THESIS

MANAGEMENT STRATEGIES FOR CYTOSPORA PLURIVORA: THE ROLE OF CANOPY SPRAYS IN WESTERN COLORADO PEACH ORCHARDS

Submitted by

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ABSTRACT

MANAGEMENT STRATEGIES FOR CYTOSPORA PLURIVORA: THE ROLE OF CANOPY SPRAYS IN WESTERN COLORADO PEACH ORCHARDS

Cytospora spp. are globally distributed pathogens on more than 120 species of woody plants, in both agricultural and natural systems. In Colorado, Cytospora canker, primarily caused by the fungal pathogen *Cytospora plurivora*, is one of the most destructive diseases affecting peach trees (*Prunus persica*), a key specialty crop with significant economic and cultural importance. The resulting yield loss and financial strain on peach growers threaten the survival of the industry and options for management are limited. No resistant varieties have been identified and no fungicides are currently registered for use on Cytospora canker in Colorado. Chemical management focuses on prevention of new infections on wounded tissue, the infection court for *C. plurivora*. Fungicides applied directly to pruning wounds have proven effective but are impractical for many growers due to the extensive labor required. Additionally, these spot treatments have not been evaluated for efficacy on wounds on main scaffold branches or trunks, which are critical infection sites. Canopy sprays, facilitated by air-blast sprayers, could be a more cost-effective option for Cytospora canker control, with the potential to target entire tree scaffolds. Air blast sprayers are currently used by growers to manage other diseases and pests and for nutrient applications; however, their efficacy on canker pathogens is not well understood. This study aims to explore the utility of canopy sprays with air blast sprayers for management of *C. plurivora* in organic and conventional peach orchards and to determine best practices for achieving optimum coverage of bark on scaffold branches.

Field trials were conducted in organic and conventional orchards, located at the CSU Agricultural Experiment stations in western Colorado, to assess both spray coverage and fungicide efficacy. Field trials to assess air-blast spray coverage were conducted in the spring and summer of 2023 to explore the effect of season of application and fan use on bark coverage. Water Sensitive Paper (WSP) cards were placed at three heights on each tree, sprayed with water, and analyzed with ImageJ software (Rasband, 1997) to determine the percent coverage of cards. Higher percent coverage was observed in the spring on bare trees, likely due to foliage blocking cards in summer trials. Fan use had variable effects on coverage within and between orchard planting blocks. Issues with uniformity in coverage were observed in all orchard blocks, with top cards receiving the lowest coverage in almost all cases.

Fungicide field trials were conducted in the fall of 2023 and spring of 2024. An Organic Materials Review Institute (OMRI) listed fungicide, lime sulfur (Lime-Sulfur Solution™, NovaSource), was tested in the organic orchard and a conventional fungicide, captan (Captan 4L, Drexel Chemical Company, Memphis, TN), tested in the conventional orchards. Trees were wounded and inoculated with mycelial plugs of *C. plurivora* at the bottom of primary scaffold branches, on mid-scaffold branches, and on top branches. Resulting lesions were measured several months post-inoculation to assess disease development. Fungicide efficacy was evaluated by comparing lesion area (mm^2) of treated and untreated trees. In fall fungicide trials, minimal lesion growth was observed overall, and treatments with lime sulfur and captan showed no efficacy in any orchard planting block. In the spring fungicide trials, treatment with lime sulfur significantly decreased lesion size in the organic orchard: on middle branches by 58% and top branches by 87%. Results of captan treatments were inconclusive but warrant future studies. No efficacy was observed in one planting block in the conventional orchard, but in the planting block with more mature trees, captan treatment was associated with a 147% decrease in lesion size.

The results of this study suggest that air blast sprayers may be an effective tool for *C. plurivora* management, but future studies are needed to confirm the extent of their efficacy. Fall canopy sprays may not be effective or necessary, while summer sprays may have limited efficacy due to low coverage on foliated trees. Efficacy was observed with spring application of lime sulfur at 3%, supporting its use in canopy sprays. Efficacy of captan applications could not be confirmed or rejected due to inconsistency of results. The rate used in this study, 3.5 liters/hectare, may be insufficient, as had been found previously. Midrate applications (7 liters/hectare) of captan should be tested in future studies. Overall, efficacy was limited, highlighting the importance of integrated pest management strategies and the need for continued research on alternatives to chemical control.

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CHAPTER 1: LITERATURE REVIEW

1.1 Fruit Trees in Colorado

Peach trees (*Prunus persica*) have been cultivated in Colorado since 1881 and have become an integral part of the state's culture (Sexton, 1996). Along with Pueblo green chiles, they are a defining specialty crop in Colorado. Colorado peaches fetch one of the highest prices in the country (United States Department of Agriculture, 2022). Often called Palisade peaches for the town of Palisade, CO where the Colorado peach industry began, they are now grown throughout Mesa, Delta, Montrose, and Montezuma counties (Colorado Department of Agriculture & National Agricultural Statistics Service, 2002). Along with most of Colorado, the land on which Palisade was established was Ute territory until the mid to late 19th century when gold miners and farmers colonized the area. Despite the dry climate and lack of groundwater, early colonists intent on growing fruit trees diverted water from the Colorado River (previously known as the Grand River) and Gunnison River to form the canals that still irrigate orchards in the region to this day (Holleran, 2005). Peach orchards were soon prolific, with more than twenty-five thousand pounds of peaches shipped annually from Palisade by the beginning of the 20th century. Peaches were not the only fruit crop; farmers also cultivated apples, pears, apricots, and cherries, and grapes (Sexton, 1996). Colorado remains a major hub of tree fruit cultivation today. In 2022, Colorado farmers produced 14,050 tons of peaches, bringing in 33.91 million dollars and ranking 5th in the US for peach production (Colorado Agricultural Statistics Service, 2023).

The dry climate in western Colorado prevents the proliferation of some fungal and bacterial diseases, but environmental factors such as early fall and late spring frosts, lack of water, and alkaline soils stress trees, leaving them vulnerable to the diseases and insects that are present

(Sharp & Cooley, 2004). Pests have played a pivotal role in the economics of fruit tree crops in Colorado. Apple trees were a once booming crop in Colorado, but an infestation of codling moths decimated the industry. Half a million apple trees were lost due to a lack of effective controls, exacerbated by the development of pest resistance to existing chemical controls (Sexton, 1996). A similar threat faces peach growers today, with a devastating fungal disease at epidemic levels: Cytospora canker. Cytospora canker is one of the most impactful diseases for peach growers in western Colorado (Stewart et al., 2022), yet management options are limited, and no fungicides are currently registered to treat the disease on peaches in Colorado.

1.2 Cytospora canker

. In 2015, Cytospora canker was observed in 100% of the orchards surveyed by the Colorado State University Western Colorado Research Station, with infection rates of 30-90% (Miller et al., 2019). Cytospora canker accounted for 20% of production loss in Colorado stone fruits in 2015, equating to over $3 million loss in crop value per year (U.S. Department of Agriculture, (USDA, 2016).

Cytospora canker has long been found in peach orchards worldwide but is most severe in cold climates, including northern fruit-growing regions of the US (Grove & Biggs, 2005, 2006). Other fruit trees are affected as well, including plum (*P. domestica*), sweet cherry (*P. avium*), and apple (*Malus domestica*) (Adams et al., 2002). Cytospora canker, known by many names, including perennial canker, Valsa canker, Leucostoma canker, and sometimes generally described as dieback or gummosis, is caused by many species within the genus *Cytospora*

Cytospora spp. affect a wide range of hosts worldwide, encompassing over 130 species of monocotyledons, dicotyledons, and gymnosperms (Adams et al., 2002, 2006; Biggs 1989; Biggs & Miles, 2005; Fan et al., 2020; Lawrence et al., 2018; Northampton et al., 2017; Pan et al., 2020, 2021; Stewart et al., 2022). It should be noted that the inconsistency of previous naming conventions causes great confusion in the discussion of fungal pathogens, and *Cytospora* spp. are no exception. Previously known as *Leucostoma* (anamorph) or *Valsa* (teleomorph), the oldest name for the genus *Cytospora* (Ehrenberg.: Fr, 1818) is now the only currently accepted name (Rossman et al., 2015). Molecular and phylogenetic analyses have revealed previously undescribed species as well as inconsistencies in the morphology of isolates. Complex and overlapping morphological characteristics make some *Cytospora* spp. particularly difficult to differentiate.

Cytospora spp. have been present on fruit trees in the genus *Prunus* within the United States since at least 1892 when *Valsa leucostoma* (anamorph *Cytospora*) was described on peach, plum, and almond trees in Pennsylvania and New Jersey (Rolfs, 1907). Early experiments at the Missouri State Fruit Experiment Station established the pathogenicity of *V. leucostoma* on *Prunus* spp. (Rolfs, 1907). These experiments describe symptoms on peach, plum, apricot, and cherry trees similar to those observed today (Rolfs, 1907).

Trunk and scaffold diseases such as Cytospora canker cause significant loss of fruit bearing wood and ultimately whole trees: reducing yield and shortening the longevity of orchards (Grove & Biggs, 2006). Early signs of infection include necrosis of woody tissue, resulting in darkening of wounded tissues or buds, localized lesions on branches, and the expulsion of amber-colored gummosis. As infections progress, large areas of dark sunken tissue known as cankers develop around the site of infection on trunks or scaffold branches. The pathogen colonizes vascular tissues, compromising vascular function and leading to girdling of branches, causing branch flagging and death. Infections can begin on any wounded woody tissue, including young branches, main scaffold limbs, and trunks. One year old branches have been thought to be the most susceptible and can serve as a route for the pathogen to spread to larger branches and the main trunk (Biggs & Grove, 2005). Cankers often show concentric rings formed by callous tissue which grows annually and is subsequently reinfected by the pathogen (Biggs & Grove, 2005). Yield loss occurs due to the loss of fruit-bearing wood, tree mortality, and potentially reduced productivity of infected branches and trees. Infection of a main scaffold branch can result in 25%- 50% loss of productivity as trees frequently have only 2-4 main scaffold branches (Biggs & Grove, 2005). Tree mortality of up to 3 to 6% per year has been recorded in infected orchards (Biggs & Grove, 2005). It has been suggested that disruption of the vascular system results in reduced productivity in branches distal to cankers (Biggs & Grove, 2005).

1.3 Pathogen Biology

The primary causal pathogen of Cytospora canker on peaches in Colorado is *Cytospora plurivora* (Stewart et al., 2022). First described in California, *C. plurivora*, along with 9 additional newly described *Cytospora* spp., was proposed as genetically distinct from *C. leucostoma* (synonym: *Leucostoma personii* (Nitschke) Höhn) and *Cytospora cincta* (synonym: *Leucostoma cinctum* (Fr.)) (Adams et al., 2002; Lawrence et al., 2018): the species previously considered the only causal pathogens of Cytospora canker on peaches in North America. Lawrence et al. (2018) did not find evidence of *C. cincta or C. leucostoma* in California orchards and suggested that the causal species of Cytospora canker in California may have been previously misidentified. While *C. plurivora* is the most common pathogen isolated from peach cankers in Colorado, many other *Cytospora* spp. are found peach as well as cherries (*P. avium*), apricots, plums (*P. domestica*), and apples (*Malus domestica*). Host range and specificity of many causal species of Cytospora canker have yet to be characterized. Observing the tremendous diversity of *Cytospora* species now recovered from fruit and nut trees in California and China, Rossman et al. (2015) suggested that environmental and geographic factors may be the major determinants of species occurrence, rather than host specificity. Additionally, the genetic diversity observed within *Cytospora* spp. suggests further differentiation may be required. Isolates within species have been observed to have varying optimum growth temperatures and secondary metabolite production: important factors in host specificity, epidemiology, distribution, and virulence (Helton and Konicek, 1962; Dhanvantari et al., 1968; Stewart et al., 2022).

