THESIS

CHEMICAL CONTROL OF *CYTOSPORA LEUCOSTOMA*, A MAJOR LIMITING FACTOR OF PEACH PRODUCTION IN WESTERN COLORADO

Submitted by

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ABSTRACT

CHEMICAL CONTROL OF *CYTOSPORA LEUCOSTOMA*, A MAJOR LIMITING FACTOR OF PEACH PRODUCTION IN WESTERN COLORADO

Cytospora canker, *Cytospora leucostoma*, is a major limiting factor of peach production in Colorado, accounting for 15-20% of crop production loss annually. Given the unique environmental factors of the western slope region of Colorado, *C. leucostoma* has developed into a severe fungal disease, reducing yields annually. Chemical measures are important for *Cytospora* control as few options currently exist for preventing new infections. The specific objectives of the following thesis are to: (1) evaluate the efficacy of conventional and organic fungicides for *C. leucostoma* control, (2) test wound sealing fungicides, embedded in paint or kaolin clay, to develop preventive and containment approaches in existing orchards. Topsin, Topsin amended in 50% latex, Captan, Captan amended in 50% latex, 50% latex, lime sulfur, and lime sulfur amended in kaolin clay (Surround WP) showed evidence of efficacy from laboratory and field trials. Of these treatments, 50% latex, Topsin amended in 50% latex, and Captan amended in 50% latex, were shown to limit pathogen growth most effectively on pruning wounds during field trials in the summer season. In all field trials, however, NuCop showed absolutely no efficacy and should be avoided for *C. leucostoma* control in western Colorado. When various chemicals were tested on existing cankers to reduce spore inoculum loads, efficacy could not be statistically confirmed. The results of this study were compicated by variable field conditions and a large range of spore produced by each canker. Thus, larger sample sizes should be used in future studies to tease a part chemical efficacy and abiotic influences.

Further, kaolin clay alone may shield fungal cankers from extreme temperatures, enhancing its growth in the field.

DEDICATION

I would like to express my deepest gratitude to Dr. Jane Stewart, for accepting me into her lab and molding me as a graduate student and researcher. Without the guidance and support of Dr. Stewart this work would not have been possible. I am fortunate to have been able to study and learn under such an accomplished plant pathologist. I would also like to thank Dr. Ioannis Minas for the extensive time and mentorship he has dedicated to both my personal advancement and to the research presented in the following thesis. In addition, I extend my gratitude to Dr. Andrew Norton and to Dr. Mark Uchanski for serving as committee members on this project. Further, I would like to thank all the funding sources for the research presented. The United States Department of Agriculture - Colorado State Department of Agriculture – Specialty Crop Block Grant Program, Upper Grand Valley Pest District, the Western Colorado Horticultural Society, and the Agriculture Experimental Station of Colorado State University. Without these recourses, this project would not have been possible.

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INTRODUCTION

Over the last one hundred years, western Colorado has developed an exceptional peach production industry, with peaches known for premium market qualities such as savor, size, color, and texture. With superior market acceptance, the value of utilized production totals in Colorado ranges from $17 to 30 million annually (USDA 2016). Cytospora canker, caused by *Cytospora leucostoma*, is one of the most concerning fungal diseases faced by peach growers on the western slope of Colorado, the major peach production area of Colorado. Despite being an important pathogen of several different cultivated crops, this pathogen has thrived in peach orchards in the Grand Junction region for more than fifty years. Given the current disease incidence in Grand Junction and the pathogen's potential to devastate entire orchards, *Cytospora* could dismantle the peach industry of Colorado if left untreated. Such an event would be reminiscent to the collapse of the apple industry on the western slope of Colorado in the early 1900's during the codling moth infestation. Codling moth developed chemical resistance, and caused the subsequent pull up and destruction of more than a half million apple trees prior Dichlorodiphenyltrichloroethane (DDT) introduction (Sexton 1995). Because of the codling moth infestation, apple production costs in the Grand Junction area became intolerable and tree fruit growers were forced to seek alternative fruit crops, such as peaches and other fruit crops that the moth was not a major pest in (Sexton 1995).

In preliminary surveys conducted by the Colorado State University Western Colorado Research Station in 2015, it was found that 100% of the orchards in western Colorado have

Cytospora canker with infection rates ranging from 33-100%, accounting for 15-20% of losses in stone fruit production annually, equating to over 3 million dollars in annual losses (Pokharel & Larsen 2009; USDA 2016). Along with the continual pathogen pressure, environmental factors unique to the high elevation of western Colorado, such as low winter temperatures, late spring freezes, diminishing water supplies, and alkaline soils, have only exacerbated the problem, weakening trees and allowing for increased pathogen aggression. Given the current incidence and severity of the Cytospora canker, this pathogen has developed into a major limiting factor of peach production in Colorado.

Currently, there are no standard cultural or chemical control methods suitable for conventional and organic production systems for this disease. Fungicides have yet to be registered and previous studies have shown conflicting results regarding pathogen control (Biggs & Grove 2005). To shield the Colorado fruit production industry from a major fruit crop production shift, integrated management practices must be developed and utilized among growers in this region. Essential to developing effective cultural practices is the uncovering of effective chemical treatments for proper control of the fungus.

Thus, the specific objectives of the research presented in this thesis include:

1. Evaluate the efficacy of conventional and organic fungicides for *Cytospora leucostoma* control,

2. Test wound sealing alternatives, with fungicides embedded in paints and kaolin clay, to develop preventive and spore containment approaches in existing orchards.

***Cytospora* Distribution.** Cytospora canker is caused by a fungal genus (*Cytospora*) that is known to infect at least sixty genera of hardwood and conifer trees (Grove 1935; Adams 2002).

Cytospora leucostoma has been reported in more than thirty-five specialty hosts around the world (Farr & Rossman 2017). As mentioned previously, one of the most troubling occurrences of this pathogen can be found in stone fruits, where large economic losses are common (Biggs 1989). Tree fruit crops where *Cytospora* poses a serious threat include, but are not limited to, peach [*Prunus persica* (L.) Batsch], cherry [*Prunus avium* (L.) L.], and plum [*Prunus domestica* (L.)] (Adams 2002; Biggs 2005). In western Colorado, this pathogen is most common and most detrimental in peach orchards. The first reports of this organism infecting peach trees in the United States were made in the early 1900's in New York and Missouri (Stewart et al. 1900; Rolfs 1909; Biggs 1989). Reports of this pathogen have since spread across the United States, and it can be found in most major peach production regions from New York to Florida and to California (Alfieri et al. 1973). Reports of Cytospora canker in stone fruits can also be found on a larger global scale as well. Despite being present globally, this pathogen is not always considered a major limiting factor in all agricultural systems. The pathogen is considered a "weak parasite," thus for it to thrive in production systems, specific biotic and abiotic factors inducing plant stress are important for pathogen establishement (Biggs 1989). The severity and prevalence of infections depend greatly on the given region and the region's influence on tree health status. Nonetheless, it has been reported as a major limiting factor in stone fruit crops in East Asia (Japan, Korea, Primorye province of Russia, and China), Europe (Hungary, Czechoslovakia, Yugoslavia, Northern Italy, and Northern France), and North America (Canada and The United States) (Vasilyeva et al. 2000; Rozsnyay 1977; Wang et al. 2016).

***Cytospora* Biology.** Cytospora canker in peach is thought to be a casual or "weak parasite" organism as it cannot invade healthy intact bark and requires a wound as a mode of entry (Biggs

1989). Once an infection has occurred, cankers are formed near the wound sites where the fungus will initially colonize the bark and cambial tissues (Biggs 1986). The most common infection sites on a given tree include both pruning and freeze damage wounds. Cankers eventually extend into vascular system of the tree, disrupting the conductivity of the xylem tissue in the areas close to the infection sites, with an active xylem infection being an essential part of the translocation of the disease (Hampson & Sinclair 1973). Girdling of the xylem eventually leads to dieback symptoms, which become especially evident on younger branches and small twigs, as rapid growth of the mycelium can cause canker lesion development on the entire circumference of a branch or small twig (Wang et al. 2016). From the time of an infection, the host will decline in production until it finally succumbs to the pathogen. Once this occurs, *Cytospora* lives as a saprophyte in the non-living host tissue and continue to produce inoculum for further spread.

Once cankers have formed, the pathogen will produce pycnidia (asexual) fruiting bodies, and can continue to sporulate year-round, providing inoculum for future infections. These fruiting bodies can be identified as small dark protrusions on the surface of a developed canker. A study conducted in Washington State found sporulation to be the highest in peach orchards during the spring and summer months when humidity was high, due to less extreme temperatures specific to the region (Grove & Biggs 2006). However, levels of sporulation are dependent on the given microclimate of an orchard in question. Despite this, spore production was shown to follow temperature and precipitation patterns, and to be present all twelve months of the year. The primary method of conidia dispersal in orchards appears to be through the presence of free water, disseminated through splashing water, wind-blown water, and through over-the-canopy and under-tree impact sprinkler irrigation systems (Luepschen et al. 1969; Barakat et al. 1995; Grove

& Biggs 2006). Though asexual spores are produced year around, it has been documented that the teleomorphic stage, the sexual stage of the fungus, rarely occurs on peach trees (Kern 1955; Wensley 1964; Adams et al. 2002).

Nomenclature Clarification. Cytospora canker on peach is known to be caused by three closely related species (Table 1.1). Due to the recent "one name for fungi" efforts by the *International Code of Nomenclature for algae, fungi and plants* (ICN; McNeill et al. 2012), which aim to consolidate those genera which may have competing generic names from their sexual and asexual morphs, Cytospora canker should be referred to by the anamorphic binomial. According to Rossman et al. (2015):

> *Cytospora* is the oldest name… and several recent accounts of *Cytospora* species have been published (Adams 2005, Fotouhifar et al. 2010, Fan et al. 2014), [thus] it seems best to use the generic name that has priority (Rossman et al. 2015).

Thus, this pathogen should be referred to as Cytospora canker, not Leucostoma canker or Valsa canker, given the guidelines of the ICN.

Table 1.1. Cytospora canker causing species on *Prunus persica.*

Anamorph Binomial	**Teleomorph Binomial**
Cytospora leucostoma [(Pers.) Sacc.]	*Leucostoma persoonii* [(Nits.) Hohn]
Cytospora cincta (Sacc.)	*Leucostoma cinctum* [(Fr.) Hohn]
Leucocytospora paraleucostoma [(Adams) Surve-Iyer & Iezzoni]	*Leucostoma parapersoonii* [(Adams) Surve-Iyer & Iezzoni]

Species Population in Western Colorado. Although there are three tentative species, which have been described as occurring on peach studies by Stewart et al. (unpublished) have revealed

species specific to the peach production area of Colorado. One species, *C. leucostoma* was identified in association with canker formation in peaches while *C. parasitica* was identified in association with canker formation in apple [*Malus domestica* (Borkh.)]. Pathogenicity assays showed that isolates of *C. parasitica* collected from apple were not pathogenic on peach (Stewart et al. unpublished). Thus, the current hypothesis is that the dominant pathogenic species in the western slope of Colorado, is *C. leucostoma*.

Chemical Control Options. As mentioned previously, there are no standard cultural or chemical control methods among conventional and organic tree fruit systems to manage this disease. Chemical control is an important component in *Cytospora* management as pruning and training (i.e. wounding) are essential to horticultural production systems (Costes et al. 2006). *Cytospora*, as a weak pathogen, requires a wound as a mode of entry, thus the biology of the fungus fits very well with the best cultural management practices of an orchard, including dormant or summer pruning. During pruning periods, tree wound exposure is highest throughout the year making proper pruning timing and preventative chemical treatments vital for control.

According to a previous chemical field study, Benomyl and Captafol, both chemicals currently unavailable for use in the United States due to teratogenic and carcinogenic defects (Kavlock et al. 1982; Patnaik 2004), were found to be effective. The same study, which applied chemicals as canopy sprays, found Captan, Dichlone, Dichloran, Ferbam, and Sulfur to be infective. In addition, it has been reported that copper based chemicals, such as the Bordeaux mixture, are phytotoxic and increase disease severity in copper susceptible cultivars (Northover 1976). In a contradicting study, conducted under laboratory conditions in petri dishes, researchers found Captan, Iprodione, and thiophanate-methyl (Topsin) to be effective in

preventing *Cytospora* colony growth. These three treatments, which were successful in plates, were also successful in preventing canker development on excised branches (Biggs et al. 1994). Thus, Captan has shown conflicting results and should be studied further to conclude the efficacy of the chemical as a potential treatment option as it is commercially available for grower use in conventional and integrated fruit production systems.

The longevity of a chemical formulation is an essential feature for preventing spore dispersal and subsequent host infections for many days after a single chemical application. Research described herein is focused on testing possible effective chemicals that are available for use in stone fruit production systems. Due to the continuous planting system of tree crop production used in western Colorado, preventive measures are critical. Containment measures that inhibit fungal sporulation become vital once infections occur within an orchard. Once an orchard develops high infection levels, it may be economically unfeasible for growers to remove all infected trees, since they can be still productive, thus spore inoculum load management becomes essential to disease control. Spore containment measures are especially important, given the high land costs of Colorado (Sharp & Cooley 2004), to avoid preventable losses and pathogen dispersal on highly valued crops. Further, the majority of tree fruit producers in western Colorado replant young trees next to older trees, increasing their chance of exposure to the pathogen. Thus, in this thesis, along with preventive shoot protection chemical control, selected chemicals were investigated as canker coverage treatments to reduce spore production on a canker. Multiple effective chemicals were studied for potential rotational use to prevent pathogen resistance. Chemicals were investigated as preventive measures and as spore containment measures on existing cankers.

