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The Effect of Adjuvants on Apple Disease Management

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Purdue University

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For the degree of Master of Science

Is approved by the final examining committee:

Janna Beckerman	_____	_____
<small>Chair</small>	_____	_____
Kiersten Wise	_____	_____
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THE EFFECT OF ADJUVANTS ON APPLE DISEASE MANAGEMENT

A Thesis
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of
Purdue University
by
Chelsi Patricia Abbott

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ABSTRACT

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The management of common apple diseases such as apple scab (*Venturia inaequalis*) and bitter rot (*Colletotrichum* spp.) relies heavily on effective fungicide applications. However, the development of fungicide resistance to newer fungicides has resulted in management failures and significant economic losses. This has led to a greater reliance on captan, an older fungicide, because there is a low risk of pathogens developing resistance. Label restrictions limit growers to 18 kg of captan per season, which may not provide sufficient control of both apple scab and bitter rot in wet years. Consequently, apple growers are faced with two equally difficult scenarios, inadequate management of diseases due to resistant pathogen strains from the use of newer fungicides or insufficient management due to restrictions on captan.

The goal of this research was to identify new approaches to reduce the amount of captan needed throughout the growing season without decreasing disease control. One potential tactic is to incorporate adjuvants into management strategies. Adjuvants are tank additives that increase the coverage and retention of sprays and correct issues with the tank water by affecting the pH. The incorporation of adjuvants into current apple disease management strategies has the potential to improve disease control by increasing the efficacy of captan sprays at reduced rates and reducing initial inoculum by enhancing urea-driven leaf litter decomposition.

To assess the improvement of captan sprays, adjuvants were combined with the lowest rate of captan and applied to apple trees every 10-14 days from bloom to harvest. Disease and phytotoxicity incidence and severity were observed on apple fruit to measure the effectiveness of the treatments. Results showed that Li700 plus captan and Bond Max plus captan consistently reduced disease incidence in high-pressure years by increasing the coverage and retention of captan and lowering the pH of the tank water. Based on the data found in this study, a grower could potentially save up to \$3,481-\$4,667 ha⁻¹ due to reduction of disease incidence.

In order to examine if adjuvants improved urea-driven decomposition of scab-infected leaves, adjuvants were combined with urea and applied to infected leaves. These leaves were then left to overwinter on the orchard floor. Leaf area decomposition and pseudothecia and ascospore reduction were observed to measure the effectiveness of the treatments. Results showed that Li700 plus urea and Wet Betty plus urea improved urea-driven leaf decomposition and pseudothecia and ascospore reduction by increasing the nitrogen content in the leaves. Based on this study, the addition of these adjuvants to urea could delay an apple scab epidemic, saving fungicide applications and postponing initial infection past the point when apples are most susceptible to *V. inaequalis*.

The addition of adjuvants to captan or urea has the potential to improve disease management by reducing fungicide rates and reducing overwintering inoculum. Together these factors may reduce the number and dose of fungicide sprays required for apple scab and bitter rot management throughout the growing season and ultimately increase a grower's net return in apple production.

CHAPTER 1. INTRODUCTION

Fresh market apples are the most important fruit crop in the North Central region of the United States, with sales exceeding \$635 million (ERS USDA, 2012). However, even the smallest of blemishes caused by diseases can make apples unacceptable for sale, resulting in significant economic loss (Lewis and Hickey, 1972). There are many diseases that threaten the profitability of apple production, and among the most important are apple scab and bitter rot (Hickey, 1991; Jones, 1994). Apple growers can experience up to 100% yield loss from apple scab (Hickey, 1991; Jones, 1994), and 90% yield loss from bitter rot if orchards are unmanaged (Hickey, 1991; Sutton, 1990b).

Management of these diseases relies on a combination of cultural and chemical practices (Sutton, 1990a,b). One important cultural practice growers may employ is sanitation (Meszka and Bielenin, 2006). Sanitation practices focus on the removal of overwintering inoculum, which reduces the initial disease pressure and the amount of chemicals needed for control during the growing season (Sutton et al. 2000; Meszka and Bielenin, 2006). Although, full benefits of sanitation are rarely attained in orchards due to variable topography and time restrictions (Vincent et al. 2004). Furthermore, due to high disease pressure, sanitation alone is an insufficient method of disease management (Sutton et al. 2000).

Another important cultural practice used to decrease fungicide applications in commercial orchards is planting resistant cultivars (Ellis et al. 1998; Meszka and Bielenin, 2006). Despite their benefits certain resistant cultivars are not commercially grown due to consumer unfamiliarity (Gianessi and Reigner, 2005; Beckerman et al. 2015).

In the United States, 93% of the acreage designated for apple orchards is treated with fungicide applications to control diseases (Gianessi and Reigner, 2005). Apple

growers in the United States may use up to 7 million pounds of fungicides per season, costing almost 70 million dollars annually (Gianessi and Reigner, 2005). This is because management of common apple diseases is heavily reliant on fungicides, requiring multiple applications per season for optimal control (Jones, 1994; MacHardy et al. 2001; Turechek, 2004).

Unfortunately for growers that rely on fungicides, there are issues with them regarding fungicide resistance and use limitations (Rosenberger, 2009). Fungicides that manage common apple diseases include dodine (now known as Syllit), methyl benzimidazole carbamates (MBC), demethylation inhibitors (DMI), quinone outside inhibitors (QoI), and succinate dehydrogenase inhibitors (SDHI) (Szkolnik and Gilpatrick, 1969; Köller, 1997; Köller et al. 2005; McKay et al. 2011). But through the extended use of these fungicides many growers now deal with resistant pathogen strains (Rosenberger, 2009). This had led to a reliance on older fungicides such as captan and ethylene bisdithiocarbamates (EBDC's), as they have low risk of developing resistance (Rosenberger, 2013). Unfortunately, both captan and EBDC's have been restricted due to their possible carcinogenicity to humans (USEPA, 2006). Only 18 kilograms of captan can be applied per growing season, restricting growers to 10 applications of captan at a 1.8 kilograms rate or less (Rosenberger, 2009). In wet years, more applications or higher rates of captan are typically needed to manage both apple scab and bitter rot throughout the entire season (Rosenberger, 2009). Mancozeb has a pre-harvest interval (PHI) of 77 days, leaving apples unprotected for two and a half months up to harvest. These factors reveal the need for methods that provide protection throughout the growing season without violating fungicide restrictions or developing resistant pathogens (Sutton, 1996; Rosenberger, 2009).

1.1 Apple scab

Apple scab, caused by the fungal pathogen *Venturia inaequalis* (Cooke) Winter, is one of the most devastating diseases on apples in regions that experience cool, wet springs (Sutton, 1990a; MacHardy et al. 2001). *V. inaequalis* infects the leaves, flowers,

and fruit (Sutton, 1990a). Early infection can be observed on the underside of developing leaves as small indistinct brown to olive green velvety lesions (Sutton, 1990a). As the disease progresses the margins of the lesions become more distinctive and the leaf tissue infected begins to deform, usually curling or distorting (Fig. 1.1A) (Sutton, 1990a). Severe infections can lead to defoliation of the tree (Sutton, 1990a). Initial infections on the fruit look similar to the early lesions observed on leaves, that is; small indistinct lesions that are near the calyx of the apple (Fig. 1.1B) (Sutton, 1990a). As the fruit and pathogen both develop the lesion becomes brown and corky, eventually forming cracks on the surface, resembling scabs (Fig. 1.1C) (Sutton, 1990a).

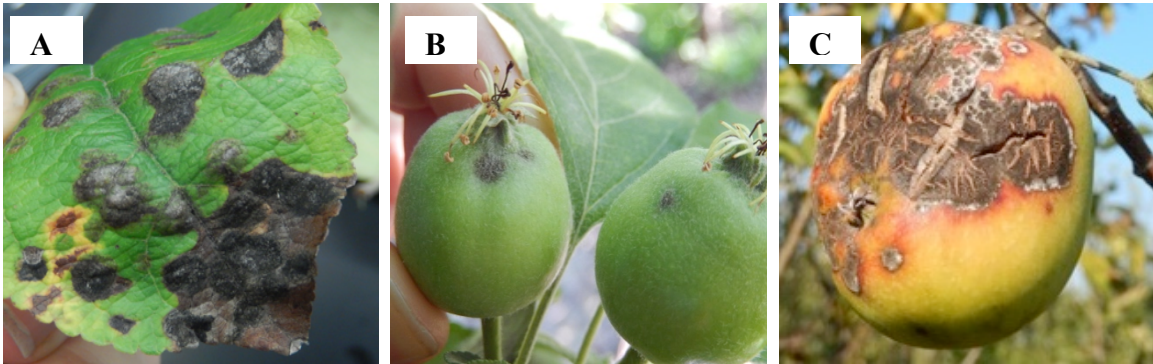


Figure 1.1: Apple scab symptoms on apple leaves and fruit. Symptoms appear as **A**, distinctive dark lesions that eventually deform the leaf, **B**, indistinct olive lesions near the calyx of the fruit, and **C**, brown and corky resembling scabs.

1.1.1 Lifecycle

V. inaequalis is a fungal pathogen that has both a saprophytic and parasitic stage (MacHardy et al. 2001). The saprophytic stage occurs over the winter in dead fallen leaves on the orchard floor (Sutton, 1990a; MacHardy et al. 2001). After the leaves die, hyphae penetrate into the plant cells and develop into stromatic spheres where fertilization between compatible mating types produces pseudothecia (MacHardy et al. 2001). Pseudothecia are the sexual structures of the fungus in which haploid (n)

ascospores are produced (Alexopoulos et al. 1996). Pseudothecia develop in the dead leaves over the winter and are forcibly ejected in the spring when the dead leaves become wet (Sutton, 1990a; MacHardy et al. 2001; Holb et al. 2004; Gianessi and Reigner, 2005). Ascospores are disseminated by wind and rain (Sutton, 1990a; MacHardy et al. 2001). The potential for infection by *V. inaequalis* is proportional to the number of pseudothecia and mature ascospores produced (Gadoury and MacHardy, 1986).

Infection occurs when the ascospores land and adheres to the emerging plant tissue in the spring (Sutton, 1990a; MacHardy et al. 2001). After the ascospore attaches to the surface the spore germinates and an appressorium develops (MacHardy et al. 2001). This is followed by the development of a penetration peg that allows the fungus to penetrate the plant cuticle and colonize the area between the epidermis and cuticle, initiating primary infection (Sutton, 1990a; MacHardy et al. 2001). After about 14 days, the fungus reproduces asexual spores called conidia that can be observed dark green lesions on the leaf surface (Sutton, 1990a; MacHardy et al. 2001). Conidia initiate secondary infections, which can continue to infect for the rest of the season (Sutton, 1990a; MacHardy et al. 2001).

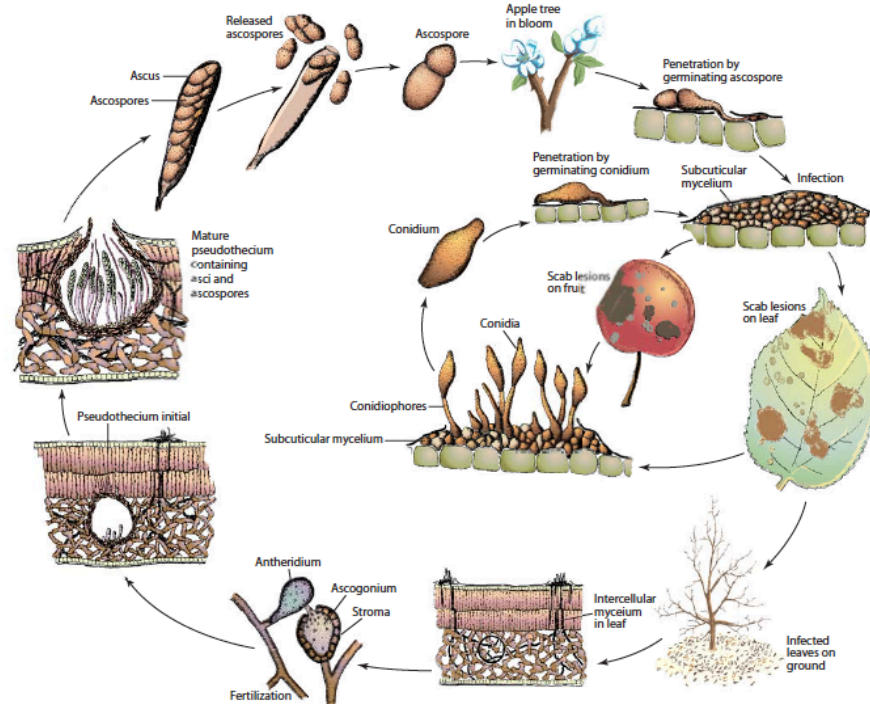


Figure 1.2: Life cycle of *Venturia inaequalis* (Agrios, 2005).

1.1.2 Management

Management of *V. inaequalis* focuses on preventing primary infection by reducing the leaf litter using sanitation and minimizing primary infection (Sutton 1990a). Sanitation practices can include leaf litter shredding, urea treatments, flaming, removal, or any combination of these (Holb et al. 2004). These methods focus on reducing initial inoculum and lowering the reliance on fungicides during the growing season by removing primary disease pressure (Holb et al. 2004; Agrios, 2005; Mac an tSaoir et al. 2010).

Cultural practices alone are not sufficient for complete disease control, resulting in reliance upon protective fungicides (Sutton et al. 2000). Protection of the fruit throughout the growing season often relies on protective fungicides applied every 10-14 days (Sutton 1990a). Historically, there have been many fungicides effective in managing apple scab, including ethylene bisdithiocarbamates (EBDC's), captan, dodine (now known as Syllit), methyl benzimidazole carbamates (MBC), demethylation inhibitors

(DMI), quinone outside inhibitors (QoI), and succinate dehydrogenase inhibitors (SDHI) (Szkolnik and Gilpatrick, 1969; Köller, 1997; Köller et al. 2005; McKay et al. 2011). Through the extended and exclusive use, *V. inaequalis* has developed resistance to the majority of these fungicides and most no longer exhibit good control (Köller et al. 2005). Apple scab can still be managed using captan and EBDC's, but label restrictions limit the applications allowed per season, which can lead to inadequate control (Rosenberger, 2009).

1.2 Bitter rot

Bitter rot is a fungal disease caused by several species of *Colletotrichum* including *C. acutatum* J.H. Simmonds, *C. gloeosporioides*, and its teleomorph *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, and *C. fioriniae* (Marcelino & Gouli) R.G. Shivas & Y.P. Tan (González & Sutton, 2004; Kou et al. 2014). These pathogens mainly affect the fruit near harvest and have the potential to cause major yield loss, particularly in areas that have hot and wet summer weather (Sutton, 1990b). These pathogens cause dark brown lesions surrounded by red halos on young fruit (Sutton, 1990b). As the apple grows, the lesions become sunken and the acervuli (asexual structures containing asexual spores) are either concentrically dispersed or scattered on the lesion (Fig. 1.3A) (Sutton, 1990b). The asexual spore masses of the fungus are generally creamy and salmon in appearance (Fig. 1.3A) (Sutton, 1990b). The lesion creates a v-shaped rot to the core of the apple, distinguishing bitter rot from white rot, which creates a cylindrical shaped rot to the core (Fig. 1.3B) (Sutton, 1990b). Fruit infected with *Colletotrichum* spp. either drop to the ground or remain on the tree as mummified fruit (Fig. 1.3C) (Sutton, 1990b). A leaf spot phase has been documented, but it is only common if the environment is very warm and humid (Sutton, 1990b).

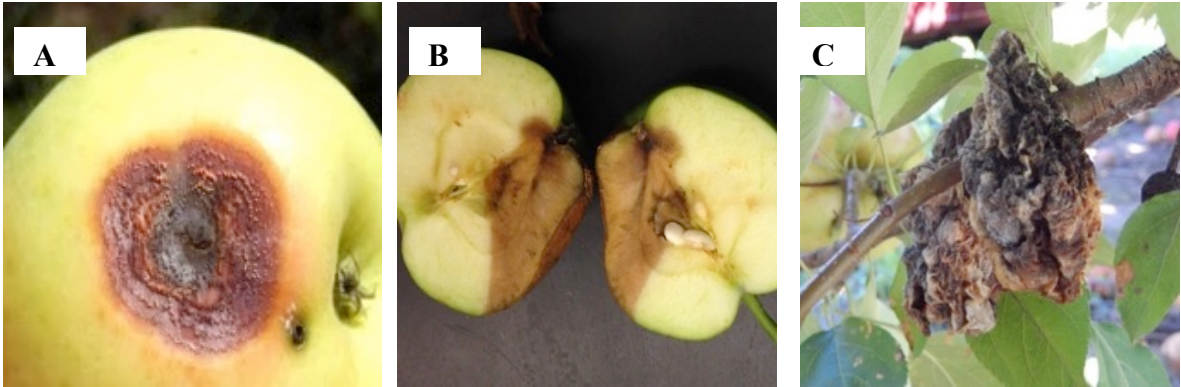


Figure 1.3: Bitter rot symptoms on apples. Symptoms appear as **A**, sunken lesions with rings of creamy salmon spores, **B**, v-shaped rot to the core of the apple, and **C**, mummified fruit that remains on the tree.

1.2.1 Lifecycle

The perithecia and the acervuli of *Colletotrichum* spp. can overwinter in mummified fruit left in the orchard or in cankers in the wood (Sutton, 1990b). Ascospores are formed inside the perithecia, and asexual conidia are borne from the acervuli (Sutton, 1990b). In rain events during the growing season, both ascospores and conidia are released (Sutton, 1990b). Both spores germinate and form appressoria that penetrate directly into the epidermal tissue of the plant (Sutton, 1990b).

After penetration, the appressorium can melanize and enter quiescence or a latent infection period (Wharton and Diéguez-Uribeondo, 2004). During this period of time the fungus exists in a brief biotrophic phase (Wharton and Diéguez-Uribeondo, 2004). The necrotrophic stage of the fungus is initiated as the fruit ripens (Wharton and Diéguez-Uribeondo, 2004). In this stage the fungus produces enzymes that degrade plant cell walls, causing the plant cells to collapse and die (Wharton and Diéguez-Uribeondo, 2004). The fungus then invades host cells beneath the initial infection site resulting in sunken brown lesions (Wharton and Diéguez-Uribeondo, 2004). Acervuli arise from these sunken lesions and produce masses of conidia that initiate the secondary lifecycle of this

pathogen, which can continually re-infect the fruit throughout the entire growing season (Agrios, 2005).