Cytospora plurivora is a necrotrophic ascomycete but can also live as a saprotroph, overwintering in dead tissues. Unable to penetrate healthy bark tissue, *C. plurivora* is deemed an opportunistic pathogen, entering stressed hosts through wounds (Willison, 1937). Trees weakened by abiotic stressors including poor soil conditions, lack of water, and extreme temperatures are particularly susceptible. Cytospora canker is common in Colorado orchards partly due to the physiological stresses presented by frequent spring frosts, fluctuating winter temperatures, and clayey alkaline soils (Sharp & Cooley, 2004; Miller et al., 2021).

The main source of *C. plurivora* inoculum is conidia, asexual spores produced throughout the year which are capable of infecting trees during all seasons (Bertrand & English, 1976). Conidia are produced through pycnidia, small dark bumps which appear on branch surfaces, releasing orange tendrils or cirri, on which the conidia are borne. The teleomorphic stage, perithecia and associated ascospores, are only produced after two or more years and are not frequently observed on peach trees (Bertrand & English, 1976; Grove & Biggs, 2006; Kern, 1955; Wensley, 1964; Adams et al., 2002). While conidia are produced year-round, spore production and growth have been found to be directly correlated with temperature and humidity and are most prolific during spring and summer (Grove & Biggs, 2006). Conidia are dispersed via water droplets, disseminated through rainfall splash, windblown rain, or irrigation (Luepschen & Rohrbach, 1969; Grove & Biggs, 2006). Aerial and insect dispersal have also been observed, although rare (Miller, 2021).

1.4 Management

Cytospora canker management in western Colorado has focused on preventive measures including cultural practices and protection of wounds with contact fungicides (Miller et al., 2019). Spread is minimized by pruning out diseased branches whenever possible and avoiding pruning during times of high inoculum load (Stewart et al., 2018). No resistant cultivars currently exist nor are there any chemical controls registered for control of *Cytospora* (Biggs & Grove, 2005, Pokharel & Larsen, 2013).

Protecting wounded tissues is of particular concern as *C. plurivora* requires wounds to infect and establish in tissues (Wilson et al., 1984). Pruning wounds are common infection courts, sites of pathogen infection. In a study of a French prune orchard, 92% of *Cytospora* infections were found on pruning wounds (Lawrence et al., 2018). Heavy annual pruning is necessary in orchards to thin fruit, increase leaf access to sunlight, and promote the growth of new fruit bearing wood, but leads to a dramatic increase in infection courts, increasing tree susceptibility. It is not unusual to remove 50% of peach branches annually (Whiting, 2018). Cultural practices such as pruning when inoculum loads are low, in mid to late winter, can minimize the incidence of pruning wound infections (Grove & Biggs, 2006; Miller et al., 2021). Additionally, sanitation of tools and removal of pruned branches and other dead tissue from orchards are critical practices as the pathogen overwinters in dead wood and perithecia can develop if dead wood is left for two or three years (Grove & Biggs, 2006). Physical barriers such as latex paint have been used to protect wounds from infection.

These paints are more effective when amended with fungicides, which can also be applied on their own as a preventative management strategy. Contact fungicides applied to wounds can prevent new infections by interfering with spore germination and fungal growth. Several modes of fungicide application are used: direct applications to wounds, backpack sprayers, or canopy sprays with air blast sprayers. Earlier studies showed mixed results on the efficacy of fungicides for Cytospora canker (Biggs & Grove, 2005) however, captan, thiophanate-methyl (topsin), and lime sulfur have shown efficacy in Colorado orchards (Miller et al., 2019, 2021, Wright, 2022). In field trials, Miller et al. (2019) demonstrated the efficacy of manual applications sprayed directly on pruning wounds. Treatment of inoculated pruning cuts prior to inoculation with *C. plurivora* significantly reduced the size of necrotic lesions (Miller et al., 2019, 2021). The most effective treatments observed in field trials were thiophanate-methyl, captan, 50% latex paint, thiophanate-methyl amended in 50% latex paint, captan amended in 50% latex paint, and lime sulfur (Miller et al., 2019, 2021). Other fungicides which have been used for Cytospora canker management are poor options due to lack of efficacy, phytotoxicity, human health risks, or concern over development of resistance. Benomyl (Benlate) and captafol were used historically, but have teratogenic and carcinogenic effects and are no longer in use (Kavlock et al., 1982; Patnaik, 2004). Copper based fungicides including Bordeaux mixture (a mixture of copper and lime sulfur) have long been popular for fungal disease management but have phytotoxic effects as well as minimal efficacy for Cytospora canker on peach (Northover et al., 1976; Miller et al., 2019). Northover et al. (1976) found that phytotoxicity varied by peach cultivar, suggesting further studies on variations in copper tolerance between cultivars would be justified.

Pathogen resistance to fungicides is of great concern and has been documented in fruit trees including apples and stone fruits (Pfeufer & Ngugi, 2012). While thiophanate-methyl was the most effective chemical tested by Miller et al., (2019, 2021) in *in vitro,* detached branch*,* and field trials, it is considered to have a high risk of resistance by the Fungicide Resistance Action Committee (FRAC Code List, 2024). Thiophanate-methyl is a single site mode of action fungicide (FRAC group B1) that inhibits spore germination by targeting the β- tubulin gene. Single site fungicides are classified as high risk because a single mutation in the targeted gene is sufficient to confer pathogen resistance, and such mutations are under strong selection pressure (Vela-Corcía et al., 2018). Resistance to Benzimidazoles, including topsin, have been reported in over 115 species of fungi in approximately 60 genera (Frac.info). This includes *Podosphaera xanthii*, a close relative of the causal species of powdery mildew on cherries (*Podosphaera clandestina*) and apples (*Podosphaera leucotricha*) in Colorado (Frac.info; Pokharel & Larsen, 2009). FRAC advocates for the use of multisite fungicides (FRAC Group M) which have multiple modes of action and therefore a low risk of resistance (FRAC, 2024). Multisite fungicides can also be mixed or rotated with single site fungicides to reduce the risk of resistance developing. Multisite fungicides are effective for a broader range of fungi. While this is beneficial for control of pathogens, it does raise potential concerns for increased off target effects.

Captan and lime sulfur are multisite fungicides (FRAC group M) with demonstrated efficacy against *Cytospora* and no reported signs of pathogen resistance (Miller et al., 2019; FRAC, 2024). Both are non-systemic contact fungicides which can serve as a protective barrier against pathogen spores, remaining on the surface of host tissues rather than spreading systemically (United States Environmental Protection Agency, 2019).

Lime sulfur, when applied with spray bottles to pruning wounds at 3%, is the most effective option found for organic production systems (Miller et al., 2019). It is one of the oldest pesticides in use, used widely as a fungicide and insecticide since the 1800s (Hassan, 2019). Lime sulfur is an aqueous solution of calcium polysulfide, produced by boiling lime (calcium oxide) or hydrated lime (calcium hydroxide) with sulfur in water. It is toxic on contact, killing spores and mycelia (EPA, 2019). Fungicidal properties are attributed to impaired electron transport in the respiratory chain and the formation of dioxide hydrogen sulfide, which is toxic to most cellular proteins (Holb & Schnabel, 2008; Beffa, 1993). According to the EPA lime sulfur poses very little-known risks to humans and other nontarget organisms and risk of exposure is low as lime sulfur dissociates rapidly in the environment to form calcium cations and sulfur, naturally present elements in ecosystems (EPA, 2019). Handling of lime sulfur can pose occupational risks such as dermal, eye, or respiratory irritation. It is highly caustic and can cause phototoxicity, particularly when applied at high temperatures. A reentry interval (REI) of 48 hours is required after application before workers can enter sprayed areas (EPA, 1991; EPA, 2019).

Captan is a phthalimide fungicide, used on a wide range of crops since 1952. Captan interferes with fungal growth by inhibiting DNA and protein synthesis, disrupting enzymatic functions, and preventing cellular respiration (Gündüz & İnanan, 2024).The half-life of captan residues on leaf tissue ranges from 10 to 43 days (EPA, 2024). It is not usually persistent in soil or water systems and not expected to leach into groundwater. Captan's half-life in soil ranges from less than one to 4 days (but in some cases up to 24 days), and in clean water it has a half-life of 5 to 19 hours (EPA, 2013). Captan is considered to have a low risk to human health and other off target organisms such as birds and mammals but has been shown to be detrimental to aquatic organisms, particularly fish. Captan has been classified as a carcinogen, causing cancer when consistently ingested at high doses, but it is not likely to cause cancer in humans with exposure. Captan is considered an eye and skin irritant, the EPA mandates PPE is during application and an REI of 24 hours (EPA, 2004).

In a recent study that evaluated winter canopy sprays against *Cytospora plurivora* in Colorado peach orchards, captan and lime sulfur were found to effectively reduce the incidence of bud infections following early season freeze injury (Wright, 2022). However, the following year, without treatment, very little *Cytospora* was recovered from bud tissues, indicating that winter sprays may not be needed outside of a major frost event. (Wright, 2022). This highlights the importance of evaluating canopy sprays in more typical conditions as well as during multiple seasons.

1.5 Canopy Sprays and Air-blast Sprayers

Air-blast sprayers allow for more efficient and economical coverage of tree canopies when compared with backpack sprayers or manual spot treatments. They are commonly used in Colorado orchards to apply canopy sprays of pesticides, dormant oils, and nutrients. Canopy sprays are used for other fungal diseases including Coryneum blight and powdery mildew, but evidence of efficacy for canker pathogens in fruit trees is lacking. The cost of labor in Colorado has increased, and practices like pruning wound applications are too labor-intensive to serve as the main method of chemical control for most growers. However, the fungicides which Miller et al. (2019, 2021) found effective in pruning wound treatments (as well as in vitro trials), could be good candidates for air-blast sprayer application.

Defining the desired coverage of fungicide sprays is critical to maximizing efficacy and avoiding the economic and environmental costs of overapplication (Bock et al., 2023). Best practices relating to spray coverage have been explored in numerous studies on foliar applications, but not on sprays targeting bark tissue, as needed for canker pathogen management. Identification of factors that impact coverage is critical as underapplication leaves exposed tissue unprotected and vulnerable to infection, while overapplication wastes fungicide and increases off-target effects, including drift (McCoy et al., 2021). While direct applications to pruning wounds have shown promising efficacy, they do not include coverage of tissues exposed by frost cracking, sunscald, insects, or bud or leaf abscission. Coverage of these areas is critical. While infections in smaller branches can spread to main scaffold branches and tree trunks, they can also be infected directly. These infections can be the most devastating, resulting in loss of main scaffold branches and whole trees.

Chen et al. (2013) determined approximately 30% coverage is ideal for foliar sprays. Studies in pecan trees found efficacy against scab at less than 20% coverage (Bock et al., 2021). Studies of foliar sprays can guide practices, but they are not directly applicable to treatment of canker pathogens as they assess the coverage of leaf and/or fruit tissue rather than bark tissue. Distribution and deposition of spray droplets vary depending on the tissues targeted. Spray droplets demonstrate distinctly different behavior on various surfaces; adhesion is influenced by surface characteristics, including morphology, hydrophobicity, surface angle, and wettability (Li et al., 2020). The ideal coverage for canker pathogens may be much higher than in foliar applications, to maximize the exposed tissue sprayed. This is critical when using a contact fungicide such as captan or lime sulfur. Studies which have evaluated canker pathogen treatment using air-blast sprayers describe spraying to runoff (until solution is dripping), a common agricultural practice which makes coverage difficult to quantify and may result in overapplication and inconsistency between applications.

Sprays must be distributed throughout the canopy to achieve maximum efficacy and reduce off target effects (EPA, 1999; Hewitt, 2000, as cited in Ferguson et al., 2020). Many factors influence coverage including driving speed, nozzle type, sprayer calibration, droplet size, canopy geometry and density, and climatic conditions (Praat et al., 1996). Tree size is a critical factor; coverage has been found to decline with height in the canopy (Bock et al., 2015; Godoy-Nieto et al., 2022). This has been well established in pecan and olive trees. In mature pecan trees, which can be up to 25m tall, overspray is common at less than 15m while top sections of the canopy have minimal coverage. This is true even when 2/3 of the volume is directed at the top of the tree, suggesting the importance of even more dramatic nozzle adjustments (Bock et al., 2015, 2021, 2023). Declining coverage in top branches correlates with reduced efficacy and greater disease severity (Bock et al., 2015, 2021).