Solutions and Relevance of Future Studies. Along with the need to uncover effective chemicals for the growers of the western slope of Colorado, epidemiology studies need to be conducted that are specific to the microclimate of the fruit production areas. Such studies will give insight to field inoculum loads, tree susceptibility levels, and spore germination and pathogen growth patterns throughout the year. Understanding the epidemiology of *Cytospora* will provide a window for proper chemical application timing. If the patterns of spore inoculum load are identified, then growers will be able to make pruning decisions and take appropriate preventive measures, to avoid exposing their trees to heightened infection levels. Orchard inoculum loads may differ by location due to the differences in given microclimates; nonetheless, variables contributing to higher spore production will be just as revealing for pathogen control. Correlation of spore production with precipitation, temperature, humidity, and other variables specific to the western slope of Colorado will provide vital information for Cytospora canker management.

While effective chemical practices are essential to the control of *Cytospora,* perhaps one of the most promising solutions would be the development of resistant cultivars that are marketable for growers in terms of current production standards. Studies have shown, based on artificial inoculation and lesion length quantification, that heritability of resistance among peach trees is viable (Chang et al. 1991). A previous study estimated the heritability of peach tree resistance to *C. leucostoma* in a population of diverse genotypes by regressing average performance of seedlings (by lesion length), on the performance of their parents (Chang et al. 1991). Chang et al. (1991) found that seedlings tended to have lower mean necrotic lengths than female parents. These findings suggest that it should be possible to select for *Cytospora* resistant individuals in a population (Chang et al. 1991).

Given our current understanding of this fungal pathogen, research addressed in this project, specific to the Colorado peach production region, focuses on strengthening the gaps of previous work. The main goal for these studies was to uncover effective chemical treatments for proper *C. leucostoma* control. Through the creation of standard cultural practices, which currently do not exist, new *Cytospora* infection rates will ideally be reduced. Future research should then focus on disease epidemiology and resistant cultivar selection, which will greatly strengthen the sustainability of peach industry in western Colorado.

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CHAPTER II. **Preventive Chemical Control of *Cytospora leucostoma* in Peaches in Western Colorado.**

SUMMARY

Cytospora spp. are hardwood and conifer tree pathogens, capable of infecting stone and other fruit tree species. In western Colorado, *Cytospora leucostoma* is ubiquitous in peach orchards, and has developed into a major limiting factor of peach production. The annual epidemiology patterns of *C. leucostoma*, specific to the western Colorado climatic conditions are not well known. *Cytospora* is considered a "weak parasite", unable to invade healthy intact bark tissue of the tree, but instead requires a wound as a mode of entry. Bark cold damage injuries plus the required pruning management practices in commercial orchards provide optimum conditions for successful fungal infections. Preventive chemical control is an integral component of pathogen infection management in tree fruit production systems. Eighteen commercial fungicides were tested for *C. leucostoma* control. All chemical treatments were initially tested *in-vitro*, in chemically amended media dishes. Successful treatments were then tested under controlled conditions on detached peach branches. The effective chemicals from these two laboratory assays, Topsin, Captan, lime sulfur, and NuCop, were further tested in the field as sprays alone at the commercial label midrates, and as wound sealant sprays with effective chemicals amended in paints and kaolin clay. Of the treatments investigated, Topsin, Captan, 50% latex, Topsin amended in 50% latex paint, Captan amended in 50% latex paint, and lime sulfur were most effective in lesion size reduction, providing evidence of efficacy from controlled laboratory conditions to variable field conditions. NuCop was consistently ineffective in all field trials and

in some instances yielded larger lesions than the non-treated positive control shoots, suggesting a link of increased pathogen growth with potential copper phytotoxicity in wounded peach shoots.

INTRODUCTION

The peach industry in western Colorado is known for producing fruit of exceptional quality considering savor, size, color, and timing of harvest. The peach industry market value of western Colorado, over the last ten years, has estimated value of 17 to 30 million dollars annually (USDA 2016). In a survey conducted by Colorado State University of the western Colorado peach production region, more specifically the Grand Valley, North Fork of the Gunnison River, and the Olathe region, it was found that 75% of trees in orchards were infected, with certain orchards having infection rates of 100%. Cytospora canker, caused by *C. leucostoma*, is estimated to account for 15-20% of losses in stone fruit production yearly within Colorado (Pokharel & Larsen 2009), accounting for an estimated $3,375,000 annual loss in peach crop value (USDA 2016).

In stone fruit production systems, tree architecture manipulation by pruning and training is an essential management component for improved light interception, increased yield and optimized fruit quality (Costes et al. 2006). The biology of *C. leucostoma* fits very well with the required pruning management practices as the fungus is considered a “weak parasite”, unable to invade healthy intact bark, requiring a wound as a mode of entry (Biggs 1989). During pruning periods, wound exposure to pathogens is highest, serving as a potentially heightened infection season. *Cytospora leucostoma* is difficult to control once an infection occurs as the fungus circumvents the vascular system, disrupting the conductivity of the xylem, with an active xylem infection being an essential part of the translocation of the disease (Hampson & Sinclair 1973). Thus, cankers forming on the trunk of a tree are very difficult to surgically remove without

permanently damaging the tree. Preventative measures are vital for pathogen control, and ideally practices should include chemical control for wounded shoot protection. Further, pathogens such as *Monilinia fruticola* should be treated for as *M. fruticola* twig infections may be invaded and enlarged by *C. leucostoma* (Biggs 1989). Such shoot protection measures are essential for disease-free plants.

Fungicides have yet to be registered for Cytospora canker and previous studies examining fungicide efficacy have yielded conflicting results regarding pathogen control (Biggs & Grove 2005). The fungicide Captan was found ineffective as a canopy spray application during the spring (Northover 1976), but effective under controlled conditions in both *in-vitro* plates and on excised branches (Biggs et al. 1994; Dhanvantari 1968). Lime sulfur was also found to be ineffective in canopy sprays in previous studies (Northover 1976). Benomyl and Captafol were found to be effective chemical options, but are no longer available for application in the United States due to teratogenic and carcinogenic effects (Kavlock et al. 1982; Patnaik 2004). *In-vitro* chemical inhibition of *Cytospora* has been studied on a wide array of chemicals in the past (Dhanvantari 1968; French 1962; Rohrbach 1965). The main objective of this research was to evaluate commercially available chemical and organic fungicides for Colorado peach growers for *C. leucostoma* control, and to test effective chemicals as wound sealing treatments to develop a preventive approach in existing orchards.

MATERIALS AND METHODS

Cytospora leucostoma **Isolation.** Symptomatic peach trees containing *C. leucostoma* infections were targeted for plant sample collections on the western slope of Colorado (Grand Junction, CO). Collected branches were transferred to Colorado State University, Fort Collins,

CO for isolations. Canker margins were cut into small pieces, no larger than 1 cm, and placed on ½ potato dextrose agar (PDA) plates. Plates were incubated at 25°C for five days, and then cultures were re-isolated onto Leonian's agar (LA) media [1 liter of media solution: peptone (0.625 g), maltose (6.25 g), malt extract (6.25 g), KH_2PO_4 (1.25 g), $MgSO_4 \cdot 7H_2O$ (0.625 g), and agar (20.0 g) (Booth 1971)] plates. Isolates were then hyphal tipped onto LA plates, and placed in glycerol broth tubes for cryopreservation at -80°C. Spore suspensions were created from selected isolates. Eight total isolates, including CP5.1, CP11.3, CP56.2, CP64.1, CP84.1, CP92, CP108.1, and CP124 were selected for use throughout the chemical studies. Six of these isolates (CP11.3, CP56.2, CP64.1, CP84.1, CP108.1, and CP124) were used for testing chemical efficacy by amending treatments in ½ potato dextrose agar (PDA) plates [1 liter of media solution: potato starch (4.0 g), dextrose (19.5 g), agar (7.5 g) (Difco^{tm})]. Two of the original eight isolates chosen (isolates CP5.1 and CP92) were selected from previous studies as the most virulent isolates, yielded the largest lesions in a given period, and thus were used for testing chemical efficacy on the detached peach branch assay. Isolate CP5.1 was further used for testing chemical efficacy in field trials, due to high pycnidium production for spore suspension creation.

In-Vitro **Chemical Assay.** Conventional and organic chemical treatments were amended in LA and observed for pathogen inhibition (Table 2.1). Media ingredients were combined and autoclaved prior to amending test chemicals in the media. Chemicals were added to LA media at the chemical label mid-rate (Table 2.1). Four-mm diameter agar plugs, with three-day old hyphal growth, were inoculated in the center of the Leonian's Agar plates and incubated at 25°C for seven days. Colonies were measured daily, after the initial 24 hours, in two locations across the diameter of the fungal growth, and fungal growth area was determined from these two daily

measurements per plate. Each of the 8 isolates was subjected to 19 treatments. Each treatment consisted of three replications per isolate and the assay was conducted twice.

Table 2.1. Chemical treatments used at label mid-rates to test fungal growth inhibition of *Cytospora leucostoma*.

Treatment name	Active ingredient	Label rate (per 200 gal.)	Rate chosen	State	Registration use
Microthiol Disperss	Sulfur	10-20 lb	15 lb	Solid	Conventional
Fontelis	Penthiopyrad	14-20 oz	17 oz	Liquid	Conventional
Torino	Cyflufenamid	3.4 oz	3.4 oz	Liquid	Conventional
Pristine	Pyraclostrobin & Boscalid	10.5-14.5 oz	12 oz	Solid	Conventional
Aliette WDG	Fosetyl	10 lb	10 lb	Solid	Conventional
Topsin M WSB	Thiopthanate-methyl	1-1.5 lb	1.25 lb	Solid	Conventional
Benlate WP	Benomyl	24-32 oz	28 oz	Solid	Conventional
Captan	N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide	3-4 qt	3.5 qt	Liquid	Conventional
Inspire Super	Difencoconazole & Cyprodinil	16-20 oz	18 oz	Liquid	Conventional
Ziram	Zinc dimethyldithiocarbamate	3 - 5.3 lb	1.15 lb	Solid	Conventional
CaCl	CaCl	48 oz	48 oz	Solid	Organic
Neem Oil	Neem Oil	3 qt	3 qt	Liquid	Organic
Mpede	Potassium salts	2-4 gal	3 gal.	Liquid	Organic
Kaligreen	Potassium bicarbonate	2.5-3 lb	2.75 lb	Solid	Organic
Serenade	*Bacillus subtilis*	14-20 oz	17 oz	Solid	Organic
NuCop WP	Copper Hydroxide	8-20 lb	10 lb	Solid	Organic
Badge X2	Copper Hydroxide & Copper Oxychloride	3.5-5.25 lb	4.25 lb	Solid	Organic
$ZnSO_4$	$ZnSO_4$	4-6 lb	5 lb	Solid	Organic
Lime sulfur	Calcium polysulfide	20-24 gal.	22 gal.	Liquid	Organic

Detached Branch Segments Chemical Assay. The most effective chemicals from the *in-vitro* assays were chosen for further investigation. Two *C. leucostoma* isolates (CP5.1 and CP92) were selected for the following assays, as these isolates were most consistently aggressive in previous experiments (Stewart et al. unpublished). Detached branch segments (20 cm long) of similar diameter (1.5 cm) were first sterilized in 10% sodium hypochlorite solution for 5 minutes and then rinsed in sterile distilled water. Once sterilized, branches were wounded with a 4 mm core borer. Detached, wounded branches were then dipped into six conventional chemical solutions including Aliette (Bayer), Topsin (United Phosphorus, Inc.), Benlate(DuPont), Captan (Loveland Products), Ziram (United Phosphorus, Inc.) and Inspire (Syngenta), while the eight OMRI approved organic chemicals included neem oil, Mpede (Gowan), Kaligreen (Toagosei), Serenade (AgraQuest), Nucop (Agri Star), Badge (Isagro), ZnSo4 (Maximo 360), and lime sulfur (Ag Formulators) for five minutes. Subsequently, branch segments were inoculated with *Cytospora* isolates CP5.1 and CP92 with two inoculation strategies (immediate and delayed). All chemical solutions were mixed at label mid-rate specifications (Table 2.1). Two controls were also included as treatments, a wounded negative control with no branch inoculation or chemical treatment, and a positive control, which included a *Cytospora* inoculation with no chemical.

Two inoculation strategies were followed; the first was an immediate inoculation that occurred the same day as the branch chemical immersions, while the second was a delayed inoculation that occurred seven days after branch chemical immersions to test residual efficacy. For the delayed inoculations to test residual efficacy, detached branch segments were placed inside plastic, one-gallon bags, stored in the greenhouse, and rotated daily to ensure even exposure to solar radiation before inoculation. The inoculation technique was identical for both strategies. Hyphal agar cores (4 mm), from three-day-old cultures, were placed at the branch

wound position and wrapped with Parafilm (American National Can). Wrapped branch segments were then placed inside plastic, one-gallon bags with two 6 cm x 6 cm moistened sponges for moisture retention and stored in a dark incubator at 28°C. Replicated branch segments were stored in different bags to avoid any potential chemical volatilization contamination. Lesion lengths were measured eight days post-inoculation, isolations were conducted on lesions to confirm Koch's postulates. Lesions were visually distinctive when compared to the negative control. Effective chemical treatments yielded wounds similar to the negative control, and preliminary isolations confirmed lack of *Cytospora* presence. Eighty millimeters was the maximum length permitted based on branch size.

