DISENTANGLING THE EFFECTS OF WEED AND DISEASE MANAGEMENT ON ARTHROPOD COMMUNITIES IN ONION SYSTEMS

by

KYLIE LENNON

(Under the Direction of Jason M. Schmidt)

ABSTRACT

Onions are intensively managed due to their high sensitivity to weed, disease, and insect pest pressure. Thrips (Thysanoptera: Thripidae) are major insect pests of onion, causing damage through feeding, and vector bacterial pathogens causing rot. Both thrips and their associated pathogens are known to survive on many weed species in onion growing regions. Combining weeding with bio-pesticides may synergistically manage thrips and disease by reducing disease prevalence and indirectly increasing onion yield. However, little is known about natural enemies in onion systems or how they may be disturbed from weeding practices. Hence, we characterize arthropod communities on commercial organic and conventional onion farms. Additionally, we estimate the effects of organic weed management and bio-pesticides on weed density, thrips and natural enemy activity, disease severity, and yield.

INDEX WORDS: Weed management, Biological control, Organic systems, Bacterial rot, Natural enemies, Thrips

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Approximately 38% of earth's land cover is utilized for agricultural purposes and with the significant land-use conversion, simultaneously, contributes to environmental challenges including greenhouse gases, pollution, soil degradation, and decrease in biodiversity (Reganold & Wachter, 2016). The overuse of synthetic fertilizers and pesticides, heavy reliance on monocultures, increased farm size among other factors have contributed to the intensification of agriculture since the "Green Revolution" in the 1960's (Crowder & Jabbour, 2014). It is projected our population will exceed 9 billion by 2050. Therefore, building environmentally friendly and economically sustainable agricultural practices is necessary to conserve our ecosystem and future food security. Organic management practices provide some benefits over conventional for building less intensive management systems that attempt to better integrate natural resources, and non-synthetic inputs (Smith et al., 2020). Organic research funding, farming land area, and market size has steadily increased (Reganold & Wachter, 2016). Organic practices include crop rotation, crop variety, conservation or augmentative biological control, diversified crops, improved soil health, and restricted use of synthetic pesticides (Reganold & Wachter, 2016; Smith et al., 2020) Additionally, pest outbreaks can be less probable in organic systems compared to more intensively managed conventional monocultures (Hole et al., 2005; Crowder & Jabbour, 2014). Intensive management may increase pest pressure by concentrating pest's resources with little offset by biological control.

Biological control is a primary ecosystem service, and natural enemies may establish higher abundance and evenness in organic systems (Crowder et al., 2010; Crowder & Jabbour, 2014). For example, a recent meta-analysis of 60 crops concluded that arthropod biodiversity tends to be higher in organically management crops (Smith et al. 2020). Predator species richness was significantly higher in organic citrus orchards compared to conventional orchards (Galloway et al., 2021). However, studies observe both positive and negative effects of organic as compared to conventional systems on within field biodiversity benefits. For example, Rosas-Ramos et al. (2022) found beetle species richness was highest in organic fruit orchards, but beetle abundance was not different between organic and conventional management, specifically for predatory and phytophagous beetle species. Organic farming benefits to arthropod biodiversity and biological control may be most prevalent in areas with larger crop fields that have been subjected to agricultural intensification (Smith et al., 2020).

Managing crops organically also comes with a few drawbacks. Although organic farming is more profitable due to market value, lower yields of high-quality marketable products result from less intensive management. A meta-analysis of 60 crops found organic vegetables yield on average 27% lower than conventional (Smith et al., 2020). Unfortunately, many organic practices do not have the same control efficacy compared to their conventional synthetic counterparts (Marrone, 2009). Weed, disease, and insect pest management are major challenges for vegetable growers in the Southeastern United States (Singh et al., 2006). The warm humid climate is suitable for rapid growth and development, and shorter overwintering periods (Price and Norsworthy, 2013). Although previous research has successfully created organic management programs of vegetables, such as sweet potato (Treadwell et al., 2007), with minimal yield loss in the southeast, additional research should be conducted integrating multiple tactics for weed, disease, and insect management in organic vegetables systems in this region.

Weeds are considered the most significant pest for most organic vegetable growers. Vegetable seedlings have shallow roots making them extremely sensitive to weed competition (Mennan et al., 2020). Therefore, managing weed seedlings early in the season is critical to reduce competition for nutrients and space between newly transplanted crops and weed seedlings (Brown & Gallandt, 2018). Since there are few effective organic herbicides to combat weeds, farmers implement a variety of weed management strategies including tillage, cover crops, mulches, flaming, and soil solarization (Singh et al., 2005; Price and Norsworthy, 2013; Brown & Gallandt, 2018; Mennan et al., 2020). Mechanical cultivation to control weeds is common, especially in organic systems. Tine-weeding is a popular type of mechanical weed management since one cultivation of tine weeding can uproot around 51% of the weed seedlings through mechanical vibrations (Kurstjens et al., 2000). Inter-row cultivation may be a useful tool for more sensitive vegetable crops by removing weeds close to the crop's roots, however, it may not be cost effective and can accelerate soil erosion (Mennan et al., 2020). Another organic weed management option is hand-weeding. Although highly effective, hand-weeding is labor intensive and costly (Johnson et al., 2012).

Natural vegetation and weeds may be important refuge sites and food-sources for pests and natural enemies in agricultural systems (Schmidt and Tscharntke, 2005; Fox et al., 2015). Many pest species are polyphagous and feed on both host and non-host crops, including weeds. For example*, Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and *Frankliniella occidentalis* Pergrande (Thysanoptera: Thripidae), two major pests of cotton were found in high abundance on flowering weed species found in cotton-growing regions (Silva et al., 2018). Weed communities tend to be significantly more diverse and abundant in organic systems averaging 1.3-1.6 times higher weed species richness compared to conventional systems (Roschewitz et al., 2005; Crowder and Jabbour, 2014), which provide both biodiversity benefits and economic risks (Wilson et al., 2008). For example, a study in the Southeastern US found spider activity higher in organic fields due to increased prey and available weed habitat, but management disturbances did not influence their activity (Fox et al., 2015). Whereas having even a few individual weeds with high seeding potential (e.g., redroot pigweed (*Amaranthus retroflexus*)), can be challenging to control and lead to future costs of weed management (Price et al., 2011). Therefore, it is crucial to understand the weed pressure and crop sensitivity to weed competition, which allows for estimating the trade-offs of biodiversity, and finding the "right" biodiversity of plants within or around the production system.

1.2 Onion systems in the Southeastern United States

A highly valued vegetable crop in the Southeastern US are onions *Allium cepa* (Asparagales: Amaryllidaceae). In 2021, 138,900 acres of US land were devoted to onion farming with a harvested value of $1,037,388,000 (NASS, 2022). Georgia is the leading producer of onions in the southeastern US valued at $143,179,000 in 2021. However, only 11,800 acres of onions were planted, and 11,600 acres harvested in Georgia. Additionally, the average yield for US production was 500 cwt lbs/acre, whereas only 270 cwt lbs/acre were yielded in Georgia. Onion yield is significantly lower in Georgia compared to the national average, and this may be due to the intense weed, disease, and insect pressure in the Southeast US. However, the price of Georgia onions ($46.50 per cwt) is astronomically higher than the US average with the price per cwt being $15.30 (Vegetable 2021 Summary NASS, 2022) due to the prestigious value of the "Vidalia onion".

Vidalia onions have national name recognition and production is characterized by the adoption of controlled atmosphere storage, mild winters, low-sulfur soils, and plentiful water supply (Boyhan & Torrance, 2002). Vidalia onions are a Granex-yellow variety grown exclusively in 12 counties in southeastern Georgia including Appling, Bulloch, Candler, Evans, Emanuel, Jeff Davis, Montgomery, Tattnall, Telfair, Toombs, Treutlen, and Wheeler and portions of Bacon, Dodge, Jenkins, Laurnes, Long, Pierce, Screven, and Wayne counties (Boyhan & Torrance, 2002). Short-day varieties are grown in this region and require only around 11 hours of day length to begin bulbs. To allow seedlings to develop sufficient leaf area, onions are seeded or transplanted in fall and bulbing begins late February to early March (Boyhan & Torrance, 2002). Additionally, transplanted onions are better equipped to combat early season weed pressure and mechanically cultivate earlier into the season (Johnson et al., 2017).

1.3 Weed Management

Southeastern United States onion (*Allium cepa* L.) growers are presented with further challenges to weed management because of the crop's structure and physiology. Onions have shallow roots, are slow-maturing, and stand upright with narrow leaves that lack a protective canopy to shade emerging weeds (Bond & Burston, 1996; Khokhar, 2017). Onions have shallow roots which can easily become damaged by blades causing crop injury (Melander et al., 1997). Hand-weeding is still widely relied on for adequate weed control for organic onion production, however, it costs approximately $3,710 per hectare in onions (Johnson et al., 2017).

Mechanical weeding is common in organic systems and has been adopted by some Vidalia onion growers since 1997. However, complete adoption of mechanical weeding has not occurred because of equipment costs and technical problems (Boyhan & Torrance, 2002). Various organic mechanical weed treatments were examined on onions in Italy and concluded a combination of inter and intra-row treatments were significantly more effective (up to 94% control) such as hoeing-ridging combined with finger-weeding compared to one type of mechanical treatment such as split-hoeing (Pannacci et al., 2020). Johnson et al. (2012) observed significantly higher onion yield when tine-weeding 2x and 4x during the season compared to the non-cultivated control, particularly controlling cutleaf evening primrose and lesser swinecress. Mechanically weeding did not cause injury to the crop leading to an inoculation source for post-harvesting pathogens. However, this machinery does not perform well in wet soil which may lead to difficulties in relying on this weeding practice in regions of high rainfall. The authors suggest implementing tine weeding as the primary source of weed removal with supplemental hand-weeding as needed may be a significant cost benefit to organic onion growers in the Southeast.

Other types of weed management have been studied in onions in other regions of the US. The use of liquefied petroleum gas (LPG) flaming was examined for intra-row weed management in onion systems (Horesh et al., 2019). Cross-flaming, where burners are staggered and perpendicular to the crop rows did not affect onion growth, suppressed weed growth, and performed better than broadcast flaming, where burners are parallel to the rows. Cross-flaming may be utilized as a pre-emergence treatment and post-emergence weed organic weed treatment. Strip tillage has been shown to increase marketable onion yield and bulb size compared to conventional tillage (Gegner-Kazmierczak & Hatterman-Valenti, 2016). Although strip tillage in this study was managed conventionally, it may be incorporated into organic systems to keep the soil undisturbed and reduce weed seedling emergence. Mulching may be an effective and less intense weed management strategy. Hay mulching had comparable weed control and onion yield to continuous mechanical treatments known as “zero seed rain” (Brown & Gallandt, 2018).

1.4 Disease Management

Iris yellow spot virus (IYSV) (Bunyaviridae: Tospovirus) is a common pathogen of *Allium cepa* transmitted by a thrips vector, commonly the onion thrips, *Thrips tabaci* or tobacco thrips, *Frankliniella fusca* Hinds (Thysanoptera: Thripidae). Viral symptoms include tan-to-bleached white irregular necrotic lesions on the foliage, tip dieback, and reduced bulb size (Nischwitz et al, 2007). IYSV was first recorded in the United States in 1989 and detected in Georgia in 2003 (Mullis et al., 2004). Although IYSV is unlikely seed-transmissible, transplants are a source of inoculation (Kritzman et al., 2001). Weeds may play a role in the epidemiology of this disease. In New York, 10 weed species adjacent to onion fields tested positive for IYSV and considered hosts of the pathogen including Redroot Pigweed, Common Burdock, Common Lambsquarters, Chicory, Prickly Lettuce, Common Purslane, Curly Dock, Green Foxtail, Spiny Sowthistle, and Dandelion (Smith et al., 2012).

Pantoea ananatis (Serrano) Mergaert is a gram-negative bacterium (Stumpf et al., 2017) in the family Enterobacteriaceae and the causal agent of onion center rot. Pathogen infestation can result in 100% yield loss if conditions are favorable for the pathogen including high humidity, higher temperatures, and increased cumulative rainfall (Gitaitis et al., 1997; Dutta et al., 2014; Tho et al., 2019). *Pantoea ananatis* inoculate leaf foliage resulting in white streaks with water-soaked lesions that eventually develop necrosis and migrate to the neck and bulb causing bulb rot (Carr et al., 2013). However, foliar disease severity is not correlated with bulb rot incidence (Gitaitis et al., 2003). Similarly, *Pantoea ananatis* is known to survive on 25 different weed species found in onion-growing regions in Georgia (Gitaitis et al., 2002). The pathogen is primarily transmitted by thrips species and is the first recorded bacterial pathogen transmitted by Thysanoptera (Gitaitis et al., 2003). *Frankliniella fusca* and *Thrips tabaci* orally ingest the pathogen, where it is localized in the gut, and transmitted via fecal contamination (Dutta et al., 2014; Dutta et al., 2016). Both larval and adult stages can acquire bacterial pathogens. Additionally, in Georgia most onion seedlings are grown in seedbeds in September and transplanted in November or December (Boyhan et al., 2002). Transplants may be another inoculation source of center rot, and if infested onions are transplanted to the field it can result in high yield loss (Munoz et al., 2014).

Disease preventative measures are required in both conventional and organic systems. Cultural control methods such as removing debris and volunteer host plants from fields reduce host inoculation sources (Gill et al., 2015). Planting early maturing varieties can reduce the reproduction span of both the pathogen and its vector. Bio-pesticides composed of naturally occurring organisms or their derivatives (Haddi et al., 2020) can successfully suppress pathogen growth in vegetable systems such as tomato (Egel et al., 2019). For example, a fungal pathogen causing downey mildew of onion was suppressed by over 50% by *Trichoderma spp.* (Akhtar & Javaid, 2018). Grode et al. (2019) used a combination of bactericides and insecticides to reduce bacterial stalk and leaf necrosis by *Pantoea agglomerans*. Bactericides alone did not significantly reduce disease severity; however, when combined with insecticides reduced diseased leaf tissue by almost 40% (Grode et al. 2019). This result reduced the number of thrips carrying *P. agglomerans,* and subsequently spreading *P. agglomerans.* A recent study also found Kocide (1.7kg/ha) significantly reduced center rot incidence in cured bulbs (Stumpf et al., 2021). However, when thrips were present, none of the bactericide treatments were effective at reducing center rot. Center rot incidence was significantly higher due to thrips transmission and feeding. Therefore, thrips feeding damage may be an inoculation source for bacterial colonization eventually leading to bulb rot. Copper fungicide sprays may not be as effective due to higher tolerance of pathogen and thrips recolonization onto onions post-spray (Dutta et al., 2014; Tho et al., 2019). Combined results from pathogen studies emphasize that successful disease management requires monitoring and attention to insect pests,

1.5 Insect Pest Management

Thrips are the most damaging pest of onion systems in the southeast (Sparks & Riley, 2008; Sparks et al., 2011; Riley et al., 2014). The combination of onion thrips' high reproductive capacity, short generation times, and ability to asexually reproduce through parthenogenesis can cause devastating yield losses (Gill et al., 2015). Feeding is completed by puncturing the leaf's surface and extracting sap. Thrips release enzymes for pre-digestion of the tissue, and consumption of the mesophyll cells causes stunted plant growth, reduction in bulb weight, and source of pathogen inoculation (Gill et al., 2015, Diaz-Montano et al., 2011). Thrips feed on the foliage resulting in silvery-white blotches and eventually curling of the leaves. Damaged foliage leads to reduction in photosynthetic and nutrient transport deficiency, resulting in nutrient-deficient bulbs (Diaz-Montano et al., 2011). Therefore, thrips presence in onion fields present multiple challenges both from feeding damage and disease spread and cannot be neglected.

Weeds may be an inoculation source for thrips and their vectored diseases. Many thrips species have a wide host range that encompasses both cultivated crops and weeds (Diaz-Montano et al., 2011). Studies in New York have observed thrips colonizing weeds early in the season before onions become more favorable to the pest (Smith et al., 2011). IYSV was also found harboring in the same weed species surrounding onion fields. Thrips were observed on numerous annual winter weeds in the southeast (Srinivasan et al., 2014). *Frankliniella fusca* surviving on these weeds were highly efficient at transmitting a vectored pathogen to cultivated crops. Weeds can enhance early thrips population growth and facilitate vector-borne pathogen transmission.

Thrips tabaci is the most devastating onion pest worldwide (Diaz-Montano et al., 2011), however, Sparks et al. (2011) found *Frankliniella fusca* to be the predominant thrips species in the Vidalia growing region. In the early 2000's *Thrips tabaci* populations were exceptionally low, around 1% of the total thrips population. Conversely, towards the latter portion of the decade the thrips species complex began to shift and higher *T. tabaci* populations were observed. The authors hypothesized this shift may be due to competitive exclusion by *F. fusca* or pyrethroid resistance by *T. tabaci*. Riley et al. (2014) confirmed *T. tabaci* abundance increases in onion production, which was estimated at over 20% of the total thrips population. Additionally, *T. tabaci* populations fluctuate drastically and appear to thrive during years of higher average seasonal temperatures, whereas *F. fusca* prefer lower average temperatures.

Currently, insecticides are the primary management tool used to control thrips and disease expansion (Diaz-Montano et al., 2012; Leach et al., 2017; Iglesias et al., 2021), with an action threshold of 5 thrips/plant in the southeast (Sparks & Riley, 2008). Spinosad in combination with an adjuvant such as neem oil was effective at reducing *T. tabaci* populations by 69%, feeding damage by 63%, and onion yield by 18% (Iglesias et al., 2021). Leach et al. (2020) hypothesized an IPM program for *Thrips tabaci* management using a thrips-resistant cultivar (Alavon) and a threshold-based insecticide program. *T. tabaci* was observed in significantly lower abundance on the Alavon cultivar, averaging 1.6 thrips/leaf compared to 6.7 thrips/leaf on the susceptible cultivar. However, bacterial rot was higher in the Alavon treatment leading to reduced marketable yeild, which was potentially a result of low *Pantoea sp*. present in the symptomatic onions screened (<5%) (Leach et al., 2017; Leach et al., 2020). Additionally, lower rates of nitrogen fertilizers may reduce *T. tabaci* populations in onion systems by 23-70% and decreased disease incidence by bacterial pathogens (Malik et al, 2009). Insect pest management practices in onion systems is critical to reducing thrips damage and pathogen spread, however, it may have a negative impact on natural enemies.

1.6 Natural enemies

Over 90 species of predatory and parasitic arthropods (i.e., natural enemies) are documented for thrips control (Loomans et al., 2003). Thrips predators encompass a range of insect orders including Orthoptera, Hemiptera, Thysanoptera, Coleoptera, Neuroptera, and Diptera (Diaz-Montano et al., 2011). Parasitoids are more selective and host-specific biological control agents compared to predators. Most *T. tabaci* parasitoids are entoparasitoids in the family Eulophidae (Diaz-Montano et al., 2011, Loomans et al., 2003). The most documented parasitoid of *T. tabaci* and *Frankliniella spp*. is *Ceranisus menes* Walker. High parasitism of *T. tabaci* by *C. memes* has been observed under greenhouse conditions (Loomans, 2003). Synchrony of the parasitoid and thrips is imperative for successful biological control. Even with high parasitism, if thrips populations build up to a threshold too large early in the season, parasitoids may not be an effective biological control agent. Predation in onion systems is more challenging since thrips tend to harbor in the inner leaves and whirl of the onion plant (Stump et al., 2021), and are only vulnerable to predation when they are on a more exposed part of the plant. Eight predator species in the families Aeolothripidae, Syrphidae, Anthocoridae, Coccinellidae, and Chrysomelidae were confirmed preying on *Thrips tabaci* in onion fields (Fok et al., 2014). Additionally, higher abundances of predators were found in organically managed onion fields in New York (Fok et al., 2014) and in the Netherlands (Booij & Noorlander, 1992). Although onions are highly managed for weeds, disease, and insects, transitioning to organic practices may aid in sustaining natural enemy populations and potentially promote biological control of thrips.

