INTEGRATED PEST MANAGEMENT OF ONION THRIPS AND FOLIAR FUNGAL PATHOGENS IN ONIONS

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ABSTRACT

An integrated pest management (IPM) program is reliant on a thorough understanding of the biology and ecology of the target pest and incorporates multiple management tactics to reduce pest pressure. Management can be further complicated when multiple pest species, such as insect vectors and plant pathogens, interact in the field. Onion thrips (*Thrips tabaci*) are the main insect pest of onions and are well documented vectors of many different types of plant pathogens. The co-occurrence of onion thrips and facultative plant pathogens in the field is well documented, but it remains unclear how these pests interact, and whether the integration of multiple management tactics can provide synergistic control of these pests. This dissertation begins to fill that gap, exploring the relationship between onion thrips and *Colletotrichum coccodes*, a fungal pathogen of onion, and testing IPM strategies for multiple pest management.

I first examined how *C. coccodes* influenced onion thrips behavior and physiology by conducting preference and performance experiments in the lab. By manipulating host plant health, I found that onion thrips can discriminate between multiple hosts and prefer hosts that were infected with *C. coccodes* compared to healthy hosts. Furthermore, adult onion thrips survived longer and reproduced at higher rates on plants infected with *C. coccodes*. Preference for an infected host can alter pathogen spread and increases in insect vector abundance often lead to more severe pathogen outbreaks. Therefore, I explored how onion thrips spread *C. coccodes* in the lab, examining density dependent and independent factors, such as temperature. At high temperatures, onion thrips reproduction increased, and they dispersed more often from an inoculated to a non-inoculated plant, leading to an increase in disease spread in the experiment. These results suggest that disease spread is likely density dependent, and that conditions favoring onion thrips outbreaks may also lead to more severe disease symptoms. Onion thrips and *C.*

coccodes exhibit a mutually beneficial relationship; with onion thrips reproducing at higher rates on *C. coccodes* inoculated plants, and subsequently disease spread increased concurrently with increased vector abundance.

I then focused on testing various IPM tactics in the field to reduce onion thrips and foliar fungal pathogens. Plastic mulches were tested as a non-chemical control method aimed at delaying onion thrips infestation in the field. I found that mulches are viable season-long alternative to conventional pesticides. Furthermore, the addition of conventional pesticides provided synergistic onion thrips control. Finally, I tested organic and conventional pesticide programs to develop a sustainable season-long control program for onion thrips and foliar fungal pathogens. Organic pesticides were not sufficient for onion thrips control, highlighting the need for alternative control methods and more sustainable conventional practices. To address this, I tested various action thresholds which reduced the number of pesticide applications throughout the season without compromising onion thrips control.

This dissertation begins to fill the gaps in our existing knowledge of insect-plant-pathogen interactions and suggests that the role of insects as vectors of facultative pathogens should not be overlooked. The mutually beneficial relationship between these pests further highlights the need to develop a comprehensive IPM program that focuses on managing both pests rather than focusing on a single species. By testing several IPM tactics, this work offers new insight into multiple pest management strategies that could be implemented in the field.

This dissertation is dedicated to my husband, Joseph, and my family for their unwavering love and support throughout this journey.

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CHAPTER 1: INTRODUCTION TO INSECT-PLANT-PATHOGEN INTERACTIONS AND MANAGEMENT STRATEGIES

Onion industry

In 2020, China led the world in onion production (~40%), followed by India (~18%), the United States of America (~3%) and Egypt (~2.5%) (FAOTSTAT 2020). Globally, people consume about 6.35 kg of onions per person per year, with Americans consuming around 8.6 kg per person per year (NOA 2020). In the United States, the onion industry is valued at $1.5 billion (USDA/NASS 2024), and Michigan produces 1% of all the onions in the United States, making the value of the onion industry in the state worth around $10 million (USDA/NASS 2024).

Onion thrips

Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), are a major insect pest of onions found throughout the world and can cause significant yield loss if unmanaged (Gill et al. 2015; Lewis 1991). They cause indirect damage in onions by feeding on the plant foliage while also creating alternative entry points for plant pathogens (Lemmetty and Lindqvist 1993; Lewis 1997; McKenzie et al. 1993; Dutta et al. 2014). Currently, conventional insecticides are the primary management strategy for onion thrips control, and maintaining insecticide efficacy is a priority for onion growers. This can be achieved through reducing the risk of resistance development by rotating insecticide class and using action thresholds to determine when to make applications (Siegwart et al. 2015). However, onion thrips are resistant to many different active ingredients and action thresholds are difficult to implement (Nault and Hessney 2008, 2010, 2011; Gill et al. 2015; Pedigo et al. 1986; Diaz-Montana et al. 2011). Therefore, non-chemical control methods must be explored, and a thorough understanding of onion thrips and their interactions with other organisms is necessary.

Onion thrips biology

Onion thrips can reproduce sexually and asexually via thelytokous parthenogenesis, causing populations to quicky increase (Nault et al. 2006). Generations of onion thrips can switch between the different modes of reproduction, and often exist in the same field simultaneously, but it is unknown what factors determine the reproductive mode (Li et al. 2014; Nault et al. 2006).

Onion thrips are holometabolous with distinct life stages. Eggs are laid by the female superficially into the plant tissue and, in laboratory studies, incubate for about 4 days, at 25 ± 1℃ (Bhonde et al. 2016; Cranshaw 2004; Gill et al. 2015). Once hatched, onion thrips have two distinct, active feeding instar larval phases before pupating in the soil (Bhonde et al. 2016; Cranshaw 2004). Onion thrips are the most mobile during their adult stage due to their ability to fly and can migrate using wind currents (Lewis 1991). Adults overwinter in or around fields, and emergence is dependent on latitude and regional temperatures (Larentzaki et al. 2007; North and Shelton 1986). In Texas, onion thrips emerge as early as February, while in New York, most emerge in May (Edelson et al. 1986; Larentzaki et al. 2007). Post emergence, adult onion thrips colonize weeds and volunteer onion plants before infesting commercial onion fields.

Feeding damage

Onion thrips feed on the plant foliage with unique mouthparts consisting of a single mandible and a pair of stylets (Diaz-Montano et al. 2011; Chisholm and Lewis 1984). The mandible probes along the surface of the foliage for a feeding site, before puncturing the epidermis and consuming the cell contents (Chisholm and Lewis 1984; Cranshaw 2004; Lewis 1991). This unique form of feeding is referred to as the “punch and suck” method and feeding can last anywhere from a few seconds to 30 minutes (Chisholm and Lewis 1984; Lewis 1991).

The punch and suck method of feeding leaves distinct, silvery scarring on the onion foliage due to the loss of chlorophyll (Boateng et al. 2014). Chlorophyll loss reduces the onion's ability to photosynthesize, resulting in smaller bulbs and yield loss for growers (Boateng et al. 2014). Even light amounts of feeding leave distinct scarring, allowing growers to identify onion thrips infestations (Chisholm and Lewis 1984). Onion thrips feed at the proximal end of the onion leaf, impacting the onions' ability to transfer nutrients to the bulb (Parrella and Lewis 1997). Feeding during the bulbing stage of onion development reduces bulb size and can cause economic loss for growers while also allowing for pathogen entrance into the plant tissue (Kendall and Capinera 1987).

Thrips as vectors of plant diseases

In addition to creating entry points for plant pathogens via feeding, onion thrips vector diseases. While larval onion thrips cause the majority of feeding damage seen on plants, adults are more mobile and thus more likely to vector pathogens over long distances (Lewis 1991; Bailey 1935). Onion thrips are well known vectors of Orthtospoviruse, but can also spread bacterial, and fungal plant pathogens (Cortes et al. 1998; Dutta et al. 2014; Leach et al. 2020; Lemmetty and Lindqvist 1993).

Onion thrips can transmit at least two economically important Orthotospoviruses: the tomato spotted wilt virus and the iris yellow spotted virus (Lemmetty and Lindqvist 1993; Cortes et al. 1998). Othrotospoviruses are persistent, circulative pathogens, acquired during the larval stage, where the virus has a latency period before being transmitted (Ullman et al. 1992). While it is possible that larval onion thrips can spread the virus, adults are more likely to be vectors due to their high mobility (Lewis 1991; Ullman et al. 1992).

Onion thrips can vector bacterial pathogens within the Pantoea genus, such *Pantoea* *ananatis*, the causal agent of center rot in onion (Dutta et al. 2014). The infection begins as blotching or spotting on the foliage before spreading to the bulb and causing infections during storage (Carr et al. 2013; Coutinho and Venter 2009). The bacteria are acquired by feeding on an infected host and spread via feces (Dutta et al. 2014; Dutta et al. 2016). High onion thrips infestations exacerbate symptoms of bacterial leaf blight and bactericides are not effective at reducing necrotic spread (Grode et al. 2017; Grode et al. 2019). Often, the most efficient way to reduce disease spread is by managing onion thrips populations with insecticides (Grode et al. 2019).

High infestations of onion thrips can also exacerbate symptoms of fungal disease (Leach et al. 2017; McKenzie et al. 1993; Marullo 1995). Stemphylium leaf blight (*Stemphylium vesicarium)* is a fungal disease first reported on onion crops in Texas in 1978 and had been reported sporadically but has recently become a dominant pathogen in the Eastern United States production region (Miller et al. 1978; Hausbeck and Werling 2018; Hay et al. 2019; Hay et al. 2021). Disease symptoms are characterized by foliar lesions ranging from light yellow to brown, and generally occur on the side of the leaf facing the prevailing winds (Miller et al. 1978). Anthracnose (*Colletotrichum coccodes)* is another onion fungal disease that was found in Michigan in 2012 and has persisted at low levels since (Rodriguez-Salamanca et al. 2012). The lesions appear tan or salmon-colored, and then dark spores begin to develop (Rodriguez-Salamanca et al. 2018). Both pathogens, if left uncontrolled, can cause significant economic loss for growers (Rodriguez-Salamanca et al. 2012; Leach et al. 2020). Developing management plans that can reduce onion thrips and pathogen pests simultaneously is a high priority for onion growers. However, understanding how these pests interact is critical to creating an effective integrated management plan.

Integrated pest management for multiple organisms

Integrated pest management requires a thorough understanding of the biology and ecology of the target pests, and the integration of multiple control tactics to create a cohesive plan (Kogan 1998). For onion thrips and fungal pathogens, such as *C. coccodes,* understanding how these pests interact with one another is critical before implementing a management program. Currently, insecticide applications are used to reduce onion thrips populations while reducing disease spread more efficiently than applying bactericides or fungicides alone (Grode et al. 2019; Leach et al. 2020). However, tank mixing insecticides and fungicides can reduce insecticide efficacy, highlighting the difficulties of multiple pest management (Nault et al. 2013). Therefore, it is important to first examine the interaction between pests, test non-chemical management strategies to delay infestation, and finally, develop a pesticide program that can target both pests.

Insect-plant-pathogen interactions

Insect pests and plant pathogens interact when they colonize the same host plant. The interactions between obligate pathogens and their vectors are well described, but these interactions are more obscure for facultative pathogens (Jeger 2020; Lowery et al. 2015; Shaw et al. 2017). Facultative pathogens do not rely on insects for dispersal, but insects can contribute to pathogen spread in multiple ways via mechanical vectoring or by creating alternative entry points through feeding (Tiberi et al. 2016; Eigenbrode et al. 2018). Plant pathogens can also affect insect behavior or physiology, and understanding the outcomes of these interactions is important for pest management (Daugherty et al. 2011; Ingwell et al. 2012).

Behavioral modifications can include changes in vector preference for or discrimination against an infected host, which can alter the rate of disease spread (Maris et al. 2004; Abe et al. 2012; Shrestha et al. 2012). However, preference is variable, and can change based on host infection stage, and may also change after pathogen exposure (Daugherty et al. 2011; Ingwell et al. 2012; Mauck et al. 2018). The vector manipulation hypothesis (VMH) suggests that insect behavior, specifically preference, changes throughout the insect's life to increase the rate of disease spread (Ingwell et al. 2012). Insects may prefer an infected host, but after pathogen exposure that preference may change and the insect may move to a healthy plant, thus spreading the pathogen (Ingwell et al. 2012; Daugherty et al. 2011; Blua and Perring 1992). Conversely, if an insect discriminates against an infected host, the contact between the insect and the pathogen is reduced, and disease spread should be decreased. The VMH was first documented in aphids, but thrips spp. exhibit preference for hosts infected with Orthotospoviruses, and a similar mechanism may be occurring (Ingwell et al. 2012; Maris et al. 2004; Abe et al. 2012).

Physiological modifications can occur when insect performance is affected by host plant infection status, such as increased reproduction or higher survival. Onion thrips on grape leaves or roses infected with downy mildew perform better than on healthy leaves (Yarwood 1943). Interestingly, preference and performance may not be linked traits, and are likely to be species specific. For example, western flower thrips (*Frankliniella occidentalis*) prefer and perform better on infected hosts, and tobacco thrips (*Frankliniella fusca)* prefer infected hosts but perform poorly on those same hosts (Maris et al. 2004; Abe et al. 2012; Shrestha et al. 2012). It is difficult to predict the effect of host plant infection status on preference and performance, therefore, species specific studies on economically important pathogens and thrips are necessary.

Non-chemical control methods

Within an integrated pest management program, the inclusion of non-chemical control methods is encouraged to reduce pest populations below economically damaging levels. Physical control methods, such as plastic mulches have been used by growers for many reasons including increased yield and soil moisture retention, weed reduction, and microclimate control (Kasirajan and Ngouajio 2012; Greer and Dole 2003). Plastic mulches are also used for pest control, but the efficacy is variable and dependent on the crop, target pest, and mulch color.

Silver reflective mulches are often used to reduce insect colonization but have had variable success in reducing thrips populations, and the effectiveness may change throughout the season (Iglesias et al. 2021; Momol et al. 1999). Western flower thrips in pepper cropping systems alighted less on peppers planted in reflective mulch beds, but by the end of the season, the peppers had higher thrips populations compared to the control (Reitz et al. 2003). It is likely that the temporal variation is due to increased canopy cover reducing the amount of exposed mulch, thus making the mulch ineffective (Reitz et al. 2003). However, in tomatoes, western flower thrips populations were deterred by reflective mulches, and the tomato plants had a lower disease incidence compared to the control (Anderson et al. 2012). The discrepancies within the literature make it difficult to predict how onion thrips will react to reflective mulches, but since mulches are used by some onion producers it is important to consider how they may directly or indirectly affect onion thrips populations.

There are multiple theories describing the mechanisms behind plastic mulches, either focusing on the indirect or direct effects (Andreotti et al. 2010; Gordon et al. 2008; Ham et al. 1993; Mathejczyk and Wernet 2017; Kelber 1999). Plastic mulches may indirectly affect thrips populations through alterations in microclimate (Andreotti et al. 2010; Gordon et al. 2008; Ham et al. 1993). Dark mulches, such as blue or black mulches, increase the air and soil temperature, potentially creating an inhospitable environment (Gordon et al. 2008). Mulches may directly affect thrips populations by reducing host finding ability. Sunlight reflected off plants can become polarized, which insects, such as butterflies, can then use to orient themselves towards a host (Kelber 1999). Highly reflective plastic mulches or colored plastic mulches may alter the wavelength and polarization of light, thus disorienting an insect (Mathejczyk and Wernet 2017). To better understand the mechanisms behind repellency it is important that researchers measure changes in the microclimate in the field, and the effect of different colored mulches on host plant selection. While plastic mulches can reduce both insect abundance and disease spread, they become less effective later in the season. Therefore, it is important to combine colored mulches with a curative measure, such as action threshold-based insecticide applications.

Chemical control of onion thrips

Insecticides are currently the cornerstone for onion thrips management despite problems with resistance to several different conventional insecticide classes (Yadav et al. 2018; Martin et al. 2003; Nault and Shelton 2010; Diaz-Montano et al. 2011; Adesanya et al. 2020). To reduce insecticide resistance development, the rotation of insecticide classes is recommended (Gill and Garg 2014). However, insecticide class rotation can be difficult because there are relatively few highly effective active ingredients for onion thrips management (Gill et al. 2015; Nault and Hessney 2008, 2010, 2011). Maintaining the efficacy of the available insecticides is a top priority for growers and can be achieved through implementing action thresholds to reduce the number of applications per season (Siegwart et al. 2015; Gill et al. 2015; Leach et al. 2017). However, for onion thrips, action thresholds vary depending on the insecticide being applied, indirect damage on leaves, field location and weather conditions, thus regular scouting of fields throughout the season is essential (Pedigo et al. 1986; Fournier et al. 1995; Rueda et al. 2007; Nault and Shelton 2010; Diaz-Montana et al. 2011). The lack of available insecticides as well as the difficulty in implementing action thresholds make resistance management a challenge for growers if appropriate extension-based education programs are not available for growers (Leach et al. 2019).

Further complexities occur when multiple pests, such as onion thrips and pathogens, co-occur in fields, requiring growers to manage both pests simultaneously. However, tank mixing insecticides with certain fungicides lowers the efficacy of insecticides, indicating that an integrated plan is necessary (Nault et al. 2013). Insecticide applications are more effective at reducing pathogen spread than fungicide or bactericide applications, indicating that insect management is critical for reducing disease spread (Grode et al. 2017; Leach et al. 2020). Therefore, developing new action thresholds that consider the interaction of onion thrips and plant pathogens is critical for pest management.

Organic insecticides or biopesticides have fewer non-target impacts, thus these may be more amenable for use in an IPM program. Multiple organic insecticides, including spinosyns and azadirachtin, have been tested for onion thrips control (Dively et al. 2020; Iglesias et al. 2021). However, whether the addition of an organic bactericide or fungicide reduces insecticide efficacy has not been tested. Organic pesticides are short lived relative to conventional pesticides and are less effective later in the season when pest pressure is high (Golec et al. 2020; Dively et al. 2020). Developing a season long organic program that manages onion thrips and fungal pathogens would require careful consideration of application timing and efficacy testing.

Research objectives

My research aimed to create an IPM program for onion thrips and fungal pathogen management, focusing on understanding the relationship between these pests, testing methods to reduce pest infestation, and finally exploring sustainable chemical control methods (Figure 1.1). The relationship between onion thrips and *C. coccodes* was explored in laboratory-based experiments. In objective 1, host plant selection was documented with paired preference and performance experiments, giving insight into how *C. coccodes* inoculated plants altered onion thrips behavior and physiology. It was unclear if onion thrips contribute to the spread of *C. coccodes*, therefore disease spread experiments were conducted to determine whether they are vectors in objective 2. By manipulating onion thrips populations and then altering environmental conditions, both density dependent and density independent factors affecting pathogen spread were tested. For objective 3, plastic mulches were tested in the field to determine if onion thrips infestations could be reduced with non-chemical control methods. Changes in plant growth and microclimate were monitored throughout the season to determine whether mulches directly or indirectly affected onion thrips populations. Finally for objective 4, organic pesticides were evaluated for their efficacy against onion thrips and pathogen spread, and the action thresholds of conventional pesticides were assessed.

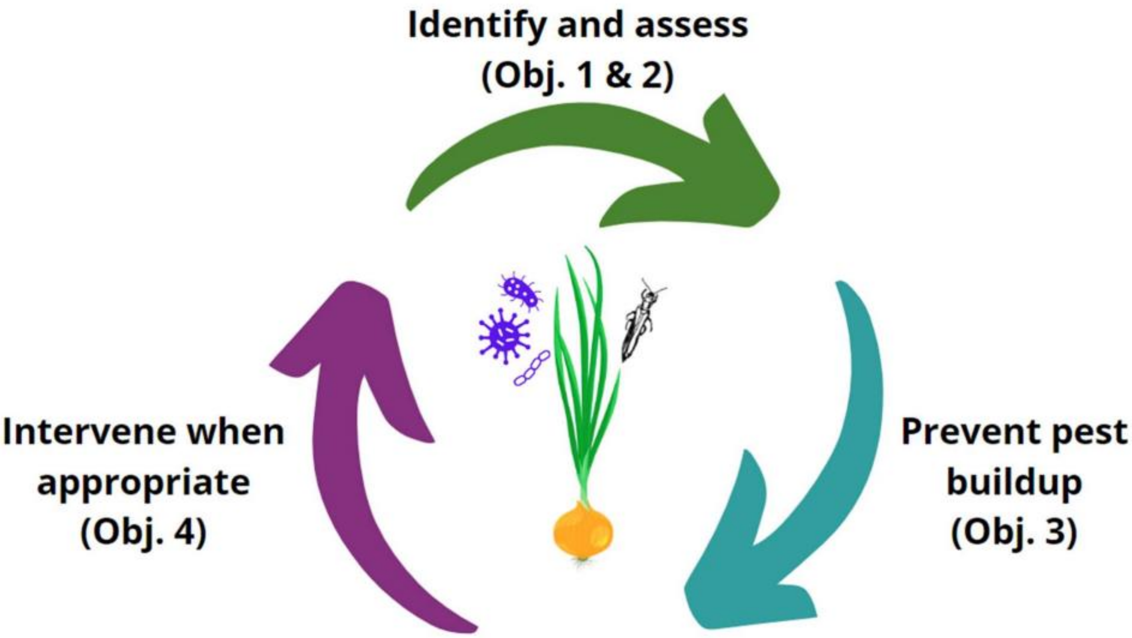

Figure 1.1. A conceptual figure outlining the research objectives within the context of developing an IPM program for onion thrips and fungal pathogens. Obj. 1 and 2: Focus on identifying and assessing the relationship between onion thrips and *Colletotrichum coccodes* in the lab. Obj. 3: Preventing pest buildup with non-chemical control methods in the field by implementing plastic mulches to reduce onion thrips pressure. Obj. 4: Focuses on the appropriate use of organic and conventional pesticides to reduce pest pressure.

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CHAPTER 2: ONION THRIPS (THYSANOPTERA: THRIPIDAE) HOST PLANT PREFERENCE AND PERFORMANCE ARE MEDIATED BY A FACULTATIVE PLANT PATHOGEN OF ONION

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Abstract

Insect vector and phytopathogen interactions are mediated by host plants. Insects interact with pathogens directly or indirectly and they may prefer host plants based on infection status. Performance on infected hosts varies depending on the type of pathogen involved. Species specific studies of economically important insects and phytopathogens are needed to understand how these interactions impact crop yields. Onion thrips, *Thrips tabaci* Lindeman, is an economically devastating insect pest of onions (*Allium cepa* L.) worldwide and it co-occurs simultaneously with many different pathogens. *Colletotrichum coccodes* (Wallr) is a generalist fungal pathogen that attacks onion foliage, causing tan lesions and decreasing yield. Onion thrips and *C. coccodes* represent two important pests of onions, but the relationship between onion thrips and *C. coccodes* infected onions has not been studied, and it is unclear if onion thrips contribute to the spread of *C. coccodes* in onion fields. A four-choice test with control, artificially injured, artificially injured + symptomatic, and inoculated-symptomatic onion suggests that onion thrips distinguish between hosts based on health status. Furthermore, a two-choice test with control, inoculated-asymptomatic, and inoculated-symptomatic onion pairings revealed that onion thrips distinguish between hosts based on infection status and prefer

inoculated-symptomatic hosts. In a no-choice test, onion thrips numbers increased on inoculated-symptomatic plants compared to control or inoculated-asymptomatic plants. Overall, we found that onion thrips preferred and performed best on *C. coccodes* infected plants.

Introduction

Herbivorous insects interact with phytopathogens when they colonize the same host. For example, insects can interact with plant pathogens through obligate and facultative vectoring (Gonella et al. 2019; Franco et al. 2017). While the relationship between insect vectors and obligate pathogens is often well defined, facultative pathogen vectoring is more ambiguous. Predicting the outcomes becomes difficult because facultatively vectored pathogens are spread by insects in multiple ways, such as creating entry points via feeding, spreading through feces, or carrying on the integument (Eigenbrode et al. 2018; Tiberi et al. 2016). In addition, plant pathogens can interact with insect vectors by modifying their behavior, physiology, and fitness (Simon and Hilker 2003). For example, a host plant’s infection status can have profound impacts on an insect vector’s preference and performance (Daugherty et al. 2011; Ingwell et al. 2012). Whether an insect prefers or discriminates against a host based on infection status is especially important for pest management.

Many thrips (Thysanoptera: Thripidae) species are plant pathogen vectors and are known for their role as tospovirus vectors; they also contribute to the spread of several fungal and bacterial plant pathogens (Ananthakrishnan and Dhileepan 1984; Whitfield et al. 2005; Diaz-Montano et al. 2011). Thrips can be obligate vectors of pathogens, for example, western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), vector the tospovirus tomato spotted wilt virus (TSWV) and onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), vector iris yellow spot virus (Wijkamp et al. 1995; Kritzman et al. 2001; Pozzer et al.

1999).Thrips can also vector pathogens facultatively as in the case of the New Zealand flower thrips, *Thrips obscuratus* Crawford (Thysanoptera: Thripidae), which mechanically vector *Botrytis cinerea* when conidia become attached to the integument (Fermaud and Gaunt 1995). Onion thrips are potential facultative vectors of powdery mildew (*Erysiphe polygoni)* with higher thrips numbers resulting in increased powdery mildew incidence (Yarwood 1943). Similarly, western flower thrips contribute to the spread of Fusarium rot (*Fusarium verticillioides*) (Farrar and Davis 1991). In cotton, flower thrips (*Frankliniella* spp.) were associated with *F. verticillioides,* and exposure to flower thrips and *F. verticillioides* resulted in severe damage (Mailhot et al. 2007). Onion thrips can mechanically vector Stemphylium leaf blight (*Stemphylium vesicarium*) (Leach et al. 2020) and bacterial leaf blight (*Pantoea agglomerans)* (Grode et al. 2017; 2019) in onions (*Allium cepa)*. In onions, onion thrips are critical to the proliferation of purple blotch (*Alternaria porri)* and prior thrips feeding damage renders the crop susceptible to infection (Bhangale and Joi 1983; McKenzie et al. 1993).

Thrips interact with a variety of plant pathogens through vectoring, but plant pathogens also interact with thrips by modifying their behavior and fitness by affecting host plant preference and performance (Abe et al. 2012; Maris et al. 2014; Shrestha et al. 2012; Yarwood 1943). Western flower thrips, for example, prefer and perform better on TSWV-infected plants when compared to healthy plants (Abe et al. 2012; Maris et al. 2004). In contrast, tobacco thrips, *Frankliniella fusca* (Hinds) preferred TSWV infected host plants, but larval performance was decreased on them (Shrestha et al. 2012). While obligate pathogens such as TSWV influence thrips preference and performance, facultative pathogens can also affect thrips. For example, onion thrips on grape leaves or roses infected with powdery mildew (*Erysiphe polygoni)* had increased survival compared to those on healthy plants (Yarwood 1943). Preference and performance of thrips on infected plant tissues is dependent on the thrips species and the pathogen involved. Therefore, it is important to study the relationships between economically important thrips species and cooccurring plant pathogens.

Onion thrips are a major insect pest of onions, they are often found in fields concurrently with fungal pathogens such as *Colletotrichum coccodes* (Wallr) Hughes, the causal agent of leaf and neck anthracnose (Gill et al. 2015; Rodriguez-Salamanca et al. 2012). In tomatoes, *C. coccodes* inoculum is spread by splash dispersal, which may come from rainfall or overhead irrigation (Sanogo et al. 1997). In onions, the most effective *C. coccodes* inoculation methods included the use of a conidial spray that targets the foliage, while soil drenches are not as effective as foliar sprays, indicating that *C. coccodes* infects onions primarily through the leaf tissue (Rodriguez-Salamanca et al. 2018). For lesions to develop on tomato fruit, *C. coccodes* requires 10 or more hours of continuous leaf wetness and temperatures between 25-31℃ (Dillard 1989). On onions, *C. coccodes* causes dry, tan to salmon colored, oval lesions on the foliage, that turn dark as the pathogen sporulates (Rodriguez-Salamanca et al. 2012; Rodriguez-Salamanca et al. 2018). Onion plots infected with *C. coccodes* that are not treated with a fungicide had significantly lower yield when compared to those treated with a fungicide (Rodriguez-Salamanca 2013). In addition to dissemination via splashing, disease can be increased through damage caused by onion thrips feeding (Dutta et al. 2014; McKenzie et al. 1993; Leach et al. 2020; Yarwood 1943; Bhangale and Joi 1983).

Onion thrips feed through a punch and suck method, destroying the epidermal plant tissues (Chisholm and Lewis 1984). The loss of integrity of the plant host’s epidermis increases its vulnerability as pathogens may enter the plant through wounds (Gill et al. 2015; Childers and Achor 1995). Disease incidence and severity is positively correlated with onion thrips populations and feeding damage, suggesting that onion thrips feeding contributes to pathogen infection (Grode et al. 2017; McKenzie et al. 1993; Yarwood 1943; Leach et al. 2020; Dutta et al. 2014). For some pathogens, such as *Alternaria porri* (Ellis), onion thrips feeding wounds provide entry points for the pathogen, facilitating infection (McKenzie et al. 1993). Other pathogens, such as *Pantoea ananatis* (Serrano) Mergaert may be spread through contaminated onion thrips feces deposited directly on feeding wounds (Dutta et al. 2014). While it is understood that onion thrips feeding promotes the infection of onion plants by some pathogens (Dutta et al. 2014; Grode et al. 2017; McKenzie et al. 1993), it is unclear how pathogen infection may mediate onion thrips behavior, such as host plant preference or performance.