Defining ideal timing of chemical treatments is crucial to achieve maximum coverage and efficacy with canopy sprays. Key factors in timing applications include weather, pathogen biology, and tree phenology. In early studies in Grand Junction, Colorado, the greatest increase in canker surface area occurred between March and June, compared with summer and fall (Jones & Luepschen, 1971; Miller et al., 2019). Interestingly, Dhanvantari (1968) found that *Cytospora* was more virulent on peach trees in summer. Sporulation follows seasonal patterns and is highest in spring and summer (Luepschen & Rohrbach, 1969; Grove & Biggs, 2006; Miller et al., 2021). In a comparison of conidia collections at different times of year, Grove and Biggs (2006) recovered the least conidia in November and the most in May.

Given the epidemic levels of Cytospora canker in western Colorado, the rapid spread of the disease, and the limited management options available, studies exploring alternative management strategies are needed to preserve Colorado's peach industry. Canopy sprays of fungicides applied with air blast sprayers may be an effective tool for managing *C. plurivora*. Furthermore, research on the efficacy of these canopy sprays against *C. plurivora* and the development of best practices for their application in orchards may have important implications for the management of other canker pathogens.

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CHAPTER 2: MANAGING CYTOSPORA PLURIVORA IN COLORADO PEACH ORCHARDS WITH CANOPY SPRAYS: ASSESSMENT OF EFFICACY AND COVERAGE

2.1 Introduction

Canker pathogens are a global threat to fruit production. In 2015, reduced productivity due to Cytospora canker was estimated to cause 15-20% of losses in stone fruit (*Prunus* spp.) production yearly (Pokharel & Larsen, 2009; USDA, 2016). Ascomycete species in the genus *Cytospora* encompass many significant canker pathogens, affecting a broad range of hosts worldwide including monocotyledons, dicotyledons, and gymnosperms (Wijayawardene et al., 2018; Liu et al., 2022). Pathogenicity has been documented in over 120 species of woody trees and shrubs in both agricultural and natural ecosystems (Stewart et al., 2022). Previously known by numerous epithets, *Cytospora,* (Ehrenberg; 1818) the earliest recorded name for the genus, became the preferred and protected name under the International Code of Nomenclature for algae, fungi, and plants (Rossman et al., 2015).

Cytospora canker has long been an important disease in peach orchards and is commonly found in cherries, apricots, plums, quince, pear (*Pyrus* spp.) and apples (*Malus* spp.)(Adams et al., 2002; Grove & Biggs, 2006). Unable to penetrate healthy bark tissue, the opportunistic pathogen enters stressed trees through openings frequently caused by pruning, frost damage, insect damage, mechanical damage, or bud or leaf abscission (Willison, 1937, as cited in Biggs, 1992). Spores germinate on these exposed tissues, producing hyphae which colonize and necrotize bark, cambium, and inner vascular tissues, resulting in dark, elliptical lesions. Vascular function is compromised by pathogen colonization and host defenses such as gum production, leading to girdling of branches or trunks (Biggs, 1992). Productivity declines due to loss of fruit bearing wood and tree mortality which can reach 3 to 6% per year (Grove & Biggs, 2006). The pathogen can live as a necrotroph or saprotroph, overwintering in cankers and other dead wood including pruned branches left in orchards (Grove & Biggs, 2006). Infection can occur in shoots, branches, or trunks, although one year old branches have been thought to be the most susceptible and can serve as a route for the pathogen to spread to larger branches and the main trunk (Grove & Biggs, 2006).

Cytospora canker is one of the most impactful diseases for peach growers in western Colorado (Stewart et al., 2022). Peach trees have been cultivated in Colorado since 1881 (Sexton, 1996) and are grown throughout Mesa, Delta, Montrose, and Montezuma counties (Colorado Agricultural Statistics Service, 2002). Colorado peaches fetch one of the highest prices per pound in the country (United States Department of Agriculture National Agricultural Statistics Service, 2002). In 2022, Colorado farmers produced 14,050 tons of peaches, bringing in 33.91 million dollars and ranking 5th in the US for peach production (CASS, 2023). Fruit trees in western Colorado are particularly susceptible to Cytospora canker due to the physiological stresses presented by early and late season frosts, fluctuating winter temperatures, lack of water, and clayey alkaline soils (Sharp & Cooley, 2004). In addition to wounding, environmental stressors play a key role in *Cytospora* spp. disease development in peaches (Miller et al., 2023).

The primary causal species on Colorado peach trees, *Cytospora plurivora*, was first described in California, where it was identified as genetically distinct from the two species previously considered to be the causal pathogens on peaches in North America: *C. leucostoma* and *C. cincta* (Lawrence et al., 2018; Stewart et al., 2022). While both sexual and asexual spores function as inoculum, the main source of *C. plurivora* inoculum is the conidia, or asexual spores, which are produced year-round, infecting trees during all seasons (Bertrand et al., 1976; Miller et al., 2021). Spores are dispersed via water droplets, often from rainfall splash, wind-blown water, or irrigation (Grove & Biggs, 2006). While conidia are produced year-round, spore production and growth are directly correlated with temperature, most prolific during spring and summer (Grove & Biggs, 2006; Miller et al., 2021; Wright, 2022). Timing of chemical treatments is an important consideration in achieving maximum coverage and efficacy with canopy sprays. Weather, pathogen, and tree phenology are important factors that must be considered in decision-making. In early studies in Grand Junction, Colorado, the greatest increase in canker surface area occurred between March and June compared with summer and fall (Jones & Luepschen, 1971). Virulence has been observed to vary with season as well; *Cytospora* was found to be more virulent on peach trees in summer (Dhanvantari, 1968). This highlights the need for explorations on management efficacy during different seasons and weather events.

Despite the prevalence and impact of Cytospora canker, options for management are lacking in number and efficacy. No resistant cultivars have been identified nor are there any chemical controls registered for control of Cytospora canker (Biggs & Grove, 2005). Cytospora canker is particularly difficult to treat due to its presence in vascular tissues (Biggs & Grove, 2005). Cytospora canker management in western Colorado has focused on preventive measures, including cultural practices that minimize wounding and exposure to inoculum and chemical control using prophylactic fungicides to protect wounded tissue from infection (Miller et al., 2019). Contact fungicides can be applied via manual spot treatments, backpack sprayers, or canopy sprays. Earlier studies showed mixed results on the efficacy of fungicides for Cytospora canker (Biggs & Grove 2005); however, captan, thiophanate-methyl (topsin) and lime sulfur have shown efficacy *in vitro*, in detached branch assays, and as direct applications to pruning wounds as demonstrated by field trials in Colorado orchards (Miller et al., 2019, 2021). While manual pruning wound applications are effective, they are labor intensive and expensive, therefore impractical for many growers. Additionally, treating only pruning cuts leaves vulnerable tissues on the main scaffold branches and trunk exposed. Alternative or additional methods of application which are cost effective and comprehensive have yet to be explored. Characterizing these methods is crucial to effectively utilize these fungicides to limit the spread of *C. plurivora.*

Among the fungicides which have demonstrated efficacy for Cytospora canker, captan and lime sulfur are among the most promising. Benomyl (Benlate) and captafol were used historically, but are no longer available to teratogenic and carcinogenic effects (Kavlock et al., 1982; Patnaik, 2004). Copper based fungicides including Bordeaux mixture have demonstrated phytotoxic effects and minimal efficacy for Cytospora canker on peach (Northover, 1976; Miller et al., 2019). Thiophanate-methyl (topsin) was the most effective chemical tested by Miller et al. (2019, 2021) but is a single site fungicide classified as high risk, with numerous reports of pathogen resistance (FRAC, 2024). In contrast, captan and lime sulfur are multisite fungicides with no reported signs of resistance (FRAC, 2024). Lime sulfur is OMRI listed and when applied at 3% is the most effective option found for organic production systems (Miller et al., 2019). Both formulations showed efficacy in reducing the incidence of bud infections in a recent study of winter canopy sprays following a late season freeze event (Wright, 2022). The study indicated potential efficacy of air-blast sprayer applications for Cytospora canker management, but was limited in scope and did not indicate the need for winter sprays independent of a freeze event (Wright, 2022). Further studies are needed to characterize the timing and methods for appropriate use of canopy sprays in the diverse range of conditions encountered in western Colorado orchards.

Air-blast sprayer applications are efficient and economical; capable of covering large orchards with minimal labor costs and investment in equipment. Modern air blast sprayers have been commonly used in orchards since the mid-1900s and are effective for insect and disease management as well as nutrient applications (Bahlol et al., 2020). Air assistance allows for deeper penetration into tree canopies by breaking the spray into small droplets that are projected through nozzles into the canopy. Sprayer fans generate the high volume of air needed for this purpose, but these droplets can travel further than intended, resulting in chemical drift and associated environmental concerns (Bahlol et al., 2020). Understanding patterns in air-assisted canopy spray deposition is crucial to optimizing application efficiency and efficacy while minimizing the consequences of overapplication (Bock et al., 2023). Underapplication leaves exposed tissue unprotected and vulnerable to infection, while overapplication results in increased off-target effects and costs due to product waste (McCoy et al., 2021). Additionally, both underapplication and overapplication impose significant selection pressure on pathogens, increasing the risk of chemical resistance development (Praat et al., 1996). In Colorado, the development of pesticide resistance and lack of alternative management options decimated the apple industry during the codling moth infestation of the early 20th century (Sexton, 1996). Peaches in Colorado now face a similar fate, with Cytospora canker at epidemic levels and limited treatment options. Risk of pathogen resistance development and environmental consequences must be carefully considered when exploring new management strategies for Cytospora canker. Elucidating optimal application practices for air-blast sprays is critical.

Best practices for optimal spray coverage have been explored in numerous studies on foliar and fruit applications but are limited regarding canker pathogens. Studies examining the application of foliar sprays can guide practices but are not directly applicable to treatment of canker pathogens as they assess the coverage of leaf or fruit tissue rather than woody tissue. For example, distribution, deposition and adhesion of spray droplets vary depending on surface characteristics such as morphology, hydrophobicity, surface angle and wettability (Li & Wang 2020). Ideal coverage for prophylactic sprays targeting canker pathogens may be much higher than in foliar applications, as efficacy may depend on maximizing the coverage of scaffold branches. Studies which have evaluated canker pathogen treatment using air-blast sprayers describe spraying to runoff (until solution is dripping), a common agricultural practice which makes coverage difficult to quantify and may result in overapplication and inconsistency between applications (Rathnayake et al., 2021). Many parameters influence the coverage and subsequent efficacy achieved by canopy sprays including driving speed, sprayer calibration, choice of nozzles and other sprayer fittings, equipment adjustments, canopy geometry and density, and climatic conditions (Praat et al., 1996). Further, best practices are nuanced; they are specific to crop canopy characteristics, sprayer type, and even geographic region (Rathnayake et al., 2021). Distribution of sprays varies even within trees and rows; coverage frequently declines with height in the canopy (Bock et al., 2015; Godoy-Nieto et al.,2022). Diminished coverage in top branches, especially for larger trees (Bock et al., 2013), correlates with reduced efficacy and greater disease severity (Bock et al., 2015, 2021). This is especially true for larger trees (Bock et al., 2015). Additional studies are needed to characterize factors influencing air blast sprayer distribution and coverage to maximize the efficacy of applications targeting canker pathogens. The results of this study will help to inform the development of protocols that optimize efficacy while limiting off-target effects and unnecessary costs caused by product waste.