Increases in arthropod diversity, abundance and promotion of biological control have been observed in many organic cropping systems (Crowder et al., 2014; Smith et al., 2020). However, studies are still needed on arthropod communities and their functional differences in conventional and organic onion systems. Natural enemies are known to aggregate near field edges where plant diversity is greater (Schmidt and Tscharntke, 2005). Previous studies have observed variations in parasitoid and predator abundance and richness in organic and conventional systems in the absence and presence of nearby natural vegetation (Galloway et al., 2021). Understanding how arthropod communities are affected by management practices, surrounding non-crop habitat, and their interaction with pests and natural enemies in the southeast should be explored.

Since the predominant pest of onions are thrips and diseases of high concern are vectored by thrips, previous research has focused on combining bactericide and insecticide treatments (Grode et al., 2019). The importance of thrips management was emphasized when bactericide efficacy was negated by thrips feeding (Stumpf et al., 2021). Studies on organic onion production in the Southeast have examined the influence of weeding on thrips-vectored diseases (Johnson et al., 2012) and the interacting effect of bio-fungicides and weed management on disease and yield. However, no studies have examined the combination of organic weed management and bio-pesticides targeting bacterial diseases in organic onion systems and the effect of management on thrips, natural enemies, disease severity, and yield. Understanding how weed management influences the interaction between weeds, pests, disease, and natural enemies may facilitate improving current organic management practices by balancing management intensity while optimizing yield.

1.7 Objectives

This project aims to understand how arthropod pests and natural enemies are influenced by organic management practices by characterizing arthropod communities found on large-scale organic and conventional onion farms. Specifically, how arthropods are affected by management intensity, with an emphasis on the effects weed and disease management practices on arthropod abundance. Additionally, we estimate the effects of organic weed management and bio-pesticides on insect and weed pest-pressure, natural enemy activity, disease incidence, and yield to provide an initial systems-based analysis of organic onion management.

Chapter 2.

1. Obtain farm survey summarizing weed, disease, and insect management to create a relative management index for each farm.
2. Characterize arthropod community composition based on functional groups
3. Compare herbivore and natural enemy abundance between management type, management intensity, and distribution within the agroecosystem.

Chapter 3.

1. Determine the effects of organic weed management and bio-pesticides on insect and weed pest-pressure, natural enemy activity, disease incidence, and yield.

Chapter 4.

1. Estimate the influence of mechanical weeding and hand-weeding on pest and natural enemy abundance, center rot incidence, and organic onion yield.

2. Determine the potential cost benefit of implementing mechanical cultivations into weed management program.

CHAPTER 2

ARTHROPOD COMMUNITY COMPOSITION AND DISTRIBUTION IN COMMERCIAL CONVENTIONAL AND ORGANIC ONION SYSTEMS.

2.1 Introduction

Agricultural intensification contributes to major environmental challenges such as greenhouse gases, pollution, soil degradation, and lower biodiversity (Reganold & Wachter, 2016), and occurs at a landscape and local scale (Tscarntke et al., 2005). Landscape intensity may have a beneficial effect on pests. For example, sorghum fields nested within a high intensity landscape had high aphid pest abundance (Emery et al., 2021). Arthropod abundance and diversity may be influenced by landscape context and composition (Tscharntke, et al., 2012). Arthropods, particularly natural enemies may be positively affected by more heterozygous landscapes (Lichtenburg et al., 2017) and immediate surrounding non-crop habitat (Zhao et al., 2013; Morandin, Long & Kremen et al., 2014) and promote biological control services. For instance, egg predation of the cabbage moth *Mamestra brassicae* (Lepidoptera: Noctuidae) in brussel sprouts was positively correlated with forest borders (Bianchi et al., 2005). Forest edges had a significant positive effect on natural enemies in conventional blueberry orchards in the southeastern US (Whitehouse, Sial, & Schmidt, 2017). However, organic orchards had an even distribution of natural enemies along the forest border and within the field interior. Positive effects of surrounding non-crop habitat may be counteracted by management practices.

Farm size and management practices are key drivers of local intensification, with conventional vs organic management being the most common metric for comparison of local intensification (Emery et al., 2021). Conventional agriculture tends to rely heavily on external synthetic outputs for high productivity and cost efficiency (Orpet et al., 2020). Organic management may provide some benefits over conventional for building less intensive management systems that attempt to better integrate natural resources, and non-synthetic inputs (Smith et al., 2020). Some widely used organic pest control practices include crop rotation, crop diversity, intercropping, cover crops, conservation or augmentative biological control, and non-synthetic insecticides (Reganold & Wachter, 2016; Smith et al., 2020). Many studies show organic management practices have a positive effect on natural enemy diversity and abundance (Crowder et al., 2014; Jacobsen et al., 2019). For example, in perennial cropping systems such as apple orchards, organic management has been observed preserving natural enemy communities and enhancing biological control of aphids (Porcel et al., 2018).

However, in crops extremely sensitive to weed competition and insect damage, such as vegetable systems, management intensity may be a more accurate scale to measure arthropod disturbance compared to two broad categories of management. Recently, vegetation surveys were used to estimate local habitat management intensity (Klein et al., 2020). They found management intensity had a significant effect on orthopterans, with the highest orthopteran abundance and richness observed in medium-low intensity meadows. Natural enemies may be more sensitive to management disturbances compared to herbivores (Sacco-Martret de Preville et al., 2022). For example, spider species composition was negatively impacted by high management intensity practices such as tillage (Diehl et al., 2013), whereas their associated aphid prey abundance was positively affected by high intensity.

One of the most highly managed vegetable crops in the Southeastern United States are onions. Onions are the leading vegetable crop in Georgia, valued at 144 million (NASS, 2020).

Although only around 11,000 acres of onions are grown in Georgia per year, the production value is over 3x higher than the national average. Therefore, onion growers are required to intensively manage their crop for maximum yield. Previous entomological research in onion systems in the Southeastern United States have primarily focused on the major pest, thrips, particularly *Thrips tabaci* (Thysanoptera: Thripidae). However, natural enemies found in commercial onion systems are unknown. The goal of our study was to characterize arthropod community composition and distribution between different management systems and management intensities on a large commercial scale. Our objectives were to i) obtain a farm survey and use results summarizing weed, disease, and insect management to create a relative management index for each farm; ii) characterize arthropod community composition based on functional groups; iii) compare herbivore and natural enemy abundance between management type, management intensity, and distribution within the agroecosystem.

2.2 Materials and Methods

Our study was conducted in Toombs and Tattnall County in the Vidalia onion growing region during March 2020. We collaborated with extension specialists at the Vidalia Onion and Vegetable Research Station and commercial growers in the region to sample the maximum number of organic onion fields possible. We obtained permission to sample at seven organic farms and located an additional seven conventional farms within close proximity (<5 miles apart) of each organic site to create a pair of organic and conventional sites (Figure 2.1). Each farm was geographically identified using a handheld GPS unit. At each farm a forest border was identified (i.e., consisting of primarily pine trees), and transects were selected in parallel to the forested border. At each field site arthropods were sampled from three, 15 meter transects: five meters into the forest border (“edge”), five meters into the onion field (“5m”), and 50 meters into the onion field (“50m”).

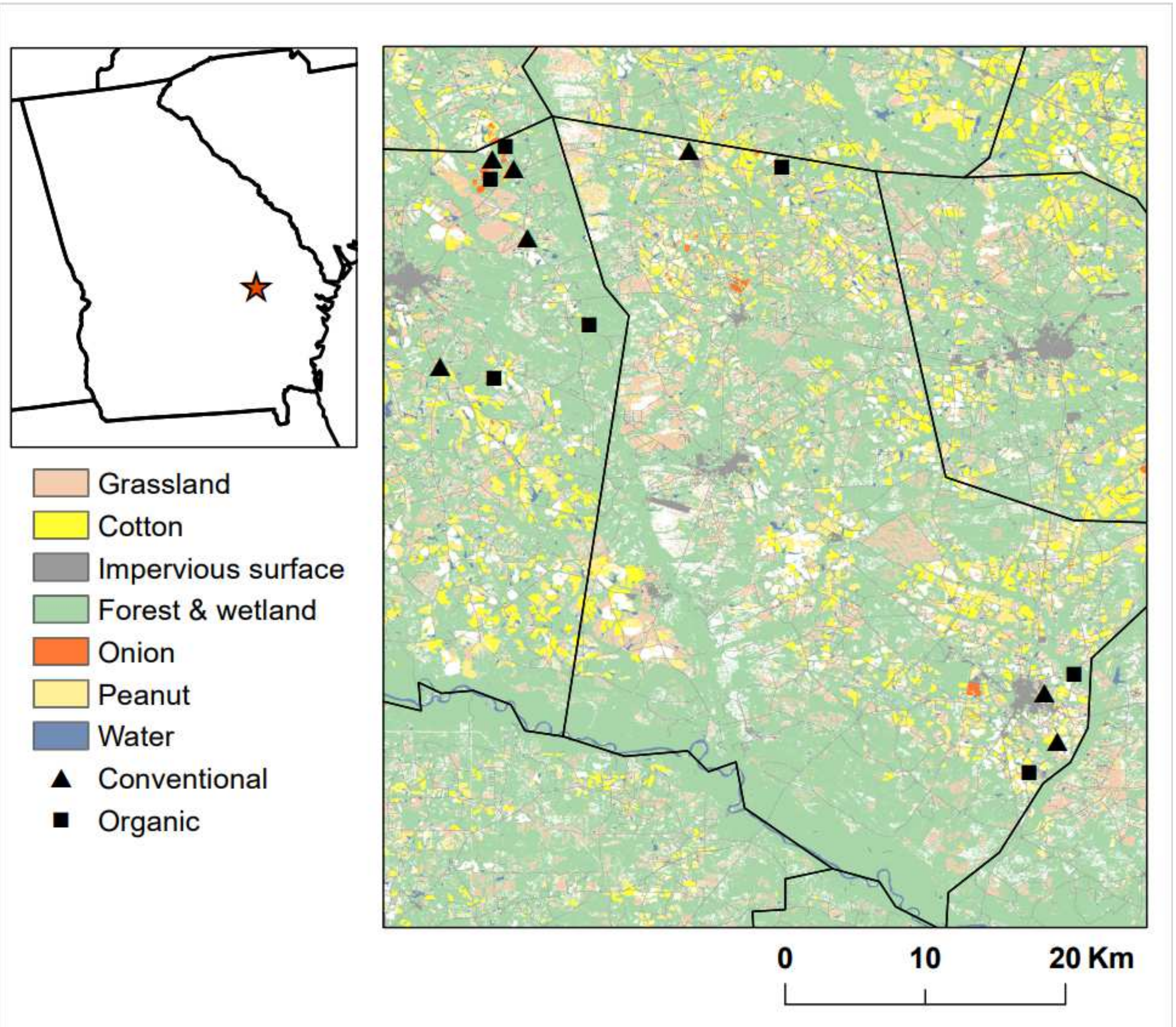

Figure 2.1. Map of field locations present within various landscape types present in Toombs and Tattnall County; Georgia during March 2020 created from the 30 pixel Cropland Data Layer (NASS 2021). Farm locations based on GPS coordinates are represented by triangles for conventional and rectangles for organic.

2.2.1 Arthropod Sampling and Identification

Within each transect, three 4x6 yellow sticky card were placed 10 to 30 cm above ground adjacent to the onion foliage (Fok et al., 2014) equidistance apart. Additionally, three pitfall traps filled with tween 80 and salt pellets were placed within each transect. All samples remained in the field for seven days. Upon collection, sticky cards were placed in a chest freezer and held on ice until frozen at -20°C. All arthropods were immediately removed from the pitfall solution and transferred to 5ml vials containing 100% ethanol. Two sample dates were conducted in 2020. Due to COVID restrictions, the study could not be repeated in spring of 2021. Overall, 246 sticky cards and pitfall traps were collected. During our sampling, the forest border of one of the organic farms was torn down during the sampling period. Therefore, we do not have edge samples for the farm.

All arthropods on sticky cards and pitfalls were identified to family or lowest feasible taxonomic group. Further, arthropods were placed into three categories based on generalized taxa functional groups: herbivores, natural enemies, and opportunists. Only taxa representative in 5% of samples were used in further analyses. All remaining families under herbivores were grouped together for analysis. Similarly, all predator taxa were grouped together for analysis and parasitoids were run separately.

2.2.2 Farm Management Survey

Management indices have been developed based on data extrapolated from landscape variables (Klein et al., 2020), vegetation variables (Sosa-Aranda et al., 2018), and insecticide inputs (Orpet et al., 2020). In Mexico, a management intensity index was developed based on inputs of fertilizer, pest, and weed control for coffee production system protocols to compare between regional production (Hernandez-Martinez et al., 2009). Our study modeled this management index by developing a numeric value to quantify local scale management intensity by combining weed, disease, and insect management protocols to further compare and understand the effects of management on arthropods. A survey was given to each farm manager of our sample sites directly post-harvest. The survey requested the names of each pesticide sprayed, their relative dates of spray, and number of applications. Additionally, the farmers were asked the type of weed management used, any diseases present, and yield if they were comfortable releasing that information. To disentangle management beyond the parameters of type (conventional and organic) we quantified the relative management intensity of each farm compared to one another. First, we created an intensity proportion for each management category (weed-Wi, disease-D_i, and insect-I_i) using the following equation

$$\sum_{i=0}^{n} \frac{(Mo_1 \times A_1) + (Mo_n \times A_n)}{Tp}$$

Mo= Mode of action (for pesticides) and type of application (weeding)
A= Number of applications performed over the growing season per mode of action or type
Tp= Total maximum intensity number for each management category

To obtain an overall index of <u>R</u>elative <u>M</u>anagement <u>I</u>ntensity (RMI) per farm, the intensity of each management category was summed and divided by the number of management categories:

$$RMI = \sum \frac{Wi + Di + Ii}{Tn}$$

Wi = Weed intensity proportion
Di = Disease intensity proportion
Ii = Insect intensity proportion
Tn = total number of management categories

2.2.3 Statistics

Linear mixed effect models (LMMs) were used to estimate the effect of management type, management intensity, and field location on arthropod responses in large-scale onion systems. The models fixed effects consisted of management type, management intensity, field location, and the interaction between management type and field location. The location of each conventional and organic pair was modeled as a random effect to help account for background variation in landscape composition. The most common arthropod taxa in <5% of samples were retained (McCune & Grace, 2002). Arthropod counts were averaged across both sampling dates and arthropod counts were log transformed to meet linear regression assumptions. Model adequacy was evaluated with residual plots and the need for interaction terms was determined using comparison of models with and without an interaction using AIC values (competing models with interaction <2 Δ AIC; Burham & Anderson 2002). Within treatment mean comparisons were conducted using least squared means ($P<0.05$). Family diversity was estimated using Simpson diversity index using vegan-community ecology package in R. All arthropods identified to family level were included in analysis. Diversity index values were combined for all dates and sampling types (i.e., pitfall and sticky card) and analyzed using generalized linear models with binomial distribution. All analyses were conducted in R statistical software version R 4.2.1 "Funny-Looking Kid" (RCoreTeam 2022).

2.3 Results

2.3.1 Management Intensity Survey

Our survey revealed high relative management intensity for both commercial conventional and organic production. All our conventional sites sprayed two pre-emergence herbicides (Goal and Prowl– active ingredients Oxyfluorfen and Pendimethalin, respectively) at transplanting (Figure 2A). Comparatively, all organic growers incorporated hand-weeding, mechanical cultivations, or a combination of both (Figure 2B). Three growers exclusively hand-weeded, one cultivated with an old Pittsburg plow and tine-weeder, and three growers cultivated with either a standard cultivator or tine-weeder and hand-weeded. Farmers did not specify the frequency of which hand-weeded was performed. Therefore, we assumed hand-weeded occurred bi-weekly beginning two weeks post transplanting until harvest (~8 weeks) based on previous Vidalia onion research (Johnson et al., 2012).

Disease management was consistent for all conventional sites, following the University of Georgia recommended onion fungicide spray program 2019-2020 (See appendix table A.4 for specifics). The spray schedule is every 7 days for 16 weeks targeting control of Botrytis neck rot, Botrytis leaf blight, Purple blotch, Rhizoctonia root rot, White mold, Pink root, and Stemphylium leaf Blight. On the other hand, disease management for organic growers varied significantly (Figure 2B). While two organic farms sprayed no pesticides for disease suppression, five organic farms sprayed copper bactericides every 10 or 21 days (Figure 2B). In addition, one farm sprayed the preventative fungicide Serenade (*Bacillus subtilis* strain QST 713) two times early in the season, and another farm incorporated four bio-pesticides for disease management (Serenade, Aviv (*Bacillus subtilis* strain IAB/BS03), Regalia (*Reynoutria sachalinensis*), and Timorex (*Melaluca alternifolia*)).

All conventional growers incorporated at least one insecticide (Lorsban- active ingredient chloropyrifos) at time of planting (Figure 2A). Two farms also sprayed Mustang maxx (Zeta-cypermethrin) in March, one sprayed two pyrethroids (Warrior and Karate- active ingredient is Lambda-cyhalothrin for both), and one farm sprayed a different undisclosed pyrethroid insecticide in April. No insecticides or management practices were targeted towards insect pest management in any organic farm in our study (Figure 2B). Moreover, we asked growers what diseases were present and their average onion yield. Center rot (bacterial disease) was the major disease present in both conventional (3) and organic (3) farms. Two conventional farms considered botrytis leaf blight as their major disease, and two sidewall decay (bacterial diseases).

One organic farm observed sour skin presence (bacterial disease). Only 9 out of 14 farms disclosed their average yield (4 conventional and 5 organic). Conventional onion bulb yield ranged from 700-750 50 lb bags/acre, averaging 715 50 lb bags/acre. Conversely, organic yield averaged 355 50 lb bags/acre ranging from 175-500 50 lb bags/acre.

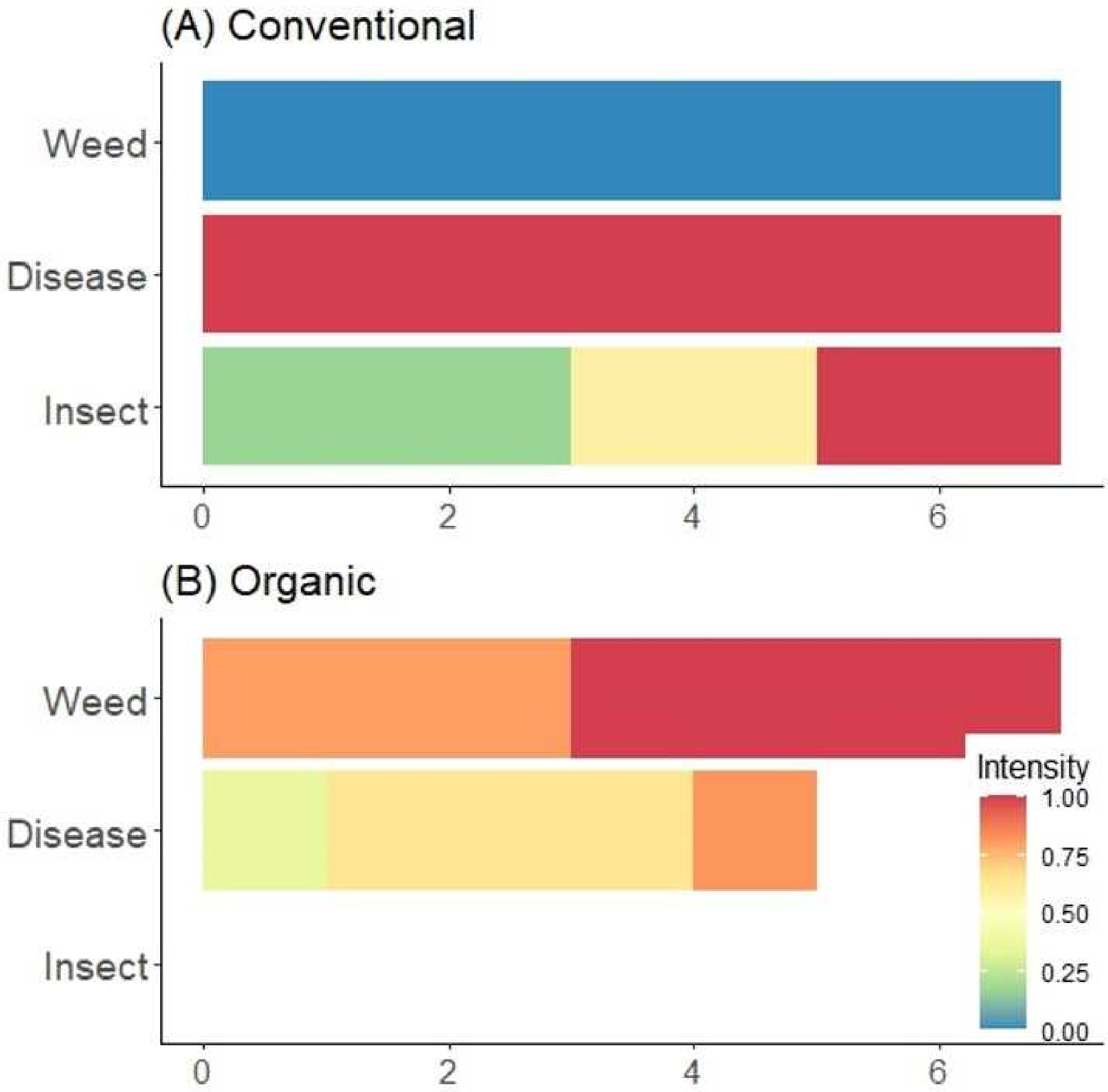

Figure 2.2 Overall management intensity proportion of conventional (A) and organic (B) weed, disease, and insect management. The y axis represents the total number of farms that performed at least one application during the onion season in each management category. The total number of farms per management type is seven. The bar colors represent the relative management

intensity of each farm per category. Intensity is a quantitative value based on the results from a survey filled out for each farm describing their management practices.