On each individual branch segment, two inoculations were made. Five branch segments were used for each chemical treatment within each time point and isolate. Thus, with two isolates used (CP5.1 and CP 92), 16 chemical treatments, two inoculation strategies (immediate and residual) per treatment, and five replications per time point, a total of 320 branches were used. The experiment was repeated twice.

Chemical Field Trials. Chemical efficacy was tested in the experimental orchard of the Western Colorado Research Center-Orchard Mesa (WCRC-OM) in Grand Junction, Colorado in the summer and fall of 2016 and the spring of 2017. The orchard block used for this study was planted in the spring of 2013 with 'Cresthaven' peach scions grafted on 'Viking' rootstock. The block was irrigated through a microsprinker irrigation system. The four most successful chemicals from the previous laboratory trials were selected for this experiment. Benlate, although it showed *C. leucostoma* control in the laboratory trials, was not chosen for further investigation due lack of a labeled use in peaches. The two conventional chemicals chosen were

Topsin and Captan, while the two registered organic chemicals chosen included lime sulfur and NuCop (Table 2.2). Chemical treatments were tested as direct sprays on wounds and as wound sealant sprays embedded in latex paint or kaolin clay. For conventional treatments, chemicals were amended in a 50% white latex paint/water mixture. For organic treatments, organic chemicals were embedded in a mid-rate kaolin clay coat mixture that is registered for organic production systems (Surround WP). Prior to the chemical applications and inoculations, one-year old tree shoots were wounded. Two wound types were used one to simulate pruning wounds and one to simulate winter freeze damage cracking wounds. Prune cuts were made with a traditional hand-pruning shear, and cut so that the wound was sloped. This method of pruning is typical among peach growers as it provides a 45°, angled surface where precipitation may more easily slide off and dry out preventing pathogen spore germination. To simulate winter freeze cracking damage of bark tissue, razor cuts were made with a generic box cutter. Cuts were 1 mm thick and 35 mm ± 1 mm long. Tree shoot treatments were randomized in orientation to minimize directional influence from solar radiation exposure and temperature. After shoot wounds were made, tree branches were flagged for chemical applications. Chemical applications were applied to all branches the same day of the wounding and at the label mid-rate (Table 2.2). Chemicals were distributed to a given branch with generic 350 ml hand-spray bottles, and sprayed until solution runoff or five hand sprays, which was about ~3ml of chemical application per branch.

Twenty-four hours after wounding and applying the chemical treatments to tree shoots, spore inoculations (isolate CP5.1) were made as to allow the chemical applications to completely dry before spore application. Inoculum was produced from 2-month-old mature fungal cultures with well-formed pycnidia grown from preserved *C. leucostoma* on filter paper. Each plate was immersed in 20-40 ml of distilled water for 3-5 minutes while being agitated slightly. Pycnidia

were then cracked open with sterilized precision tweezers and conidia were then spread throughout the plate with a sterilized inoculation loop. Spore concentration per ml was then quantified using a hemocytometer. The spore suspension was diluted or concentrated accordingly to an estimated 10^5 spores per ml. A final hemocytometer measurement was conducted to confirm the desired concentration. The spore suspension was then applied to each branch individually using a pipette (Figure 2.1). A one hundred µl spore suspension was applied to each wound, and wounds were then wrapped in Parafilm.

Two inoculation strategies were used in the summer 2016 trials (immediate and delayed), however the fall and spring trials only consisted of one inoculation strategy, immediate inoculations (Table 2.3). Immediate inoculation occurred 24 hours after chemical application. Delayed inoculations in summer 2016 trials occurred seven days after chemical applications to test chemical residual activity. Once inoculated, shoot infections were allowed to grow for 60 days in the summer trials. In the fall trials, shoot infections were allowed to grow for 150 days due to slower pathogen growth and subsequent smaller lesion sizes, confirmed in preliminary studies using untreated positive control shoots. Similarly, in the spring trials, shoot infections were left to grow for 90 days prior final lesion evaluation. After the fungal growth period, branches were harvested, and evaluated for canker lesion size. Canker sizes were measured using a digital caliper (Figure 2.2). A volume measurement (mm^3) was used for pruning cuts as the canker encompassed the entire diameter and depth of the branch. The tissue was infected entirely and the lesion could not be scraped out. The volume was calculated by measuring the branch diameter and the canker length. For the razor wounds, a 2-dimensional area (mm^2) was calculated as cankers did not encompass the entire diameter of the branch. Branches had to be very carefully opened as to not scrape away the 2-d lesion plane, which extended directly below

the razor wound. Koch's postulates were satisfied by confirming lesions were caused by *C. leucostoma* through the plating of symptomatic plant tissue on ½ PDA media.

A randomized complete block design was created for each season, wound type, and inoculation time point. For the 2017 summer trials, six blocks (trees) were used for each experimental group. For the 2016 fall and spring trials, four blocks (trees) were used for each experimental group. Each block contained all treatments. Within each tree, each chemical treatment was assigned 5 one-year-old shoot replications.

Statistical Analysis. RStudio was used for statistical analyses and packages used included lme4, lmertest, pbkrtest, and lsmeans (R Core Team 2017; Lenth 2016; Halekoh & Højsgaard 2014; Kuznetsova et al. 2016; Bates et al. 2015). Summary statistics and graphical depictions were created using the plyr package and the ggplot2 package (Wickham 2011; Wickham 2009). A mixed model was used for the analysis of each experiment. For the *in-vitro* trials, a continuous response variable of fungal growth area (mm^2), with a fixed chemical treatment predictor variable, and two random effect predictor variables of isolate and run were assigned in the mixed model. Isolate and run were treated as random effects to account for the variance effect between the isolates used and runs conducted. For the detached branch immediate and delayed inoculation trials, the continuous response variable for both models included lesion length (mm), with a fixed predictor variable comprised of 16 treatment levels, and two random effect variables including isolate and run. To correct for assumptions of normality and equal variances, the data from the immediate inoculation time point were log transformed. For the field trials, a mixed model was built for each experimental group (wound type, spray type, inoculation strategy) (Table 2.3). The continuous response variable for prune wounds equaled lesion volume (mm^3), while the

continuous response variable for razor wounds equaled lesion area (mm^2). The mixed model predictor variables consisted of fixed chemical treatment predictor variables, and a random effect blocking variable. A linear model analysis of variance (ANOVA) was run on all mixed models, followed by Tukey adjusted pairwise comparisons ($\alpha = 0.05$) (Tukey's HSD adjusted p-values are $P < 0.05$).

Table 2.2. Chemical treatments and corresponding concentrations chosen for application on 1-year old peach shoots.

Treatment Name	Active Ingredient	Recommended Rate per 200 gallons	Rate Chosen	State	System
Captan	N-Trichloromethylthio-4-cyclohexene-1, 2-dicarboximide	3-4 qt	3.5 qt	Liquid	Conventional
Topsin M WSB	Thiopthanate-methyl	1-1.5 lb	1.25 lb	Solid	Conventional
Latex	Latex paint	10.5-14.5 oz	12 oz	Solid	Conventional
Lime Sulfur	Calcium polysulfide	20-24 gal	22 gal	Liquid	Organic
NuCop WP	Copper hydroxide	8-20 lb	3.4 oz	Liquid	Organic
Surround WP	Kaolin clay	10 lb	10 lb	Solid	Organic

Table 2.3. Investigations conducted in the field at the Western Colorado Research Center. Various trials were conducted in different seasons.

Season	Experiment Group[a] (wound type, spray type, inoculation strategy)	Sample Size[b]
Summer	pruning, standard chemical spray, immediate inoculations	n = 180
Summer	pruning, wound sealing spray, immediate inoculations	n = 210
Summer	pruning, standard chemical spray, delayed inoculations	n = 180
Summer	pruning, wound sealing spray, delayed inoculations	n = 210
Summer	razor, standard chemical spray, immediate Inoculations	n = 180
Summer	razor, wound sealing spray, immediate inoculations	n = 210
Summer	razor, standard chemical spray, delayed inoculations	n = 180
Summer	razor, wound sealing spray, delayed inoculations	n = 210
Fall	pruning, standard chemical spray, immediate inoculation	n = 120
Fall	pruning, wound sealing spray, immediate inoculation	n = 140
Fall	razor, standard chemical spray, immediate inoculation	n = 120
Fall	razor, wound sealing spray, immediate inoculations	n = 140
Spring	pruning, standard chemical spray, immediate inoculation	n = 120
Spring	pruning, wound sealing spray, immediate inoculation	n = 140
Spring	razor, standard chemical spray, immediate inoculation	n = 120
Spring	razor, wound sealing spray, immediate inoculations	n = 140

[a] Experimental group refers to the experimental trial conducted. Two wound types were investigated, pruning and razor wounds. Two spray types were investigated, standard chemical sprays without 50% latex or kaolin clay and wound sealing chemical sprays amended in 50% latex or kaolin clay. Inoculation strategy refers to the time point of inoculation, immediate inoculations (24 hours post chemical applications) or delated inoculations (7 days post chemical applications).

[b] Sample size varies due to number of treatments used. Sprays embedded with paints and kaolin clay coating (wound sealing sprays) contained more treatments as a latex control and a Surround WP control were also used.

a. ***b.***

Figure 2.1. *Cytospora leucostoma* spore suspension on 1-year old peach shoots, field trials.
[a] *C. leucostoma* spore suspension (1 x 10^5 ul) inoculation on pruning wound on peach shoots.
[b] *C. leucostoma* spore suspension (1 x 10^5 ul) inoculation on razor wound on peach shoots.

a. ***b.***

c.

Figure 2.2. *Cytopspora leucostoma* lesion assessment on peach shoots.
[a] Opening wound on peach for *C. leucostoma* lesion assessment.
[b] Razor wound canker on peach to simulate freeze damage (2-D).
[c] Prune wound canker on peach to simulate pruning technique (3-D).

RESULTS

***In-Vitro* Growth of Fungal Cultures on Chemical-Amended Petri-Dishes.** Effective *in-vitro* chemical treatments were identified for *C. leucostoma* colony growth inhibition. After seven days of isolate growth, the conventional fungicides Aliette, Topsin, Benlate, Captan, and Inspire Super were all found to significantly inhibit colony area when compared to the positive control ($P < 0.0001$) (Figure 2.3). The organic system chemicals Mpede, Kaligreen, Serenade, NuCop, Badge, ZnSO4, and lime sulfur were also found effective in mycelium growth inhibition when compared to the positive control ($P < 0.0001$) (Figure 2.3). While not as effective as those treatments in the statistical "f" group, conventional chemicals Pristine, Torino, Fontellis, and Microthiol Disperss also showed inhibition activity when compared to the positive control ($P < 0.0001$) (Figure 2.3). CaCl and neem oil did not yield significant differences when compared to the positive control, and were ineffective in colony growth inhibition after seven days of incubation ($P = 0.9823$; $P = 0.9910$, respectively) (Figure 2.3).