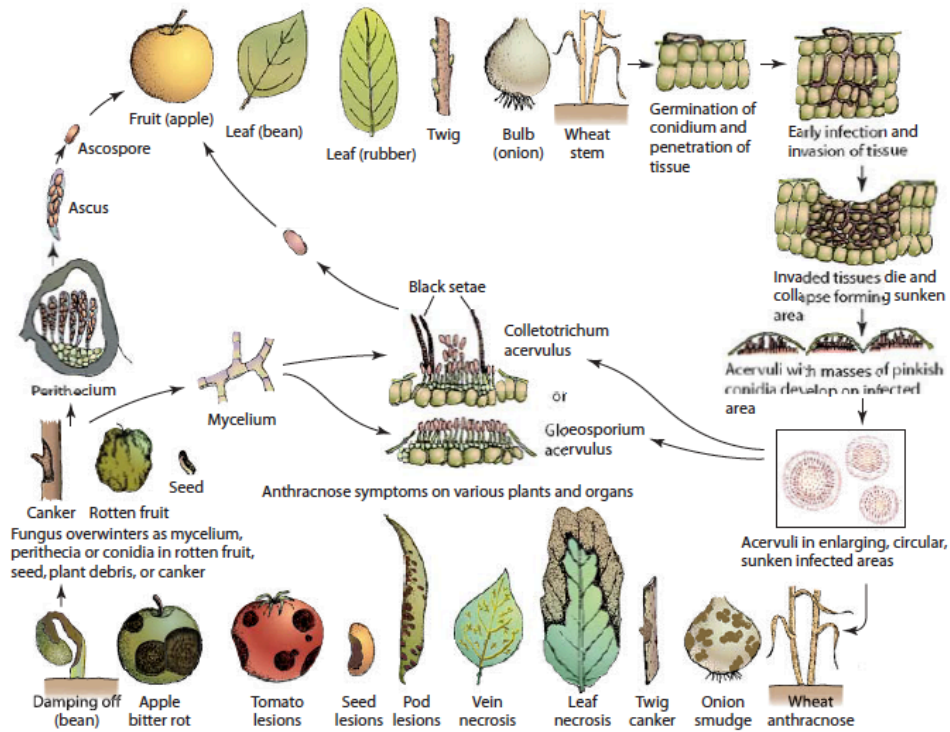


Figure 1.4: Life cycle of *Colletotrichum* spp. (Agrios, 2005).

1.2.2 Management

Major epidemics of bitter rot can occur when primary infections are extensive and produce large amounts of secondary inoculum (Sutton, 1990b). Current management of this disease is a combination of sanitation (removal of mummified fruit) and the use of fungicides on a 10-14 day schedule (Sutton, 1990b). Reliance on fungicides can be problematic as many fungicides that manage bitter rot are restricted due to public concern of pesticide residues on food (Biggs, 1999). Another form of bitter rot management is the use of resistant cultivars (Wharton and Diéguez-Uribeondo, 2004). Despite the benefits of using resistant cultivars, growers generally tend to prefer highly susceptible cultivars

like Honeycrisp and Ginger Gold because consumers are more familiar with them (Biggs and Miller, 2001). As a result, the cultivation of highly susceptible apple cultivars requires more intensive management (Sutton 1996) in the form of additional fungicide sprays and thorough sanitation. It is also important to note that severe bitter rot infections are more of an issue in southern states due to the consistently warmer climate (Turechek, 2004). As northern states have warmer summers there can be a potential for increasingly severe disease epidemics (Hirshi et al. 2012).

1.3 Fungicides and their importance in apple production

Both apple scab and bitter rot have the potential to reduce the economical value of apple crops (Hickey, 1991; Jones et al. 1996). While the management of both diseases relies on a multi-faceted approach, fungicides are heavily relied upon in disease management strategies in apple orchards (Bower et al. 1993; Penrose, 1994). In 2006, 93% of orchards in the United States used fungicides in their apple production and those orchards experienced an 86% increase in yield compared to orchards that did not (Gianessi and Reigner, 2006). It is estimated that apple growers in the United States experience a \$1.22 billion profit gain from using fungicides in their orchards compared to orchards that do not use fungicides (Gianessi and Reigner, 2006), with the majority of fungicide used for the control apple scab (Merwin et al. 1994).

1.3.1 Fungicide restrictions

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was first passed in 1947 and concentrated on the registration and labeling of pesticides, but did not regulate their use (EPA, 2012). Since then it has been amended multiple times but currently mandates that the Environmental Protection Agency (EPA) is to regulate pesticides to protect human and environmental health (EPA, 2012). When setting regulations of pesticides, the EPA considers the adverse effects the pesticide has on

human, animal, and environment (Gullino, 1994). Due to their potential to cause harmful effects, fungicides are restricted due to issues of toxicity, leaching, persistence, or bioaccumulation (Gullino, 1994). The consequences of these issues can be limitations in application rates and frequency, reductions in number of treatments allowed, pre-harvest intervals, and restrictions to certain areas or crops (Gullino, 1994).

Fungicide restrictions have had negative effects in disease management in orchards (Gullino, 1994; Sutton, 1996; Rosenberger, 2009). Captan and mancozeb are fungicides that are effective at controlling apple scab and bitter rot (Biggs 1999; Rosenberger, 2009). Unfortunately, both are classified as potential human carcinogens after prolonged, high-exposure (USEPA, 2006; Beckerman et al. 2015). Consequently, there are restrictions on captan and mancozeb applications in orchards (Rosenberger, 2009; Beckerman et al. 2015). Only 18 kilograms of captan can be applied per growing season, restricting growers to 10 applications of captan at a 1.8 kilograms rate or less. In wet years, more applications or higher rates of captan are typically needed to manage both apple scab and bitter rot throughout the entire season (Rosenberger, 2009). Secondly, mancozeb has a pre-harvest interval (PHI) of 77 days, leaving apples unprotected when apples are most susceptible to bitter rot.

These restrictions have made captan and mancozeb less suitable for use in disease management and thus were eventually replaced by environmentally benign fungicides that were more effective at managing diseases (Gullino, 1994; Beckerman et al. 2015). These newer fungicides were safer and had curative qualities, allowing growers to make applications up to 72 hours after infection occurred resulting in better disease control with fewer applications (Beckerman et al. 2015). But shortly after their release, these newer fungicides were found to be high risk for pathogens developing resistance (Rosenberger, 2009; Beckerman et al. 2015). The development of fungicide resistance in orchards led to new obstacles in disease management programs.

1.3.2 Fungicide resistance

There have been many fungicides that are classified to control apple scab and bitter rot, including dodine (now known as Syllit), methyl benzimidazole carbamates (MBC), demethylation inhibitors (DMI), quinone outside inhibitors (QoI), and succinate dehydrogenase inhibitors (SDHI) (Szkolnik and Gilpatrick, 1969; Köller, 1997; McKay et al. 2011). However, the extended use of these fungicides has resulted in resistant strains of *V. inaequalis* (Jones and Walker, 1976; Kuck et al. 1995; Russell, 1995; Bartlett et al. 2002; Köller et al. 2005) and *Colletotrichum* spp. (Jones et al. 1996) within a couple years of their respective release. While there are factors that make pathogens high risk for developing resistance, such as a shorter lifecycle and abundant sporulation, there are also qualities fungicides have that increase the risk for resistance development (Brent and Hollomon, 2007).

Fungicides can be classified as single site or multi-site, which affects their specificity and resistance risk (McGrath, 2004). The MBC, DMI, QoI, and SDHI fungicides have a single-site or specific targeted function (Damicone and Smith, EPP-7663; FRAC, 2015). This means that they target one critical enzyme or protein required by the fungus (McGrath, 2004; Brent and Hollomon, 2007). For example, MBC fungicides target β -tubulin assembly in mitosis, which disrupts microtubule synthesis in fungal cells and arrests cell division (Mueller and Bradley, 2008; FRAC, 2015). While DMI fungicides obstruct the enzyme C14-demethylase from producing sterols that are essential to fungal cell walls, resulting in irregular cell walls and cell death (Mueller and Bradley, 2008; FRAC, 2015). QoI fungicides target the quinol outer binding site of the cytochrome bc1 complex, which halts energy production resulting in cellular death (Mueller and Bradley, 2008; FRAC, 2015). Finally, SDHI fungicides obstruct the ubiquinone binding sites in the mitochondrial complex II, disrupting cellular respiration in the fungus (Avenot and Michailides, 2010).

While these fungicides can be more specific in toxicity, they can also have higher risk of pathogens developing resistance, as only a single gene mutation that alters the target site—is needed to overcome the function of the fungicide (Dekker, 1985; Sisler,

1988; McGrath, 2004; Brent and Hollomon, 2007). If this mutation occurs, the resistant individual survives and continues to reproduce, so the proportion of the population that is resistant steadily increases until the fungicide is no longer effective (Fig. 1.5) (Brent and Hollomon, 2007).

Once a pathogen becomes resistant to a specific mode of action, that fungicide group is no longer reliable method of disease control (Brent and Hollomon, 2007). Furthermore, improper use of these fungicides or reduction of their rates may actually increase resistance incidence in the field (Beckerman et al. 2015). These factors have left growers with limited management options (Rosenberger, 2009; Beckerman et al. 2015). As a solution, alternative management strategies including sanitation, scouting, and the use of resistance cultivars have been developed or reintroduced (De Waard et al. 1993; Beckerman et al. 2015). These methods are primarily based on the notion of reducing reliance on fungicides that are high risk for developing resistance and focusing on more sustainable management strategies (De Waard et al. 1993; Cooley and Autio; 1997; Beckerman et al. 2015).

Despite these multiple tactics available for apple disease management, none are as effective as fungicides, so apple scab and bitter rot are still heavily reliant on fungicides, requiring multiple applications per season for optimal control (Jones, 1994; MacHardy et al. 2001; Turechek, 2004). Therefore, it is imperative to explore effective fungicides that are at low risk for pathogen-developed resistance without violating their label restrictions (De Waard et al. 1993; Knoche, 1994; Penrose, 1994; Rosenberger, 2009; Rosenberger and Cox, 2012).

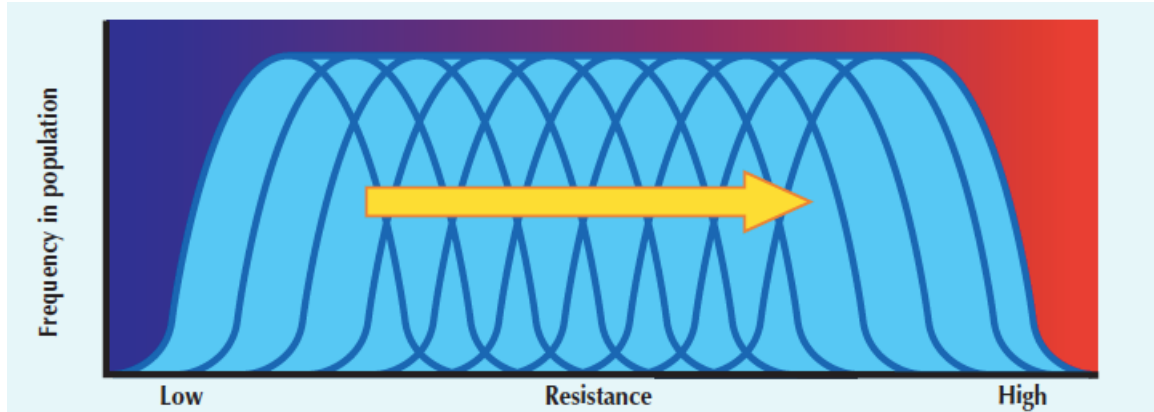


Figure 1.5: Shift in the sensitivity of pathogen populations over time from sensitive to resistant as the number of fungicide applications increase. Each bell curve represents a successive pathogen population (Brent and Hollomon, 2007).

1.3.3 Captan

The evolution of fungicide resistance in *V. inaequalis* and *Colletotrichum* spp. to newer fungicides has left growers with few options for disease control (De Waard et al. 1993; Brent and Hollomon, 2007). This has forced growers to rely on fungicides such as captan (N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide), a chloroalkyl thio fungicide which disrupts multiple cellular division pathways of the fungus (Richmond et al. 1967; Turecek, 2004; Rosenberger, 2013). In order for a fungus to become resistant to captan, it would have to simultaneously develop multiple mutations for all blocked pathways, which is unlikely (Brent and Hollomon, 2007; Rosenberger, 2013). Furthermore, captan is effective at controlling apple scab and bitter rot at a lower application rate than other fungicides such as the EBDC, mancozeb (Xu et al. 2008; Turecek, 2004; Rosenberger, 2013). High disease control coupled with its low risk of fungal resistance, makes captan one of the most effective fungicides to manage many apple diseases including scab, leaf spots, blights, and rots (Lewis and Hickey, 1972; Frank et al. 1985; Turecek, 2004).

Though there are benefits of using captan in apple disease management, there are several drawbacks (Rosenberger, 2013). When suspended in alkaline conditions, captan

has a half-life of 20 minutes, meaning it could degrade significantly before applications are made (Gradis and Sutton, 1981; Frank et al. 1985). Captan also cannot persist if one inch or more of rain occurs within 24 hours after application, leaving the plant surface unprotected against fungal infection (Smith and MacHardy, 1984). Furthermore, the EPA has classified captan as a group B2 carcinogen, or a potential human carcinogen (EPA, 1999). This classification means that while captan has shown sufficient evidence for carcinogenicity in animal subjects, little to no data on carcinogenicity of humans has been recorded (EPA, 1999). Although not confirmed, based on studies performed with animals, captan is likely to be carcinogenic after prolonged, high-exposure, but unlikely if exposure is at low dosage (USEPA, 2006). Lastly, there are restrictions on captan applications that limit growers to 40 pounds per season, which may be inadequate to provide sufficient disease control throughout the entire season (Rosenberger, 2009).

While this information addresses the negative consequences and limitations of using captan, it also highlights a potential area of research that focuses on improving captan's efficacy at reduced rates without decreasing disease control. Research in this area should address lowering the pH of tank water to prevent degradation of captan and increasing the retention and coverage to improve efficacy of captan. These enhancements would improve captan's disease control and allow growers to apply captan at lower rates or extended intervals so control can be obtained the entire season (Knoche, 1994). Reducing the rate of fungicides needed to control disease would be an economic benefit to the growers while also lowering the amount of chemicals introduced into the environment and to food products (Knoche, 1994). The latter has increasingly become the focus of research because of the growing public concerns surrounding chemical residue on food (Knoche, 1994; Penrose, 1994; Berrie and Xu, 2003; Flint et al. 2003; Ahouangninou et al. 2012).

1.4 Sanitation in apple production

Fungicides are the primary tools used to manage apple diseases (Bower et al. 1993; Penrose, 1994; Jones, 1994; MacHardy, 1996; Turechek, 2004; Meszka and

Bielenin, 2006). But fungicides have limitations that may reduce their efficacy, requiring the addition of other strategies such as sanitation for more complete disease control (Rosenberger, 2009). Sanitation practices remove the overwintering stage of the pathogen and thereby decrease the primary inoculum, which ultimately reduces fungicide applications required during the growing season (Scribner, 1888; Sutton 1990a,b; Sutton et al. 2000; Meszka and Bielenin, 2006). This may be especially beneficial in seasons where disease pressure is so high that fungicides alone are not effective.

There have been several studies exploring the reduction of initial inoculum of *V. inaequalis* by implementing sanitation practices (Sutton et al. 2000). Since the pathogen overwinters in leaves on the orchard floor, these practices focus on eradicating the leaf litter by removal, shredding, chemical treatments, or urea applications (Sutton et al. 2000; Vincent et al. 2004). Shredding the leaf litter can reduce scab incidence by 90% in a controlled environment (Sutton et al. 2000). Though in actuality, the reduction of inoculum from shredding or removal of the leaf litter may range from 50-65% due the variable topography and obstructions in true orchards (Vincent et al. 2004). In addition to reducing the efficacy of shredding the obstacles in true orchards make this practice time consuming and difficult (Vincent et al. 2004).

Alternatively spray applications to eradicate leaf litter may be an effective substitute to shredding and removal (Vincent et al. 2004). Chemical compounds such as mercury, in the past have shown promise in reducing primary inoculum and the number of fungicide treatments needed during the growing season (Keitt, 1930; Kadow and Hopperstead, 1941; Goldsworthy et al. 1949; Burchill, 1968). But due to their harmful effects to the environment and other organisms, many of the chemicals used in these studies have been taken off the market (Sutton et al. 2000). This led to research of other treatments using compounds that are more acceptable by today's safety standards (Sutton et al. 2000). From this research, urea spray applications were found to be the most effective at reducing overwintering inoculum (Sutton et al. 2000).

1.4.1 Urea

Urea is a synthetic crystalline solid containing 46% nitrogen (Overdahl et al. 1991). The high nitrogen content, low price, and ease of handling and storage has made urea an ideal source of nitrogen for agriculture over other dry nitrogen sources (Overdahl et al. 1991). However, because urea is synthesized it is not approved for organic agriculture, so the benefits of using urea can only be realized in conventional agriculture.

In conventional orchards, one use of urea in agriculture is to apply it to apple leaves to enhance litter decomposition by promoting growth of saprophytic microorganisms in the soil (Crosse et al. 1968; Beresford et al. 2000; Sutton et al. 2000). Enhanced decomposition of the leaves decreases substrate available to *V. inaequalis* needed to develop overwintering inoculum in the spring (Burchill, 1968). The high nitrogen content in urea also directly inhibits pseudothecia, or fruiting body, development of *V. inaequalis* (Crosse et al. 1968; Beresford et al. 2000). *V. inaequalis* requires depletion of all nitrogen before pseudothecia development can occur and excess nitrogen can delay or prevent this next step in the lifecycle (Ross, 1961). Urea applications have also been found to inhibit pseudothecia development by stimulating bacteria that is antagonistic to *V. inaequalis* (Crosse et al. 1968; Ross and Burchill, 1968; Meszka and Bielenin, 2006).

Urea is applied post-harvest before or after leaf-fall in early autumn, preventing the overwintering stage of *V. inaequalis* (Burchill, 1968; Vincent et al. 2004). Urea treatments made before leaf-fall can have adverse effects to the health of the tree (Rosenberger, 1996). Additional nitrogen may prolong the growth period of the tree, which delays the hardening of the tissues (Schupp et al. 2001). Prolonged growth exposes the plant tissue to lower temperatures, which can increase the cold damage to the buds and consequently affect the following season's yield (Wood and Beresford, 2000; Schupp et al. 2001). Alternatively, urea can be applied to the orchard floor after leaves have abscised from the tree (Rosenberger, 1996). Issues with post leaf-fall applications include inadequate coverage of the leaf surfaces and reduced treatment efficacy (Rosenberger, 1996). Furthermore, ground applications may also be insufficient in reducing inoculum if

the ground freezes soon after the urea treatment is made (Ciecierski et al. 1995). This is because low temperatures slow the urea-driven decomposition rate of the leaf litter (Ciecierski et al. 1995; Rosenberger, 1996; Sutton et al. 2000; Mac an tSoir et al. 2010).

These facts outline the limitations of urea applications, but they also reveal a potential area of research. Too few studies have looked at remedying the limitations of the ground applications, as they are safer regarding tree health. Furthermore, to date there has been no research to explore compounds that increase the coverage and penetration of urea from a sanitation standpoint. Such improvements would enhance urea-driven leaf litter decomposition and thus further reduce initial inoculum of *V. inaequalis*. Lastly, few studies have researched alternative compounds to substitute urea applications in organic orchards. Increasing urea treatment efficacy or exploring different compounds may result in a lower amount of primary inoculum in conventional and organic orchards, which may potentially reduce the amount of fungicides needed to control apple scab during the growing season (Sutton et al. 2000; Meszka and Bielenin, 2006).