2.3.2 Overall arthropod community composition

We found high abundance and diversity of arthropods on sticky cards in this study totaling 32,733 arthropods across 1 sub-class, 14 orders, 3 sub-orders, 1 infra-order, 1 super-family, and 31 families (Table 2.1). High abundances of both suborders of flies (Diptera) were observed Nematocera (7,215) and Brachycera (3,485) on sticky cards. However, since they were not identified to family (except for a few key natural enemies), and contain taxa with a wide functionality range, we did not include them in further analysis. Additionally, since a substantial portion of opportunists were Collembola (Table 1) were found primarily on a few cards located in the edge, opportunists were not included in analyses.

We collected 11,005 arthropods in pitfalls in 3 classes, 1-sub class, 13 orders, 2 sub-orders, 1 infra-order, and 29 families (Table 2.2). Most arthropods collected were considered opportunists, with over half of the total arthropod count being isopods found in the field edge (6,473). Collembola and Formicidae were also found in high numbers in pitfalls, with total counts of 1,399 and 901, respectively. Due to their high abundance in the edge and unknown role in onion ecosystems, they were not further analyzed. Additionally, herbivore and parasitoid abundance were extremely low in pitfalls (Table 2). Therefore, we excluded herbivore and parasitoid abundance from pitfalls and estimated their abundance exclusively from sticky cards. Overall, 1,216 predators were collected in pitfalls (Table 2.) and grouped together for further analysis. Arthropod diversity at the family level was not significantly different between field location (X^2 (2) = 1.645, P= 0.4393), management type (X^2 (1) = 0.035, P= 0.8509), or their interaction (X^2 (2) = 0.224, P= 0.8939) using Simpson diversity index. Additionally, arthropod diversity was not significantly different between management intensity (X^2 (1) = 0.161, P= 0.6879).

2.3.3 Herbivores

We categorized 16 families on sticky cards as herbivores, totaling 11,699 and 10,964 in conventional and organic fields, respectively (Table 2.1). Aphididae (Hemiptera), Thysanoptera, and Cicadellidae (Hemiptera) were the predominant herbivores collected across both management types (Table 2.1). For further analysis herbivores known to be pests of onion or in other cropping systems in the southeast US were grouped together as “herbivores”. This group consisted of the following taxa: Aphididae, Thysanoptera, Cicadellidae, Aleyrodidae (Hemiptera), Chrysomelidae (Coleoptera), Curculionidae (Coleoptera), Membracidae (Hemiptera), and Miridae (Hemiptera). Herbivore abundance was significantly affected by field location (Table 2.3) Herbivore abundance was significantly higher 5m into the field compared to 50m into the field and the edge (Figure 2A). Similar herbivore abundance was found on sticky cards in the edge and 50m. Although no difference in herbivore abundance was observed between organic and conventional farms, management intensity had a significant effect on herbivore abundance (Table 2.3). Mean herbivore abundance was weakly correlated with management intensity (Figure 2B) (Multiple R-squared = 0.2104). Specifically, intensity value 0.622 was significant (t-value= -3.270, P= 0.0014).

2.3.3 Parasitoids

All parasitoids on sticky cards were identified to Parasitica due to their minute size and difficulty to accurately identify on sticky cards. Parasitoid abundance was significantly different between field locations (Table 2.3.) with significantly higher abundance in the edge compared to both within onion field locations (Figure 3A). However, there was a significant interaction between management type and field location (Table 2.3). Parasitoid abundance was significantly higher in the organic edge compared to conventional edge (Figure 3A). Additionally, management intensity had no effect on parasitoid abundance (Table 2.3).

2.3.4 Active Flying Predators

Overall, 710 predators were collected on sticky cards with Coccinellidae (Coleoptera) and Staphylinidae (Coleoptera) being the predominant families (Table 2.1). For analysis, the following groups were summed together for analysis of predators collected on sticky cards and referred to as "active flying predators": Coccinellidae, Staphylinidae, Araneae, Zygoptera (Odonata), Syrphidae (Diptera), Dolichopodidae (Diptera), Carabidae (Coleoptera), and Anthocoridae (Hemiptera). Predators were significantly affected by field location (Table 2.3). The highest abundance of predators was found in the edge, followed by 5m (Figure 3B). Only predator abundance in the edge and 50m were statistically significant. Neither management type nor management intensity had a significant effect on active flying predators (Table 2.3).

2.3.5 Ground Predators

Over half of ground predators collected in pitfalls were spiders, of which Lycosidae was the predominant family. For analysis purposes, all predators collected in pitfalls were summed and referred to as "ground predators" from the following taxa: Lycosidae, other Araneae, Dermaptera, Carabidae, and Staphylinidae. Ground predator abundance was significantly affected by field location (Table 2.3), with higher abundance in the edge (Figure 4A). Management intensity had a significant influence on management intensity (Table 2.3). Mean ground predator abundance weakly correlated with management intensity (Figure 2B) (Multiple R-squared = 0.192). Specifically, intensity values 0.479, 0.622 and 0.733 were significant (t-value= -2.919, P= 0.0042; t-value= -2.275, P=0.0248; t-value= -4.079, P=<0.0001).

2.4 Discussion

Our study is the first to provide an initial assessment of the arthropod community composition in commercial conventional and organic onion fields in the southeastern US. Overall, a high diversity of arthropods was observed in onion systems, however surprisingly there was no difference in arthropod diversity between organic and conventional farms. As expected, onions in this region are intensely managed due to their high sensitivity to weeds, disease, and insects in both conventional and organic systems. However, management practices between each system varied greatly. Conventional growers emphasized disease management, whereas organic growers allocated lots of time and labor into hand-weeding and cultivating. Weed management is considered of the main challenges for organic farmers and typically managed by hand-weeding, mechanical cultivations, transplanting, or mulching (Gomiero et al., 2012). Although mechanical cultivation is becoming more widely accepted in onion systems in this region, most organic growers still rely heavily on hand-weeding (Johnson et al., 2012). This reliance was reflected in our survey results, with 6/7 of organic growers either exclusively hand-weeding or hand-weeding in tandem with mechanically weeding. Disease management intensity varied between organic farms, ranging from 0-5 bio-pesticides over the season. However, organic growers only disclosed the bio-pesticides used for fungal and bacterial diseases in this study. Cultural control tactics may have been incorporated to aid in disease suppression including removing debris and volunteer plants, and planting early or resistant varieties (Gill et al., 2015). All conventional growers incorporated at least one insecticide into their management regime. Whereas organic growers strictly targeted management towards weeds and disease. Therefore, you would anticipate a reduction of pests or overall arthropod abundance in conventional systems.

Interestingly, we observed no difference in arthropod abundance between organic and conventional systems for herbivores or natural enemies. A similar result was observed for pests and natural enemy abundance in apple orchards (Orpet et al., 2020). Although many previous studies have shown organic systems increase natural enemy abundance and promote biological control of pests, some studies have observed contrasting results. Letourneau & Goldstein (2001) observed overall higher biodiversity and abundance of natural enemies in commercial organic tomato systems compared to conventional. However, there was no difference between predominant pests and their natural enemies between system types. A recent study found pest aphid abundance and their associated parasitoids remained similar between conventional and conservation soil management practices, but carabid diversity and abundance were significantly reduced by conventional practices (Sacco-Martret de Preville et al., 2022). Ground-predators may be more sensitive to disturbances caused by my management practices, such as hand-weeding compared to active herbivores and parasitoids that can relocate more efficiently under unfavorable conditions. Therefore, management practices targeted towards weed and disease management may have a direct effect on pests and natural enemies.

To explore the potential impacts of management intensity within each system type, we gave each farm a relative management intensity index score. Management intensity had a significant effect on herbivores and ground-dwelling predators. Herbivore abundance was weakly correlated with management intensity, as RMI increases herbivore abundance increased in conventional farms and decreased in organic. Whereas ground predator abundance was weakly negatively correlated to RMI for both systems. A study in papaya found management intensity based on a similar quantitative index had no effect on herbivore abundance but had a negative effect on natural enemy abundance (Flores-Gutierrez et al., 2020). Similar to our study, organic growers did not apply insecticide, while conventional growers did. Additionally, low intensity farming increased natural enemy abundance in papaya and led to significantly greater yield. Similarly, leaf damage was significantly reduced in low intensity coffee systems compared to high intensity, and management intensity had a negative effect on caterpillar abundance (Sosa-Aranda et al., 2018). The authors suggested either low insecticide efficiency or high efficiency of other management practices that subsequently reduced pest damage without insecticides. Insecticides may not be effective at reducing pest abundance under highly managed agroecosystems. Although management practices at a local scale impact natural enemy abundance, the surrounding landscape may also play a role (Puech et al., 2015).

Arthropod abundance was highest in the edge for natural enemies. This suggests a positive edge effect of non-crop vegetation on natural enemy establishment (Schellhorn et al., 2014). This edge effect was strongest for ground predators and parasitoids in our study. A recent study found non-crop habitat increased syrphid larvae abundance and their predation on thrips in onion systems (Sekine et al., 2022). Syrphid larvae and parasitoids abundance was higher in non-crop habitats in peas (Hatt et al., 2017), but coccinelids and aphids were not impacted by adjacent non-crop habitats. A significant interaction between management type and field location was observed for parasitoid abundance. Parasitoids were highest in the organic edge. This may be due to the fauna biodiversity in the edge. However, we did not include vegetation data in our study. Therefore, there are limitations to our understanding of the edge effect. Contrarily, herbivore abundance was highest 5m into the field. Previous studies in this region have pest abundance highest around crop field margins (Aigner et al., 2017).

2.5 Conclusions

Conventional and organic management is not dichotomous. Rather, management intensity is a scale within certain parameters. Our study attempted to quantify relative management intensity incorporating weed, disease, and insect management over the entire season. However, there are some caveats to our results. For instance, all management categories were weighed equally in our index. However, each variable may vary in their impact on pests and natural enemies. Although we reported the active ingredients of each pesticide used, we did not rate each pesticide based on its target or potential toxicity to natural enemies. Each pesticide was given the same value. Additionally, we only have approximate spray times (i.e., at transplanting, or mid-March) and do not have exact dates of application. Hence, we are uncertain if pesticide applications or weeding practices that occurred prior to insect sampling had any immediate influence on pest abundance or dispersal. Future research incorporating management intensity index should consider dates of spray and weeding, as well as considering the weight of each category on its potential effect on the response variable of interest. Additionally, we excluded ants from our ground predator analysis. However, recent studies show ants are important biological control agents in vegetable systems (Frizzo et al., 2020), and should be included in future biological control research in onion systems. Here, we provide evidence organic management practices in highly managed onion crops do not increase natural enemy abundance or diversity at a local scale; rather, the forest edge appears to have the greatest influence on natural enemy establishment.

Chapter 2: Tables and Figures

Table 2.1. Rank abundance of arthropods collected on sticky cards. Of all the taxonomic groups were identified to the lowest taxonomic level. Group abundances were separated based on management type. Taxa is separated into three functional groups: herbivores, natural enemies, and opportunists. Taxonomic groups not present in 5% of samples were removed.

	Taxonomy	Conventional	Organic	Total
Herbivores	Nematocera	4031	3184	7215
	Aphididae	3363	3705	7068
	Brachycera	1794	1691	3485
	Thysanoptera	1223	1474	2697
	Cicadellidae	784	436	1220
	Pscoptera	149	136	285
	Aleyrodidae	135	83	218
	Latrididae	78	64	142
	Chrysomelidae	51	76	127
	Curculionidae	40	39	79
	Membracidae	18	16	34
	Cucujoidea	8	25	33
	Heteroptera	10	10	20
	Miridae	13	7	20
	Lepidoptera	2	18	20
	Bostrichidae	7	11	18
Natural enemies	Parasitica	3048	4115	7163
	Coccinellidae	181	161	342
	Staphylinidae	59	61	120
	Araneae	41	34	75
	Zygoptera	21	26	47
	Syrphidae	23	23	46
	Dolichopodidae	5	35	40
	Carabidae	11	10	21
	Anthocoridae	10	9	19
Opportunists	Collembola	1508	229	1737
	Mites	255	151	406
	Formicidae	16	20	36
	Total	**16884**	**15849**	**32733**

Table 2.2. Rank abundance of arthropods collected in pitfalls. Group abundances were separated based on management type. Taxa is separated into three functional groups: herbivores, natural enemies, and opportunists. Taxonomic groups not present in 5% of samples were removed.

	Taxonomy	Conventional	Organic	Total
Herbivores	Thysanoptera	14	34	48
	Cydnidae	20	10	30
	Aphididae	7	12	19
	Curculionidae	5	10	15
Natural enemies	Araneae (other)	183	189	372
	Lycosidae	108	130	238
	Dermaptera	35	185	220
	Carabidae	92	109	201
	Staphylinidae	97	88	185
	Parasitica	41	20	61
Opportunists	Isopoda	676	5797	6473
	Collembola	659	740	1399
	Formicidae	419	482	901
	Acari	9	10	19
	Blattodea	14	5	19
	Chilopoda	3	11	14
	Total	**2382**	**7832**	**10214**

Table 2.3. Results from generalized linear mixed effect models to interpret the effect of management intensity, field location, management, and their interaction on arthropod groups. Herbivores, parasitoids, and active flying predators were estimated using sticky cards, and pitfalls were used for ground predator estimation. All arthropod groups were log transformed prior to analysis.

Arthropod group	Treatment	Sum sq	Mean sq	Df	F	P>F
Herbivores	Intensity	0.79	0.13	6	3.835	**0.0305**
	Field Location[a]	1.18	0.59	2	17.148	**<0.0001**
	Management[b]	0.06	0.06	1	1.796	0.2343
	Location * Management	0.05	0.03	2	0.777	0.4620
Parasitoids	Intensity	0.42	0.07	6	1.324	0.3503
	Field Location	7.88	3.94	2	74.781	**<0.0001**
	Management	0.00	0.00	1	0.000	0.9841
	Location * Management	0.42	0.21	2	3.976	**0.0216**
Active Flying Predators	Intensity	0.24	0.04	6	1.053	0.447
	Field Location	0.71	0.36	2	9.275	**0.0002**
	Management	0.00	0.00	1	0.101	0.762
	Location * Management	0.05	0.03	2	0.685	0.506
Ground Predators	Intensity	1.57	0.26	6	4.669	**0.0145**
	Field Location	1.96	0.98	2	17.472	**<0.0001**
	Management	0.36	0.36	1	6.396	**0.0468**
	Location * Management	0.02	0.01	2	0.135	0.8739

[a] Field location categories- Edge, 5m, 50m into field

[b] Management- conventional, organic

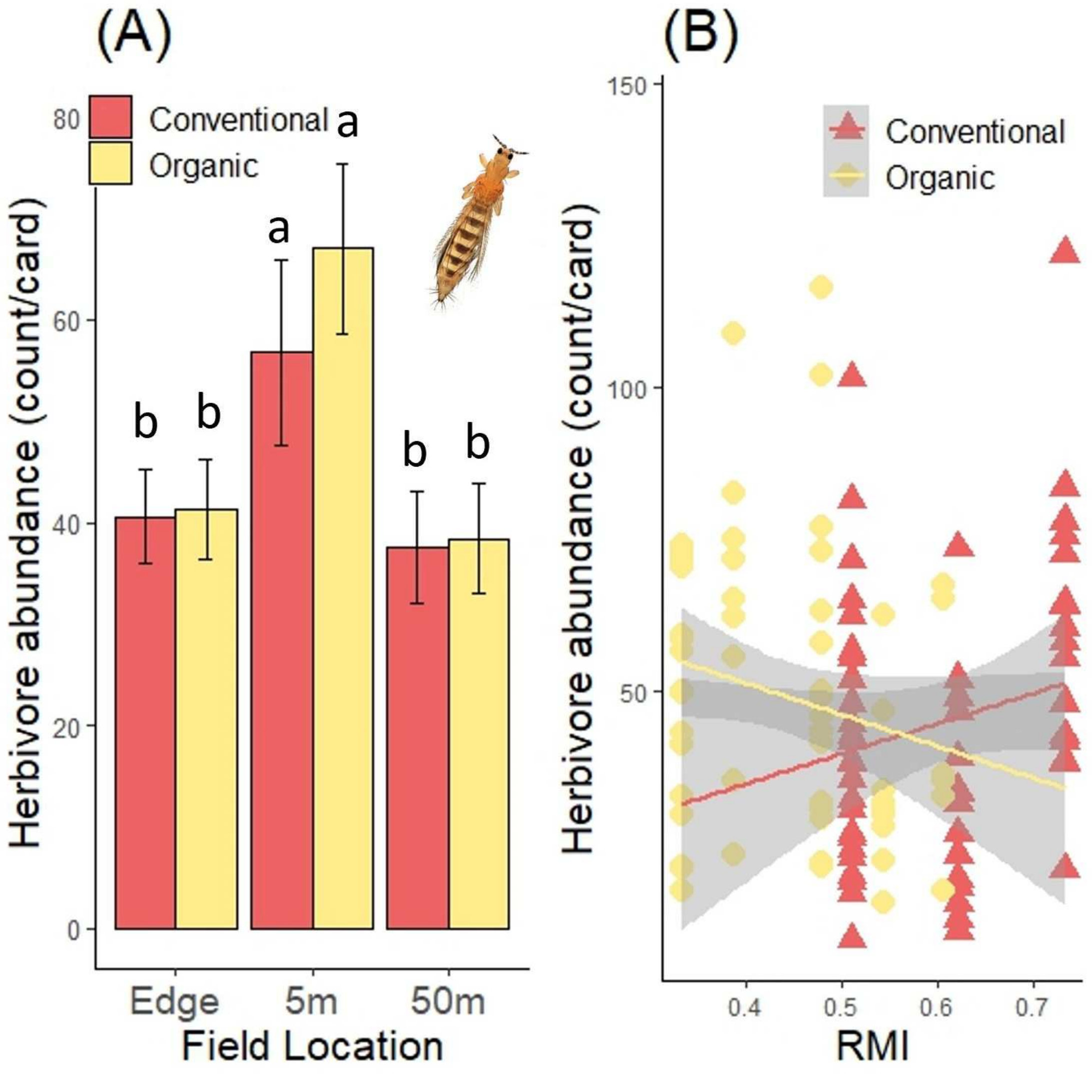

Figure 2.3 Significant effects of field location and management type on herbivore count mean (± 1SE) (A). Herbivore counts were estimated using yellow sticky cards and averaged across sample dates. Herbivores consists of counts from the following generalist herbivore families: Aphididae (Hemiptera), Thysanoptera, Cicadellidae (Hemiptera), Aleyrodidae (Hemiptera), Chrysomelidae (Coleoptera), Curculionidae (Coleoptera), Membracidae (Hemiptera), and Miridae (Hemiptera). Error bars represent standard error of mean. Means sharing the same letter are not significantly different using least squared means (P<0.05). (B) Shows the correlation between relative management intensity and herbivore abundance in conventional and organic onion systems. The grey line represents a 95% confidence interval of the model.