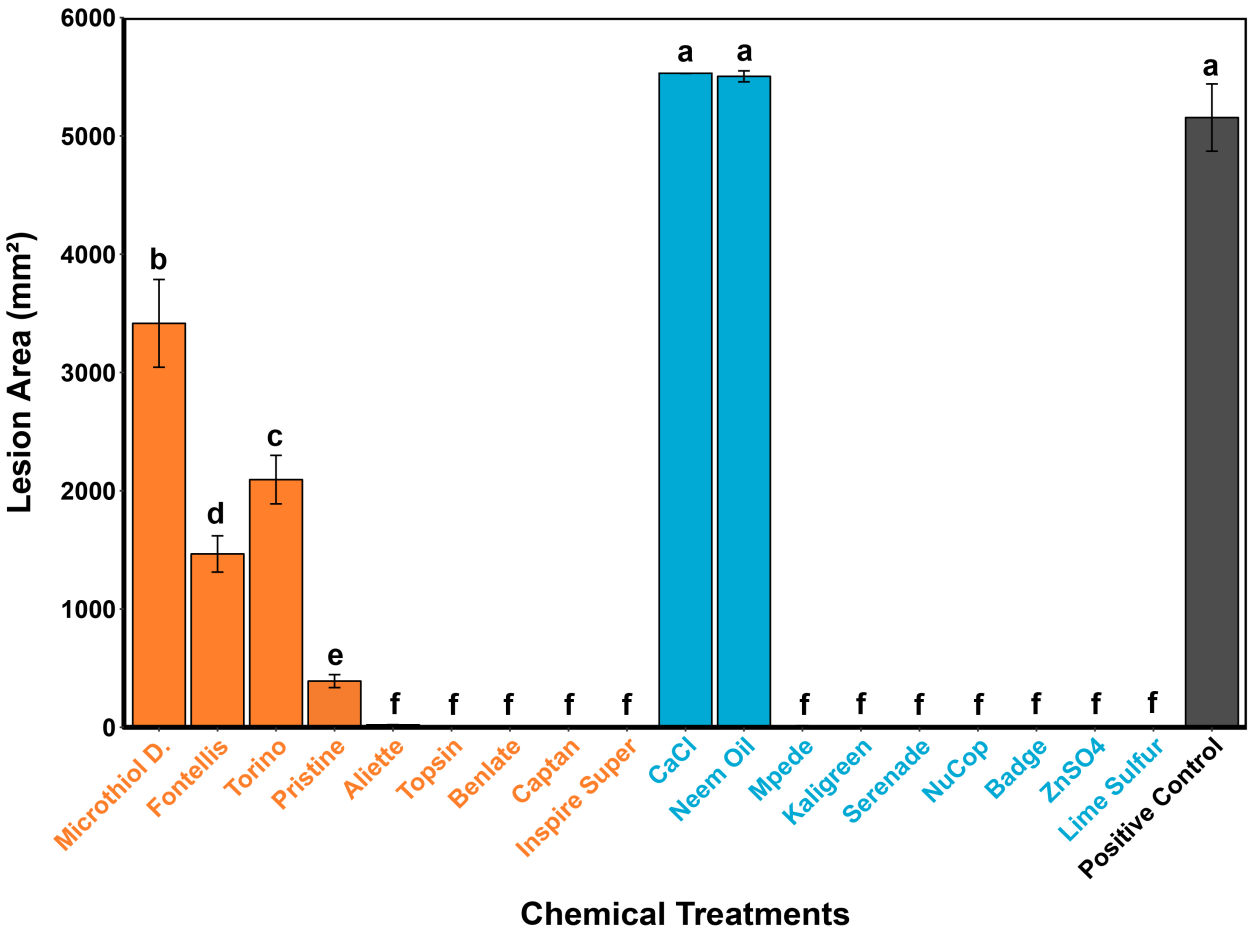

Figure 2.3. *In-vitro* chemical efficacy of *Cytospora leucostoma* colony growth after 7 days of incubation at 25°C on a Leonian's Agar (LA) amended with conventional chemicals (orange, Microthiol D. – Inspire Super) and registered organic chemicals (blue, CaCl - lime sulfur). Fungal colony area is plotted by chemical treatment. The positive control (grey bar) consists of a *C. leucostoma* inoculation in LA nutrient agar with no chemical amended. Significance of treatments with the lower-case letter display are reflective of Tukey adjusted values ($P < 0.05$) of the developed mixed model.

Detached Branch Segments Chemical Assay. Effective chemical treatments from the *in-vitro* assay were chosen for further investigation on detached peach branches. As mentioned, two inoculation strategies were investigated, one testing immediate chemical efficacy and the other testing 7-day residual chemical efficacy. For the immediate inoculations (Figure 2.4), the conventional chemicals Topsin, Benlate, and Captan were most significantly different than the positive control ($P < 0.0001$). Further, these three chemicals were not significantly different than

the negative control and wounds were similar in appearance to the negative control ($P > 0.9$) (Figure 2.4). The two organic chemicals NuCop and lime sulfur were also significantly different than the positive control ($P < 0.0001$) (Figure 2.4), and were also not significantly different than the negative control ($P = 0.5153$; $P = 0.5524$) (Figure 2.4). The conventional chemical Ziram was significantly different than the positive control ($P < 0.0001$) indicating pathogen inhibition, yet it was also significantly different than the negative control ($P = 0.0003$) suggesting the control activity of Ziram is not equivalent to a wounded but non-inoculated branch. All other chemicals Aliette, Inspire, neem oil, Mpede, Kaligreen, Serenade, Badge, and ZnSO were ineffective compared to the positive control ($P > 0.90$; $P = 0.3343$; $P = 0.7466$; $P = 0.3819$; $P = 0.4888$; $P = 0.4652$; $P = 0.9271$; $P > 0.90$, respectively) (Figure 2.4).

For the delayed inoculations (Figure 2.5), conventional chemicals Topsin, Benlate, Captan, and Ziram were significantly different than the positive control ($P = 0.0059$; $P < 0.0001$; $P < 0.0001$; $P = 0.0012$, respectively) (Figure 2.5), and were not significantly different than the negative control, suggesting control activity of these chemicals was equivalent to a wounded but non-inoculated branch ($P = 0.4515$; $P > 0.90$; $P > 0.90$; $P = 0.7221$, respectively) (Figure 2.5). The organic system chemical NuCop was also significantly different than the positive control ($P = 0.0001$) and not significantly different compared to the negative control ($P = 0.9741$) (Figure 2.5). All other chemicals Aliette, Inspire, neem oil, Mpede, Kaligreen, Serenade, Badge, and ZnSO4 were ineffective in residual activity for delayed inoculations as no significant differences were detected between the chemicals and the positive control ($P > 0.95$) (Figure 2.5). Lime sulfur was also found ineffective in residual *C. leucostoma* growth inhibition when compared to the positive control (Figure 2.5; $P = 0.3253$)

The conventional chemicals Captan and Topsin, along with the organic chemicals NuCop and lime sulfur, were chosen for further investigation in field trials. Captan, Topsin, and NuCop were selected based on consistency in *C. leucostoma* growth inhibition in both the immediate and residual chemical efficacy trials. While lime sulfur was not effective in the residual chemical efficacy trial, it was chosen for further investigation due to its effective control in the immediate chemical efficacy trial.

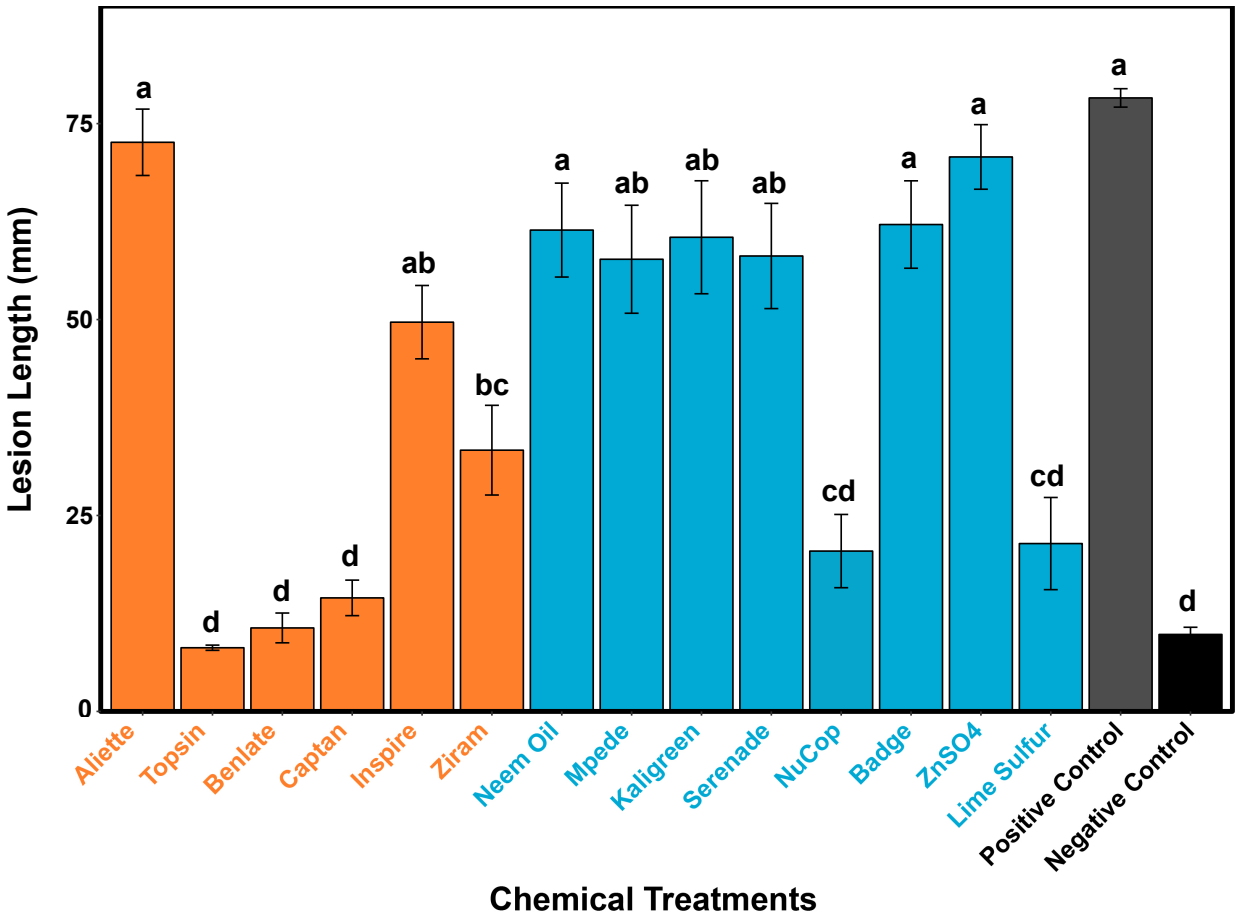

Figure 2.4. Chemical efficacy on immediate *Cytospora leucostoma* inoculations on detached peach branch segments incubated at 28°C for 8 days. Fungal lesion length is shown in the plot by chemical type. Efficacy of conventional chemicals (orange bars, Aliette – Ziram) and organic chemicals (blue bars, neem oil - lime sulfur) are shown. The positive control (grey bar) consists of a *Cytospora leucostoma* inoculation on detached branch segments with no chemical application. The negative control (black bar) consists of a core wounding with no *Cytospora* inoculation and no chemical application. Significance of treatments with the lower-case letters are reflective of Tukey adjusted values ($P < 0.05$) of the developed mixed model.

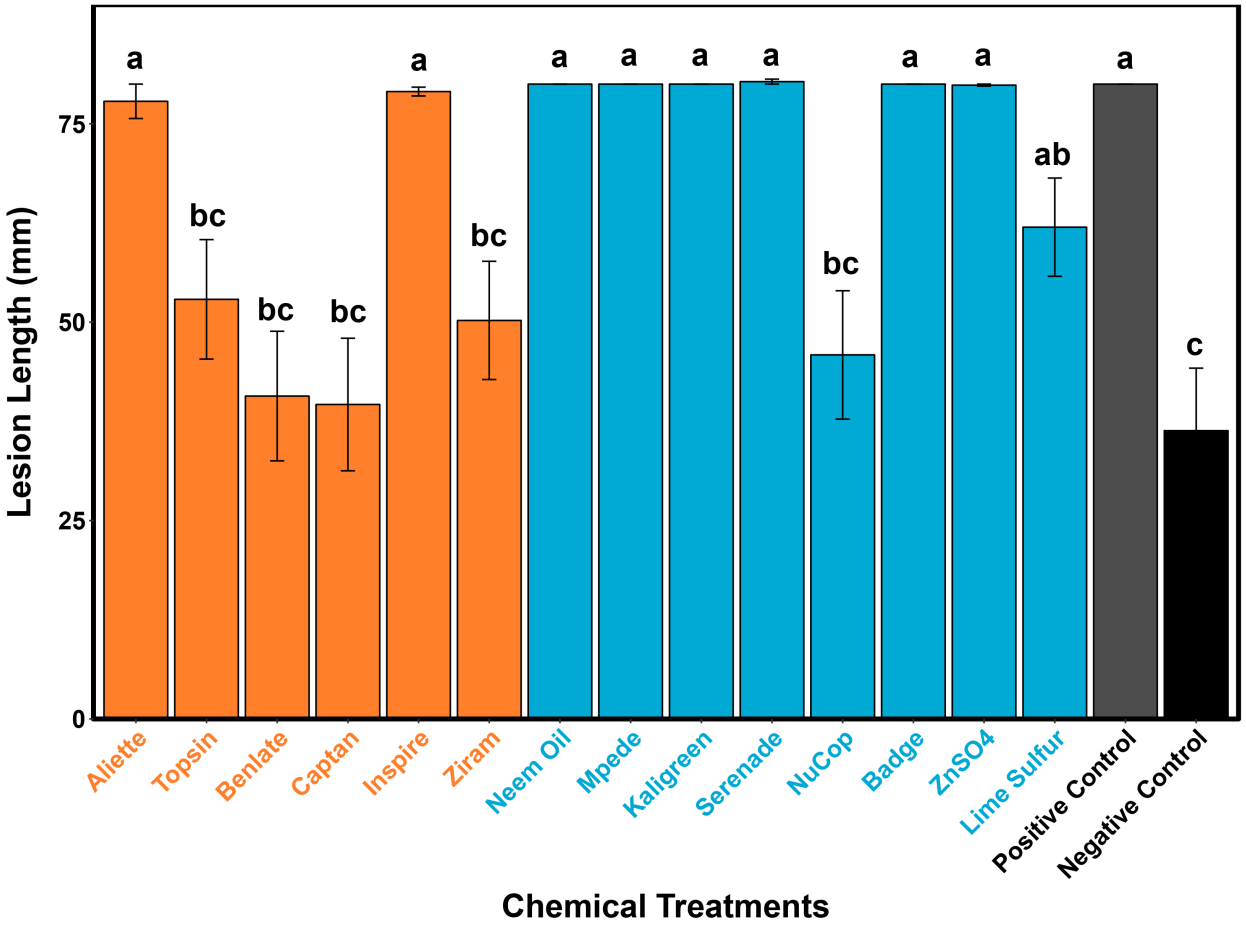

Figure 2.5. Chemical efficacy on delayed *Cytospora leucostoma* inoculations on detached peach branch segments. Chemical treatments were applied to branches and inoculations were made 7 days post chemical application. Branches were then inoculated and incubated at 28°C for 8 days. Fungal lesion length is shown in the plot by chemical type. Efficacy of conventional chemicals (orange bars, Aliette – Ziram) and organic chemicals (blue bars, neem oil - lime sulfur) are shown. The positive control (grey bar) consists of a *Cytospora leucostoma* inoculation on detached branch segments with no chemical application. The negative control (black bar) consists of a core wounding with no *Cytospora* inoculation and no chemical application. Significance of treatments with the lower-case letters are reflective of Tukey adjusted values ($P < 0.05$) of the developed mixed model.