We examined the effects of varying host plant health status (control, artificially injured, artificially injured + symptomatic, and inoculated-symptomatic) on onion thrips preference in a laboratory setting using a four-choice test. The pathogen chosen for all experiments was *C. coccodes*, a relatively new pathogen in onions first found in Michigan in 2012, and more recently found in Ohio in 2014, and New York in 2016 (Rodriguez-Salamanca et al. 2012; Baysal-Gurel et al. 2014, Hay et al. 2016). After 13 days, the number of adult and larval onion thrips were recorded on each plant. To better understand the effect of *C. coccodes* on host plant selection, we examined the effects of varying host infection status (control, inoculated-asymptomatic, inoculated-symptomatic) on onion thrips preference in a two-choice test. In both the four-choice and two-choice tests, we assessed the correlation between the number of onion thrips per plant and estimated damage severity (%). In previous research, other thrips species preferred infected hosts but whether host preference was indicative of insect performance varied based on the thrips species (Abe et al. 2012; Maris et al. 2004; Shrestha et al. 2012). Therefore, to understand the implications of the four-choice and two-choice tests, a no-choice test was conducted, and the number of onion thrips in the adult and larval life stage was recorded. We hypothesized that in the four-choice test, onion thrips would prefer inoculated-symptomatic plants, in the two-choice test, onion thrips would prefer inoculated-asymptomatic plants when compared to control or inoculated-symptomatic plants, and that preference would be indicative of performance.

Materials and Methods

Onion thrips colony

Onion thrips collected from a commercial onion field (Grant, MI) managed with conventional pesticides and fertilizer were used to start a colony at Michigan State University (East Lansing, MI) in May 2021. The onion thrips colony was maintained in a mesh cage (50 cm^3; MegaView, Taichung, Taiwan), and kept at room temperature with ambient humidity and a 16h:8h L:D cycle. Onion thrips were maintained on plants grown from certified organic onion seeds (*A. cepa* cv. 'New York Early'; Johnny Seeds, Winslow, ME) and allowed to feed *ad libitum*. Three to four onions at the 5-7 leaf stage were added to the cage every week to provide fresh foliage for continuous rearing. The onion thrips colony was maintained for 8 weeks prior to the initiation of experiments.

Colletotrichum coccodes inoculation

Colletotrichum coccodes was isolated from a conventional onion field (Allegan County, MI) in August 2020. The isolate was maintained on 25% potato dextrose agar (PDA) media and morphologically identified based on key characteristics including sclerotia and conidia shape (Cano et al. 2004). Isolate identity was confirmed using the ribosomal internal transcribed spacer region in a nested PCR (Cullen et al. 2002). DNA was extracted using the DNeasy Total DNA Isolation Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. A total volume of 25 µl PCR mixture contained PCR grade H_20 (Ambion, Austin, TX), 10x PCR Buffer

(Invitrogen, Waltham, MA), 50 mM $MgCl_2$ (Invitrogen), 10 mM dNTP (Promega, Madison, WI), 10 µM of each primer (Invitrogen), 5 u/µl Taq polymerase (Invitrogen) and 6.44 µg/ml of the template DNA. The PCR reactions were conducted in a Mastercycler Pro thermocycler (Eppendorf, Hauppauge, NY) with the genus specific primers (Cc1F1/Cc2R1) conditions set to an initial denaturation at 95℃ for 2 minutes, followed by 35 cycles of denaturation at 95℃ for 45 seconds, annealing for 61℃ for 1 minute and extension at 72℃ for 90 seconds, and a final elongation step at 72℃ for 5 minutes (Cullen et al. 2002). The product of the first PCR was used as template in the second PCR with species-specific primers (Cc1NF1/Cc2NR1). This had an initial denaturation at 95℃ for 2 minutes, followed by 35 cycles of denaturation at 95℃ for 45 seconds, and annealing and extension at 72℃ for 135 seconds, with a final elongation at 72℃ for 5 minutes (Cullen et al. 2002). PCR products were then visualized on a 1% agarose gel to confirm identification.

Prior to creating the inoculum, *C. coccodes* isolates were morphologically assessed for possible cross contamination by other pathogens (Cano et al. 2004). *C. coccodes* inoculum was grown on a full-strength PDA for 7 days after which the petri dish was flooded with double distilled water and the conidia removed by scraping a sterile tongue depressor across the top of the media. Onion plants were inoculated with a conidial suspension (2.5 x 10^5 conidia / ml) sprayed onto the foliage until it was fully wetted (i.e. dripping) (Rodriguez-Salamanca et al. 2018). The spray applications were made using a 250 ml plastic hand spray bottle (Meijer Inc., Grand Rapids, MI). *C. coccodes* conidial sprays were made without abrasive agents because they do not increase disease progression for this disease (Rodriguez-Salamanca et al. 2018).

Four-choice test

Organic onion (cv. ‘New York Early’) seeds were planted in a 50-cell seedling starter tray filled with perlite soil mix (Suremix Perlite, Michigan Grower Products Inc., Galesburg, MI) and kept in an environmental chamber (25℃, 50% RH, 16h:8h (L:D)). Plants were fertilized (2-3-1 N-P-K, Ocean Crest Seafood's Inc., Gloucester, MA) once a week at a rate of 74 ppm N and watered as needed. After 4 weeks, four onion seedlings at the 3-5 leaf stage were transplanted 10 cm apart into a rectangular aluminum tray containing slits in the bottom for drainage that were filled with perlite soil mix (30 cm x 25 cm x 8 cm, Handi-Max®, Wheeling, IL). Seedlings were then returned to the environmental chamber for 1 week and watered every other day.

A cylindrical plastic cage constructed out of transparency film (School Smart, Greenville, WI; 26 cm tall, 15 cm wide) was placed over all arenas and buried 2-3 cm into the soil (Figure 1A-C). The top of the cage was covered with a nylon mesh material (160 µm, MegaView, Lehigh Acres, FL) that allowed ventilation and prevented onion thrips from moving out of the arenas. The aluminum trays were placed randomly within the environmental chamber under condition as described for onion planting. Each tray was watered to saturation every other day.

Each of the four plants in a tray was randomly assigned one of four treatments: 1) control (sprayed with water until runoff), 2) artificially injured, 3) artificially injured + *C. coccodes* inoculated-symptomatic and, 4) *C. coccodes* inoculated-symptomatic (Figure 2.1A). Trays (N = 34) were arranged randomly on the shelves in the environmental chamber. To ensure that *C. coccodes* inoculated plants presented symptoms at the time of exposure to onion thrips, they were inoculated 7 days prior to onion thrips exposure. Plants were inoculated in the aluminum trays. To prevent cross contamination of treatments, conidial sprays were applied from the top to individual onion plants surrounded by a narrow cylindrical-plastic tube made of transparency film (26 cm tall x 6 cm wide). Early inoculation resulted in the development of small tan lesions with a 1-9% disease severity rating (Figure S2.1). One day before onion thrips exposure, plants assigned to the artificially injured or artificially injured + symptomatic treatments were pricked 10 times in a vertical line with a size 1 insect pin (Hamilton Bell Co, Montvale, NJ) on each leaf to differentiate between mechanical and biological damage. Additionally, three trays that contained only control onions were used to assess if there was a directional bias within the environmental chamber that may influence onion thrips' choice.

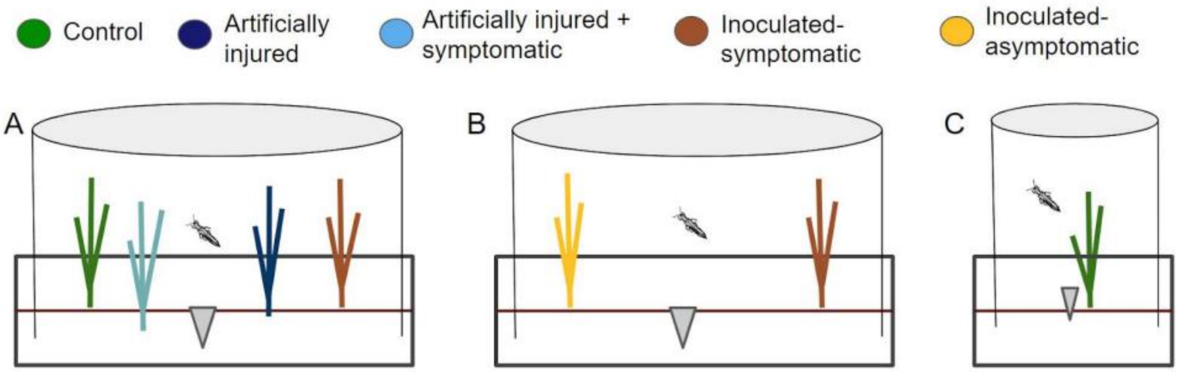

Figure 2.1. The experimental arenas for choice test with onion thrips and *C. coccodes*. A) Four-choice test arena with onions that were either 1) control, 2) artificially injured, 3) artificially injured +*C. coccodes* symptomatic, or 4) *C. coccodes* symptomatic. B) Two-choice test arena for onion thrips with the following choices: 1) control vs *C. coccodes* inoculated-asymptomatic, 2) control vs *C. coccodes* inoculated-symptomatic, 3) *C. coccodes* inoculated-asymptomatic vs *C. coccodes* inoculated-symptomatic. C) No-choice performance test arena with one onion of varying infection status: 1) control, 2) *C. coccodes* inoculated-asymptomatic, 3) *C. coccodes* inoculated-symptomatic. All onions were placed randomly inside a plastic, cylindrical cage made from transparency film with a mesh top. Five adult onion thrips were placed in the center of the arena and after 13 days, adult and larval onion thrips per plant were recorded for each experiment, and the damage severity (%) was recorded for the four-choice and two-choice experiments.

Five randomly selected adult onion thrips from the colony were placed in a 1.7 ml microcentrifuge tube (Thomas Scientific, Swedesboro, NJ) with a 3 cm piece of fresh onion leaf for nourishment. The vials with onion thrips were kept in a cooler until they were released into the arenas. After all onion thrips were collected, one vial was randomly assigned to each arena, and opened, allowing onion thrips free movement within the arena. The number of onion thrips per plant and plant damage severity (%) were recorded 13 days after onion thrips exposure.

Onion thrips numbers were counted by destructively sampling each arena by removing the cylindrical cages and uprooting the onions to pull apart the leaves to check the neck for onion thrips. After removing the plant from the arena, we counted the number of larvae and adult onion thrips on each plant. Thirteen days after thrips exposure, all plants that received *C. coccodes* inoculum were exhibiting disease symptoms. Damage severity was visually rated on each plant from 0 to 100% by estimating the amount of green vs. necrotic tissue caused by both onion thrips feeding and *C. coccodes*. To account for inherent observer bias when taking visual assessments (James 1974), all plants within the arenas were assigned an identification number that was used for data collection and the observer did not know which treatment they were observing.

Two-choice test

Onions for the two-choice test were grown following the procedure described above for the four-choice test. After 4 weeks, two onions in the 3-5 leaf stage were transplanted 10 cm apart into rectangular aluminum trays and covered with transparent cylindrical plastic cages (Figure 2.1B). Aluminum trays were considered the experimental unit and were placed in a randomized design within an environmental chamber under the same conditions previously described.

All onion seedlings were randomly assigned a treatment: 1) control, 2) *C. coccodes* inoculated-asymptomatic, and 3) *C. coccodes* inoculated-symptomatic. Seedlings were then placed in pairings so that each tray held one of the following combinations of seedlings: 1) control vs. inoculated-asymptomatic (n = 30), 2) control vs. inoculated-symptomatic (n = 30), or 3) inoculated-asymptomatic vs. inoculated-symptomatic (n = 30). To check for directional bias within the environmental chamber, 23 aluminum trays contained a pairing of only control plants. A *C. coccodes* conidial suspension was applied to each inoculated plant as described above using the same conidia concentration as above. Inoculated-symptomatic plants were inoculated 7 days before onion thrips exposure. Inoculated-asymptomatic plants were inoculated 3 days before onion thrips exposure. Inoculation timing was staggered so that plants would be at different stages of symptom progression when they were exposed to onion thrips. At the time of onion thrips exposure, inoculated-symptomatic seedlings had early disease symptoms of small tan lesions ranging from 1-7% symptom severity.

Onion thrips were added to the experimental units following the same procedure used in the four-choice test. Onion thrips per plant and the percentage of damage severity (onion thrips feeding and *C. coccodes* symptoms) were recorded 13 days after the onion thrips were added as described in the four-choice methods.

No-choice test

Onions were grown following the same procedure as described in the four-choice test. After 4 weeks, individual plants were transplanted into plastic pots (8.5 cm deep, 10 cm wide; The HC Companies: ITML, Middlefield, OH) and maintained in the growth chamber under the same conditions as described above for one week. An experimental unit consisted of one potted onion plant surrounded by a cylindrical-plastic cage made from transparency film (26 cm tall x 6 cm wide) with nylon mesh on top to allow airflow while preventing escape of the onion thrips (Figure 2.1C). Each experimental unit received one of the following treatments: 1) control, 2) *C. coccodes* inoculated-asymptomatic, and 3) *C. coccodes* inoculated-symptomatic onion plants. Experimental units were then placed in a randomized complete block design within the environmental chamber with 30 blocks. Inoculation methods and timing were the same as described in the two-choice test. Onion thrips were added following the same procedures as in the four-choice test. After 13 days, the arenas were destructively sampled as described in the four-choice methods and onion thrips numbers and life stage were recorded for each plant.

Data analysis

To determine onion plant preference, Chi-square tests were performed on the total number of onion thrips per plant for the four- and two-choice tests. The observed number of onion thrips per plant was compared to the expected number of onion thrips per plant specified by the null hypothesis (i.e., the number of onion thrips per plant between treatments is the same) (Franke et al. 2012). For the four-choice test, significant Chi-square tests with Bonferroni adjustment for multiple comparison were used to determine significant differences between treatments ($\alpha = 0.01$) (Sharpe 2015). To determine damage severity differences among treatments an analysis of variance (ANOVA) was performed for the four- and two-choice tests. R-squared values were used to determine if there was a correlation between onion thrips numbers and damage severity. For the no-choice experiment, to determine the effect of treatment (control, *C. coccodes* inoculated-asymptomatic, and *C. coccodes* inoculated-symptomatic) on onion thrips performance, the mean larval and adult onion thrips per plant were compared using an ANOVA with treatment as a fixed factor and block as random factor (Quinn and Keough 2002). The dependent variables met the assumptions of ANOVA. Tukey's HSD test ($\alpha = 0.05$) was used to determine the differences between treatment means. All data analyses were completed in R (RStudio Team 2021).

Results

Four-choice test

Overall, there were significantly more onion thrips on artificially injured plants and inoculated-symptomatic plants when compared to control plants or artificially injured + symptomatic plants ($\chi^2 = 101.23$, df = 3, p-value < 0.001; Figure 2.2). Compared to control plants, there were twice as many onion thrips on artificially injured + symptomatic plants (χ^2 = 25.04, df = 1, p-value < 0.001), approximately three times more onion thrips on artificially injured plants (χ^2 = 64.51, df = 1, p-value < 0.001), and four times as many onion thrips on inoculated-symptomatic plants (χ^2 =93.44, df = 1, p-value < 0.001). There were 50% more onion thrips on artificially injured plants compared to artificially injured + symptomatic plants (χ^2 = 10.82, df = 1, p-value < 0.001) and twice as many onion thrips on inoculated-symptomatic plants compared to artificially injured + symptomatic plants (χ^2 = 26.04, df = 1, p-value < 0.001). There was no difference between the number of onion thrips on artificially injured plants compared to inoculated-symptomatic plants (χ^2 = 3.44, df = 1, p-value = 0.064).

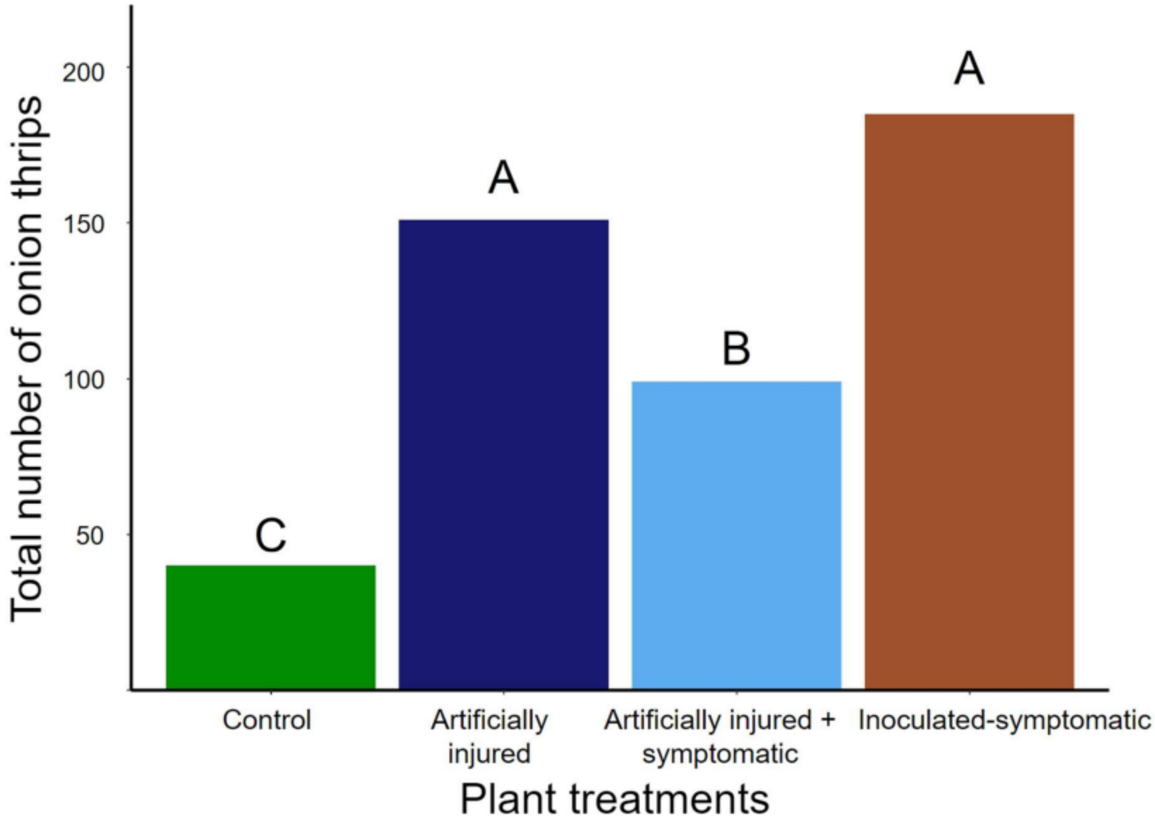

Figure 2.2. Total number of onion thrips per onion plant. The four plants were either healthy or infected with *C. coccodes* in a four-way choice test (N = 34). Four 6-week-old onions were arranged in an experimental arena and 5 adult onion thrips were added from a colony to each arena. After 13 days, the total number of adult and larval onion thrips were counted on each plant. Different letters above bars denote significant differences among means (χ^2-test; $\alpha = 0.01$).

The damage severity (%) on each plant varied by plant treatment (F = 30.21, df = 3, 132, p-value < 0.001; Figure 2.3). There was a weak positive correlation between damage severity (%) and onion thrips numbers on control plants ($R^2 = 0.09$, p-value = 0.028), on inoculated-symptomatic plants ($R^2 < 0.01$, p-value = 0.027) and on artificially injured plants ($R^2 = 0.31$, p-value < 0.001). There was no correlation between damage severity (%) and onion thrips numbers on artificially injured + symptomatic plants ($R^2 < 0.01$, p-value = 0.091).

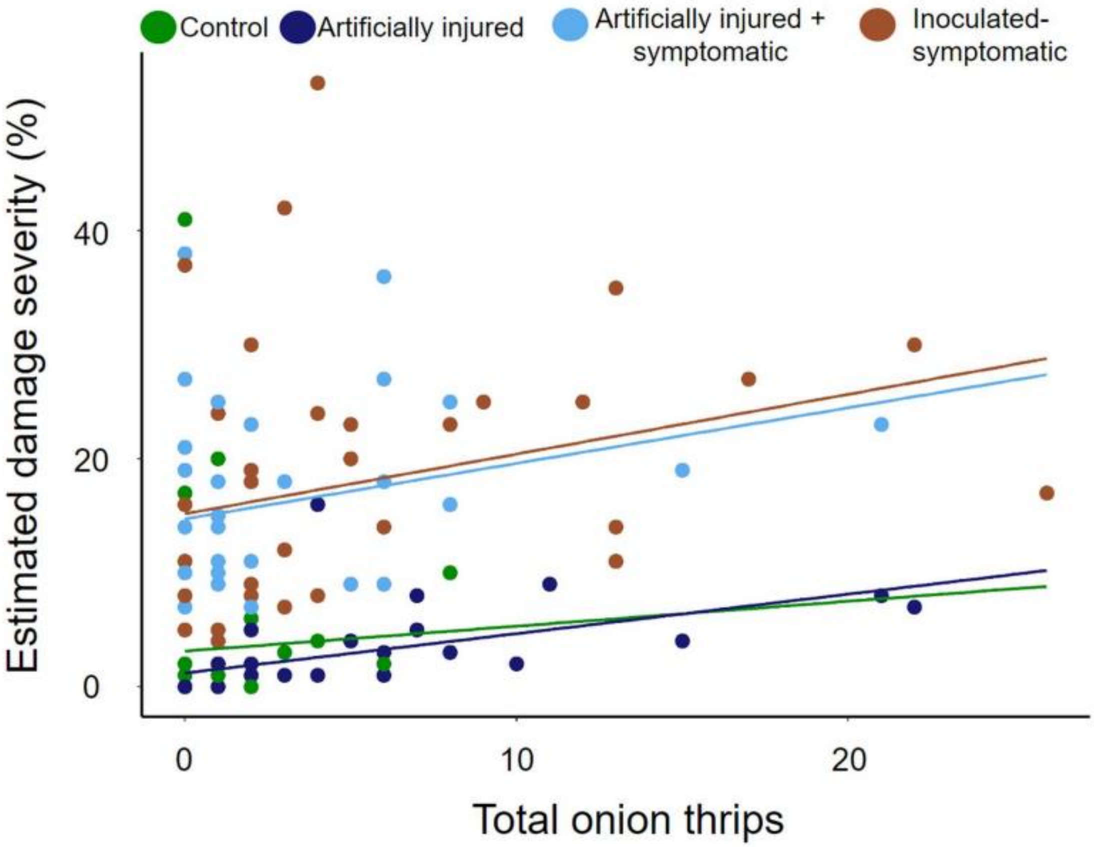

Figure 2.3. Correlation between onion plant damage severity (%) and the number of onion thrips on plants that were either healthy or infected with *C. coccodes*. Five adult onion thrips were added to a four-way choice test and after 13 days, the number of onion thrips were counted on each plant and the damage severity (%) was visually estimated.

Two-choice test

The number of onion thrips did not differ on inoculated-symptomatic and inoculated-asymptomatic onions (χ^2 = 2.61, df = 1, p-value = 0.106; Figure 2.4). When given a choice, there were 61% more onion thrips on inoculated-symptomatic onion compared to control plants (χ^2 = 30.72, df = 1, p-value < 0.001). There were approximately 66% more onion thrips on inoculated-asymptomatic onions when compared to control onions (χ^2 = 19.08, df = 1, p-value < 0.001).

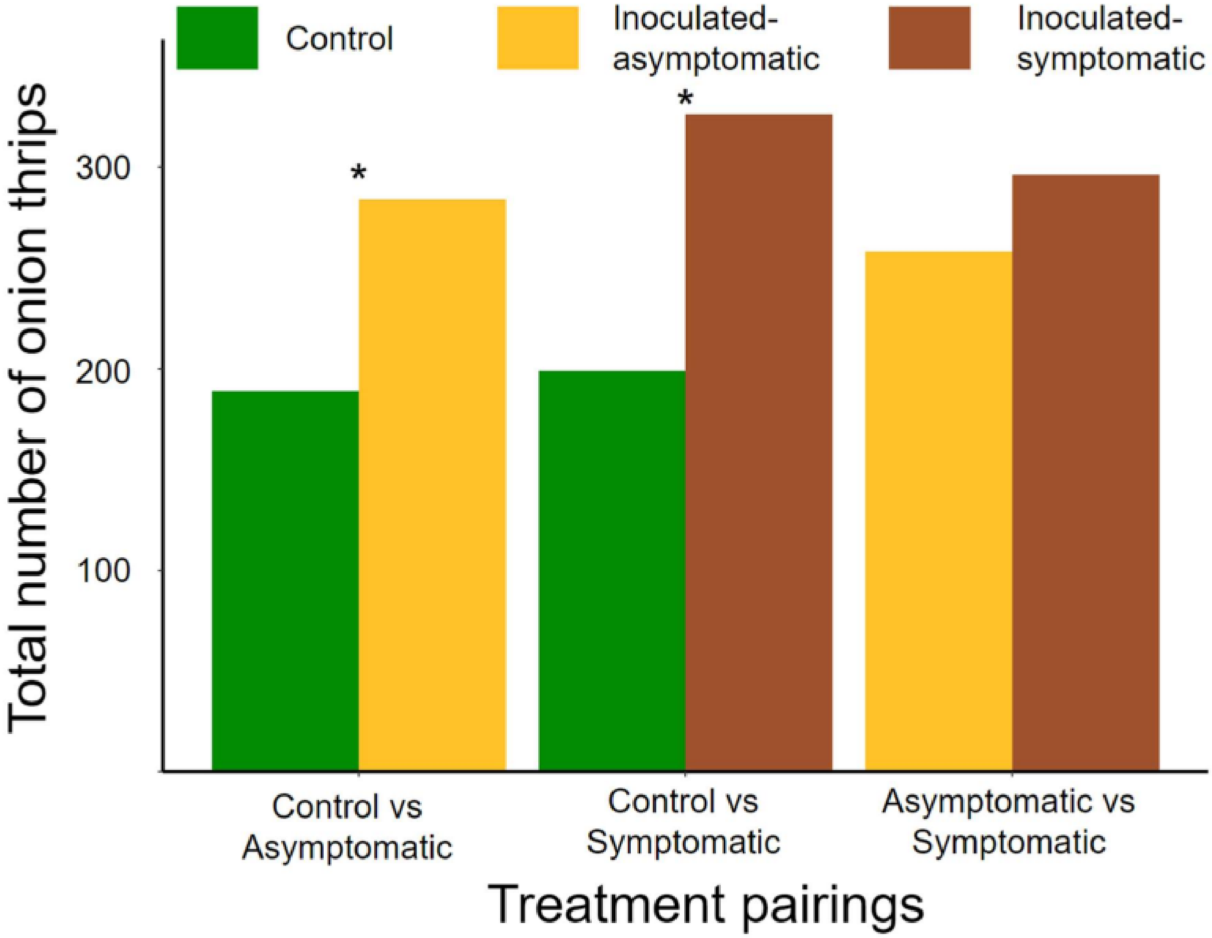

Figure 2.4. Total number of onion thrips on onion plants with three different levels of *C. coccodes* symptoms in two-way choice-tests (N = 30). Two 5-week-old plants were set up in experimental arenas and 5 adult onion thrips were added from a colony to each choice-test at the start of the experiment. Significant differences between choices are indicated with an asterisk (χ^2-test; $\alpha = 0.05$).

The damage severity (%) on each plant varied by plant treatment (F = 55.80, df = 2, 181, p-value < 0.001; Figure 2.5). There was a positive correlation between the number of thrips and damage severity (%) on control plants ($R^2 = 0.22$, p-value < 0.001). There was no correlation between onion thrips numbers and damage severity (%) on inoculated-asymptomatic plants ($R^2 = 0.18$, p-value = 0.095), or inoculated-symptomatic plants ($R^2 < 0.01$, p-value = 0.089).

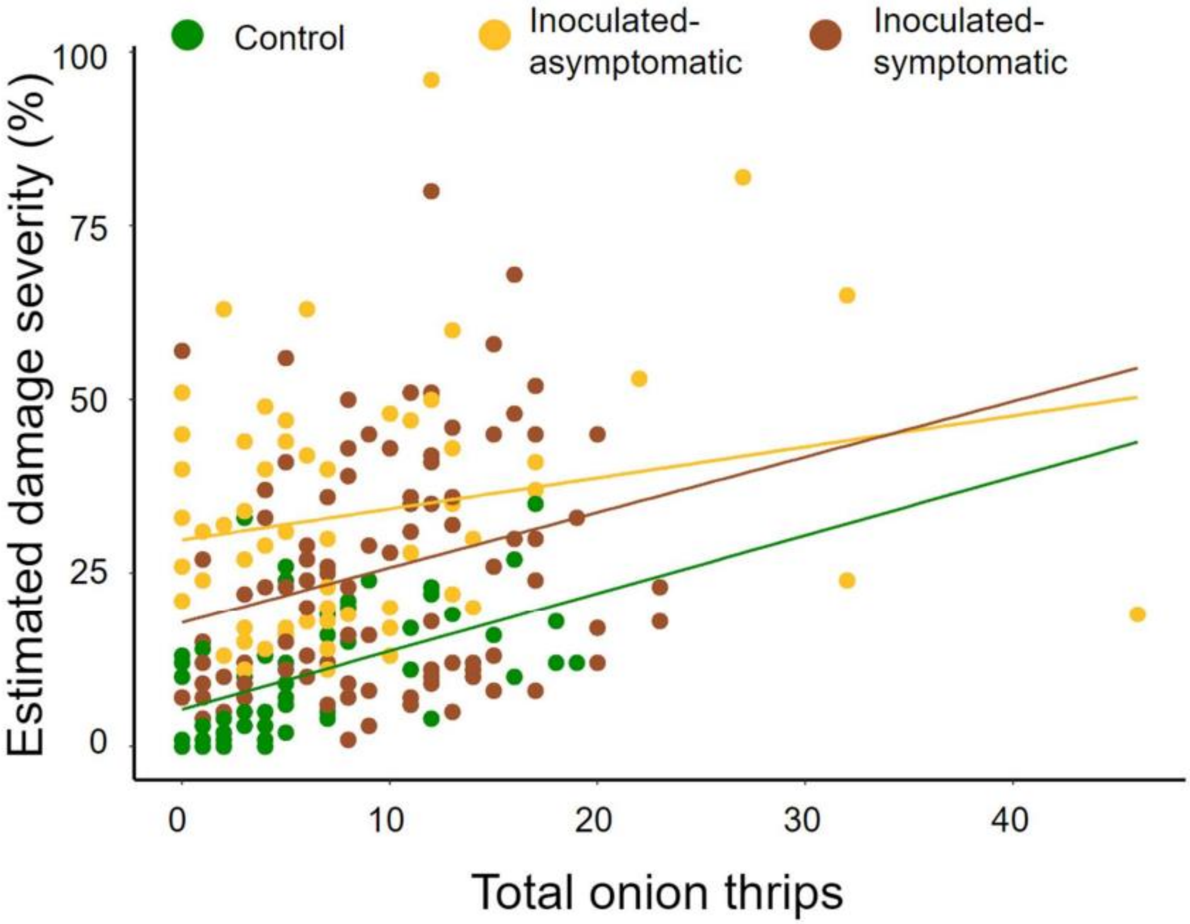

Figure 2.5. Correlation between onion plant damage severity (%) and the number of onion thrips per plant. Five onion thrips were added to a two-way choice test and plants were visually assessed for damage and the number of onion thrips were counted 13 days after the start of the experiment.