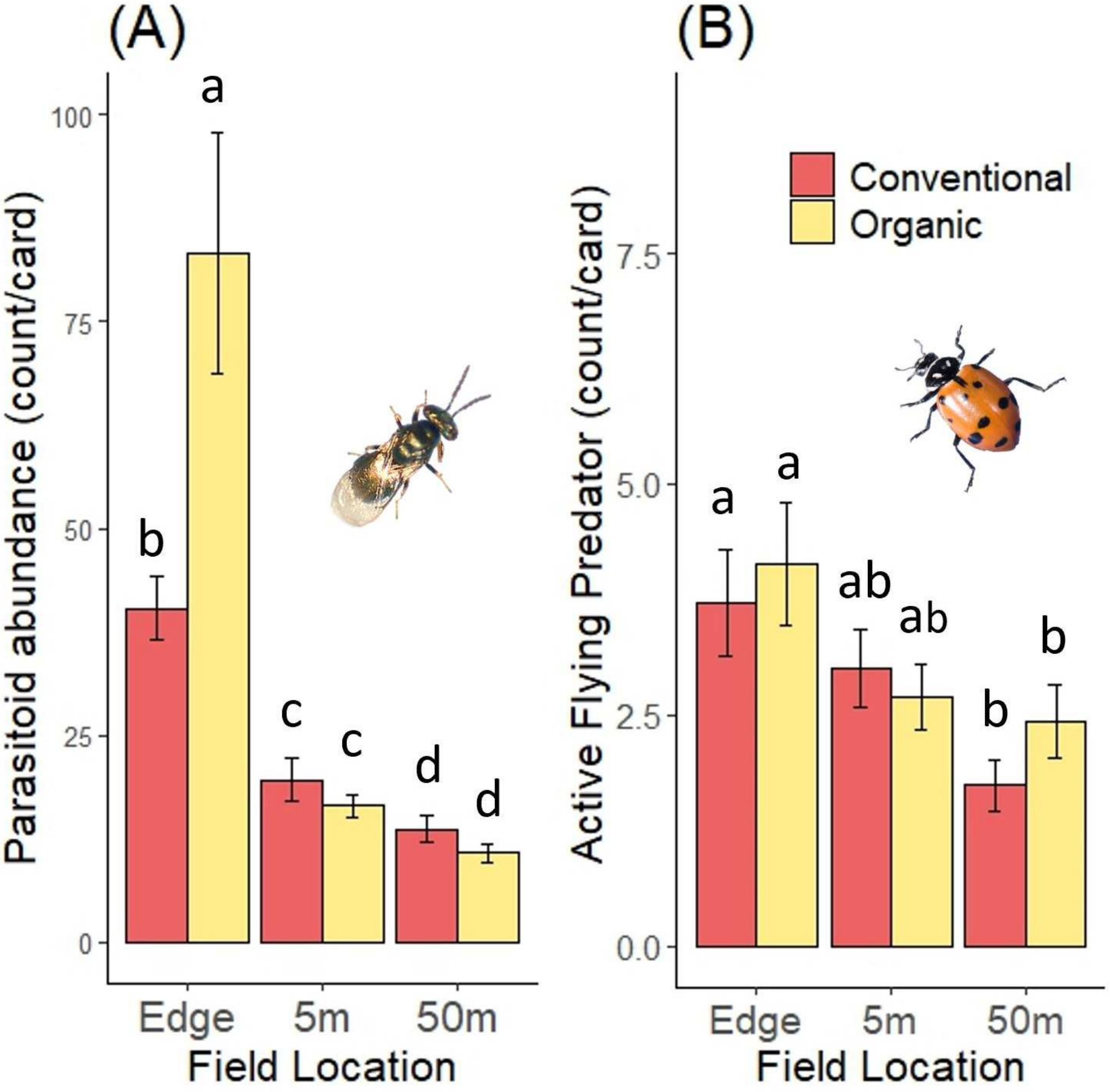

Figure 2.4. Significant effects of field location and management type on parasitoid count mean (± 1SE) (A) and active flying predators (B). Counts were estimated using yellow sticky cards and averaged across sample dates. Active flying predators consists of counts from the following generalist predator families: Coccinellidae (Coleoptera), Staphylinidae (Coleoptera), Araneae, Zygoptera (Odonata), Syrphidae (Diptera), Dolichopodidae (Diptera), Carabidae (Coleoptera), and Anthocoridae (Hemiptera). Error bars represent standard error of mean. Means sharing the same letter are not significantly different using least squared means (P<0.05

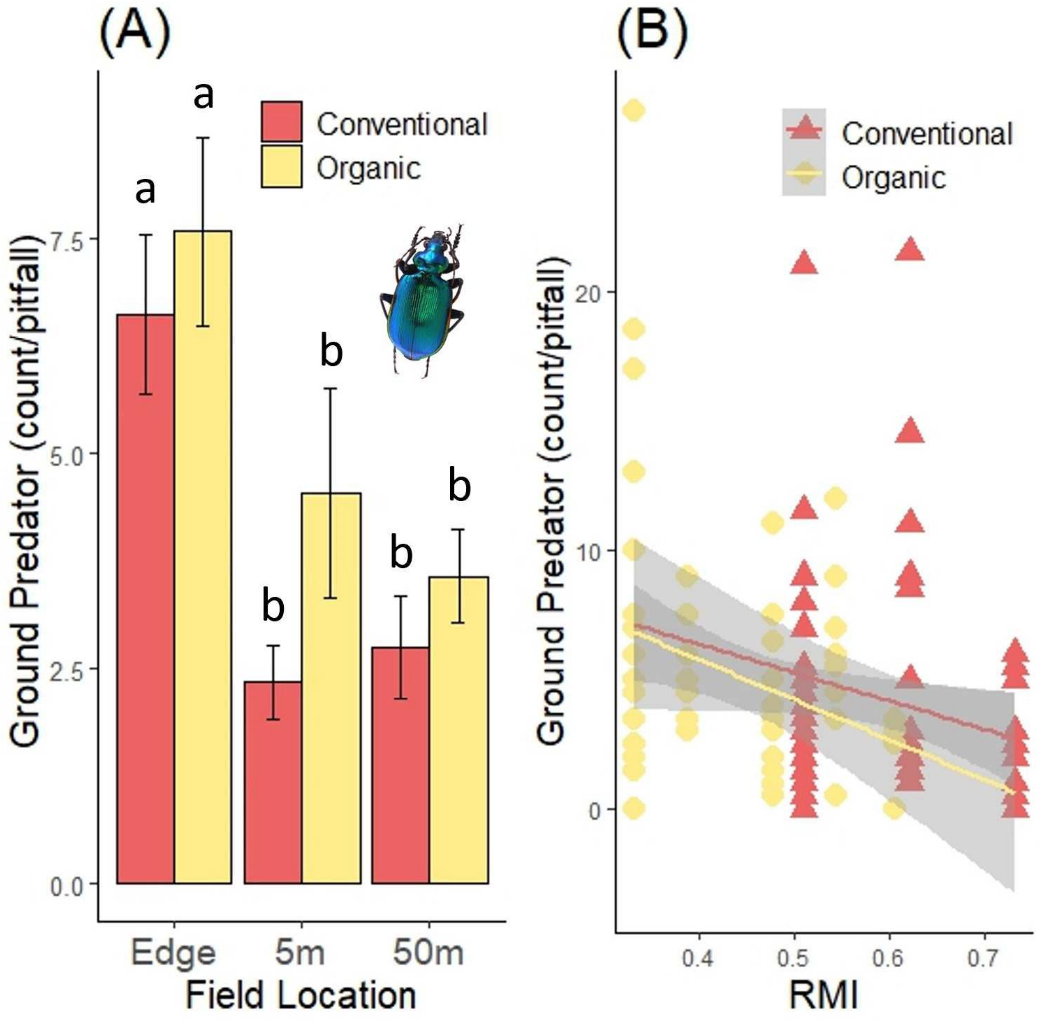

Figure 2.5. Significant effects of field location and management type on ground predator count mean (± 1SE) (A). Ground predator counts were estimated using pitfall traps and averaged across sample dates. Ground predators consists of counts from the following generalist predator taxa: Lycosidae (Araneae), Araneae (other), Dermaptera, Carabidae (Coleoptera), and Staphylinidae (Coleoptera). Error bars represent standard error of mean. Means sharing the same letter are not significantly different using least squared means ($P<0.05$). (B) Shows the correlation between relative management intensity and ground predator abundance in conventional and organic onion systems. The grey line represents a 95% confidence interval of the model.

CHAPTER 3

FROM WEEDS TO NATURAL ENEMIES: IMPLICATIONS OF WEED CULTIVATION AND BIO-PESTICIDES FOR ORGANIC ONION PRODUCTION.

3.1 Introduction

Effective weed management is challenging for farmers in organic vegetable production (Bond & Burston, 1996; Chen et al., 2017). Weeds compete with crops for nutrients, sunlight, and space resulting in reduced crop quality and yield (Madden et al., 2021). Commonly in organic systems, weeds are managed using mechanical cultivation or hand-weeding in conjunction with cultural control tactics, such as crop rotation, alternative planting dates, and cover crops (DeDecker et al., 2014; Chen et al., 2017; Wortman et al., 2018; Bessette et al., 2019). The onion (*Allium cepa* L.) growers in the southeastern United States are presented with further challenges to weed management because of the crop's structure and physiology. Onions have shallow roots, are slow-maturing, and stand upright with narrow leaves that lack a protective canopy to shade emerging weeds (Bond & Burston, 1996; Kokhar, 2017). Organic farmers commonly hand-weed, but labor costs are high (Johnson et al., 2012; Johnson et al., 2017; Brown & Gallandt, 2018). Mechanical weeding can be more cost effective for managing weed seedlings (Chen et al., 2017; Brown & Gallandt, 2018). For example, a tine-weeder with rows of tines that vibrate weeds is effective for uprooting weed seedlings (Kurstjens et al., 2000; Johnson et al., 2012). Reducing weed competition is likely only one benefit of effective weed management programs, as polyphagous insect pests may colonize weeds for food, shelter, and overwintering sites, resulting in increased pest abundance and associated arthropod-transmitted pathogens (Eigenbrode et al., 2018; Barbercheck et al., 2021).

Suppressing weeds, therefore, may synergize with insect pest management in onions by reducing pests, such as thrips (Thysanoptera: Thripidae). Thrips feed on a variety of non-crop plants (Smith et al., 2011), and due to damage caused through feeding and pathogens vectored, thrips are the most economically important pest of onions (Diaz-Montano et al., 2011). Thrips feeding is completed by puncturing the leaf's surface and extracting sap. Thrips punctures create inoculation points for pathogens (Gill et al., 2015), and onion thrips (*Thrips tabaci)* transmit at least two bacterial bulb rot pathogens that are known to survive on numerous weed species found in onion-growing regions in Georgia (Gitaitis et al., 2002). Furthermore, weed management may indirectly reduce disease severity by decreasing available alternative hosts for thrips, but even one feeding event could transfer pathogens through fecal contamination (Dutta et al., 2014), therefore, organic growers must apply foliar bio-pesticides to aid in disease suppression. Foliar bio-pesticides composed of naturally occurring organisms or their derivatives (Haddi et al., 2020) are proposed to help manage bacterial diseases and fugal pathogens in onion (Johnson et al., 2017; Akhtar & Javaid, 2018).

To advance weed and thrips management in onion, our current study assesses the efficacy of combining weed management practices with bio-pesticides (Johnson et al., 2017). We predicted that combining weeding with bio-pesticides may synergistically man

age thrips and disease by reducing pathogen inoculation, thereby decreasing disease severity and indirectly increasing crop yield/quality. However, with the disturbances from weed management, there is potential for negative impacts on beneficial arthropods. Weeds often have positive effects on arthropod biodiversity by attracting natural enemies into cropping systems (Root, 1973; Amaral et al., 2016; Madden et al., 2021). Over ninety species of beneficial arthropods are documented as biocontrol agents of pest thrips species, including *Thrips tabaci*

(Loomans, 2003), but few studies have assessed diversity of natural enemies in onion fields in the southeast US (Fok et al., 2014). Hence, we determine the effects of organic weed management and bio-pesticides on insect and weed pest-pressure, natural enemy activity, disease incidence, and yield to provide an initial systems-based analysis of organic onion weed management.

3.2 Materials and Methods

3.2.1 Experimental Design

The study was conducted during 2019 and 2020 at the Vidalia Onion and Vegetable Research Center, Toombs County (32.018801°N, 82.220101° W), which is located in the heart of Vidalia sweet onion production. The experimental design was a randomized complete block design, with four replications of each weed and bio-pesticide combination. The plot design consisted of a factorial arrangement of four weed cultivations (non-cultivated control, tine-weeded 2x, tine-weeded 4x, hand-weeded) and three bio-pesticide (non-treated control, OxiDate 2.0, Serenade). Plots were 6.10 m long by 1.83 m wide, each containing four onion rows that were spaced out 0.30 m apart with one onion every 10.16 cm (Boyhan & Kelley, 2008). Sweet Magnolia (Seminis) variety was chosen due to its high yield in a study by Tyson et al. (2019) in a trial examining 45 Vidalia onion varieties, where Sweet Magnolia had the highest total yield (18.14 kg bags/acre) and third highest in marketable yield. Onions were transplanted by hand in mid-December and weed cultivations and bio-pesticides applications were performed from late-December until early-February.

Weed cultivation treatments consisted of non-treated control, hand-weeding, tine-weeding 2x, and tine-weeding 4x (Aerostar Tined Weeder; Einbo¨ck, Schatzdorf, Austria). No weeding was performed post transplanting for the non-treated plots. Hand-weeding was performed bi-weekly for the 8 weeks following transplanting during the critical weed period. Managing weed seedlings during the “critical period” where competition for nutrients and space early in the crop season has been successful at reducing management intensity while effectively managing weeds in previous studies (Brown & Gallandt, 2018). Tine-weeding 2x was completed bi-weekly for the 4 weeks following transplanting and 4x for 8 weeks.

Serenade ASO (Bayer) and OxiDate 2.0 (Biosafe Systems) are certified organic bio-pesticides proposed to control for bacterial rot of onions. Serenade is a preventative fungicide, and its active ingredient is the beneficial soil bacterium *Bacillus subtilis* strain QST 713. The bacterium creates a biofilm on the plant’s roots creating a barrier that excretes antibacterial and fungal metabolites to give nutrients to the plant while simultaneously being harmful to pathogens. OxiDate is a curative and preventative broad-spectrum bactericide and fungicide using hydrogen peroxide as its primary active ingredient. Treatments were conducted bi-weekly three times beginning in late-January and ending in late-February. All applications were completed with a sprayer calibrated at 189.27 L/acre with concentrations of 9.46 ml/L mixture for Serenade and 71.92 ml/L of OxiDate. Bio-pesticides were sprayed using a CO^2-pressurized tractor-mounted plot sprayer calibrated to apply 468 L ha−1 at 414 kPa using high volume spray tips (Turbo TeeJet® 11006 tips, Spraying Systems, Wheaton, IL) (Johnson et al., 2017).

3.2.2 Arthropod Sampling and Identification

Yellow sticky cards were chosen to monitor activity of thrips and other arthropods in onion plots. Sticky cards are an effective thrips and parasitoid sampling method (Broughton & Harrison, 2012; Bockmenn & Mayhofer, 2017). One yellow sticky card (4x6) was placed vertically in the center of each plot 25-75 cm above ground, so the card was level with onion foliage (Fok et al., 2014). Cards were deployed one week following the final weed cultivation and collected after seven day of field exposure on two dates. All arthropods were identified to family or lowest possible taxonomic level. We counted all known biocontrol agents (Loomans et al., 2003). Parasitoids tend to be very minute and difficult to identify to family or lower on sticky cards; therefore, we identified them currently to Parasitica. All arthropods observed on cards were counted; however only thrips, generalist predator families observed in more than 5% of samples, and parasitoids were used in analyses.

3.2.3 Sequencing Parasitoid Communities from Sticky Cards

Sticky cards were randomly chosen per year, taking into consideration all weed cultivation and bio-pesticide treatments. Twenty cards were selected per year for parasitoid removal. Organisms were removed individually with a flattened pin tip and placed directly into 1.5 ml micro-centrifuge tube with 100% alcohol. The pin was cleaned with bleach, deionized water, and 100% ethanol in between each parasitoid transfer to reduce DNA contamination from other organisms from the card. All parasitoids removed from a single card were placed in 1 tube. Dneasy Blood and Tissue kit (Qiagen, Hilden, Germany) were used to extract DNA from parasitoid communities following the manufacturer's instructions. We included an extraction blank that did not contain parasitoid material as a negative control to account for background DNA. The same negative blank control from extractions was also used during PCR and through sequencing. No amplification was seen for the negative control, and less than 4 reads were recovered for minibarcodes assigned to the negative control. We used a common two-step nested DNA metabarcoding approach to diagnose the composition of parasitoid communities from sticky cards (Kitson et al., 2019; Grabarczyk et al., 2022). Following the PCR conditions of previous work (Grabarczyk et al., 2022, Lefort et al. 2017), in the first PCR, a 157 bp region of

Cytochrome Oxidase I (COI) was amplified with the primers ZBJ-ArtF1c/ZBJ-ArtR2c (Zeale et al., 2011), or 350 bp region using mlep/lep (Hajibabaei et al., 2006; Lefort et al., 2017), and the primers contained Illumina bridging primers and dual-tags of unique combinations of eight forward and eight reverse tags for labeling each sample (Kitson et al., 2019). PCR products were cleaned with magnetic beads 1X AmpureBeads XP (Beckman Coulter Brea, CA USA), and 1 ul of clean PCR products was used as the template for the second PCR with primers for Illumina adaptors and again dual-tagged (Kitson et al., 2019; Grabarczyk et al., 2022). PCR products were cleaned, concentration estimated with Qiaxcel Advanced system, and equimolar concentration of amplicons were pooled to prepare our library. Pooled samples were submitted to the Georgia Genomics and Bioinformatics Core lab (GGBC-UGA) for sequencing on an Illumina MiSeq (Illumina, San Diego, CA, USA) with MiSeq Reagent Kit v3 (2x300bp with 600 cycles). Raw sequence data is available in the NCBI Sequence Read Archive under the BioProject XXXXX.

Quality of raw reads (multiplexed fastq files) was checked using FastQC (Andrews & Bittencourt, 2010) on forward and reverse pools. Following sequencing, sequences were demultiplexed using the *cutadapt* software (Martin, 2011) The same software was used to trim primers from forward and reverse read, which were subsequently merged with *PEAR* (Zhang et al., 2014), setting a cut-off Phred score of 30. The remaining filtering pipeline was used using *vsearch v2.8.2* (Rognes et al., 2016). We used quality rate filtering (*fastq_maxee = 1*) to keep the reads containing only one potential nucleotide mistake. This step was following by dereplication, single filtering (removal of amplicons), indel filtering and "de novo" chimera removal. At this point, a fasta file with Amplicon Single Variants (ASVs) was obtained which were clustered using a 97% of similarity through the greedy algorithm to obtain Operational Taxonomic Units (OTUs). An © table was obtaining through mapping to the initial filtered reads. Taxonomic classification of the sequences was performed against NCBI Genbank nr/nt using BLAST algorithm (Johnson et al., 2008) and R package *taxonomizr* (RCoreTeam 2022) in order to infer the species level classification when possible. Moreover, a complementary taxonomic classification was obtained against The Barcode of Life Data System (BOLD) database (Ratnasingham & Herbert, 2007) using the python package bold identification. Based ©OTU tables for reads of different taxa recovered from parasitoids removed from sticky cards, we filtered the data to include Hymenoptera, and Thripidae, and included only the clusters that could be identified to Genus with >95% identity, and > 300 bp length for mlep and >120bp length overlap for zbj. We then removed rare reads and set our threshold for detection of any taxa to >10 reads, and less than ten reads was adjusted to zero (i.e., absent) (McClenaghan et al., 2019; Sullins et al., 2018). For estimating richness of parasitoids recovered from sticky cards, we first rarefied the sample to standardize read depth across samples (Deagle et al., 2019, Grabarczyk et al., 2022).