Standard Chemical Spray Field Trials. Four chemical treatments, two effective conventional and two effective registered organic system chemicals from the *in-vitro* and the detached branch segments assays, were chosen for further investigation in field trials. As mentioned, several experimental groups were investigated in the field trials (Table 2.3). These groups were separated by season, wound type, spray type, and inoculation strategy. Spray type refers to the four chemicals selected applied as either standard isolated sprays or wound sealant sprays embedded in latex paint or kaolin clay coat, which is reported in the next section.

When applied as standard isolated sprays on pruning wounds during the summer trial, Captan was the only chemical found to have immediate chemical efficacy, as lesion volume was significantly lower than the positive control inoculation (Figure 2.6a; $P = 0.0085$). Topsin did not show evidence of immediate chemical efficacy during the summer trial on pruning wounds, as the treatment was not significantly different than the positive control and was significantly different than the negative control (Figure 2.6a; $P = 0.2296$; $P = 0.0359$). Lime sulfur and NuCop were also found ineffective during the summer trials on pruning wounds (Figure 2.6a; $P = 0.4991$; $P = 0.7724$, respectively). When chemicals were tested for residual efficacy during the summer on pruning wounds, Topsin was the only treatment that showed evidence of efficacy in comparison to the positive control (Figure 2.7a; $P = 0.0084$). Further, treatments Captan, lime sulfur, and NuCop did not show evidence of residual chemical efficacy when compared to the positive control (Figure 2.7a; $P = 0.7329$; $P = 0.8845$; $P > 0.95$, respectively).

For the summer trial, treatments were also investigated on razor wounds, to simulate freeze damage cracking of the bark tissue. No treatments were effective on immediate inoculation razor wounds during this trial. Captan immediate chemical efficacy could not be confirmed or rejected as no significant differences were found between Captan and the positive or the negative controls

(Figure 2.6d; $P = 0.5228$; $P = 0.5930$). Topsin also did not show evidence of immediate chemical efficacy on razor wounds during the summer when compared to the positive and negative controls (Figure 2.6d*;* $P = 0.7501$; $P = 0.3825$). Similarly, lime sulfur was ineffective when compared to the positive control (Figure 2.6d*;* $P = 0.9101$; $P = 0.2136$). Interestingly, NuCop yielded lesions significantly larger than the positive control (2.6d; $P = 0.0012$), showing evidence of increased pathogen growth with this treatment. When chemicals were tested for residual efficacy on razor wounds during the summer, Topsin, Captan, and lime sulfur all showed evidence of chemical efficacy when compared to the positive control (Figure 2.7c; $P = 0.0167$; $P = 0.0006$; $P = 0.016$; respectively). NuCop was not effective, as it did not show significant differences in lesion area compared to the positive control (Figure 2.7c; $P = 0.9686$).

Chemical efficacy was also investigated in the fall on pruning and razor wounds. Captan, lime sulfur, and NuCop did not show evidence of immediate chemical efficacy on pruning wounds when compared to the positive control (Figure 2.6b; $P = 0.2100$; $P = 0.9545$; $P > 0.95$, respectively). Topsin, on the other hand, was effective showing significantly smaller lesion volumes when compared to the positive control (Figure 2.6b; $P = 0.0193$). Further, Topsin did not differ significantly from the negative control (Figure 2.6b; $P = 0.5258$). When chemical treatments were tested on razor wounds during the fall, no immediate significant differences were found because of little branch infection that occurred.

As in the fall, during the spring investigations, Captan, lime sulfur, and NuCop did not show evidence of immediate chemical efficacy on pruning wounds when compared to the positive control (Figure 2.6c; $P = 0.2170$; $P = 0.0694$; $P = 0.9738$; respectively). However, Topsin did inhibit pathogen growth as lesion volumes were significantly smaller when compared to the positive control and did not differ significantly from the negative control (Figure 2.6c; $P =$

0.0068; $P = 0.9235$). When chemical treatments were tested on razor wounds in the spring, no chemicals yielded effective results. Interestingly, NuCop yielded lesions significantly larger than the positive control (Figure 2.6e; $P = 0.0023$) showing evidence of increased pathogen growth with this treatment. NuCop was consistently ineffective in preventive control in all trials (Figures 2.6-2.8).

Wound Sealing Spray Amended in Latex Paint and Kaolin Clay Coating Field Trials. Chemical efficacy was also tested by amending chemicals with latex paint and kaolin clay (Surround WP) as a means of covering tree wounds to avoid pathogen infection. Conventional chemicals Topsin and Captan were combined with 50% white latex paint, while NuCop and lime suflur were combined with kaolin clay at the midrate concentration to represent an organic disease management strategy. Organic production systems cannot use synthetic latex paint thus kaolin clay (Surround WP) was chosen as the barrier film component for the two, registered organic, chemical options.

During the summer field trial, immediate inoculation pruning trials, chemicals combined with 50% latex paint were all effective in lesion inhibition when compared to the positive non-chemically treated, inoculations (Figure 2.8a; $P < 0.0001$). Latex paint alone was equally as effective as was latex paint combined with Topsin or Captan, and significantly different than the positive control inoculation ($P < 0.0001$). Lime sulfur combined with kaolin clay was also effective in immediate chemical efficacy when compared to the positive control ($P = 0.0005$), although it was not as effective as the latex paint combination treatments (Figure 2.8a). When treatments were investigated for residual chemical activity during summer, all chemical treatments combined with latex paint were effective (Figure 2.7b & d; $P < 0.0001$). Lime sulfur

combined with kaolin clay did not show residual activity on pruning wounds (Figure 2.7b; P = 0.4079). When latex paint- and kaolin clay- amended chemical treatments were tested on razor wounds, during the summer, no significant differences were observed (Figure 2.8d). NuCop in combination with kaolin clay was found to have significantly larger lesions when compared to the positive control, suggesting increased pathogen growth (Figure 2.8d; P = 0.0005). When the paint- kaolin clay-amended treatments were tested for residual efficacy during summer on razor wounds, Topsin and 50% latex, Captan and 50% latex, and 50% latex alone were effective and yielded significantly reduced lesion sizes compared to the positive control (Figure 2.7d; P = .0095; P = 0.033; P = 0.001; respectively). Lime sulfur combined with kaolin clay exhibited residual activity in the field on razor wounds during the summer (Figure 2.7d; P = 0.0058). Kaolin clay (Surround WP) applied alone or with NuCop, was consistently ineffective in all trials (Figure 2.8).

Chemically amended latex paint and kaolin clay were also tested for immediate efficacy in the fall of 2016 and the spring of 2017. During the fall trials, no immediate chemical efficacy was found on pruning wounds as no treatments were significantly different than the positive control inoculations (Figure 2.8b). However, during the spring trials, Topsin and Captan, in combination with 50% latex paint, were effective in pathogen growth inhibition as lesion sizes were smaller on pruning wounds compared to the positive control (Figure 2.8c; P = 0.0003; P = 0.0182). Due to the lack of plant tissue infection, no chemicals were found effective during the fall. During spring trials, however, all chemical treatments were effective with the exception of NuCop combined with kaolin clay (Figure 2.8e).

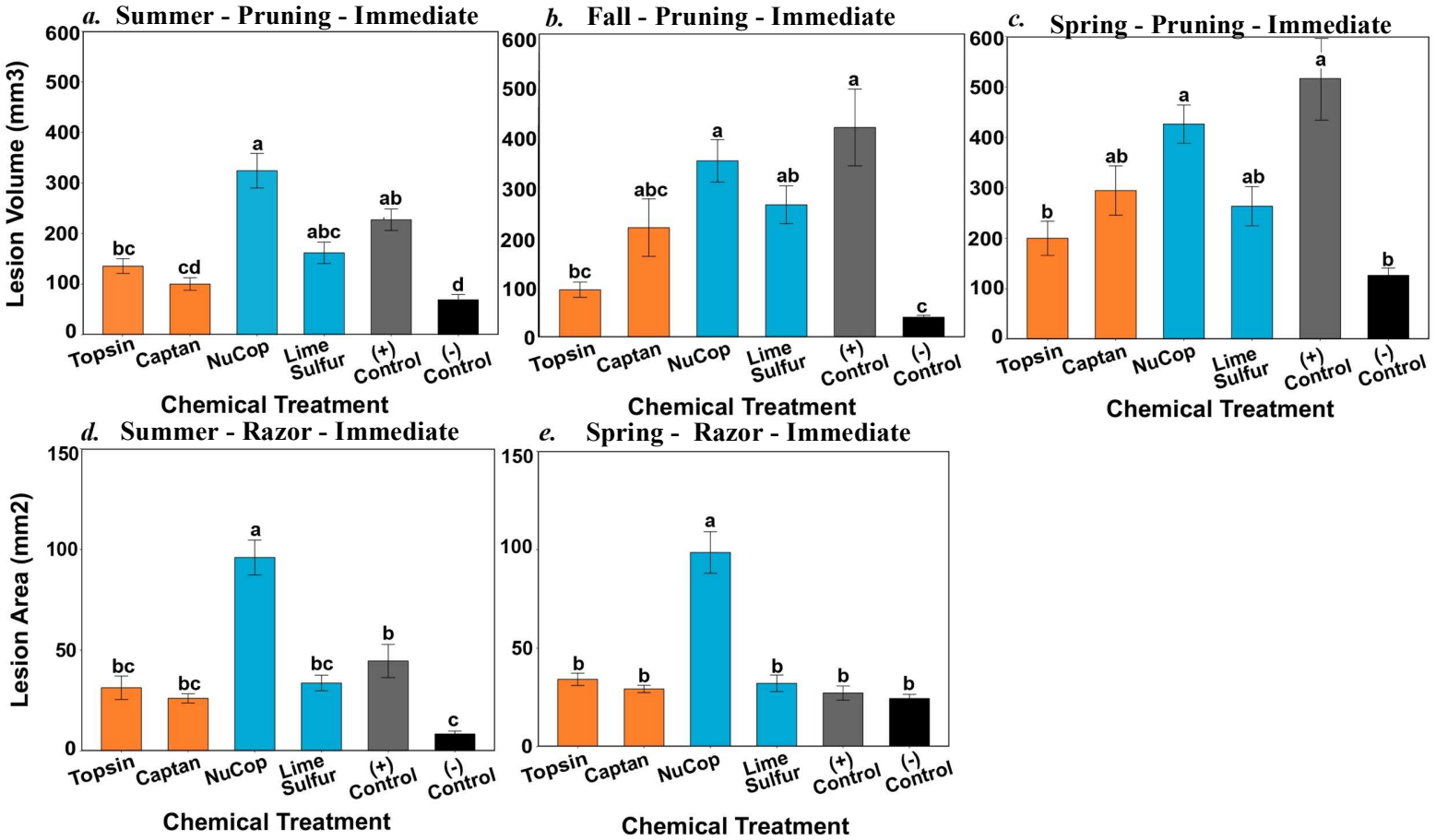

Figure 2.6. Field Trials: Immediate chemical efficacy sprays in the field by season on *Cytospora leucostoma* inoculations (100 µl of 1 x 10^5 spores ml) (a-c, inoculated pruning wounds; d-e, inoculated razor wounds). Plots portray fungal lesion size by conventional (orange bars) and organic (blue bars) chemical treatments. The positive control (grey bar) consists of *C. leucostoma* inoculations on detached branches with no chemical applications. The negative control (black bar) consists of a wounding with no pathogen inoculation. (a) Summer trial, pruning wounds and immediate inoculations. (b) Fall trial, pruning wounds and immediate inoculations. (c) Spring trial, pruning wounds and immediate inoculations. (d) Summer trial, razor wounds and immediate inoculations. (e) Spring trial, razor wounds and immediate inoculations. Significance of treatments with lower-case letters are reflective of Tukey adjusted values ($P < 0.05$) of the developed model.

