POPULATION DYNAMICS OF THE BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS* (STAL) IN THE VENETO REGION OF ITALY AND ITS DAMAGE POTENTIAL TO KIWIFRUIT AND CHERRY

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(Under the Direction of George Vellidis and Alberto Pozzebon)

ABSTRACT

The brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae), is an invasive insect pest that has established populations in both Europe and North America. This polyphagous insect feeds on a wide array of crops, including both kiwifruit and cherry. The objectives of this study were to 1) characterize the BMSB feeding damage on cherry in regards to insect density and duration of exposure of the fruit to feeding BMSB 2) characterize the feeding damage on kiwifruit in regards to insect density and fruit maturation stage 3) evaluate the spatial and temporal patterns of BMSB in kiwifruit orchards in the Veneto region of Italy, taking proximity to the Brenta River, field surroundings, and location within the orchard, and kiwifruit maturation stage into consideration. We found strong evidence that BMSB is a viable candidate to becoming a severe pest in both cherry and kiwifruit production in Italy. We also found that proximity to the Brenta River had a strong effect on BMSB abundance in kiwifruit orchards.

INDEX WORDS: BMSB, *Halyomorpha halys,* Damage, Kiwifruit, Landscape, Cherry, Insects, Entomology

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B.S., The University of Georgia, 2016

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment
of the Requirements for the Degree

MASTER OF SCIENCE

Athens, GA

2018

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August 2018

ACKNOWLEDGEMENTS

The author expresses gratitude toward his major professors, George Vellidis and Alberto Pozzebon, for their guidance, advice, and professional criticism throughout this graduate program. He also expresses sincere appreciation towards Paola Tirello and Davide Scaccinni for their help with the data collection processes and graduate mentorship. Appreciation is also shown to all of the farm owners that allowed their property to be used for these experiments. He would also like to thank his family, friends, and wife for their patience and support throughout his academic career. Finally, the author expresses his gratitude to Professor Francesco Morari who facilitated his participation in the Dual Master’s Degree in Sustainable Agriculture at the University of Padova.

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1. GENERAL INTRODUCTION

1.1 Insect Overview

Halyomorpha halys (Stål, 1855), or the brown marmorated stink bug (BMSB), is an insect in the order Hemiptera and family Pentatomidae. This insect is phytophagous and has piercing/sucking mouthparts that it uses to probe for food and to extract nutrients from fruits, seeds, leaves, stems, and other plant tissues (Hoebeke & Carter, 2003). The BMSB's piercing and sucking mouthparts cause damage to fruits and other plant tissues by penetration. This penetration may allow entrance of disease through the open wounds; causing increasing economic damage to the crops that it feeds upon (Kuhar et al., 2012)

The BMSB is a relatively new invasive species to North America and Europe. Native to eastern Asia, the BMSB has spread to parts of Europe and North America presumably by trade and increased globalization (Zhu et al., 2012). The BMSB was discovered in the U.S. in Allentown, Pennsylvania in 1996 and has caused severe agricultural damage throughout its stay (Hamilton, 2009; Rice et al, 2014). In 2010, BMSB damage resulted in $37 million USD of losses in apple in the mid-Atlantic region of the U.S. alone (Rice et al, 2014). The BMSB has currently been recorded in 44 states and 4 Canadian provinces in North America (www.stopbmsb.org for updates). The global spread of this insect is threatening agricultural production of many different crops all throughout North America, Asia, and Europe.

Because this pest is relatively new and has potential to cause severe damage, the overall goal of this project was to understand the BMSB's crop host range, impact potential, colonization processes, and how this knowledge may facilitate managing this pest in the future.

Three specific objectives were used to achieve the project's overall goal. The first was to assess the damage that the BMSB inflicts upon cherry fruits, and to determine if this pest may become economically significant on this crop. The second objective was to evaluate the damage the BMSB inflicts on kiwifruits, and to determine if this pest may become economically significant on this crop. The third objective was to evaluate landscape effects on BMSB abundance and to evaluate the distribution of these insects within the orchards. This helped with the understanding of the pest's population dynamics within orchards and which landscapes may be more at risk of invasion.