3.2.4 Weed Density, Yield and Pathogen Estimates

Weeds were counted in two 0.5-m^2 quadrats (0.5 · 1.0 m) in each plot, centered over a pair of onion rows. Visual estimates of weed control and weed counts were determined after all mechanical cultivations ceased in late-February. Onion yields were measured by mechanically undercutting and lifting onions at physiological maturity. One week after field curing, the roots and onion tops were clipped and graded according to organic USDA standards. Marketable yield [total yield at harvest – cull (pre-harvest+post-harvest)] and quality were determined for each treatment. Bulbs were stored and later evaluated for the presence of center rot (caused by *Pantoea* spp.) and sour skin (caused by *Burkhol43epacianepacia* (Burkholder)). Post-harvest evaluation of bulbs was estimated after 30 days of storage at 2^{o}C on a sub-sample of 20 and 40 bulbs per plot in 2019 and 2020, respectively. After storage, bulbs were sliced, and diseased bulbs counted. Due to COVID restrictions, no yield data was taken for 2020.

3.2.5 Statistical analyses

Generalized linear mixed models were used to detect significant differences in weed density, thrips, parasitoid wasps, predators and yield. Initially we incorporated an interaction term; however, removing the non-significant interaction between cultivation and bio-pesticide reduced the AIC value, and provided better spread of residuals from fitted models for all response variables. Therefore, models without an interaction term better model the response variables. Block was modeled as a random effect to account for potential variation associated with blocks in our design. To meet assumptions of linear models, weed density was transformed using sqrt. Thrips, parasitoid wasps, predators, and yield were natural log transformed. Separate analyses were performed for each year due to differences between years. Disease severity was analyzed as a binomial distribution (n positive bulbs for center rot/ n total bulb number). For analysis of disease, we used a general linear model (GLM) for proportion of center rot observed from stored bulbs. Within treatment comparisons were analyzed using least squared means ($P<0.05$). All analyses were conducted in R version 4.1.2 “Bird Hippie” (R Core Team 2021).

3.3 Results

3.3.1 Weed Density

Cutleaf evening-primrose (*Oenothera laciniata*) was the prominent weed species observed for both years (Table A.1.). The next most common weeds were henbit (*Lamium amplexicaule*) and lesser swincress (*Coronopus didymus)* in 2019 and wild radish (*Raphanus raphanistrum*) in 2020 (Table A.1.). We provide a summary of weeds (see Table A.1.), and given the overwhelming dominance of cutleaf evening-primrose, it was the only species included in formal analysis. Given no significant interaction between cultivation and bio-pesticide treatments, and lower AIC value for model lacking an interaction term (Δ8.364, $F_{6,33} = 0.351$, P = 0.9044; Δ7.043, $F_{6,33} = 0.114$, P = 0.8659, in 2019 and 2020, respectively), we interpret the main effects of cultivation and bio-pesticides (Table 1A). Cultivation had a significant effect on weed density in 2019 (Table 1A) and 2020 (Table 1A). Weed density was lowest in the hand-weeded plots both years (Table 1A). Plots that were tine-weeded four times post-planting (i.e. TW 4x treatment) had significantly higher weed density compared to the hand-weeded plots in both years (Table 1A, Figure 1A,B). Weed density was not significantly different between the plots managed with only two tine-weeding events post-planting (i.e. 2x treatment) and non-cultivated control plots across both years (Table 1A, Figure 1A,B). Although the effect of cultivation on weed density was consistent between years, weed density was higher overall in 2020. Bio-pesticides did not have significant effects on weed density in 2019 (Table 1B) or in 2020 (Table 1B).

3.3.2 Thrips Activity

Similar to weed density results, we observed no significant interaction between weed cultivation treatments and bio-pesticides, so we interpret the main effects (Δ11.495, $F_{6,33} = 0.351$, P = 0.9044; Δ5.787, $F_{6,33} = 1.931$, P = 0.1049, in 2019 and 2020, respectively, Table 1B). Weed cultivation significantly influenced thrips abundance in 2019 (Table 1A). The lowest abundance of thrips was observed in the hand-weeded plots (Figure 1C). Comparatively, thrips activity was higher in the tine-weeded 4x plots in 2019 (Figure 1C). Although thrips abundance in the tine-weeded 2x plots was significantly higher than in the 4x plots (Figure 1C), thrips abundance was not significantly different in the non-cultivated plots compared to either mechanical treatment

(Figure 1C). Weed cultivation had a significant effect on thrips activity in 2020 (Table 1A). Consistent with 2019, lower thrips abundance was observed in the hand-weeded plots (Figure 1D). There was no significant difference of thrips abundance in the non-cultivated and tine-weeded 2x plots (Figure 1D). No difference in thrips abundance was observed in the tine-weeded 4x plots relative to all other treatments. Bio-pesticides had no significant effect on thrips activity in 2019 or in 2020 (Table 1B).

3.3.3 Natural Enemy Activity

Of the 8,398 natural enemies collected on sticky cards combined across both years, 7,383 were parasitoid wasps (Table A.3.). The primary hosts of the parasitoid wasps collected is unknown. However, due to their high abundance, parasitoids were included in a separate analysis. Since all predator groups were found in low abundance, counts of predator families were combined to form the variable "predators" for analysis. The following generalist predators were observed: Geocoridae: Hemiptera, Anthocoridae: Hemiptera, Carabidae: Coleoptera, Staphylinidae: Coleoptera, Syrphidae: Diptera, Coccinellidae: Coleoptera, and spiders (for complete taxa abundance summary see Table A.3.).

Parasitoid abundance was not significantly influenced by interactive effects of cultivation and bio-pesticides ($\Delta 15.061$, $F_{6,33} = 0.518$, $P = 0.7904$; $\Delta 12.583$, $F_{6,33} = 0.761$, $P = 0.6057$, in 2019 and 2020, respectively) or the individual main effects of cultivation Table 1A, Figure 2A) or bio-pesticide; Table 1B, Figure 2A) in 2019. However, in 2020 cultivation did have a significant effect on parasitoid activity (Table 1A, Figure 2B). Parasitoid abundance was significantly lower in the hand-weeded plots compared to the non-cultivated and tine-weeded 2x plots. There was no statistically significant difference between parasitoid abundance in the tine-weeding 4x plots and the other cultivations. Bio-pesticides had no significant effect on parasitoid abundance in 2020 (Table 1B, Figure 2B). Predator abundance in 2019 was not influenced by bio-pesticide (Table 1B, Figure 2C) or the interactive effects of cultivation and bio-pesticides (Δ5.856, $F_{6,33} = 1.259$, P= 0.3028) but was significantly affected by cultivation (Table 1A, Figure 2C). Predator activity was significantly reduced in the hand-weeded plots compared to the other cultivation treatments. However, in 2020 no significant difference was observed in predator abundance across weed cultivation (Table 1A, Figure 2D), bio-pesticide treatments (Table 1B, Figure 2D) or their interaction (ΔAIC=12.354, $F_{6,33} = 0.814$, P= 0.3455).

3.3.4 Sequencing Parasitoid and Thrips Communities

Parasitoids were removed from sticky cards and extracted as whole communities by sticky card. A total of 40 parasitoid communities (20 per year) and 20 thrips (10 per year) were prepared for extraction and sequencing. On average our sequencing depth was 1064/ sample (MLEP) and 3288/sample (ZBJ). The average richness of parasitoids recovered was 1.8/sticky card (MLEP), and 2.1/sticky card (ZBJ), and total richness 22 (MLEP), and 26 (ZBJ) (Table 2). There were many thrips on the sticky cards, and both primers revealed similar thrips families and genera on the cards. For MLEP/LEP primers, we recovered a total of 10,687 reads for *Microcephalothrips sp*. and 3,898 *Frankiniella sp*., respectively. For ZBJ, 70,280 we recovered reads for *Microcephalothrips sp.* (Thysanoptera: Thripidae), and 70 for *Frankliniella sp.* (Table 2).

3.3.5 Disease Severity

Due to the low prevalence of sour skin observed in this study, and the known thrips ability to vector *Pantoea spp*., only center rot incidence was analyzed in stored bulbs. Given the significant interaction between weed cultivation and bio-pesticides both years (Table 3), we focus the results on interpreting the interactions (Table 3). In 2019, the interaction suggests the effect of bio-pesticides on center rot severity were similar among all treatments, except when Serenade was combined with tine-weeding 2x. This treatment combination resulted in significantly lower disease severity (0.18 ± 0.05) compared to all bio-pesticide treatments (Table 3). When non-treated, hand-weeding (0.18 ± 0.08) resulted in significantly lower disease incidence compared to tine-weeding 2x (0.53 ± 0.10). Contrastingly, in 2020 both OxiDate and Serenade resulted in significantly lower incidence of center rot compared to the non-treated plots. Non-treated × uncultivated plots resulted in 100% center rot incidence. Similar to the previous year, non-treated × hand-weeding and tine-weeding 2x treatments resulted in significantly different disease prevalence. However, tine-weeding 2x produced lower center rot incidence (0.43 ± 0.22) compared to hand-weeding (0.68 ± 0.08) in 2020. A significant interaction was observed, which is explained by significantly lower than expected disease incidence in onions under a management of OxiDate when combined with tine-weeding 4x (0.03 ± 0.03; Table 3). There were no significant interactions between Serenade and any weed cultivation treatment in 2020.

3.3.6 Yield Estimates per Treatment

Onions were graded based on organic onion USDA standard size constraints of medium, jumbo, and colossal categories. However, no colossal onions were harvested during this study. Weed cultivation had a significant effect on total bulb yield (Table 1A). The total onion yield for the non-cultivated plots was 2.65 kg/plot, which was less than 1/9 of the hand weeded plot yield of 24.65 kg/plot (Table A.2.). There were no significant interactive effects of cultivation and bio-pesticides (Δ5.910, $F_{6,33}$ = 1.167, P= 0.3455), and the total bulb yield was not significantly different between the non-cultivated plots and either tine-weeded treatment. (Figure 3; Table 1A). Significantly higher onion yield of both medium and jumbo onions were harvested from the hand-weeded plots (Figure 3; Table A.2.). Bio-pesticides did not have a significant effect on total onion yield (Table 1

3.4 Discussion

Weed management is a major concern for organic onion production in the Southeast US due to onions high sensitivity to weed competition and the potential role of weeds as hosts for important pathogens and pests. This study aimed to assess the efficacy of various weed management practices and bio-pesticides on weed density, thrips and natural enemy abundance, disease severity, and yield. Prolonged weeding (exceeding 4 weeks post-transplanting), including hand-weeding and mechanically weeding resulted in lower weed density. Furthermore, thrips abundance was negatively affected by intensive weeding, where low thrips activity was observed in hand-weeded plots. Although previous studies have shown the presence of weeds in cropping systems may reduce pest prevalence while having a minimal negative impact on yield (Penagos et al., 2003), our study found hand-weeding produced 9x higher onion yield compared to non-cultivated plots. Surprisingly, neither tine-weeding treatment significantly affected onion yield compared to the control. The first 8 weeks post-transplanting have previously been determined the most critical for onion establishment and weed competition (Brown & Gallandt, 2018), our results suggest hand-weeding is required to efficiently manage weeds for maximum yield while providing additional suppression of thrips activity.

Our study observed a significant reduction in weed density when prolonged weeding occurred post-transplant. Hand-weeding provided the highest weed suppression in both years. Other studies observed high weed control through hand-weeding in onion systems (Ghosheh and Al-Shannag, 2000; Johnson et al., 2012; Johnson et al., 2017). Due to the high labor inputs of hand-weeding, this study examined weed management exclusively using a tine-weeder. Cultivating bi-weekly 4x significantly reduced weed abundance compared to tine-weeding 2x and the non-cultivated control. However, tine-weeding did not have the same weed control efficacy compared to hand-weeding. Pannacci et al. (2020) observed equivalent results with mechanical cultivations reducing weed density relative to the non-cultivated control, but not as much weed suppression as hand-weeding. Consequently, hand-weeding had a negative impact on thrips abundance followed by tine-weeding 4x. Thrips are polyphagous herbivores known to survive on a variety of non-allium crops, including weeds. Therefore, the reduction in available host crops due to intensive weed management might have a beneficial effect on pest suppression (Horton et al., 2003). Studies in other cropping systems have seen similar reduction of major pests under intensive mechanical weeding regimes with minimal impact on predators and parasitoids (Penagos et al., 2003).

Weed management had a slight negative impact on natural enemies in our study depending on year. In 2019, no weeding treatment had a significant effect on parasitoids. However, in 2020, parasitoid abundance was significantly lower in hand-weeded plots. Alternatively, predator abundance was significantly affected by weed management in 2019 only. Predator activity was lowest in the hand-weeded plots, but only significantly lower compared to the tine-weeded treatments. A study in orchards found frequently mowing significantly reduced predator and parasitoid wasp abundance (Horton et al., 2003). A recent study in vegetable crops found comparable results with 30% higher predator density observed under weedy conditions (Madden et al., 2021). This may be due to the absence of weeds during the early portion of the growing season when natural enemies were unable to colonize weed vegetation for shelter and alternative food sources. Also, disturbances caused by frequently weeding and alterations in the microenvironment may deter natural enemy establishment. Interestingly, tine-weeding had no effect on either predators or parasitoids in this experiment. Multiple studies have observed intensive cultivations that alter soil structure and distribution of plant residue such as conventional tillage have a negative impact on ground-dwelling predator abundance (Muller et al., 2022) but not active aerial predators and parasitoids (Jasrotia et al., 2021). Sticky cards were used to approximate natural enemy abundance, which mainly trap aerial predators and parasitoids. Potentially, weeding may have a negative effect on more polyphagous ground-dwelling predators. Additional studies should incorporate pitfall traps to monitor the impact of organic management practices on ground predator activity.

Two bio-pesticides targeted for management of bacterial diseases showed only moderate impacts on center rot incidence. Our current study is consistent with previous findings in this system (Johnson et al., 2017) in that both studies did not observe consistent disease severity within weed cultivation or bio-pesticide treatment of onions. Our study observed a significant interaction between Serenade and tine-weeding 2x in 2019. Serenade is a preventative fungicide, where beneficial bacteria colonize the plants roots to protect against plant pathogens (Lahlali et al., 2011). If the crop is dealing with too much competition from weeds or disease, then Serenade might not be as effective. Additionally, if the crop becomes damaged from excessive weeding, wounds provide potential pathways for plant pathogens to enter plant cells and may also make Serenade less effective. Tine-weeding 2x may have been an optimal amount of weeding to allow Serenade to protect the crop from center rot with little crop damage. On the other hand, OxiDate is a biochemical pesticide that kills all microflora particularly bacterial microflora on contact through oxidation reactions. In a previous study, OxiDate suppressed numerous bacterial pathogens, including pathogens of vegetable crops in the family *Enterobacteriaceae* (Mahovic et al., 2013). In our study, OxiDate showed inconsistent results across years, this may be a result of the limitations of small experimental plots. Nevertheless, Serenade has shown significant reduction of disease severity in other crops such as blueberry (Abbey et al., 2021) and lower pathogen colonization in canola (Lahlali et al., 2011). In cucurbits, powdery and downy mildew were reduced only when OxiDate and Serenade soil were rotated with copper-based bio-fungicides (Champ WG), and resulted in similar yield compared to copper only (Marine et al., 2016).

Neither bio-pesticide appeared to effect pests or natural enemies, which is consistent with results of insect exposure to Serenade (Maebe et al., 2020). Interestingly, recent studies have found combining insecticides and bio-pesticides are required to reduce disease incidence and severity by *Pantoea spp*. (Grode et al., 2019). A copper-based bio-pesticide known as Kocide was effective in reducing center rot incidence when thrips were absent (Stumpf et al., 2021). However, when thrips feeding and pathogen transmission occurred, Kocide was ineffective. Thrips feeding damage may be an inoculation source for bacterial colonization eventually leading to bulb rot. Moreover, thrips feeding may directly affect onions resulting in lower bulb size and quality (Diaz-Montano et al., 2012), especially when thrips populations are high (Ghosheh & Al-Shannag, 2000). Insecticides are the most widely used management strategy to control for thrips pests conventionally (Diaz- Montano et al., 2010). Controlling for onion thrips using insecticides increased onion bulb weight by 86% compared to untreated plants (Leach et al., 2020). Since there are few organic insecticide alternatives, organic techniques such as straw mulch have been effective at reducing thrips abundance by 18-45% and subsequently increasing onion yield by 13% (Schwartz et al., 2009).

Only two thrips species, *Franklinella fusca* and *Thrips tabaci,* are capable of transmitting *Pantoea spp* (Dutta et al., 2014; Dutta et al., 2016). Due to thrips minute size, they are difficult to accurately identify on sticky cards, and were only identified to order. In an attempt to further identify thrips collected and whether species observed are capable of vectoring *Pantoea spp*., we used DNA metabarcoding. Successful DNA barcoding of thrips extracted from sticky cards in onion fields has been performed previously with approximately 79% identification to genus or species level (Marullo et al., 2020). Both ZBJ and MLEP/LEP primers reveled similar results with *Microcephalothrips sp.* and *Frankliniella spp*. being the predominant genera based on rarefied read estimates. Both genera are observed in high abundance on weed species surrounding cotton fields (Silva et al., 2018). In addition, *Frankliniella spp.* populations in the surrounding weeds are linked to increased thrips infestations in the onions. In our study, cutleaf evening-primrose was the most abundant weed species collected for both years followed by henbit and lesser swinecress. Johnson et al. (2017) found comparable weed species results in the Vidalia onion region. Although *F. fusca* can survive on cutleaf evening-primrose, previous studies have not observed high numbers of thrips on this weed species (Chamberlin et al., 1992). Furthermore, neither cutleaf evening-primrose, henbit, nor lesser swinecress are known hosts of *P. ananatis.* From the weed species observed, only wild radish (*Brassica spp*.) has been reported to host the bacterial pathogen (Gitatis et al., 2002). Although wild radish was present in 2020, cutleaf primrose was the most common, and is not known to harbor *Pantoea spp.. Further,* the species of thrips that appeared to be common on sticky cards, were not species that are known to vector *Pantoea spp.* The combined results of non-transmitting thrips species and weed species present likely contributed the low disease severity across treatments.

To gain a general understanding of the natural enemies found in onion ecosystems in the Southeast US, common predators and parasitoids were identified from sticky cards. Approximately, 12% of natural enemies collected were polyphagous predators, predominantly Geocoridae, Coccinellidae, and spiders. Our results were consistent with other vegetable cropping systems in this region (Madden et al., 2021). The overwhelming majority of natural enemies (88%) collected were parasitoid wasps. Since parasitoids are difficult to accurately identify to genera or lower on sticky cards, they were identified only to Parasitica. To resolve these unknown communities, DNA metabarcoding was performed to estimate the parasitoid species-complex in onion systems. Our findings showed a high number relative rarefied reads for multiple genera containing important known biological control agents of aphids such as *Aphelinus spp*. controlling the soybean aphid (Hopper et al., 2014), *Diaeretiella spp.* controlling aphids on cruciferous plants (MacDonald et al., 2003), and *Lysiphlebus spp.* in wheat and grain sorghum (Tomanovic et al., 2018). Moreover, *Diadegma spp.* are important biocontrol species of diamondback moth larvae (Nam et al., 2022), and *Telenomus spp*. are known to parasitize multiple families of arthropods such as Bombycidae, Geometridiae, Chrysopidae, and Pentatomidae. Unfortunately, little is currently known about parasitoids that parasitize thrips (Stopar et al., 2021). Most known thrips larvae parasitoids are in the Eulophidae family, with *Ceranisus* being the predominant genera for biological control of Thripidae pests (Loomans et al., 2003). However, some species of Trichogrammidae and Mymaridae have been observed parasitizing thrips eggs. Both egg parasitoid families were collected in relatively high abundance during this study and may be an avenue to explore for thrips parasitism in the southeastern US. The metabaroding approach to communities does have limitations, and known species biases, but we provide initial foundational information on parasitoid communities and the data were standardized by rarefying the samples prior to summarizing the overall results (see Deagle et al. 2019). Overall, this study revealed high parasitoid abundance and diversity of parasitoids with a wide host range. These parasitoids may be providing ecosystem services through biological control of pests in the crop or surrounding non-crop habitats.