The variability observed between sprayer types, geographic regions, application timing, and orchard characteristics, including tree age, size, and phenology, highlight the need for specific studies of efficacy and best practices for air-blast applications on peach trees in western Colorado. The objectives of this research were to 1) investigate factors impacting the coverage of scaffold branches achieved in air-blast sprayer applications and 2) evaluate the efficacy of air-blast sprayer fungicide applications in preventing new infections of *C. plurivora.*

2.2 Methods

General Experimental Design. Spray coverage and fungicide efficacy were evaluated at two sites: an organic orchard at the Western Colorado Research Center at Rogers Mesa (WCRC-RM), in Hotchkiss, Colorado, and a conventional peach orchard at the Western Colorado Research Center at Orchard Mesa (WCRC at Orchard Mesa), in Orchard Mesa, Colorado. Coverage trials were conducted in the spring, (March) 2023, and summer (July) of 2023. In coverage trials, water was applied to trees via air-blast sprayers and spray coverage quantified using water sensitive paper (WSP) cards (TeeJet Technologies, Glendale Heights, IL) placed in trees via bamboo stakes. The average percent coverage of WSP cards was used as a proxy to estimate spray deposition on scaffold branches. Fungicide efficacy trials were conducted in fall (November 2023-March 2024) and spring (April-June of 2024). Trees were wounded, treated with fungicides via air blast sprayers, then inoculated with mycelial plugs of *C. plurivora*. Resulting lesions were measured after several months. Resulting lesions were measured several months later and compared with unsprayed inoculated and uninoculated controls to quantify differences in the spread of infection, as measured by lesion area (mm^2).

Field Trial Design. Trials were conducted at two sites: an organic orchard at Colorado State University, Western Colorado Research Center - Rogers Mesa (RM) in Hotchkiss, CO and a conventional orchard at - Colorado State University, Western Colorado Research Center - Orchard Mesa (OM) in Orchard Mesa, CO. Three orchard planting blocks were used: one at WCRC-RM (RM) and two at WCRC-OM (OM-1 and OM-2). The second planting block at OM, OM-2, was included to explore any differences in coverage or fungicide efficacy associated with air-blast sprays on larger, more mature trees. Experimental trees were selected in two sets of two neighboring rows at RM (RM-A and RM-B) and OM-1 (OM-1A and OM-1B, Table 1). In OM-2, the layout of the planting block necessitated changes in trial design. For the coverage trials, one full row of trees at OM-2 was used, while for the fungicide trials, trees were spread between three rows. Trees were spaced 1.5 m apart at RM and 1.8 m apart in OM-1 and 2, with 3.6 m spacing between rows, and pruned with the perpendicular V system (resulting in two to three main scaffold branches). Trees in RM and OM-2 were irrigated via micro sprinklers, and in OM-1, rows were irrigated with micro sprinklers in the northern half of rows and drip lines in the southern half.

Table 1. Characteristics of trees in planting blocks RM, OM-1, and OM-2. RM-A, RM-B, OM-1A, and OM-1B each represent two neighboring rows within planting blocks. Estimates of mean height were calculated from a subset of trees measured in August or September of 2024.

Site Name	Year Planted	Approximate Height (m)	Cultivar and Rootstock
RM			
RM-A	2018	2.45	Mixed[1] on Lovell
RM-B	Unknown	2.96	Unknown
OM-1			
OM-1A	2019-2020	3.15	Redhaven on Lovell or Red Lovell
OM-1B	2020	3.66	Mixed[2] on Lovell
OM-2	2014	3.52	Cresthaven on Viking

[1] Cultivars in RM-A: Angelus, Blushingstar, Cresthaven, Glohaven, Glowingstar, Newhaven, O' Henry, Starfire, Suncrest, FF19, FF23, FF19, and FF24.

[2] Cultivars in OM-1B: Cresthaven, GloHaven, Glowing Star, Starfire, and Suncrest

Equipment. Canopy sprays were applied with air blast sprayers for both coverage and fungicide efficacy trials. At WCRC-OM, a 400-gallon (1514.16 L) Rears Pul-Blast Sprayer (Rears Manufacturing, Coburg, OR) fitted with a 91.44 cm diameter fan was used, pulled by a

Holder A-track 5.58 tractor (Holder Tractors Inc., Embrun, Ontario, CA). At RM, a 50-gallon (189.47 L) Rears Pak-Blast Sprayer (Rears Manufacturing, Coburg, OR) fitted with a 60.96 cm diameter fan was used, pulled by a John Deere 2355N tractor (Deere &Co, Moline, IL). Sprayers were fitted with Tee Jet Disc-Core Type Full Cone Spray nozzles (TeeJet Technologies, Glendale Heights, IL) each fitted with a D8 ceramic disc and a DC56 ceramic core with a diameter of 0.318 cm. The nozzles were operated at a pressure of 80 PSI, delivering a flow rate of 10.33 liters per minute (LPM).

Coverage Trials. Coverage trials were conducted on 27 March 2023 when trees were bare and on 11 July 2023 (OM) or 12 July 2023 (RM) when the trees had full canopies. In all planting blocks, half of each row was sprayed with the air-blast sprayer fans on, and half with the fans turned off. The same trees were used for both trials.

Tree Selection. Trees were selected arbitrarily with consideration of good V perpendicular form and adequate spacing from one another, the edge of the planting block and the transition point for fan treatments selected. At RM and OM-1, eight trees in the south half of each row and eight trees in the north half each row were selected. In OM-2, four trees in the north half of the row and four trees in the south half of the same row were selected. Trees used for control cards (unsprayed) were located several rows away from the sprayed rows.

WSP Cards. Water was applied via air-blast sprayers to trees with WSP cards, which served as a proxy to quantify scaffold branch coverage. WSP cards were cut in half (to 5.08cm x 3.81 cm) and secured to bamboo stakes tied to each of the two main scaffold branches on trees, one facing east and one west. Six cards were taped to each bamboo stake at three heights with one facing east and one west at each height. Bamboo stakes were placed in trees with the bottom cards at the base of the main scaffold branches, near the tree crotch, and top cards at the height of the highest branches (Figure 1). Once sprayed, cards were allowed to dry then collected and transported to Colorado State University, Fort Collins, CO for analysis. WSP cards were scanned and transformed into digital images which were trimmed to 1.35” x 2” in Adobe Photoshop (San Jose, CA) to standardize cards with uneven edges. Percent coverage, as measured by the area of blue on each card, was analyzed using ImageJ software (Rasband, 1997).

Weather. Weather conditions at the time of each spray were recorded using the weather stations located at OM (ORM02: latitude 39.04 °N, longitude: -108.5 °E, elevation 4700 ft) and RM (HOT02: latitude 38.8 °N, longitude -107.8 °E, elevation 5631 ft). The weather stations were accessed through the Colorado Agricultural Meteorological Network (COAGMET) managed by the Colorado Climate Center at Colorado State University (https://coagmet.colostate.edu/). Weather conditions for each experiment are shown in Tables 1 and 2. Precipitation was not included as no precipitation took place during the sprays; however, minimal precipitation did occur after sprays and before or during inoculations in some planting blocks. Inoculations in block OM-2 were completed on the evening of November 15th, prior to any precipitation. OM-1 was inoculated the following morning, during 3.8mm of accumulated precipitation. RM blocks were inoculated on the afternoon of the 16th, after 4.6mm accumulated precipitation. Additional precipitation occurred in the following days: November 18th 0.3mm at OM and 0.5mm at RM, November 19th 4.8mm at OM and 5.3mm at RM.

Table 2. Weather data recorded at the associated COAGMET weather stations during coverage trials.

	Date	Start Time	Temp (°C)	Relative Humidity	Wind Speed (m)	Direction [1]	Gusts (m)	Direction[1]
RM	5/28/23	1:35 PM	6.5	20.1%	1.1	56°	2.1	63°
OM	5/28/23	12:30 PM	3.5	40.8%	2.4	350°	4.2	15°
RM	7/11/23	11:15 AM	29.9	19.0%	1.4	307°	2.1	289°
OM	7/12/23	11:10 AM	32.1	24.3%	2.0	42°	2.8	35°

[1] Direction relative to true north

Table 3. Weather data recorded at the associated COAGMET weather stations during fungicide trials.

	Date	Start Time	Temp (°C)	Relative Humidity	Wind Speed (m)	Direction[1]	Gusts (m)	Direction[1]
RM	11/14/2023	10:00 AM	9.6	31.5%	0.9	168°	2.0	154°
OM	11/14/2023	4:55 PM	13.3	26.0%	0.8	238°	1.0	236°
RM	4/9/2024	1:00 PM	11.3	28.1%	2.1	267°	3.9	273°
OM	4/11/2024	9:10 AM	10.3	31.0%	3.4	120°	4.3	117°

[1] Direction relative to true north

Fungicide Trials

Tree Selection. Thirty trees in each orchard block, 15 in each set of rows, were selected arbitrarily, excluding trees with extensive existing infections when possible and allowing for adequate spacing. In each planting block, five trees were selected in the northern half, where canopy sprays took place, and ten trees in the southern half, which was left unsprayed for controls. The same design was used in both sets of rows in each planting block to minimize any risk of fungicide drift reaching the unsprayed controls. The design for the fall and spring fungicide trials was identical, although different trees were selected so that none were used twice.

Treatments. All trees were wounded with 9 mm diameter cork borers at six locations per tree: three heights on each of the main scaffold branches: “bottom” (approximately 10-20 cm above tree crotch), “middle” (approximately 1.5 meters from the ground), and “top,” one year old branches towards the top of the canopy (Figure 1). Wound depth was determined by the depth at which cambial tissue was reached (but not removed). In trees with a third scaffold branch, the two branches closest to perpendicular to the rows were chosen.

Fungicides were applied with air blast sprayers, using captan at OM (Captan 4L, Drexel Chemical, Memphis, TN) at a rate of 3.5 liters/hectare and 3% lime sulfur at RM (Lime Sulfur

Solution, NovaSource Tessenderlo Kerley, Inc. Phoenix, AZ). Air blast sprayers and nozzles were the same as those used in coverage trials. Trees in the northern half of rows were sprayed with fungicides while trees in the southern half of rows were left unsprayed for use as positive and negative controls. Wounds on sprayed trees were subsequently inoculated with *C. plurivora* mycelial plugs, as were half of the unsprayed trees which served as positive controls. The other half of the unsprayed trees were left uninoculated to serve as negative controls. Negative controls were unreceived no further treatment beyond wounding in the fall trial, but in the spring trial were covered with parafilm due to the high rate of infections in negative controls observed in the fall trial, presumably from natural inoculum, as described in Miller et al. (2019).

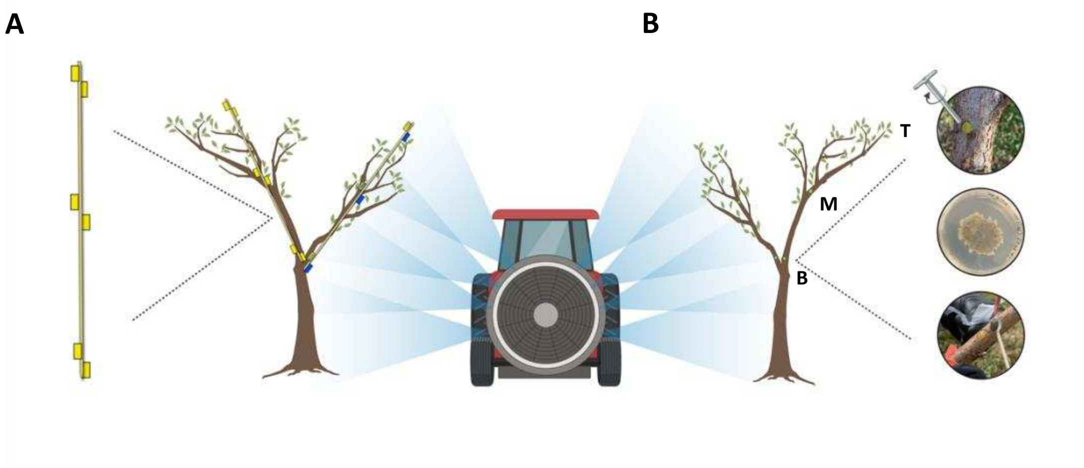

Figure 1. **(A)** Experimental design for coverage trials. Six WSP cards were attached to bamboo stakes at three heights, “bottom(B)”, “middle(M)” and “top(T).” Two bamboo stakes were placed in each tree, on each of the main scaffold branches. **(B)** Experimental design for fungicide trials. Trees were wounded in three locations on each scaffold branch: “bottom” “middle” and “top.” Photographs of branch wounding, a culture of *C. plurivora* used for inoculations, and the application of a mycelial plug to a wound are shown.