1.2 BMSB Morphology and Biology

Throughout literature, there have been some inconsistencies in nomenclature for the brown marmorated stink bug; it has been referred to as *Pentatoma halys, Poecilometis mistus, Dalpada brevis, Dalpoda remota, Halyomorpha brevis, Halyomorpha mista, Halyomorpha remota*, and, most recently, *Halyomorpha halys* (Rider et al., 2002; Rider, 2005). Female BMSB produce eggs that are light yellow or green in color 14 to 25 days after their final molt. These egg masses are typically laid in clusters with 20 to 32 eggs per cluster. These oval-shaped eggs are typically 1.3 mm in diameter and 1.6 mm in length and are affixed to a host surface using an adhesive produced by the female (Hobeke & Carter, 2003). Four to seven days after the eggs are laid, nymphs emerge from the eggs. The freshly hatched nymphs are oval in shape, yellow/brown in color, moddled with red and black on the abdomen, and have a tick-like appearance. There are a total of 5 instars before the BMSB reaches adult stage. The first instar is approximately 2.4 mm in length and the abdomen is mostly yellowish red. The second instar is approximately 3.7 mm in length and the abdomen is whitish with reddish spots and reddish junctions. The third instar is approximately 5.5 mm in length, and the body becomes pear shaped. The fourth instar is approximately 8.5 mm in length and the body is pear shaped. The fifth instars are approximately 12 mm in length and the body remains pear shaped (Hoebeke & Carter, 2003). The later instars are darker in color with bands on the antennas and legs. The period from hatching to adult takes 33 to 55 days on average. Nymphal mortality from egg to adult is typically 23% to 50%. Adults are typically a greyish brown and, like other pentatomids, have a shield-shaped body. The distinguishing characteristics of the BMSB from other stink bugs is the alternating dark and white bands on the legs and on the last two segments of the antennae and the smooth edges of the pronotum. Adult BMSB morphology is similar to other stink bug species, such as *Euschistus conspersus, Euschistus variolarius, Podisus maculiventris, Menecles insertus, Arma custos* and *Dolycoris baccarum* and may be misidentified by an inexperienced individual. Nymphal BMSB have morphological similarities to nymphal *Rhaphigaster nebulosa* (Herbert et al., 2011). BMSB adult males are, on average, 1.2 cm in length, while adult females are, on average, 1.44 cm in length (Medal et al., 2013). Width across humeral angles is typically 7 to 10 mm (Hoebeke & Carter, 2003). Males are identified by a rear ventral scoop that is present on the tip of the abdomen. Females differ in their egg laying capacity depending on many different factors including temperature, photoperiod, genetic variation, and rearing method (Medal et al., 2013). Nielsen et al. (2008a) found that, on average, the BMSB has the capacity to produce 212 eggs and 8 egg masses during their lifetime. In 1983, Kawada & Kitamura found that each female produced, on average, 487 eggs in their lifetime. Medal et al. (2013) found that, on average, females laid 6 egg masses and a total of 168 eggs in their lifetime. Adult female's lifespan ranges from 9 to 16weeks, and the adult male lifespan ranges from 8 to 18 weeks. BMSB has been recorded having 1-5 generations per year in its native host range (Hoffman, 1931). The BMSB may be univoltine or bivoltine in the United States and in Europe depending on environmental conditions such as photoperiod and temperature (Hoebeke & Carter, 2003).