<u>No-choice test</u>

Across all life-stages, onion thrips had the greatest survival and reproduction on inoculated-symptomatic plants (F = 13.84; df = 2, 87, p-value < 0.001; Figure 2.6). Adult onion thrips survival rate was twice as high on inoculated-symptomatic plants (53%) than control plants (21%) (t-value = 3.02, df = 87, p-value =0.009). There was no difference in adult onion thrips survival rate when comparing control to inoculated-asymptomatic plants (t-value = 1.51, df = 87, p-value = 0.291) or inoculated-symptomatic to inoculated-asymptomatic plants (t-value

= 1.51, df = 87, p-value = 0.291). Inoculated-symptomatic onion plants had over twice as many larvae than control onion plants (t-value = 5.933, df = 87, p-value < 0.001) and 1.5 times more larvae than inoculated-asymptomatic plants (t-value = 3.567, df = 87, p-value = 0.006). There was no difference in the number of larvae on control and inoculated-asymptomatic plants (t-value = 2.109, df = 87, p-value = 0.094).

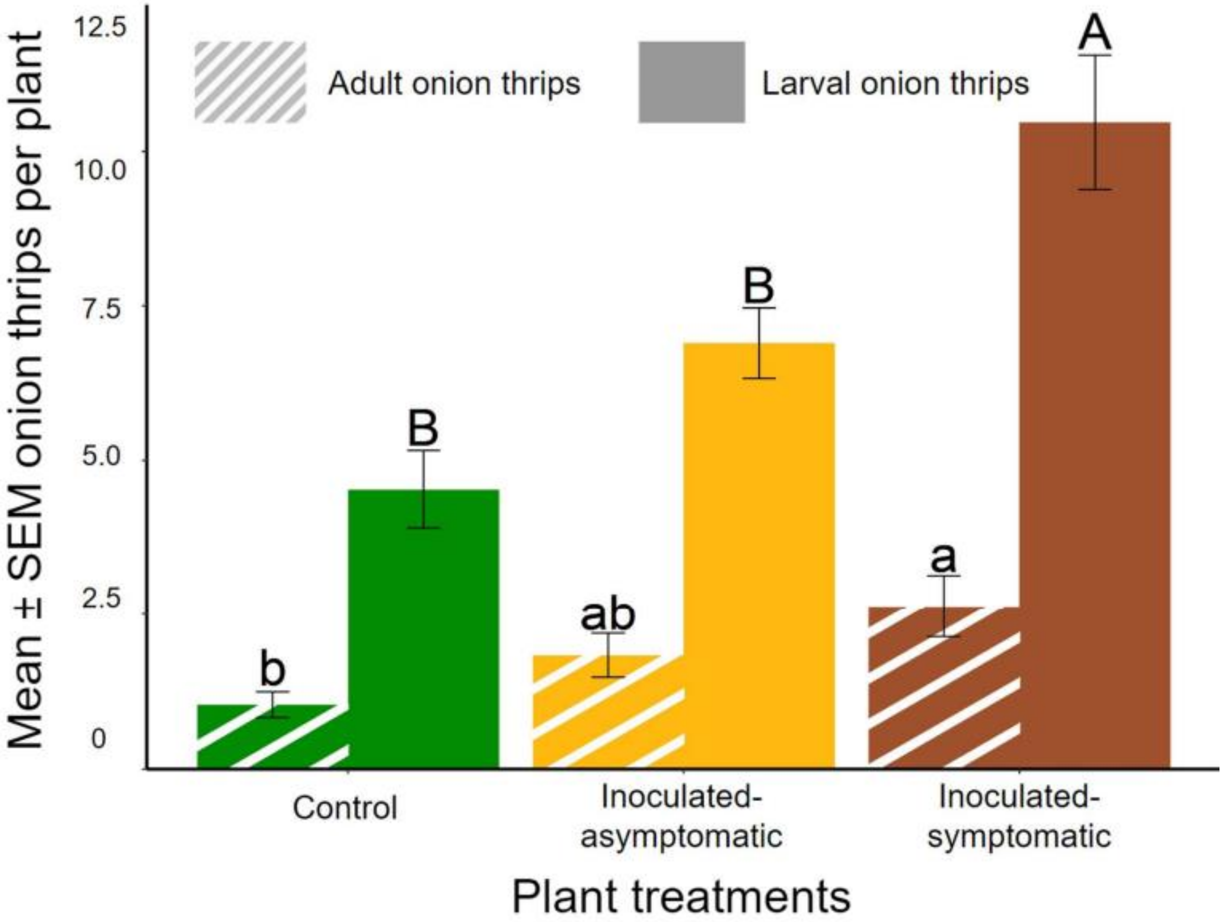

Figure 2.6. Mean (± SEM) adult and larval onion thrips per onion plant with three different levels of *C. coccodes* symptoms. Solid bars represent larvae and hashed bars represent adult onion thrips. Five adult onion thrips were added to a no-choice test with one plant of each treatment (N = 30). Thirteen days after the start of the experiment, adult and larval onion thrips were counted on each plant. Bars with different letters of the same case indicate significant differences (Tukey's HSD, α = 0.05).

Discussion

Onion thrips are an economically devastating pest of onions, and along with plant pathogens, cause major crop losses (Gill et al. 2015). The relationship between onion thrips and plant pathogens, such as *A. porri* and *P. ananatis,* is well documented, with onion thrips feeding often leading to increased pathogen spread and severity (McKenzie et al. 1993; Dutta et al. 2014; Grode et al. 2017, 2019). However, the impact of facultatively vectored pathogens on onion thrips behavior and fitness has not been well described compared to obligately vectored pathogens. Onion thrips can distinguish between onion plants based on phenotypic and biological attributes (Basri and Ansari 2021; Mo et al. 2008; Pobozniak et al. 2021). Our results further demonstrate that onion thrips can distinguish between onion plants based on plant health and infection status and they perform best on inoculated-symptomatic plants. These results suggest that onion thrips do not randomly transfer and open plants for pathogens to enter, but they may accelerate plant damage by preferentially feeding on plants that are already stressed by pathogens and then spread the pathogen to healthy plants.

In the four-choice test, onion thrips exhibited a strong preference for artificially injured onions and *C. coccodes* inoculated-symptomatic onions. Artificially injured + symptomatic onions were secondarily preferred, and control onions were the least preferred. The reasons for this may be due to changes in plant chemistry as many pathogens are known to induce defensive pathways that make plants more attractive to insects (Franco et al. 2017). Pathogens within the *Colletotrichum* genera are hemibiotrophic, and when infecting plant cells, they develop biotrophic hyphae that induce the salicylic acid pathway, but the hyphae become necrotrophic after successful colonization (Mendgen and Hahn 2002). Necrotrophic hyphae can trigger the jasmonic acid pathway (Boddy 2016) which is also induced by mechanical damage and onion thrips feeding (Abe et al. 2008; Howe and Jander 2008). Onion thrips may prefer artificially injured onions and inoculated-symptomatic onions because they induce the same response in onions as thrips feeding, thus signaling that the plant is an appropriate host. Onion thrips may have not preferred artificially injured + symptomatic plants when compared to artificially injured and inoculated symptomatic plants because the combination of multiple stressors may have primed plant defense responses thus making the plant an inadequate host. In addition, the artificial injury may be preferred because the injury itself causes increased stress responses and allows the pathogen to enter the plant more quickly than without any injury to the plant.

In the two-choice tests, onion thrips preferred *C. coccodes* inoculated host plants when compared to control plants but did not differentiate between inoculated-symptomatic and inoculated-asymptomatic hosts. This lack of difference may have been because by the end of the experiment all plants inoculated with *C. coccodes* exhibited signs of disease. Preference for infected hosts may be explained by both chemical and visual cues (Harris and Miller 1983; Visser 1988). While we did not measure changes in plant metabolites, all the inoculated plants looked visibly different than control plants towards the end of the experiments. However, there was no visible difference in damage severity between the inoculated-asymptomatic and inoculated-symptomatic plants (Figure S2.2). Plants inoculated with *C. coccodes* exhibited phenotypic changes like leaf necrosis, the development of tan lesions on leaves, had begun to sporulate and were visually different from the control plants (Supp. Figure S2.3). Changes in the plant phenotype may be important for host selection as some species of thrips orient based on visual cues (Teulon et al. 1999). For example, onion thrips prefer sticky traps that are blue or yellow, indicating that visual cues are important in pre-alighting behavior (Devi and Roy 2017). Thus, the change in leaf color in our study may have made the plant more attractive to onion thrips. In the future, increasing the frequency of sampling experimental units throughout the duration of the experiment to estimate the effect of disease progression on insect preference will be important in understanding the impact of time on their interaction.

In our four-choice test, there was a weak positive correlation between onion thrips numbers and damage severity (%) on control plants, artificially injured plants and inoculated-symptomatic plants. Similarly, in the two-choice tests, there was a positive correlation between onion thrips numbers and damage severity (%) on control plants, but a weak correlation on inoculated-asymptomatic and inoculated-symptomatic plants. In both experiments, all plants that were inoculated with *C. coccodes* were exhibiting signs of leaf necrosis 13 days after onion thrips exposure. It is possible that the pathogen is driving damage severity symptoms on leaves, while masking thrips damage.

The results from our no-choice experiment demonstrated that onion thrips survival and reproduction increased on inoculated-symptomatic plants. These results support the preference-performance hypothesis which suggests that adult preference regarding oviposition, correspond with larval performance (Jaenike 1978). Larval thrips have low mobility compared to adults, thus host plant selection by the mother is critical to larval growth (Gill et al. 2015; Clark et al. 2011). It is unclear why onion thrips development increases on inoculated-symptomatic onions compared to the control onions, but it may be due to food quality. In general, thrips are polyphagous feeders, and some consume pollen and fungal spores (Ananthakrishnan and Dhileepan 1984). It is possible that the increased performance on inoculated-symptomatic plants may be due to onion thrips consuming the *C. coccodes* mycelium, which may have provided an alternative food source. Onion thrips have been reported potentially feeding on fungal material from plants infected with powdery mildew, while it was not confirmed whether fungal material was ingested, onion thrips appeared to have destroyed established mildew colonies (Yarwood 1943). In our experiments, onion thrips may be consuming *C. coccodes* conidia or mycelia, resulting in overall increased performance, but we do not have evidence that such feeding behavior occurs in this species. Onion thrips are often associated with fungal pathogens (Leach et al. 2020; McKenzie et al. 1993; Yarwood 1943; Fermaud and Gaunt 1995), and when fungal pathogens infect host plant tissue, changes in metabolic profiles occur (Medina-Melchor et al. 2022). Future research that focuses on the relationship between onion thrips performance and facultatively vector pathogens, should investigate whether onion thrips are mycophagous and compare the metabolic profiles of healthy hosts to inoculated hosts.

Epidemiological theory suggests that vector preference based on host infection status may have a direct impact on pathogen spread (Kingsolver 1987; Sisterson 2008). For example, once *Rhopalosiphum padi* Linnaeus (Hemiptera: Aphididae) acquire Barley yellow dwarf virus (BYDV), the infective aphid prefers to feed on healthy plants, promoting transmission (Ingwell et al. 2012). Another aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), orient towards cucumber mosaic virus infected plants, but after feeding, disperse and prefer to feed on a non-infected plant (Ziebell et al. 2011). In both cases, the insect vectors initially orient towards infected plants until they acquired the virus, and then exhibit a feeding preference for healthy plants, thus increasing the probability of disease transmission. Onion thrips are not obligate vectors of *C. coccodes,* yet they exhibit a preference for infected and injured hosts. Previous studies regarding onion thrips and facultative pathogens (Leach et al. 2020; Yarwood 1943; McKenzie 1993; Fermaud and Gaunt 1995; Grode et al. 2017; Dutta et al. 2014) indicated a close association between pests, thus further experiments investigating how onion thrips preference affects fungal pathogen spread in the field should be conducted. While we observed a preference for and increased performance on *C. coccodes* inoculated plants, different fungal, bacterial, or viral pathogens may interact with onion thrips differently. Therefore, we cannot assume that results with one fungal pathogen are generally applicable, and research should continue to look at insect vectors and pathogens on species specific levels.

In conclusion, a clear understanding of the relationship between host preference and disease dynamics may provide valuable insight into pest management for growers. When considered independently, both onion thrips feeding and foliar plant pathogens reduce the amount of photosynthetic tissue, resulting in lower onion yields, but in the field, it is difficult to differentiate between damage types as they occur simultaneously (Kendall and Capinera 1987; Fournier et al. 1995; Diaz-Montano et al. 2010; Rodriguez-Salamanca 2013; Gill et al. 2015). Therefore, to maximize onion yields, we need to develop a pest management strategy that considers the interactions between pests, rather than focusing on managing pests in isolation. Possible management strategies could include the disruption of host plant selection by the insect through either chemical or visual cues, therefore reducing how frequently the insect and the pathogen are in contact. Additionally, the development of new pest management thresholds which consider multiple pests simultaneously may also help increase crop yield. Understanding the interactions between multiple pests is key to optimizing integrated pest management strategies.

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APPENDIX 1: CHAPTER 2 SUPPLEMENTARY MATERIALS

Supplementary material

Figure S2.1. A photo of a symptomatic onion leaf prior to onion thrips exposure. Plants designated as inoculated-symptomatic were inoculated with a *Colletotrichum coccodes* foliar spray 7 days prior to onion thrips exposure. All plants assigned as inoculated-symptomatic had typical signs of *C. coccodes* infection, such as developing tan lesions.

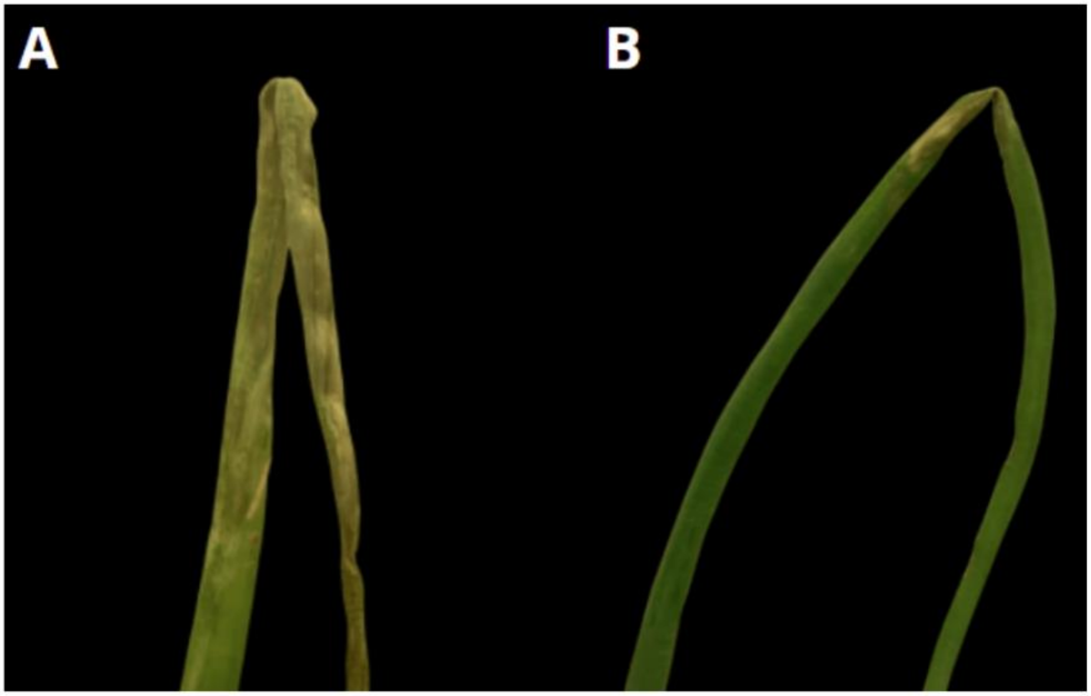

Figure S2.2. A comparison of inoculated-symptomatic (A) and inoculated-asymptomatic (B) onion leaves 7 days after onion thrips exposure. A) Symptomatic plants had tan lesions and leaf necrosis. B) Inoculated-asymptomatic plants had small tan lesions with less severe symptoms compared to the symptomatic plants. Thirteen days after onion thrips exposure, the inoculated-symptomatic and inoculated-asymptomatic plants had similar levels of disease severity.

Figure S2.3. Inoculated-symptomatic onion leaf at the end of the choice tests, 13 days after onion thrips exposure. Inoculated-symptomatic plants at the start of the experiment had severe signs of *Colletotrichum coccodes* infection, including necrosis and tan lesions that were sporulating.

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CHAPTER 3: ENVIRONMENTAL CONDITIONS CAN INDIRECTLY AFFECT FACULTATIVE PATHOGEN SPREAD BY ALTERING INSECT VECTOR DISPERSAL

Abstract

Climate change impacts agriculture through shifts in regional environmental conditions, significantly altering insect vector and plant pathogen interactions. These pests often have a positive density dependent relationship, but for pathogens that do not rely on insect vectors, the relationship can be more difficult to predict. Furthermore, environmental conditions, such as temperature and relative humidity, can indirectly alter pathogen spread by impacting vector reproduction or behavior. Therefore, studies examining the interactions between pathogens and insect vectors under varying environmental conditions are critical to understanding pathogen spread. Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) are the main insect pest of onion (*Allium cepa*) and co-occur with many pathogens including *Colletotrichum coccodes* Wallr (Glomerellales: Glomerellaceae), a fungal pathogen of onion. We conducted experiments to understand how different densities of onion thrips and environmental conditions affect pathogen spread. To determine how onion thrips density affects pathogen spread, we released 0, 5 or 25 onion thrips on *C. coccodes* inoculated plants, that were symptomatic for disease, and allowed them to move between the symptomatic and healthy plants. To determine the effects of environmental conditions on pathogen spread, we altered temperature and relative humidity and added either 0 or 5 onion thrips to the experiment. Onion thrips dispersal, damage distribution, and pathogen spread 13 d after onion thrips were released were assessed for each study. Our results indicate that *C. coccodes* was only disseminated in the presence of onion thrips and that onion thrips dispersed more at high population densities. Pathogen spread also increased at high temperatures, regardless of relative humidity. Overall, our study highlights how changes in insect abundance, which can be directly affected by environmental conditions, can alter pathogen

spread.

Introduction

Environmental conditions can have a significant influence on insect vector and plant pathogen interaction (Jones & Barbetti 2012; Peters et al. 2022). One impact of climate change on agriculture is a shift in regional environmental conditions that can alter pathogen-vector dynamics. However, studies that investigate these interactions in agricultural systems are lacking, particularly for facultative pathogens (Anderson et al. 2020; Peters et al. 2022; Jones & Barbetti 2012; Jones 2016). Depending on the pathosystem, climate change features like unpredictable changes in temperature and relative humidity, are expected to have a significant influence disease severity, which could have a direct impact or indirect impact on pathogen spread by altering vector traits, including abundance.

An increase in insect vector abundance often leads to increases in disease severity and incidence through direct density dependent mechanisms (Grode et al. 2017, 2019; Leach et al. 2020; Yarwood 1943). For obligate plant pathogens, the positive relationship between vector abundance and pathogen spread is predictable (Jeger 2020; Lowery et al. 2015; Shaw et al. 2017). In the case of facultative pathogens that do not exclusively rely on insect vectors, this density dependent relationship between insects and pathogen spread is less predictable because the pathogen can be disseminated with or without the vector. For example, insect feeding wounds provide entry points for infection and insects can mechanically vector pathogens; in the absence of an insect vector, mechanical wounding by soil particles and spread via rain splash provide an alternative infection route (Tiberi et al. 2016; Eigenbrode et al. 2018). Thus, reducing insect vector numbers will reliably reduce disease severe and occurrence when pathogen spread is insect dependent. However, when pathogens do not rely on insect vectors, it is less clear how much insects contribute to pathogen spread and disease severity. In agricultural studies, controlling insect vector populations through insecticides often leads to reduced pathogen spread and disease severity; this has been demonstrated for some fungal pathogens, highlighting the importance of understanding the strength of the relationship between vector and pathogen (Constancio et al. 2023; Leach et al. 2020; Grode et al. 2019; Mailhot et al. 2007). The addition of a fungicide along with the insecticide treatments often further reduces disease symptom severity and incidence without further reducing insect populations, suggesting that while insects contribute to pathogen spread, there are other factors contributing to increased disease occurrence and severity within these systems (Constancio et al. 2023; Mailhot et al. 2007). While insect population dynamics are important for determining plant pathogen dynamics, the role of factors that impact insects and their relationship with the pathogen needs further investigation.

As ectotherms, insect development is dependent on ambient temperature, with warmer temperatures leading to positive changes in growth, developmental rates, increased plant material consumption, and reduced time between generations (Cornelissen 2011; Jamieson et al. 2012; Lemoine et al. 2013). Consequently, in agroecosystems, higher temperatures may indirectly lead to pathogen spread via increases in insect vector abundance. Moreover, temperature and humidity often increase concurrently, with high relative humidity often creating conditions that may favor high disease severity (Velásquez et al. 2018; Singh et al. 2023). For some fungal pathogens, moisture is necessary for conidial release from the acervulus, and splashing rain can spread conidia to nearby susceptible plants (Dillard 1989). Additionally, high relative humidity is important for increased fecundity and pupation success of some insects (Steiner et al. 2011; Garrick et al. 2016). Therefore, increased relative humidity may have an indirect effect on pathogen spread by altering the vector reproduction. Overall, the importance of relative humidity and temperature in the case of plant pathogens, like foliar fungal pathogens, is not well understood and studies examining its indirect impact on pathogen spread are lacking.

The fungal pathogen, *Colletotrichum coccodes,* is a pathogen of potatoes and tomatoes, and more recently has been found in onions (*Allium cepa*) (Rodriguez-Salamanca et al. 2012). It was first identified in Michigan onion fields in 2012, since then it has continued to occur and was later identified in Ohio and New York (Rodriguez-Salamanca et al. 2012; Baysal-Gurel et al. 2014; Hay et al. 2016). *Colletotrichum coccodes* is spread through water-splash, which can occur during rain events or overhead irrigation, both events leading to increases in disease severity (Dillard 1989). Onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae), are the main insect pests of onions, and vector many different plant pathogens (Yarwood 1943; Bhangale & Joi 1983; Ulman et al. 1997; Dutta et al. 2014; Eigenbrode et al. 2018). While onion thrips and *C. coccodes* may be found concurrently in onion fields, it is unclear how onion thrips contribute to the spread of *C. coccodes* and the impact of environmental conditions.

We determined the effects of onion thrips populations and environmental conditions on disease dynamics using laboratory-based experiments by first manipulating initial onion thrips densities and then altering temperature, and relative humidity. To better understand the density dependent relationship between onion thrips and *C. coccodes* transmission, we placed 0, 5 or 25 adult onion thrips in experimental units consisting of one *C. coccodes* inoculated onion and three non-inoculated onions. We hypothesized that more onion plants would become infected by *C. coccodes* at higher onion thrips intial densities due to increased mechanical vectoring of *C. coccodes* conidia. To address how a change in environmental conditions affects onion thrips behavior and pathogen spread, we used a similar experimental design and released 0 or 5 adult onion thrips on one *C. coccodes* inoculated onion and two non-inoculated onions, while manipulating temperature and relative humidity. We hypothesized that increased temperature and high relative humidity would lead to increased pathogen transmission among the non-inoculated plants.

Methods and materials

Onion thrips colony

Onion thrips from a commercial, conventionally managed onion field (Allegan, MI) were collected and used to start a colony at Michigan State University (East Lansing, MI) in August 2022. The colony was maintained in a mesh cage (50 cm^3; MegaView, Taichung, Taiwan), and kept at 22℃ and a 16:8 hr L:D cycle. Onion thrips were allowed to feed *ad libitum* on onion grown from certified organic onion seeds (*A. cepa* cv. 'New York Early'; Johnny Seeds, Winslow, ME). Each week, ten onions at the 5-7 leaf stage were added to the cage to provide fresh foliage for continuous rearing. The onion thrips colony was maintained in these conditions prior to the experiment initiation.

Colletotrichum coccodes inoculation

Colletotrichum coccodes was isolated from plants grown in the same field (Allegan, MI) in August 2020. Prior to the experiments, the isolate was maintained on 25% strength potato dextrose agar (PDA) media. The isolate was first identified morphologically based on sclerotia and conidial shape (Cano et al. 2004) and confirmed using the ribosomal internal transcribed spacer region in a nested PCR (Cullen et al. 2002). The culture plates were visually assessed for cross contamination by other pathogens (Cano et al. 2004). Isolates were grown on full-strength PDA for 7 d prior to inoculation. The Petri dish was flooded with double distilled water and conidia were dislodged from the media by scraping a sterile tongue depressor across the top. The onion seedlings were then inoculated with the suspension (2.5×10^5 conidia/ml) 7 d prior to onion thrips exposure. The conidial suspension was sprayed onto the onion foliage until run off (Rodriguez-Salamanca et al. 2018). All applications were made with a 250 ml plastic hand spray bottle (Meijer Inc., Grand Rapids, MI).

Experiment 1: Effect of onion thrips initial density on pathogen spread

Organic onion seeds (cv. ‘New York Early’) were planted in a 50-cell seedling tray and fertilized (125 ppm, 20-20-20 N-P-K, Jack’s General Purpose Water-Soluble Fertilizer; JR Peters, Inc., Allentown, PA) once a week. The seedlings were grown for 4 wk, until the 3-leaf stage. Four seedlings were transplanted into aluminum trays (30 x 25 x 8 cm, Handi-Max, Wheeling, IL) approximately 10 cm apart in a straight line, arranged so that the foliage was not touching, and assigned a location from 1 to 4 (1 = first seedling in the line; 4 = last seedling) (Figure 3.1). After transplanting to aluminum trays, seedlings were allowed to grow for an additional 14 d, until the 5-leaf stage, prior to onion thrips exposure to ensure survival.

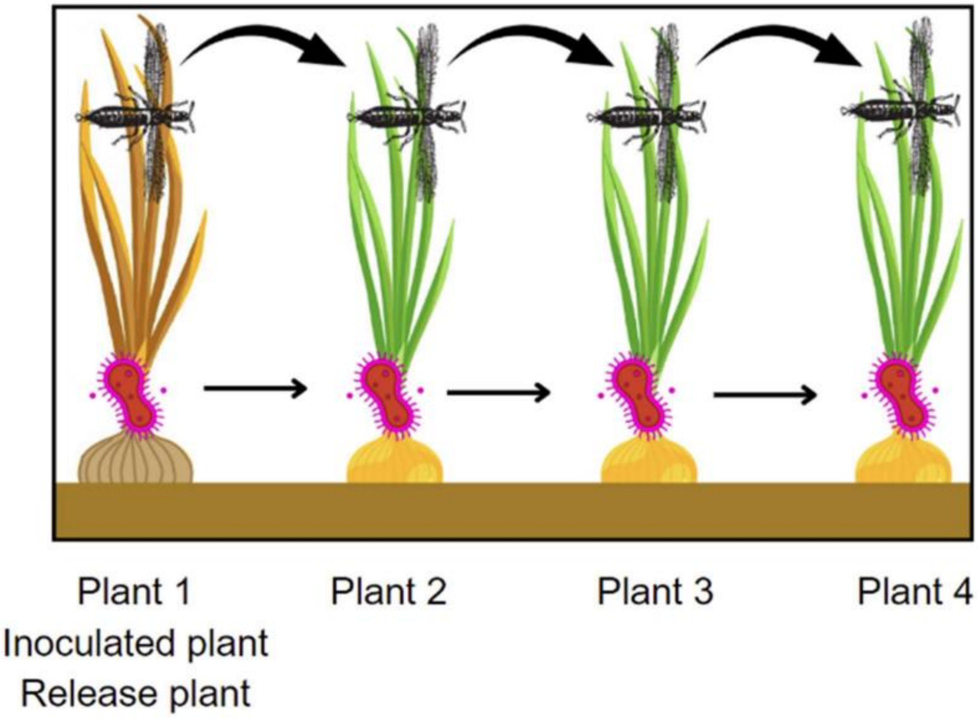

Figure 3.1. Onion plants at the 3-4 leaf stage were placed in a straight line, approximately 10 cm apart in a tray. Plants in location 1 were inoculated with *C. coccodes* and onion thrips were released on this plant. Onion thrips movement and pathogen spread was assessed at the end of the 13 d experiment. A cylindrical cage with a mesh top was placed over the top of the plants, preventing onion thrips movement among experimental units.