1.5 Adjuvants

The efficacy of spray applications is dependent on even deposition of the active ingredient on the plant surface (Holloway, 1993; Wagner et al. 2003; Brink et al. 2004; Hunche et al. 2006; Balardin et al. 2010). But because of the hydrophobic nature of plant surfaces, spray deposits are generally inconsistent leading to varying levels of protection (Holloway, 1970; Steurbaut, 1993; Tang et al. 2008). Plant species with thick cuticles or hairy surfaces, like apples, can be difficult to wet resulting in a decrease the amount of active ingredient present on the surface (Holloway, 1970; Steurbaut, 1993; Gaskin et al. 2005). Spray efficacy can be improved by modifying the chemical and physical properties of the spray solution to better cover and adhere to hydrophobic surfaces (Abbott and Van Dyk 1990; Green, 2000; Gent et al. 2003; Ryckaert et al. 2007).

Adjuvants are “materials that are added to a tank mix to aid or modify the action of an agrichemical, or physical characteristics of the mixture” (American Society for Testing and Materials ASTM, 1999). Adjuvants have been found to increase the spread,

retention, penetration, and spray efficiency of materials being applied to plant surfaces (Percich and Nickelson, 1982; Steurbaut, 1993; Thompson et al. 1996; Gent et al. 2003). Improvement of these qualities can lower the rate of chemicals applied and extend the period between applications, without decreasing yield (Gent et al. 2003; Ryckaert et al. 2007; Rosenberger and Cox, 2012). This benefits the environment by reducing the chemical load and benefits the growers as it may decrease costs (Knoche, 1994; Gent et al. 2003; Gaskin et al. 2004; Balardin et al. 2010; Baseeth and Sebree, 2010). Adjuvants are typically priced at 3% of the cost of chemicals used in agriculture making the initial financial expense relatively low (Green, 2000; Hazen, 2000). The issue is there are many types of adjuvants available to growers and choosing the correct one can present a challenge, as different adjuvants can have distinctive effects on sprays.

1.5.1 Wetter-Spreaders

Wetter-spreaders are adjuvants that are classified as surfactants, or surface acting agents (Hazen, 2000). All surfactants such as wetter spreaders are considered adjuvants, but not all adjuvants can be considered surfactants. Surfactants are specific adjuvants that reduce the surface tension and lower the contact angle of water droplets, making the droplet lay flat (Hazen, 2000; Rosen and Kunjappu, 2012). This increases the spreading and coverage of chemicals on hard-to-wet surfaces (Hazen, 2000). Improved coverage and spread on a hard to wet surfaces by wetter-spreaders are due to the amphiphilic nature of surfactants (Cserháti, 1995; Hazen, 2000; Adamczak, 2013). Wetter-spreaders consist of molecules with a hydrophilic head and a hydrophobic tail, much like the phospholipid membrane of the cell (Adamczak, 2013). The hydrophobic portion interacts with the water molecules by breaking the intermolecular hydrogen bonds (Adamczak, 2013). Ultimately this reduces the surface tension of the water droplet and allows the droplet to flatten and cover more surface area (Fig. 1.6) (Hazen, 2000). The amphiphilic structure has a similar effect on the contact angle of the water droplet (Lee et al. 2008). On hydrophobic surfaces, like waxy leaves, the hydrophobic tails of the surfactant are

absorbed and a hydrophilic bilayer is created, which causes the water droplet's contact angle to decrease, increasing the spread over the waxy surface (Fig. 1.6) (Lee et al. 2008).

The addition of wetter-spreaders to spray mixtures is important as many leaves have waxy cuticles making leaf surfaces hydrophobic which reduces the coverage of water droplets (Wagner et al. 2003). This is because hydrophobic surfaces strengthen water molecules cohesive nature (Wagner et al. 2003). Strong cohesive properties and high surface tension due to intermolecular forces between the hydrogen and oxygen molecules of water causes the formation distinct water droplets (Ehlers and Goss, 2003; Wagner et al. 2003). Furthermore, water molecules also have a strong affinity for each other because of the partial positive charge on hydrogen and the partial negative charge on oxygen, creating a strong “pulling effect”, which causes additional beading (Ehlers and Goss, 2003). Like cohesion and intermolecular forces, “pulling effects” can be influenced by the nature of the surface water is upon, depending on if the surface is highly hydrophobic or rough (Vogler, 1998). In the case of apple leaves, their surface is not classified as a difficult to wet species, but trichomes that exist on apple leaves can greatly reduce spreading of the water droplet (Yu et al. 2009). The waxy cuticle of apple fruit has been classified as a difficult to wet surface (Gaskin et al. 2005). The overall combination of hairy leaves and waxy fruit can become obstacles in protective sprays that need optimal coverage to be effective (Wagner et al. 2003; Yu et al. 2009; Gaskin et al. 2005).

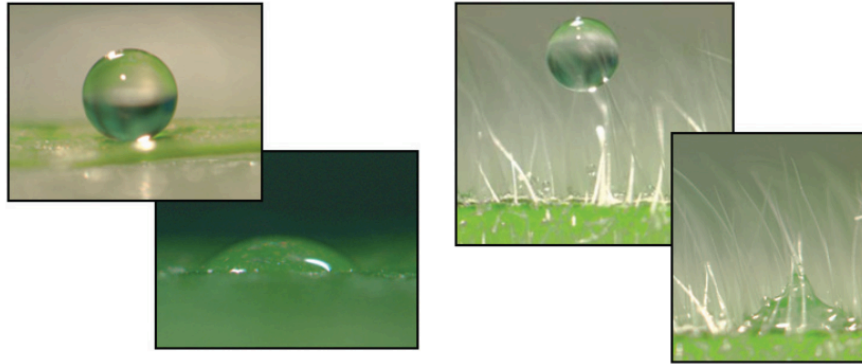


Figure 1.6: Effect of wetter-spreader adjuvants on water droplets. By reducing the surface tension and contact angle, adjuvants allow water droplets to flatten on waxy and hairy leaves (Whitford et al. 2014).

1.5.2 Stickers

The waxy cuticle of the leaf and fruit on the apple tree is an obstacle not only for coverage as previously mentioned, but can also cause other issues such as droplet run-off, bounce, or shatter off (Wagner et al. 2003). Stickers are adjuvants that help adhere droplets to the target surface and attempt to resist removal by rain and wind (Hazen, 2000). Adhesive qualities of this type of adjuvant are due to its viscous nature (Hazen, 2000). Stickers act like oils or thickening agents, containing anionic materials such as fatty acids or synthetic polymer latexes (Wasan et al. 1988). These substances alter the nature of the spray mixture and allow it to adhere to the leaf surface and resist wash-off, run-off, or evaporation and consequently may improve the performance of fungicides (Hazen, 2000).

Stickers can have multiple effects when coupled with fungicides such as an increased retention of the fungicide on the surface (Hazen, 2000), extension of the chemical activity (van Zyl, 2009), and decrease drift of the fungicide (Wasan et al. 1988). An addition of sticking agents to a tank mixes aids in deposition as well (Green and Beestman, 2007). Ultimately, the addition of sticker adjuvants can decrease fungicide runoff or fungicide displacement by wind or rain (Fig. 1.7) (Hazen, 2000; Balardin et al.

2010). This increases fungicide efficacy, while reducing residue accumulation in the soil (Reddy and Locke, 1996).

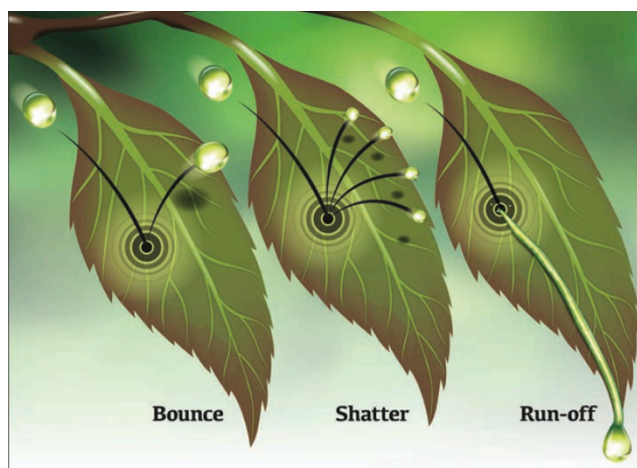


Figure 1.7: Effect of sticker adjuvants on spray applications. By increasing the viscosity of the water droplet these adjuvants can reduce the bounce, shatter and run-off of spray applications (Whitford et al. 2014).

1.5.3 Acidifiers

In some cases, it is not the plant that acts as the main deterrent to pesticide efficacy. In many cases it is the tank water, specifically water that is too alkaline (Whitford et al. 2012). Alkaline water degrades select pesticides making them less soluble or eliminating their treatment efficacy (Whitford et al. 2012). Acidifiers are adjuvants composed of weak acids that lower the pH of alkaline solutions (Baseeth and Sebree, 2010). This can reduce chemical degradation from alkaline hydrolysis (Bakke, 2007). Acidifiers can also increase the solubility of pesticides and induce penetration by neutralizing weakly acidic pesticides (Green and Beestman, 2007). Acidifying adjuvants would be especially useful to use with captan to prevent degradation by alkaline water (Gradis and Sutton, 1981; Frank et al. 1985).

1.5.4 Penetrants

The cuticle of leaves and fruit act as a barrier to help protect the plant from the environment (Whitford et al. 2012). While important to plant health waxy cuticles can decrease the efficacy of pesticide applications by reducing pesticide solubility and penetration (Whitford et al. 2012). Penetrant adjuvants induce the movement of chemicals into the plant tissue by disrupting or dissolving the waxy cuticle on the surface of the leaf (Manthey and Nalewaja, 1992) or inducing uptake through the stomata (Fig. 1.8) (Stevens et al. 1991; Gaskin, 1995; Gottwald et al. 1997; Hazen, 2000). Unfortunately, by increasing uptake of chemicals, some penetrants combined with certain fungicides could produce undesirable results such as phytotoxicity or increased disease severity (Tomlinson and Faithfull, 1979; Cowgill et al. 2013; Rosenberger, 2013). Phytotoxicity is the localized burning of cells resulting from inappropriate mixtures of adjuvants and agrichemicals (Steurbaut, 1993; Stock and Holloway, 1993; Cowgill et al. 2013). Phytotoxicity damage can cause major decreases in yield by blemishing fruit finish (Rosenberger, 2014).

In regards to apple disease management there are some chemicals such as captan, sulfur, and copper that are prone to phytotoxicity (Rosenberger, 2013). These products are biocidal, meaning they kill any cell upon uptake (Cowgill et al. 2013). If combined with adjuvants that have penetrant qualities, damage from phytotoxicity may be severe (Rosenberger, 2013). There are thousands of adjuvants available to growers and few experiments testing the combinations of penetrants with fungicides. This can lead to inappropriate combinations of fungicides and penetrants, resulting in yield loss due to adverse effects.

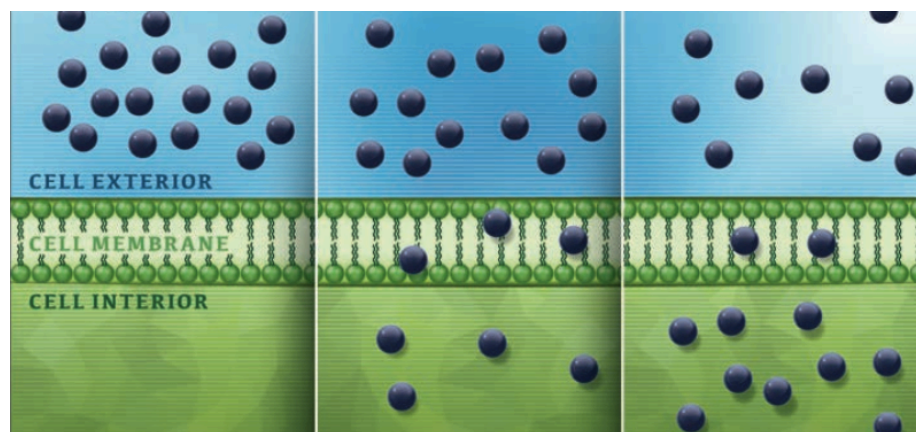


Figure 1.8: Effect of penetrating adjuvants on pesticides. Penetrants increase the fluidity of the cell membrane and induce movement of the chemical into the plant cells (Whitford et al. 2014).

1.6 Adjuvants and fungicides

The majority of research concerning adjuvant efficacy in field environments has concentrated on the improvement of herbicides (Foy, 1993; Steurbaut, 1993; Gent et al. 2003). Unlike herbicides, very few publications relate to combinations of fungicides and adjuvants and even fewer of these experiments are conducted in field environments (Steurbaut, 1993; Gent et al. 2003). In addition, the few field experiments that have been performed show inconsistent results of adjuvant combinations with fungicides, making practical use a risky endeavor for growers (Steurbaut, 1993).

The addition of nonionic alcohol-based surfactant, a nonionic polymer-based surfactant, and an experimental hard resin-based adjuvant were found to enhanced the over all efficacy of mancozeb after two applications in the field by increasing the control of brown spot of wild rice (Percich and Nickelson, 1982). Adjuvants, particularly a non-ionic alcohol-based surfactant, a nonionic oil-based surfactant, and an anionic oil-based surfactant alone were comparable to the fungicide azoxystrobin in managing white rust on spinach (Irish et al. 2002). Furthermore, when combined with azoxystrobin, these adjuvants plus a cationic glyceride-based adjuvant and another nonionic alcohol-based

surfactant enhanced coverage of the fungicide and further decreased disease incidence (Irish et al. 2002). The authors noted that more research on adjuvants was needed to determine the volumes and frequency of application needed for adequate disease control and the economic risk/benefit of using adjuvants (Irish et al. 2002). A polymer-based nonionic and anionic surfactants have shown the potential to decrease foliar fungal diseases by increasing the retention of fungicides such as propaconazole (Ryckaert et al. 2007). Lastly, a nonionic alcohol-based surfactant with penetrant qualities enhanced the control of *Phytophthora collar rot* on apple trees by increasing the penetration of the fungicide Ridomil Gold 4EC (47.6% mefenoxam) into the bark (Beckerman and Deford, 2007).

These studies confirmed that the conceptual claims of adjuvants had practical merit, but other studies were performed that showed contrary results. The management of coffee rust with the fungicide parazate was not enhanced by the addition of several resin or latex-based surfactants with sticker qualities (Valdez et al. 1959). While another study found that neither alcohol or oil-based sticker adjuvants significantly improved fungicide retention after rainfall (Ogawa et al. 1977). To further demonstrate the contrast in adjuvant performance between controlled and field studies, Gent et al. (2003) directly compared adjuvants in and out of the laboratory. In controlled conditions, two organosilicone adjuvants significantly increased the coverage and absorption of water droplets, while a nonionic latex-based surfactant and an oil-based adjuvant only increased absorption (Gent et al. 2003). All other adjuvants, including a nonionic resin-based surfactant did not significantly increase either measure (Gent et al. 2003). In the field study using the same adjuvants, it was found that only the nonionic resin-based surfactant, the nonionic latex-based surfactant, and one of the organosilicone adjuvants increased disease control, and only when disease pressure was high. The authors hypothesized that these varied results in disease management were highly dependent on the plant species, pathogen biology, and disease pressure (Gent et al. 2003).

The inconsistent results of adjuvants improvement of fungicides in field studies could be attributed to factors that also affect the productivity of other agrichemicals in the field (Sturbaut, 1993; Gent et al. 2003). These factors include harsher environments

such as wind and rain that can increase wash-off (Ragsdale, 1991), increased canopy density that can decrease deposition (Ellis et al. 2004), low disease pressures, and increased complexity of plant-pathogen-environment interactions (Steurbaut, 1993; Gent et al. 2003). So thorough knowledge of the activity of the fungicide and how it interacts with the fungus is needed to understand how the addition of adjuvants would affect the efficiency of fungicides (Steurbaut, 1993).

1.6.1 Phytotoxicity

Appropriate combinations of adjuvants and fungicides may improve disease management by reducing the rate used (Steurbaut, 1993; Gent et al. 2003; Beckerman and Deford, 2007). Alternatively, if inappropriate mixtures are used, growers can run the risk of yield reduction due to disease or phytotoxicity (Steurbaut, 1993; Cowgill et al. 2013). Phytotoxicity is the localized burning of plant tissues resulting in cellular death (Stock and Holloway, 1993). It can cause blemishes that affect the fruit and the leaf, decreasing the quality and value of the apple (Fig. 1.9) (Cowgill et al. 2013). Phytotoxicity can also negatively affect the activity of the fungicide and increase the plant's vulnerability to further infection (Steurbaut, 1993; Stock and Holloway, 1993). Environmental factors including temperature and humidity can affect phytotoxicity (Cowgill et al. 2013). For example, if temperatures exceed 85°F and humidity is higher than 70%, the cuticle permeability increases and there is a greater likelihood of burning resulting from sprays (Crassweller, 2011; Cowgill et al. 2013). Alternatively, cool temperatures can be just as damaging by increasing the solubility of chemicals like copper, resulting in phytotoxicity (Crassweller, 2011). Cooler temperatures can also slow the drying time of fungicides on the plant surface, which increases uptake and can cause phytotoxicity (Zabkiewicz et al. 1988). Therefore, growers should be cautious in applying sprays during cloud cover as the lower temperature and increased relative humidity could result in an environment conducive to phytotoxicity.

In general, most adjuvants have been found to be chemically inert (Parr and Norman, 1965), though some have been observed to cause phytotoxicity in certain cases

due to inducing movement of fungicides into the plant (Furmidge, 1959; Gottwald et al. 1997). In addition to penetrants, wetter-spreaders comprised of carbon-chained alcohols of 7-10 carbons in length have been found to induce movement of chemicals into the leaf by increasing the fluidity of the cuticle (Schonherr, 1993; Stock and Holloway, 1993; Hess and Foy, 2000).

The potential risk caused by the uncertainty of adjuvant and fungicide combinations can pose problems for growers in selecting combinations to use in field applications (Sturbaut, 1993). This issue was discussed by Rosenberger (2013) in a publication that highlighted captan's potential for phytotoxicity. As described above captan is a very effective fungicide in regards to apple scab and bitter rot control (Rosenberger, 2013). But if captan penetrates a cell wall it is very toxic to the cell and will cause cell death (Rosenberger, 2013). This could be an issue if the fungicide was paired with adjuvants or with other fungicides that have adjuvants or oils in their formulations that promote cell penetration (Rosenberger, 2013). For example, when captan is combined with the fungicide Fontelis, which has oils in its formulation that induce penetration, this results in phytotoxicity (Rosenberger, 2013). Phytotoxicity can have drastic economic impacts, as the effects are not observed until after the application is made, so a grower could spray an entire field without realizing the damage until too late (Gent et al. 2003).