BMSB mouthparts are composed of a long beak, which encompasses the stylets inside. The stylet bundle inside of the beak includes 4 stylets: 2 outer mandibular stylets and 2 inner maxillary stylets. During feeding, the stylets extend out of the beak and pierce the plant tissue to access food (Peiffer & Felton, 2014; Mitchell, 2004). Of the two maxillary stylets, saliva is pumped down one and liquid food travels up the other. This saliva helps break down plant tissue and further liquefy the tissues in order to make them more accessible for feeding. The saliva is largely responsible for the damage that occurs to the plant tissues during feeding (Mitchell, 2004). The BMSB has two types of saliva, including watery saliva and a gel-type saliva. The watery saliva aides with the digestion of plant materials and contains digestive enzymes and other protein components (Ramzi & Hosseininaveh, 2010). It is believed that the watery saliva is produced by the accessory salivary glands. The gel-type saliva is the part of the saliva that forms the salivary sheath, or the “stylet sheath.” This stylet sheath forms a hardened lining around the insect’s stylet and the plant tissues that it is feeding upon. The sheath forms a seal around the stylet and plant tissues to prevent any plant fluid losses (Medrano et al., 2011). The stylet sheath is released from the salivary canal and will harden quickly once it is secreted and exposed to the atmosphere. These stylet sheaths are visible under the use of a dissecting microscope and may be used to observe feeding intensity (Peiffer & Felton, 2014). Some examples of experiments that have used stylet sheath presence to determine feeding intensity include Niva & Takeda, 2003; Hedstrom et al., 2014; Wiman et al., 2015; Walton et al., 2016.

In addition to being a polyphagous crop pest, the BMSB is also an urban nuisance because of its overwintering aggregation behaviors. Similar to other species of stink bugs

(Saulich & Musolin, 2014), BMSB experiences facultative diapause and overwinters as sexually immature adults (Nielsen & Hamilton, 2009). In the fall, the BMSB searches for overwintering sites to stay for the duration of the winter and is unlikely to leave unless stored nutrients in the fat body are depleted (Funayama, 2012). Li et al. (2007) found that most BMSB become inactive below 9ºC. With the presence of warm weather in spring, BMSB emerges from overwintering sites and moves to host plants in order to feed prior to laying eggs of the first summer generation (Bergmann et al., 2013; Inkley, 2012). Its tendency is to choose dead trees and man-made structures. Lee et al. (2014) used detector canines to characterize the overwintering sites of the BMSB in natural landscapes and found that the insects typically choose dead trees with dry crevices and thick bark, particularly oak and locust trees. In addition to being an arboreal species that overwinters in trees, the BMSB has also developed the behavior to overwinter in man-made structures. Using gaps in the windows, door trims, roof flashings, and other openings around homes, the BMSB will enter structures and become a nuisance to the inhabitants (Welty et al., 2008). In 1990, Qin reported that more BMSB flew to the west-facing portion of buildings rather than other cardinal directions, however the reason is not well understood (Qin, 1990). Post-diapause, the BMSB becomes highly active and takes flight in search of nutriment. Lee and Leskey (2015) found that flight behavior picks up immediately following diapause and the mean flight distances over a 22-h period were 2442 m and 2083 m for male and female BMSB, respectively, although temperature had a major effect on distance flown. Lee et al. (2014) monitored dispersal capacity of all stages of BMSB in the laboratory and in field conditions. They found that there were significant differences among the different life stages that were tested. They found that the older nymphal stages walked and climbed significantly farther distances than the first through third instars; again, temperature had a major effect on travel distance. Mating and oviposition do not begin until approximately 2 to 3 weeks after the adults emerge from their diapause sites. The adults require 14 to 15 days from imaginal ecdysis to become sexually mature (Hoebeke & Carter, 2003).