A large cylindrical plastic cage made from transparency film (School Smart, Greenville, WI; 26 cm tall, 15 cm radius) was placed over each tray so that onion thrips could move freely among plants within a tray but not between trays. To prevent onion thrips movement between the aluminum trays but still allow air movement, the top of the cage was covered with a nylon mesh (160 µm, MegaView, Lehigh Acres, FL). Each tray was assigned to one of four treatment combinations: 1) mock inoculated + 5 onion thrips (N = 20), 2) mock inoculated + 25 onion thrips (N = 20), 3) inoculated + 5 onion thrips (N = 20), 4) inoculated + 25 onion thrips (N = 20), 5) mock inoculated + 0 onion thrips (N = 10) and, 6) inoculated + 0 onion thrips (N = 10) (Table 3.1). Aluminum trays were placed in a randomized unbalanced block design in the environmental chamber with a reduced number of control treatments (treatments 5 and 6 in Table

3.1, N = 10) and watered every 3 d. The 5-onion thrips initial density corresponds to the action threshold (one onion thrips/leaf) for most insecticides used for onion thrips management (Rueda et al. 2007; Nault & Shelton 2010; Atia et al. 2021). For treatments that included inoculated plants (treatments 3 and 4), 7 d before onion thrips exposure, onion plants in location 1 were inoculated with *C. coccodes*, and visually assessed disease symptomatic when exposed to onion thrips. To prevent pathogen exposure to the remaining three plants within the tray, the onion plant was isolated within a cylindrical plastic tube (30 cm tall, 3 cm radius) with an open top, and the inoculum was applied to the onion foliage through the top of the tube. The mock-inoculated plants were sprayed with double distilled water until the foliage was fully wetted.

Table 3.1: List of treatments in each onion thrips experiment. The temperature column is the temperature of the growth chamber the experiment took place in, RH is the relative humidity within the experimental arenas, inoculation status is whether the plant in location 1 was inoculated with *C. coccodes* and the onion thrips added column is the number of onion thrips added at the start of the experiment. N represents the total number of replicates for each treatment.

Experiment	**Treatment number**	**Temperature (°C)**	**RH**	**Inoculation status**	**Onion thrips added**	**N**
Effect of onion thrips initial density on pathogen spread	1	25	NA	Mock	5	20
	2	25	NA	Mock	25	20
	3	25	NA	Inoculated	5	20
	4	25	NA	Inoculated	25	20
	5	25	NA	Mock	0	10
	6	25	NA	Inoculated	0	10
Effects of environmental conditions on pathogen spread	1	22	Low	Inoculated	5	20
	2	22	High	Inoculated	5	20
	3	33	Low	Inoculated	5	20
	4	33	High	Inoculated	5	20
	5	22	Low	Inoculated	0	5
	6	22	Low	Mock	5	5
	7	22	High	Inoculated	0	5
	8	22	High	Mock	5	5
	9	33	Low	Inoculated	0	5
	10	33	Low	Mock	5	5
	11	33	High	Inoculated	0	5
	12	33	High	Mock	5	5

Five or 25 adult onion thrips were randomly selected from the colony and placed in a 1.7 ml microcentrifuge tube (Thomas Scientific, Swedesboro, NJ) with a fresh piece of onion foliage (3 cm) for nourishment. The onion thrips vials were kept in a cooler for a maximum of 1 h prior to being released into the aluminum trays. Vials with onion thrips were randomly assigned to treatments, empty vials were placed in treatments without onion thrips. All vials, regardless of treatment, were placed at the base of plants in location 1, and opened, allowing onion thrips free movement. Adult and larval onion thrips per plant and estimated damage severity (i.e. amount of tissue damage per plant, rated 0-100%) was recorded 13 d after onion thrips exposure. To count the number of adult and larval onion thrips per plant, the cages were removed, the onions were uprooted, and the foliage was pulled apart to check the neck of the plant for onion thrips. Damage severity was visually estimated on a scale of 0-100% by estimating the amount of necrotic tissue on the plant caused by onion thrips feeding and pathogen damage.

To determine whether onion thrips had spread *C. coccodes* from the inoculated onions to the non-inoculated onions, experimental units were randomly selected for pathogen testing (N=5). A small portion of damaged onion foliage (3 cm) was removed from the onion and surface sterilized by placing in a 10% bleach solution for 20 s and then placed in double distilled water for 20 s to rinse the bleach. Each onion sample was surface sterilized twice to ensure that only pathogens within the onion tissue were being sampled. After surface sterilizing, onion tissue was placed on 25% strength PDA Petri dish and any fungal growth that occurred was isolated and placed on a fresh Petri dish to create pure cultures. Pure cultures were then assessed for the target pathogen by visually comparing the culture morphology to a known *C. coccodes* isolate to confirm pathogen identity. The presence or absence of *C. coccodes* was then recorded for each plant that was sampled.

Experiment 2: Effects of environmental conditions on pathogen spread

Onion plants were grown from seed as previously described. Three seedlings were transplanted in the same aluminum trays as described above and assigned location numbers 1-3. After transplanting to the trays, the seedlings were grown for 14 d prior to onion thrips exposure and covered with cylindrical plastic cages. The trays were placed in a randomized unbalanced block design with treatments 1-4 replicated 20 times and treatments 5-12 replicated five times (Table 3.1).

Temperature and relative humidity (RH) were modified using two environmental chambers set to either a high (33℃) or low temperature (22℃). To mimic fluctuating temperatures throughout the day, each growth chamber was set to the following schedule: 0801-2000 hr hold at 33 or 22℃, 2001hr – 2200 hr decrease to 23 or 12℃, 2201-0600 hold at 23 or 12℃, 0601-0800 increase to 33 or 22℃. Relative humidity was increased by covering the top of the cage with a plastic film to retain high moisture levels. Relative humidity was monitored by placing a hygrometer (AikTryee; Shenzhen Youkeshu Technology, Shenzhen, China) within the cages and recording the value every 48 hr. The high relative humidity treatments fluctuated between 80-95%, and the low relative humidity treatments fluctuated between 30-50%, regardless of temperature.

There were 12 treatments which varied in temperature, relative humidity, inoculation status and onion thrips presence or absence (Table 3.1). The following treatments were included: 1) low temperature + low RH, 2) low temperature + high RH, 3) high temperature + low RH, and 4) high temperature + high RH. Each treatment had 0 or 5 thrips. Treatments without onion thrips served as a control. Plants in location 1 were inoculated or mock inoculated as previously described. Including inoculated and mock inoculated controls allowed the differentiation of plant damage due to onion thrips feeding or the pathogen. Any damage observed on mock inoculated plants was attributed to onion thrips feeding.

Thirteen d after onion thrips exposure, adult and larval onion thrips numbers and the estimated damage (%) per plant were recorded following the same procedure as above. Additionally, *C. coccodes* presence or absence was recorded for all plants from each treatment by collecting the plants and following the same protocol as described above to identify the pathogen in culture.

Data Analysis

To determine how varying onion thrips density or environmental conditions affect onion thrips movement and estimated plant damage among plants, we used Generalized Linear Mixed Models (GLMMs). All models were created in R (R Core Team 2023) using the glmmTMB package (Brooks et al. 2017), and the ‘drop1’ function for evaluating significant main and interactive fixed effects. For models with significant interactive effects ($\alpha = 0.05$), the ‘lstrends’ function (Lenth et al. 2024) was used to determine if there were significant differences between treatments by comparing the slopes of the lines. Slopes closer to 0 indicate that the distribution of the dependent variable is more evenly distributed among plants.

Effect of onion thrips initial density on pathogen spread

Thrips dispersal — We first tested whether onion thrips distribution is different between life stages by creating two models, with either adult or larval onion thrips per leaf as the dependent variable. We then compared models with the onion thrips initial density (0, 5 or 25 adult onion thrips) and inoculation status interacting with plant location as the fixed effects to determine whether initial density changed thrips distribution among the four plants. Additionally, including a variable representing inoculation status interacting with plant location allowed us to determine if pathogen presence or absence altered onion thrips distribution among the four plants. Finally, we included a fixed effect that was an interaction between inoculation status and initial onion thrips density to determine whether pathogen presence or absence affected onion thrips distribution at varying onion thrips numbers. For all models, block was used as a random effect, and a negative binomial distribution was used.

Pathogen spread and plant damage — A Chi-square test was performed to determine if there were differences in the total number of plants positive for *C. coccodes* at varying onion thrips densities. The total number of plants positive for *C. coccodes* was compared to the expected number of plants positive (the total amount of plants positive for *C. coccodes* was the same between treatments).

To assess how varying onion thrips initial density impacted the plant damage, we built a model that compared how the damage per plant varied by plant location interacting with onion thrips initial densities or inoculation status, as well as an interaction between onion thrips initial density and inoculation status. For the model, block was the random effect, and we used a beta distribution.

Effects of environmental conditions on pathogen spread

To understand how varying environmental conditions affected onion thrips distribution, we evaluated models with adult or larval onion thrips per leaf as the dependent factor and plant location interacting with temperature or relative humidity as the independent factors. Additionally, we included inoculation status (inoculated or mock inoculated) as a fixed factor, interacting with plant location to assess whether *C. coccodes* presence or absence significantly affected the dispersal of adult or larval onion thrips. For both models, block was used as the random factor, and we used a negative binomial distribution.

To assess whether varying environmental conditions impact the damage distribution among plants within experimental arenas, we evaluated models with estimated damage as the dependent factor and plant location interacting with temperature or relative humidity as the independent variables. We also included inoculation status interacting with plant location to determine if *C. coccodes* presence or absence affected the damage distribution among the three plants. Finally, we assessed whether onion thrips presence or absence affected damage distribution by comparing models with or without onion thrips interacting with plant location. For all models, block was used as the random factor, and we used a beta distribution.

A Chi-square test was performed to determine if there were differences in the total number of *C. coccodes* positive plants at varying temperatures and relative humidity. The total number of *C. coccodes* positive plants was compared to the expected number of plants positive. For each Chi-square, the first plant (i.e., the inoculated plant) was removed from the analysis because that plant was experimentally inoculated with *C. coccodes* and positive for *C. coccodes.*

Results

Effect of onion thrips initial density on pathogen spread

Increasing onion thrips initial density significantly changed the dispersal patterns of onion thrips among the four plants (Table 3.2, Models 1, 2). In treatments where 5 adult onion thrips were released on the first plant (i.e. low onion thrips initial density), the adult onion thrips dispersed evenly across all plants (slope = 0.02), while in treatments with 25 onion thrips released, adult onion thrips moved away from the release plant and colonized plants in locations 2, 3, and 4 (slope = 0.32) at a three times higher rate comparatively (Figure 3.2A; t-value = -1.978, df = 80, p-value = 0.048). Interestingly, the opposite patterns were observed for the larval onion thrips. In low onion thrips initial density treatments, larval onion thrips were distributed primarily on plants in the first location, and the larval populations were reduced by as much as 60% on the fourth plant (slope = -0.40). In contrast, in the high onion thrips initial density treatments, larval onion thrips were relatively evenly distributed among all four plants (Figure 3.2B; slope = -0.05; t-value = -3.897, df =80, p-value < 0.001). Inoculation status did not significantly affect onion thrips dispersal among plants within the trays, and the interaction between inoculation status and onion thrips initial density was not significant (Table 3.2, Models 1,2).

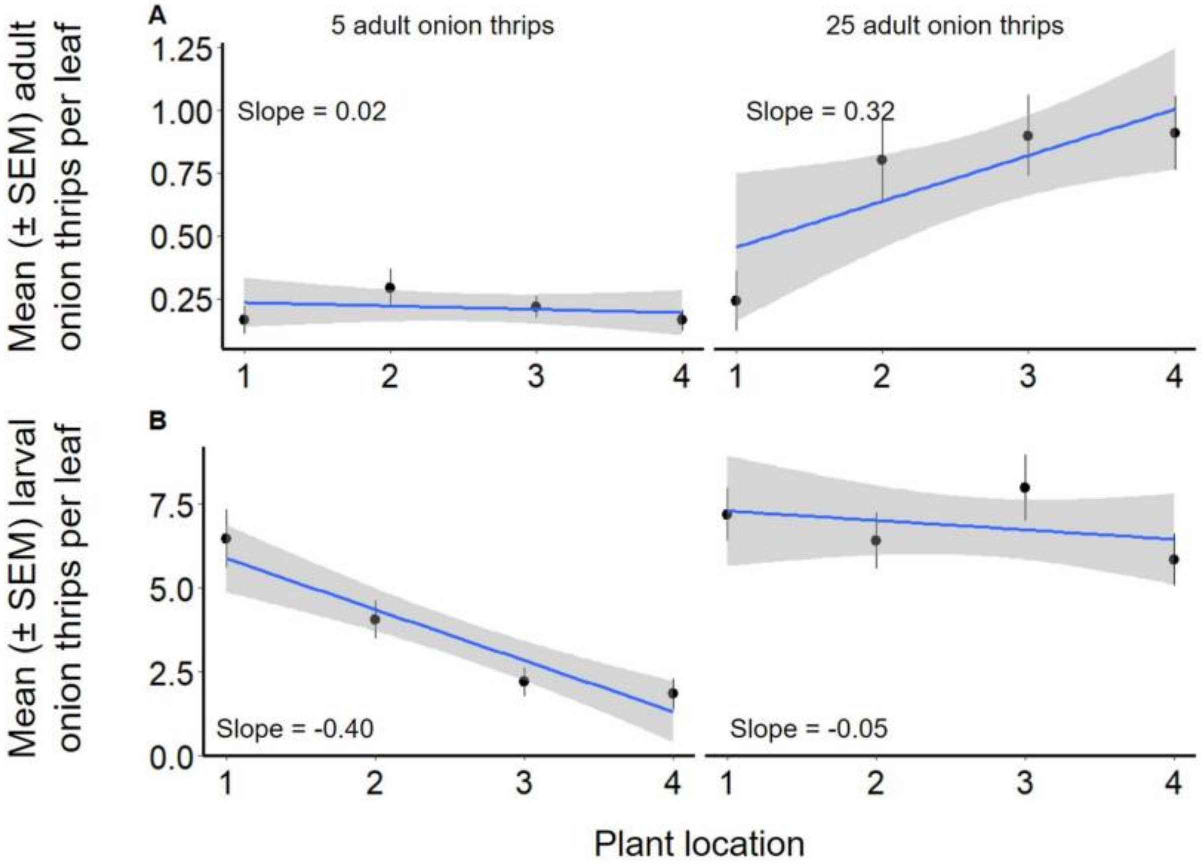

Figure 3.2. Varying numbers of onion thrips (5 or 25 adult onion thrips) were released on plant in location 1, and 13 d later, the number of adult and larval onion thrips per leaf was recorded along with the location of the plants. The shaded areas represent 95% confidence intervals created using a linear regression. A) Mean (± SEM) adult onion thrips dispersal among the four plants at different onion thrips starting densities. B) Mean (± SEM) larval onion thrips dispersal among the four plants at different onion thrips starting densities.

There were significant differences in the distribution of plant damage among the four plants within the experimental arenas based on onion thrips initial density (Table 3.2, Model 3). Treatments that received no onion thrips (pathogen only) had the most damage on the first plant and no damage on the remaining plants (slope = -0.24). Treatments with five onion thrips had about 50% damage reduction between the first and second plants, and almost a 65% reduction in damage on third and fourth plants when compared to the plant in the first location (slope = -0.88). The damage distribution of the treatment without onion thrips was 72% more evenly distributed compared to the damage distribution of the five onion thrips treatment, indicating a more even damage distribution among plants in the treatment without thrips (Figure 3.3A; t-value = 6.153, df = 60, p-value < 0.001). Similarly, the same pattern was observed in the high onion thrips density compared to the no onion thrips treatments, with damage more evenly distributed among plants in treatments without thrips (t-value = 4.057, df = 60, p-value < 0.001). Damage was about 33% more evenly spread among all four plants at the 25 onion thrips initial density (slope = -0.66) compared to plants in the 5 onion thrips treatments (t-value = -2.545, df = 80, p-value = 0.030).

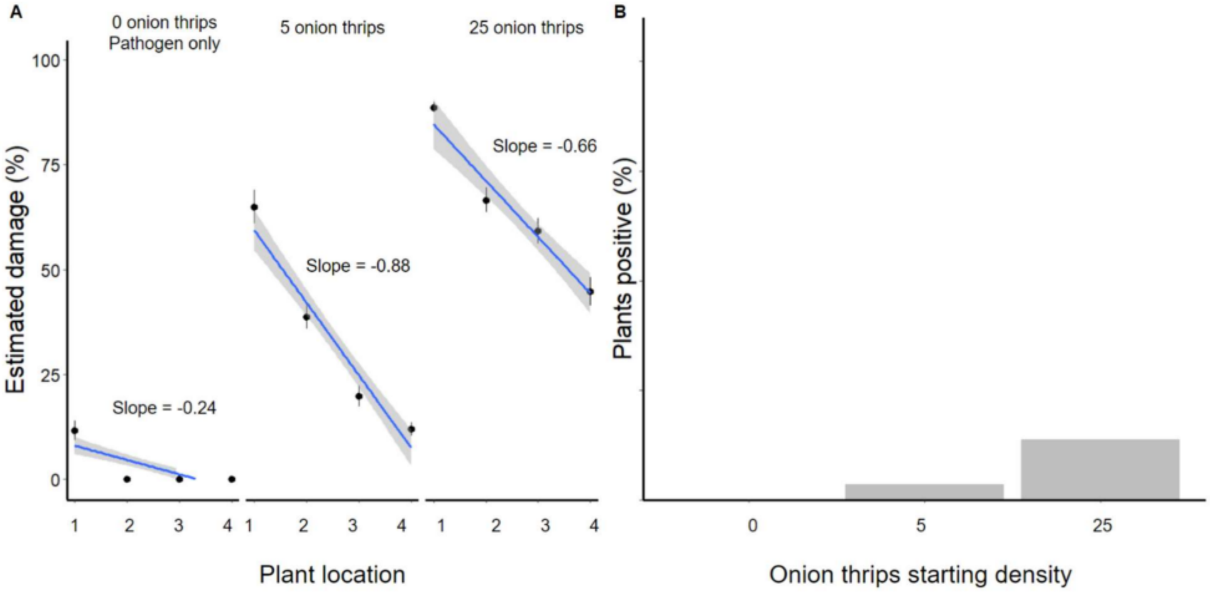

Figure 3.3. Varying numbers of onion thrips (0, 5 or 25 adult onion thrips) were released on plants in location 1 and 13 d later, the amount of damage per plant by location was visually estimated on a 0-100% scale. A subset of plants (N=5) was then tested for *C. coccodes* to determine if infection occurred. A) The estimated damage (%) on each of the four plants in the experiment. The shaded areas represent 95% confidence intervals created using a linear regression. B) The percentage of plants positive for *C. coccodes* at each starting onion thrips density.

Table 3.2: The models considered in the analysis of varying onion thrips initial density in the first experiment. Fixed effects included: 1) location: location of plant within the aluminum tray, 2) inoculation: inoculation status with/without *C. coccodes*, and 3) initial density: initial number of onion thrips added. The full model was compared with the model resulting from the single term deletion (i.e. removal of the specific interactive effect). The AIC (Akaike information criterion), Chi square, and P value (α = 0.05) were used to determine significant interactions.

Model #	Full model	Fixed effects single term deletion	AIC	Chi square	P value
1	Adult thrips ~ location*inoculation + location*density+ inoculation*density + (1|block)	Location*inoculation	827	0.12	0.72
		Location*density	831	3.89	0.04
		Inoculation* density	827	0.00	0.98
2	Larval thrips ~ location*inoculation + location*density + inoculation* density + (1|block)	Location*inoculation	1850	2.29	0.13
		Location*density	1863	14.73	< 0.01
		Inoculation*density	1850	1.78	0.18
3	Damage ~ location*inoculation + location*density + inoculation*density + (1|block)	Location*inoculation	-2801	0.06	0.80
		Location* density	-2766	37.06	< 0.01
		Inoculation* density	-2800	2.52	0.28

There were approximately three times more plants positive for *C. coccodes* in the high initial density treatments compared to the low density treatments (Figure 3.3B; $\chi^2 = 5.2$, df = 2, p-value = 0.07). In all treatments, 100% of the inoculated plants tested positive for *C. coccodes,* indicating a successful inoculation. When onion thrips were absent, none of the plants were positive for *C. coccodes.* When 5 onion thrips were added, 4% of the plants were positive for *C. coccodes* compared to the 14% of plants that were positive when 25 onion thrips were added.

Effects of environmental conditions on pathogen spread

Relative humidity and inoculation status were not significant factors in adult or larval onion thrips distribution among the three plants (Table 3.3, Model 1). Temperature significantly affected adult onion thrips distribution (Table 3.3, Model 1; t-value = 4.032, df = 120, p-value < 0.001). At the high temperature, there were similar numbers of adult onion thrips on location 1 plants when compared to location 2 plants, and about 30% less onion thrips on plants in location 3 compared to locations 1 and 2 (Figure 4A; slope = -0.08). At the lower temperature, adult onion thrips remained primarily on plants in location 1 and a few dispersed to the plants in locations 2 or 3 (Figure 3.4A; slope = -0.75). Temperature also had a significant impact on larval onion thrips distribution among the three plant locations (t-value = 7.493, df = 120, p-value < 0.001). At high temperatures, larval onion thrips were dispersed evenly across all plant locations (Figure 4B; slope = -0.09). At low temperatures, larval onion thrips were found primarily on location 1 plants, with an 80% reduction in the number of larval onion thrips on location 2 plants compared to location 1, and a 90% reduction on location 3 plants compared to location 1 (Figure 3.4B; slope = -1.33).

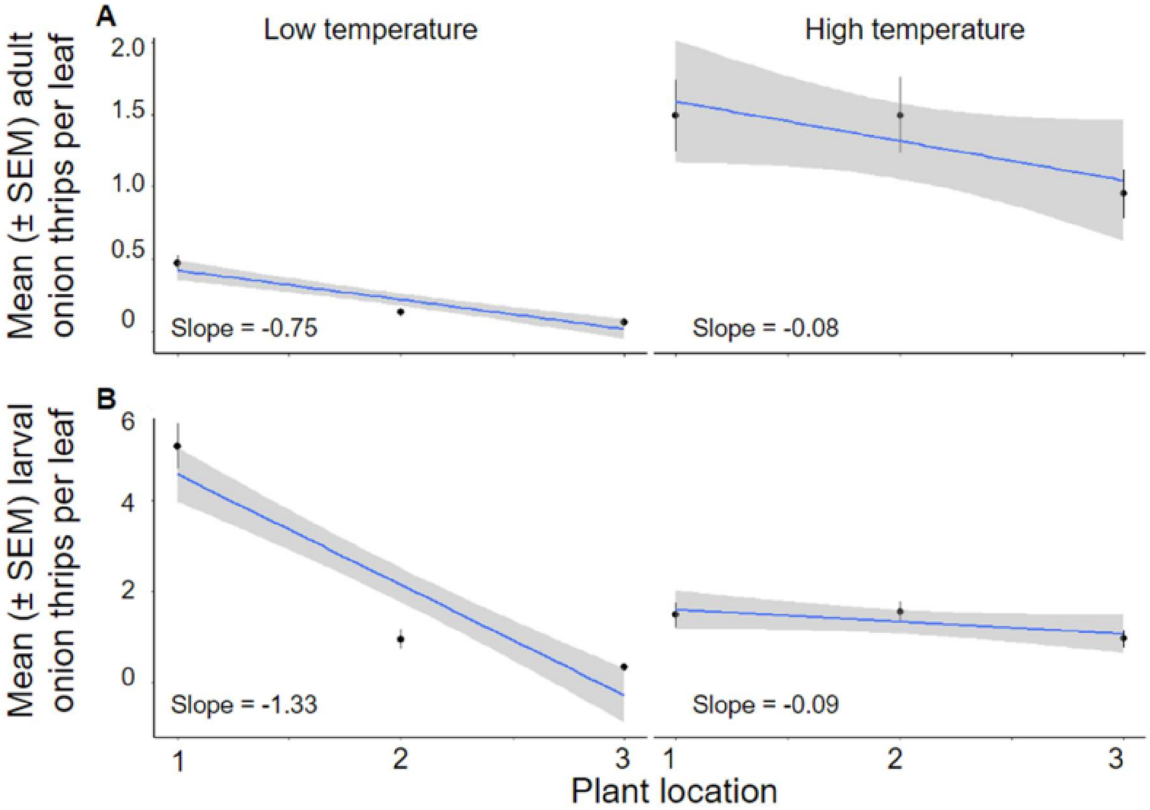

Figure 3.4. At varying temperature treatments (22°C and 33°C), 5 adult onion thrips or no thrips were released on plants in location 1, 13 d later, the adult and larval onion thrips per plant were recorded on each plant. The shaded areas represent 95% confidence intervals created using a linear regression. A) Mean ± SEM adult onion thrips dispersal under high compared to low temperatures. B) Mean ± SEM larval onion thrips dispersal under high compared to the low temperatures.

Relative humidity and inoculation status did not significantly affect damage distribution among the three plants (Table 3.3, Model 3). Additionally, temperature did not significantly affect the distribution of damage among the three plants (Table 3.3; Figure 3.5A). However, onion plants in the high temperature treatments had an increased incidence of *C. coccodes* positive plants when compared to plants in the low temperature treatments (Figure 3.5B; χ^2 = 4.76, df = 1, p-value = 0.03). In all temperature treatments, 100% of plants in the first location (i.e., the experimentally inoculated plant) were *C. coccodes* positive. In the high temperature treatments, 56% of subsequent plants were *C. coccodes* positive compared to 37% of plants in the low temperature treatments. There was no difference in the number of *C. coccodes* positive plants at differing humidity levels (χ^2 = 0.44, df = 1, p-value = 0.51).

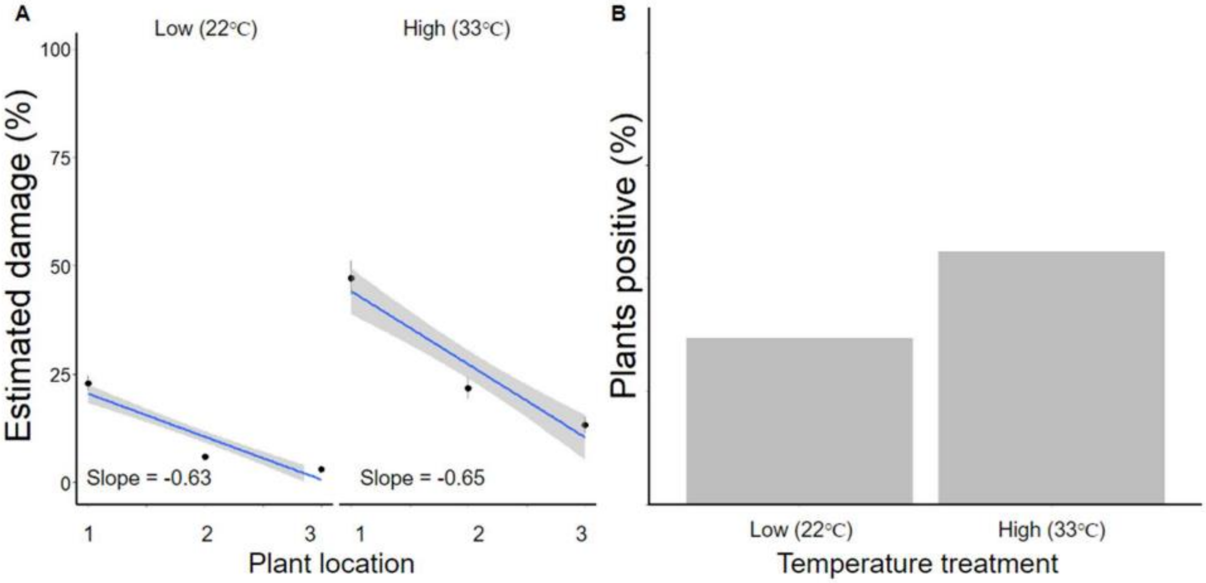

Figure 3.5. At varying temperatures (22°C and 33°C), 5 adult onion thrips were released on plants in location 1 and 13 d later, the amount of damage per plant in each location was visually estimated on a 0-100% scale. All plants were then tested for *C. coccodes* to determine if infection occurred. A) The estimated damage (%) on each plant at varying temperatures. The shaded areas represent 95% confidence intervals created using a linear regression. B) The percentage of plants positive for *C. coccodes* at varying temperatures.

Table 3.3: The models considered for testing the effect of varying environmental conditions on onion thrips dispersal. Fixed effect included: 1) location, location of plant within aluminum tray, 2) inoculation: inoculation status with/without *C. coccodes*, 3) relative humidity (RH), and 4) temperature. The full model was compared with the model resulting from the single term deletion (i.e. removal of the specific interactive effect). The AIC (Akaike information criterion), Chi square, and P value (α = 0.05) were used to determine significant interactions.

