topsin-M) exhibited a statistically significant lower disease incidence (P<0.0001; Table 1).

An additional inoculation time-point meant to simulate early season fruit infection was conducted during the second year of this experiment, (eighteen weeks before harvest). A significantly greater proportion of fruit were infected when inoculations were conducted at two weeks before harvest compared to five and eighteen weeks before harvest (P<0.0001). A statistically greater incidence of infection attributed to *N. perennans* was observed compared to inoculations with *C. kienholzii* (P=0.0193). Similar to the previous year, the only pre-harvest fungicide that effectively reduced *N. perennans* and *C. kienholzii* infections was thiophanate-methyl (P<0.0001; Table 1).

Efficacy of postharvest fungicide drenches

Inoculations conducted during the first year of this study yielded a greater incidence of bull's eye rot when *N. perennans* and *C. kienholzii* were applied at two weeks prior to harvest rather than five weeks (P<0.0001). In general, greater incidence of disease was attained when fruit inoculations were conducted using a spore suspension of *N. perennans* instead of *C. kienholzii* (P=0.0002). Bull's eye rot recovery for fruit treated with fludioxonil (Scholar) was not statistically different from recovery rates observed for the no fungicide control. Applications of thiabendazole (Mertect) and pyrimethanil (Penbotec) to *N. perennans* and *C. kienholzii* inoculated fruit prior to storage resulted in significantly less bull's eye rot (P<0.0001), with thiabendazole treated fruit exhibiting less disease on average compared to fruit treated with pyrimethanil (Table 2).

During the second year of this study, bull's eye rot incidence was significantly higher when pathogen inoculum was applied to fruit two weeks prior to harvest rather than five and eighteen weeks before harvest (P<0.0001). Inoculations using a spore suspension of *N. perennans* conidia yielded significantly more bull's eye decayed fruit compared to *C. kienholzii* inoculations (P<0.0001). Bull's eye rot incidence for fruit treated with fludioxonil prior to storage was proportionally as high as fruit left untreated. Application of difenoconazole plus fludioxonil (Academy) to pathogen inoculated fruit only slightly reduced bull's eye rot incidence compared to the no fungicide control. Only fruit treated with thiabendazole or pyrimethanil demonstrated a significant reduction in bull's eye rot due to *N. perennans* and *C. kienholzii* inoculations (P<0.0001; Table 2).

DISCUSSION

In light of the temporary suspension of shipment of Washington grown apples to the Chinese market and subsequent strict phytosanitary regulations established in response to postharvest decay pathogens (Warner, 2014), bull's eye rot has become an economically important disease for pome fruit growers and packers of the Pacific Northwest region. Once considered a minor disease in Washington State, bull's eye rot outbreaks have become increasingly more common over the past decade. In effort to curtail the occurrence of bull's eye rot in this area, various fungicides registered for use on pome fruit were selected and their efficacy against bull's eye rot was tested. Results from this study indicate that among the pre-harvest and postharvest fungicides evaluated, only thiophanate-methyl (Topsin-M), pyrimethanil (Penbotec) and thiabendazole (Mertect) were effective in providing adequate control of bull's eye rot in stored apple relative to a no treatment control. While results from this study obviously provide growers and packers with invaluable information pertaining to bull's eye management, it also highlights the complexities encountered by members of the apple industry in dealing with postharvest decay issues.

As benzimidazole fungicides, thiophanate-methyl and thiabendazole are classified as having moderate to high risk of resistance developing in pathogen populations (Fungicide Resistance Action Committee). This fact has already been demonstrated by the appearance of thiabendazole-resistant strains of *Penicillium expansum* (Li and Xiao, 2008) and *Botrytis cinerea* (Zhao et al., 2010) throughout cold storage facilities in Washington State. The short life cycle and high sporulation capacity of *P. expansum* make it a likely candidate for developing resistance to high risk fungicides. *Neofabraea* species, however, are comparatively slow growing, and in requiring water splash for spore dissemination, are less capable of spreading at a high rate thus posing less of a risk for developing fungicide resistance. Nevertheless, thiophanate-methyl-resistant strains of *N. perennans* and *N. alba* have already appeared in pathogen populations originating from Northern Germany, partially in response to excessive use of this fungicide (Weber and Palm, 2010). The fact that two of the most effective fungicides available to pome fruit growers for bull's eye rot control share a common mode of action, should be of concern for the Washington apple industry. In order to minimize the potential for resistance, chemistries with differing modes of action should be alternated regularly. As a fungicide belonging to the anilinopyrimidine class, pyrimethanil seemingly presents a useful alternate fungicide as part of a bull's eye rot management program. Unfortunately, pyrimethanil resistance has also appeared in *P. expansum* populations originating in Washington State (Xiao et al., 2011), further confounding bull's eye rot management. While the primary aim of this project was to provide growers with information concerning fungicides that can be used to successfully manage bull's eye rot, the results from this work further highlight the resounding need for additional fungicides registered for use in pome fruit production systems.