Figure 1.9: Phytotoxicity burns on apple leaves and fruit (photos courtesy of Dr. Janna Beckerman).

1.7 Adjuvants and sanitation

There have been several studies addressing the significance of sanitation in apple production (Beresford et al. 2000; Sutton et al. 2000; Mac an tSoir et al. 2010), but few publications have studied the combination of adjuvants and urea to improve leaf litter decomposition. The addition of adjuvants has the potential increase the coverage, retention, and penetration of urea applications. An organosilicone-based surfactant improved the coverage of urea and induced stomatal infiltration, increasing the nitrogen content in the leaves by 15% (Leece and Dirou, 1977). From a sanitation standpoint, the increased nitrogen in the leaves could increase leaf decomposition, reduce inoculum development, and thereby reduce disease. In another study, anionic, ionic and cationic surfactants reduced *V. inaequalis* ascospore dose by 95% (Burchill and Swait, 1977). In this study, urea was not used in conjunction with the surfactants, so their reduction of inoculum may be attributed to the increase in leaf decomposition (Burchill and Swait, 1977).

These studies suggest that the addition of certain adjuvants can work synergistically to increase urea-driven decomposition of the leaves (Burchill and Swait, 1977). This is important as urea alone may have insufficient coverage and penetration into leaves (Rosenberger, 1996). Therefore, the incorporation of adjuvants with urea can increase the efficacy of sprays in which and thus further reduce the leaf litter and primary inoculum. Decreasing the inoculum further may effectively reduce the amount of fungicide sprays needed for sufficient control during the growing season.

1.8 Research objectives

The objective of this research was to test the effect of various adjuvants when combined with captan and urea in order to improve the management of *V. inaequalis* and *Colletotrichum* spp.. We examine how the addition of adjuvants to captan during season and to urea post-harvest could improve management of common apple diseases. We

hypothesize that the incorporation adjuvants may reduce the rate of fungicides, such as captan, needed throughout the growing season without affecting yield.

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CHAPTER 2. INCORPORATING ADJUVANTS WITH CAPTAN TO MANAGE COMMON APPLE DISEASES

2.1 Introduction

Apple scab, caused by the fungus *Venturia inaequalis* and bitter rot, caused by the fungi *Colletotrichum* spp. are economically important diseases of apple trees (*Malus domestica*) in regions that experience cool, wet springs and warm, wet summers (Sutton, 1990a,b). Fungicides are currently the most heavily relied upon method of disease management (Gianessi and Reigner, 2005). Beginning in the 1940's, the most effective fungicides used in apple disease management were captan and mancozeb (Brandes, 1953; McHardy et al. 2001; Gianessi and Reigner, 2005). These fungicides are classified as protectants that must be applied prior spore germination, as they are only active on plant surfaces (Köller et al. 2005). Later, in the 1960's, fungicides were introduced that provided enhanced disease management (Köller et al. 2005; Beckerman et al. 2015). These included dodine (now known as Syllit), methyl benzimidazole carbamates (MBC), demethylation inhibitors (DMI), quinone outside inhibitors (QoI), and succinate dehydrogenase inhibitors (SDHI) (Szkolnik and Gilpatrick, 1969; Köller, 1997; McKay et al. 2011). Many of these products had curative qualities that allowed growers to apply fungicides post-infection (Köller et al. 2005; Beckerman et al. 2015). This “kick back” or ability to limit pathogen growth after penetration of the leaves or fruit differentiated the newer fungicides from the protectants (Köller et al. 2005). These newer fungicides also differed from certain protectants in their ability to control other important apple diseases

such as rust and powdery mildew (Köller et al. 2005). Ultimately these factors improved disease management and reduced the number of applications needed to control apple scab and bitter rot to grower satisfaction (Köller et al. 2005; Beckerman et al. 2015).

Although modern fungicides contribute to more effective disease management, their site-specific mode of action coupled with the extended and exclusive use of these fungicides resulted in the evolution of resistant strains of *V. inaequalis* (Kuck et al. 1995; Russell, 1995; Bartlett et al. 2002; Köller et al. 2005) and *Colletotrichum* spp. (Jones et al. 1996) within a couple years of their respective release. Inadequate control due to fungicide resistance led to the greater reliance on older protectant fungicides, such as captan (Richmond et al. 1967; Turechek, 2004).

Captan is a chloroalkyl thio fungicide, which disrupts multiple cellular division pathways of sensitive fungal strains (Richmond et al. 1967; Turechek, 2004; Rosenberger, 2013). Because of its multi-site inhibitor, a pathogen would have had to simultaneously develop multiple mutations for all blocked pathways to become resistant to captan, which is unlikely to occur (Brent and Hollomon, 2007; Rosenberger, 2013). This is why captan remains an important option in apple disease management.

Unfortunately, there are several drawbacks that reduce captan efficacy. When suspended in alkaline tank water ($\text{pH} > 7$), captan has a half-life of 20 minutes, meaning it could degrade significantly before applications are made (Gradis and Sutton, 1981; Frank et al. 1985). Captan also cannot persist on plant surfaces if greater than one inch of rain occurs within 24 hours after application, leaving the leaves and fruit unprotected during high infection periods (Smith and MacHardy, 1984). Lastly, the U.S. Environmental Protection Agency (U.S. EPA) has classified captan as a group B2 carcinogen, or a potential human carcinogen (EPA, 1999). Consequently, there are restrictions on captan applications that limit growers to 18 kg per season. Since the management of apple scab and bitter rot in high disease pressure years may require up to 14 applications, this limits growers to either lower rates of captan or fewer sprays. This often results in unacceptable levels of apple scab and bitter rot and can lead to significant yield loss (Rosenberger, 2009).

As such, captan is low-risk for development of resistant strains and remains a dependable product for control of common apple diseases (Köller et al. 2005). Therefore, research that focuses on improving the performance of captan applications is necessary. One potential solution for improving captan efficacy is utilizing adjuvants. Adjuvants are “materials that are added to a tank mix to aid or modify the action of an agrichemical, or physical characteristics of the mixture” (American Society for Testing and Materials ASTM, 1999). Adjuvants have been found to increase the spread, retention, penetration, and spray efficiency of materials being applied to plant surfaces (Percich and Nickelson, 1982; Steurbaut, 1993; Thompson et al. 1996; Gent et al. 2003). Additionally, adjuvants may also correct issues with mixing and application by affecting spray drift, the pH, or the foaming of the tank water (Bakke, 2007).

The majority of research concerning adjuvants in field environments has concentrated on the improvement of herbicide activity (Foy, 1993; Steurbaut, 1993; Gent et al. 2003). Very few publications relate to combinations of fungicides and adjuvants, and even fewer published works involve experiments conducted in field environments (Steurbaut, 1993; Gent et al. 2003). This is problematic because inappropriate mixtures of fungicides and adjuvants could result in yield reduction due to phytotoxicity (Steurbaut, 1993; Cowgill et al. 2013). Phytotoxicity is the localized burning of plant tissues resulting in blemishes that decrease the quality and value of the apple (Cowgill et al. 2013). Phytotoxicity occurs when certain adjuvants, such as penetrants and some surfactants, cause the uptake of toxic chemicals into the plant (Coupland et al., 1989; Gaskin, 1995; Cowgill et al. 2013). Captan is biocidal or toxic to any cell it enters, if combined with adjuvants that increase uptake there is a high potential for phytotoxicity (Cowgill et al. 2013).

Although there are many adjuvants available, growers are reluctant to use them in conjunction with captan, as their impact on efficacy and risk of phytotoxicity remains uncertain. Identification of compatible adjuvant and captan combinations may contribute to improved management of apple scab and bitter rot, providing more options for growers confronted with fungicide resistance issues.

2.2 Materials and Methods

Adjuvants were tested for their ability to improve captan's control of apple scab and bitter rot throughout the growing season (Table 2.1). Concentrations of all adjuvants were based on recommended rates taken from the commercial label. The concentration of captan (Captan 80WDG; Arysta LifeScience, Cary, NC) used was based on the lowest recommended label rate (2.8 kg/ha).

2.2.1 Field tests

In the summers of 2013, 2014, and 2015, studies were conducted to evaluate the effect of adjuvants when combined with captan to manage apple scab and bitter rot throughout the growing season. These experiments were executed in field plots at Meig's Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN. The treatment blocks were set up in a randomized complete block design in 16-yr-old plots of Golden Delicious and Honeycrisp apple trees on M.7 rootstocks planted in Toronto-Millbrook complex soil. Golden Delicious trees were planted 6.1-m apart with 6.1-m between each row in a 0.5-ha field containing 130 trees. Honeycrisp trees were planted 3-m apart with 3.7-m between each row in a 0.2-ha field containing 104 trees. All trees were infected with naturally occurring inoculum of each disease.

2.2.2 Experimental design

In 2013 the design in the Honeycrisp plot was set up in a completely randomized design of 10 treatments containing uneven replications of 4 trees. In 2014 and 2015 the trees in the Honeycrisp plot design were set up as a randomized complete block design of 10 treatments containing 3 replications of 3 trees. In 2013, 2014, and 2015 the design of the trees in the Golden Delicious plot was a randomized complete block design of 10 treatments containing 3 replications of 4 trees.

2.2.3 Treatment applications

All adjuvants were used in combination with captan and applied to trees with an air blast sprayer (John Bean DP10P 50P) with John Bean nozzles with ceramic whirl plates and discs on flip over bodies that produced a solid cone spray pattern. Treatments were applied at 8.9 kph and 1,724-2,068 kPa (935.2 l/ha rate) every 10-14 days beginning at 24 May in 2013, 30 April in 2014, 23 April in 2015, and ending at harvest. In 2013, Honeycrisp trees received 8 applications made on 24 May; 4, 17, 28 June; 11, 23 July; 6, and 16 August. Golden Delicious trees received 11 applications made on 24 May; 4, 17, 28 June; 11, 23 July; 6, 16, 30 August; 13, and 25 September. In 2014, Honeycrisp trees received 10 applications made on 30 April; 12, 22 May; 5, 18 June; 2, 14, 25 July; 8, and 19 August. Golden Delicious trees received 12 applications made on 30 April; 12, 22 May; 5, 18 June; 2, 14, 25 July; 8, 19 August; 2 and 16 September. In 2015, Honeycrisp and Golden Delicious trees both received 11 applications made on 23 April; 6, 19, 29 May; 9, 23 June; 2, 16, 30 July; 11, 24 August. After 24 August 2015, no further applications were made to Golden Delicious because the trees did not produce apples. In 2015, water sensitive spray cards (Syngenta, Greensboro, NC) were placed in a single tree for each treatment approximately 1.5-m from the ground before treatment applications were made to assess adjuvant effect on water droplets.

2.2.4 Sampling and disease rating

For all years, apples from only the inner trees of each replicate were scored to avoid fruit that may have been exposed to overlapping spray of treatments that could result in possible biased phytotoxicity scoring. In all years, fruit of each cultivar were scored for incidence and severity of apple scab, bitter rot, and phytotoxicity.

In 2013, Honeycrisp trees were scored on 22 August by arbitrarily selecting five branches from the two inner trees in each replicate. Disease and phytotoxicity incidence

were recorded by counting the total number of infected apples. Golden Delicious trees were scored on 30 September by arbitrarily selecting 5 branches from the two inner trees in each replicate. Disease and phytotoxicity incidence was scored by recording the number of infected/blemished apples out of the total number of fruit on the branch.

In 2014 and 2015, Honeycrisp trees were scored on 24 August and 8 September respectively, by arbitrarily selecting 10 branches from the inner tree in each replicate. In 2014, Golden Delicious trees were scored on 23 September, by arbitrarily selecting 5 branches from the two inner trees in each replicate. Incidence was scored by recording the number of infected/blemished apples out of the total number of fruit on the branch. Severity was measured the same between all the years by estimating the surface area of the infection/blemish using the template published by Croxall et al. 1952. In 2015, no scores for Golden Delicious apples were recorded as no apples were produced due to biennial bearing.

2.2.5 Statistical analysis

For all years, disease and phytotoxicity incidence and severity scores were averaged within treatments, subject to arcsin transformation and analyzed with a one-way ANOVA using a generalized linear model procedure (PROC GLM) of SAS v9.3 (SAS Institute). Means were then separated according to Fisher's protected LSD test at $P=0.05$, using SAS v9.3 (SAS Institute). In 2014, apple scab severity data for Honeycrisp were not included in statistical analysis due to an abundance of zero variances. Additionally, for all years there was no phytotoxicity for "captan only" and "no treatment" treatments in either cultivar, so they were not included in statistical analysis in order for data to meet the homogeneity of variance assumption.

2.3 Results

Disease incidence varied among all three experimental seasons. In all years, conditions were favorable for apple scab infection. However, in 2013, a timely application of dodine in the early season fungicide regimen resulted in low apple scab incidence. Also, in 2015, due to biennial bearing, no Golden Delicious apples were produced. Environmental conditions were less conducive for bitter rot infection in 2013 than in 2014 and 2015. Cultivar susceptibility to each disease differed as well. Golden Delicious apples were more susceptible to apple scab infection and less susceptible to bitter rot infection, while Honeycrisp apples were more susceptible to bitter rot and less susceptible to apple scab. Temperature and rainfall among the years of the experiment varied as well, the highest temperatures and lowest amount of rainfall occurred in 2013 (Fig. 2.1A) and the lowest temperatures and highest amount of rainfall occurred in 2015 (Fig. 2.1C).

2.3.1 2013 trial results

For Golden Delicious no adjuvants combined with captan reduced apple scab incidence (Fig. 2.2A). In terms of scab severity, most treatments had no effect compared to the untreated control but adjuvants Bond Max and Latron B-1956 when combined with captan had significantly higher scab severity (Fig. 2.2B). No adjuvants combined with captan reduced the incidence of bitter rot compared to captan alone (Fig. 2.2C). Bond Max plus captan and Latron B-1956 plus captan decreased severity of bitter rot compared to captan alone (Fig 2.2D). Furthermore, Attach alone, Attach plus captan, and Latron B-1965 plus captan had significantly higher incidences of phytotoxicity (Fig. 2.2). For Honeycrisp apples, there was no reduction in scab incidence or severity or bitter rot incidence among the captan and adjuvant plus captan treatments (Fig. 2.3A,B,C). Attach alone and Bond Max plus captan sustained increased levels of bitter rot severity (Fig.

2.3D). Bond Max plus captan, Attach plus captan, and Latron B-1956 plus captan had significantly higher severity of phytotoxicity (Table A1, see appendix).

2.3.2 2014 trial results

For Golden Delicious, Li700 plus captan, Bond Max plus captan, and Latron B-1956 plus captan reduced incidence and severity of apple scab (Fig. 2.4A,B). No addition of adjuvants to captan reduced bitter rot incidence or severity compared to captan alone (Fig. 2.4C,D). Li700 plus captan, Bond Max plus captan, and Latron B-1956 plus captan had significantly higher incidence and severity of phytotoxicity (Fig. 2.4; Table A2, see appendix). For Honeycrisp, no adjuvant and captan combinations significantly reduced apple scab or bitter rot incidence or severity compared to captan alone (Fig. 2.5). Bond Max plus captan significantly increased the incidence and severity of phytotoxicity, and Latron B-1956 plus captan had significantly higher incidence of phytotoxicity (Fig. 2.5; Table A2, see appendix).

2.3.3 2015 trial results

In Honeycrisp apples, there was no significant reduction of apple scab incidence or severity in any adjuvant and captan combinations (Fig. 2.6A,B). Li700 plus captan and Bond Max plus captan significantly reduced the incidence of bitter rot compared to captan alone (Fig. 2.6C). All adjuvant and captan combinations significantly reduced the severity of bitter rot compared to captan alone (Fig. 2.6D). Attach plus captan had a significantly higher incidence of phytotoxicity while Bond Max plus captan had a significantly higher severity of phytotoxicity (Fig. 2.6; Table A3, see appendix).

2.4 Discussion

In this study we identified that certain adjuvants have the potential to improve captan management of apple scab and bitter rot when environmental conditions were favorable for disease and the host cultivar was susceptible. Adjuvants applied alone were not comparable to captan, suggesting that the enhanced captan performance was potentially due incorporation of adjuvants with the fungicide.

Visual inspection of water sensitive spray cards suggests that Li700 and Latron B-1956 increased the coverage of the water droplet (Fig. 2.7). Using ASSESS 2.0 (Lamari, 2008), we found that a spray containing only captan covered 27% of the the water sensitive card surface, where Li700 plus captan covered 92% and Latron B-1956 plus captan covered 71% of the card surface (Fig. 2.7). Both adjuvants are composed of chemical components that increase the spread of water droplets by reducing the surface tension, which resulted in increased coverage of captan (Hazen, 2000). An even deposition of the active ingredients on plant surfaces is among the most important factors determining the efficacy of fungicide applications (Holloway, 1993; Wagner et al. 2003; Balardin et al. 2010). But even or complete deposition of sprays rarely occurs on apple fruit as they are moderately difficult to wet because of their waxy cuticles (Gaskin et al. 2005). This becomes an issue as thorough coverage is especially important when applying captan, as it is a protectant, requiring nearly complete coverage of the plant surface in order to obtain suitable disease management (Köller et al. 2005). Both Li700 and Latron B-1956 improved captan coverage but only the addition of Li700 consistently resulted in less disease. This may be due to the differences in the chemical composition of Li700 and Latron B-1956. Where the polyoxyethylene in Li700 reduces surface tension much like many surfactants that have long chain of hydrophobic carbons (Hazen, 2000), the modified phthalic molecules in Latron B-1956 due to their compacted hydrophobic portion may act more like organosilicone surfactants, or super-spreaders (Ananthpadmanabhan et al. 1990). This means that the addition of Latron B-1956 may cause excessive spreading, resulting in unexpected run-off of fungicides resulting in decreased efficacy (Holloway, 1994; Hess, 1999). Another factor that could have resulted

in differences between Latron B-1956 and Li700 is that the latter is an acidifier. The addition of Li700 would reduce the pH of the basic tank water and possibly prevent alkaline hydrolysis of captan which could prevent the breakdown of the active ingredient into less efficacious compounds (Wofle et al. 1976; Somerville et al. 2012). The addition of Li700 to captan could have implications to growers in places like Indiana, where water has a pH of 8 and higher.

Bond Max moderately increased the coverage of captan, covering 47% of the water sensitive spray card and Attach did not increase the coverage of the water droplet, covering 27% of the spray card (Fig. 2.7). Both adjuvants have surfactant qualities, likely due to the alcohol ethoxylated and oxyethylene present in their chemistry (Hazen, 2000). These adjuvants also have sticking qualities due to the latex (Bond Max) and pinene (Attach) found in their chemistry (Hazen, 2000). These compounds may counteract the spreading caused by the surfactants and result in reduced droplet coverage. Alternatively, increased “stickiness” of the water droplets could have improved disease management by increasing the retention of captan (Hazen, 2000; Gaskin et al. 2014). Improved retention of captan is important as apple fruit retain less fungicides due to run-off and because a certain amount of captan residue is required to remain on the plant surface to ensure good disease management for the entire interval between applications (Frank et al. 1985; Gaskin et al. 2005). Though both adjuvants may have improved the retention of captan, only Bond Max improved disease management likely because latex-based stickers improve retention significantly better than pinene-based stickers (Gaskin and Steele, 2009; Gaskin et al. 2014).