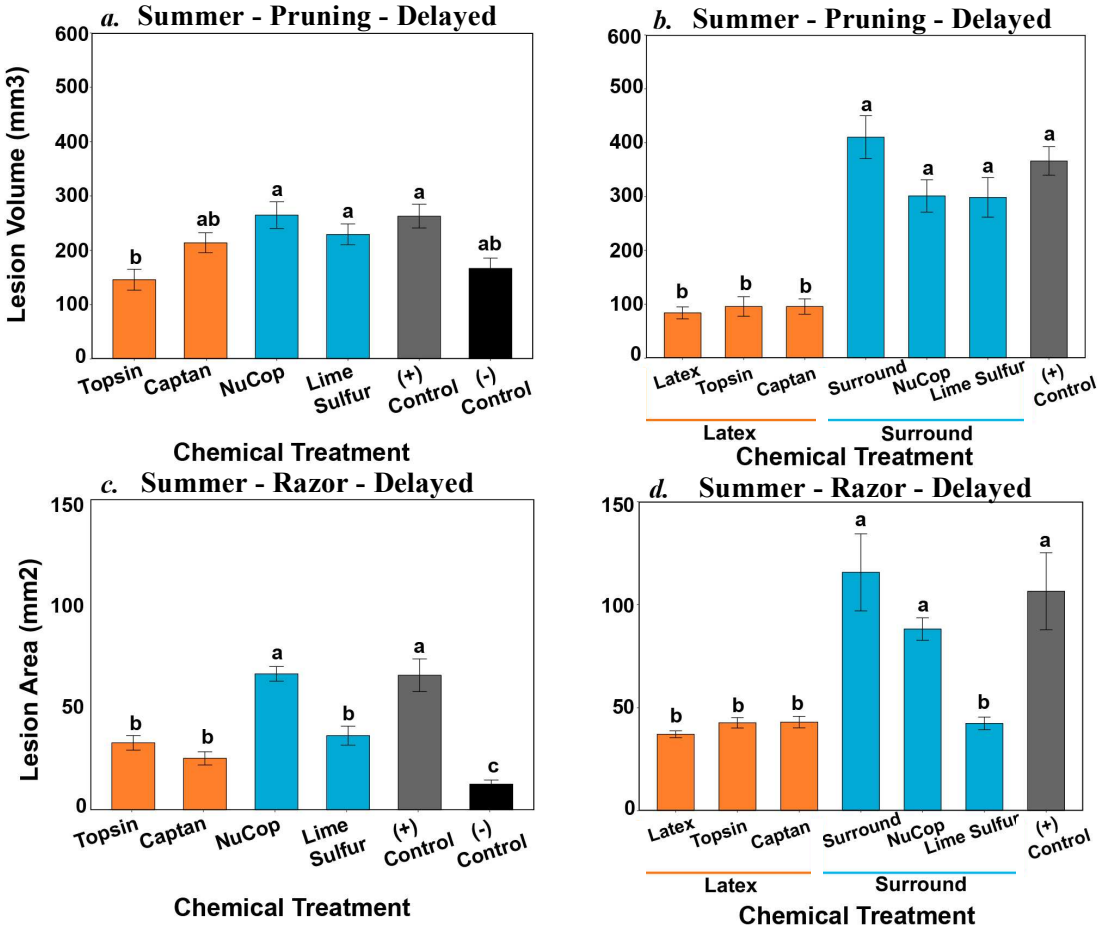

Figure 2.7. Field trials: Summer residual chemical efficacy by wound type on *Cytospora leucostoma* inoculations (100 µl of 1 x 10^5 spores ml). Plots portray fungal lesion size by chemical treatment, conventional (orange bars) and organic (blue bars). The positive control (grey bar) consists of *C. leucostoma* inoculations on detached branches with no chemical applications. The negative control (black bar) consists of a wounding with no pathogen inoculation. (a) Summer trial, standard chemical sprays, pruning wounds, delayed inoculations. (b) Summer trial, chemicals embedded in paint and kaolin clay, pruning wounds, delayed inoculations. (c) Summer trial, standard chemical sprays, razor wounds, delayed inoculations. (d) Summer trial, chemicals embedded in paint and kaolin clay, razor wounds, delayed inoculations. Significance of treatments with lower-case letters are reflective of Tukey adjusted values ($P < 0.05$) of the developed model.

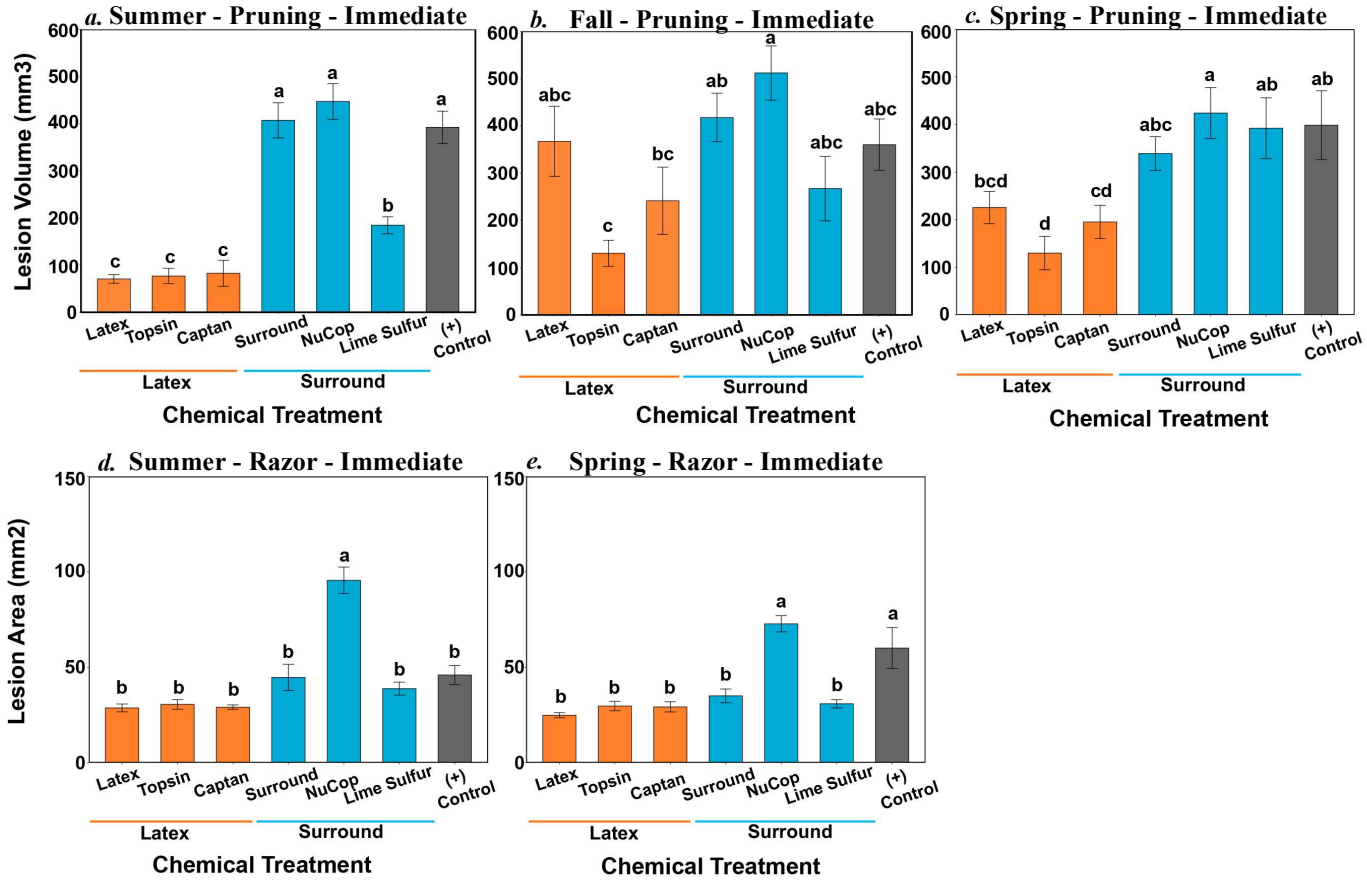

Figure 2.8. Field trials: Wound sealant sprays with chemicals amended in latex paint or kaolin clay immediate efficacy on *Cytospora leucostoma* inoculations (100 µl of 1 x 10^5 spores ml) (a-c, inoculated pruning wounds by season; d-e, inoculated razor wounds by season). The plots above portray fungal lesion size by chemical treatment, conventional (orange bars) and organic (blue bars). The positive control (grey bar) consists of *C. leucostoma* inoculations on detached branches with no chemical applications. (a) Summer trial, pruning wounds, immediate inoculations. (b) Fall trial, pruning wounds, immediate inoculations. (c) Spring trial, pruning wounds, immediate inoculations. (d) Summer trial, razor wounds, immediate inoculations. (e) Spring trial, razor wounds, immediate inoculations. Significance of treatments with lower-case letters are reflective of Tukey adjusted values ($P < 0.05$) of the developed model.

DISCUSSION

The efficacy of several chemicals from controlled laboratory studies to field trials for the control of *C. leucostoma*, a major limiting factor of peach production in Colorado, is reported. Of the chemicals tested, Captan, Topsin and lime sulfur alone, Captan and Topsin amended in 50% white latex paint, and lime sulfur amended with kaolin clay were the most effective chemical treatments for preventive *C. leucostoma* control. While no chemical was consistently effective in all seasons where field trials performed (summer, fall, spring), the seven aforementioned chemicals showed evidence of efficacy under controlled laboratory conditions, and also under variable field conditions.

The chemicals Aliette, Topsin, Benlate, Captan, Inspire Super, Mpede, Kaligreen, Serenade, NuCop, Badge, ZnSO4, and lime sulfur all successfully inhibited hyphal growth in chemically amended nutrient agar (Figure 2.3). This confirms previous *in-vitro* studies of the chemical efficacy of Captan and Topsin amended in nutrient agar against *C. leucostoma* hyphal growth (Biggs et al. 1994). This list was reduced further in laboratory studies to Topsin, Benlate, Captan, Ziram, NuCop, and lime sulfur by immersing detached branch segments in chemical solutions and inoculating with fungal cores (Figure 2.4). Although these six chemicals were effective on removed branch segments in terms of reducing pathogen growth, they were not as effective as when amended in nutrient agar. This likely must do with the woody tissue composition of a detached branch and the distribution of a given chemical when compared to continuous contact in a nutrient agar chemical solution. Nonetheless, results were consistent with previous studies, which reported Captan and Topsin to be effective in preventing *C. leucostoma* growth on excised branches immersed in chemical solutions (Biggs et al. 1994).

Field trials (Table 2.3) revealed evidence of chemical efficacy for Captan, Topsin and lime sulfur alone, Captan and Topsin amended in 50% white latex paint, and lime sulfur amended with kaolin clay. Contrary to previous studies, which found Captan and lime sulfur to be ineffective in canopy field applications, evidence of efficacy for these two chemicals was found (Figures 2.6a & 2.7c) (Northover 1976). Previous studies did not apply targeted pathogen inoculations on targeted sprays. Instead, previous work applied 14 liters of a fungicide to individual trees and evaluated one year old shoots for disease incidence without applying targeted pathogen inoculations. Lack of chemical efficacy may have been reported due to infections already being present, thus the study did not report preventive chemical measures. Results reported in this thesis include targeted chemical sprays and targeted pathogen inoculations of 1 x 10^5 spores ml, to evaluate preventive measures. Further, this study confirms earlier reports (Biggs 1990) that claim the efficacy of latex as a temporary sealant on wounded tree shoots (Figure 2.7b & d; Figure 2.8a & e).

Interestingly, by conducting chemical trials in multiple seasons, it was evidenced that pathogen growth rates (post-inoculation) varied depending on the season. In previous studies investigating seasonal growth rates of *C. leucostoma* in Grand Junction, Colorado, researchers found that the greatest increase in canker surface area occurred between the months of March and June, while periods of least increase occurred in the consecutive summer and fall months (Jones & Luepschen 1971). In the field trials conducted in this thesis, similar to the results of the previous studies, inoculations made during the March and June months yielded the quickest increase of lesion size, shoots being harvested 3 months post-inoculation and 2 months post-inoculation, respectively. Inoculations made during November yielded much slower lesion size

growth thus shoots were harvested 5 months post-inoculation. Pathogen growth rate periods were consistent in this study with previous investigations conducted in western Colorado.

As a result of the varied seasonal growth periods in the field trials, chemical efficacy of paint treatments was only found during the summer and spring pruning wound sealing trials. This concurs with previous research suggesting that latex paint may only prevent infections for a short period of time (Biggs 1990). Fall trials did not result in effective pruning wound sealing efficacy (Figure 2.8 b), likely due to the slowed pathogen growth and extended five-month post-inoculation harvest time. The latex paint failed to provide long term (five-month) protection, splitting open and revealing the underlying tissue for infection (Biggs 1990). Spores were confirmed viable through laboratory isolations, below wrapped Parafilm, for at least three months and likely were able to infect once the latex had worn off. Thus, one can conclude that effective latex paint treatments provide no more than three months of protection, given the efficacy of the summer and spring paint trials with a two to three-month pathogen growth period when compared to the lack of efficacy in the five-month pathogen growth period of the fall trials (Figure 2.8a & c versus 2.8b).

In the research presented, NuCop (50% copper hydroxide) was found to be consistently ineffective, and in three experimental field trials, yielded larger lesion sizes than the positive control (Figures 2.6d-e; Figure 2.8d). Previous reports suggest that copper hydroxide is phytotoxic to peach trees with susceptibility varying depending on variety (Lalancette & McFarland 2007). Studies testing Cytospora canker control in peach have reported that the copper and the lime based Bordeaux mixture is ineffective and phytotoxic, thus increasing severity of the disease (Northover 1976). Results of the present study are consistent with previous claims of copper phytotoxicity and suggest that lesion sizes may be larger due to

phytotoxic effects. Isolations were made from cankers treated with NuCop and *Cytospora* infections were confirmed.