Model #	Full model	Fixed effects single term deletions	AIC	Chi Square	P value
1	Adult thrips ~ location*RH + location*temperature + location*inoculation + (1|block)	Location*RH	1342	0.23	0.63
		Location*temperature	1360	18.37	< 0.01
		Location*inoculation	1343	0.81	0.37
2	Larval thrips ~ location*RH + location*temperature + location*inoculation + (1|block)	Location*RH	1799	0.20	0.65
		Location*temperature	1855	56.20	< 0.01
		Location*inoculation	1799	0.30	0.58
3	Damage ~ location*RH + location*temperature + location*inoculation + location*population + (1|block)	Location*RH	-708	1.49	0.22
		Location*temperature	-709	0.04	0.84
		Location*inoculation	-709	0.15	0.70
		Location*population	-702	7.50	<0.01

Onion thrips presence or absence (*C. coccodes* only) significantly affected the damage distribution among the three plants within the experimental arenas (Figure 3.6; t-value = 2.762, df = 120, p-value = 0.006). When onion thrips were present, damage was distributed across all three plants, with the most damage present in the first location with a ~60% and 75% reduction in the amount of damage on the second and third plants, respectively, compared to the first (slope = -0.89). In treatments that did not receive onion thrips (*C. coccodes* only), damage was isolated on plants in location 1, and no subsequent plants were damaged (slope = -0.39).

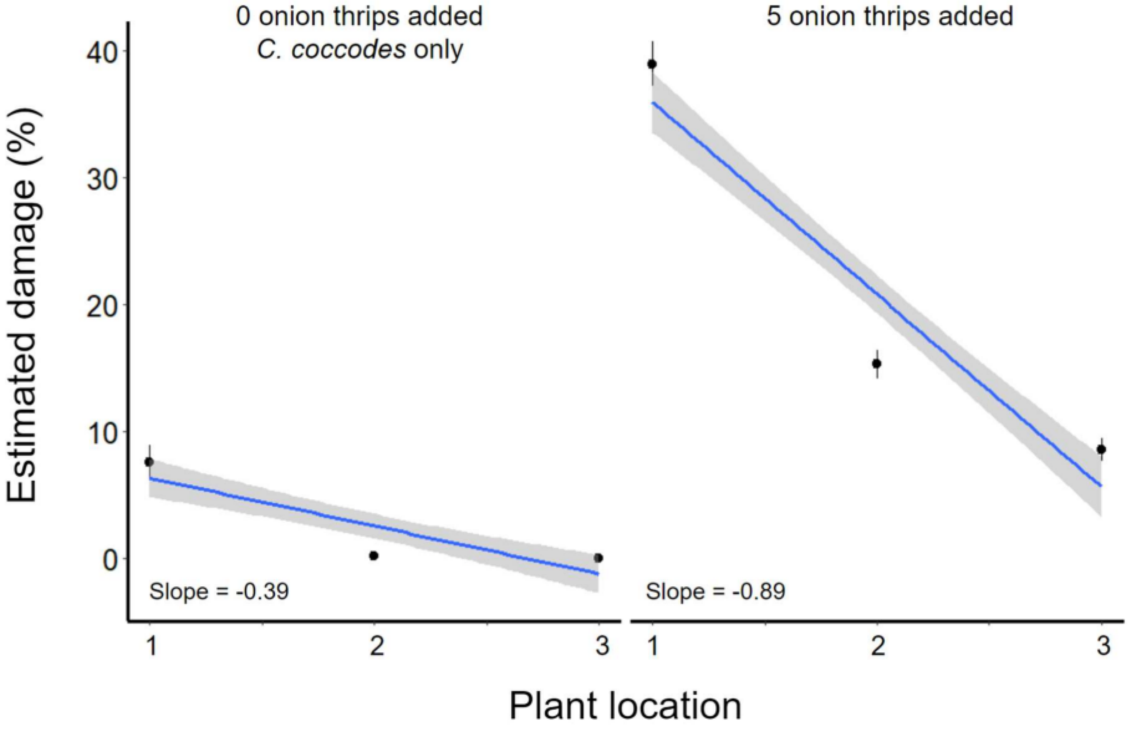

Figure 3.6. Varying numbers of onion thrips (0 or 5 adult onion thrips) were released on plants in location 1, 13 d later, the estimated damage (%) was visually assessed and rated on a 0-100% scale. The shaded areas represent 95% confidence intervals created using a linear regression.

Discussion

Our results demonstrated that the foliar pathogen *C. coccodes* can be spread by onion thrips and could be indirectly modified by a high temperature, potentially via an increase in the vector population, which may be injuring the plant tissue or mechanically vectoring conidia. Plants at high temperatures had increased disease incidence (i.e., number of plants positive for *C. coccodes*) and more onion thrips at the end of the experiment than plants at low temperatures. Relative humidity did not affect pathogen spread in our experiment.

Increasing the initial density of onion thrips altered adult and larval distribution patterns. Adults are more mobile than larval onion thrips and in our experiments, adults moved more among plants than immatures, especially when their initial densities were high (Gill et al. 2015). In treatments with high onion thrips initial densities, 9% of adult onion thrips were found on the first plant, compared to 25% of adults in the low initial density treatment. Western flower thrips (*Frankliniella occidentalis*) density similarly influences dispersal rate, and subsequent pathogen dispersal (Shaw et al. 2017; Rhainds & Messing 2005). Larval onion thrips distribution was likely reflective of adult onion thrips dispersal; in high density treatments, larval thrips were distributed relatively evenly across all plants, while they were primarily on the first plant in the low-density treatments. Larval onion thrips are the most damaging life stage and may be good predictors of where disease is likely to develop due to increased feeding damage (Coudriet et al. 1979; Leach et al. 2017). It's likely that at high densities, the increase in pathogen spread is due to higher larval populations on the healthy plants increasing the entry points for the pathogen, and increased adult dispersal to the healthy plants increasing mechanical vectoring rate. Previously, we found that onion thrips adults prefer *C. coccodes* inoculated onions over non-inoculated onions, but the high initial density may trigger dispersal away from the inoculated onion (Rhainds & Messing 2005; Constancio et al. 2022). Vector dispersal from an infected to a healthy plant has been documented to increase disease incidence in other systems as well (Daugherty et al. 2011; Ingwell et al. 2012).

In our bioassays, *C. coccodes* spread exclusively in the presence of onion thrips, and although a statistical difference was not detected, pathogen spread was reduced with lower onion thrips populations. In field-based studies, insecticide applications reduced necrotic tissue in onions, but it was unclear if this was due to reduced pathogen spread or feeding damage because of the difficulty in differentiating between damage types (Constancio et al. 2023; Leach et al. 2020; Grode et al. 2019; Mailhot et al. 2007). Our models indicated that the damage distribution was predicted by onion thrips densities interacting with plant location (Table 3.3, Model 3), regardless of *C. coccodes* suggesting that onion thrips feeding is the primary damage type. However, it is unknown how in a field setting, where precipitation or overhead irrigation often associated with severe disease outbreaks, this outcome would change (Tiberi et al. 2016; Rodriguez-Salamanca et al. 2018; Eigenbrode et al. 2018). Conversely, high precipitation and overhead irrigation lead to a reduction in onion thrips populations due to being washed away from the plants, potentially leading to a decreased amount of feeding damage on plants (North and Shelton 1986). In the future, it will be important to understand how the differing effects on onion thrips populations and disease severity affect pathogen spread.

We predicted that relative humidity would influence pathogen spread indirectly by altering vector reproduction. We found that relative humidity did not affect onion thrips, likely because temperature was more important in influencing their development (Bergant et al. 2005). However, it is also possible that the relative humidity in our experiment was not outside of onion thrips' tolerance range (Shipp & Gillespie 1993). Onion thrips are thigmotactic and may have been able to move to microclimates where relative humidity was favorable, thus avoiding the extreme conditions we created (Childers & Achor 1995; Kirk 1997). In onions, larval onion thrips are often found in the neck of the onion, where the leaves are close together, potentially exposing them to high relative humidity (Mo et al. 2008). Finally, to prevent plant desiccation within our experiment units, our plants were watered every 3 d by soaking the soil around the experimental unit to prevent water from splashing onto the plant foliage. In the field, *C. coccodes* conidia are spread via water splash, and in our experiment, we avoided overhead watering to prevent pathogen spread (Dillard 1989). Thus, the only way for *C. coccodes* conidia to move among plants was through mechanical vectoring by onion thrips. Therefore, because relative humidity did not affect onion thrips reproduction or dispersal, we did not observe effects on pathogen spread.

Increasing temperature altered both adult and larval onion thrips dispersal among the plants, likely due to increased densities and dispersal rates at high temperatures. At high temperatures, about 37% of adult onion thrips remained on the first onion plants, compared to nearly 100% of adult onion thrips in the low temperature treatment. High temperatures increase the rate of aerial adult onion thrips dispersal in the field, and a similar pattern was observed in our experiment (Smith et al. 2016). Additionally, although both temperatures had the same onion thrips initial densities (5 onion thrips per leaf), high temperature treatments had more adults at the end of the experiment than at the beginning, indicating a positive change in the development rate. At 30℃, onion thrips take approximately 10 d to develop from egg to adult, compared to 17 d at 21℃, so at our high temperatures, the development rate increased, and onion thrips completed their full lifecycle during the experiment (Jamieson et al. 2012; Mound 1997). Larval onion thrips followed a similar distribution pattern as adults, with approximately 30% of larval onion thrips found on the first plant at high temperature, compared to nearly 100% on the first plant in the low temperature. There were more larval onion thrips found at low than high temperature, further supporting that onion thrips at low temperatures had not yet completed a full lifecycle (Cornelissen 2011; Jamieson et al. 2012). Likely corresponding with increased onion thrips populations at high temperatures, we found more plants positive for *C. coccodes* at high temperatures. These results together indicate that onion thrips density is an important factor in pathogen spread, therefore altering developmental rate, decreasing generation time, and increasing dispersal rate via increased temperature indirectly affected pathogen spread in our experiments.

Neither temperature nor relative humidity affected the damage distribution. Without thrips, damage was found only on the first (inoculated plant), but in the presence of thrips, damage occurred on all plants, similar to our results in the first experiment. Relative humidity did not alter onion thrips dispersal; therefore, it also did not indirectly alter damage distribution, nor did it affect disease incidence (i.e., the number of plants positive for *C. coccodes*). Yet, in the field, relative humidity may increase with precipitation and overhead irrigation, facilitating conidial dissemination, potentially altering pathogen spread (Dillard 1989; Tiberi et al. 2016; Eigenbrode et al. 2018). Temperature altered onion thrips dispersal but did not alter damage distribution, but we found that more plants were positive for *C. coccodes* at high temperatures. This may be due to the increased development rate at high temperatures (Cornelissen 2011; Jamieson et al. 2012). The larvae we recorded were second instar and had not spent enough time feeding on subsequent plants to significantly alter damage patterns. It’s possible that if the experiment had continued, the damage distribution pattern would have been reflective of the larval onion thrips distribution.

The interactions between fungal pathogens and insects can be overlooked because insect vectors are not necessary for pathogen spread to occur. However, we found that temperature, but not relative humidity, indirectly affected *C. coccodes* spread by affecting insect behavior and reproduction. Our results provide important information about how environmental changes may indirectly affect pathogen spread in a controlled setting, but field-based experiments are needed to investigate this further. Understanding the effect of changing environmental conditions on pathogen spread is critical for agriculture, especially as climate change causes unpredictable shifts in regional environmental conditions.

Acknowledgments

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CHAPTER 4: PLASTIC MULCHES PROVIDE ALTERNATIVE MANAGEMENT OPTIONS FOR ONION THRIPS

Abstract

An integrated pest management program (IPM) relies on multiple management methods to reduce pest populations. In particular, onion thrips (*Thrips tabaci*) have relatively few effective control methods, and especially non-chemical management methods, like plastic mulches, need to be developed. We tested 7 different plastic mulches in the field, including 3 silver mulches, to determine their effect on thrips populations. A subset of the mulches was tested in a second field trial with or without a pesticide program. Thrips populations were recorded once a week for both field trials and spectral data was collected using a spectrophotometer. Plant growth, temperature above and below the mulch were recorded for the second field trial. Finally, we conducted a no-choice lab experiment to determine the potential mechanisms of mulches on thrips population regulation. Thrips numbers, mulch and soil temperature, and plant growth were recorded after 13 days. In the first field trial, we found that not all the silver mulches were equally effective at reducing thrips populations. In the second field trial, red and diamond silver mulch reduced thrips populations similar to the pesticide program. Furthermore, the addition of a pesticide program provided synergistic control in most treatments. Finally, the no-choice experiment revealed that mulches likely affect thrips populations through wavelength-specific induced changes. Overall, we found that silver and red mulches suppressed thrips populations as effectively as a season-long pesticide program, providing options for an integrated pest management program.

Introduction

Integrated pest management (IPM) requires the synergistic use of available techniques, such as biological, cultural, physical, and chemical control methods, to manage a pest (Ehler and

Bottrell 2000). Within an IPM framework, chemical control should only be used as a last resort with resistance management guidelines, such as pesticide class rotation and action thresholds, guiding their use (Siegwart et al. 2015). Especially in the case of insect pests, such as onion thrips (*Thrips tabaci* Lindeman, Thysanoptera: Thripidae), that rapidly develop resistance to insecticide classes, increasing the number of effective non-chemical management options is paramount (Sparks 2013; Pedigo et al. 1986; Ehler and Bottrell 2000; Devi and Roy 2017).

Plastic mulches are used as a physical control method in IPM, they can be deployed either alone or in combination with other pest control options. Historically, plastic mulches have been used to increase soil moisture retention and plant yield, control the microclimate around plants, and reduce weed pressure (Ham et al. 1993; Greer and Dole 2003; Job and Bhakar 2018). Plastic mulches are also used as a physical control strategy for insect pests, but they have had mixed success in reducing pest populations. Reflective mulches have had some success in reducing aphids (Aphidae), whiteflies (Aleyrodidae), spotted-wing drosophila (*Drosophila suzukii*) and pear psylla (*Cacopsylla pyricola*) (Brown et al. 1993; Csizinszky et al. 1995, 1999; Greer and Dole 2003; Nottingham and Beers 2020; McIntosh et al. 2022). However, silver or reflective mulches have had variable success in deterring onion thrips, either reducing numbers or having no effect (Lu 1990; Csizinszky et al. 1995; Greer and Dole 2003; van Toor et al. 2004; Iglesias et al. 2021). The reason for variable success is unclear, and pest population patterns may be driven either indirectly through changes in the microclimate around mulches which may make the area inhospitable to pests, or by wavelength-specific color preferences which can affect behavior (Andreotti et al. 2010; Gordon et al. 2008; Ham et al. 1993; Larentzaki et al. 2008; Bégin et al. 2001; Vincent et al. 2003; Diaz and Fereres 2007). Therefore, it is important to evaluate the indirect and direct effects of mulches both on the crop and on the insect.

Mulches alter the microclimate around plants throughout the growing season, and these changes can also affect insect populations (Andreotti et al. 2010; Gordon et al. 2008; Ham et al. 1993; Larentzaki et al. 2008). For example, dark-colored mulches, such as black or red, are recommended in the early season because they increase soil temperature around the plant (Job and Bhaker 2018; Csizinszky et al. 1995; Aguyoh et al. 1999; Greer and Dole 2003). These increases may potentially push insects to the upper limits of their thermal tolerance, thus reducing pest populations (Gordon et al. 2008; Job and Bhakar 2018). However, dark mulches are not recommended for summer or fall crops because high heat retention may be detrimental to plant health (Aguyoh et al. 1999; Lai et al. 2024). Conversely, light mulches, such as white or highly reflective silver mulches, decrease temperature around the plant and are recommended for cool weather crops to reduce soil temperatures (Ham et al. 1993; Greer and Dole 2003; Lai et al. 2024). However, white mulch can increase pest numbers, for example, it is highly attractive to onion thrips, and therefore may not be suitable for fields with historically high onion thrips infestations (Brown and Brown 1992; Iglesias et al. 2021; Scott et al. 1989). Mulch color not only affects microclimate and potentially indirectly insect physiology but may also affect insect behavior (Bégin et al. 2001; Vincent et al. 2003; Diaz and Fereres 2007).

Plastic mulches reflect different wavelengths of light based on their color, providing additional photobiological benefits to plants while potentially inducing wavelength-specific behaviors in onion thrips (Decoteau et al. 1988, 1989; Csizinszky et al. 1995; Lopez-Reyes et al. 2022). Light within the UV range (300 – 400 nm) increases leaf pigmentation and thickness, but many thrips species prefer light within that range (Csizinszky et al.1995; Lopez-Reyes et al. 2022). Red and near-infrared light (620 – 800 nm) increases photosynthesis and yield in onion plants but is one of the least attractive colors to thrips, likely due to their low sensitivity to wavelengths within that range (Walker 1974; Alam et al. 2007; Egri et al. 2020; Lopez-Reyes et al. 2022). White mulch reflects across the entire visible spectrum (400 – 800 nm) and therefore should provide multiple photobiological benefits (Csizinszky et al. 1995; Courbier and Pierik 2019; Dănăilă-Guidea and Delian 2020). Conversely, black mulch reflects minimal light, thus plants may not receive additional photobiological benefits but it is attractive to thrips (Csizinszky et al. 1995; Ham et al. 1993; Streck et al. 1995). In addition to composition, wavelength intensity (i.e., amount of light reflected) can also alter insect behavior. For example, increasing the intensity of blue light often correlates with increased attractiveness for western flower thrips (*Franklinella occidentalis)*, and onion thrips prefer highly reflective colors within the same hue (Otani et al. 2014; Otieno et al. 2018; Westmore et al. 2019; Stukenberg et al. 2020). Due to the varying and potentially conflicting effects of plastic mulches on plants and insect pests, it remains unclear whether mulches in their associated colors are a viable alternative management strategy.

The goal of our study was to test various plastic mulches to determine which colors were most effective in controlling onion thrips populations and to determine if plastic mulches could be a viable management strategy to reduce insecticide sprays. Onion thrips are a significant pest of onions (*Allium cepa* L. Aspragales: Amaryllidaceae) and can cause substantial economic losses if left uncontrolled (Kendall and Capinera 1987; Fournier et al. 1995; Waiganjo et al. 2008). In our first field experiment, we tested 7 different treatments, including 3 silver mulches, to identify the most promising colors for onion thrips control. We hypothesized that all 3 silver mulches would be equally effective in reducing onion thrips populations when compared to the control. In our second field experiment, we investigated the level of onion thrips control provided by a subset of mulches from the first experiment alone or combined with pesticide programs. We hypothesized that mulches alone would not be as effective as a grower-standard pesticide program for onion thrips control, but the addition of a pesticide program to the mulch treatments would provide synergistic control. To determine the potential mechanisms of mulch effects on onion thrips, we conducted a no-choice experiment in an environmental chamber to determine thrips performance in response to mulch color.

Methods

Field mulch trials

We conducted two field experiments with different colored mulches to evaluate their effectiveness in deterring onion thrips. The first field experiment took place at Michigan State University's Entomology Research Farm (Lansing, MI) in a mowed grassy area in the summer of 2022. The plastic mulch treatments included: 1) control (mowed grass), 2) black mulch, 3) reflective silver mulch, 4) metallic silver mulch, 5) diamond silver mulch, 6) red mulch, and 7) white mulch (N = 15, Table 4.1). All mulches were cut into 1.2 m x 1.8 m sections and laid in a randomized complete block design in the field. To secure the mulch to the ground, each side of the rectangular sheet was rolled around a rebar and held in place with yard staples (High Tech Pet, Ventura, CA). Onions (cv. 'New York Early') were grown by placing one seed in a plastic pot (8.5 cm deep, 10 cm wide; ITML Horticultural Products Inc., Brantford, Ontario) filled with a perlite soil mix (Suremix Perlite, Michigan Grower Products Inc., Galesburg, MI). The seedlings were maintained in an environmental chamber (25°C, 50% RH, 16:8 h (L:D)) and were covered during germination to prevent soil dehydration. The cover was removed 7 days after seeding once germination occurred in > 75% of seedlings. The seedlings were watered 3 times a week and fertilized at 75 ppm N (20-20-20 Jack's General Purpose Water-Soluble Fertilizer, JR Peters Inc., Allentown, PA) once a week.

Table 4.1: The mulches selected for testing to determine their effectiveness in reducing onion thrips populations in 2022 and 2023.

Mulch color	Experiment	Thickness (mil)	Manufacturer		Light reflection	
			Location	Mulch description	Range (nm)	Reflectiveness (%)
Control (bare ground)	Field experiment 1,2 Lab experiment				300 – 800	< 10
Black	Field experiment 1,2 Lab experiment	1.0	Ken-Bar, Rochester NY	Black	300 – 800	< 5
Reflective silver	Field experiment 1	1.25	Ken-Bar, Rochester NY	Metallic plastic	300 – 800	~ 30
Metallic silver	Field experiment 1	Not provided	Dubois Agrinovation, ON	Metallic reflective	300 – 800	~ 65
Diamond silver	Field experiment 1,2 Lab experiment	6.0	Viagrow, East Point, GA	Mylar diamond film	300 – 800	~ 85
Red	Field experiment 1,2 Lab experiment	1.0	Ken-Bar, Rochester NY	Red	620 – 800	~ 35
White	Field experiment 1,2 Lab experiment	1.25	Ken-Bar, Rochester NY	White on black	400 – 800	~ 35

Onions were placed into the field when plants were approximately 8 weeks old. Small holes were dug into the ground beneath the mulch and slits were cut in the mulch through which pots were inserted, ensuring that the tops of the pots were flush with the mulch surface. Three seedlings per plot were positioned in the center of the mulch sheets, equidistant (36 cm) from each other, and watered 2-3 times a week. Each week from 4 August to 8 September 2022, onion thrips were counted by visually inspecting all the leaves, and the number of leaves per plant was also recorded.

In the second field experiment, we tested a subset of the plastic mulches from 2022, in combination with a pesticide program. This experiment took place at the Southwest Michigan Research and Extension Center (SWMREC; Benton Harbor, MI) on Spinks Loamy Fine Sand (USDA Soil 13B). To prepare the soil for mulches, the ground was rototilled to 15 cm depth, raised beds (0.6 m wide) were created using a tractor-mounted bed shaper, and a drip fertigation line was laid in the center of the beds. After mulches were laid, approximately 2 cm diameter holes were cut into the mulch every 20 cm so that onion transplants (cv. ‘Ailsa Craig’; Dixondale Farms, Carrizo Springs, TX) in the 2-4 leaf stage could be transplanted. After transplanting, fertilizer was immediately applied at 125 ppm N (20-20-20 N-P-K, Jack’s General Purpose Water-Soluble Fertilizer), and reapplied 3 times throughout the growing season (9, 30 June, and 21 July), onions were watered once a week.

All treatments were set up in a randomized complete block design on 17 May 2023. Split plot factors were the presence or absence of a pesticide program, initiated when the 1 thrips/leaf threshold was reached on 6 June 2023, for a total of 10 treatments. Full plots measured 0.6 m x 10 m and subplots were 0.6 m x 5.0 m. The treatments were: 1) bare ground (no mulch) + no pesticide, 2) bare ground + pesticide, 3) black mulch + no pesticide, 4) black mulch + pesticide,

5) red mulch + no pesticide, 6) red mulch + pesticide, 7) diamond silver mulch + no pesticide, 8) diamond silver mulch + pesticide, 9) white mulch + no pesticide, 10) white mulch + pesticide (N = 4). The pesticide program consisted of two back-to-back applications 7 days apart of spirotetramat (Movento; 0.78ml/l, Bayer Crop Science, Leverkusen, Germany) tank mixed with pydiflumetofen and fludioxonil (Miravis Prime; 1.8ml/l, Syngenta, Basel, Switzerland) on 6 and 14 June 2023, and two back-to-back applications 7 days apart of abamectin and cyantraniliprole (Minecto Pro; 1.5ml/l, Syngenta) on 11 and 18 July 2023. Each insecticide was tank mixed with a nonionic surfactant (1% v:v, Syl-Tac, Wilbur-Ellis, Aurora, CO). All insecticides were applied as a foliar spray with a CO_2 backpack sprayer equipped with a single nozzle boom with an XR8003 flat-fan nozzle (TeeJet Technologies, Wheaton, IL), calibrated to 241 kPa to deliver 246 l/ha.

Each week, the field was scouted for onion thrips by randomly selecting 10 plants per subplot and recording the total number of onion thrips per plant. Additionally, 5 plants per subplot were randomly selected to record plant height and the total number of leaves each week. For height measurements, a ruler was placed on the soil surface, and height was measured from the base of the plant to the tip of the tallest leaf. We used a probing digital thermometer (Sovarcate, Hunan, China) to record soil temperature 2.5 cm below the soil surface, at the base of 5 onions per subplot. Temperature on the surface of the mulch was measured by aiming an infrared thermometer (Sovarcate) near the base of 5 randomly selected onions in each plot, approximately 5 cm from the top of the mulch. All temperature, plant and insect measurements were taken each week between 24 May and 8 August 2023.

No-choice lab experiment

An onion thrips colony was established at Michigan State University (East Lansing, MI) beginning in August 2022. Onion thrips were collected from a commercial onion field (Grant, MI) where conventional pesticides and fertilizers were used. Mesh cages (50 cm^3, MegaView, Taichung, Taiwan) were kept at room temperature with ambient humidity and a 16:8 h L:D cycle. To sustain continuous rearing, 6-8 onions at the 5-7 leaf stage, grown from certified organic onion seeds (cv. 'New York Early'; Johnny's Selected Seeds, Winslow, ME) were added into each cage every week so onion thrips could feed *ad libitum*.

To evaluate the impact of different colored mulches on onion thrips performance, a no-choice laboratory experiment was conducted. Experimental units consisted of one 6-week-old onion seedling grown as described in the previous section. One seedling was transplanted into a rectangular aluminum tray (30 cm x 28 cm x 8 cm, Handi-Max, Wheeling, IL) filled with perlite soil mix (Suremix Perlite) to a depth of 6 cm with drainage slits along the bottom and was watered immediately after transplanting. There were five different treatments: 1) control – no mulch, 2) black mulch, 3) diamond silver mulch, 4) red mulch, and 5) white mulch (N = 27). Mulches were cut into squares (30.48 cm x 30.48 cm) to cover the entire surface of the experimental units, small slits were made in the center of each mulch square for inserting the onion seedling into the soil in the tray, and the edges were tucked underneath the soil to hold the mulch in place. Cages made of transparency film (School Smart, Greenville, WI; 26 cm tall x 28 cm x 26 cm) and topped with a nylon mesh (160 μm mesh size) were placed over each tray to prevent onion thrips from moving between experimental units while allowing ventilation. Experimental units were organized within environmental chambers (25°C, ambient RH, 16:8 h L:D) in a randomized complete block design.

Three adult onion thrips were removed from the colony and transported in a 1.7 ml microcentrifuge tube (Thomas Scientific, Swedesboro, NJ) to the environmental chamber; a 3 cm piece of fresh onion foliage was provided to thrips during handling and transportation. The experimental units were set up approximately 1 hour before introducing onion thrips into the experiment. Onion thrips vials were randomly assigned to experimental units; vials were then opened and placed at the base of the onion seedling to allow thrips to move to the plants. The transparent cage was then placed over the arena, and additional soil was added between the bottom of the plastic cover and the aluminum tray to prevent onion thrips from escaping. After 6 days, the experimental units were watered by immersing the bottom of aluminum trays into water for approximately 40 s, allowing water to soak the soil through the slits in the tray. After 13 d the number of adult and larval onion thrips, estimated feeding damage severity (%), plant growth (height and number of leaves), temperature on the surface of the mulch, and soil temperature were recorded for each plant. Destructive sampling was used to count the number of onion thrips; the onions were uprooted so that the neck of the onion could be examined for onion thrips. Feeding damage severity was visually rated by estimating the amount of damaged tissue per plant between 0 to 100%. Mulch surface temperatures were measured with an infrared thermometer (Sovarcate) 5 cm from the mulch surface, and soil temperatures were measured with a digital probe thermometer (Sovarcate) at a depth of 2.5 cm once at the end of the experiment.

Spectrophotometry

A handheld spectrophotometer (FLAME-T-UV-VIS-ES; Ocean Insight, Orlando, FL) was used to measure the light reflectance (%) of mulches between 300 and 800 nm. Each measurement averaged 20 readings with a scan time of 3 s each, and a resolution of 0.2 nm, in the ultraviolet–visible light (UV-VIS) range. The spectrophotometer was connected to a pulsed xenon lamp as the light source (PX-2; Ocean Insight) so that light conditions remained consistent. In the field, the reflectance of the mulches was measured with a handheld probe (QR400-7-SR; Ocean Insight) positioned perpendicular 10 cm from the mulch surface. Reflectance readings were obtained in the field on 28 July 2022, 17 July, and 14 August 2023, and in the laboratory experiment on 21 August 2023.

Data analysis

In our first field experiment, to determine how different mulch colors affected onion thrips populations, we constructed generalized linear mixed models (GLMMs) using the ‘glmmTMB’ package with onion thrips per leaf as the response variable and mulch color as the fixed factor (Brooks et al. 2017). Block and date were random factors, and a negative binomial distribution was used. In all models, the ‘drop1’ function was used to identify significant fixed effects of mulch color compared to the null model ($\alpha = 0.05$). When the model had a significant fixed effect, Tukey’s HSD test was conducted using the ‘multcomp’ package (Hothorn et al. 2008) to determine significant differences ($\alpha = 0.05$) among treatments.

In our second field experiment, we were interested in how mulch color combined with an insecticide program affected onion thrips populations, plant growth, soil temperature and temperature of the surface of the mulch. We used GLMMs to determine if there was an interactive effect of mulch color and spray program on onion thrips populations, with block and date as the random factors, and a negative binomial distribution. The ‘drop1’ function was used to determine significant treatment interactions, followed by a Tukey’s HSD to determine significant differences among treatments ($\alpha = 0.05$). For plant growth data, leaf number and plant height were analyzed only from the date when plants were at their peak growing stages. To determine how treatment affected the number of leaves per plant and plant height, we created linear mixed effects models with the ‘lme4’ package (Bates et al. 2015) with both mulch color and spray treatment as fixed effects, and block as the random factor. To determine how mulch treatment affected temperature at the mulch surface and below the soil, we compared the mean temperatures above and below the mulch to the mean ambient temperature (MSU Enviroweather, 2024). Linear mixed effects models were created for both soil and mulch surface temperature with mulch color as the fixed factor and date as the random factor. To determine significant treatment effects in models for onion thrips per leaf, leaves per plant, average plant height, and temperature, the ‘drop1’ function was used, followed by a Tukey’s HSD to determine significant differences among treatments ($\alpha = 0.05$).