While the addition of certain adjuvants improved disease management on susceptible cultivars in years with high disease incidence, there were large differences among years in this study. Adjuvant effects on incidence of apple scab and bitter rot were highly dependent upon conducive environment conditions for infection and susceptibility of the cultivar. A favorable environment and susceptible host may have attributed to a high disease incidence, which allowed for more detectable separation of the treatments. This result supports a previous study where the differences between adjuvant efficacy in laboratory and field experiments showed that there were differences in adjuvants between

controlled and field environments, with the latter showing fewer adjuvant effects (Gent et al. 2003). The authors hypothesized that lack of significance in the field experiments was due to a low disease incidence (Gent et al. 2003). Furthermore, there are also several other factors that could reduce the efficacy of adjuvants in the field including harsher environments caused by wind and rain that can increase wash-off and increased canopy density that can decrease deposition (Steurbaut, 1993). Since in this study adjuvants that increased the retention and deposition of captan improved disease management the most, these are very important factors to consider when using adjuvants in field environments.

The variation in adjuvant effects on disease management among the years highlights the difficulty in making practical interpretations from this research. In many cases, growers are reluctant to use adjuvants because they are unaware of how they will contribute to disease management or if the addition of adjuvants will result in phytotoxicity. Significant incidence phytotoxicity was observed when Li700 or Bond Max was combined with captan. This result was expected as Li700 is classified as a penetrant, increasing penetration of spray chemicals into the plant cell (Hazen, 2000). Furthermore, studies have shown that Bond Max improves pesticide absorption in some plant species, potentially due to the long carbon-chained alcohols (Hess and Foy, 2000; Gent et al. 2003). Allowing captan to enter the plant cell and result in phytotoxicity.

To demonstrate the potential value of incorporating certain adjuvants with captan, we quantified the hypothetical benefits and costs of managing apple scab and bitter rot. We assumed that the potential gross value of fresh market apples in Indiana is \$22,163 ha⁻¹ (NASS USDA, 2014). That is, if an orchard had 0% incidence of disease the grower would earn \$22,163 ha⁻¹ for their apples. However, growers could lose up to 100% of yield due to apple scab and bitter rot infection, resulting in \$0. Using this concept, applying captan to manage apple scab on Golden Delicious in 2014 resulted in 36.9% disease incidence. This means that the grower would still have the potential to earn 63.1% of \$22,163 ha⁻¹ or \$13,983 ha⁻¹ (Fig 2.8). In 2015, captan alone resulted in 24.5% bitter rot incidence in Honeycrisp. This means the grower could potentially earn 75.5% of \$22,163 ha⁻¹ or \$16,733 ha⁻¹. Since this research addresses the improvement of captan

with adjuvants, the potential earnings of using adjuvants were compared with the potential earnings of using captan in that year.

Using this, we were able to calculate the theoretical savings and losses a grower could experience if certain adjuvants were combined with captan in management strategies. In 2014, the addition of Li700 or Bond Max to captan resulted in 11% or 18.2% incidence of scab in Golden Delicious. This corresponds to 89% or 81.8% of \$22,163 ha⁻¹ or potential earnings of \$19,726 ha⁻¹ or \$18,128 ha⁻¹. Compared to the potential earnings of using captan alone in 2014 (\$13,983 ha⁻¹) the addition of Li700 or Bond Max to captan could potentially have saved up to \$5,743 ha⁻¹ (\$19,726 - \$13,983) or \$4,146 ha⁻¹ (\$18,128 - \$13,983). In 2015, the addition of Li700 or Bond Max to captan resulted in 4% or 3.3%. This corresponds to 96% or 96.7% of \$22,163 ha⁻¹ or potential earnings of \$21,277 ha⁻¹ or \$21,432 ha⁻¹. Compared to the potential earning of applying captan alone in 2015 (\$16,733 ha⁻¹), the addition of Li700 or Bond Max to captan could potentially have saved a grower up to \$4,543 ha⁻¹ (\$21,277 - \$16,733) or \$4,699 ha⁻¹ (\$21,432 - \$16,733).

Though phytotoxicity incidence in our study was statistically significant in some years, the incidence of phytotoxicity overall may not be economically significant. In 2014, on Golden Delicious apples, the addition of Li700 or Bond Max to captan resulted in 4.8% or 4.7% incidence of phytotoxicity. This corresponds to potentially losing 4.8% or 4.7% of \$22,163 ha⁻¹ or \$1,064 or \$1,042 ha⁻¹ respectively. While in 2015 on Honeycrisp apples, the addition of Li700 or Bond Max to captan resulted in 0.4% or 1.4% incidence of phytotoxicity. Therefore, a grower could potentially lose 0.4% or 1.4% of \$22,163 ha⁻¹ or \$89 or \$311 ha⁻¹ respectively.

Taking into account losses due to phytotoxicity and the costs of applying Li700 or Bond Max (\$12 or \$20 ha⁻¹, respectively), the overall benefit of using Li700 or Bond Max could potentially have been up to \$4,442-\$4,667 or \$3,481-\$4,388 ha⁻¹ depending on the susceptibility of the cultivar, environmental favorability, and the target pathogen. These values assumed that all apples were equally valued, which is not the case as certain cultivars are more highly valued than others and may have higher impacts on the benefit of adjuvants.

Overall results showed that certain adjuvants have the potential to improve captan disease management compared to captan alone when conditions are favourable for disease and the host is susceptible. With the addition of these adjuvants this study achieved good management of diseases at reduced rates of captan on favourable disease years by increasing the coverage and retention of the water droplet, and lowering the pH of the tank water. Although the results varied yearly, this study highlights the potential of adjuvants to improve disease management, while emphasizing the need for more practical studies like this one to better understand the role of adjuvants and fungicides in field environments.

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2.5 Tables and Figures

Table 2.1: List of materials used in this study to manage apple scab and bitter rot.

Trade Name	Active Ingredients	Concentration per 379 L	Manufacturer	Classification
Captan 80WDG	Captan: N-Trichloromethylthio-4-cyclohexene-1,2-dicarboximide	1.1 kg	Arysta LifeScience	Multi-site fungicide
Li700	Phosphatidylcholine, methylacetic acid, and alkyl polyoxyethylene ether	0.30 L	Loveland Industries	Adjuvant: Acidifier, penetrant, drift reduction
Bond Max	Alcohol ethoxylate, 1,2-propanediol, and synthetic latex	0.44 L	Loveland Industries	Adjuvant: Spreader-sticker, deposition aid
Attach	Pinene (terpene) polymers, petrolatum, (p-Dodecylphenyl), omega-hydroxypoly (oxyethylene)	0.30 L	Loveland Industries	Adjuvant: Spreader-sticker
Latron B-1956	Modified phthalic glycerol alkyd resin	0.30 L	Simplot Grower Solutions LLC	Adjuvant: Spreader-sticker

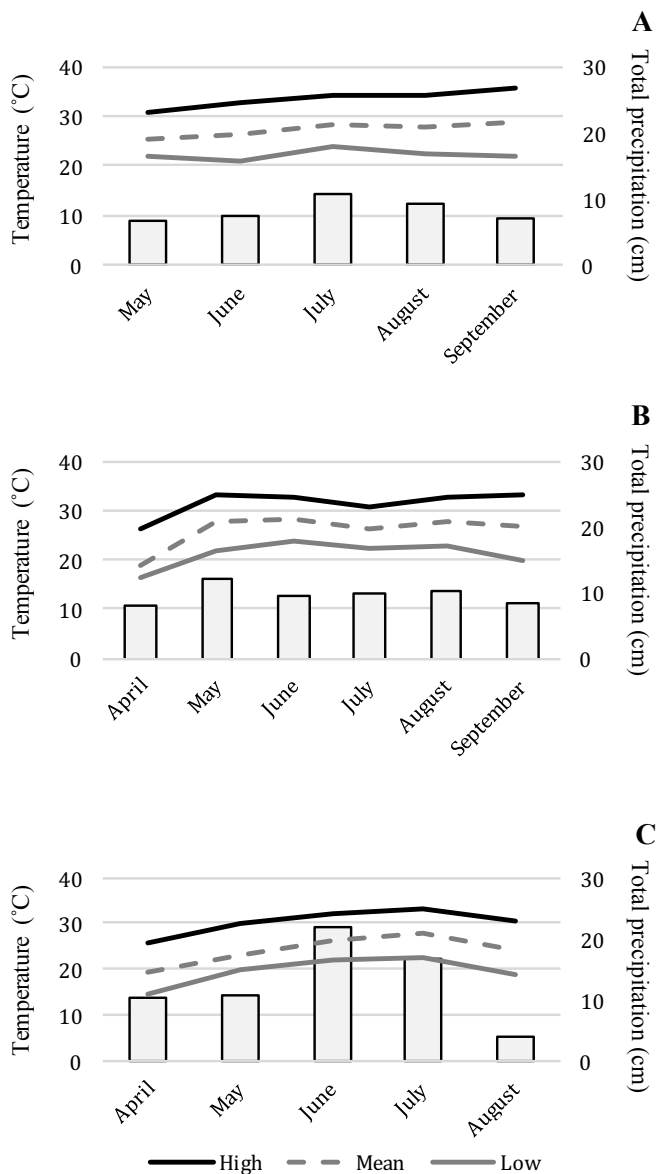


Figure 2.1: Average monthly high, mean, and low temperatures ($^{\circ}\text{C}$) and total monthly precipitation (cm) throughout the experiments conducted in **A**, 2013, **B**, 2014, and **C**, 2015. Weather data was collected by the Meig's Weather Station at the Throckmorton-Purdue Agricultural Center.

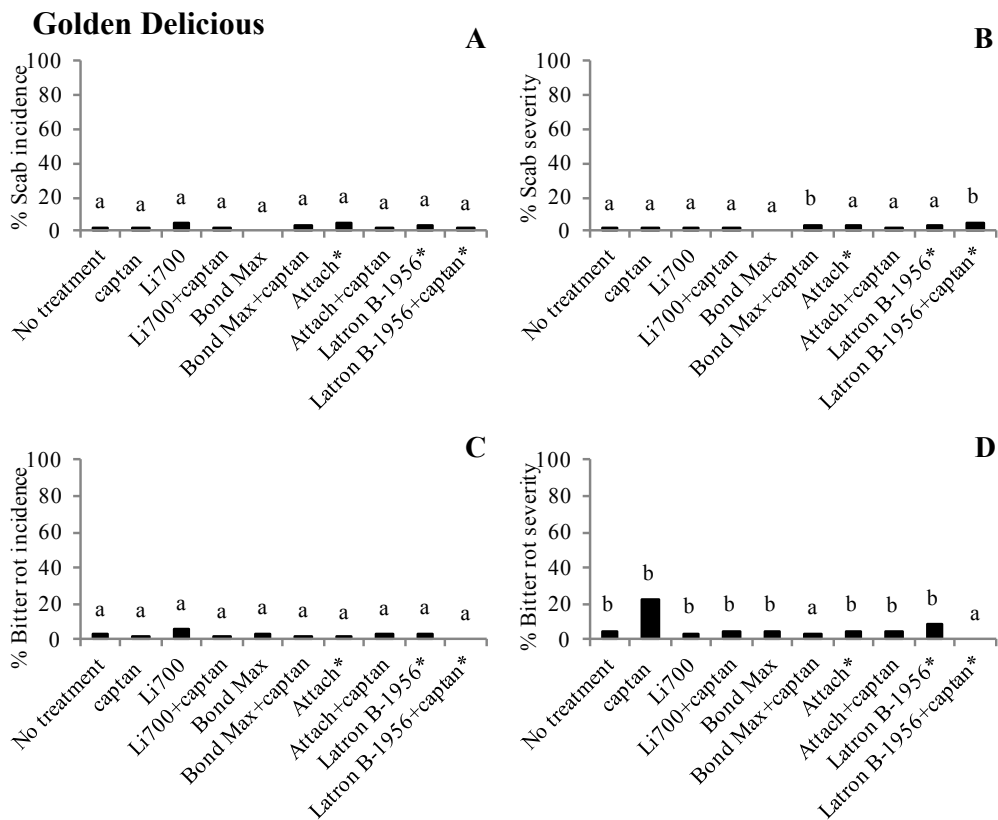


Figure 2.2: Effect of adjuvants on captan management of apple scab (*V. inaequalis*) **A**, incidence and **B**, severity and bitter rot (*Colletotrichum* spp.) **C**, incidence and **D**, severity on Golden Delicious apples at Meig's Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2013¹. "Captan" refers to Captan 80WDG.

*Treatment had significant incidence of phytotoxicity.

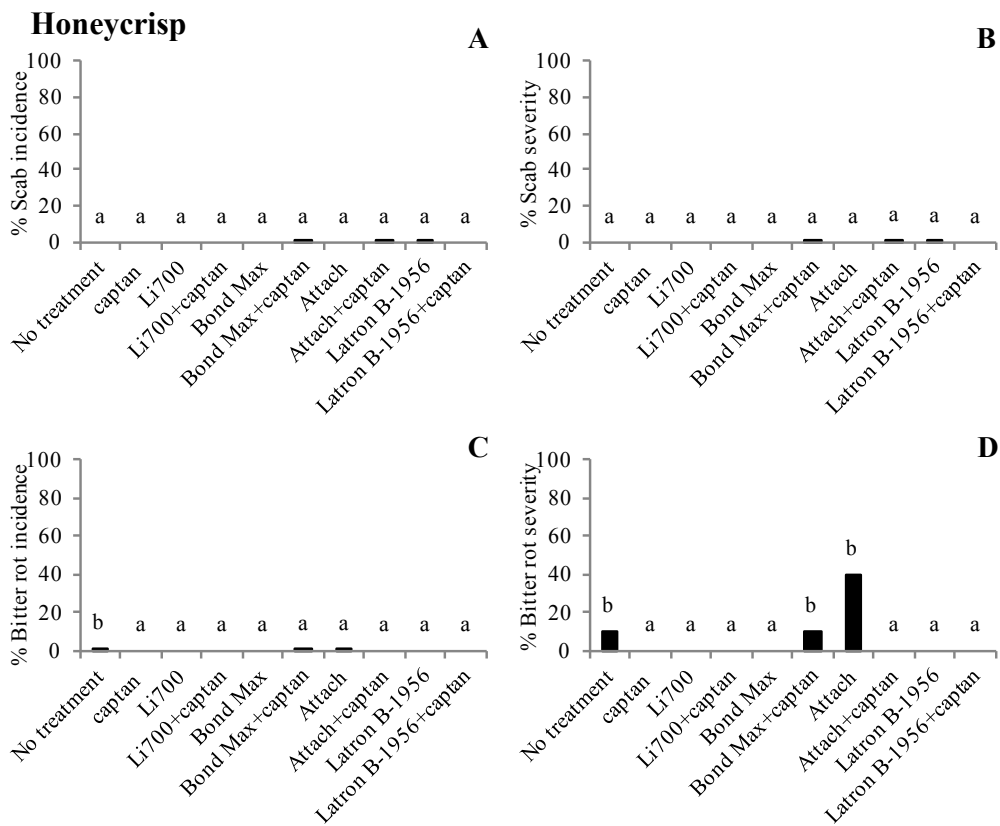


Figure 2.3: Effect of adjuvants on captan management of apple scab (*V. inaequalis*) **A**, incidence and **B**, severity and bitter rot (*Colletotrichum* spp.) **C**, incidence and **D**, severity on Honeycrisp apples at Meig’s Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2013¹. “Captan” refers to Captan 80WDG. *Treatment had significant incidence of phytotoxicity.

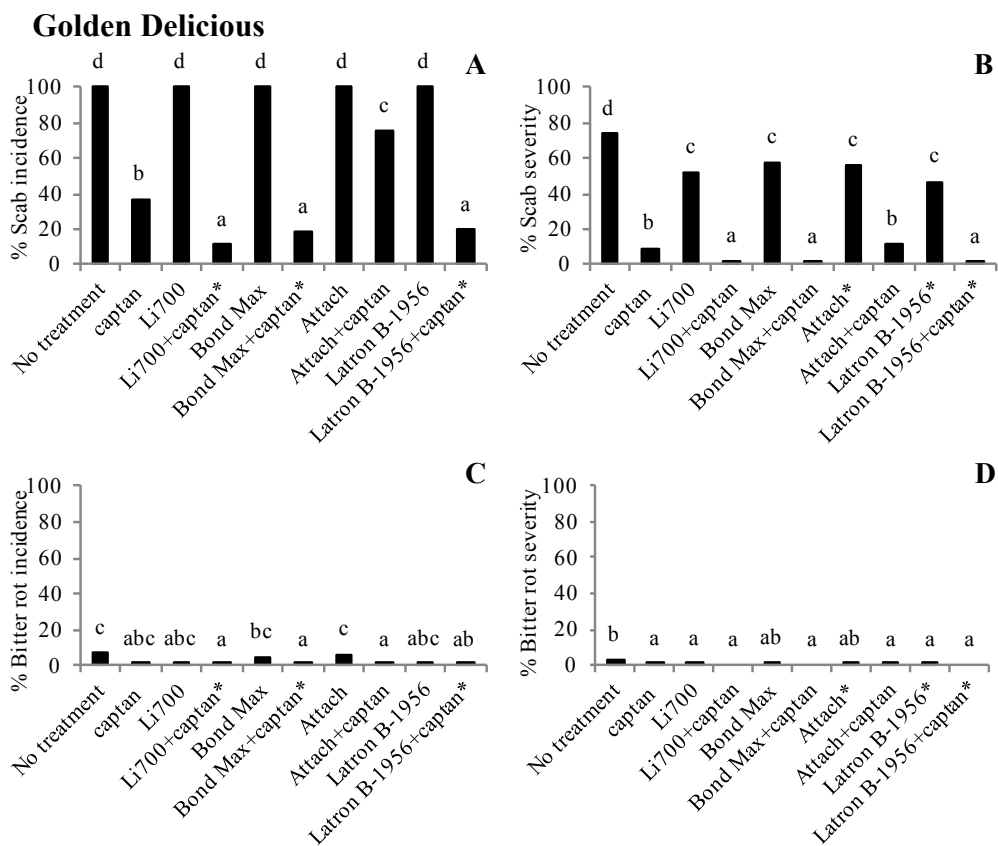


Figure 2.4: Effect of adjuvants on captan management of apple scab (*V. inaequalis*) **A**, incidence and **B**, severity and bitter rot (*Colletotrichum* spp.) **C**, incidence and **D**, severity on Golden Delicious apples at Meig's Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2014¹. "Captan" refers to Captan 80WDG. *Treatment had significant incidence of phytotoxicity.