3.5 Conclusions

Transitions to organically managed vegetable systems have demonstrated the importance of interdisciplinary approaches. Our study suggests hand-weeding throughout the season is crucial to suppress weeds allowing adequate onion development. Overall, this study observed a significant interaction between bio-pesticides and weed cultivations, with both OxiDate and Serenade effective at controlling center rot incidence in bulbs when combined with mechanical cultivations. Nonetheless, a portion of thrips collected can transmit important bacterial pathogens of onions. Under high thrips pressure, implementation of bio-insecticides should be considered. Although tine-weeding did not produce high yield in this study, implementing cultivations using a tine-weeder is promising due to lowering costs, and the potential interaction with bio-pesticide treatments and insignificant impact on natural enemy abundance. To gain a better understanding of the potential biological control of thrips by predators and parasitoids, future research should examine natural enemy communities at a large-scale with higher sampling effort considering the influence of management practices on these communities.

Chapter 3: Tables and Figures

Table 3.1. Results from generalized linear mixed models to interpret the effect of (A) weed cultivation treatments (uncultivated, tine-weeding 2x, tine-weeding 4x, hand-weeded) on weed density, thrips, parasitoids, predators, and yield. (B) Results from generalized linear mixed models to interpret the effect of bio-pesticides treatments (non-treated, Serenade, OxiDate) on weed density, thrips, parasitoids, predators, and yield. Separate models were fit for each year. Weed density was estimated using counts (no./m^2) of Cutleaf evening-primrose. Thrips, parasitoids, and predator counts are estimated using yellow sticky cards. Weed density was transformed using sqrt for analysis. All other response variables were logarithmically transformed. Separate models were fit for each response variable by year. Yield was only measured in 2019.

(A) Weed cultivation treatments

Year	Response variable	Sum sq	Mean sq	$F_{3,39}$	*P*
2019	Weed Density	60.11	20.03	76.16	**<0.0001**
	Thrips	7.33	2.44	14.77	**<0.0001**
	Parasitoids	0.44	0.15	1.90	0.1458
	Predators	1.95	0.65	3.40	**0.0272**
	Yield	28.63	9.54	46.77	**<0.0001**
2020	Weed Density	52.76	17.59	40.37	**<0.0001**
	Thrips	1.13	0.38	3.27	**0.0311**
	Parasitoids	4.20	1.40	14.73	**<0.0001**
	Predators	0.55	0.18	1.94	0.1393

(B) Bio-pesticide treatments

Year	Response Variable	Sum sq	Mean sq	$F_{2,39}$	*P*
2019	Weed Density	0.43	0.21	0.81	0.4520
	Thrips	0.60	0.30	1.81	0.1778
	Parasitoids	0.39	0.20	2.53	0.0929
	Predators	0.06	0.03	0.15	0.8637
	Yield	0.02	0.01	0.05	0.9521
2020	Weed density	0.34	0.17	0.39	0.6768
	Thrips	0.09	0.05	0.40	0.6707
	Parasitoids	0.07	0.03	0.36	0.6994
	Predators	0.11	0.06	0.61	0.5497

Table 3.2. Summary of metabarcoding parasitoids extracted from sticky cards within and around onion plots. Reads were summed (total reads) across samples to estimate parasitoid taxa recovered from sticky cards in the experimental onion plots. In separate reactions, two COI metabarcoding primer systems were used to amplify DNA extractions of parasitoids removed from sticky cards, MLEP/LEP and ZBJ (Zeale et al. 2011, Lefort et al. 2017).

Taxonomic description	Total reads	
Hymenoptera	mlep	zbj
Aphelinidae: *Aphelinus sp.*	**22321**	**9071**
Aphelinidae. Aphytis	-	159
Aphelinidae. Encarsia	-	275
Braconidae: *Aphidius sp.*	-	446
Bracon sp.	130	-
Chelonus sp.	-	414
Cotesia sp.	754	6204
Diaeretiella sp.	**8813**	**22690**
Dinotrema sp.	699	-
Heterospilus sp.	1347	-
Lysiphlebus sp.	2292	**16036**
Meteorus sp.	833	2488
Peristenus sp.	2645	-
Crabronidae: Ectemnius sp.	593	-
Andricus sp.	-	154
Cynipidae: *Belonocnema treatae*	175	40
Callirhytis	-	60
Encyrtidae: *Copidosoma sp.*	719	1351
Eupelmidae: *Brasema sp.*	341	-
Eurytomidae: *Sycophila sp.*	3040	-
Neochrysocharis sp.	-	2547
Figitidae: *Alloxysta sp.*	-	242
Kleidotoma sp.	366	-
Ichneumonidae: *Diadegma sp.*	-	**15053**
Syrphoctonus sp.	**7199**	**30364**
Mymaridae: *Cosmocomoidea sp.*	2105	-
Lymaenon sp.	-	74
Ooctonus sp.	-	101
Platygastridae: *Inostemma sp.*	69	-
Platygaster sp.	-	1063
Synopeas sp.	71	556
Pteromalidae: *Asaphes sp.*	448	3790
Chlorocytus sp.	-	52
Scelionidae: *Telenomus sp.*	**14248**	**92677**
Trissolcus sp.	328	4905
Trichogrammatidae: *Trichograma sp.*	-	**20237**

Table 3.3 Summary of generalized linear models of the proportion of center rot present post-harvest in onion bulbs under various weed cultivation and bio-pesticide treatment combinations. Mean (±SE) proportion of center rot prevalence in each weed cultivation and bio-pesticide treatment combination. Significance between all treatment combinations were conducted using least squared means (P<0.05). Separate analyses were conducted for each year.

Bio-pesticide	Cultivation	2019	2020
Non-treated	Uncultivated	0.35 ± 0.16ab	1.00 ± 0.00a
	TW 2x	0.53 ± 0.10b	0.43 ± 0.22c
	TW 4x	0.29 ± 0.08ab	0.50 ± 0.12bc
	Hand-weeded	0.18 ± 0.08a	0.68 ± 0.08b
OxiDate	Uncultivated	0.35 ± 0.22a	0.23 ± 0.05c
	TW 2x	0.55 ± 0.18a	0.30 ± 0.06c
	TW 4x	0.28 ± 0.18a	0.03 ± 0.03d
	Hand-weeded	0.33 ± 0.06a	0.35 ± 0.15c
Serenade	Uncultivated	0.38 ± 0.21ab	0.25 ± 0.06c
	TW 2x	0.18 ± 0.05b	0.35 ± 0.10c
	TW 4x	0.40 ± 0.21a	0.23 ± 0.08c
	Hand-weeded	0.50 ± 0.09a	0.48 ± 0.03c
Cultivation		df= 3, X^2=2.61, P= 0.4552	df=3, X^2= 41.49, P**<0.0001**
Bio-pesticide		df= 2, X^2= 0.64, P= 0.7249	df=2, X^2= 101.09, P**<0.0001**
Cultivation * Bio-pesticide		df= 6, X^2= 25.48, P=**0.0003**	df=6, X^2=52.09, P**<0.0001**

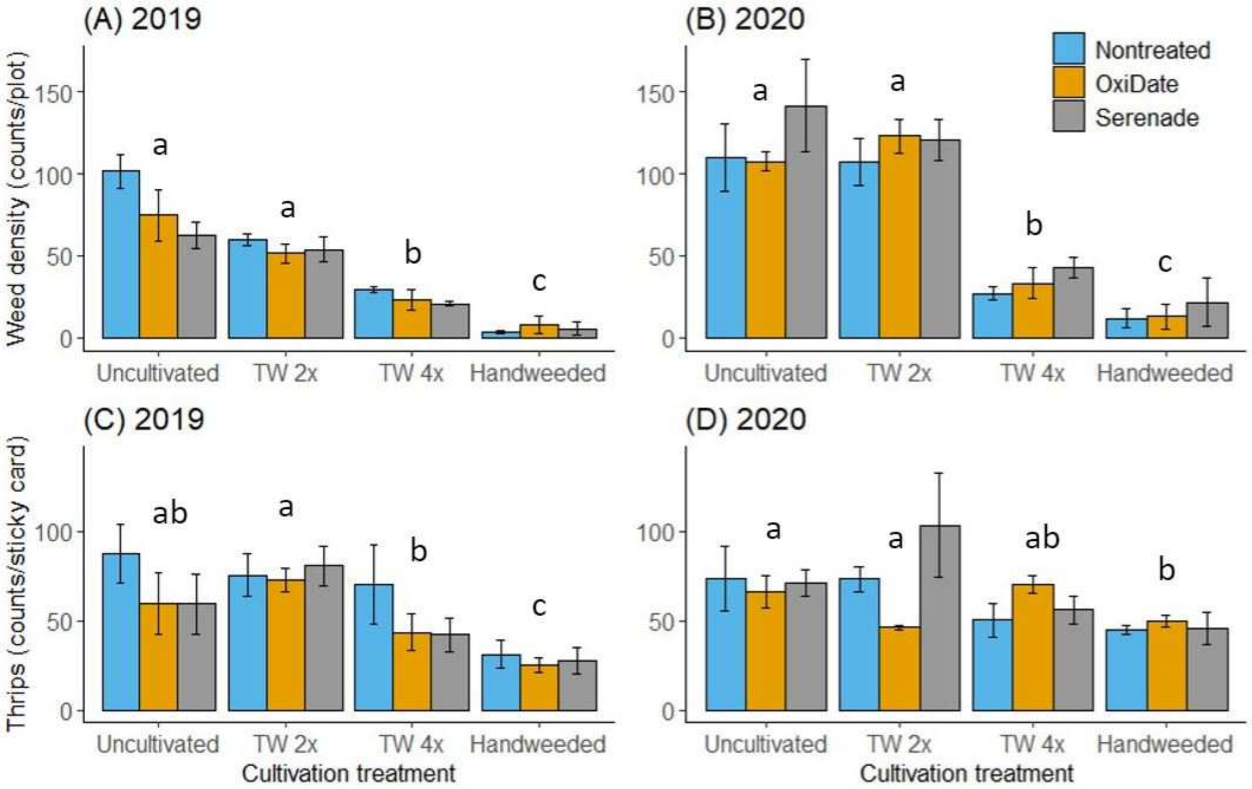

Figure 3.1. Significant effects of weed cultivation and bio-pesticide treatments on weed density mean (± 1SE) in 2019 (A) and 2020 (B) and thrips count mean (± 1SE) in 2019 (C) and 2020 (D). Weed density was estimated using counts (no./m^2) of Cutleaf evening-primrose. Weed counts were taken after all mechanical cultivations ceased in late-February both years.
Thrips counts are estimated using yellow sticky cards and combined for both sample dates. Error bars represent standard error of mean. Means sharing the same letter are not significantly different using least squared means ($P<0.05$).

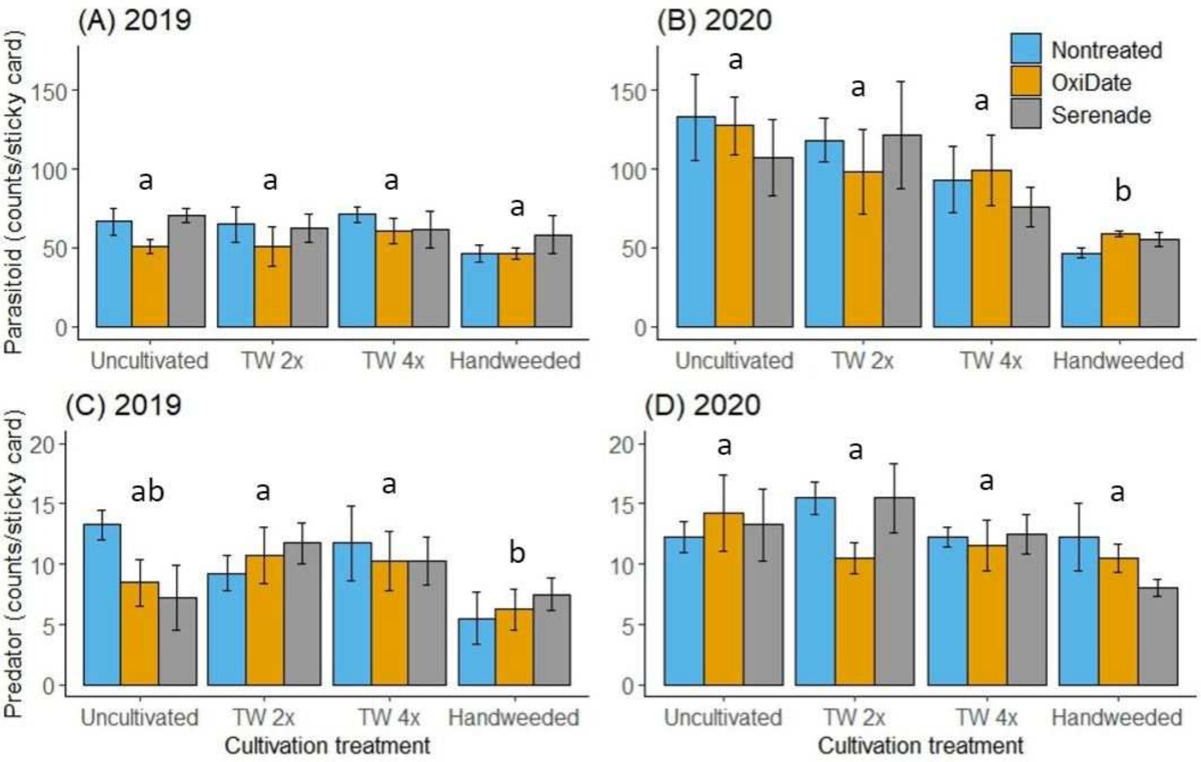

Figure 3.2 Significant effects of weed cultivation and bio-pesticide treatments on parasitoid count mean (± 1SE) in 2019 (A) and 2020 (B) and predator count mean (± 1SE) in 2019 (C) and 2020 (D). Parasitoid and predator counts were estimated using yellow sticky cards and combined for both sample dates. Predators consists of counts from the following generalist predator families: (Geocoridae: Hemiptera), (Anthocoridae: Hemiptera), (Carabidae: Coleoptera), (Staphylinidae: Coleoptera), (Syrphidae: Diptera), (Coccinellidae: Coleoptera), and spiders. Error bars represent standard error of mean. Means sharing the same letter are not significantly different using least squared means (P<0.05).

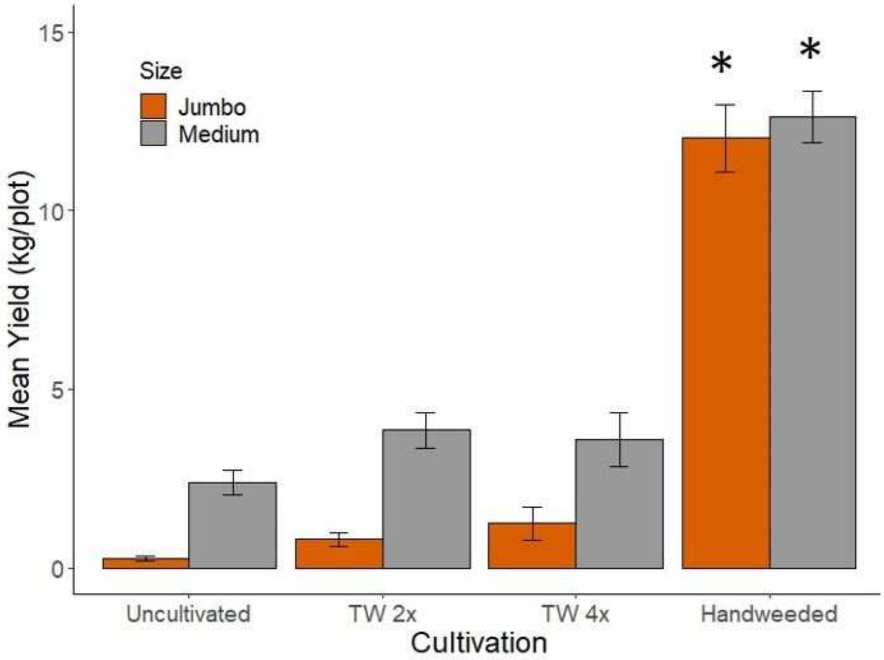

Figure 3.3 Marketable onion bulb yield (mean ± 1SE) by weed cultivation treatments in 2019. Yield was separated by marketable bulb categories (medium and jumbo). * Symbol represents significant effects of cultivation on onion bulb yield. Analyses were only conducted on size categories in relation to cultivation, no comparisons between medium and jumbo bulb size were conducted.

CHAPTER 4

CAN TINE-WEEDING BE AN ALTERNATIVE TO EXCLUSIVELY HAND-WEEDING IN ORGANIC ONION PRODUCTION?

4.1 Introduction

Onions *Allium cepa* (Asparagales: Amaryllidaceae) are the most highly valued vegetable crop in Georgia (Southern Vegetable Summary NASS, 2020). In 2021, 11,800 acres of land were devoted to onion farming with a production value of $143,179,000 (NASS, 2022). However, onion yield in Georgia is almost half of the national average (270 cwt lbs/acre and 500 cwt lbs/acre, respectively). The major reduction in yield may be contributed by the recent shift towards more organic production and the intense weed, disease, and insect pressure in Georgia.

Weed management is one of the greatest obstacles for organic onion growers due to the crop's structure and physiology. Onions have shallow roots, are slow-maturing, and stand upright with narrow leaves that lack a protective canopy to shade emerging weeds (Bond and Burston, 1996; Khokhar, 2017). In Georgia, most all organic onions are transplanted in fall (Boyhan & Torrance, 2002) since transplanted onions are better equipped to combat early season weed pressure and mechanically cultivate earlier into the season (Johnson et al., 2017). Hand-weeding is still widely relied on for adequate weed control for organic onion production in Georgia. However, hand-weeding is very labor intense and costs approximately $3,710 per hectare of onions (Johnson et al., 2017).

Mechanical weeding is common in many organic systems (Kurstjens, 2007) and has been adopted by some onion growers in Georgia since 1997. However, complete adoption of mechanical weeding has not occurred because of equipment costs, technical problems, and poor performance in wet soil (Boyhan & Torrance, 2002). Additionally, onion roots may easily become damaged during mechanical cultivations, causing crop injury and potential yield loss (Melander et al., 1997). Mechanical weed treatments combining inter and intra-row treatments, such as hoeing-ridging combined with finger-weeding have shown high weed control efficacy in onion systems in other regions (Pannacci et al., 2020). Hence, we determine the effect of combining mechanical cultivations and hand-weeding at different durations over the season. We also estimate the effects of weeding on thrips, parasitoids, generalist predators, center rot incidence, and yield. Additionally, in 2022 we document the amount of time spend hand-weeding to determine the potential cost benefit of implementing mechanical cultivations into weed management program.

4.2 Materials and Methods

4.2.1 Experimental Design

The study was conducted in 2020, 2021, and 2022 at a University of Georgia research farm in Tifton, GA (31.472548°N, 83.527839° W). In 2020, the experiment was a randomized complete block design, with four replications of each hand-weeding and tine-weeding treatment combination (Johnson et al., 2012) (Figure 1A). There was a total of six weeding treatment combinations: non-cultivated control, tine-weeding 2x (TW2x), tine-weeding 2x + hand-weeding (TW2x + HW), tine-weeding 4x (TW4x), tine-weeding 4x + hand-weeding (TW4x + HW), and hand-weeded. The same experimental design was repeated for 2021, however, an additional weeding treatment of tine weeding 8x (TW8x) was included to explore the effects of a more intensive mechanical treatment (Figure 1B). Plots were 20 ft long by 6 ft wide, each containing four rows that were spaced out 12 inches apart with one onion every 4 inches (Boyhan & Kelley, 2008). The onion variety Sweet Magnolia (Seminis) will be used due to its high marketable yield in a study by Tyson et al (2019) in a trial. Onions will be transplanted by hand in mid-December and weed cultivations will be performed bi-weekly following transplanting. Tine-weeding will be performed two- or four-times following transplanting for treatments. Hand-weeding will be performed for the 8 weeks following transplanting.