This study presents chemical efficacy on two wound types, pruning and razor wounds. Razor wounds were selected to mimic cold damage that causes phloem tissue cracking and splitting. The results herein are not without limitations. Razor wound results may have been limited, as razor cuts were not infected consistently. Limitations due to lack of pathogen infection were likely caused by inconsistent razor wound depths in different plant tissue layers, as the wounds were applied by hand with a razor-sharp box cutter. Razor cut application pressure, in terms of cut depth, were not consistent as pressure of cut for each wound would vary since they were applied by hand, and there is no way to standardize the hand force consistently. Further, razor wounds may not be as easily infected when compared to larger pruning wounds given the differences in exposure area of the underlying tissue and wound size. Varying spore inoculum concentrations may also have played a role in the variation of razor wound infections, despite creating a suspension of 1×10^5 spore ml for each inoculation. Spore viability in extreme temperatures while applying inoculations may have altered the original concentration. Further limitations in the field trials include difficulty in differentiation between oxidized plant tissue and actual lesion areas. Plant samples were extracted to confirm Koch's postulates, but differences between tissue oxidation and pathogen lesions where indistinguishable.

Of the fungicides tested in this study, Captan, Topsin and lime sulfur alone, Captan and Topsin amended in 50% latex paint, and lime sulfur amended with kaolin clay (Surround WP) showed evidence of efficacy from controlled laboratory experiments to variable field studies. Of these treatments, 50% latex paint, Topsin amended in 50% latex paint, and Captan amended in 50% latex paint, provided the most evidence of efficacy on pruning wounds. In the summer

application, with a 2-month pathogen growth period, all chemical combinations combined with 50% latex paint were effective on pruning wounds (Figure 2.8a). Even though efficacy for Captan and Topsin was also found without 50% latex paint in different pruning trials, all treatments were effective for the paint-embedded chemical trials on pruning wounds during the summer. Further, the differences between the canker size and the positive control canker size for the latex - chemical combinations were large for every single treatment in the trial (Figure 2.8a). However, efficacy was not consistent in all field trials for any of the chemicals. NuCop showed absolutely no efficacy and was shown to potentially have phytotoxic effects in the trials. Future directions should include the testing of novel chemicals as well as outlining the epidemiology patterns of *C. leucostoma* specific to the western slope of Colorado region. This will allow for effective chemical application timing to decrease heavy field inoculum pressure and provide preventive infection measures on peach trees.

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CHAPTER III. **Limiting Spore Production from Existing *Cytospora leucostoma* Cankers.**

SUMMARY

Cytospora leucostoma is a known pathogen on *Prunus* spp., and a major limiting factor in peach production in western Colorado accounting for 15-20% of crop loss annually. From the time of an infection, cankers will develop and cause the tree to decline in production until it finally succumbs to the pathogen. During the gradual decline of the tree, peach production is still possible even though yield loads are significantly reduced. Thus, the disease may persist in orchards as growers may not be economically inclined to remove symptomatic trees that are still able to produce fruit. Containment chemical control, or reducing spore production on existing cankers via chemical measures, is an integral component of pathogen management as it decreases spore inoculum loads within orchards. Four chemicals, chosen from laboratory studies described in chapter II and confirmed as effective *in-vitro* chemicals, were tested as chemical amendments with latex paint and kaolin clay for spore inoculum reduction on existing cankers within orchards. The treatments consisted of 50% white latex, Topsin amended in 50% latex, Captan amended in 50% latex, kaolin clay (Surround WP), NuCop combined with kaolin clay, and lime sulfur combined with kaolin clay. On all the treatments tested, efficacy could not be statistically confirmed or denied due to variable field conditions; nonetheless, 50% white latex alone and combined with Captan showed the most evidence of spore inoculum reduction. Kaolin clay (Surround WP), NuCop combined with kaolin clay, and lime sulfur combined with kaolin clay did not show evidence of efficacy. Furthermore, spore production levels, on non-treated cankers, fluctuated in response to temperature and relative humidity changes.

INTRODUCTION

Peaches are a specialty crop in Colorado, with values of utilized production totals that range from $17-30 million annually (USDA 2016). According to surveys conducted by Colorado State University in 2015, *Cytospora leucostoma* is a ubiquitous pathogen in western Colorado with 100% of orchards surveyed containing 33-100% of planted trees infected by *C. leucostoma*. Previous work estimated that Cytospora canker reduced crop yields by 15-20% annually in Colorado (Pokharel & Larsen 2009). Further, the pathogen has been shown to sporulate year-round in western Colorado (Miller et al. unpublished), making new infections possible all twelve months of the year. Levels of spore production depend on the environmental factors of the region and microclimate in question. Previous studies conducted in Washington State also found year-round sporulation with the highest rates of spore production occurring during months with higher precipitation (Grove & Biggs 2006). Grove and Biggs (2006) also reported that *Cytospora* spores were dispersed up to 60 centimeters when exposed to water splash.

Given the constant pathogen pressure, preventive management measures are vital to orchard protection. Especially given the infection cycle of *C. leucostoma*, a pathogen that consistently releases inoculum throughout the year readily infecting tree wounds, translocating via the xylem tissue, and persisting in dead wood once the host declines (Hampson & Sinclair 1973; Biggs 1989). Management practices could include planting new trees in isolated sites away from diseased areas, sanitation, avoidance of interplanting young trees near disease harboring trees, and protection of tree plant material from wound openings caused by insects, winter damage, prune damage, rodent injury, and more (Biggs 1989). Along with these measures, containment measures must be incorporated for a complete integrated pest management plan. Containment measures refer to chemical applications applied over a fruiting canker that seal and dry out the

pathogen, thereby limiting spore production on existing cankers and reducing inoculums loads with an orchard. If producers are economically unable to remove infected trees, then canker cover to reduce spore inoculum load is an integral component to reducing pathogen spread.

White latex paint has been used historically on tree bark as a protective measure to prevent sunburn and secondary pathogens such as *Cytospora* spp. from colonizing sunscald wounds. Latex paint has been reported as one of the most practical methods in stabilizing bark temperatures and preventing abiotic temperature damage (Litzow & Pellett 1983). Thus, in the presented research 50% latex was investigated as a potential agent in canker desiccation. Cytospora cankers flourish under humid conditions; thus, by covering cankers, spore release may be inhibited through the drying of the cankers and through the sealing of the pyncidia fruiting bodies. As latex paint is a synthetic product, kaolin clay (Surround WP) was also investigated as an alternative canker cover for organic production systems.

The objectives of this study were to evaluate the efficacy of conventional and organic fungicides as chemical covers over fruiting cankers to limit *Cytospora leucostoma* spore production. Temperature and relative humidity in study orchards are also reported, to better understand spore production patterns during the study.

MATERIALS AND METHODS

Location and Design of Study. Chemical efficacy on existing Cytospora canker spore loads was evaluated in an eleven-year-old conventional peach orchard in Palisade, Colorado planted with 'Zee Lady' peach trees on rootstock Hansen 536. One canker per tree was randomly selected, with a total of 56 cankers. Six conventional and organic treatments were studied with one non-treated positive control for a total of seven treatments. Sample sizes included eight

cankers per treatment. The conventional chemicals included 50% white latex paint, Topsin amended in 50% latex paint, and Captan amended in 50% latex paint (Table 3.1). The organic chemicals included Surround WP, which is a protective film of kaolin clay, lime sulfur amended in kaolin clay, and NuCop amended in kaolin clay (Table 3.1).

Chemical Applications. Chemical applications were made on marked cankers in the study orchard. Cankers were treated with the chemical solutions and mixtures one month after initial spore collections were taken. Initial spore counts were taken as pre-chemical treatment collections on fruiting *Cytospora* cankers. The initial spore collections were taken to account for spore reduction differences in pre-chemical collections and post chemical collections. Chemical treatments were added to either 50% latex or kaolin clay (Surround WP) at the commercial label mid-rate at the final volume (see table 3.1 for chemical midrates used). Kaolin clay mixtures were created by using also the Surround WP label mid-rate (Table 3.1). Chemical solutions and mixtures were applied using 32-ounce spray bottles. Cankers were sprayed with treatments until fully covered or runoff (Figure 3.1b). The 50% latex solutions were created by combining 50% distilled water with 50% latex.

S**pore Collections.** Pre-treatment spore collections were made one month prior to chemical applications (October 6th, 2016) and once a month for seven consecutive months post chemical application (through May 20th, 2017). Canker effluent was collected using a funnel and a laboratory wash bottle (Figure 3.1a). Cankers were washed, and 10 ml of canker effluent was collected into 15 ml tubes. Once spore effluent was collected from each canker, the effluent was then centrifuged and concentrated to 5 ml. Spore concentrations were then calculated for the 5 ml

sample using a hemocytometer, and the estimated number of spores per ml were calculated and extrapolated for the number of spores in 10 ml effluent.

Statistical Analysis. RStudio was used for statistical analyses. Packages used included lme4, lmerTest, car, pbkrtest, and lsmeans (R Core Team 2017; Lenth 2016; Fox & Weisberg 2011; Halekoh & Højsgaard 2014; Kuznetsova et al. 2016; Bates et al. 2015). Summary statistics and graphical depictions were created using plyr and ggplot2 packages (Wickham 2011; Wickham 2009). A two-factor repeated measure model was designed for the analysis of the experiment. Spore counts (spores per ml) was treated as the response variable. Predictor variables included two fixed effect variables of treatment and time, along with two random effect predictor variables of canker and interaction between chemical and time. Canker was treated as a random effect to account for the variance effect between the cankers chosen. Statistical analyses were conducted on spore production differences from pre-treatment measurements. Pre-treatment measurements were subtracted from the post-treatment measurements from each treatment group to standardize the count data. The assumptions of normality were satisfied and therefore data were not transformed. An outlier test was conducted, using the car package, due to the large variability from canker to canker (Fox & Weisberg 2011). No significant outliers were found. A linear model analysis of variance (ANOVA) was run on the created repeated measure model, followed by Tukey adjusted pairwise comparisons ($\alpha = 0.05$) (Tukey's HSD adjusted p-values are $P < 0.05$). 95% confidence intervals were used to evaluate differences (Figure 3.2; Table 3.2), since the pre-treatment was subtracted from the analysis. Thus, treatments were non-significant from the baseline when confidence intervals included 0 (Figure 3.2).

Table 3.1. Chemical treatments and corresponding concentrations chosen for application on existing *Cytospora leucostoma* cankers.

Treatment Name	Active Ingredient	Recommended Rate per 200 gallons	Rate Chosen	State	System
Captan	N-Trichloromethylthio-4-cyclohexene-1, 2-dicarboximide	3-4 qt	3.5 qt	Liquid	Conventional
Topsin M WSB	Thiopthanate-methyl	1-1.5 lb	1.25 lb	Solid	Conventional
50% Latex	Latex	10.5-14.5 oz	12 oz	Solid	Conventional
Lime sulfur	Calcium polysulfide	20-24 gal	22 gal	Liquid	Organic
NuCop WP	Copper Hydroxide	8-20 lb	3.4 oz	Liquid	Organic
Surround WP	Kaolin Clay	10 lb	10 lb	Solid	Organic

a.

b.

c.

Figure 3.1.
a. Collection of *Cytospora leucostoma* spore effluent from an existing canker on peach tree
b. Existing *Cytospora leucostoma* cankers on peach tree treated with 50% latex
c. Existing *Cytospora leucostoma* cankers on peach tree treated with kaolin clay (Surround WP)

RESULTS

Spore Counts of Treated Cankers. Spores were observed in all collections and all treatments over the eight-month period from October 6th, 2016 through May 5th, 2017. Spore counts ranged from 100 to 70,000 spores per ml. At each specific collection date, following the pre-treatment collection, significant differences could not be confirmed when comparing different chemical treatments for the most effective chemical at one time point ($P > 0.707$, for all values). However, significant fluctuations in spore counts were found by date when comparing the collection time points of a specific chemical treatment to the pre-treatment collection time point (Figure 3.2; Table 3.2). There were significant differences in the positive controls, cankers with no chemical applications, when comparing pre-treatment (October) measurements with spore counts from December (9th, 2016), January (17th, 2017), February (27th, 2017), March (15th, 2017), and May (20th, 2017) (Figure 3.2; Table 3.2). A similar trend was observed with collection time points in the latex combined with Topsin treatment. In the latex only treatment, significant decreases in spore counts occurred during December, January, February, and March (Figure 3.2; Table 3.2), when compared to the pre-treatment October collection date. When latex was combined with Captan, significant fluctuations were only observed during January, February, and March (Figure 3.2; Table 3.2). However, no significant decreases in spore counts were observed in the kaolin clay (Surround WP) alone treatment. However, when kaolin clay was amended with lime sulfur, a significant decrease in spore counts was observed for February. Surround combined with NuCop yielded significant decreases in spore counts in February, March, and May (Figure 3.2; Table 3.2).

Weather Data. During the 8-month trial period, fluctuations in temperature and humidity were observed. December of 2016, January of 2017, and February of 2017 had the lowest monthly temperature averages of 31°F, 32°F, and 43°F; respectively (Figure 3.3a). Despite these low temperatures, monthly percent relative humidity averages were highest for December and January at 62% RH, and 66% RH; respectively (Figure 3.3a). In February 2017, the day prior to and the day of collecting spore samples had the lowest temperature and relative humidity combinations when compared to all other time points (37°F; 41% RH; respectively; Figure 3.3b). March 2017 and April 2017 contained high average temperature averages one day prior to and on the same day of making collections at 57°F and 60°F; respectively (Figure 3.3b). Further, these two months had high average monthly temperatures at 52°F and 55°F, respectively (Figure 3.3a). Despite the high temperatures, % relative humidity was lowest for March of 2017 and April of 2017 compared to all other months observed (37% RH; 34% RH; respectively; Figure 3.3a).