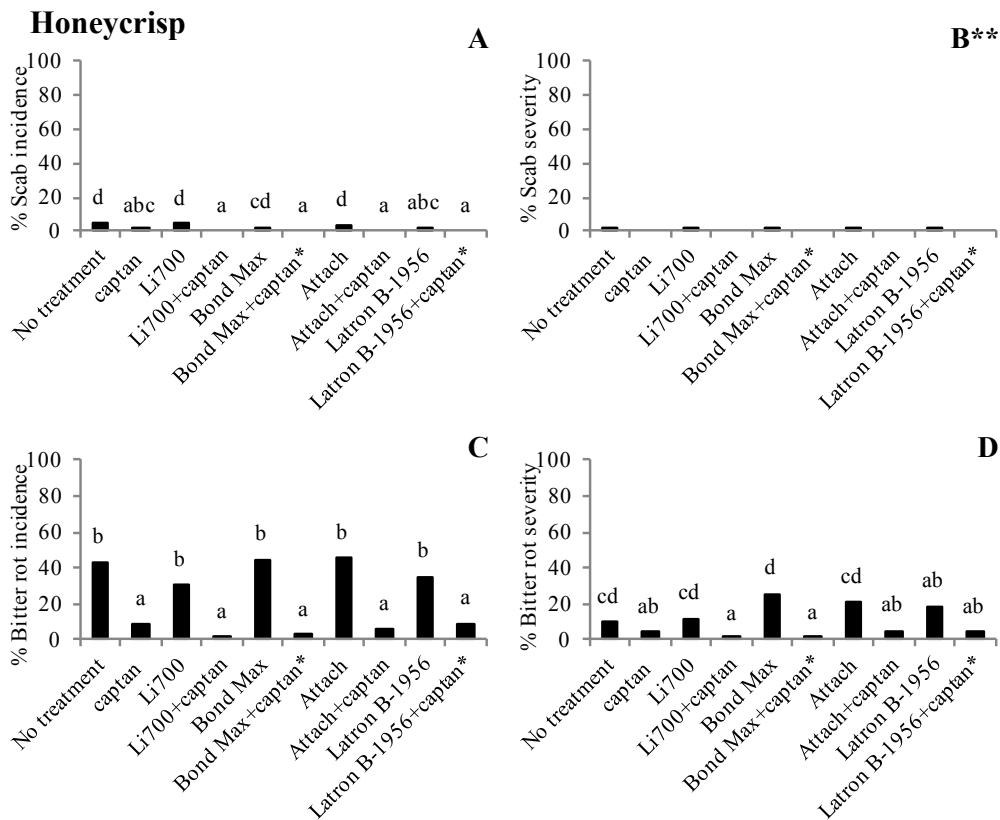


Figure 2.5: Effect of adjuvants on captan management of apple scab (*V. inaequalis*) **A**, incidence and **B**, severity and bitter rot (*Colletotrichum* spp.) **C**, incidence and **D**, severity on Honeycrisp apples at Meig’s Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2014¹. “Captan” refers to Captan 80WDG. *Treatment had significant incidence of phytotoxicity. ** Due an abundance of zero’s data was not statistically analyzed.

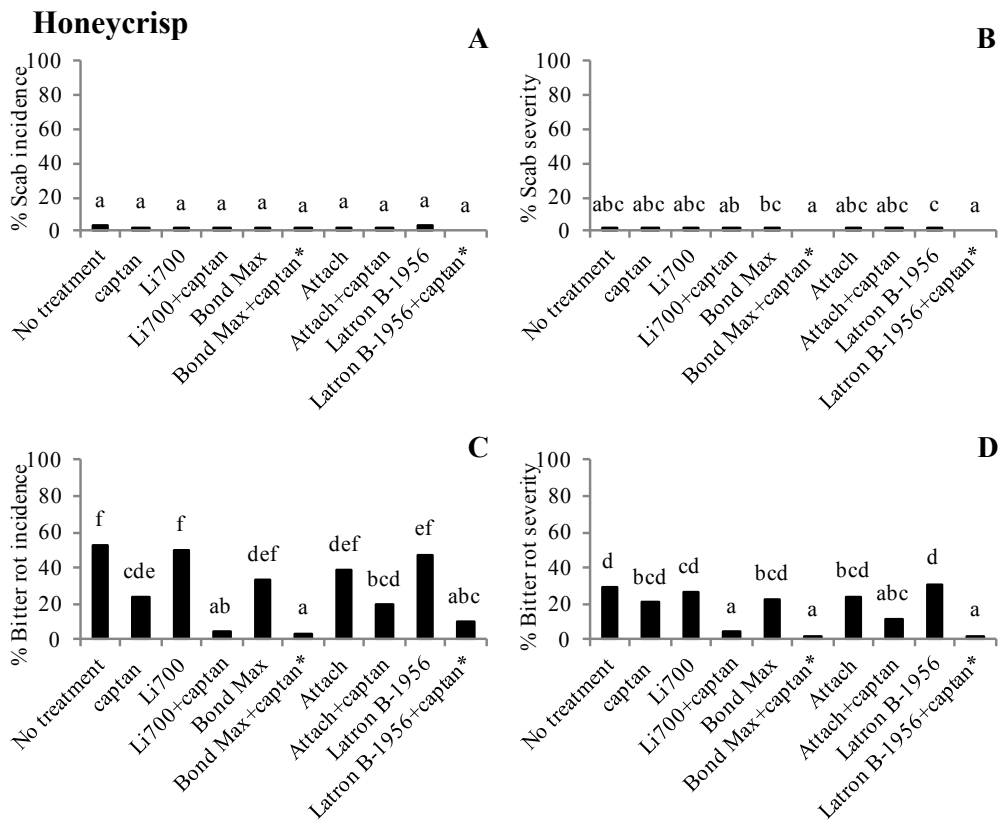


Figure 2.6: Effect of adjuvants on captan management of apple scab (*V. inaequalis*) **A**, incidence and **B**, severity and bitter rot (*Colletotrichum* spp.) **C**, incidence and **D**, severity on Honeycrisp apples at Meig’s Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2015¹. “Captan” refers to Captan 80WDG. *Treatment had significant incidence of phytotoxicity.

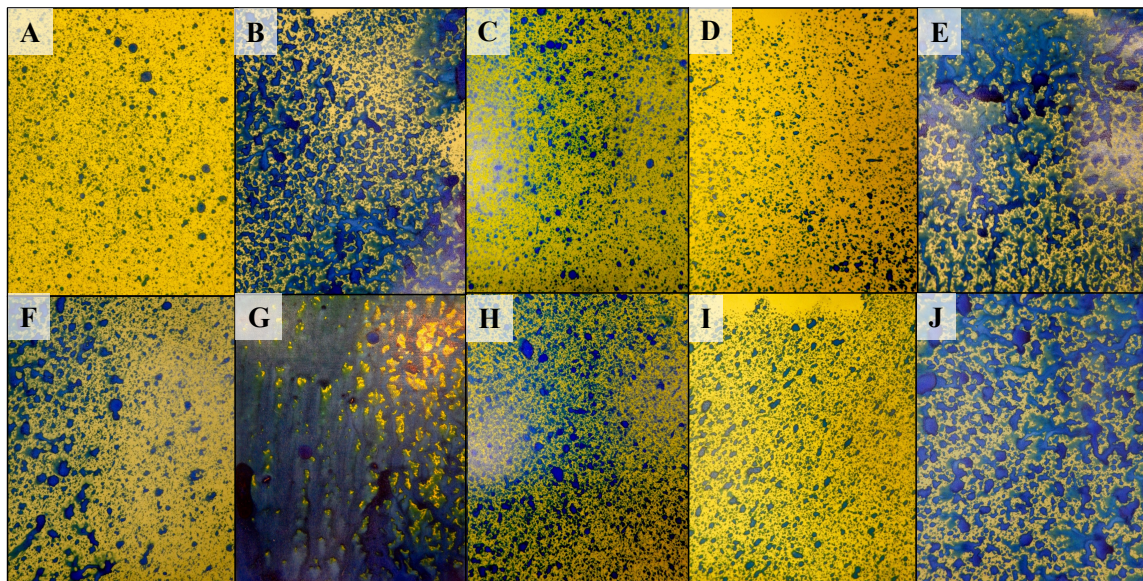


Figure 2.7: Water sensitive spray cards showing the effect of adjuvants on water droplets after various treatments were applied using an air blast sprayer. The blue colouration indicates water coming into contact with the card. Treatments are **A**, Control (H_2O) = 13% coverage; **B**, Li700 = 68% coverage; **C**, Bond Max = 27% coverage; **D**, Attach = 16% coverage; **E**, Latron B-1956 = 74% coverage; **F**, captan = 27% coverage; **G**, Li700 + captan = 92% coverage; **H**, Bond Max + captan = 47% coverage; **I**, Attach + captan = 27% coverage; **J**, Latron B-1956 + captan = 71% coverage. Droplet coverage was computed using image analysis software APS Assess (Lamari, 2008).

CHAPTER 3. INCORPORATING ADJUVANTS WITH UREA TO REDUCE THE INOCULUM OF *VENTURIA INAEQUALIS*.

3.1 Introduction

Apple scab, caused by the fungus *Venturia inaequalis* (Cke.) Wint., is one of the most important apple (*Malus domestica*) diseases in regions that experience cool, wet springs (Sutton, 1990a; MacHardy et al. 2001). Growers rely heavily on fungicides for disease management, often applying as many as 14 sprays per season (Sutton, 1990a; MacHardy, 1996; Beresford et al. 2000). Fungicides within several classes are registered for controlling apple scab, including dodine (now known as Syllit), methyl benzimidazole carbamates (MBC), demethylation inhibitors (DMI), quinone outside inhibitors (QoI), and succinate dehydrogenase inhibitors (SDHI) (Szkolnik and Gilpatrick, 1969; Köller, 1997; Köller et al. 2005; McKay et al. 2011). However, extended use of these fungicides has resulted in the development of resistant strains of *V. inaequalis* within a couple years of their respective releases (Köller et al. 2004). Inadequate control led to the greater reliance on older protectant fungicides, such as captan, that have low risk of developing pathogen resistance (Nicholson and Beckerman, 2008). Unfortunately, growers are restricted to ten applications of captan at a 4.5 kg/ha rate per season due to environmental and human health concerns related to captan use (Rosenberger, 2009). In wet years this provides insufficient management of apple scab, and can result in significant yield loss (Rosenberger, 2009).

Failures in disease management due to increasing fungicide resistance and restrictions has made the addition of cultural practices like sanitation necessary

(Beckerman et al. 2015). Sanitation focuses on removing the overwintering inoculum of *V. inaequalis* and has been shown to reduce the initial disease pressure and thereby the amount of chemicals needed for control during the growing season (Sutton et al. 2000; Meszka and Bielenin, 2006). A 20% reduction of disease incidence present in the spring could significantly limit inoculum present in the orchard (Rosenberger and Cox, 2012). This reduction could delay the epidemic of apple scab and potentially result in fewer sprays to control disease (Gadoury and MacHardy, 1986).

There have been many studies exploring the reduction of initial inoculum of *V. inaequalis* by implementing sanitation practices (Sutton et al. 2000). Since the pathogen overwinters in leaves on the orchard floor, practices removing the leaf litter by removal, shredding, chemical treatments, or urea applications are essential (Sutton et al. 2000; Vincent et al. 2004). Shredding the leaf litter can reduce scab incidence by 90% in a controlled environment (Sutton et al. 2000). Though in actuality, reduction of inoculum from shredding or removal of the leaf litter may range from 50-65% due the variable topography and obstructions in orchards, making this practice time consuming and difficult (Vincent et al. 2004).

Alternatively, spray applications to decrease the leaf litter may be an effective substitute to shredding and removal (Vincent et al. 2004). Previously used chemical compounds, such as mercury, have shown promise in reducing primary inoculum and the number of fungicide treatments needed during the growing season (Keitt, 1930; Kadow and Hopperstead, 1941; Goldsworthy et al. 1949; Burchill, 1968). But due to their harmful effects to the environment and other organisms, many of the chemicals used in these studies have been taken off the market (Sutton et al. 2000). This led to research of other methods such as the use of urea or biological control (Sutton et al. 2000; Rosenberger, 2003). Biological control using fungi that are antagonistic to ascospore production has shown promise, but field efficacy, registration, commercialization, and costs have been major obstacles in the practicality of these products (Rosenberger, 2003). Thus urea, with its relatively low application costs and accessibility to growers, is a more suitable product (Vincent et al. 2004).

Urea is a synthetic nitrogen fertilizer that promotes litter decomposition and inhibits *V. inaequalis* pseudothecia development by maintaining high nitrogen content in the leaves (Beresford et al. 2000; Sutton et al. 2000). Unfortunately, as urea is a synthesized compound, organic growers are not permitted to use it in their management programs. This reduces apple scab management options exclusively removal or shredding the leaf litter (Gomez et al. 2007). As described above, these methods are often time consuming and difficult to implement (Vincent et al. 2004). And as a consequence, organic orchards often have poor primary scab management, resulting in epidemics (Holb et al. 2005). This high disease pressure may ultimately reduce the efficacy of summer management techniques, leading to management failures and consequently causing major economic loss (Jamar, 2011).

In conventional orchards, where urea is permitted, treatments may be applied before or after leaf-fall after harvest (Beresford et al. 2000). It is important when applying urea to understand that applications made before leaf-fall can have adverse effects to the health of the tree as additional nitrogen may prolong the growth period of the tree, ultimately delaying the hardening of the tissues (Rosenberger, 1996; Schupp et al. 2001). This exposes the plant tissue to lower temperatures, which can increase the cold damage to the buds and consequently affects the following season's yield (Wood and Beresford, 2000; Schupp et al. 2001). Alternatively, urea can be applied to the orchard floor after leaves have abscised from the tree (Beresford et al. 2000). Issues with ground applications include inadequate coverage and reduced efficacy if the ground freezes soon after the treatment is applied (Ciecierski et al. 1995; Rosenberger, 1996; Mac an tSoir et al. 2010).

These factors highlight limitations in primary inoculum management of apple scab in both organic and conventionally managed orchards. Therefore, research that explores new compounds that can be used in organic orchards and research that focuses on improving the limitations of ground applications of urea is necessary. Specifically compounds that increase the coverage and penetration of urea from a sanitation standpoint are necessary. One potential solution for improving or potentially substituting for urea applications involves utilizing adjuvants. Adjuvants are “materials that are added

to a tank mix to aid or modify the action of an agrichemical, or physical characteristics of the mixture” (American Society for Testing and Materials ASTM, 1999). Adjuvants have been found to increase the spread, retention, penetration, and over all spray efficiency of materials being applied to plant surfaces (Percich and Nickelson, 1982; Steurbaut, 1993; Thompson et al. 1996; Gent et al. 2003).

The addition of adjuvants may work synergistically with urea to increase urea-driven decomposition of the leaves and reduce primary inoculum of *V. inaequalis* (Burchill and Swait, 1977). To date, no studies have explored the effect of adjuvants in combination with urea on urea-driven leaf litter decomposition and inoculum reduction. Additionally, it has yet to be explored if organic adjuvants may be comparable alternatives to urea for use in organic orchards. This demonstrates the need to identify if the incorporation of adjuvants with urea or adjuvants alone could reduce leaf litter and primary inoculum of *V. inaequalis*.

3.2 Materials and Methods

Adjuvants were tested for their ability to improve urea-driven leaf litter decomposition of scab-infected leaves and pseudothecia reduction of *V. inaequalis* (Table 3.1). Scab-infected leaves were collected from apple trees then placed on the orchard floor to overwinter after treatments were made. Concentrations of all adjuvants were based on recommended rates on commercial product labels. In congruence with the literature, a 5% urea (Urea, Granular 46-0-0) solution was used (Sutton et al. 2000).

3.2.1 Field tests

In the fall of 2013, a preliminary study was conducted to test the effect of adjuvants when combined with urea to reduce the primary inoculum of *V. inaequalis*. This study was performed in field plots at Meig’s Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN. Treatment blocks were set up in a randomized

complete block design in a 5-yr-old block of McIntosh (*Malus domestica* ‘McIntosh’) apple trees on B9 rootstocks planted in Toronto-Millbrook soil (soil pH: 6.7). McIntosh trees were planted 3-m apart with 5-m between each row in a 0.2-ha field containing 80 trees. Naturally infected McIntosh leaves were used for this study. Data from this study were acquired and analyzed in the spring of 2014 and henceforth will be referred to as the 2014 trial. In the fall of 2014, a larger study was conducted in the same field plot. The treatment blocks were expanded to include two rows of the 5-yr-old McIntosh apple trees containing B9 and Elma 26 rootstocks. Naturally-infected McIntosh leaves and Gala (*Malus domestica* ‘Gala’) leaves were used for this study. Data from this study were acquired and analyzed in the spring of 2015 and henceforth will be referred to as the 2015 trial.

3.2.2 Experimental design

The 2014 trial the treatment blocks were arranged in a randomized complete block design of 10 treatments with 4 replications containing 10 leaves each, with a total of 400 leaves. The 2015 trial the treatment blocks were arranged in a randomized complete block design of 10 treatments with 4 replications each containing 50 leaves. This design was applied to both McIntosh and Gala leaves, with a total of 2,000 McIntosh leaves and 2,000 Gala leaves.

3.2.3 Sanitation treatments

In the 2014 trial, 400 scab-infected McIntosh leaves were collected after harvest. On 13 October 2013, all scab-infected leaves were immersed in their respective treatments for 10 seconds and then placed under wire mesh for overwintering on the orchard floor. At the time of treatment application, the temperature was 20°C and after the trial began, no precipitation was recorded until 24 October (0.28 cm). In the 2015 trial, 2,000 scab-infected McIntosh leaves and 2,000 scab-infected Gala leaves were collected

after harvest. On 23 October 2014, all scab-infected leaves were immersed in their respective treatments for 10 seconds and then placed under wire mesh for overwintering on the orchard floor. At the time of treatment application, the temperature was 17°C and after the trial began, rain was recorded on 24 October (0.05 cm), 28 October (0.84 cm), and 31 October (0.51 cm). For both years, an additional 100 untreated leaves were overwintered similarly to serve as a check group.

3.2.4 Quantifying the leaf litter

For both years, all overwintered leaves were collected in the spring (April to May) and scanned on a flat bed scanner connected to a PC. A digital image assessment program (Image J, Rasband 1997-2014) was used to quantify leaf litter area associated with each replication of each treatment. In the 2014 trial, the degradation of leaf litter was determined by comparing the area of all leaves recovered from the wire mesh after overwintering. In the 2015 trial, leaves were scanned before and after overwintering on the orchard floor to analyze total leaf percent area lost.