REFERENCES

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Zhao, H., Kim, Y. K., Huang, L., and **Xiao, C. L.** 2010. Resistance to thiabendazole and baseline sensitivity to fludioxonil and pyrimethanil in *Botrytis cinerea* populations from apple and pear in Washington State. Postharvest Biology and Technology 56:12-18.

Table 1. Average recovery of bull's eye rot from cv. Fuji apples inoculated with spores of either *Neofabraea perennans* or *Crytosporiopsis kienholzii* at various pre-harvest periods and treated with select pre-harvest applied fungicides.

Year	Pathogen	Inoculation period (weeks before harvest)	Pre-harvest fungicide	Average bull's eye rot recovered (%)
2014	*Neofabraea perennans*	5 wbh	No fungicide control	63.75%
			Zinc (Ziram)	62.50%
			Pyraclostrobin + boscalid (Pristine)	58.75%
			Thiophanate-methyl (Topsin-M)	15.00%
		2 wbh	No fungicide control	78.75%
			Zinc (Ziram)	81.25%
			Pyraclostrobin + boscalid (Pristine)	81.25%
			Thiophanate-methyl (Topsin-M)	27.50%
	Cryptosporiopsis kienholzii	5 wbh	No fungicide control	49.25%
			Zinc (Ziram)	37.50%
			Pyraclostrobin + boscalid (Pristine)	60.00%
			Thiophanate-methyl (Topsin-M)	7.50%
		2 wbh	No fungicide control	88.75%
			Zinc (Ziram)	65.00%
			Pyraclostrobin + boscalid (Pristine)	90.00%
			Thiophanate-methyl (Topsin-M)	17.50%

Table 1 (continued). Average recovery of bull's eye rot from cv. Fuji apples inoculated with spores of either *Neofabraea perennans* or *Crytosporiopsis kienholzii* at various pre-harvest periods and treated with select pre-harvest applied fungicides.

Year	Pathogen	Inoculation period (weeks before harvest)	Pre-harvest fungicide	Average bull's eye rot recovered (%)
2015	*Neofabraea perennans*	18 wbh	No fungicide control	17.50%
			Zinc (Ziram)	22.50%
			Pyraclostrobin + boscalid (Pristine)	23.75%
			Thiophanate-methyl (Topsin-M)	8.75%
		5 wbh	No fungicide control	31.25%
			Zinc (Ziram)	22.50%
			Pyraclostrobin + boscalid (Pristine)	32.50%
			Thiophanate-methyl (Topsin-M)	14.00%
		2 wbh	No fungicide control	69.75%
			Zinc (Ziram)	38.75%
			Pyraclostrobin + boscalid (Pristine)	57.50%
			Thiophanate-methyl (Topsin-M)	16.25%
	Cryptosporiopsis kienholzii	18 wbh	No fungicide control	36.25%
			Zinc (Ziram)	10.00%
			Pyraclostrobin + boscalid (Pristine)	21.25%
			Thiophanate-methyl (Topsin-M)	12.50%
		5 wbh	No fungicide control	12.50%
			Zinc (Ziram)	17.50%
			Pyraclostrobin + boscalid (Pristine)	17.50%
			Thiophanate-methyl (Topsin-M)	11.00%
		2 wbh	No fungicide control	52.50%
			Zinc (Ziram)	37.50%
			Pyraclostrobin + boscalid (Pristine)	43.75%
			Thiophanate-methyl (Topsin-M)	10.00%

Table 2. Average recovery of bull's eye rot from cv. Fuji apples inoculated with spores of either *N. perennans* or *C. kienholzii* in the field at various pre-harvest periods and treated with select postharvest applied fungicides.