Inoculations. Cultures of *Cytospora plurivora* (isolate CP 5.3) were grown on ½ strength potato dextrose agar plates (PDA, Hardy Diagnostics; Santa Maria, CA) for 5 weeks at 25°C. The isolate used was originally isolated from a symptomatic peach tree in western Colorado and stored on filter paper at -20°C. Inoculations took place 24 hours (captan) or 48 hours (lime sulfur) post-fungicide application, using an established protocol modified to use larger wounds and mycelial plugs (Miller et al., 2019). Briefly, 9 mm diameter cork borers were used to cut agar plugs which were subsequently transferred to wounds, fungal side down, using toothpicks and secured with ParaFilm M (Bemis MFG, Sheboygan Falls, Wisconsin).

Sample Collection. Lesions were measured and collected approximately 120 days post-inoculation (dpi) in the fall-winter trial (Table 4) and 75 dpi in the spring-summer trial (Table 5). Infections were allowed to develop for a longer duration in the fall-winter trial than in the spring-summer trial due to seasonal differences in pathogen growth rates (Miller et al., 2019).

Table 3. Fall-winter fungicide trial dates.

	Wounded	Sprayed	Inoculated	Collected	Dpi
RM	11/13/2023	11/14/2023	11/16/2023	3/13/2023	119
OM-1	11/14/2023	11/14/2023	11/16/2023	3/14/2023	120
OM-2	11/14/2023	11/14/2023	11/15/2023	3/15/2023	122

Table 4. Spring-summer fungicide trial dates.

	Wounded	Sprayed	Inoculated	Collected	Dpi
RM	4/09/2023	4/09/2023	4/11/2023	June 24th	75
OM-1	4/10/2023	4/11/2023	4/12/2023	June 25th	75
OM-2	4/10/2023	4/11/2023	4/12/2023	Jane 25th	76

Bark was scraped at the wound sites using a pocket knife to expose lesions. Lesion length and width were measured with a digital caliper (General, Secaucus, NJ) and used to calculate lesion area (mm^2). Tissue was scraped away around and below lesions to measure the full extent of lesion development below the surface. Tissue at the margin of lesions was collected for re-isolation. Bottom and middle lesions were measured and sampled in the field and top branches were harvested and transported before measurement. All samples and top branches were placed in a cooler with cold packs for transportation, then stored at 4°C during transportation to Colorado State University.

Re-isolation and ID Verification. To confirm that the lesions were caused by *C. plurivora*, thereby fulfilling Koch's postulates, isolates were cultured from lesion samples and gDNA extracted for PCR amplification and Sanger sequencing. Lesion samples at the interface of healthy and diseased tissue were cut into pieces no larger than 3cm x 3cm, surface sterilized in 10% sodium hypochlorite solution for 2 minutes, rinsed in sterile distilled water, and placed on ½ strength PDA plates. Cultures were incubated at 25°C for 2 weeks prior to DNA extractions. The same process of DNA extraction, PCR amplification, and Sanger sequencing was used prior to each fungicide trial to verify the identity of the cultures used for inoculations.

Extraction protocols. Genomic DNA (gDNA) extractions were performed. Briefly, a small portion of mycelia was scraped from cultures using a sterile pipette tip, then suspended in a microcentrifuge tube containing 100µl of a 5% Chelex® 100 resin (Biorad, Hercules, CA) solution. Samples were heated to 98ºC for 20 minutes using an Eppendorf Mastercycler PRO 3 Thermal Cycler (Enfield, CT). The resulting supernatant (ca. 30 µl) containing the eluted gDNA was transferred to a new microcentrifuge tube and stored at -20°C.

PCR protocols. The partial translation elongation factor 1-α (*TEF*1) gene was amplified using the primer pair EF1-728F and EF1-986R (Carbone & Kohn, 1999; Rehner & Buckley, 2005; forward primer: EF1-728F: 5'-CATCGAGAAGTTCGAGAAGG-3'and reverse primer: EF1-986R: 5'-TACTTGAAGGAACCCTTACC-3'). PCR amplifications were performed using a 20-µl reaction system comprised of 1 µl template DNA (or 1 µl molecular grade water in the negative control), 7 µl of autoclaved molecular grade water, 1 µl each of 10 µM primer, and 10 µl GoTaq® Green Master Mix (Promega Corporation, Madison, WI). Amplifications were performed using an Eppendorf Mastercycler pro S Thermal Cycler (Eppendorf, Hamburg, Germany) set to the following program: One cycle of 1 minute at 94°C, 40 cycles of 30s at 95°C, 30s at an annealing temperature of 57°C, and 45s at 72°C, followed by a final elongation cycle of 10 minutes at 72°C and a perpetual hold at 4°C. Gel electrophoresis was used to confirm amplification. PCR products were run on a 1.5% agarose gel with 0.5X TBE buffer and stained with GelRed™ Nucleic Acid Gel Stain (Gold Biotechnology, St. Louis, MO). Gels were visualized under UV. PCR products with bands of the desired size, approximately 350 base pairs, were purified using ExoSAP-IT® PCR Product Cleanup (Applied Biosystems by Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions and subsequently Sanger sequenced at Eurofins (MWG Operon USA, Louisville, KY). Reisolations from the spring fungicide trials were identified following the same protocol other than the following modifications. Amplifications were performed using a T100 thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA) was used for amplification. PCR products (15µl per well) were excised from 1.5% agarose gels with 0.5X TBE buffer and purified using the Zymoclean™ Gel DNA Recovery Kit according to manufacturer specifications (Zymo Research, Irvine, CA) prior to Sanger sequencing at Eurofins. Sequences were uploaded to Geneious Prime 2024.0 (https://www.geneious.com) and identified as *C. plurivora* using the BLASTn (Basic Local Alignment Search Tool) search engine (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the GenBank database at NCBI (National Center for Biotechnology Information).

Data Analysis. Statistical analyses were run in RStudio *2023.06.1+524* "*Mountain Hydrangea*" using the following packages: lme4, lmertest, pbkrtest, and emmeans (Bates et al., 2015; Kuznetsova et al., 2017; Halekoh and Højsgaard 2014; Kuznetsova et al., 2017; Lenth, 2024).

Coverage Trials. Generalized linear mixed models (GLMM) were built using the lmer package in RStudio. Orchard blocks RM, OM-1, and OM-2 were analyzed separately. Data were arcsine transformed to improve heterogeneity of variance and normality of residuals which were assessed to confirm the assumptions of the analysis were met. Transformed data were normally distributed and residual spread improved although some evidence of heterogeneity of variance was still observed. Percent coverage of cards was defined as the dependent variable, with fan use, trial season, card height, and the interactions of fan x season, height x fan, height x season, and height x season x fan as fixed effect predictor variables. Two random predictor variables, tree and rows, were included to account for multiple measurements per tree and any unknown variability between sets of rows within orchard blocks.

Fungicide Trials. Similarly, generalized linear mixed models (GLMM) were fitted for the fall and spring fungicide trials. As in the coverage trials, the orchard blocks were analyzed separately to improve heterogeneity of variance and normality. The data were log transformed to meet the assumptions of the model. Transformations improved distribution and residual spread, although some evidence of heterogeneity of variance was still observed. Treatment and wound height were treated as fixed effects. The dependent variable was defined as lesion area (mm^2) with fungicide treatment, height and the interaction of treatment x height as fixed effect predictor variables and tree and rows included as random effect predictor variables. Treatment levels included negative controls, positive controls, and treatment (lime sulfur at RM and captan at OM). A linear model analysis of variance (ANOVA) was run on each model ($\alpha = 0.05$) for both coverage and fungicide trials. The denominator degree of freedom was protected from bias through the inclusion of the Kenward-Roger adjustment for GLMMs (Kenward and Roger, 1997). Tukey adjusted pairwise comparisons were conducted on main effects and interactions ($\alpha = 0.05$).

2.3 Results

Coverage Trials. The GLMMs showed significant interactions between the main effects of season, fan, and height. Mean percent coverage of cards in planting blocks OM-1 and OM-2 was influenced by the interactive effects between season, fan and height ($P<0.001$, $P=0.022$). At RM, however, the three-way interaction was not significant ($P=0.715$). The main effects of season, fan, and height and the interaction between fan and height ($P<0.001$), were significant, and the interaction between season and height at RM trended towards significance ($P=0.051$).

Height. Coverage varied by height; cards placed at the top of the canopy consistently showed the lowest mean percent coverage: in every orchard block, spring or summer, with fan on or off (Figure 1). No significant differences in between middle and bottom card coverage were observed in the summer applications. In the spring trial in OM-2, spraying without the fan resulted in significantly (26%) less coverage on the bottom than on the middle cards ($P=0.03$). However, no other significant difference in coverage between bottom and middle branches was observed in the spring trial.

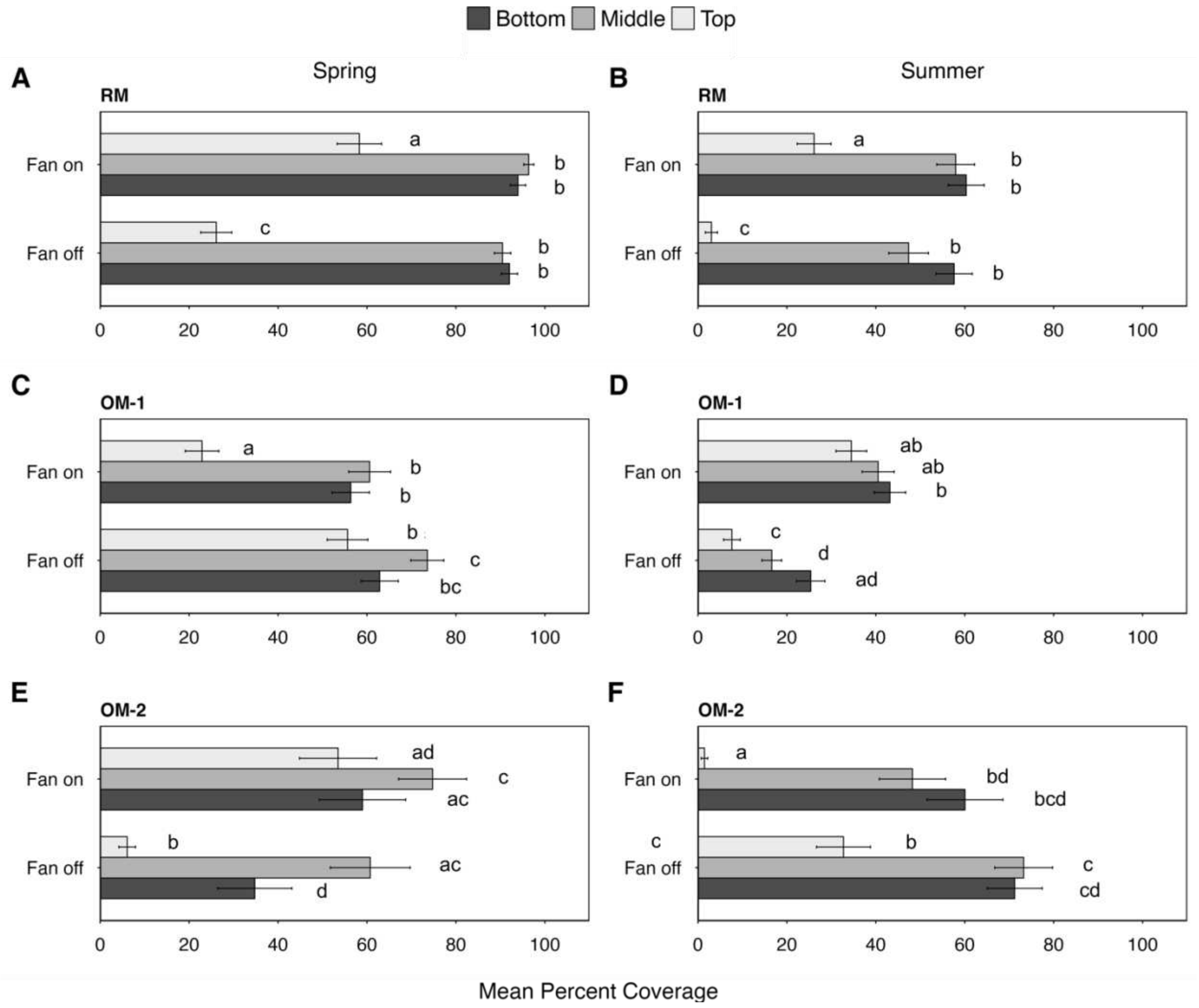

Figure 2. Mean percent coverage of WSP cards by height (bottom, middle, or top) in tree scaffolds, with air blast sprayer fan on or off. Spring trials at orchard blocks **(A)** RM, **(C)** OM-1, and **(E)** OM-2 and summer trials at orchard blocks **(B)** RM, **(D)** OM-1, and **(F)** OM-2. Error bars denote one standard error of the mean. Letters indicate statistically significant differences in mean percent coverage based on model-based pairwise comparisons (Tukey-Kramer, α=0.5); letters that are not shared indicate significant differences in mean percent coverage.