3.2.5 Assessing pseudothecia and ascospore development

For the 2014 trial, assessment of pseudothecia and ascospore development began when the first ascospore discharge was microscopically observed from untreated check leaves. Total amount of pseudothecia present on leaves in all treatments were counted and recorded from each replicate. Throughout May of 2014, eight randomly selected pseudothecia from each replicate, totaling 32 pseudothecia per treatment, were removed, crushed on glass microscope slides, and inspected for ascospore development. The number of pseudothecia selected per treatment was chosen to eliminate possible treatment bias as one treatment only had 32 pseudothecia present. Asci and ascospore development were assessed using a modified version of the rating system published by Gadoury and MacHardy (1982). The modified version of asci and ascospore development

involved a 1-5 scale where 1= no asci; 2= asci with no developed ascospores; 3= asci with 1-50% developed ascospores; 4= asci with more than 50% developed ascospores; and 5= mature ascospores. Rankings were then used to determine the percentage of fertile pseudothecia per treatment by counting the number of pseudothecia with a rank of 3 or higher and dividing that value by the total number of pseudothecia assessed per treatment. For the 2015 trial, when 50% of pseudothecia in the untreated check group were producing mature ascospores (9 April), all leaves were collected and stored at 4°C. Collection differed from the 2014 trial due to the larger number of sampled leaves in the 2015 trial. In the 2015 trial 25 pseudothecia from each of the four replicates were arbitrarily selected for the asci/ascospore development ratings. Total number of pseudothecia, asci/ascospore development, and consequently percentage of fertile pseudothecia were assessed similar to the 2014 trial.

3.2.5 Statistical analysis

In both 2014 and 2015 trials, leaf area data were averaged within treatments and analyzed with a one-way ANOVA using a generalized linear model procedure (PROC GLM) of SAS v9.3 (SAS Institute). Means were separated according to Fisher's protected LSD test at $P=0.05$ using SAS v9.3 (SAS Institute). Total pseudothecia data were averaged within means, subjected to log transformation ($\log x+1$), and analyzed with a one-way ANOVA using a generalized linear model procedure (PROC GLM) of SAS v9.3 (SAS Institute). Means were separated using a Fisher's protected LSD test at $P=0.05$ using SAS v9.3 (SAS Institute).

3.3 Results

Naturally infected leaves collected for both trial years all had greater than 20% scab severity in the fall. Weather during the experiment (October to May) was relatively

similar between the years (Fig. 3.1). Though in the 2015 the month of treatment application (October) had much more rain recorded than the 2014 trial (Fig. 3.1).

3.3.1 2014 trial results

Visual inspection of scanned images (Fig 3.2) suggested that select adjuvants enhanced leaf litter decomposition. However, none of the adjuvant/urea combinations resulted in a statistically significant reduction in the leaf litter compared to urea alone (Fig. 3.3A). With the exception of Latron B-1956 alone, all adjuvants alone reduced the leaf litter compared to urea alone (Fig. 3.3A). Adjuvants when combined with urea did not significantly reduce the amount of pseudothecia compared to urea alone (Fig. 3.3B). Li700 alone was comparable to urea in reducing pseudothecia numbers (Fig. 3.3B). Li700 plus urea, Bond Max plus urea, and Wet Betty plus urea all significantly reduced the percentage of fertile pseudothecia compared to urea alone (Fig. 3.3C). Most notable is the result that when Bond Max and Wet Betty were added to urea, asci were not developed (0% fertile pseudothecia, Fig. 3.3C, 3.4).

3.3.2 2015 trial results

Leaf litter decomposition differed slightly between apple cultivars. For McIntosh, all adjuvants alone reduced the leaf litter as well as urea alone (Fig. 3.5A). Li700 plus urea and Wet Betty plus urea significantly reduced the leaf litter compared to urea (Fig. 3.5A). For Gala, all adjuvants except Latron B-1956 reduced the leaf as well as urea alone (Fig. 3.5B). Li700 plus urea, Bond Max plus urea, and Wet Betty plus urea significantly reduced the leaf litter compared to urea alone (Fig. 3.5B). For both McIntosh and Gala cultivars, all adjuvants in combination with urea reduced the number of pseudothecia compared to urea alone (Fig. 3.5C,D). For McIntosh, only Latron B-1956 reduced the number of pseudothecia as well as urea alone (Fig. 3.5C). Wet Betty when combined with urea significantly decreased the number of pseudothecia more than the

other adjuvant and urea combinations (Fig. 3.5C). For Gala, only Bond Max alone was comparable to urea in reduction of number of pseudothecia (Fig. 3.5D). For both cultivars Li700 plus urea, Bond Max plus urea, and Wet Betty plus urea decreased the percentage of fertile pseudothecia greater than urea alone (Fig. 3.5E,F). For Gala, Li700, Latron B-1956, and Wet Betty alone reduced the percentage of fertile pseudothecia as well as urea alone (Fig. 3.5F).

3.4 Discussion

Though the preliminary study conducted in 2014 lacked statistical significance, likely due to the small sample size, the visual data suggests that the incorporation of adjuvants to urea may improve apple scab management. The larger study performed in 2015 found that select adjuvants have the potential to hasten urea-driven leaf litter decomposition and reduction of *V. inaequalis* inoculum. Most notably, adjuvants Wet Betty or Li700 when combined with urea significantly increased leaf decomposition and reduced number and fertility of pseudothecia. Wet Betty and Li700 are classified as penetrants, which means they induce movement of chemicals into plant tissue (Hazen, 2000). Because urea is effective at decomposing leaves and inhibiting pseudothecia development due to maintaining high nitrogen content in the leaves (Beresford et al. 2000; Sutton et al. 2000), increasing penetration of urea into leaves would enhance treatment efficacy. It was shown by Leece and Dirou (1977), that an organosilicone surfactant with penetrant qualities increased the penetration of urea into leaves, which resulted in a 15% higher nitrogen content in leaves compared to adjuvants without penetrant qualities.

Increased nitrogen content in leaves could be only one reason why Wet Betty and Li700 had the most significant effect on urea. Temperature may have played a role in differentiating adjuvant effects on urea-driven leaf litter decomposition and inoculum reduction. In both years the average temperature was low, with temperatures falling below 0°C within three weeks of initiation of the field tests. Urea treatments are ineffective if the ground freezes too soon after application (Rosenberger, 1996; Sutton et

al. 2000; Mac an tSoir et al. 2010). This may have slowed the leaf litter decomposition or reduced microbial populations that are antagonistic to *V. inaequalis* (Ciecierski et al. 1995), rendering treatments that did not significantly increase nitrogen content ineffective. Thus the penetrant adjuvants may have increased uptake of urea and maximized the treatment efficacy before the ground froze.

Unexpected, but interesting, results in this study were that adjuvants were comparable to urea alone in reducing leaf litter and pseudothecia development. Burchill and Swait (1977) found similar results using anionic, ionic and cationic surfactants to reduce *V. inaequalis* ascospore dose. Their effect on inoculum reduction was attributed to the increase in leaf litter decomposition (Burchill and Swait, 1977). It may be hypothesized that certain adjuvants work synergistically with soil microorganisms that decompose leaves and are antagonistic to *V. inaequalis*. More information regarding the method in which adjuvants do this is needed to better understand these results.

Among other adjuvants, Wet Betty was comparable to urea in both years. In fact, in 2015, Wet Betty alone decomposed McIntosh leaf litter to a greater extent than urea (Fig. 3.4C). Since Wet Betty is an organic adjuvant, these results suggest that this adjuvant could be an acceptable alternative to urea applications in organic orchards. These findings are consistent with observations reported by Bengsston et al. (2009) that found yucca, a plant extract and the active ingredient in Wet Betty, significantly reduced germination and penetration of conidia of *V. inaequalis* in apple leaves. The authors did note that yucca had a lesser effect on post-penetration stages, such as stroma formation (Bengsston et al. 2009). Since pseudothecia eventually develop from stroma (MacHardy et al. 2001), this could explain why Wet Betty had a lesser effect on pseudothecia reduction in comparison to leaf litter decomposition. The greater effect on leaf litter reduction could also be due to the organic plant material present in Wet Betty promoting microorganism populations in the soil that decompose leaf litter. However, this is speculative and more research into this method is needed to better understand the benefits of using Wet Betty in organic orchards.

Overall, the addition of certain adjuvants, such as Wet Betty or Li700, to urea may reduce the initial inoculum of *V. inaequalis*, which could potentially delay an apple

scab epidemic (Gadoury and MacHardy 1986). A delay of a scab epidemic may have several benefits to apple disease management. First by postponing the initial infection past the point when apples and leaves are most susceptible to *V. inaequalis* (Rosenberger and Cox 2012), and secondly, a delay in a scab epidemic could save fungicide sprays (Gadoury and MacHardy 1986; MacHardy et al. 1993). Delaying infection past the point of high susceptibility would decrease primary and consequently secondary infections (Rosenberger and Cox 2012). This would decrease disease incidence during the growing season and potentially reduce fungicides needed for adequate scab management throughout the summer (Rosenberger and Cox 2012).

Delaying an apple scab epidemic would be important to growers who own high inoculum orchards because these growers typically need to begin fungicide applications earlier and use higher rates of fungicides throughout the season (Rosenberger and Cox 2012). If these growers rely on captan for scab management during the season, they may run the risk of exceeding the ten applications at a 4.5 kg/ha rate per season restriction that is set on captan (Rosenberger 2009). Therefore, the incorporation of select adjuvants with urea could decrease the management problems associated with high inoculum orchards by postponing the initial infection of *V. inaequalis* and reducing the primary and consequently secondary inoculum. This would reduce disease pressure and decrease the need for frequent applications of high rates of fungicides. Ultimately this may allow growers to use restricted fungicides, like captan, all season and still maintain adequate control.

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3.5 Tables and Figures

Table 3.1: List of materials used in this study to reduce *V. inaequalis* inoculum.

Trade name	Active ingredient	Concentration /379 L	Manufacturer	Classification
Urea, Granular 46-0-0	Nitrogen	20 kg	Agrium	Fertilizer
Li700	Phosphatidylcholine, methylacetic acid, and alkyl polyoxyethylene ether	0.3 L	Loveland Industries	Acidifier, penetrant, drift reduction
Bond Max	Alcohol ethoxylate, 1,2-propanediol, and synthetic latex	0.4 L	Loveland Industries	Spreader-sticker, deposition aid
Latron B-1956	Modified phthalic glycerol alkyd resin	0.3 L	Simplot Grower Solutions LLC	Spreader-sticker
Wet Betty	Yucca extract, saponin	0.7 L	Advanced Nutrients	Organic, wetter, spreader, penetrant

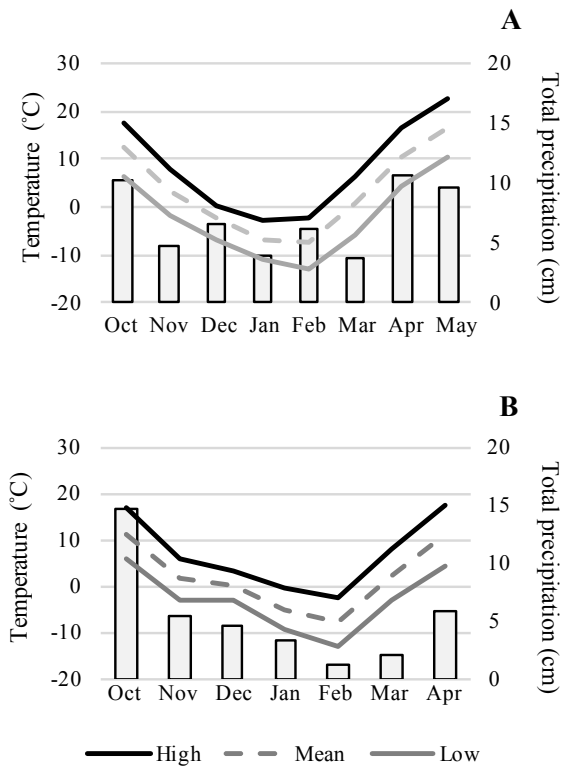


Figure 3.1: Average monthly high, mean, and low temperatures ($^{\circ}\text{C}$) and total monthly precipitation (cm) throughout the experiments conducted in **A**, 2014-15 and **B**, 2014-15. Weather data were collected by the Meig's Weather Station at the Throckmorton-Purdue Agricultural Center.

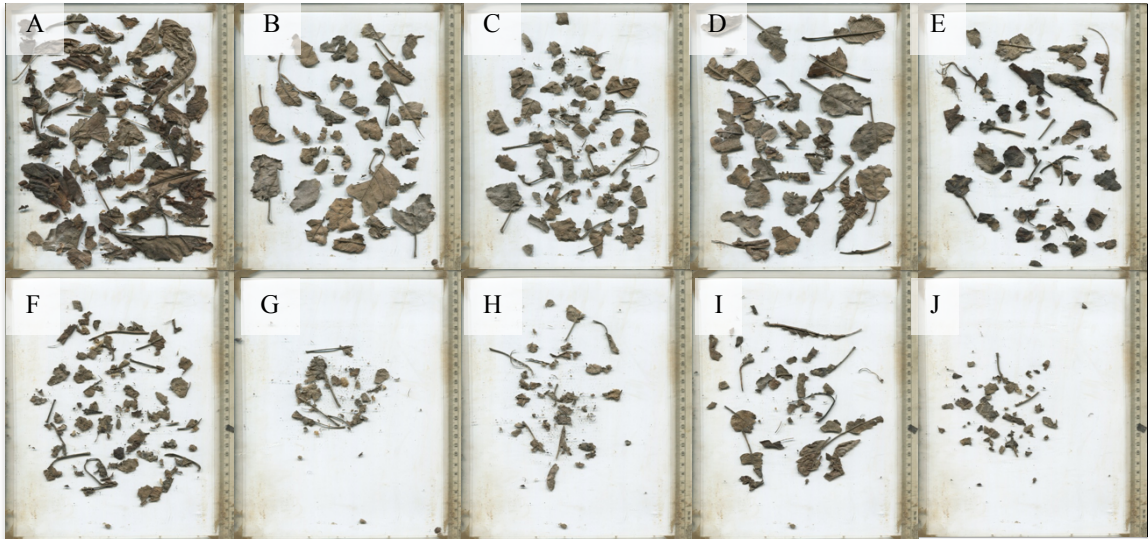


Figure 3.2: Images showing the leaf litter degradation of 40 leaves per treatments in response to various adjuvant and urea treatments in 2014. Treatments are **A**, Water; **B**, Li700; **C**, Bond Max; **D**, Latron B-1956; **E**, Wet Betty; **F**, Urea; **G**, Li700 + urea; **H**, Bond Max + urea; **I**, Latron B-1956 + urea; and **J**, Wet Betty + urea.

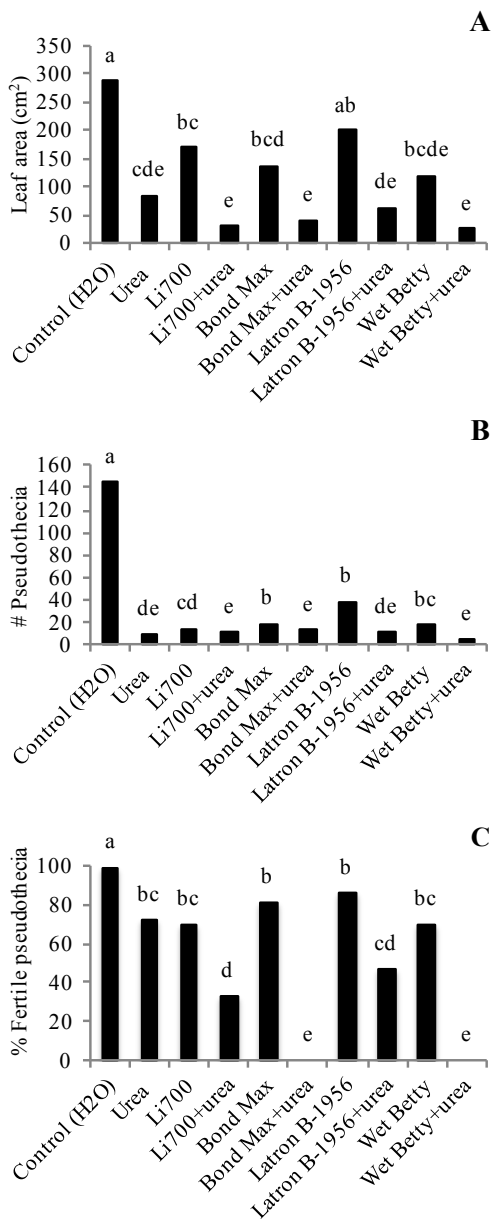


Figure 3.3: Effect of adjuvants on **A**, leaf area of overwintered scab-infected McIntosh leaves, **B**, *V. inaequalis* pseudothecia present in sampled leaves, and **C**, percent fertile pseudothecia on scab-infected McIntosh leaves overwintered at Meig's Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2014¹.

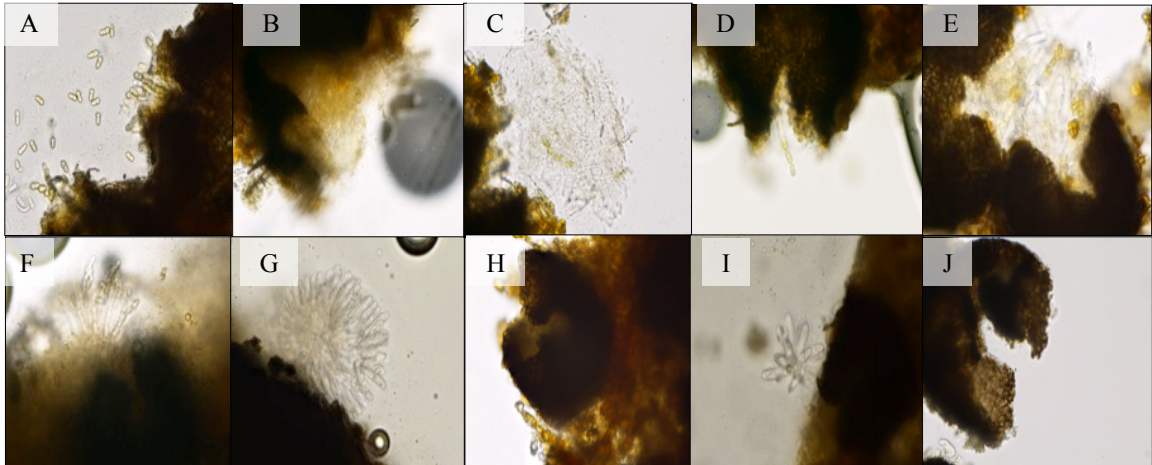


Figure 3.4: Micrographs showing the development of the pseudothecia and ascospores of the fungus *Venturia inaequalis* in response to various adjuvant and urea treatments in 2014. Treatments are **A**, Water; **B**, Li700; **C**, Bond Max; **D**, Latron B-1956; **E**, Wet Betty; **F**, Urea; **G**, Li700 + urea; **H**, Bond Max + urea; **I**, Latron B-1956 + urea; and **J**, Wet Betty + urea.