Year	Pathogen	Inoculation period (weeks before harvest)	Postharvest fungicide	Average bull's eye rot recovered (%)
2014	*Neofabraea perennans*	5 wbh	No fungicide control	61.25%
			Fludioxonil (Scholar)	52.50%
			Pyrimethanil (Penbotec)	17.50%
			Thiabendazole (Mertect)	8.75%
		2 wbh	No fungicide control	60.00%
			Fludioxonil (Scholar)	73.75%
			Pyrimethanil (Penbotec)	61.25%
			Thiabendazole (Mertect)	20.00%
	Cryptosporiopsis kienholzii	5 wbh	No fungicide control	51.25%
			Fludioxonil (Scholar)	62.50%
			Pyrimethanil (Penbotec)	2.50%
			Thiabendazole (Mertect)	1.25%
		2 wbh	No fungicide control	89.00%
			Fludioxonil (Scholar)	86.25%
			Pyrimethanil (Penbotec)	1.25%
			Thiabendazole (Mertect)	2.50%

Table 2 (continued). Average recovery of bull's eye rot from cv. Fuji apples inoculated with spores of either *N. perennans* or *C. kienholzii* in the field at various pre-harvest periods and treated with select postharvest applied fungicides.

Year	Pathogen	Inoculation period (weeks before harvest)	Postharvest fungicide	Average bull's eye rot recovered (%)
2015	*Neofabraea perennans*	18 wbh	No fungicide control	17.50%
			Fludioxonil (Scholar)	27.50%
			Pyrimethanil (Penbotec)	6.25%
			Thiabendazole (Mertect)	10.00%
			Difenoconazole + Fludioxonil (Academy)	20.00%
		5 wbh	No fungicide control	48.75%
			Fludioxonil (Scholar)	36.25%
			Pyrimethanil (Penbotec)	17.50%
			Thiabendazole (Mertect)	12.50%
			Difenoconazole + Fludioxonil (Academy)	25.00%
		2 wbh	No fungicide control	68.75%
			Fludioxonil (Scholar)	41.25%
			Pyrimethanil (Penbotec)	14.00%
			Thiabendazole (Mertect)	28.00%
			Difenoconazole + Fludioxonil (Academy)	52.50%
	Cryptosporiopsis kienholzii	18 wbh	No fungicide control	26.25%
			Fludioxonil (Scholar)	26.25%
			Pyrimethanil (Penbotec)	1.25%
			Thiabendazole (Mertect)	5.00%
			Difenoconazole + Fludioxonil (Academy)	18.25%
		5 wbh	No fungicide control	21.25%
			Fludioxonil (Scholar)	15.00%
			Pyrimethanil (Penbotec)	1.00%
			Thiabendazole (Mertect)	0.00%
			Difenoconazole + Fludioxonil (Academy)	10.00%
		2 wbh	No fungicide control	60.00%
			Fludioxonil (Scholar)	52.50%
			Pyrimethanil (Penbotec)	1.00%
			Thiabendazole (Mertect)	6.00%
			Difenoconazole + Fludioxonil (Academy)	37.50%

EXECUTIVE SUMMARY

The primary objectives of this research were to evaluate the efficacy of various pre-harvest and postharvest applied fungicides for control of bull's eye rot caused by *Neofabraea perennans* and *Cryptosporiopsis kienholzii*. To accomplish this goal, fungicide evaluations were conducted in the orchard at multiple pathogen inoculation intervals and the trials were replicated across two years. Data from both years consistently indicated that among the fungicides tested, thiophanate-methyl is the only pre-harvest fungicide capable of adequate bull's eye rot control while pyrimethanil and thiabendazole were the only two postharvest chemistries providing acceptable control of these two pathogens.

These data come at a pivotal time as bull's eye rot and other postharvest diseases native to the Pacific Northwest now present quarantine concerns for international trade. During the year prior to China's temporary trade closure of Washington grown apples, the value of Washington apples shipped to China was estimated at $6.5 million. Interception of Sphaeropsis rot, speck rot and bull's eye rot on apples exported from Washington resulted in a two year shut down that potentially cost the Washington apple industry $13 million. The need to identify fungicides that can effectively manage bull's eye rot is high, and while this research accomplishes this need, it also emphasizes the need for additional research in this area.

The three fungicides identified as effective against bull's eye rot only provide temporary relief for this complex situation. Issues regarding fungicide resistance are a major concern that can only exacerbate the future of bull's eye rot management. Newly registered fungicides for use in pome fruit production have become available during the course of this study. These new fungicides provide a great opportunity for additional work to be completed in this research area.

Currently, *in vitro* spore germination and mycelial growth assays using fungicide amended media are being conducted in the Mazzola laboratory for control of bull's eye rot and other fungi causing economically important postharvest disease. The outcome of this work appears promising, and should contribute much needed information to strengthen bull's eye management.