Season. Spring sprays resulted in higher percent coverage than summer sprays in every instance at RM and most instances at OM-1, but less consistently in OM-2 (Figure 3). At RM, cards sprayed during the spring trials had higher percent coverage than those in summer trials at every height, with fan on or off (*P*<0.001). At OM-1, spring sprays resulted in significantly higher percent coverage than summer sprays on all bottom and middle cards, but in top cards spring only resulted in higher coverage when the fan was off (Figure 3). When the fan was on, top cards in

OM-1 received better coverage in summer than in spring (*P*=0.020). In OM-2, spring sprays resulted in higher percent coverage than summer sprays only on middle and top cards sprayed with the fan on (*P*=0.020 and *P*<0.001, respectively). On bottom and top cards sprayed with the fan off, summer sprays resulted in higher coverage (*P* =0.004 and *P*=0.016). Bottom cards sprayed with the fan on and middle cards sprayed with the fan off showed no significant difference in coverage between spring and summer applications (*P*=0.999 and *P*=0.286, respectively).

Fan. Sprayer fan use had significant, but inconsistent effects on percent coverage of cards (Figure 3). At RM, fan use was associated with higher percent coverage on top cards in both spring (*P*<0.001) and summer trials (*P*<0.001), but no significant differences on bottom or middle cards. In OM-1 and OM-2, the effect of fan use varied by season and showed opposing trends between the two blocks (Figure 3). In OM-1, fan use was associated with increased percent coverage on middle and top cards in spring (*P*=0.026, *P*<0.0001), but decreased coverage at all heights in summer (bottom, *P*=0.003, middle, *P*<0.0001, top, *P*<0.0001). In OM-2, fan use decreased coverage on bottom and top cards in spring (*P*=0.030, *P*=0.0002), but increased coverage on middle and top cards in summer (*P*=0.043, *P*=0.002).

Sprayer fans were associated with improved uniformity of coverage at OM-1; during the summer trial when fans were on, no significant differences in percent coverage between heights was observed (Figure 3). Top cards had higher percent coverage when sprayer fans were used (*P*<0.001), resulting in more uniform coverage between heights. In the spring, however, fan use was associated with decreased coverage of top cards in OM-1 (*P*<0.001).

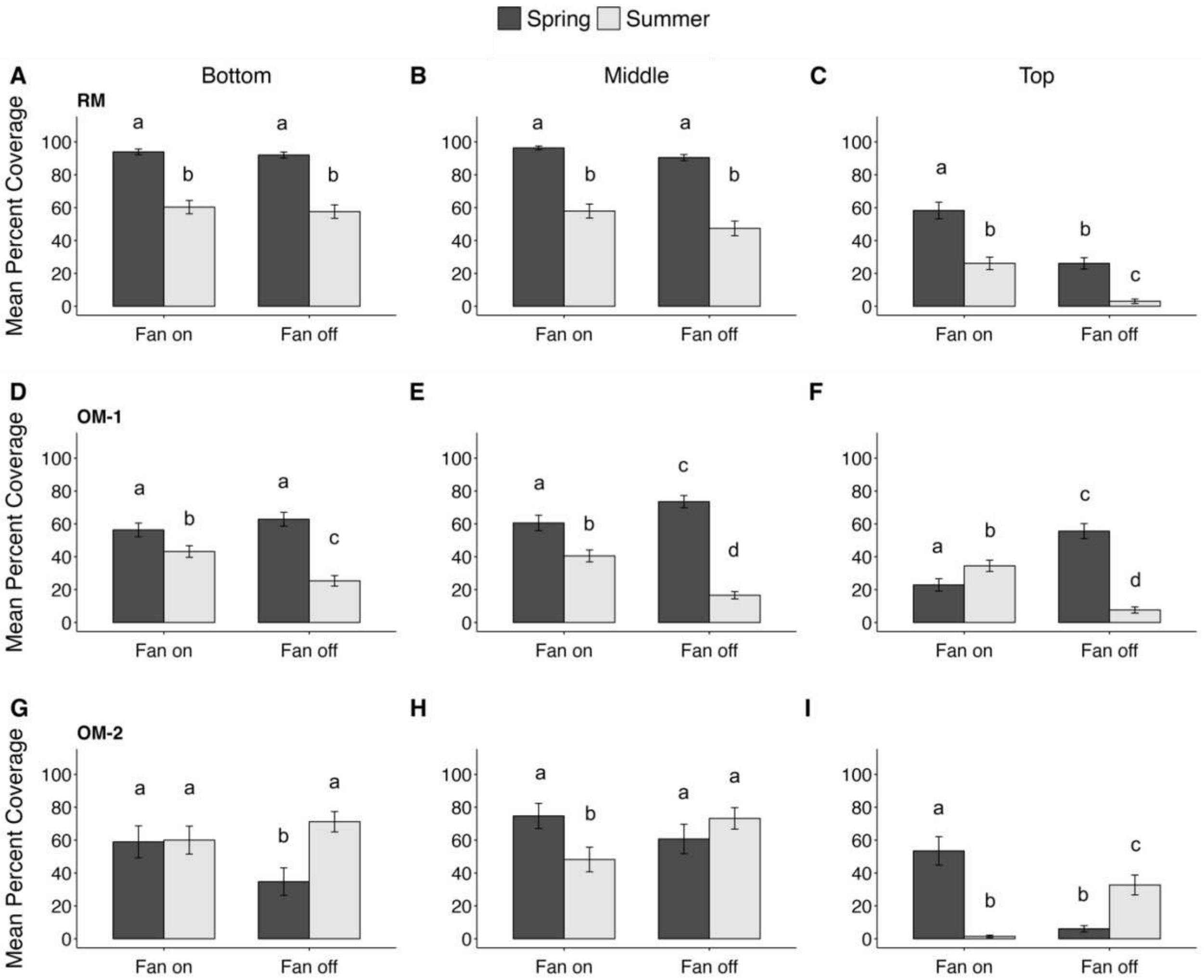

Figure 3. Effects of season of application and sprayer fan use on mean percent coverage of cards sprayed in spring and summer. Results for each orchard planting block are shown at the three scaffold heights where cards were placed in trees: bottom, middle, and top at orchard block RM **(A, B, C),** OM-1 **(D, E, F)** and OM-2 **(H, I, J)**. Error bars denote one SE of the mean. Letters indicate statistically significant differences in mean percent coverage based on model-based pairwise comparisons (Tukey-Kramer, α=0.5); letters that are not shared indicate significant differences in mean percent coverage.

Fall Fungicide Trials

Treatment. Fungicide treatments applied in the fall did not significantly decrease lesion area in any of the orchard blocks (Figure 4). Negative controls, positive controls, and treatments all yielded similar results. Negative control lesions were 4.36 mm^2 larger than middle wounds on trees treated with lime sulfur at RM (*P*=0.049) and negative controls at OM-1 were 22.10 mm^2 larger than lesions on trees treated with captan (*P*=0.014).

Height. Top branches were associated with higher lesion areas in some cases in each orchard block (Figure 4). At RM, in treated branches, top branches had significantly larger lesions than middle branches (*P*=0.0049). At OM-1, in positive control branches, lesions were significantly larger on top than on bottom branches (*P*=0.041). At OM- 2, in treated branches, top lesions were significantly larger than bottom lesions (*P*=0.034).

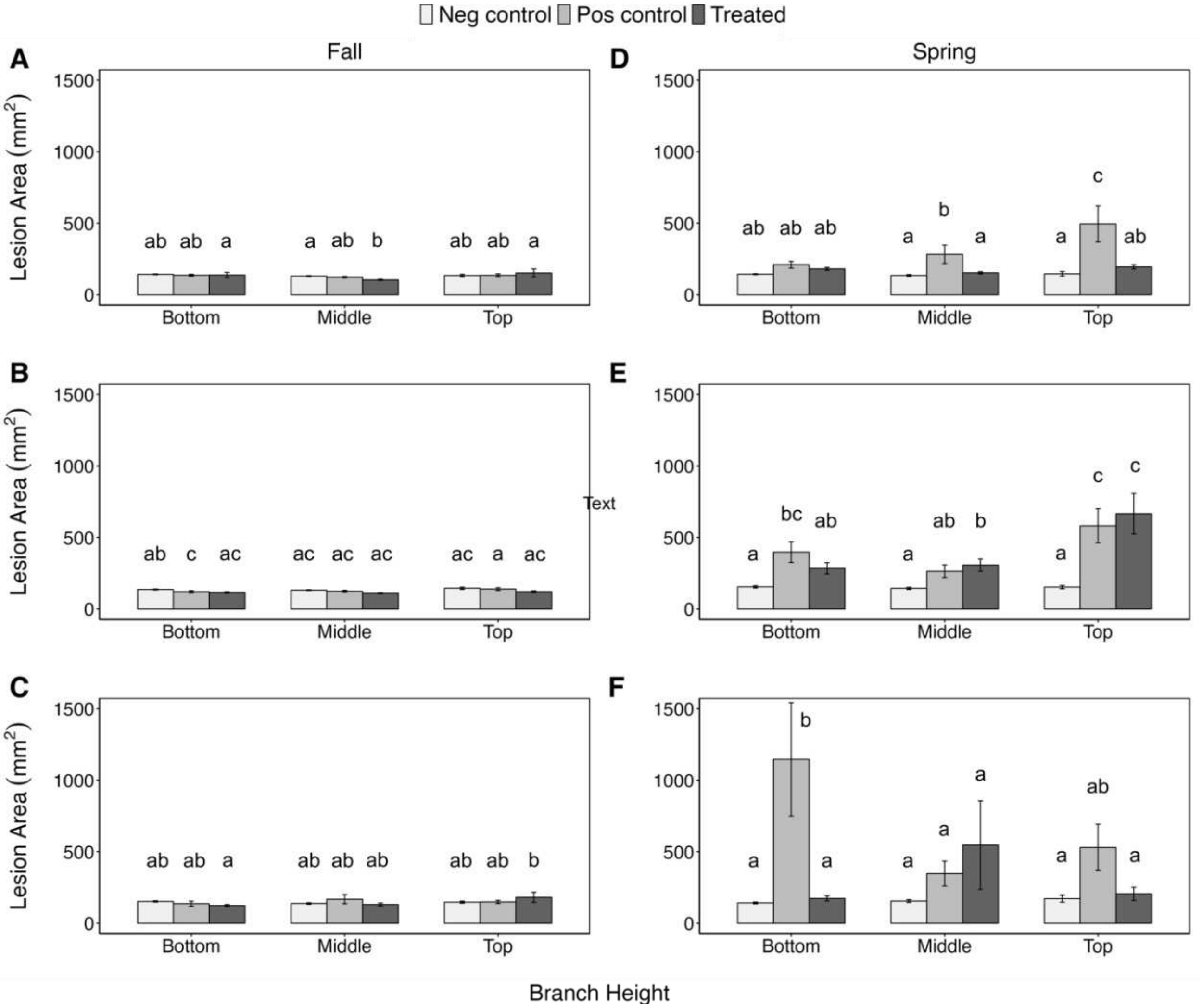

Figure 4. Efficacy of fungicide sprays on wounded branches inoculated with *C. plurivora*, measured by mean lesion area (mm^2). Branches at the bottom, middle, and top of tree scaffolds were wounded prior to fungicide application via air-blast sprayers and inoculated 24 hours (captan) or 48 hours (lime sulfur) post treatment, as determined by re-entry intervals. Inoculated unsprayed branches were used as positive controls and uninoculated unsprayed branches as negative controls. Mean lesion areas are shown for fall trials at orchard blocks **(A)** RM, **(C)** OM-1, **(E)** OM-2, and spring trials at **(B)** RM, **(D)** OM-1, **(F)** OM-2. Error bars denote one SE of the mean. Letters indicate statistically significant differences in mean percent coverage based on model-based pairwise comparisons (Tukey-Kramer, α=0.5); letters that are not shared indicate significant differences in mean percent coverage.