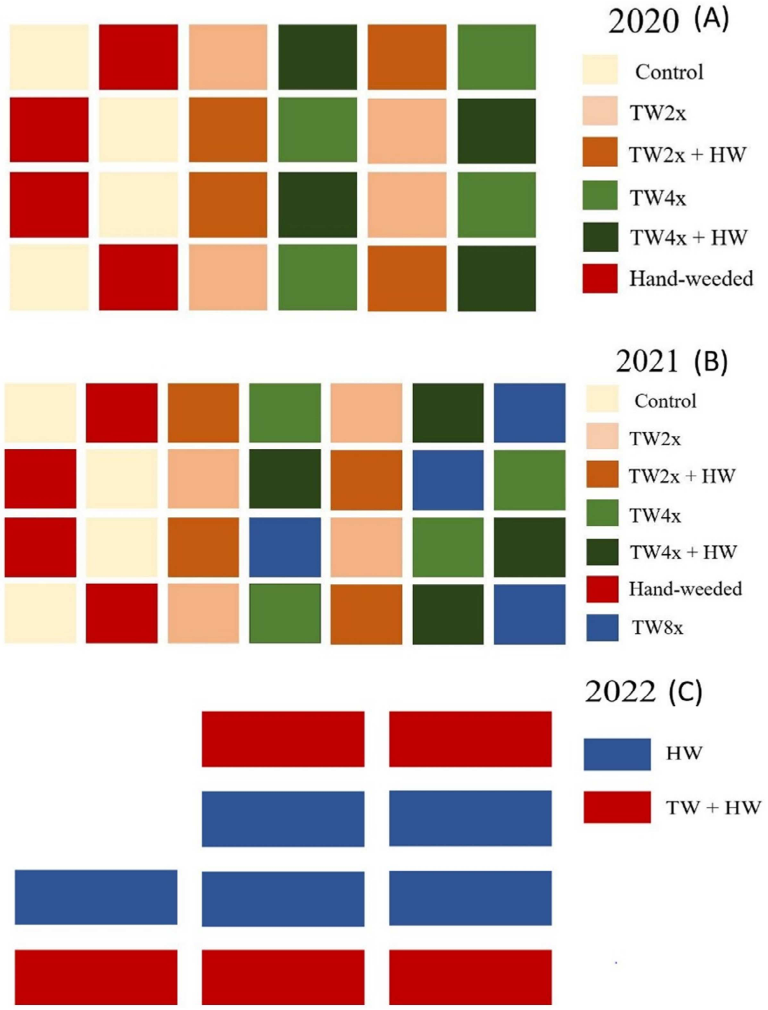

Figure 4.1. Represents the experimental design for 2020 (A), 2021 (B), and 2022 (C). Each colored block represents a different weed treatment combination. 2020 and 2021 are randomized complete block designs (n=4 per treatment) with 6 treatments in 2020 and 7 in 2021. In 2022, only two treatments were compared.

In 2022, only two treatments (exclusively hand-weeded and hand-weeded + tine-weeded) were compared. Each treatment was replicated 5 times for a total of ten plots. Onion plots were 25 ft by 6 ft separated by 15 ft by 6 ft non-crop alleys. All weeding was performed bi-weekly two weeks post-transplanting. Tine-weeding was performed until onion bulbs and foliage growth became too large and began damaging onion bulbs. Hand-weeding was performed until harvest (End of April).

4.2.2 Sampling

One yellow sticky card (4x6) was placed vertically in the center of each plot 10-30 inches above ground, so the card is level with onion foliage. In 2020 and 2021 sticky cards were placed out the week following the last weed cultivation treatment to avoid arthropod disturbance by weeding and collected after seven days of field exposure for three dates. In 2022, sticky cards were placed out 3 days post-weeding and picked up seven days later prior to the next round of weeding. All arthropods were identified to family or lowest possible taxonomic level. We counted all known biocontrol agents (Loomans et al., 2003), including parasitoids and generalist predators. Parasitoids tend to be very minute and difficult to identify to family or lower on sticky cards; therefore, they were only identified down to Parasitica.

Wild radish counts were taken for each plot in a 5000cm^2 area early season (01/09/2020 and 01/11/2021) and mid-season prior to harvest (03/18/2020 and 03/09/2021). Onion yields were measured by mechanically undercutting and lifting onion at physiological maturity (Late April). One week after field curing, the roots and onion tops were clipped and graded according to USDA standards. Marketable yield [total yield at harvest – cull (pre-harvest+post-harvest)] and quality were determined for each treatment. Bulbs were stored and evaluated for the presence of

Pantoea spp. Post-harvest evaluation of bulbs was estimated after 30 days of storage at 2°C on a sub-sample of 10 and 20 bulbs per plot in 2020 and 2021, respectively.

4.2.3 Statistical Analysis

Generalized linear models (GLM) were used to examine the effect of different combinations of tine-weeding and hand-weeding on early and mid-season weed density, thrips and natural enemy abundance, and yield. To meet normal distribution assumptions, all response variables were averaged per date and log transformed (except disease). Center rot incidence was analyzed as a binomial distribution (n positive bulbs for center rot/ n total bulb number). For analysis of disease, we used a Generalized Linear Model (GLM) for proportion of center rot observed from stored bulbs. Within treatment comparisons were analyzed using least squared means ($P<0.05$). Due to variation in plot design, separate analysis was conducted by year. All analyses were completed in R version 4.1.2 “Bird Hippie” (R Core Team 2.

4.3 Results

4.4 .1 Arthropods

Thrips abundance was significantly affected by weed cultivation in 2020 and 2022 (Table 4.1). In 2020, thrips abundance was highest in the TW2x + HW plots, followed by TW4x + HW, and hand-weeded. Whereas the lowest thrips abundance was observed in the control and TW4x treatments (Table 4.2). In 2022, weeding significantly affected thrips abundance (Table 4.1) with hand-weeding having the significantly lower abundance compared to TW + HW (Table 4.2). However, weeding did not have a significant effect on thrips abundance in 2021 (Table 4.1).

Parasitoid abundance was significantly affected by weeding in 2020 (Table 4.1). TW2x and TW4x had the highest parasitoid abundance (Table 4.2). There was no significant difference between parasitoid abundance between weeding treatments in 2021 or 2022 (Table 4.1; Table 4.2). Similarly, predator abundance was significantly influenced by weed treatments in 2020 but not in 2021 or 2022 (Table 4.1). In 2020, predator counts were highest in the hand-weeded plots followed by Tw4x + HW and TW2x + HW, and the lowest abundance was found in the control and TW2x treatments (Table 4.2).

4.3.2 Weed, Disease, and Yield

In 2020, early and late season weed counts were significantly affected by weeding treatments (Table 4.3). For both early and late-season, the highest weed counts were observed in the control, TW2x, and TW4x treatments, and the lowest weed counts in the hand-weeded, TW4x + HW, and TW2x + HW (Table 4.4). In 2021, weeding had a significant effect on early-season weeds but not late-season counts (Table 4.3), where weed counts were significantly higher when TW2x + HW compared to exclusively hand-weeding (Table 4.4).

Center rot incidence was not affected by weeding treatments (Table 4.3) and observed in low abundance in both 2020 and 2021 (Table 4.4). In 2020, yield was significantly impacted by weeding (Table 4.3). Onion yield was highest in the hand-weeded plots, followed by TW4x + HW, TW2x + HW, and TW4x, and the lowest yield was in the control and TW2x treatments (Table 4.4). In 2021, onion yield was not affected by weeding (Table 4.3). In 2022, onion yield was significantly higher when exclusively hand-weeded compared to TW + HW ($F_{1,8} = 12.74$, $P=0.007$; Figure 4.2). However, the amount of time taken to hand-weed over the 2022 season was significantly higher in the exclusively hand-weeded plots ($F_{1,8} = 50.14$, $P<0.0001$; Figure 4.2).

4.4 Discussion

We found inconsistent effects of organic weeding treatments for onion management in Georgia. Thrips abundance was the highest in hand-weeded plots. Previous studies have found thrips and other pest abundance was reduced under high weed management intensity regimes (Penagos et al., 2003). Similarly, predator abundance was highest in the hand-weeded plots in 2020. This was unexpected since generalist ground dwelling predators are known to use surrounding weeds and other non-crop habitat for alternative food and shelter (Madden et al., 2021), and tend to be sensitive to intensive soil management (Sacco-Martret de Preville et al., 2022). Contrastingly, parasitoid abundance was highest in the tine-weeding treatments. Studies in other cropping systems have found frequent mowing led to reduction in parasitoid abundance (Horton et al., 2003). Overall, thrips and predators were highly abundant under high intensity weed management practices. Whereas parasitoids were highest under strictly mechanical cultivation regimes. However, the high density of wild radish in the rows between plots and within some treatments (Figure 4.3C) may have deterred arthropods, and our data in 2020 and 2021 may not be an accurate depiction of how weeding impacts thrips and natural communities.

High weed pressure in this region led to additional fluctuations in weeding efficacy. In 2020, hand-weeding and tine-weeding 4x were sufficient at reducing weed counts. However, in 2021, wild radish pressure was so high, all weed treatments led to similar weed counts late season. However, a previous study in this region found that tine-weeding 2x and 4x during the season reduced weed abundance, particularly cutleaf evening-primrose and lesser swinecress. Wild radish was the predominant weed observed in this study and is known to cause high yield loss when not managed (Blackshaw, Lemerle, & Young et al., 2002). In our study, wild radish caused severe yield loss and reduction in bulb size, particularly in 2021 (Figure 4.3B).

Additionally, wild radish may be a reservoir host for arthropod-transmitted viruses in vegetable crops in the southeast (Kavalappara et al., 2022), and is a known host of *Pantoea spp* (Gitiatis et al., 2002). However, we observed low abundance of disease overall with no impact of weeding in this study.

4.5 Conclusions

Due to the high weed pressure in 2020 and 2021, we altered the experimental design in 2022 to maximize the effect of additional mechanical treatments (Figure 4.3D). We tine-weeded with supplemental hand-weeding and exclusively hand-weeded bi-weekly post-transplanting until harvest, for a total of 8 weeding treatments. To determine a potential cost benefit of labor reduction, we recorded the amount of time hand-weeded over the season. We found tine-weeding significantly reduced the amount of time needed to hand-weed (Figure 4.2A). Furthermore, mechanically cultivating resulted in a reduction in overall yield (Figure 4.2.B). Johnson et al. (2012) did not observe tine-weeding causing crop injury resulting in yield reduction. However, cultivating late in the season when bulbs begin to emerge above the soil and overgrown foliage may cause crops to be torn out of the ground and cause crop injury and create a source of pathogen inoculation. At this time, we do not have 2022 weed count or disease data. Overall, our study demonstrated the importance of intensive weed management practices in Georgia. Due to the high weed pressure in this region, hand-weeding is still recommended for maximum yield. However, there are potential benefits to implementing tine-weeding into a weed management program. It may reduce pest abundance with little negative impact on natural enemies. Furthermore, tine-weeding with supplemental hand-weeding may be a significant labor savings (i.e., cost benefit) to organic onion growers. Future research should examine this combination on a large scale.

Chapter 4: Tables and Figures

Table 4.1. Results from generalized linear model to interpret the effect of weed combination treatments on thrips, parasitoids, predators, and yield. Separate models were fit for each year. Arthropod counts are estimated using yellow sticky cards. Arthropod counts were logarithmically transformed.

			Cultivation		
Year	Arthropod	Df	Deviance	F	P(>F)
2020[a]	Thrips	5	16.12	4.955	**0.0007**
	Parasitoids	5	7.60	9.314	**<0.0001**
	Predators	5	14.26	9.936	**<0.0001**
2021[b]	Thrips	6	0.62	0.480	0.8215
	Parasitoids	6	1.45	2.560	**0.0261**
	Predators	6	0.85	0.677	0.6683
2022[c]	Thrips	1	3.02	11.078	**0.0021**
	Parasitoids	1	0.47	3.932	0.0553
	Predators	1	0.15	0.948	0.3370

[a]- Control, TW2x, TW2x + HW, TW4x, TW4x + HW, Hand-weeded
[b]- Control, TW2x, TW2x + HW, TW4x, TW4x + HW, TW8x, Hand-weeded
[c]- TW + HW, HW

Table 4.2 Mean (±1SE) thrips, parasitoid, and predator per weed cultivation treatment. Separate models were fit for each year. Arthropod counts are estimated using yellow sticky cards. Significance between all treatment combinations were conducted using least squared means (P<0.05).

Year	Cultivation	Thrips	Parasitoids	Predators
2020	Control	11.83 ± 7.40b	10.83 ± 1.62b	2.33 ± 0.45b
	TW2x	21.50 ± 8.26ab	18.92 ± 2.82a	3.08 ± 0.65b
	TW2x + HW	55.67 ± 21.57a	9.83 ± 1.17b	9.42 ± 1.59a
	TW4x	12.92 ± 3.46ab	22.25 ± 2.31a	4.08 ± 0.51bc
	TW4x + HW	39.00 ± 13.63a	10.58 ± 1.50b	9.08 ± 2.16ac
	Hand-weeded	34.42 ± 11.58a	9.00 ± 1.26b	10.50 ± 2.18a
2021	Control	7.75 ± 1.87a	12.42 ± 1.05a	2.75 ± 0.63a
	TW2x	6.75 ± 1.79a	8.75 ± 1.07a	2.58 ± 0.50a
	TW2x + HW	8.67 ± 2.04a	8.17 ± 0.72a	2.00 ± 0.48a
	TW4x	6.67 ± 1.47a	9.00 ± 0.88a	2.42 ± 0.43a
	TW4x + HW	7.08 ± 1.66a	9.75 ± 1.40a	2.50 ± 0.44a
	TW8x	9.08 ± 3.16a	8.42 ± 0.84a	2.83 ± 0.64a
	Hand-weeded	6.92 ± 2.04a	11.00 ± 1.13a	1.92 ± 0.50a
2022	TW + HW	11.50 ± 2.08b	10.20 ± 1.19a	4.80 ± 0.53a
	Hand-weeded	19.05 ± 2.44a	12.00 ± 0.77a	5.70 ± 0.71a

Table 4.3. Results from generalized linear model to interpret the effect of weed combination treatments on weed counts (early and late-season), yield, and disease proportion. Separate models were fit for each year. Weed counts were estimated using count/5000cm^2. Weed count and yield were logarithmically transformed.

			Cultivation	
Response	Year	df	F	P(>F)
Weed Count (Early-season)	2020	5	23.474	<0.0001
	2021	6	3.959	0.0377
Weed Count (Late-Season)	2020	5	12.015	<0.0001
	2021	6	1.523	0.2194
Yield	2020	5	13.239	<0.0001
	2021	6	1.481	0.2327
		df	X^2	P(>Chi)
Disease	2020	5	3.136	0.6791
	2021	6	0.079	0.3922

Table 4.4. Mean (±1SE) weed counts (early and mid-season), disease, and yield per weed cultivation treatment. Separate models were fit for each year. Weed counts were estimated using count/5000cm^2. Significance between all treatment combinations were conducted using least squared means (P<0.05).

Year	Cultivation	Weed (Early-season)	Weed (Late-season)	Disease	Yield
2020	Control	26.50 ± 4.73a	77.50 ± 13.26a	0.45 ± 0.10a	18.33 ± 1.90c
	TW2x	16.50 ± 1.55a	36.75 ± 10.41a	0.025 ± 0.025a	53.63 ± 2.92bc
	TW2x + HW	3.25 ± 0.95b	23.75 ± 1.60b	0.15 ± 0.05a	128.05 ± 21.72ab
	TW4x	14.75 ± 2.25a	36.25 ± 4.01a	0.05 ± 0.05a	74.08 ± 23.65ab
	TW4x + HW	2.50 ± 1.32b	8.75 + 1.70c	0.175 ± 0.08a	158.60 ± 39.41ab
	Hand-weeded	0.75 ± 0.25b	20.75 ± 5.01bc	0.20 ± 0.09a	146.70 ± 16.58a
2021	Control	98.22 ± 17.07ab	158.77 ± 50.32a	0.05 ± 0.04a	18.35 ± 3.40a
	TW2x	96.88 ± 12.62ab	161.46 ± 28.05a	0.09 ± 0.04a	22.98 ± 2.11a
	TW2x + HW	111.68 ± 14.47a	230.08 ± 54.99a	0.03 ± 0.01a	27.94 ± 4.01a
	TW4x	87.46 ± 8.89ab	138.56 ± 40.00a	0.04 ± 0.01a	27.33 ± 2.50a
	TW4x + HW	86.11 ± 28.05ab	255.64 ± 17.92a	0.09 ± 0.04a	38.64 ± 5.71a
	TW8x	75.35 ± 11.83ab	121.09 ± 11.73a	0.10 ± 0.02a	29.58 ± 9.43a
	Hand-weeded	40.36 ± 11.09b	166.84 ± 31.99a	0.06 ± 0.03a	33.93 ± 8.27a

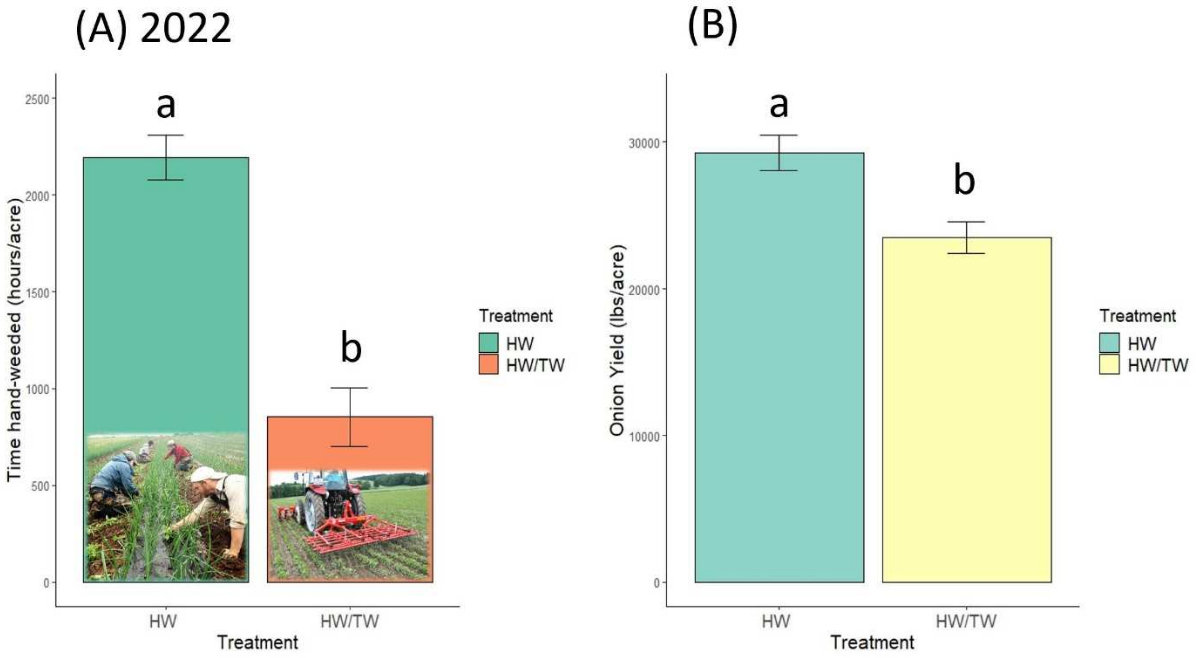

Figure 4.2. Significant effects of weed cultivation treatment on the amount of time hand-weeded (hours/acre) (A) and onion yield (B). Time is summed up for all dates over the season.
Error bars represent standard error of mean. Means sharing the same letter are not significantly different using least squared means (P<0.05).