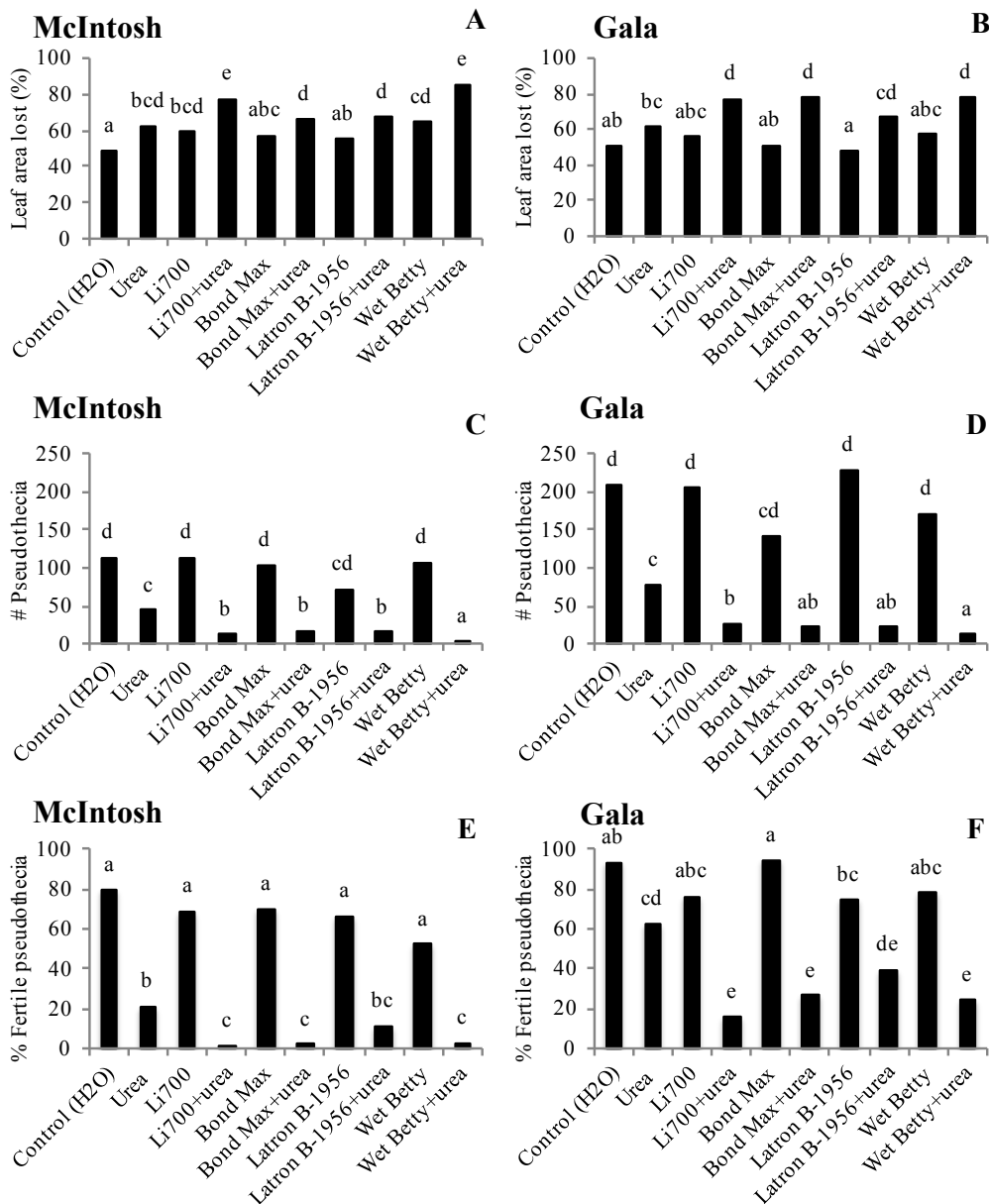


Figure 3.5: Effect of adjuvants on percent leaf area of overwintered of **A**, scab-infected McIntosh leaves and **B**, scab-infected Gala leaves, on *V. inaequalis* pseudothecia present in sampled in **C**, scab-infected McIntosh leaves and **D**, scab-infected Gala leaves, and percent fertile pseudothecia on **E**, scab-infected McIntosh leaves and **F**, scab-infected Gala leaves overwintered at Meig's Farm, Throckmorton Purdue Agricultural Center in Lafayette, IN, 2015¹.

APPENDIX

Figures 2.1; 2.2; 2.3; 2.4; 2.5;

¹ Data was subject to arcsin transformation before statistical analysis and are displayed as back transformed units of means. Treatments denoted with the same letter are not significantly different at $P=0.05$ as determined by a Fisher's Protected LSD test performed after a one-way ANOVA.

Figures 3.1; 3.2;

¹ Number of pseudothecia data was subject to log transformation before statistical analysis and are displayed as back transformed units of means. Percent fertile pseudothecia was subject to arcsin transformation before statistical analysis and are displayed as back transformed units of means. Treatments denoted with the same letter are not significantly different at $P=0.05$ as determined by a Fisher's Protected LSD test performed after a one-way ANOVA.

Table A1: Effect of adjuvants and captan on apple scab (*V. inaequalis*), bitter rot (*Colletotrichum* spp.), and phytotoxicity incidence and severity on Golden Delicious and Honeycrisp apples at Meig's Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2013.

Cultivar	Treatment	Timing ^z	Scab		Bitter rot		Phytotoxicity	
			% incidence	% severity	% incidence	% severity	% incidence	% severity
Golden Delicious								
	No treatment	---	2.2a ^{yx}	1.0a	2.6a	5.0b	0.0	0.0
	Captan	1C->11C	0.6a	1.0a	1.8a	22.5b	0.0	0.0
	Li700	1C->11C	4.7a	1.5a	5.7a	3.8b	3.3a	10.0a
	Bond Max	1C->11C	0.0a	0.0a	3.3a	5.0b	3.3a	10.0a
	Attach	1C->11C	5.0a	3.0a	0.6a	5.0b	15.0b	6.7a
	Latron B-1956	1C->11C	3.3a	2.8a	3.5a	8.3b	3.3a	10.0a
Captan plus:								
	Li700	1C->11C	2.0a	1.0a	1.3a	5.0b	6.0a	12.5a
	Bond Max	1C->11C	3.9a	3.7b	1.7a	3.5a	7.5a	6.3a
	Attach	1C->11C	1.5a	2.3a	2.8a	4.7b	15.6b	10.8a
	Latron B-1956	1C->11C	0.8a	5.0b	0.0a	0.0a	18.0b	12.5a
Honeycrisp ^w								
	No treatment	---	0.0a	0.0a	1.5b	10.0b	0.0	0.0
	Captan	1C->8C	0.0a	0.0a	0.0a	0.0a	0.0	0.0
	Li700	1C->8C	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	Bond Max	1C->8C	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	Attach	1C->8C	0.0a	0.0a	1.0a	40.0b	0.0a	0.0a
	Latron B-1956	1C->8C	0.1a	0.5a	0.0a	0.0a	0.0a	0.0a
Captan plus:								
	Li700	1C->8C	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	Bond Max	1C->8C	0.1a	0.2a	1.0a	10.0b	0.4a	4.0b
	Attach	1C->8C	0.1a	1.0a	0.0a	0.0a	0.8a	3.8b
	Latron B-1956	1C->8C	0.0a	0.0a	0.0a	0.0a	0.2a	1.5b

^z Treatments applied: 1C (1st cover) = 24 May; 2C (2nd cover) = 4 Jun; 3C (3rd cover) = 17 Jun; 4C (4th cover) = 28 Jun; 5C (5th cover) = 11 Jul; 6C (6th cover) = 23 Jul; 7C (7th cover) = 6 Aug; 8C (8th cover) = 16 Aug; 9C (9th cover) = 30 Aug; 10C (10th cover) = 13 Sept; 11C (11th and final cover) = 25 Sept.

^y All data are displayed as means and were subject to arcsin transformation prior to statistical analysis using the statistical software SAS 9.3

^x Column numbers followed by the same letter are not significantly different at $P=0.05$ as determined by Fisher's Protected LSD test performed after a one-way ANOVA.

^w Honeycrisp apple scab, bitter rot, and phytotoxicity incidence means are count data not percentages.

Table A2: Effect of adjuvants and captan on apple scab (*V. inaequalis*), bitter rot (*Colletotrichum* spp.), and phytotoxicity incidence and severity on Golden Delicious and Honeycrisp apples at Meig's Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2014.

Cultivar	Treatment	Timing ^z	Scab		Bitter rot		Phytotoxicity	
			% incidence	% severity	% incidence	% severity	% incidence	% severity
Golden Delicious								
	No treatment	---	100.0d ^{yx}	73.4d	6.7c	2.5b	0.0	0.0
	Captan	B->10C	36.9b	8.9b	0.8abc	0.2a	0.0	0.0
	Li700	B->10C	99.9d	51.9c	1.0abc	0.2a	0.0a	0.0a
	Bond Max	B->10C	100.0d	57.7c	4.8bc	0.9ab	0.0a	0.1ab
	Attach	B->10C	100.0d	55.9c	6.1c	0.7ab	0.0a	0.0a
	Latron B-1956	B->10C	99.8d	46.9c	0.6abc	0.1a	0.3a	0.0a
Captan plus:								
	Li700	B->10C	11.0a	1.7a	0.1a	0.0a	4.8b	0.6bc
	Bond Max	B->10C	18.2a	1.3a	0.1a	0.0a	4.7b	0.9c
	Attach	B->10C	74.9c	11.2b	0.1a	0.1a	1.0ab	0.3abc
	Latron B-1956	B->10C	19.4a	1.0a	0.4ab	0.0a	3.6b	0.5bc
Honeycrisp								
	No treatment	---	4.7d	0.1	43.1b	9.6cd	0.0	0.0
	Captan	B->8C	0.1abc	0.0	8.3a	4.2ab	0.0	0.0
	Li700	B->8C	4.2d	0.1	30.5b	11.9cd	0.3a	0.3a
	Bond Max	B->8C	2.5cd	0.1	44.5b	25.7d	0.3a	0.3a
	Attach	B->8C	3.6d	0.3	45.0b	20.8cd	0.4ab	0.1a
	Latron B-1956	B->8C	1.9abc	0.1	35.3b	18.6ab	0.4ab	0.4ab
Captan plus:								
	Li700	B->8C	0.0a	0.0	1.5a	0.3a	1.2abc	0.4ab
	Bond Max	B->8C	0.0a	0.0	2.7a	1.0a	2.1bc	1.4b
	Attach	B->8C	0.0a	0.0	5.6a	5.1ab	1.0abc	0.5ab
	Latron B-1956	B->8C	0.0a	0.0	9.3a	4.1ab	3.0c	0.7ab

^z Treatments applied: B (bloom) = 30 Apr; PF (petal fall) = 12 May; 1C (1st cover) = 22 May; 2C (2nd cover) = 5 Jun; 3C (3rd cover) = 18 Jun; 4C (4th cover) = 2 Jul; 5C (5th cover) = 14 Jul; 6C (6th cover) = 25 Jul; 7C (7th cover) = 8 Aug; 8C (8th cover) = 19 Aug; 9C (9th cover) = 2 Sept; 10C (10th and final cover) = 16 Sept.

^y All data are displayed as means and were subject to arcsin transformation prior to statistical analysis using the statistical software SAS 9.3.

^x Column numbers followed by the same letter are not significantly different at $P=0.05$ as determined by Fisher's Protected LSD test performed after a one-way ANOVA.

Table A3: Effect of adjuvants and captan on apple scab (*V. inaequalis*), bitter rot (*Colletotrichum* spp.), and phytotoxicity incidence and severity on Honeycrisp apples at Meig's Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2015.

Treatment	Timing ^z	Scab		Bitter rot		Phytotoxicity	
		% incidence	% severity	% incidence	% severity	% incidence	% severity
No treatment	---	2.7a	0.2abc	52.3f	29.0d	0	0
Captan	B->9C	2.4a	0.2abc	24.5cde	20.4bcd	0	0
Li700	B->9C	1.0a	0.2abc	49.9f	26.4cd	0.1ab	0.0ab
Bond Max	B->9C	1.7a	0.4bc	33.1def	22.3bcd	0.0a	0.0a
Attach	B->9C	2.2a	0.1abc	38.7def	24.2bcd	0.0a	0.0a
Latron B-1956	B->9C	2.7a	0.7c	47.4ef	31.2d	0.4abc	0.1ab
Captan plus:							
Li700	B->9C	0.8a	0.1ab	4.0ab	4.2a	0.4abc	0.1ab
Bond Max	B->9C	0.1a	0.0a	3.3a	2.4a	1.4bc	0.2b
Attach	B->9C	0.5a	0.1abc	19.0bcd	11.5abc	0.1ab	0.1ab
Latron B-1956	B->9C	0.0a	0.0a	9.6abc	2.4a	1.5c	0.2b

^zTreatments applied: B (bloom) = 23 Apr; PF (petal fall) = 6 May; 1C (1st cover) = 19 May; 2C (2nd cover) = 19 May; 3C (3rd cover) = 9 Jun; 4C (4th cover) = 23 Jun; 5C (5th cover) = 2 Jul; 6C (6th cover) = 16 Jul; 7C (7th cover) = 30 Jul; 8C (8th cover) = 11 Aug; 9C (9th cover) = 24 Aug.

^yAll data are displayed as means and were subject to arcsin transformation prior to statistical analysis using the statistical software SAS 9.3.

^xColumn numbers followed by the same letter are not significantly different at $P=0.05$ as determined by Fisher's Protected LSD test performed after a one-way ANOVA.

Table A4: Effect of adjuvants on urea-driven reduction of *V. inaequalis* pseudothecia and urea-driven leaf litter decomposition of scab-infected McIntosh leaves at Meig's Farm, at the Throckmorton Purdue Agricultural Center in Lafayette, IN, 2014.

Treatment	No. pseudothecia^z	Leaf area (cm²)^y
Control (H ₂ O)	145a ^x	286.86a
Urea	10de	85.73cde
Li700	15cd	169.11bc
Bond Max	19b	137.85bcd
Latron B-1956	39b	199.16ab
Wet Betty	18bc	119.94bcde
Urea plus:		
Li700	11e	30.54e
Bond Max	13e	40.49e
Latron B-1956	11de	63.89de
Wet Betty	5e	24.95e

^z Data was subject to log transformation before statistical analysis. Data are displayed as back transformed units of means.

^y Data are displayed as means and were analyzed using the statistical software SAS 9.3.

^x Column numbers followed by the same letter are not significantly different at $P=0.05$ as determined by a Fisher's Protected LSD test performed after a one-way ANOVA.

Table A5: Effect of adjuvants on urea-driven reduction of *V. inaequalis* pseudothecia and on percent urea-driven leaf litter decomposition of scab-infected McIntosh leaves and scab-infected Gala leaves at Meig's Farm, Throckmorton Purdue Agricultural Center in Lafayette, IN, 2015.

Cultivar		
Treatment	No. pseudothecia^z	Leaf area lost (%)^y
McIntosh		
Control (H ₂ O)	114d ^x	48.5a
Urea	44c	62.3bcd
Li700	114d	59.0bcd
Bond Max	104d	57.3abc
Latron B-1956	72cd	55.2ab
Wet Betty	107d	64.3cd
Urea plus:		
Li700	13b	77.5e
Bond Max	18b	66.4d
Latron B-1956	15b	67.3d
Wet Betty	3a	85.5e
Gala		
Control (H ₂ O)	210d ^x	50.8ab
Urea	77c	61.0bc
Li700	205d	55.7abc
Bond Max	142cd	51.2ab
Latron B-1956	229d	48.0a
Wet Betty	172d	56.9abc
Urea plus:		
Li700	27b	77.2d
Bond Max	22ab	77.9d
Latron B-1956	22ab	66.4cd
Wet Betty	13a	77.8d

^z Data was subject to log transformation before statistical analysis. Data are displayed as back transformed units of means.

^y Data are displayed as means and was obtained by measuring leaf area before and after overwintering on the orchard floor to analyze total leaf percent area lost.

^x Column numbers followed by the same letter are not significantly different at $P=0.05$ as determined by a Fisher's Protected LSD test performed after a one-way ANOVA performed using the statistical software SAS 9.3.

Chapter 3: Quantifying the effect of adding adjuvants to urea using PAD.

To quantify the effect that adjuvant additions to urea could have on apple scab management, the number of days a scab epidemic could be delayed due to treatments was calculated. This was calculated using the potential ascospore dose (PAD) previously published (Gadoury and MacHardy, 1986). However, because the calculation of PAD included factors not measured in this study we were unable to calculate the true PAD. Instead we extrapolated from data used in the studies conducted by Gadoury and MacHardy (1986) and MacHardy et al. (1993). In these studies, PAD is a measure of the potential ascospores that may present in an orchard (Gadoury and MacHardy, 1986), thus we used percent fertile pseudothecia per treatment as a similar measurement to PAD in our study. Using our modified ranking system of percent fertile pseudothecia, any pseudothecia that had developing ascospores present were considered fertile under the assumption that in the future they had the potential to produce mature ascospores and contribute to primary infection. This may overestimate the potential inoculum present, so actual reduction of inoculum due to the addition of adjuvants to urea may be greater than what we calculated in this study.

Using percent fertile pseudothecia produced in each treatment, we calculated the percent decrease of fertile pseudothecia between treatments. For example, in the 2015 trial on McIntosh leaves, the percent fertile pseudothecia produced in the control treatment when scored was 79%; 22% of the pseudothecia in the urea treatment were fertile; 3% of the pseudothecia in the Li700 plus urea treatment were fertile; and 6% of the pseudothecia in the Wet Betty plus urea treatment were fertile (Table 3.2). Therefore, the amount urea, Li700 plus urea, and Wet Betty plus urea treatments reduced fertile pseudothecia compared to the control were 72%, 96%, and 92%, respectively. Using PAD we may be able to quantify how much reduction in fertile pseudothecia, or potential ascospores, may influence disease epidemics and consequently disease management.

PAD is used to estimate the onset of scab epidemics and adjust fungicide spray programs (Gadoury and MacHardy, 1986). To calculate the number of days an epidemic could be delayed, the authors used a formula published by Van der Plank (1963) that defined the mathematical relationship between inoculum and disease development

(Gadoury and MacHardy, 1986). One of the variables in this formula was the ratio of the amount of inoculum before eradication to the amount of inoculum surviving eradication (Gadoury and MacHardy, 1986). In their study they used a constant PAD of 98,388 ascospores per square meter per year as the amount of inoculum before eradication and recorded PAD's from real orchards as their amount of inoculum surviving eradication (Gadoury and MacHardy, 1986). We aligned our variables similarly to assign the percent fertile pseudothecia in the control treatment as our amount of inoculum before eradication, and the percent fertile pseudothecia in urea, adjuvant, and adjuvant plus urea treatments as the amount of inoculum surviving eradication.

Using data published by MacHardy et al. (1993), we used the delay of epidemic values corresponding to the percent reduction of fertile pseudothecia calculated in our study from both Gala and McIntosh trials. For example, 72%, 96%, and 92% reduction of PAD corresponds to the amount of fertile pseudothecia were reduced by urea, Li700 plus urea, and Wet Betty plus urea treatments compared to the control in our 2015 McIntosh trial. We were able to extrapolate that urea alone would delay apple scab epidemic 0-3 days, whereas Wet Betty plus urea would delay an epidemic 3-6 days, and Li700 plus urea would cause a delay of 4-8 days, depending on cultivar.