Spring Fungicide Trials

Treatment. In the spring fungicide trials, significant differences were observed between treatments (negative controls, positive, controls, and sprayed trees) in all orchard blocks and the effect of treatment on lesion size varied by height (Figure 4). Analyses revealed significant interactions between treatment and height in RM (P=0.044) and OM-1 (P=0.0495), and a trend towards significance in block OM-2 (P=0.0509).

At RM, branches treated with lime sulfur developed significantly smaller lesions than positive control branches on both middle and top branches (Figure 4). Lime sulfur treatment was associated with a 58% decrease in lesion area on middle branches (P=0.034) and an 87% decrease in lesion area on top (P<0.001) branches. No significant difference in lesion size was observed in bottom branches treated with lime sulfur (P=0.815). Lesions on lime sulfur treated branches were not significantly different from negative controls at any height (Figure 4).

At OM-1, captan treatment was not associated with any significant difference in lesion size when compared to positive controls (Figure 4). Lesions were significantly larger on captan treated branches than on negative controls on both middle and top branches (P=0.008, P<0.0001). Positive control lesions were significantly larger than negative control lesions on both bottom and top branches (P=0.005, P<0.0001).

In OM-2, bottom branches treated with captan developed lesions which were 147% smaller than positive control lesions (P=0.001) and not significantly different from negative controls (P=0.872). Positive controls on bottom branches in OM-2 were significantly larger than negative controls (P<0.001) and significantly larger than the positive controls at middle heights (P=0.007). No significant differences between treatments were observed on top or middle branches at OM-2 (Figure 4). While no statistical analysis between orchard blocks was done, the lesions on bottom branches at OM-2 were the largest observed at any of the orchard blocks.

Height. Within treatments, lesion sizes varied by height and showed different trends in the three orchard blocks (Figure 4). At RM, on positive controls, lesions were larger on top branches than on middle or bottom branches (P=0.013, P=0.001). At OM-1, lesions were larger on top branches than middle branches in both positive controls and captan treatments. In contrast, at OM-2, bottom positive controls were significantly larger than middle positive controls (P=0.018).

2.4 Discussion

The results of this study indicate that season of application and fan use are key factors in air blast sprayer coverage of scaffold branches and that spray coverage is not uniform between scaffold heights. Significant differences in percent coverage were observed between cards placed at the bottom, middle, or top of scaffold branches. Unequal distribution of coverage by height was observed in all orchard blocks, with top cards consistently receiving the least coverage. This aligns with findings in foliar sprays, which show that coverage by air-blast sprayers declines with height in the canopy (Bock et al., 2015; Godoy-Nieto et al., 2022). Uniformity of coverage is an important consideration in disease management as lower coverage in top branches is associated with increased disease severity (Bock et al., 2021).

Percent coverage was significantly higher in spring applications than in summer in almost all cases, regardless of height or fan use, likely due to the lack of foliage present on the trees. Significantly higher coverage was observed in spring at all heights at RM, regardless of fan use, and at most heights in OM-1 and OM-2. In similar studies, Bock et al. (2015) and Bock and

Hotchkiss (2020) confirmed that cards placed within canopies received less spray coverage than when in the open, particularly at the top of canopies where fans are less effective at displacing leaves. This suggests that sprays applied when trees are bare are superior for maximizing coverage and may be the best option for canker pathogen management. However, it may not be practical for growers to limit sprays to seasons when trees are bare, particularly when managing *Cytospora plurivora* which has high inoculum and fast growth rates in spring and summer (Miller et al., 2021). The efficacy period of fungicides is an important determinant of application timing and should be explored further in canopy sprays for Cytospora canker. Growers must consider both the seasonal phenology of trees and pathogens in determining application timing for canker pathogens.

Sprayer fans did not have a consistent effect on coverage or distribution of coverage by height, even when season of application was taken into consideration. Findings in RM and OM-3 were variable and did not follow any discernable trend. Axial fans on air blast sprayers are used to improve the uniformity of coverage but have been observed to decrease coverage in smaller crops such as grapes, or in trees when foliage is not present (McCoy et al., 2022). This trend was observed in OM-1, coverage and uniformity between card heights improved with fan use in the summer, but decreased with fan use in the spring (when trees were bare). Interestingly, OM-1 was also the only orchard block with an instance of better coverage in summer; coverage of top cards with fan use was significantly higher in summer than in spring, possibly due to the fan projecting spray excessively for application on bare trees and missing the target.

Measurements of light interception (PAR), revealed slightly higher interception in OM-1 than in OM-2, indicating Anecdotally, trees in OM-1 were observed to be more vigorous and have more dense foliage, when compared with trees in OM-2. This could explain why fan use was more effective at increasing coverage in OM-1 in the summer while decreasing coverage in the spring, and why this trend was not observed in OM-2. Overall, the different results between orchard blocks highlight the importance of considering tree characteristics including age, height, architecture, and vigor. Future studies should elucidate the impact of orchard characteristics on the coverage and efficacy of air-blast sprayer applications.

Results from the fungicide efficacy trials indicate that canopy sprays may not be effective or necessary during fall and winter. During the fall applications, neither lime sulfur or captan significantly reduced lesion size, and positive control inoculations did not surpass the size of negative controls. All lesions, on both sprayed and untreated control trees, increased minimally in size from November to March. This was likely due to the slow growth rate of *C. plurivora* which has an optimum temperature of 28°C and minimal growth at or below 10°C (Stewart et al., 2022). In fall and winter, temperatures in western Colorado are frequently below 10°C, and Cytospora has previously been observed to grow minimally during these seasons in comparison to spring and summer (Miller et al., 2019; Jones & Luepschen, 1971).

Also as in these previous findings, lesions appeared to grow at a much faster rate in the spring. During the spring fungicide trials, treatment with lime sulfur resulted in significantly smaller lesion sizes in mid-scaffold and top branches at RM. These lesions were not significantly larger than uninoculated controls, indicating lime sulfur reduced lesion sizes to that of uninfected wounds. Efficacy varied by height in the canopy, which is likely related to the decrease in coverage observed in the coverage trials. These findings also concur with numerous previous studies which have found lime sulfur at 3% effective in limiting *C. plurivora* growth *in vitro*, on detached branch assays, in direct applications, and in canopy sprays (Miller et al., 2021; Wright, 2022). The findings of this study contradict earlier studies which found no efficacy of lime sulfur canopy sprays (Northover, 1976).

The results of the spring fungicide trials also demonstrate potential efficacy of captan treatments, but findings are too inconsistent to draw a firm conclusion regarding recommended use. Lesions on trees sprayed with captan were significantly smaller on bottom branches in OM-2, but larger on top branches in OM-1 and not significantly smaller at any other height. Captan has shown mixed efficacy in past studies as well. Numerous studies have established the efficacy of captan in limiting *C. plurivora* growth *in vitro*, in detached branch assays and in field trials (Dhanvantari, 1968; Biggs et al., 1994; Miller et al., 2019, 2021), but early studies found no efficacy in shoot inoculations or spring canopy sprays (Northover, 1976). A previous study identified captan, lime sulfur, and thiophanate-methyl as the most effective fungicides for *C. plurivora* control, but none were consistently effective in all field trials conducted (Miller et al., 2019). The inconsistencies observed in captan treatments at OM-1 and OM-2 may have been related to the rate used (3.5 liters per hectare). Previous studies indicated higher efficacy of captan when sprayed at 7 liters per hectare (Wright, 2022).

Future studies are needed to better characterize the efficacy of canopy spray use for management of Cytospora canker, in particular, the most effective formulations and best practices for their application. Further characterization of the optimum coverage for canker pathogen treatment will be key in achieving maximum efficacy of canopy sprays. Many factors beyond the scope of this study impact the coverage achieved by air-blast sprayers. Direct comparisons of sprayer types, calibrations, driving speeds, spray volume, and nozzle orientation have been conducted in other crops, with significant findings to inform foliar spray practices. Continuing studies on air-blast sprayer use for canker pathogens should include similar comparisons to elucidate best practices of air blast sprayer use for optimal coverage. The differences in coverage and efficacy observed between seasons highlight the need for further characterization of treatments during different times of year, including studies over the course of multiple years.

2.5 Conclusion

Overall, the results of this study indicate potential utility of canopy sprays in management of Cytospora canker, but conflicting results necessitate future studies before formal recommendations can be made. Fall applications may not be needed in average years, but could be effective in extreme weather events, as was observed on bud infections after a deep freeze (Wright, 2022). Spring applications of lime sulfur show promising efficacy; however, this varies by height in tree scaffolds. Inconsistent efficacy of captan in spring trials warrants continued study, which should include mid-rate applications (7 liters/hectare) as 3.5 liters/hectare may be insufficient.

Coverage of woody tissues varied significantly by season of application, regardless of fan use. Higher percent coverage was observed during the spring trial, likely due to the presence of foliage in summer, which blocks spray from reaching bark. This should be taken into consideration when determining application timing and seasonal differences in spray protocols. Season of application has a significant effect on both coverage and efficacy of canopy sprays, therefore, timing for optimal coverage must be considered along with pathogen pressure.

Characterizing optimal coverage for treatment on canker pathogens and contributing factors should be a focus of future research. Direct comparisons of sprayers, calibration, driving speeds, spray volume, and nozzle orientation have been conducted in other crops, resulting in improvements of foliar spray practices. Continued explorations of the applicability of these methods for canker pathogens are needed. To achieve maximum efficacy of air-blast sprayer applications while minimizing waste and environmental consequences, understanding the impact of equipment, calibration, and orchard characteristics on coverage is critical.

Fungicides should be used carefully to prevent the development of resistance; further study on rate of use, frequency of application, and additional formulations is needed. FRAC recommends rotating formulations with different modes of action or mixing fungicides, as well as utilizing an integrated approach to management (FRAC, n.d.). Alternatives to traditional fungicides, such as biocontrols, are a growing area of research. *Trichoderma* spp. have demonstrated efficacy for control of canker pathogens, including *Cytospora plurivora,* at or surpassing the efficacy of thiophanate-methyl (Travadon et al., 2023). *Trichoderma*-based product Vintec is now registered in California for management of *Cytospora* spp. on almonds (EPA, 2020). Future research on non-traditional management methods may provide more sustainable management in the long-term. Continued study to identify resistant cultivars and rootstocks, use of biocontrols and treatments that enhance host defense, and cultural practices that reduce susceptibility should all be pursued along with traditional chemical management strategies.