In our no-choice lab experiment, we were interested in how mulch color affected onion thrips populations, plant growth, and temperature around the plants under controlled conditions. We used GLMMs to determine the effect of mulch color on onion thrips numbers, with block as a random factor and a Poisson distribution. To determine how mulch color affected plant growth, we used linear mixed-effects models with block as the random factor. Finally, for soil and mulch surface temperature, we created linear mixed-effects models with mulch color as the fixed factor and block as the random factor. Models were checked for significance in the same way as described in the first experiment.

Results

Field mulch trials

In 2022, mulch color had a significant effect on onion thrips numbers (Figure 4.1A; $\chi^2 = 161.56$, df =6, p-value < 0.01). Compared to the control treatment, there were about twice as many onion thrips per leaf in the diamond silver mulch (t-value = 6.05, df = 180, p-value < 0.01), the red mulch (t-value = 7.36, df = 180, p-value < 0.01) and the white mulch (t-value = 5.07, df = 180, p-value < 0.01). Onion thrips per leaf tripled on the black mulch (t-value = -9.58, df = 180, p-value < 0.01), the reflective silver mulch (t-value = 8.99, df = 180, p-value < 0.01) and the metallic silver mulch (t-value = 10.16, df = 180, p-value < 0.01) compared to the control treatment. Among the silver mulches, the reflective silver and metallic silver mulch performed similarly to each other (t-value = 1.44, df = 180, p-value = 0.79), but the diamond silver mulch significantly reduced onion thrips populations compared to the reflective silver mulch (t-value = 3.33, df = 180, p-value = 0.01) and the metallic silver mulch (t-value = 4.70, df = 180, t-value < 0.01).

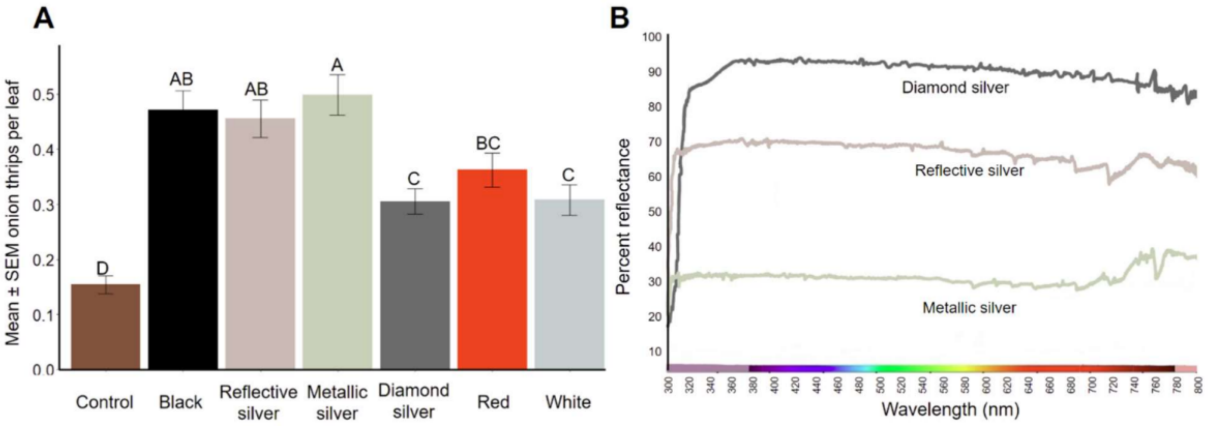

Figure 4.1. In 2022, 6 different plastic mulches and a no mulch control were tested in the field, including 3 silver mulches, to determine how they affect onion thrips populations. Three onions in plastic pots were placed on mulch sheets and onion thrips were counted weekly for 6 weeks. A) The seasonal mean ± SEM onion thrips per leaf for each mulch. Bars with different letters indicate significant differences (Tukey's HSD, α = 0.05). B) A handheld spectrophotometer was used to determine the differences in the amount of light reflected from the 3 silver mulches.

In the 2022 field experiments, all 3 silver mulches reflected light within the UVA range (300-400 nm), but none of the other mulches reflected light within this range (Figure 4.1B). The 3 silver mulches reflected light within the same range (UV-VIS), but the amount of light reflected was different between each silver mulch. The diamond silver mulch was the most reflective (70-90% reflectance), reflective silver mulch was in the middle (60%), and metallic silver mulch reflected the least amount of light (25%). White mulch did not reflect any light in the UV range but reflected between 65-70% of light in the visible range, similar to the reflective silver mulch in the visible range. Red mulch reflected less than 10% of light within the UV and 400 – 600 nm range, but reflectance increased to about 20% in the 600-800 nm range. Black mulch reflected less than 10% of light across the UV-VIS spectrum.

In 2023, there was a significant interactive effect between mulch color and insecticide treatment on onion thrips populations (Figure 4.2; $\chi^2 = 25.83$, df = 4, p-value < 0.01). In general, across most mulch treatments, adding a spray program provided significant additional onion thrips suppression when compared to the mulch alone (t-value > 3.29, df = 80, p-value < 0.03), except for the diamond silver mulch where thrips numbers were similar between the insecticide-treated and untreated plots (t-value = 2.76, df = 80, p-value = 0.15). White mulch was the least effective treatment overall, regardless of the presence of an insecticide program, with about twice as many thrips per leaf than in the other treatments (t-value < -7.41, df= 80, p-value < 0.01). The addition of a pesticide program on the white mulch significantly reduced onion thrips populations (t-value = 3.29, df = 80, p-value = 0.03), but there were still 2.5 times more thrips when compared to the bare ground control treatment with an insecticide program (t-value = -14.29, df = 80, p-value < 0.01). The red mulch without an insecticide had similar numbers of onion thrips as the control treatment with insecticides (t-value = -2.15, df = 80, p-value = 0.49). Red mulch with an insecticide program was the most effective at reducing onion thrips populations, with 62% fewer onion thrips when compared to the control and black mulch with insecticide (t-value > 8.88, df = 80, p-value < 0.01), and 20% fewer onion thrips compared to the diamond silver with an insecticide program (t-value = -4.84, df = 80, p-value < 0.01). Black mulch, with or without a pesticide program, performed similarly to the control treatment, with or without a pesticide program (t-value < 1.25, df = 80, p-value > 0.97).

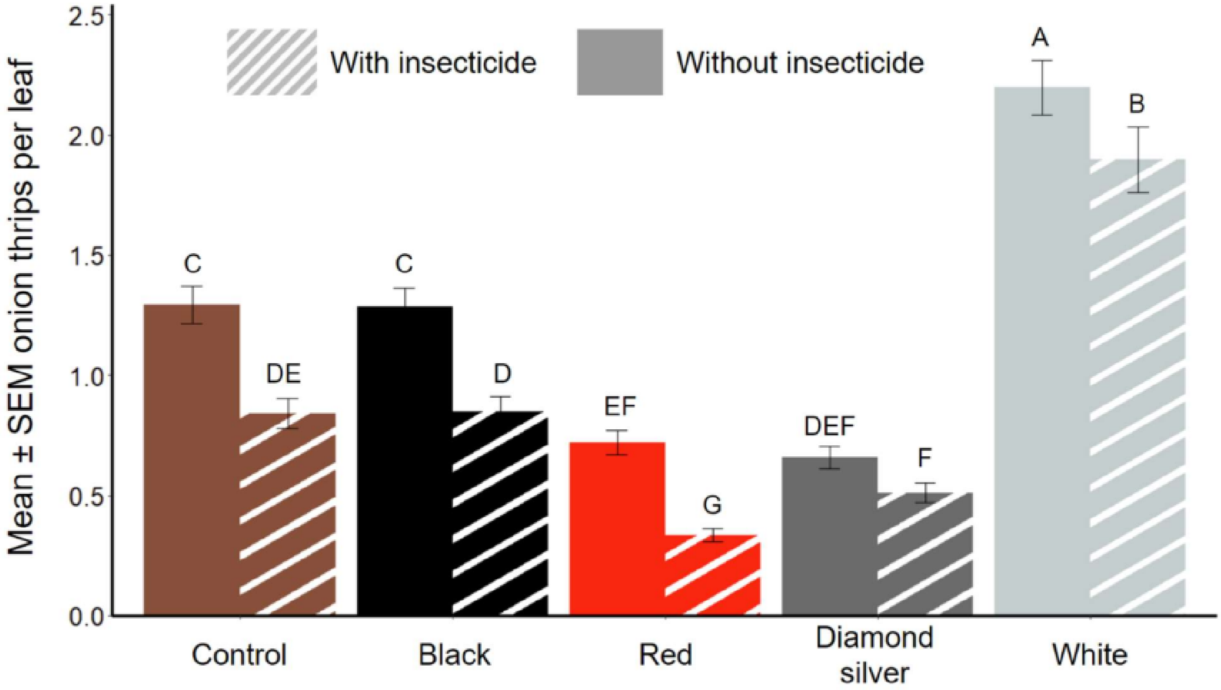

Figure 4.2. The 2023 seasonal mean ± SEM onion thrips per leaf for 4 mulches and a bare ground control treatment in the field, either with or without the addition of a conventional pesticide program. The pesticide program consisted of two back-to-back applications 7 d apart of spirotetramat tank mixed with pydiflumetofen + fludioxonil on 6 and 14 of June, and two back-to-back applications 7 d apart of abamectin and cyantraniliprole on 11 and 18 of July. Onion thrips were counted on 10 plants per subplot each week. Solid bars represent mulches without a pesticide program, and hashed bars are mulches with a pesticide program. Bars with different letters indicate significant differences (Tukey's HSD, $\alpha = 0.05$).

Mulch color had a significant effect on the average number of leaves (Figure 4.3A; F= 5.93, df = 4, 191, p-value < 0.01), but insecticide treatment did not influence leaf numbers (F = 0.08, df = 1, 191, p-value = 0.77). There were more leaves on plants grown on the black, red, and white mulch compared to plants in the control treatment (t-value > 2.86; df = 8, p-value < 0.03). There were similar numbers of leaves per plant in the diamond silver mulch and the control treatments (t-value = -0.08, df = 8, p-value = 0.99). Mulch color also significantly affected plant height (Figure 4.3B; F = 3.37, df = 4, 191, p-value = 0.01), but insecticide treatment did not (F = 0.67, df = 1, 191, p-value = 0.41). Onions grown on the black and red mulches were significantly taller than plants on the diamond silver mulch (black: t-value = -2.89, df = 8, p-value = 0.03, red: t-value = -3.09, df = 8, p-value = 0.02).

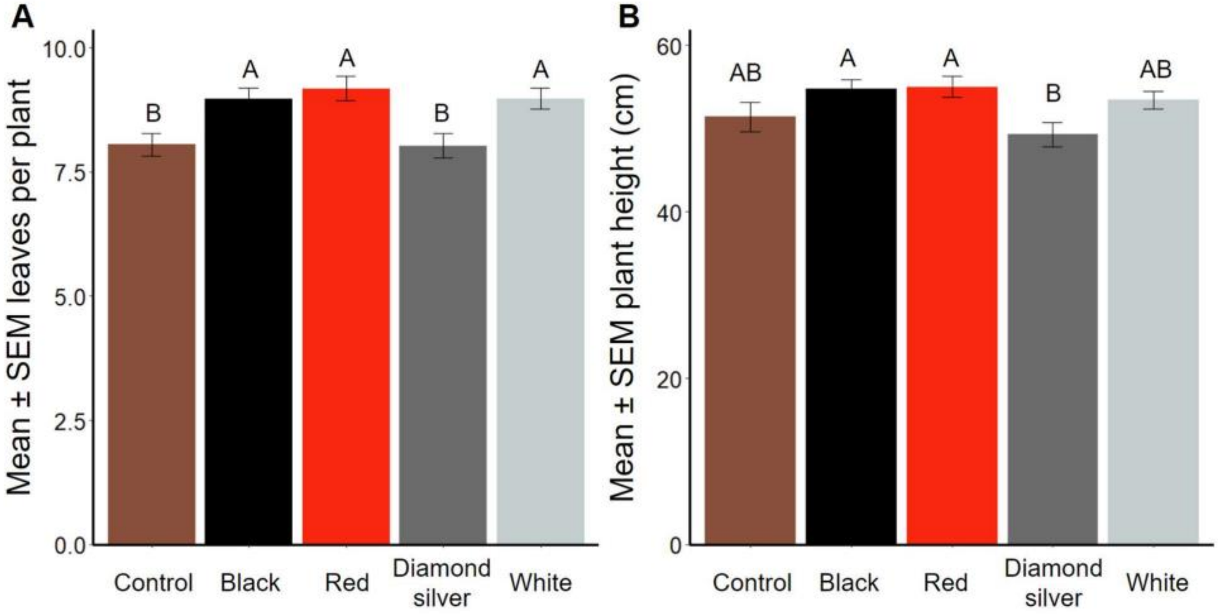

Figure 4.3. In the 2023 field experiment, onion plant data was collected every week to determine how mulch color impacts plant growth. Ten plants per plot were measured each week throughout the growing season. A) Mean ± SEM number of leaves per plant on 10 July 2023. B) Mean ± SEM plant height (cm) on 10 July 2023. Bars with different letters indicate significant differences (Tukey's HSD, α = 0.05).

The temperature of the mulch surface was significantly altered by mulch color (Figure 4.4A; F = 301.94, df = 5, 129.47, p-value < 0.01). Black mulch was the only treatment to significantly increase the mulch surface temperature compared to the ambient temperature, (14°C above ambient, t-value = 4.00, df = 18, p-value < 0.01). When compared to the bare ground control, black, red, and white mulch significantly increased the temperature at the surface of the mulch by approximately 9°C, 3.5°C, and 2°C respectively (t-value > 2.79, df =18, p-value < 0.05). Diamond silver mulch significantly reduced the temperature at the surface of the mulch compared to the bare ground treatment by 9°C on average (t-value = -17.09, df = 18, p-value < 0.01). Mulch color also significantly affected soil temperature (Figure 4.4B; F = 366.57, df = 5, 78.86, p-value < 0.01). The diamond silver mulch was the only treatment where the soil temperature was not significantly higher compared to the ambient temperature (t-value = 2.26, df = 18, p-value = 0.18). Red and black mulch significantly increased the soil temperature compared to the bare ground control treatment by 2℃ (t-value > 15.58, df = 18, p-value < 0.01). Compared to the bare ground control, both diamond silver and white mulch reduced soil temperature by approximately 2℃ (t-value > -9.41, df =18, p-value < 0.01).

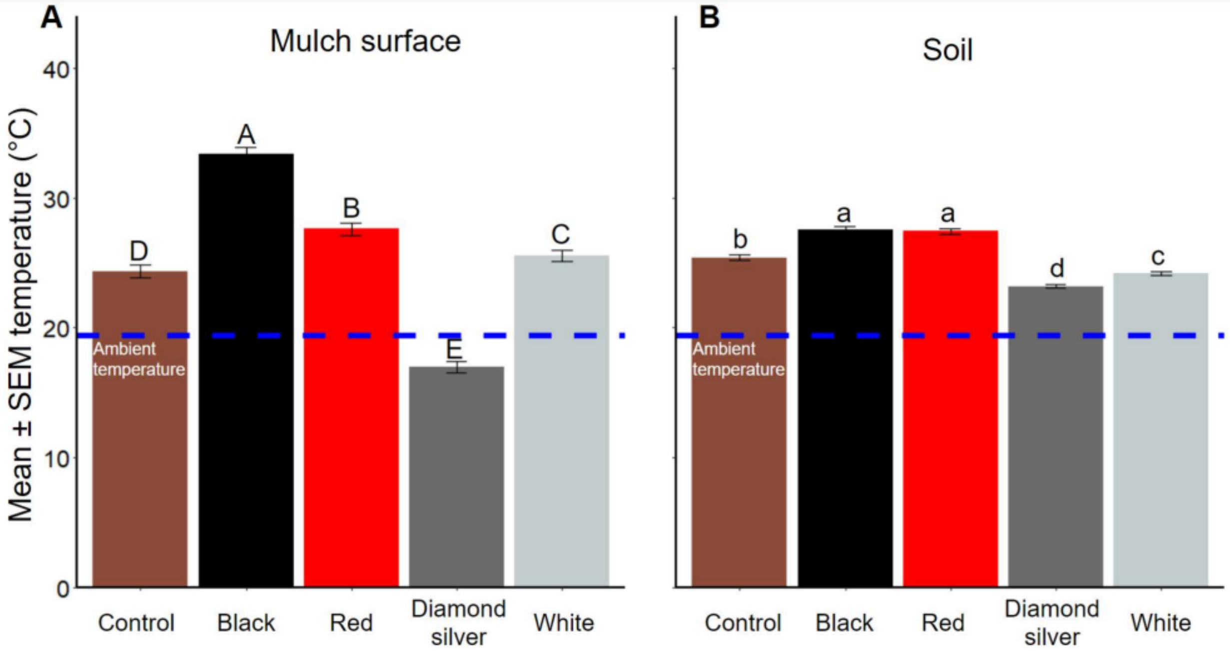

Figure 4.4. The mean ± SEM temperature (°C) at (A) the surface of the mulch and (B) 2.5 cm below the soil surface of four different plastic mulches and a bare ground control tested in an experimental onion field. Each week, mulch surface and soil temperature were measured 10 times per plot. The blue dashed line represents the seasonal mean ambient air temperature, which was obtained from MSU Enviroweather. Bars with different letters indicate significant differences (Tukey's HSD, α = 0.05).

In the 2023 field trials, we found similar reflectance patterns than in 2022 (Figure 4.5). Within the UV range, diamond silver mulch reflected approximately 65% of light, significantly more than any other mulch color (t-value > 86.29, df = 956, p-value < 0.01). Within the visible range, the diamond silver mulch reflected 70% of light across the spectrum, and white mulch reflected 40% of light, significantly less than diamond silver (t-value = -195.17, df = 4190, p-value < 0.01). Black mulch and the control treatment only reflected 4% of light across the VIS spectrum and were not significantly different from each other (t-value = 2.44, df = 4190, p-value = 0.11). Red mulch reflected significantly less light (approximately 8%) within the 400 - 620 nm range (VIS excluding red) spectrum compared to diamond silver and white mulch (t-value < -453.49, df = 2190, p-value < 0.01). However, within the red range (620 – 800 nm), red reflected approximately 35% of light, which was significantly more than the white mulch (30% of light reflected; t-value = 70.93, df = 2000, p-value < 0.01), but significantly less than diamond silver mulch (66% of light reflected; t-value = 363.98, df = 2000, p-value < 0.01).

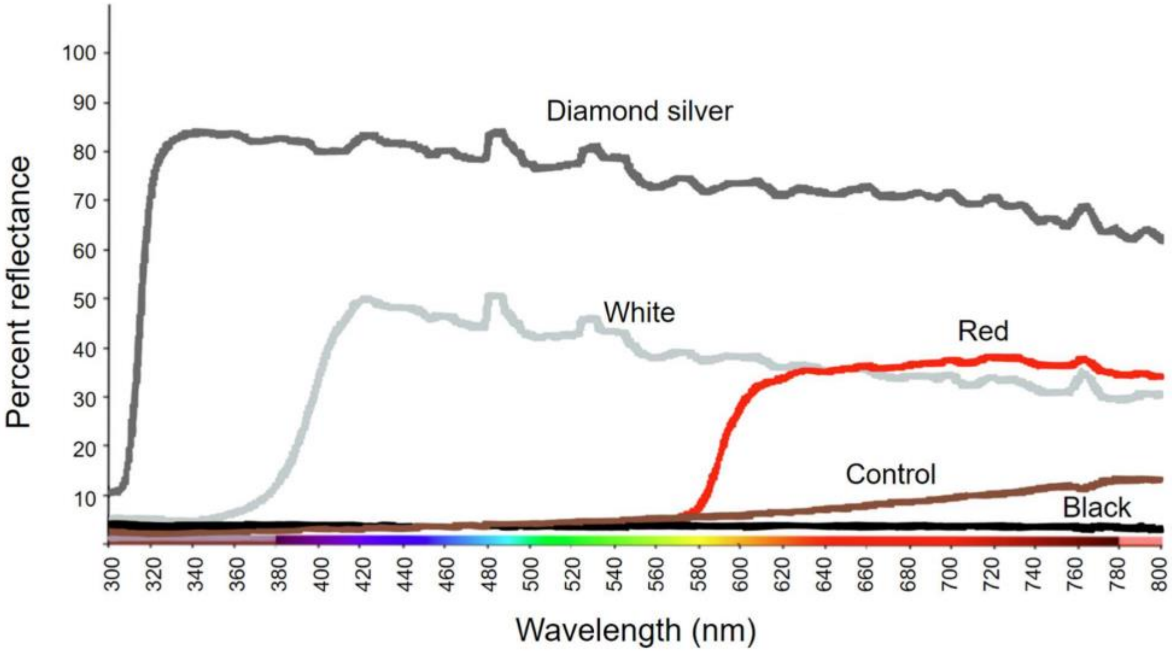

Figure 4.5. Light reflectiveness for 4 mulches and a bare ground control treatment in an experimental onion field. A handheld spectrophotometer was used to measure the light on 14 August 2023 within the 300 – 800 nm (UV-VIS) range. The spectrophotometer was connected to a pulsed xenon light source to maintain standard light conditions during data collection.

<u>No-choice lab experiment</u>

In our no-choice experiment, mulch color significantly affected larval onion thrips numbers ($\chi^2 = 22.67$, df = 4, p-value < 0.01) but not adult numbers (Figure 4.6; $\chi^2 = 1.91$, df = 4, p-value = 0.75). There were no significant differences in larval onion thrips numbers between any of the mulch treatments compared to the no mulch control treatment (t-value < 2.34, df =54, p-value > 0.13). However, there were approximately half as many larval onion thrips on the red (2.44 larval onion thrips/leaf) compared to the black mulch (4.75 larval onion thrips/leaf; t-value = -3.25, df = 54, p-value = 0.01), and the white mulch (4.25 larval onion thrips/leaf; t-value = 2.89, df = 54, p-value = 0.03). Mulch color did not significantly affect plant growth (F = 0.92, df = 4, 104, p-value = 0.46).

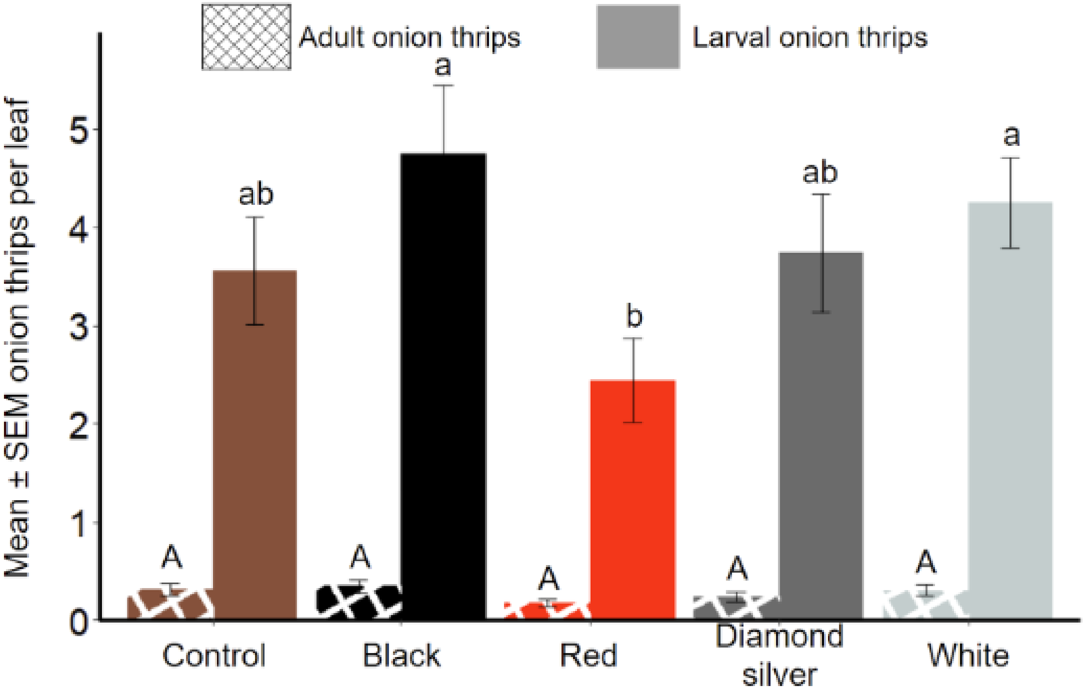

Figure 4.6. Mean ± SEM adult and larval onion thrips per leaf on 4 different mulches and a bare ground control treatment in a no-choice experiment in the lab. Solid bars represent larval onion thrips and crosshatch bars represent adult onion thrips. Three adult onion thrips were added to each experimental unit, and after 13 days, the number of adult and larval thrips was recorded. Bars with different letters of the same case indicate significant differences (Tukey's HSD, α = 0.05).

Mulch color significantly affected mulch surface temperature (F = 25.4, df = 4, 104, p-value < 0.01). All treatments increased the mulch surface temperature compared to the control treatment by approximately 1°C (t-value > 6.09, df = 54, p-value < 0.01). There were no significant differences between the different colored mulches when compared to each other (t-value < -2.5, df = 54, p-value > 0.09). Soil temperature was significantly affected by mulch color (F = 7.18, df = 4, 104, p-value < 0.01). All mulch treatments significantly increased soil temperature compared to the control treatment by approximately 2°C (t-value > 2.86, df = 54, p-value < 0.03). There were no significant differences in soil temperature between the mulches when compared to each other (t-value < -2.50, df = 54, p-value > 0.08).

Discussion

Our results demonstrate that some plastic mulches can be used as an effective alternative management strategy for onion thrips. Red mulch and diamond silver mulch without a pesticide program were as effective at managing onion thrips populations as a pesticide program, potentially offsetting spray application costs and the negative impacts associated with conventional pesticide use. Furthermore, we found that combining a pesticide program with plastic mulches can provide additional onion thrips control. Our lab-based performance experiment suggests that mulch impacts thrips directly, rather than through indirect effects, such as changes in the microclimate.

In our first field experiment, we tested various plastic mulches to determine which colors were most likely to be successful in reducing onion thrips populations. All of our mulch treatments had increased onion thrips populations compared to the control, but this could be due to the lack of color contrast between the grassy background and onion foliage, or associational resistance provided by the grass (Barbosa et al. 2009). Western flower thrips are attracted to objects that have contrasting background, and this might be the case for onion thrips as well (Vernon and Gillespie 1995; Mainali and Lim 2010). We expected that all tested types of silver reflective mulches would be equally effective at reducing onion thrips numbers on onions, but this was not the case, with inconsistent performance across silver reflective mulches, as reported in previous studies (Lu 1990; Csizinszky et al. 1995; Greer and Dole 2003; van Toor et al. 2004; Iglesias et al. 2021). Although the reflectance range was similar across the silver reflective mulches in our experiment, the amount of reflected light (% reflectiveness) was different depending on the mulch brand. Alterations in the reflectiveness or intensity of light change the attractiveness/repellence of mulches, and this could be why our silver reflective mulches performed differently from one another, with the most reflective mulch, diamond silver, performing the best for onion thrips suppression (Otani et al. 2014; Otieno et al. 2018; Stukenberg et al. 2020). The results highlight the need to investigate spectral information so that the differences between wavelength composition (chromatic) and reflectiveness or intensity (achromatic) cues can be identified.

In our second field experiment, we selected a subset of the mulches and tested them with and without a pesticide program, with the goal of understanding if the integration of a pesticide program could provide synergistic control. Surprisingly, we found that red (without a pesticide program) and diamond silver mulch (with or without a pesticide program) alone achieved similar levels of onion thrips control compared to the control (bare ground) with a pesticide program. Onion thrips are notorious for developing insecticide resistance to the limited number of active ingredients available for their control (Martin et al. 2003; Shelton et al. 2006; Nault and Shelton 2010; Diaz-Montano et al. 2011; Adesanya et al. 2020) and having non-insecticide-based management options, such as mulches, that can suppress their populations to the same levels as a season-long pesticide program can be an important tool in their integrated pest management program.

The integration of a pesticide program with the mulches provided synergistic control in all our treatments, except for the diamond silver mulch. Onion thrips numbers in these plots were already some of the lowest across our treatments, well below the action threshold of 1 onion thrips/leaf for the seasonal average and below the economic injury level of 2.2 onion thrips/leaf (Fournier et al. 1995). With the addition of a pesticide program, the black mulch (0.85 onion thrips/leaf) and the control treatment (0.84 onion thrips/leaf) suppressed onion thrips populations below the threshold. The red mulch with a pesticide program was the most effective in suppressing onion thrips populations (0.33 onion thrips/leaf). Although the diamond silver mulch with a pesticide program reduced onion thrips (0.5 onion thrips/leaf) below the action threshold, the addition of the pesticide program did not provide additional control. In our study, the diamond silver mulch was the only mulch that reflected light within the UV range, and since pesticides can degrade in the presence of UV light, it's possible that this led to decreased pesticide efficacy (Leach et al. 2017; Lakshmipathy et al. 2024).