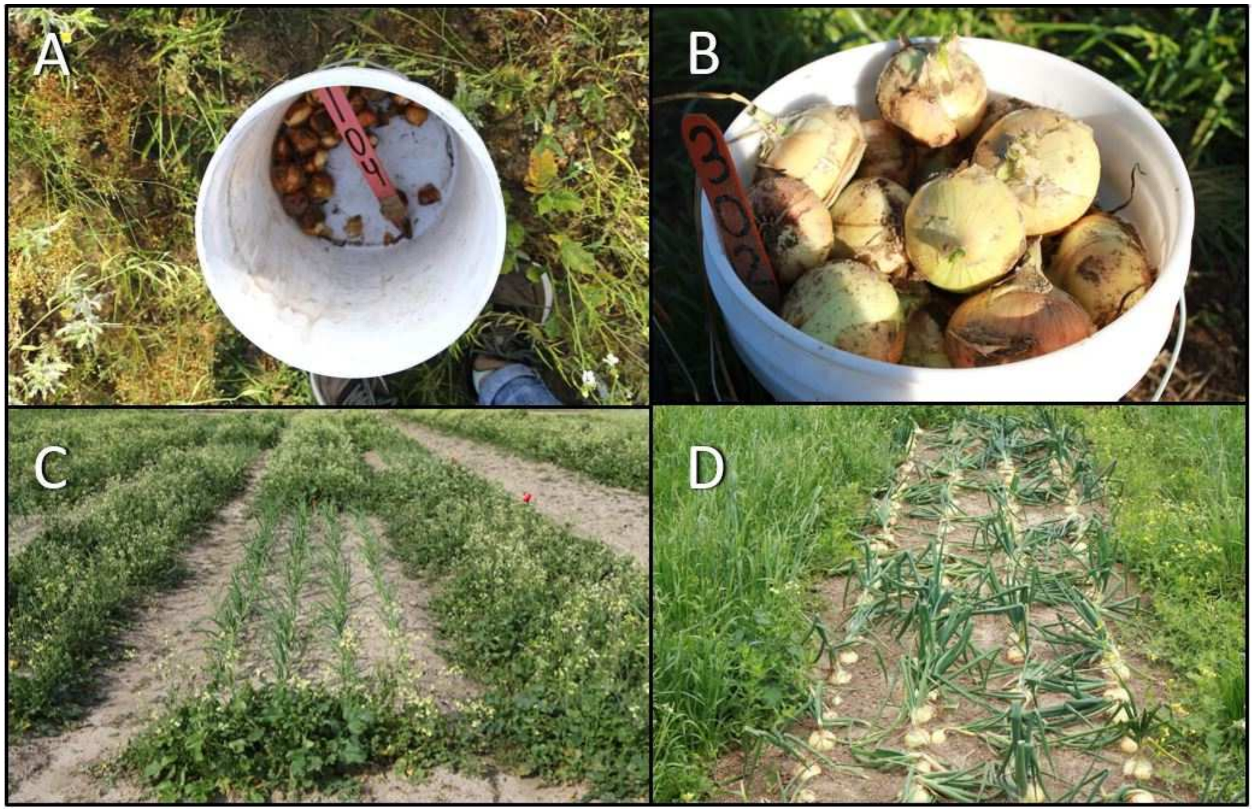

Figure 4.3. (A) 2021 yield from a TW4x plot (n=20). (B) 2022 yield from an exclusively hand-weeded plot (n= entire plot). (C) 2020 hand-weeded plot taken mid-season (D). 2022 exclusively hand-weeded plot taken late-season right before harvest.

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APPENDICES

A. CHAPTER 2.

Table A.1. Mean (± 1SE) of herbivore counts on sticky cards per management type (organic and conventional) and field location (edge, 5m, 50m) separated by taxa.

	Edge		5m		50m	
Herbivore	Conventional	Organic	Conventional	Organic	Conventional	Organic
Aphididae	17.05 ± 3.42	22.75 ± 3.04	39.26 ± 5.72	44.98 ± 2.82	23.88 ± 2.75	24.55 ± 2.76
Thysanoptera	6.76 ± 1.04	9.33 ± 1.50	11.86 ± 1.70	17.50 ± 2.66	10.79 ± 1.49	9.64 ± 1.25
Cicadellidae	14.19 ± 2.66	7.89 ± 1.45	3.17 ± 0.62	2.43 ± 0.40	1.53 ± 0.36	1.19 ± 0.22
Aleyrodidae	0.88 ± 0.42	0.22 ± 0.09	1.45 ± 0.40	0.74 ± 0.14	0.88 ± 0.22	1.05 ± 0.26
Chrysomelidae	0.57 ± 0.17	0.47 ± 0.14	0.48 ± 0.12	0.74 ± 0.19	0.17 ± 0.07	0.71 ± 0.19
Curculionidae	0.36 ± 0.14	0.47 ± 0.22	0.33 ± 0.12	0.38 ± 0.10	0.26 ± 0.09	0.14 ± 0.05
Membracidae	0.24 ± 0.09	0.11 ± 0.05	0.10 ± 0.06	0.12 ± 0.06	0.10 ± 0.06	0.17 ± 0.05
Miridae	0.19 ± 0.08	0.00 ± 0.00	0.12 ± 0.07	0.12 ± 0.06	0.00 ± 0.00	0.05 ± 0.03
Total	40.24 ± 5.12	41.25 ± 3.94	56.76 ± 7.20	67.00 ± 4.86	37.60 ± 3.99	37.50 ± 3.79

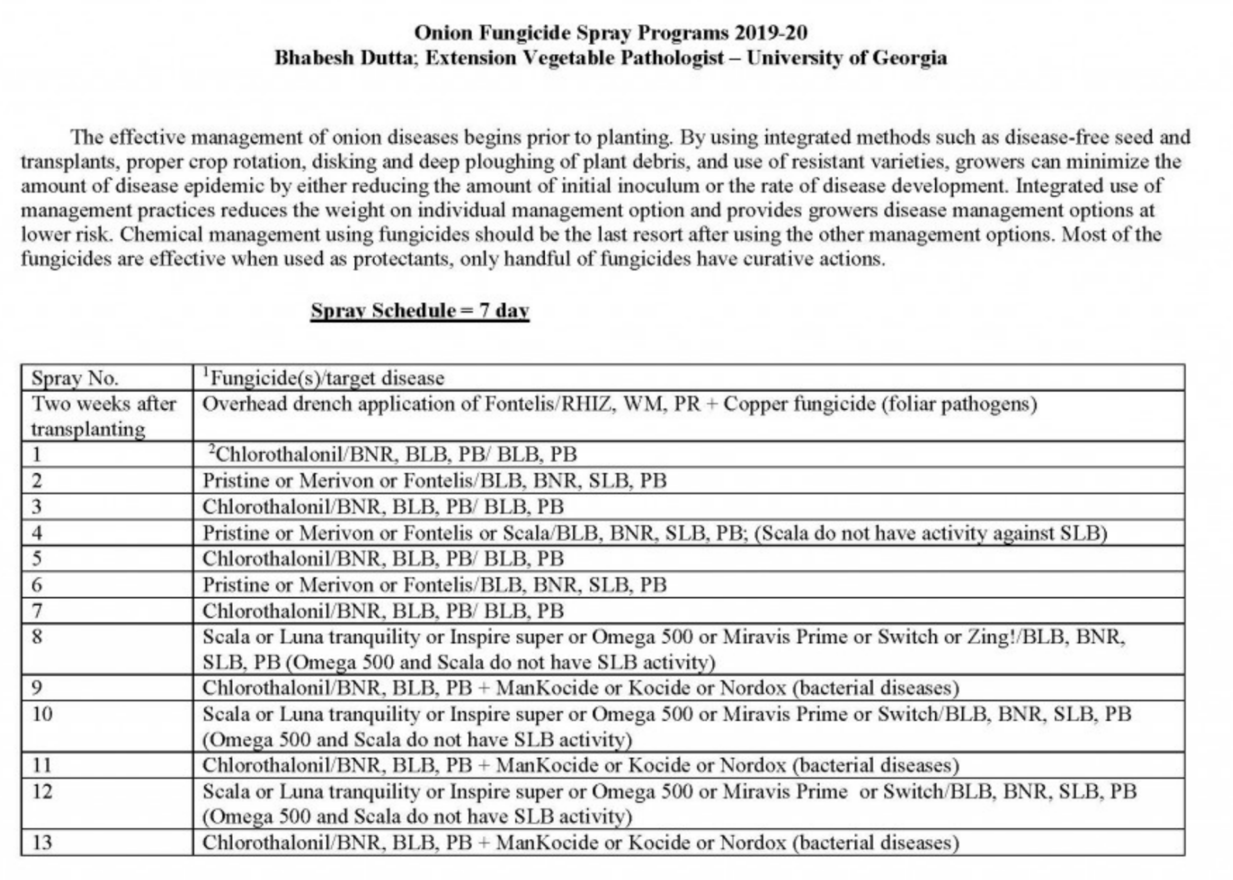
Table A.2. Mean (± 1SE) of natural enemy counts on sticky cards per management type (organic and conventional) and field location (edge, 5m, 50m) separated by taxa.

	Edge		5m		50m	
Natural Enemies	Conventional	Organic	Conventional	Organic	Conventional	Organic
Parasitoids	40.69 ± 4.24	83.22 ± 19.03	19.57 ± 2.66	16.45 ± 1.59	13.60 ± 2.12	10.40 ± 1.48
Active Flying Predators						
Coccinellidae	1.55 ± 0.41	2.11 ± 0.42	2.02 ± 0.32	1.40 ± 0.27	0.74 ± 0.16	0.62 ± 0.13
Staphylinidae	0.60 ± 0.18	0.64 ± 0.17	0.45 ± 0.11	0.31 ± 0.08	0.36 ± 0.10	0.64 ± 0.13
Araneae	0.69 ± 0.17	0.64 ± 0.29	0.17 ± 0.06	0.14 ± 0.08	0.17 ± 0.06	0.14 ± 0.06
Zygoptera	0.24 ± 0.14	0.14 ± 0.09	0.10 ± 0.06	0.10 ± 0.07	0.17 ± 0.12	0.40 ± 0.23
Syrphidae	0.43 ± 0.16	0.14 ± 0.05	0.12 ± 0.05	0.29 ± 0.11	0.05 ± 0.03	0.17 ± 0.07
Dolichopodidae	0.00 ± 0.00	0.25 ± 0.12	0.00 ± 0.00	0.40 ± 0.19	0.12 ± 0.08	0.21 ± 0.15
Carabidae	0.19 ± 0.06	0.19 ± 0.08	0.05 ± 0.03	0.00 ± 0.00	0.02 ± 0.02	0.07 ± 0.04
Anthocoridae	0.02 ± 0.02	0.03 ± 0.03	0.10 ± 0.07	0.05 ± 0.03	0.12 ± 0.06	0.14 ± 0.09
Predator Total	3.71 ± 0.50	4.14 ± 0.82	3.00 ± 0.41	2.69 ± 0.33	1.74 ± 0.26	2.40± 0.40

Table A.3. Mean (± 1SE) of ground predator counts in pitfalls per management type (organic and conventional) and field location (edge, 5m, 50m) separated by taxa.

	Edge		5m		50m	
Ground Predator	Conventional	Organic	Conventional	Organic	Conventional	Organic
Araneae (non-Lycosidae)	3.00 ± 0.46	3.77 ± 0.45	0.71 ± 0.18	0.48 ± 0.14	0.93 ± 0.20	0.79 ± 0.21
Lycosidae	0.62 ± 0.17	1.89 ± 0.63	1.18 ± 0.25	0.74 ± 0.17	1.47 ± 0.73	0.74 ± 0.18
Dermaptera	0.26 ± 0.15	0.14 ± 0.09	0.23 ± 0.09	2.93 ± 1.28	0.43 ± 0.16	1.36 ± 0.47
Carabidae	1.83 ± 0.54	1.75 ± 0.44	0.32 ± 0.12	0.62 ± 0.17	0.29 ± 0.13	0.48 ± 0.18
Staphylinidae	1.98 ± 0.63	1.31 ± 0.49	0.18 ± 0.07	0.33 ± 0.09	0.21 ± 0.11	0.64 ± 0.17
Total	6.48 ± 1.08	7.58 ± 1.07	2.66 ± 0.49	4.55 ± 1.28	3.29 ± 0.97	3.57 ± 0.60

Table A.4. Recommended fungicide spray program from the University of Georgia.



Onion Fungicide Spray Programs 2019-20
Bhabesh Dutta; Extension Vegetable Pathologist – University of Georgia

The effective management of onion diseases begins prior to planting. By using integrated methods such as disease-free seed and transplants, proper crop rotation, disking and deep ploughing of plant debris, and use of resistant varieties, growers can minimize the amount of disease epidemic by either reducing the amount of initial inoculum or the rate of disease development. Integrated use of management practices reduces the weight on individual management option and provides growers disease management options at lower risk. Chemical management using fungicides should be the last resort after using the other management options. Most of the fungicides are effective when used as protectants, only handful of fungicides have curative actions.

Spray Schedule = 7 day

Spray No.	[1]Fungicide(s)/target disease
Two weeks after transplanting	Overhead drench application of Fontelis/RHIZ, WM, PR + Copper fungicide (foliar pathogens)
1	[2]Chlorothalonil/BNR, BLB, PB/ BLB, PB
2	Pristine or Merivon or Fontelis/BLB, BNR, SLB, PB
3	Chlorothalonil/BNR, BLB, PB/ BLB, PB
4	Pristine or Merivon or Fontelis or Scala/BLB, BNR, SLB, PB; (Scala do not have activity against SLB)
5	Chlorothalonil/BNR, BLB, PB/ BLB, PB
6	Pristine or Merivon or Fontelis/BLB, BNR, SLB, PB
7	Chlorothalonil/BNR, BLB, PB/ BLB, PB
8	Scala or Luna tranquility or Inspire super or Omega 500 or Miravis Prime or Switch or Zing!/BLB, BNR, SLB, PB (Omega 500 and Scala do not have SLB activity)
9	Chlorothalonil/BNR, BLB, PB + ManKocide or Kocide or Nordox (bacterial diseases)
10	Scala or Luna tranquility or Inspire super or Omega 500 or Miravis Prime or Switch/BLB, BNR, SLB, PB (Omega 500 and Scala do not have SLB activity)
11	Chlorothalonil/BNR, BLB, PB + ManKocide or Kocide or Nordox (bacterial diseases)
12	Scala or Luna tranquility or Inspire super or Omega 500 or Miravis Prime or Switch/BLB, BNR, SLB, PB (Omega 500 and Scala do not have SLB activity)
13	Chlorothalonil/BNR, BLB, PB + ManKocide or Kocide or Nordox (bacterial diseases)

B. CHAPTER 3.

Table B.1. Mean (± 1SE) of each weed species counts per meter of area for each weed cultivation and bio-pesticide treatment combination. Weed counts were taken after all mechanical cultivations ceased in late-February both years. In 2019, we observed CEP, HT and LSC, and in 2020, CEP and WR.

	2019			2020	
	Weed Density (no./m2)				
Treatment	CEP	HT	LSC	CEP	WR
Handweeded	5.50 ± 2.18	0.08 ± 0.08	0 ± 0	15.33 ± 5.51	0 ± 0
Oxidate	7.75 ± 5.45	0.25 ± 0.25	0 ± 0	11.75 ± 5.56	0 ± 0
Serenade	5.50 ± 4.17	0 ± 0	0 ± 0	12.75 ± 7.60	0 ± 0
Untreated	3.25 ± 1.31	0 ± 0	0 ± 0	21.50 ± 15.04	0 ± 0
No **cult**	78.58 ± 7.83	3.25 ± 0.60	1.75 ± 0.75	119.67 ± 11.60	3.25 ± 0.64
Oxidate	74.75 ± 15.55	4.25 ± 0.95	2.0 ± 2.0	110.0 ± 20.22	4.0 ± 1.68
Serenade	62.50 ± 7.84	3.75 ± 1.25	2.50 ± 1.19	107.50 ± 5.69	3.25 ± 0.75
Untreated	101.50 ± 10.34	1.75 ± 0.63	0.75 ± 0.48	141.50 ± 28.27	2.50 ± 0.87
Tines **2X**	54.92 ± 3.24	2.67 ± 0.89	0 ± 0	116.92 ± 6.87	2.50 ± 0.53
Oxidate	51.25 ± 5.79	1.25 ± 0.95	0 ± 0	107.25 ± 14.20	3.75 ± 1.03
Serenade	53.75 ± 7.59	3.75 ± 2.25	0 ± 0	123.0 ± 10.52	2.0 ± 0.82
Untreated	59.75 ± 3.42	3.00 ± 1.29	0 ± 0	120.50 ± 12.61	1.75 ± 0.75
Tines **4X**	24.33 ± 2.32	1.58 ± 0.58	0 ± 0	34.33 ± 4.06	1.75 ± 0.33
Oxidate	23.25 ± 6.32	1.25 ± 0.75	0 ± 0	27.0 ± 3.74	2.25 ± 0.63
Serenade	20.50 ± 1.50	0.25 ± 0.25	0 ± 0	33.0 ± 9.31	1.75 ± 0.75
Untreated	29.25 ± 1.93	3.25 ± 1.25	0 ± 0	43.0 ± 6.12	1.25 ± 0.25

Superscripts are abbreviations for the following plants: [a] Cutleaf evening-primrose (*Oenothera laciniata*); [b] Henbit (*Lamium amplexicaule);*[c] Lesser swinecress (*Coronopus didymus);*[d] Wild Radish (*Raphanus raphanistrum)*

Table B.2. Mean (± 1SE) of medium bulb yield, jumbo bulb yield, and total marketable onion yield in 2019 per weed cultivation and bio-pesticide treatment combination. Due to COVID-19 restrictions, we were unable to harvest onions in 2020.

	Yield (g/bulb)		
Treatment	Medium	Jumbo	Total
Handweeded	12.63 ± 0.72	12.02 ± 0.95	24.65 ± 1.28
Oxidate	11.91 ± 0.99	11.11 ± 1.32	23.02 ± 1.76
Serenade	12.02 ± 1.64	12.25 ± 1.58	24.27 ± 2.53
Untreated	13.95 ± 1.09	12.70 ± 2.29	26.65 ± 2.50
No **cult**	2.38 ± 0.34	0.26 ± 0.07	2.65 ± 0.39
Oxidate	1.93 ± 0.43	0.23 ± 0.13	2.16 ± 0.54
Serenade	2.16 ± 0.60	0.23 ± 0.13	2.38 ± 0.70
Untreated	3.06 ± 0.70	0.34 ± 0.11	3.40 ± 0.75
Tines **2X**	3.86 ± 0.50	0.79 ± 0.18	4.65 ± 0.54
Oxidate	3.74 ± 0.68	1.02 ± 0.43	4.76 ± 0.77
Serenade	3.18 ± 0.59	0.57 ± 0.22	3.74 ± 0.65
Untreated	4.65 ± 1.23	0.80 ± 0.29	5.44 ± 1.30
Tines **4X**	3.60 ± 0.76	1.25 ± 0.46	4.84 ± 1.07
Oxidate	4.08 ± 1.41	2.16 ± 1.26	6.24 ± 2.28
Serenade	4.53 ± 1.52	0.91 ± 0.41	5.44 ± 1.87
Untreated	2.15 ± 0.99	0.58 ± 0.39	2.84 ± 1.30

Table B.3. The sum of each arthropod taxonomic group per weed cultivation and bio-pesticide combination. Listed are the sums of all individuals counted on sticky cards over all plots (replication of 4 per treatment combination), sampling dates and years for each taxa. Arthropod groups are separated into two major categories: herbivores and natural enemies. Only taxonomic groups collected in greater than 5% of samples are included below. Where, cultivation treatments are indicated by: HW= hand weeding, NC=No weeding, TW2= Tine weeding 2 times post planting, TW4= Tine weeding 4 times post planting, and bio-rational treatments are listed as: S=Serenade, O=Oxidate, and NT = not treated.

Description	HW x S	HW x O	HW x NT	NC x S	NC x O	NC x NT	TW 2 x S	TW 2 x O	TW 2 x NT	TW 4 x S	TW 4 x O	TW 4 x NT	Total
Herbivores													
Thysanoptera	294	301	305	522	505	646	735	475	594	394	455	482	**5708**
Aphididae	433	396	422	214	123	157	194	174	204	254	298	268	**3137**
Aleyrodidae	61	55	57	5	7	17	22	24	18	31	34	37	**368**
Cicadellidae	29	50	32	99	84	96	78	56	66	58	58	40	**746**
Membracidae	2	4	5	11	6	6	5	7	4	2	2	8	**62**
Miridae	26	11	11	61	23	47	48	14	39	42	57	18	**397**
Curculionoidea	3	6	2	8	6	7	3	2	6	1	4	4	**52**
Natural enemies													
Parasitica	453	420	372	522	714	799	736	598	733	548	640	658	**7193**
Geocoridae	9	7	7	15	13	20	21	15	16	21	13	18	**175**
Anthocoridae	4	8	15	13	19	6	17	16	12	12	13	17	**152**
Carabidae	14	15	17	13	15	22	22	20	18	27	21	24	**228**
Staphylinidae	13	16	16	14	23	15	16	7	13	7	9	10	**159**
Syrphidae	0	0	1	5	3	10	2	3	4	2	4	2	**36**
Araneae	4	7	3	5	3	3	4	3	5	4	2	4	**47**
Coccinellidae	18	14	12	17	15	26	27	21	31	18	25	21	**245**
Total	1363	1310	1277	1524	1559	1877	1930	1435	1763	1421	1635	1611	**18705**