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APPENDIX

Table A1. Mean percent coverage and standard error (SE) of WSP cards in spring and summer trials

	Spring				**Summer**			
	On		*Off*		*On*		*Off*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
RM								
Bottom	93.95	1.71	92.03	1.83	60.33	4.04	57.61	4.08
Middle	96.37	1.13	90.48	1.84	57.96	4.26	47.38	4.47
Top	58.28	5.00	26.11	3.47	26.13	3.81	3.03	1.37
OM- 1								
Bottom	56.35	4.20	62.85	4.19	43.18	3.54	25.38	3.18
Middle	60.60	4.72	73.58	3.71	40.53	3.61	16.60	2.22
Top	22.90	3.78	55.63	4.57	34.49	3.45	7.64	1.88
OM- 2								
Bottom	59.00	9.71	34.77	8.35	60.05	8.52	71.23	6.19
Middle	74.75	7.65	60.73	8.98	48.24	7.46	73.25	6.51
Top	53.50	8.67	6.08	1.87	1.47	0.74	32.73	6.06

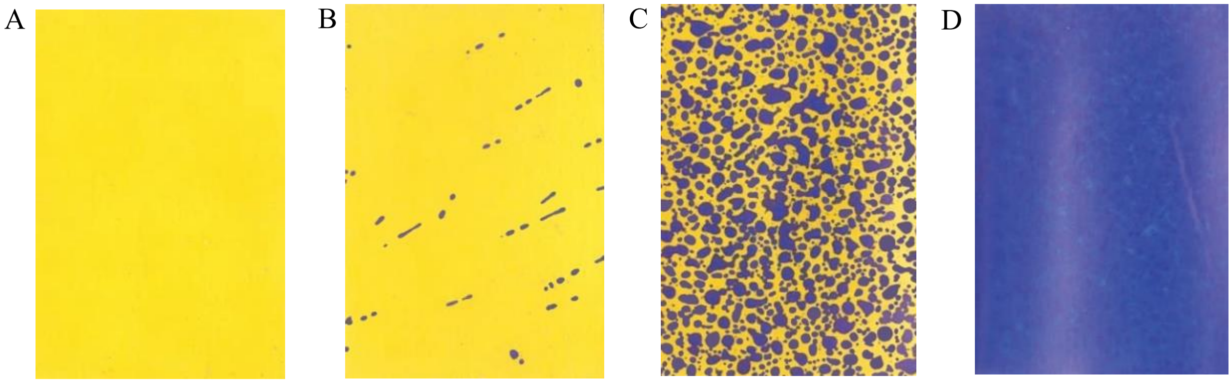

Figure A1. Water sensitive paper (WSP) card samples from spring coverage trials in OM-1 (A) Control (B) 1.49% (C) 63.7% (D) 100.00%.

Table A2. Mean lesion area (mm^2) and standard error (SE) at each height by treatment in fall fungicide trials. Negative controls were unsprayed and uninoculated, positive controls were unsprayed and inoculated.

RM	**Bottom**		**Middle**		**Top**	
Treatment	Mean	SE	Mean	SE	Mean	SE
Negative	142.55	3.11	129.90	3.26	134.07	8.86
Positive	136.25	6.93	122.68	4.80	135.40	10.56
Lime sulfur	137.59	18.00	104.24	4.48	151.88	28.69

OM-1	**Bottom**		**Middle**		**Top**	
Treatment	Mean	SE	Mean	SE	Mean	SE
Negative	136.10	3.64	131.69	3.05	144.95	8.18
Positive	120.28	7.73	123.72	6.58	139.11	10.09
Captan	115.53	4.99	110.28	3.32	120.63	6.23

OM-2	**Bottom**		**Middle**		**Top**	
Treatment	Mean	SE	Mean	SE	Mean	SE
Negative	152.68	4.75	137.89	5.66	147.33	7.69
Positive	136.57	17.71	168.11	32.05	149.17	11.50
Captan	123.45	7.41	130.59	10.73	181.49	35.38

Table A3. Mean lesion area (mm^2) and standard error (SE) at each height by treatment in spring fungicide trials. Negative controls were unsprayed and uninoculated, positive controls were unsprayed and inoculated.

RM	**Bottom**		**Middle**		**Top**	
Treatment	Mean	SE	Mean	SD	Mean	SE
Negative	143.63	3.63	134.33	6.71	145.84	15.97
Positive	209.66	23.68	277.75	64.34	495.01	125.53
Lime sulfur	180.98	10.36	153.23	14.30	194.62	14.30

OM-1	**Bottom**		**Middle**		**Top**	
Treatment	Mean	SE	Mean	SE	Mean	SE
Negative	154.92	8.18	144.28	7.56	153.24	11.99
Positive	397.43	72.46	264.30	44.09	581.94	118.77
Captan	284.21	39.53	307.13	42.54	666.06	141.35

OM-2	**Bottom**		**Middle**		**Top**	
Treatment	Mean	SE	Mean	SE	Mean	SE
Negative	142.08	6.39	155.48	10.11	172.02	24.84
Positive	1145.69	396.43	347.82	87.14	530.26	162.24
Captan	173.63	17.31	546.45	309.36	205.81	46.40

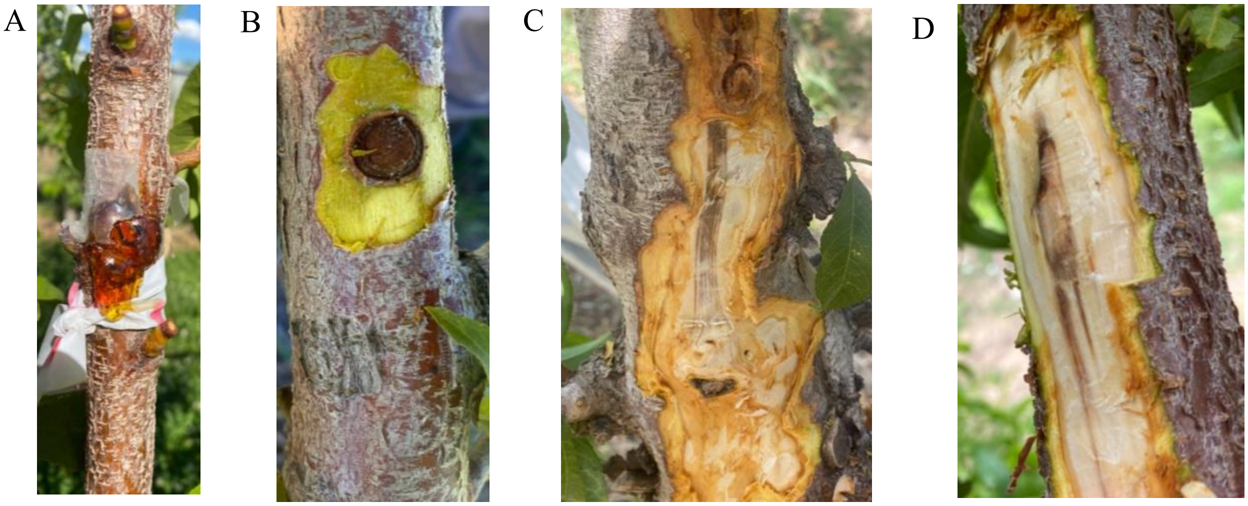

Figure A2. Photographs of lesion sites on branches. **(A)** top positive control with gummosis, **(B)** middle treated branch with minimal lesion growth, **(C&D)** bottom positive controls.

Table 8. Coverage trial ANOVA tables (A) RM (B) OM-1) (C) OM-2.

(A) Rogers Mesa

Predictors	**Sum of Sq**	**Mean Sq**	**Num *df*[a]**	**Den *df*[b]**	***F*[c]**	***P*[d]**
Season	13.26	13.26	1	59	139.24	< 2.2e-16 ***
Fan	1.88	1.88	1	59	19.74	3.945e-05 ***
Height	64.87	32.44	2	693	340.73	< 2.2e-16 ***
Season: Fan	0.01	0.01	1	59	0.07	0.795
Season: Height	0.57	0.29	2	693	3.00	0.05053.
Fan: Height	4.41	2.21	2	693	23.17	1.824e-10 ***
Season:Fan:Height	0.06	0.03	2	693	0.34	0.715

(B) Orchard Mesa 1

Predictors	**Sum of Sq**	**Mean Sq**	**Num *df*[a]**	**Den *df*[b]**	***F*[c]**	***P*[d]**
Season	12.44	12.44	1	59	96.87	4.651e-14 ***
Fan	0.14	0.14	1	59	1.12	0.294
Height	9.79	4.90	2	696	38.15	< 2.2e-16 ***
Season:Fan	6.93	6.93	1	59	53.98	6.992e-10 ***
Season:Height	2.11	1.05	2	696	8.20	0.0003012 ***
Fan:Height	0.45	0.22	2	696	1.74	0.177
Season:Fan:Height	2.20	1.10	2	696	8.55	0.0002149 ***

(C) Orchard Mesa 2

Predictors	**Sum of Sq**	**Mean Sq**	**Num *df*[a]**	**Den *df*[b]**	***F*[c]**	***P*[d]**
Season	0.01	0.01	1	12	0.05	0.827
Fan	0.04	0.04	1	12	0.29	0.598
Height	11.31	5.66	2	168	44.65	2.818e-16 ***
Season:Fan	2.66	2.66	1	12	20.97	0.0006334 ***
Season:Height	1.41	0.71	2	168	5.58	0.0045042 **
Fan:Height	0.21	0.10	2	168	0.82	0.441
Season:Fan:Height	0.98	0.49	2	168	3.88	0.0224730 *

[a] numerator degrees of freedom; [b] denominator degrees of freedom; [c] F-value; [d] p-value

Table 9. Fall fungicide trial ANOVA tables (A) RM (B) OM-1) (C) OM-2.

(A) Rogers Mesa

Predictors	**Sum of Sq**	**Mean Sq**	**Num dfa**	**Den *df*[b]**	**Fc**	**Pd**
Treatment	0.16	0.08	2	27.12	1.31	0.285
Height	0.55	0.28	2	143.26	4.59	0.012 *
Trt:Height	0.33	0.08	4	143.26	1.38	0.245

(B) Orchard Mesa 1

Predictors	**Sum of Sq**	**Mean Sq**	**Num *df*[a]**	**Den *df*[b]**	***F*[c]**	***P*[d]**
Treatment	0.81	0.40	2	26.20	4.84	0.016 *
Height	0.35	0.18	2	143.44	2.10	0.126
Trt:Height	0.27	0.07	4	143.43	0.80	0.526

(C) Orchard Mesa 2

Predictors	**Sum of Sq**	**Mean Sq**	**Num *df*[a]**	**Den *df*[b]**	***F*[c]**	***P*[d]**
Treatment	0.03	0.02	2	12.76	0.2376	0.792
Height	0.25	0.12	2	70.25	1.8357	0.167
Trt:Height	0.44	0.11	4	70.26	1.6421	0.173

[a] numerator degrees of freedom; [b] denominator degrees of freedom; [c] F-value; [d] p-value

Table 10. Spring fungicide trial ANOVA tables (A) RM (B) OM-1) (C) OM-2.

(A) Rogers Mesa

Predictors	**Sum of Sq**	**Mean Sq**	**Num *df*[a]**	**Den *df*[b]**	**Fc**	**Pd**
Treatment	8.99	4.49	2	27.40	22.68	1.544e-06 ***
Height	1.53	0.76	2	143.01	3.86	0.023 *
Trt:Height	1.98	0.49	4	143.00	2.4957	0.045 *

(B) Orchard Mesa 1

Predictors	**Sum of Sq**	**Mean Sq**	**Num *df*[a]**	**Den *df*[b]**	***F*[c]**	***P*[d]**
Treatment	20.97	10.49	2	168.01	26.68	8.69e-11 ***
Height	4.91	2.45	2	168.00	6.24	0.002 **
Trt:Height	3.8230	0.96	4	168.00	2.43	`0.0490 *

(C) Orchard Mesa 2

Predictors	**Sum of Sq**	**Mean Sq**	**Num *df*[a]**	**Den *df*[b]**	***F*[c]**	***P*[d]**
Treatment	8.70	4.35	2	12.28	9.01	0.004 **
Height	0.48	0.24	2	67.57	0.49	0.613
Trt:Height	4.82	1.20	4	67.55	2.49	0.050 .

[a] numerator degrees of freedom; [b] denominator degrees of freedom; [c] F-value; [d] p-value\