Mulches may directly impact onion thrips; for other thrips species, the irradiation of red light in a greenhouse reduced the density of adults (Katai et al. 2015; Shibao and Tanaka 2015; Murata et al. 2018a). Although we did not directly irradiate the onions, it's possible that the increase in red light reflecting off the red mulch was enough to deter onion thrips from landing on the plants. The diamond silver mulch has a broad range wavelength composition, and it is possible that this combined with high reflectiveness reduced thrips colonization and population growth (Lopez-Reyes et al. 2022). Interestingly, in the first field experiment, white mulch performed similarly to the red and diamond silver mulch but was not effective in reducing onion thrips populations in subsequent experiments. In the second field experiment white mulch had the greatest onion thrips pressure in our plots (2.2 onion thrips/leaf in the untreated plots; 1.9

onion thrips/leaf in the pesticide treated plots); these results are supported by other studies although it is unknown why this occurs, it may be because it does not reflect light within the UV range (Iglesias et al. 2021; Teulon and Penman 1992). The differences in the effectiveness of the white mulch between experiments are potentially due to manufacturing changes, changes in latitude and timing, or differences in the length of the experiments. It's been suggested that white mulch degrades throughout the season, or becomes dirty, causing it to appear more yellow which attracts thrips (Mutetwa and Mtaita 2014). However, our spectral data suggests that the white mulch continued to reflect within the entire spectrum rather than just the yellow range (570 – 580 nm), therefore it is unlikely that this alone explains the increases in onion thrips populations compared to the other mulches.

In addition to affecting onion thrips populations, mulch color affected plant growth and microclimate. Onion plants grown on the red and black mulches had more leaves compared to the control plants. Red and black mulches are recommended to increase early season growth because they increase the temperature around the plants, and our data supports these findings (Job and Bhaker 2018; Csizinszky et al. 1995; Greer and Dole 2003). Interestingly, onions growing on white mulch had a similar number of leaves compared to those on the red and black mulch, despite a low soil temperature and having about twice as many onion thrips on average. In tomatoes, white mulch induces foliage growth and similar patterns are seen in our experiment (Decoteau et al. 1989). In contrast to the red and black mulch, white mulch had a lower soil temperature compared to the control treatment, so it is unlikely that for the white mulch, microclimate changes were the mechanism behind plant growth. Onions grown on the diamond silver mulch were the smallest, had fewer leaves than those grown on the red, black, and white mulch, and were shorter than onions grown on the red and black mulch. The surface of the diamond silver mulch was cooler than the ambient temperature, and the soil temperature was lower compared to the other mulches, thus it’s possible that the lower temperatures on the diamond silver mulch reduced plant growth (Daymond et al. 1997). Although we were unable to measure yield, leaf number and plant height are positively correlated with bulb size (Nourbakhsh and Cramer 2022), suggesting that there is a potential for reduced yields when using diamond silver mulch. Overall, the effects of mulches on plant growth and microclimate were variable, and they did not translate into predictable changes in onion thrips densities, indicating that direct rather than indirect mulch effects modulate onion thrips numbers on plants.

Our no-choice lab experiment also suggested that mulch color directly affects onion thrips, especially the immature stages. Although there were no differences in adult populations at the end of our lab experiment, there were significant differences in the number of larval onion thrips which could be a result of changes in fecundity or survival. Other insects, such as predatory pirate bugs (*Orius sauteri* Poppius; Hemiptera: Anthocoridae), have reduced fertility and fecundity and took longer to lay eggs under red (678.5 nm) light, and it’s possible we were observing a similar pattern with onion thrips (Wang et al. 2013). In the field, the red and the diamond silver mulch had the lowest onion thrips populations, but it is unclear whether that was due to physiological changes causing reductions in population growth. Melon thrips, *Thrips palmi* (Thysanoptera: Thripidae), do not exhibit an adverse physiological response when exposed to different wavelengths of light, including red light (660 nm), suggesting that responses to light are likely species-specific (Murata et al. 2018a). However, melon thrips exhibit behavioral changes and disperse away from plants irradiated with red light to search for alternative hosts (Murata et al. 2018b). In our lab experiment, alternative hosts were not available, which may explain similar adult numbers across treatments in the lab experiment, but they may have spent less time on the plants, resulting in lower larval numbers. In the field, it's possible that adult onion thrips either avoided plants in the red and diamond silver mulches, or they landed on plants within those treatments but quickly dispersed; in future experiments, we need to determine if mulches provide pre- or post-alighting cues. Overall, it is unclear whether the changes in populations are due to wavelength-induced physiological or behavioral changes, or a combination of both. All the mulch treatments had significantly higher temperatures on the mulch surface and in the soil compared to the control, but there were no differences between the mulch treatments. This further supports our field experiments indicating that mulches directly affect onion thrips populations through wavelength-specific induced changes rather than indirectly through changes in the microclimate or host plant quality.

In conclusion, our results suggest that mulches can be a viable alternative management strategy to pesticide programs, and the combination of mulches with a pesticide program can provide further onion thrips suppression resulting in reduced reliance on insecticides. Furthermore, we found that silver reflective mulches are not equally effective against onion thrips, the diamond silver mulch being the most effective, likely due to the differences in achromatic (i.e. % reflectiveness) cues. In the field, mulches had inconsistent effects on onion thrips populations, plant growth, and microclimate, therefore growers should consider how these affect plant growth before selecting a mulch. Finally, wavelength likely had a direct effect on onion thrips, especially apparent in a reduction in immature stages in the lab. Overall, our study highlighted how multiple IPM tactics, such as chemical and cultural control, can be combined to create an integrated pest management program for onion thrips.

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CHAPTER 5: MANAGING INSECT AND PLANT PATHOGEN PESTS WITH ORGANIC AND CONVENTIONAL PESTICIDES IN ONION

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Abstract

Onion thrips (*Thrips tabaci* Lindeman, Thysanoptera: Thripidae) is a significant insect pest of onions (*Allium cepa* L., Asparagales: Amaryllidaceae). In addition to feeding on onion foliage, they may spread plant pathogens. Currently, onion thrips and pathogens are managed as separate pests with insecticides and fungicides. It may be beneficial to manage these pests simultaneously as limiting onion thrips may reduce pathogen damage. We tested combinations of bio- and conventional pesticides in a season-long management program in Michigan onion fields. From 2020-2022, we counted onion thrips weekly and visually estimated plant foliage necrotic damage (%) in experimental plots each year. In 2020, we tested six treatment programs including: azadirachtin, spinosad, a copper-based fungicide, azadirachtin + copper-based fungicide, spinosad + copper-based fungicide, and an untreated control. The thrips populations were not significantly reduced compared to the control, but necrotic damage was reduced significantly in spinosad-treated plots. In 2021, we tested a combination of 8 bio- and conventional pesticide programs. Compared to the control, the bioinsecticides did not reduce onion thrips populations, but the conventional pesticide programs reduced both onion thrips numbers and necrotic damage. In 2022, we tested only conventional insecticide programs but included three different action thresholds for initiation and applied them with or without a

fungicide, for 8 treatments. All insecticide programs reduced onion thrips compared to the control, the action threshold did not impact thrips numbers significantly. Overall, the use of action thresholds can lead to fewer insecticide applications and lower incidence of leaf damage.

Introduction

Overreliance on pesticides in agriculture has numerous negative effects related to public health, non-target impacts, and pesticide resistance (Pimentel 2005; Rolim et al. 2020; Serrao et al. 2022; Silva et al. 2020). Despite these negative impacts, pesticides continue to be one of the most effective tools used in Integrated Pest Management (IPM) to control pests (Reddy 2016), and the incorporation of biopesticides into conventional agricultural practices could lead to greater sustainability. In organic agriculture, pesticides are limited to those approved by the Organic Materials Review Institute (OMRI) and can only be used if all other management options have been exhausted (USDA 2023). The sustainable use of pesticides in both conventional and organic management systems relies on strategies that limit their use. These strategies include the use of action thresholds, a thorough understanding of the biology and ecology of the system, the correct timing and dose of applications, and the use of appropriate active ingredients for specific pest species (Siegwart et al. 2015).

The complexities of pest management are exacerbated when different pest species co-occur and affect each other on the host plant. Management tactics that focus on a single pest may compound other pest problems, or in some instances, the suppression of one pest leads to a decrease of another. When plant pathogens are transmitted by insect pests, controlling the insect may reduce pathogen symptoms because insect feeding can provide entry points for plant pathogens (Grode et al. 2017, 2019; Leach et al. 2017; McKenzie et al. 1993). Action thresholds developed for one pest may not apply to multiple interacting pests and thresholds may need to be revised to optimize management.

Farmers are facing new pest management challenges, including the overall rising costs of pesticides, partially due to increases in the cost of development of pesticides that favor highly selective products, which are safer for non-target organisms (Sparks 2013). Overall, there are few active ingredients to control insects, increasing the need to understand the biology of the pest complex (Sparks 2013). Prophylactic applications of insecticide are increasingly unsustainable and action thresholds are a critical part of many insect management programs (Anon., 2001; Leach et al. 2017, 2019; Ternest et al. 2020). Farmers are increasingly interested in incorporating IPM tools to reduce reliance on pesticides and delay pest resistance (Iglesias et al. 2021b; Leach et al. 2017). Generally, biopesticides are short-lived and more effective against immature than adult insects (Golec et al. 2020). If biopesticides are to be adopted more widely in agriculture, their efficacy and application timing must be carefully evaluated as they do not perform well when insect population pressure is high (Dively et al. 2020).

Onion thrips (*Thrips tabaci* Lindeman, Thysanoptera: Thripidae) is a significant insect pest of onions, (*Allium cepa* L., Asparagales: Amaryllidaceae), and if not controlled, can result in severe crop loss (Diaz-Montano et al. 2011; Fournier et al. 1995; Gill et al. 2015; Lewis 1991). Onion thrips is resistant to several classes of insecticides; rotating active ingredients and using action thresholds sustain insecticide efficacy (Adesanya et al. 2020; Diaz-Montano et al. 2011; Leach et al. 2019; Martin et al. 2003; Nault and Shelton 2010; Shelton et al. 2006). One of the most effective insecticides for onion thrips control is spinetoram (Gill et al. 2015; Moretti et al. 2019). Onion growers consider it a high priority to maintain its efficacy (Leach et al. 2017, 2019). Despite efforts to adopt IPM strategies for onion thrips management, insecticides remain the cornerstone for management (Devi and Roy 2018; Iglesias et al. 2021a, b; Leach et al. 2017, 2020, Yadav et al. 2018). Among the bioinsecticides, spinosad is the most effective for onion thrips control (Dively et al. 2020; Iglesias et al. 2021a, b; Nault and Hessney 2005) and it has the same mode of action as spinetoram (spinosyns). Other biopesticides such as azadirachtin and pyrethrin have been tested for onion thrips control with and without adjuvants (Dively et al. 2020; Iglesias et al. 2021b)

Onion thrips damage the onion foliage by feeding and transmitting plant pathogens (Bhangale and Joi 1983; Dutta et al. 2014; Leach et al. 2017; McKenzie et al. 1993). Two important fungal pathogens of onion in Michigan include *Colletotrichum coccodes* (Wallr) (Glomerellales: Glomerellacaea), first identified in 2012 (Rodriguez-Salamanca et al. 2012), and *Stemphylium vesicarium* (E.G. Simmons) (Pleosporales: Pleosporaceae) a destructive pathogen of the eastern production region (Hausbeck and Werling 2018; Hay et al. 2022). The relationship between facultative onion pathogens and onion thrips has been the focus of various studies (Constancio et al. 2022; Grode et al. 2017, 2019; Leach et al. 2020) but the influence of bio- and conventional pesticides on onion foliage and yields is not well understood. Managing two organisms simultaneously may be complicated by tank mixing insecticides and fungicides which may reduce insecticide efficacy (Nault et al. 2013). The action thresholds used to control onion thrips (Nault and Shelton 2010) have been developed without considering the interaction between thrips and onion plant pathogens.

The goal of this study was to evaluate the efficacy of bio- and conventional pesticides in a season-long program to control multiple onion pests and develop a combined onion thrips and Stemphylium leaf blight management pesticide program, with resistance management practices in mind. Our first goal was to broaden the insecticide options currently used in onion thrips management by testing bioinsecticides. In 2020 we evaluated the efficacy of biopesticide programs by comparing six different treatments. Building off these results in 2021, we then compared eight different bio- and conventional pesticide programs side-by-side. We repeated some of the biopesticide programs from 2020 and added two new conventional pesticide programs to address the differences in the efficacy of bio- and conventional pesticides. The conventional pesticide programs we evaluated in 2021 follow the recommended pesticide programs but are not appropriate for insecticide resistance management. Therefore, we made the decision to change pesticide programs to address the overarching goal of our study. In 2022, we tested varying thrips action thresholds of the conventional pesticide program from 2021. We did this to reduce the total number of applications of conventional pesticides and allow better coherence to insecticide resistance management guidelines. To measure the impact of our treatments on onion pests, we counted onion thrips per leaf weekly, and visually estimated leaf necrosis to measure foliar health.

Materials and Methods

Field Site

Experiments were conducted on muck soil, previously cropped to celery, from June to August from 2020 to 2022 on a commercial conventional onion farm located in Allegan County, MI. Onions are rotated to a new field every year and experimental plots were moved to a new location within the same farm each year. ‘Bradley’ onions were direct seeded in early April of each year on raised plant beds at a density of approximately 625,000 seeds/ha. Each plant bed was comprised of 8 rows spaced 0.15 m apart; onions were direct seeded 5 cm apart. Fertilization, herbicide application, and irrigation were managed by the grower cooperator, and weeds were removed manually to supplement commercial practices. Herbicide treatments were applied to the whole field by the grower using a broadcast sprayer prior to experimental applications. Treatment plots (6 m long x 1.5 m wide) were separated by a 0.6 m buffer and arranged in a randomized complete block design, with 4 replicates. All pesticides were applied as a foliar spray with a CO_2 backpack sprayer and a broadcast boom. The boom was equipped with three XR8003 flat-fan nozzles (TeeJet Technologies, Wheaton, IL) spaced 45 cm apart, calibrated to 241 kPa to deliver 467 L/ha.

Onion Thrips

Onion thrips counting began on 8 July 2020, 14 June 2021, and 14 June 2022 and included counting all thrips on 10 randomly selected plants per plot from the center 6 rows of the beds. Selected plants including the neck of the onion were visually assessed in a non-destructive manner for the total number of adult and larval onion thrips. Thrips were counted weekly until 27 July 2020, 9 August 2021, and 8 August 2022. In addition to recording onion thrips numbers, the number of leaves was counted weekly for 20 plants randomly selected from the center 6 rows of the field plots.

Necrotic Plant Tissue

Necrotic plant tissue was assessed by visually estimating the percentage of necrotic tissue (0 – 100%) per plot on 28 July, and 3 and 10 August in 2020; 23 and 30 July and 6 and 17 August in 2021. In 2022, plots were assessed weekly from 20 June to 29 August, for a total of 10 assessments except for the first week of August. If there were signs of Stemphylium leaf blight, the pathogen was isolated from 50 plants and identified based on conidial morphology (Woudenberg et al. 2017).

Pesticide Treatments

Treatments in 2020 included: 1) untreated control, 2) azadirachtin (neem oil), 3) spinosad (Entrust SC), 4) azadirachtin + copper (Kocide 3000), 5) spinosad + copper, and 6) copper

(Table 5.1). Pesticides were applied weekly from 9 July until 4 August, and were tank mixed with a nonionic surfactant (Dyne-Amic, 0.05% v/v), for a total of 5 pesticide applications.

Table 5.1: Pesticides used in the onion thrips and onion disease management field experiments, 2020-2022. Group number refers to the mode of action of the pesticide.

Group #	Product name	Manufacturer	Active ingredient	Rate	Chemical type	OMRI[a] certified	Year applied
UN	Neem oil	Platonix	Azadirachtin	7.8 ml/L	Insecticide	Yes	2020
UN	Neemix 4.5	Certus Biologicals	Azadirachtin	7.8 ml/L	Insecticide	Yes	2021
5	Entrust SC	Corteva	Spinosad	1.1 ml/L	Insecticide	Yes	2020, 2021
	Kocide 3000-O	DuPont	Copper hydroxide	3.6 ml/L	Bactericide/ Fungicide	Yes	2020, 2021
23	Movento	Bayer CropScience	Spirotetramat	0.78 ml/L	Insecticide	No	2021, 2022
6, 28	Minecto Pro	Syngenta	Abamectin, Cyantraniliprole	1.5 ml/L	Insecticide	No	2021, 2022
5	Radiant SC	Corteva	Spinetoram	1.5 ml/L	Insecticide	No	2021, 2022
1	Lannate LV	Corteva	Methomyl	7.5 ml/L	Insecticide	No	2021
3	Warrior II	Syngenta	Lambda-cyhalothrin	0.3 ml/L	Insecticide	No	2021
6	Agri-Mek	Syngenta	Abamectin	0.5 ml/L	Insecticide	No	2021
7, 12	Miravis Prime	Syngenta	Pydiflumetofen, Fludioxonil	1.8 ml/L	Fungicide	No	2021, 2022
M5	Bravo Weather Stik	Adama Agricultural Solutions	Chlorothalonil	3.8 ml/L	Fungicide	No	2021, 2022
	Dyne-Amic 90	Helena Chemical Company	Methylated seed oils Organisilicone non-ionic surfactant	0.05% v/v	Surfactant	No	2020
	Activator 90	Loveland	Non-ionic surfactant	0.05% v/v	Surfactant	No	2021
	Syl-Tac	Wilbur-Ellis	Organosilicone surfactant Modified vegetable oil concentrate	0.10 % v/v	Surfactant	No	2022

[a] OMRI: Organic Materials Review Institute

Treatments in 2021 included: 1) untreated control, 2) azadirachtin (Neemix 4.5), 3) spinosad, 4) azadirachtin + copper, 5) spinosad + copper, 6) copper, 7) conventional insecticide program + copper (CI + copper), and 8) conventional insecticide and fungicide programs (CI + CF). All pesticides were tank-mixed with a nonionic surfactant (Activator 90, 0.05% v/v). Conventional insecticide treatments 7 and 8 contained the following applied twice, 7 days apart before rotating to the next insecticide: spirotetramat (Movento), abamectin + cyantraniliprole (MinectoPro), spinetoram (Radiant SC), methomyl + lambda-cyhalothrin (Lannate LV + Warrior II), and a single application of abamectin (Agri-Mek) (Table 5.1). The conventional fungicide program consisted of pydiflumetofen + fludioxonil (Miravis Prime) and chlorothalonil (Bravo Weather Stik), applied 7 days apart and rotated weekly for 6 weeks, before applying chlorothalonil. All pesticide programs began on 15 June and continued until 10 August.

In 2022, pesticide treatments were applied according to various thrips action thresholds and included: 1) untreated control, 2) fungicide only, 3) low threshold + fungicide, 4) moderate threshold + fungicide, 5) high threshold + fungicide, 6) low threshold, 7) moderate threshold, and 8) high threshold. Pesticides were tank mixed with an organosilicone surfactant (Syl-Tac, 1% v/v). All programs containing fungicides alternated between pydiflumetofen + fludioxonil and chlorothalonil weekly, starting on 5 July. The low action threshold was 0.5 thrips/leaf (6 applications) and included: spirotetramat, abamectin + cyantraniliprole, and spinetoram; each applied twice, 7 days apart. Spirotetramat's recommended action threshold for onion thrips is 0.6 onion thrips per leaf, therefore, to test the efficacy of the low threshold and to ensure all 6 pesticide applications could be applied to the field, we reduced the action threshold to 0.5 onion thrips per leaf. The moderate threshold was 0.6 onion thrips/leaf (4 applications) and included abamectin + cyantraniliprole and spinetoram; each applied twice, 7 days apart. Abamectin +

cyantraniliprole is typically the second product used in onion thrips management (applied after spirotetramat), however, the moderate threshold did not receive a spirotetramat application, and so abamectin + cyantraniliprole was applied using the action threshold commonly recommended for spirotetramat. The high threshold was 1.0 thrips/leaf (2 applications) and included spinetoram applied 7 days apart. The low, moderate, and high threshold programs began on 5 July, 26 July, and 9 August, respectively.

Data Analysis

Data from each year were analyzed separately. To determine the effect of treatment on onion thrips populations and the estimated necrotic plant tissue, the mean number of onion thrips per leaf was log-transformed, and the estimated percent necrotic plant tissue was logit-transformed to meet the assumptions of an analysis of variance (ANOVA) and compared with the ‘lme4’ package (Bates et al. 2015) using treatment as the fixed factor and date and plot as a random factor. Tukey’s HSD test ($\alpha = 0.05$) was used to determine the differences between treatment means using the ‘multcomp’ package (v1.4-22; Hothorn, Bretz and Westfall, 2023). All analyses were completed in R (R Core Team 2023).

Results

2020

Onion Thrips

In the control treatment, onion thrips numbers averaged 9.4 per leaf and ranged from 7.6 to 6.9 onion thrips per leaf in the treated plots. Although numerically different, there were no statistically significant differences in onion thrips numbers among the treatments (F = 0.53, df = 5, 77.49, p-value = 0.76; Figure 5.1A).

Necrotic Plant Tissue

In adjacent experimental plots, the first plant with *Stemphylium*-like conidia was observed on 7 July. There was a significant effect of treatment on necrotic damage on 3 August 2020 (F = 12.33, df = 5, 15, p-value < 0.01, Fig. 5.1B; Fig. S5.1). Compared to the control treatment, there was an approximately 20% reduction in necrosis in plots treated with spinosad (t-value = 5.73, df = 6, p-value < 0.01) and spinosad + copper (t-value = 6.09, df = 6, p-value < 0.01). There was no difference among the control, azadirachtin, azadirachtin + copper, and copper-only treatments.

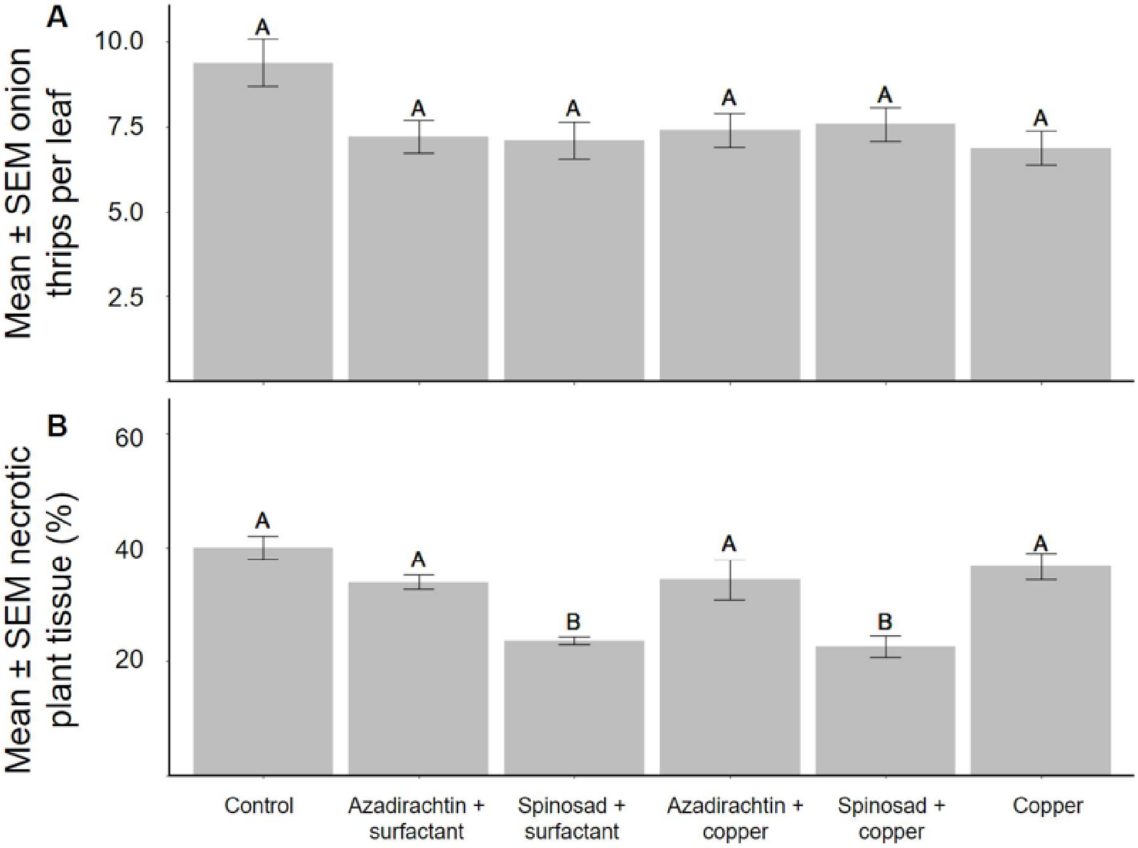

Figure 5.1. In 2020, pesticide treatments were applied weekly to an onion field. Each week, onion thrips were counted on 10 plants per plot and the % of necrotic tissue per plot was visually estimated. A) The seasonal mean ± SEM onion thrips per leaf for each treatment. B) The mean ± SEM estimated percent necrotic tissue on 3 August 2020. Different letters above the bars indicate significant differences (Tukey's HSD, α = 0.05).

2021

Onion Thrips

Onion thrips numbers were significantly different among pesticide treatments (F = 5.34, df = 7, 27.85, p-value < 0.01; Fig. 5.2A). Compared to the control (1.4 onion thrips per leaf), there was no significant difference in the mean number of onion thrips per leaf in any of the biopesticide programs (ranging from 1.0 to 1.5 onion thrips per leaf). The conventional insecticide + fungicide program (0.5 onion thrips per leaf) reduced onion thrips numbers by 65% when compared to the control (t-value = 4.64, df = 72, p-value < 0.01). There was no statistical difference in onion thrips numbers between the conventional insecticide + copper treatment (0.7 onion thrips per leaf) and the conventional insecticide + fungicide program (t-value = -1.91, df = 72, p-value = 0.54).

Necrotic Plant Tissue

In adjacent experimental plots, the first plant with Stemphylium-like conidia was observed on 24 June and the pathogen was isolated and identified from 50 plants. A moderate incidence of pink root rot and low incidence (<10%) of anthracnose was also observed in adjacent experimental plots. We found that the estimated necrotic damage was significantly different among treatments on 17 August 2021 (F = 9.76, df = 7, 21, p-value < 0.01; Fig. 5.2B; Fig S5.2). Compared to all treatment programs, the fully conventional (insecticide + fungicide) program significantly reduced the amount of necrotic tissue in the field (t-value > 3.84, df = 8, p-value < 0.02). There were no significant differences in necrotic tissue among the other treatments.

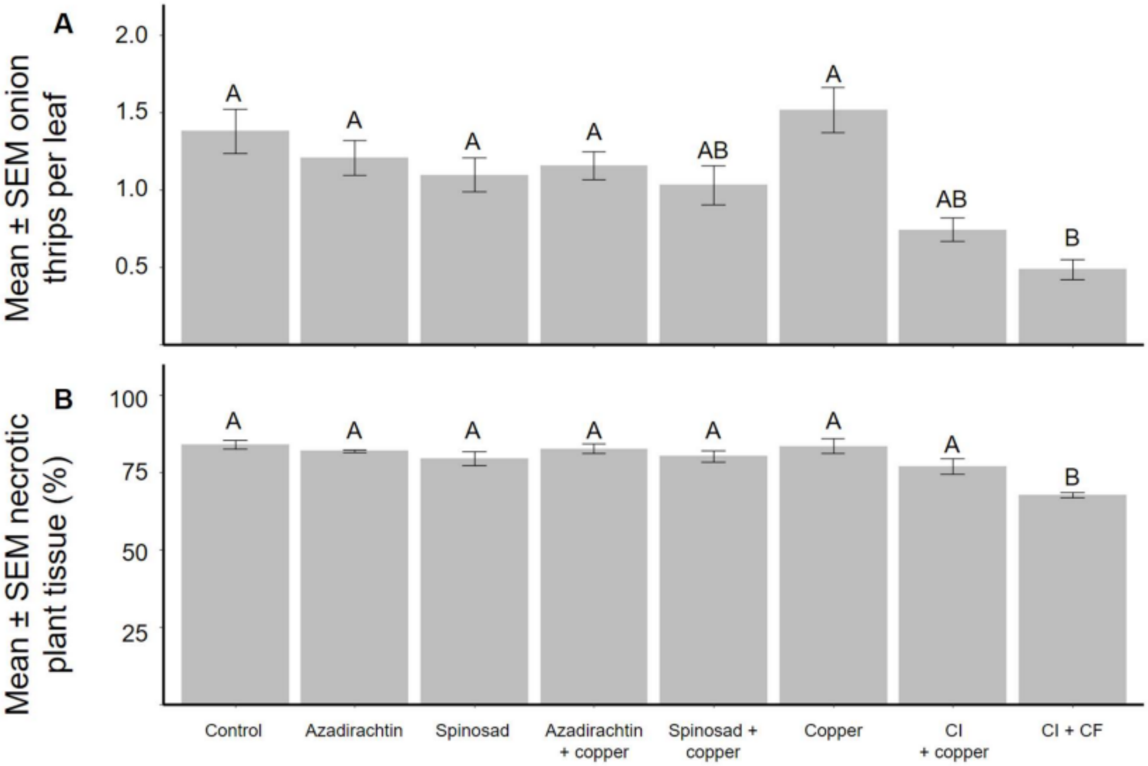

Figure 5.2. In 2021, 8 different pesticide treatments consisting of a mix of bio- and conventional pesticides were applied weekly in an onion field. Treatments labeled CI + copper represent the conventional insecticide program + copper fungicide, while the CI + CF treatment is the conventional insecticide program with a conventional fungicide program. Each week, onion thrips were counted on 10 plants per plot and the % necrotic tissue was visually estimated for each plot. A) The seasonal mean ± SEM onion thrips per leaf for each treatment. B) The mean ± SEM estimated percent necrotic tissue on 17 August 2021. Different letters above the bars indicate significant differences (Tukey's HSD, $\alpha = 0.05$).