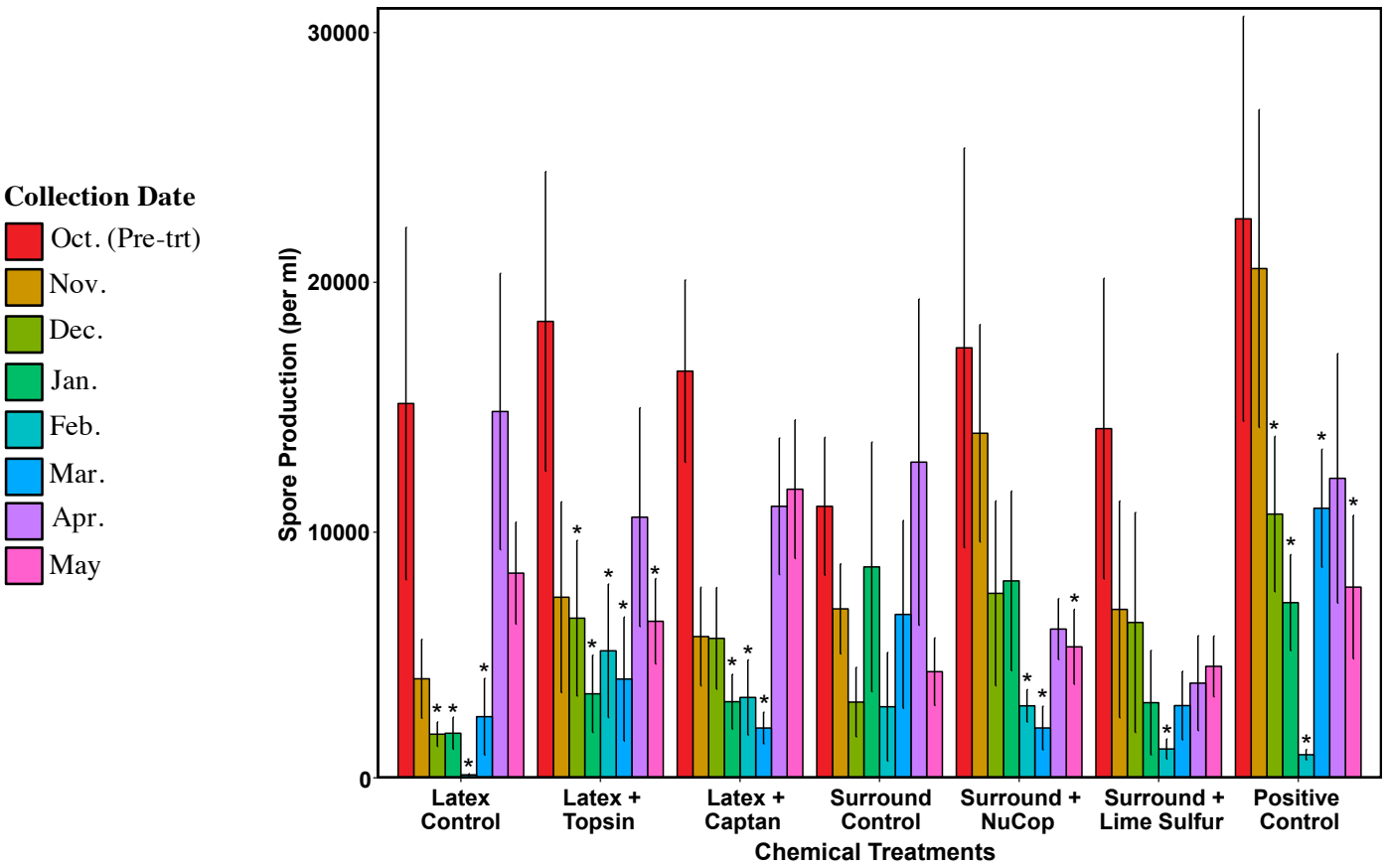

Figure 3.2. Spore counts by collection date of chemically treated *Cytospora* cankers on 11 year-old 'Zee lady' peach trees. Monthly spore counts are presented over an 8-month period (7 months post-treatment). Chemical treatment (x-axis) colors represent spore collections for monthly time points. The positive control consists of spore count data from non-chemically treated cankers. The asterisk shows treatments that are significantly different from the pre-treatment (red) measurement collection within each chemical group. Statistical differences are reflective of 95% confidence intervals of a multi factor repeated measures model.

Table 3.2. Significant confidence intervals (CL) of spore count differences between pre-treatment measurement date and post-treatment measurement dates are show below. Pre-treatment spore counts were subtracted from post-treatment spore counts thus significant confidence intervals below do not include 0. The pre-treatment measurement is 0, confidence intervals including 0 (not shown) did not have a significant reduction in spore counts. As the significant confidence intervals below are based on the difference; larger intervals show higher levels of decreased spore production.

Treatment	Date	[a]Lower CL	[a]Upper CL
Latex	12/9/16	1175.77	25424.24
Latex	1/17/17	1137.62	25386.1
Latex	2/27/17	2811.77	27060.24
Latex	3/15/17	1329.87	26552.46
Latex + Topsin	12/9/16	596.32	23278.68
Latex + Topsin	1/17/17	3637.94	26320.31
Latex + Topsin	2/27/17	1908.94	24591.31
Latex + Topsin	3/15/17	3588.43	26595.41
Latex + Topsin	5/20/17	721.32	23403.68
Latex + Captan	1/17/17	1955.69	24638.1
Latex + Captan	2/27/17	2243.6	25259.2
Latex + Captan	3/15/17	3850.97	27293.5
Surround + NuCop	2/27/17	3058.2	25740.56
Surround + NuCop	3/15/17	3938.33	26945.65
Surround + NuCop	5/20/17	1181.6	24188.9
Surround + Lime Sulfur	2/27/17	1544.32	24226.7
Positive Control	12/9/16	533.82	23216.2
Positive Control	1/17/17	4096.32	26778.7
Positive Control	2/27/17	10212.94	32895.31
Positive Control	3/15/17	2838.9	26860.1
Positive Control	5/20/17	3471.32	26153.7

[a]95% confidence interval

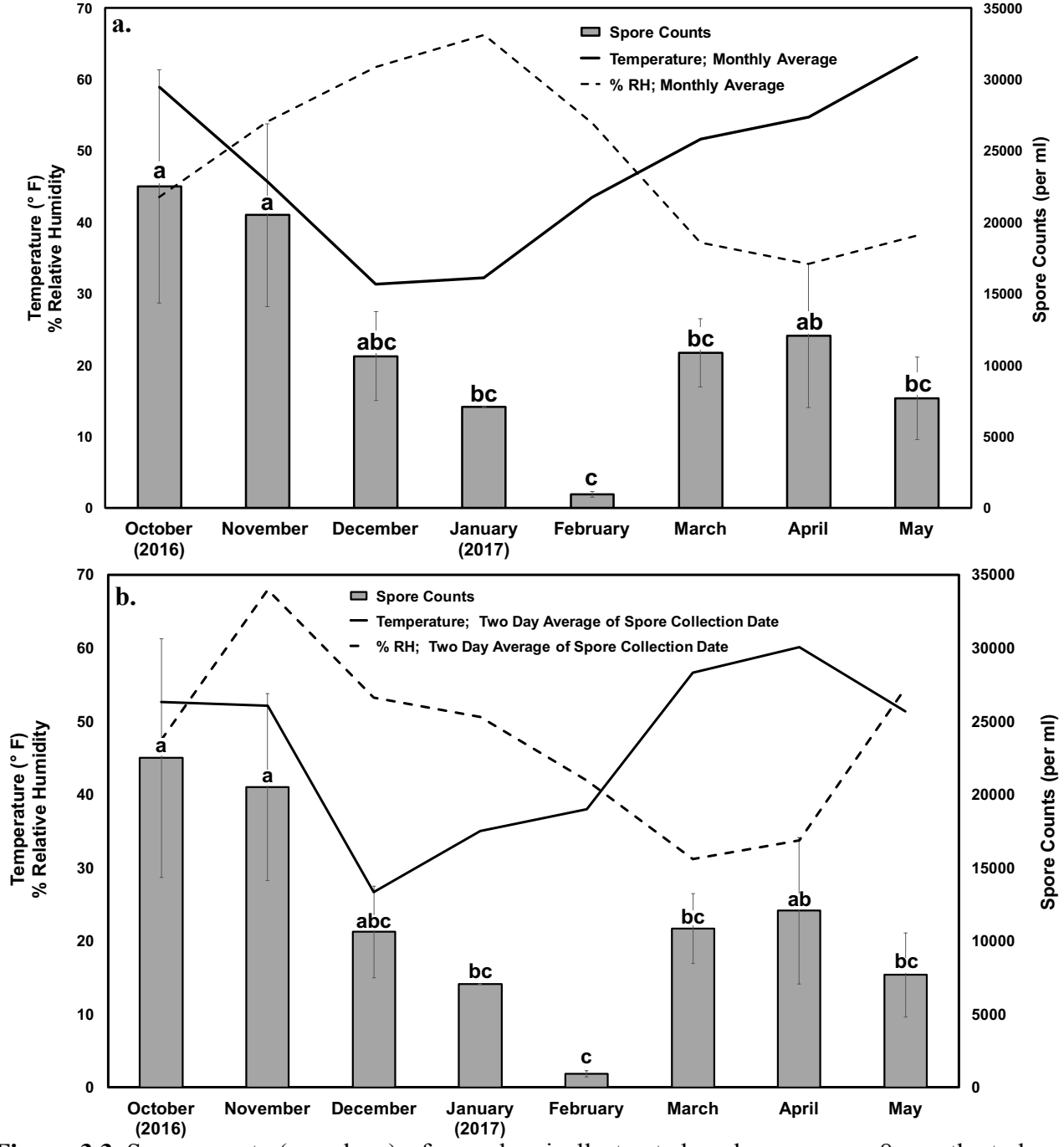

Figure 3.3. Spore counts (grey bars) of non-chemically treated cankers over an 8-month study period, and relationships to temperature (solid lines) and % relative humidity (% RH, dotted lines) in a 'Zee Lady' peach orchard in Palisade, CO. **Plot (a)** shows monthly averages of temperature and monthly averages of % RH over the study period. Spore production is presented as one collection per month, n = 8 cankers per collection time point. **Plot (b)** shows two-day averages of temperature and % RH one day prior and the day of spore collection. Spore production is presented as one collection per month. Significance of dates with the lower-case letters are reflective of 95% Confidence level and Tukey adjusted values ($P < 0.05$) of the developed repeated measures model.

DISCUSSION

The results of this study have shown that *C. leucostoma* spores are continuously produced on cankered peach trees despite chemical cover. Chemical efficacy could not be confirmed or denied as all significant differences between baseline measurements and following time points were also evident in the positive control (no chemical application) (Figure 3.2). The inability to tease apart the factor responsible for the spore count decreases was likely due to the large variation of spore production between cankers. This variation could be due to canker age and size, number of pyncidia present on canker, canker position on tree, canker moisture, and other similar variables. The sample size of 8 cankers per chemical treatment was likely not enough given the large variation in spore production within treatments. Further, drastic seasonal changes from October to May could have also obscured possible differences in chemical efficacy across treatments (Figure 3.2). Nonetheless, there is evidence of spore inoculum levels fluctuating with changes in temperature and humidity (Figure 3.3b). The largest drop in spore production was observed in the positive control in February 2017 (2/27/17) (Figure 3.2; Figure 3.3b). This decrease in spore production is consistent with the temperature and relative humidity data showing that the two-day average, one day prior and day of collections, yielded the lowest combined temperature and humidity averages when compared to all other months (Figure 3.3b). It is evident that temperature and humidity together play a role in canker spore activity. This is consistent with previous literature monitoring spore production in peach orchards in eastern Washington. This research also found spore production to be lowest when temperature and precipitation were lower (Grove & Biggs 2006).

Despite temperature and relative humidity likely playing a major role in canker spore inoculum load, the 50% latex treatment may provide control. During November of 2016 (6^{th},

2016), there was a large drop in spore production when comparing the pre-treatment measurement (October) to the first post-treatment measurement (November) for the latex control and latex + Captan treatments (Figure 3.2). However, this drop was not statistically significant. As mentioned, variable field conditions, along with inadequate sample sizes may have obscured statistical significance. Interestingly, there was no statistical significance observed in the kaolin clay (Surround WP) control treatment for any of the monthly measurements (Figure 3.2). Thus, kaolin clay may not inhibit spore production and may in fact protect the pathogen from extreme environmental variables such as temperature and humidity, enhancing canker growth.

Future experiments should be conducted on a year-round basis to have less abiotic influence. Further, sample size should be increased to better account for the large variation observed from canker to canker. Cankers vary drastically in spore production thus future studies should also focus on canker selection based on number of pycnidia fruiting bodies per canker. In this manner, variation from canker to canker can be reduced and chemical efficacy significance can be better targeted. Additionally, multiple applications of effective chemical combinations should be tested as the covering film might not be as effective over the time post application.

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