2022

Onion Thrips

Overall, treatment had a significant effect on onion thrips numbers (F = 4.33, df = 7, 21, p-value < 0.01; Fig. 5.3A). Treatments with or without fungicides had similar numbers of onion thrips throughout the growing season (0.81 onion thrips per leaf vs 0.63 onion thrips per leaf). All insecticide-only treatments, regardless of action threshold, reduced onion thrips populations by about 54% when compared to the control (1.2 onion thrips per leaf; t-value < -3.44, df = 56, p-value < 0.02). When comparing treatments across all insecticide programs, with or without fungicide, all of them reduced onion thrips similarly, regardless of the action threshold.

Necrotic Plant Tissue

There was a significant effect of treatment on necrotic damage on 29 August (F = 5.93, df = 7, 24, p-value < 0.01; Fig. 5.3B, Fig. S5.3). When compared to the control treatment the low action threshold + fungicide significantly reduced the amount of necrotic tissue (t-value = 5.2, df = 21, p-value < 0.01). Similarly, the low action threshold + fungicide treatment also significantly reduced necrotic tissue in the field when compared to the high action threshold without fungicide (t-value = -4.14, df = 21, p-value < 0.01). When compared to the control treatment, the moderate action threshold without a fungicide and the high action threshold without a fungicide did not reduce the amount of necrotic tissue.

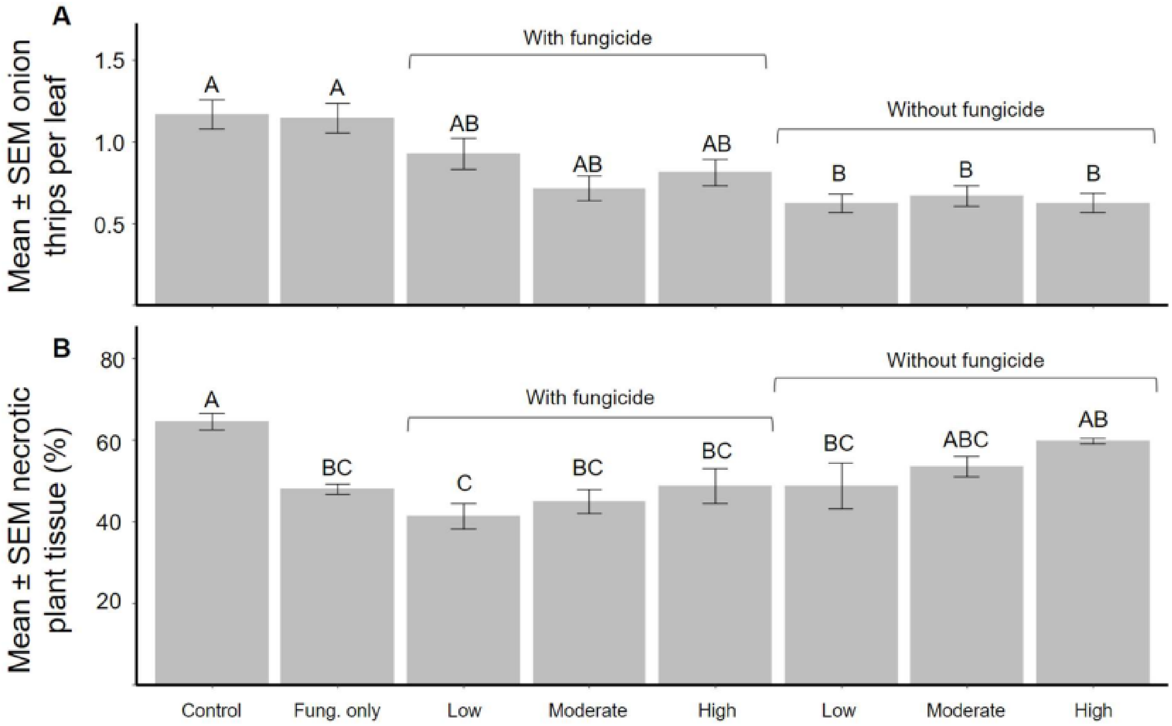

Figure 5.3. In 2022, 8 different conventional pesticide programs, with or without a fungicide, were applied to an onion field timed at three onion thrips action thresholds. Each week, onion thrips were counted on 10 plants per plot and the % necrotic tissue was visually estimated for each plot. Low threshold applications began when the onion thrips populations reached 0.5 onion thrips per leaf, the moderate threshold was 0.6 onion thrips per leaf, and the high threshold was 1.0 onion thrips per leaf. The fungicide-only treatments were applied weekly and began when the low onion thrips action threshold was reached. A) The seasonal mean ± SEM onion thrips per leaf for each treatment. B) The mean ± SEM estimated percent necrotic tissue on 29 August 2022. Different letters above the bars indicate significant differences (Tukey's HSD, α = 0.05).

Discussion

Our results demonstrated that the bioinsecticides we tested cannot reduce onion thrips sufficiently to keep their populations below the commonly used action threshold of 1 thrips/leaf (Nault and Shelton 2010) even under different pest pressures observed in 2020 (7-10 thrips/leaf) and 2021 (1-1.5 thrips/leaf). We expected the bioinsecticides to perform better in the low insect-pressure year (2021), but this was not the case. The 2021 growing season had nearly twice as much rainfall as 2020 (average rainfall June-August: 18.4 cm = 2020, 34.8 cm = 2021, MSU Enviroweather), so bioinsecticides or onion thrips may have washed off the plants. Biopesticides are often more susceptible to extreme weather conditions than conventional pesticides (Fenibo et al. 2021; Fronk 2022; Gill et al. 2015). Our results represent the best-case scenario for the performance of biopesticides: there were 5 and 9 weekly applications in 2020 and 2021, respectively. These types of calendar-based applications are not representative of typical organic pest management programs, indicating that even these frequent applications were not able to provide sufficient thrips control.

Interestingly, azadirachtin and spinosad performed similarly in both years for thrips suppression relative to the control. Based on previous research, we expected that spinosad would perform significantly better than azadirachtin for onion thrips suppression (Dively et al. 2020; Iglesias et al. 2021a). The reason that spinosad did not perform as expected (Dively et al. 2020) is unlikely to be the result of the surfactant used as two different products were used in 2020 and 2021, and adjuvants were not a significant factor in onion thrips numbers in a previous study (Iglesias et al. 2021a). The sensitivity of onion thrips to spinetoram has been evaluated in New York due to concerns that thrips are developing resistance to this insecticide (Moretti et al. 2019). Despite a variation in LC_{50} levels among different field populations, New York growers reported that spinetoram performed as expected. This is supported by spray trial results, including our 2022 results which indicate that it remains effective in the field (Moretti et al. 2019). In Michigan, spinetoram has been used by growers extensively for the past >10 years so the lack of efficacy for spinosad could be due to an increase in resistance to spinosyn insecticides (Siegwart et al. 2015). It is possible that low levels of resistance are detected when applying the bioinsecticide formulation of this insecticide which may have lower longevity and thus efficacy in the field than the conventional version. Monitoring onion thrips susceptibility to spinosyns using appropriate bioassays should be a focus for geographic regions that rely on these insecticides for onion production (Yannuzzi et al. 2021).

Copper alone did not reduce the amount of necrotic tissue, but the amount of necrotic tissue was lower in spinosad + copper-treated and spinosad-treated plots in 2020. In the case of spinosad-treated plots, the average thrips numbers were similar to those in other treatments that did not reduce necrotic tissue. We did not count adult and immature thrips separately, but it is possible that spinosad was more effective against immature than adult stages, which has been observed for some bioinsecticides (Dively et al. 2020). Overall, copper did not contribute to reducing necrotic tissue and it is possible that the presence of onion thrips feeding reduced the efficacy of copper to control diseases (Stumpf et al. 2021). In 2021, the conventional treatment (CI + CF) had lower amounts of necrotic tissue relative to the other treatments. Leaf necrosis and thrips action thresholds increased in concert from low to high, in 2022, thus the measure of leaf necrosis better predicted our expected thrips performance than average thrips numbers. This may have been because we were measuring relatively small differences among treatments that may have been masked by the variability in insect numbers on plants but were captured in data that reflects cumulative season-long effects. In the future, it will be important to increase sample size and reduce variation.

The conventional insecticide programs significantly reduced the average number of thrips in both years below the action threshold, regardless of tank mixing with fungicides. However, chlorothalonil can interfere with insecticide efficacy in some instances (Nault et al. 2013). In 2022, thrips levels were numerically higher when insecticides were applied with rather than without a fungicide. In 2022, the conventional fungicide-only treated plots significantly reduced necrotic tissue relative to the control, while the average numbers of thrips were similar to the untreated plots. This is likely due to the fungicides providing control against common leaf blight pathogens. The different thresholds we tested in 2022 did not significantly influence average thrips numbers; insect pressure overall was low in 2022 and our thresholds were relatively similar to each other therefore it was difficult to separate these treatments statistically. In the future, thresholds that span a larger range should be tested for their efficacy to determine how thrips and onion diseases could be managed together effectively.

In conclusion, our results suggest that biopesticides have low efficacy for controlling onion pests and their relatively high cost may hinder their adoption in large-scale agriculture. We were not able to conclusively determine in this study if tank mixing of fungicides and insecticides lead to reduced amounts of necrotic tissue, but this issue and the different action thresholds should be further tested in years when onion thrips pressure is high. In addition, using the recommended action threshold is likely to lead to significant savings for growers. In a year when onion thrips pressure was low (2022), two insecticide applications were as effective as six at managing onion thrips, indicating that a threshold-based management program could significantly contribute to reducing economic inputs and insecticide resistance risks (Leach et al. 2018). Indeed, onion growers who adopted threshold-based onion thrips management reduced their insecticide use by 1 to 4 applications leading to appreciable savings (Leach et al. 2018).

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Confederacy of Ojibwe, Odawa and Potawatomi peoples. We advocate for the sovereignty of Michigan’s 12 federally recognized Indian nations, for historic Indigenous communities in Michigan, for the individuals and communities who live here now, and those who were forcible removed from their homeland. By offering this Land Acknowledgement, we affirm indigenous sovereignty, and we will work to hold Michigan State University accountable to the needs of American Indian and Indigenous peoples.

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APPENDIX 2: CHAPTER 5 SUPPLEMENTAL MATERIALS

METHODS: AREA UNDER THE DISEASE PROGRESSION CURVE (AUDPC)

To compare how the pesticide programs affected the amount of necrotic onion tissue, we calculated the area under the disease progress curve (AUDPC) for each year. The AUDPC is used to combine multiple observations of the necrotic tissue progression throughout the season to compare across treatments using a single value (Jeger and Viljanen-Rollinson 2000). The AUDPC values were compared with an ANOVA with treatment as the fixed factor using the 'lme4' package. The 'multcomp' package was used for separating treatment means (Tukey's HSD test, $\alpha = 0.05$). The AUDPC was calculated using the 'agricolae' package (v1.3-5; de Mendiburu, 2022) in R (R Core Team 2023).

RESULTS

In 2020, there was a significant effect of treatment on disease progression ($F = 8.03$, df = 5, 18, p-value < 0.01). Compared to the control, treatments with spinosad (t-value = 4.19, df = 6, p-value < 0.01) and treatments with spinosad + copper (t-value = 4.48, df = 6, p-value < 0.01), significantly reduced disease progression throughout the season.

There was a significant effect of treatment on disease progression ($F = 9.96$, df = 7, 24, $P < 0.01$) in 2021. Compared to the control, the conventional insecticide + copper (CI + copper) (t-value = 4.79, df = 8, p-value < 0.01) and the combined conventional insecticide and conventional fungicide program (CI + CF) (t-value = 6.36, df = 8, p-value < 0.01) significantly reduced disease progression throughout the season. There was no difference in disease progression between spinosad and conventional insecticide + copper spray programs, but the fully conventional (insecticide + fungicide) spray program significantly reduced disease progression compared to the spinosad treatment (t-value = 3.23, df = 8, p-value = 0.016). There was no significant difference in disease progression in the conventional insecticide + copper and the fully conventional program.

In 2022, treatment significantly affected disease progression (*F* = 4.3, df = 7, 24, p-value < 0.01). Compared to the control treatment, the low action threshold + fungicide significantly reduced disease progression throughout the season (t-value = 4.15, df = 14, p-value < 0.01). The low action threshold + fungicide also significantly reduced disease progression compared to the moderate action threshold without fungicide (t-value = -3.43, df = 14, p-value = 0.04) and the high action threshold without fungicide (t-value = -4.19, df = 14, p-value < 0.01).

FIGURES AND FIGURE CAPTIONS

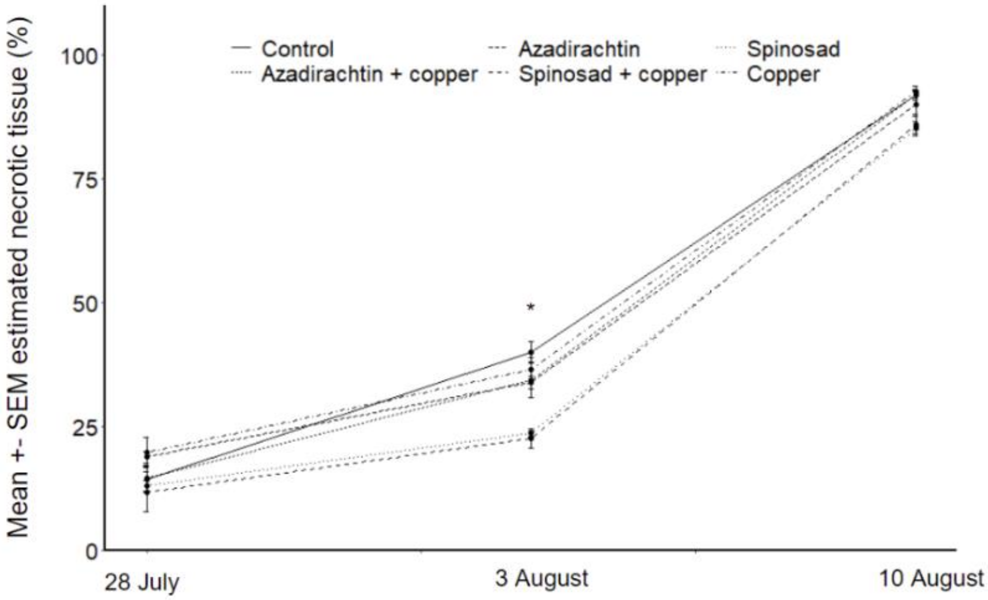

Figure S5.1. In 2020, we tested 6 different pesticide programs in an onion field. The amount of necrotic tissue per plot was visually estimated 3 times throughout the growing season. On July 28th, less than 25% of the plot had necrotic tissue, on August 10th, approximately 80% of the plots were necrotic. Asterisks represent significant differences among treatments, within a date (Tukey's HSD, α = 0.05).

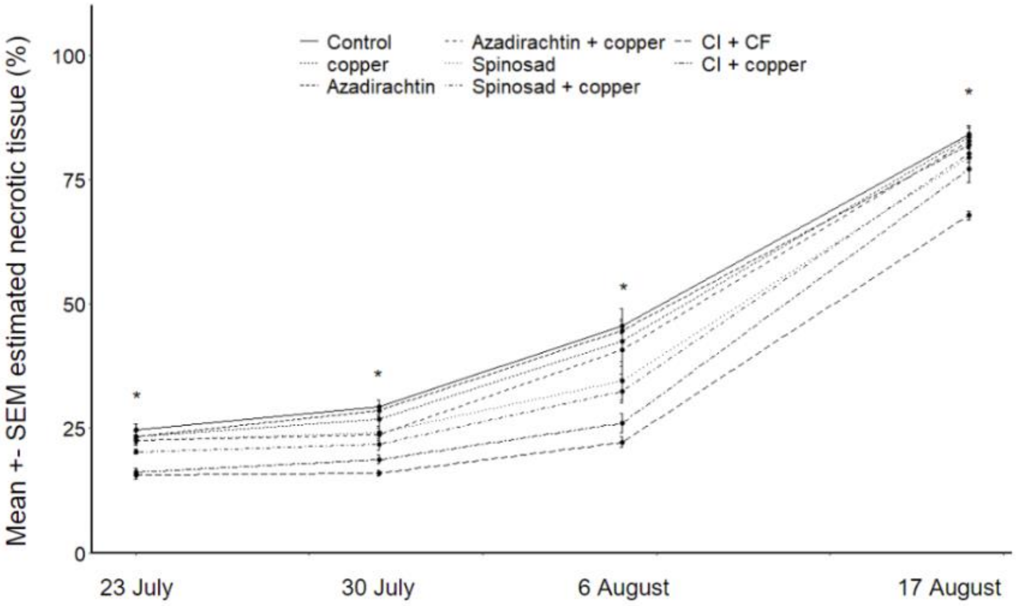

Figure S5.2. In 2021, we tested 8 different pesticide programs in an onion field. Treatments labeled CI + copper represents the conventional insecticide program + copper fungicide, while the CI + CF treatment is the conventional insecticide program paired with a conventional fungicide program. The amount of necrotic tissue per plot was visually estimated 4 times at the end of the growing season. Asterisks represent significant differences among treatments, within dates (Tukey's HSD, α = 0.05).

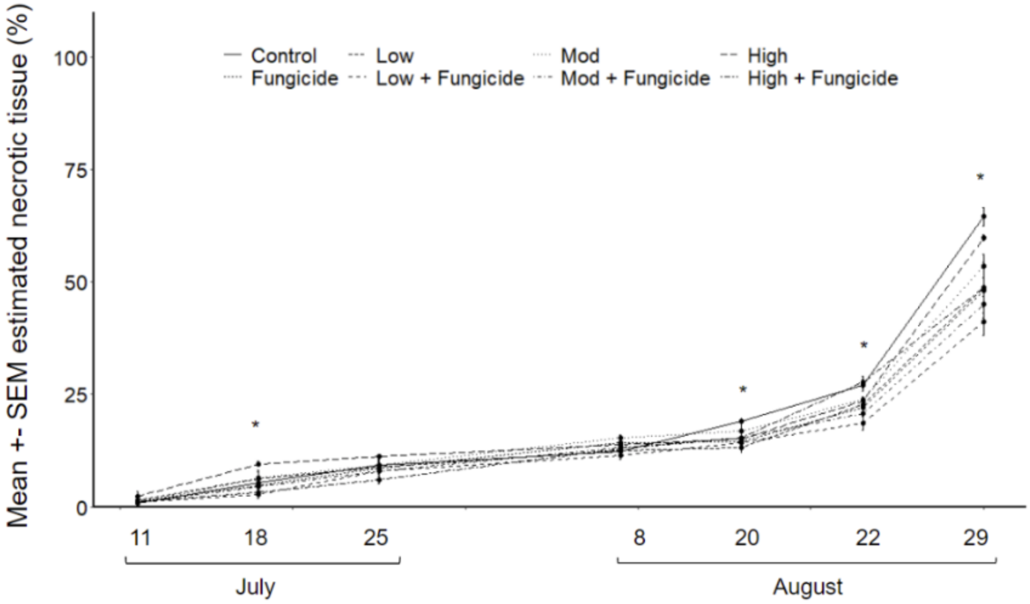

Figure S5.3. In 2022, we tested 8 different pesticide programs in an onion field, these included a combination of pesticides applied when an onion thrips action threshold (Low = 0.5 thrips/leaf; Mod = 0.6 thrips/leaf; High = 1.0 thrips/leaf) was reached. The amount of necrotic tissue per plot was visually estimated weekly throughout the growing season. Asterisks represent significant differences among treatments, within dates (Tukey's HSD, α = 0.05).

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CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

In this dissertation, I examined insect-plant-pathogen interactions, first investigating how *Colletotrichum coccodes* influences onion thrips host preference and performance, then investigating how onion thrips contribute to *C. coccodes* spread with density dependent and independent mechanisms. I tested an integrated pest management (IPM) program to determine potential synergistic methods for onion thrips control and established a multiple pest management pesticide program to reduce onion thrips and foliar fungal pathogens in the field. The occurrence of thrips and facultative pathogens are well documented in the field (Bhangale and Joi 1983; Farrar and Davis 1991; McKenzie et al. 1993; Fournier et al. 1995; Fermaud and Gaunt 1995; Marullo 1995; Mailhot et al. 2007; Leach et al. 2020), but there is a gap in the understanding of how these pests directly influence each other and how these interactions affect pest management. My research has begun to fill that gap, providing a better understanding of insect-plant-pathogen interactions and potential IPM strategies for multiple pest management.

In Chapter 2, my research focused on the influence of *C. coccodes* on onion thrips host preference and performance, manipulating host health through artificial damage or infection stage (asymptomatic or symptomatic). The effect of host plant infection on preference and performance has been documented for obligate pathogens and their insect vectors, and the vector manipulation hypothesis (VMH) suggests that obligate pathogens alter vector behavior to increase disease spread (Abe et al. 2012; Shrestha et al. 2012; Maris et al. 2004; Daugherty et al. 2011; Ingwell et al. 2012). This relationship has not been well studied with facultative pathogens, such as *C. coccodes*, despite previous work indicating that powdery mildew (*Erysiphe polygoni)* increases onion thrips survival rate (Yarwood 1943). I determined that plants infected with *C. coccodes* or plants that were artificially injured, were preferred over healthy plants or plants that were both infected and artificially injured. Furthermore, onion thrips preferred *C. coccodes* infected plants compared to healthy plants but did not differentiate between hosts at different infection stages (asymptomatic vs symptomatic). Finally, onion thrips performed best on symptomatic hosts compared to healthy hosts. This chapter demonstrated that *C. coccodes* affects onion thrips preference and performance, indicating that facultative pathogens may also manipulate vector behavior.

Epidemiological theory suggests that the preference for *C. coccodes* infected hosts will directly impact pathogen spread, likely increasing the rate of disease spread (Kingsolver 1987; Sisterson 2008). Preference for infected hosts may increase the rate at which onion thrips and *C. coccodes* conidia or mycelia are in contact with one another, thus if onion thrips disperse away from these infected plants, they may be moving propagative material to healthy hosts. Furthermore, higher reproductive rates and survival on *C. coccodes* infected onions indicate that there is potential for simultaneous pest outbreaks, particularly because these pests already cooccur. Increases in survival rate and reproduction may be due to consumption of fungal material as an alternative food source, or changes in the metabolic profile due to the infection (Yarwood 1943; Medina-Melchor et al. 2022). Future research should focus on determining the mechanisms of preference and performance, as better understanding may help disrupt host plant selection in the field, providing alternative control methods for onion thrips.

In Chapter 3, I focused on understanding how onion thrips spread *C. coccodes* in the lab, examining potential density dependent and independent mechanisms. Increasing onion thrips populations often correlates with increases in disease spread and severity within the field (Yarwood 1943; Grode et al. 2017, 2019; Leach et al. 2020). However, because *C. coccodes* does not rely on an insect vector for dispersal, it is unclear how much onion thrips contribute to disease spread, and whether environmental conditions alter the rate of disease spread. I determined that onion thrips are vectors of *C. coccodes,* and that temperature, but not relative humidity can indirectly alter disease spread. At higher temperatures, onion thrips reproduced more rapidly and were more likely to disperse from a *C. coccodes* inoculated plant to a healthy plant, increasing the rate of disease spread in the experiment.

This chapter provided novel insight into how environmental changes can affect onion thrips behavior and reproduction, indirectly affecting disease spread. The controlled conditions in the environmental chamber allowed me to better understand the indirect effect, but field-based experiments will be necessary to better understand how these patterns change under realistic conditions. For example, relative humidity did not affect disease spread in my experiment, but in the environmental chamber, airflow and water-splash were reduced. However, in the field, relative humidity corresponds with precipitation, and disease can be spread through wind and rain-splash, therefore, it is likely that disease spread patterns will change under field conditions (Tiberi et al. 2016; Eigenbrode et al. 2018). Understanding the indirect effect of climate on pathogen-vector dynamics is important as climate change causes shifts in regional environmental conditions (Anderson et al. 2020; Peters et al. 2022). Furthermore, increases in the number of onion thrips at high temperatures compared to low temperatures, and the corresponding higher disease spread, likely indicates that disease spread is density dependent. These findings, along with the previous chapter, suggest that there may be a mutually beneficial relationship between onion thrips and *C. coccodes,* which will have significant impacts on pest management. Therefore, the remainder of my research focuses on reducing pest populations in the field.

In chapter 4, I focused on the comparison of plastic mulches to the grower standard pesticide program, and tested onion thrips performance on the mulches in the lab to determine potential mechanisms. Silver or highly reflective mulches have been used to reduce pest populations, with success for aphid, whitefly, spotted-wing drosophila, and pear psylla control in field-based studies, but the success has been variable for thrips control (Wolfenbarger and Moore 1968; Powell and Stofella 1990; Csizinszky et al. 1995, 1999; Nottingham and Beers 2020; McIntosh et al. 2021; van Toor et al. 2004; Iglesias et al. 2021). I first tested various colored mulches, including 3 different silver mulches. I found that silver mulches performed inconsistently, with a certain type of silver mulch outperforming the others. Furthermore, when comparing mulches to the standard grower pesticide program, I determined that mulches are a viable alternative management strategy, and the addition of a pesticide program to the mulch treatments provided synergistic control of onion thrips. Finally, a lab-based performance test suggested that mulch color impacts onion thrips directly, likely through wavelength specific behavioral changes.

The results of this chapter provides growers with an effective non-chemical control strategy for reducing onion thrips populations. Onion thrips are resistant to many different insecticides, therefore having a season long, non-chemical control method is critical to their integrated pest management program. Furthermore, it was unclear whether mulches affect pest populations directly through behavioral changes, or indirectly through changes in physiology due to changes in the microclimate around plants (Bégin et al. 2001; Vincent et al. 2003; Diaz and Fereres 2007). The findings from this chapter provide support to suggest that mulches are directly altering insect behavior and potentially physiology. It remains unclear whether mulches reduced onion thrips populations by being unattractive to onion thrips, reduced population growth on certain colors, or if they landed on the least preferred mulches at the same rate as the others but then dispersed away afterwards. Future field research could focus on the differences in adult and larval onion thrips numbers on each mulch to determine if the population patterns are driven by adult color preference or larval survival.

Chapter 5 focused on testing sustainable chemical control methods for onion thrips and foliar fungal pathogens. Onion thrips and foliar fungal pathogens are primarily managed with pesticides despite the negative impacts on non-target organisms, and the development of pesticide resistance (Pimentel 2005; Rolim et al. 2020; Silva et al. 2020). Pesticides will likely remain a critical component of pest management, therefore, it is important that both conventional and organic pesticides are used sustainably within the guidelines of resistance management (Reddy 2016). I determined that organic pesticides, despite weekly applications, did not reduce onion thrips populations compared to the control treatments, but plant necrosis was reduced in some treatments. Furthermore, a side-by-side comparison of organic and conventional pesticide programs revealed that organic pesticides cannot be used to reduce onion thrips populations or necrotic damage, but conventional pesticides reduced both pests simultaneously. I tested various action thresholds for the conventional pesticide program to determine whether the number of pesticide applications per season can be reduced. I found that using an action threshold did not significantly affect onion thrips populations, although the seasonal onion thrips populations were lower than expected, which may have made treatment effects difficult to separate.

In this chapter, organic pesticides were not successful in reducing onion thrips populations despite weekly applications. Organic pesticides are expensive in comparison to conventional ones, and the low efficacy indicates that organic growers need to consider other control methods, like silver or red plastic mulches, to reduce onion thrips and necrotic damage. Using the action thresholds for the conventional pesticide program is likely to reduce the amount of pesticides applied in the season, as 2 pesticide applications were as effective as 6 when onion thrips populations were low. Reducing the number of applications throughout the season can lead to reduced economic input for growers and a lower risk of resistance development. Future research should focus on continuing to test action thresholds in years when onion thrips populations and disease pressure are high to determine how these pests can be effectively managed together.

Overall, this dissertation explored insect-plant-pathogen interactions for facultative pathogens and economically important insect vectors, and tested IPM programs in the field to manage both pests. When considered together, Chapters 2 and 3, suggest that onion thrips and *C. coccodes* exhibit a mutually beneficial relationship. Onion thrips prefer *C. coccodes* infected plants and reproduce at higher rates on these hosts; subsequently, *C. coccodes* spread appears to be density dependent, and higher onion thrips populations increased disease spread. The relationship between onion thrips and obligate pathogens is well studied, but this research highlights the importance of conducting facultative pathogen studies as well. Chapters 4 and 5 focused on developing sustainable management practices for onion thrips and foliar fungal pathogens. Red and diamond silver plastic mulches without pesticide programs were as effective as conventional pesticide programs in managing onion thrips populations below the action threshold, and implementing action thresholds reduced the number of pesticide applications throughout the season. Grower adoption of these IPM tactics is going to be important for sustainable and long-term onion thrips and pathogen management. Overall, future research should continue to explore insect-plant-pathogen interactions, particularly for facultative pathogens that have been historically overlooked. Many of the experiments in this dissertation focused on insect behavior and future work could focus on manipulating these behaviors to enhance integrated pest management.

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APPENDIX 3: VOUCHER SPECIMEN RECORD

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2024-04

Author and Title of thesis:
Author: Natalie Constancio
Title: Integrated pest management of onion thrips and foliar fungal pathogens in onions

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Table S.1: Voucher specimens deposited at the Albert J. Cook Arthropod Research Collection (Michigan State University).

Family	Genus-Species	Life Stage	Quantity	Preservation
Thripidae	*Thrips tabaci*	Adult	10	ethanol