1.3 Impact of BMSB on Crops

The BMSB is a highly polyphagous insect that has more than 120 known host plants, with new host plants being identified annually (Haye et al., 2015). For this reason, the impact of this pest on different crops has been evaluated in detail on a number of major crops in U.S. agriculture including apple, peach, pear, wine grape, soybean, corn, cotton, ornamentals, and many different vegetables among others (Leskey et al., 2012; Kamminga et al., 2012). In 2010, BMSB caused more than $37 million in damage to apple production in the mid-Atlantic states. The growers retaliated by increasing their insecticide applications to 4 times the usual rate. This increase in insecticide usage resulted in secondary pest outbreaks of aphids, mites, and scales in apple orchards (Leskey et al. 2012a). Kamminga et al. (2014) used insect-proof mesh cages on cotton plants to determine temporal damage level on the cotton bolls. They found that, as cotton boll size increases, the damage level caused by the BMSB also increases. Owens et al. (2013) also used cage studies, however, their experiment was to find if BMSB damage significantly reduces soybean yield in comparison to other stink bug species. Their findings indicate that the threshold densities for the BMSB should be similar to those of the native stink bugs and damage level by BMSB does not significantly differ from that of native stink bugs in soybean production. Soybeans are sensitive to BMSB feeding from stages R3 to R6 (Nielsen et al., 2011; Koch et al., 2015). Rahman & Lim (2017) evaluated soybean pods as a viable food source for the BMSB as compared to the seeds. They discovered that nymphal BMSB can reach maturity and adults can both survive and successfully reproduce on a diet of soybean pods alone, making it a viable food source, however, BMSB lived longer on, fed more intensively on, and preferred a diet of soybean seeds as opposed to pods. On one particular farm, BMSB damage resulted in 100% yield losses for two consecutive years. Even regular insecticide applications between April and August did not prevent the damage (Bernon, 2004).

Kuhar et al. (2012) observed BMSB feeding and damage on many different vegetable crops including okra, asparagus, cucurbits, brassicas, sweet corn, and pepper. Okra, sweet corn, and pepper appeared to be highly preferred host plant species for adult colonization and reproduction. Green bean and eggplant also proved to be suitable for oviposition and development of all nymphal stages. Tomato seems less suitable for reproduction, but the fruit experiences high feeding damage, especially in late August (Rice et al., 2014; Kuhar et al., 2012). On sweet corn, Cissel et al. (2015) conducted cage studies to assess the damage potential of the BMSB at different ear developmental stages. They found that the greatest yield losses occurred when infestations occurred on earlier ear development and severe yield loss can occur because of BMSB feeding. Both adults and nymphal stages were found on pear fruits with highest population densities occurring in July and mid-August, when pit hardening and mid-season swell period occurs. At every farm sampled, the damage that occurred to pear fruit was greater than 25% (Nielsen & Hamilton, 2009). Many orchards with stone fruits such as peach and nectarine experienced greater than 90% crop yield losses (Leskey & Hamilton, 2010).

On many fruits, BMSB may cause indirect damage such as chemical/physical contamination and open wounds that lead to fungal infestation of the plant tissue; however, studies on this topic are limited (Wiman et al., 2014). In Japan, BMSB is known to transmit witches broom, a phytoplasma disease, on princess trees (*Paulownia tomentosa*) (Dara, 2011). With wine grape, the physical damage to the crop is not the main concern; however, feeding may result in deformation and necrosis at the feeding site (Rice et al., 2014). Through both mechanical and hand harvesting, failure to detect BMSB in grape clusters may occur and has proven to taint the taste and lower the quality of the wine that is produced. It was found that a very low amount of BMSB taint has a negative impact on Pinot noir quality (Wiman et al., 2014). The stress volatiles produced by BMSB that are responsible for the taint are identified as tridecan and (E)-Z-decenal. Some preliminary data show that the taint does not survive fermentation and bottling, however, there are other studies with conflicting results that suggest that the taint may survive these processes (Mohekar et al., 2014; Tomasino et al., 2014).

Martinson et al. (2015) tested whether fruit availability influences the seasonal abundance of BMSB. They found that even though different life stages of BMSB were found on 3884 trees of 223 cultivars in woody plant nurseries when fruits were present, the absence of fruits significantly decreases the population densities within the plant nurseries. With this conclusion, they claim that designing landscapes with fruitless varieties of trees may help with control of BMSB. BMSB has the ability to damage the bark of woody trees and shrubs, which causes the liberation of sugars, which then attracts other pests, including several hymenoptera species (Martinson et al., 2013). It has been determined through experimental study that the BMSB is a viable candidate for becoming an economically significant pest on blueberry (Wiman et al., 2015). In blackberries and raspberries, BMSB can cause two types of injury. Earlier in the growing season, BMSB feeds on and may destroy buds. Later in the season, BMSB feeds on mature berries, inserting their stylets in-between drupelets and possibly contacting the receptacle; this type of feeding causes change in fruit color and collapse of drupelets. BMSB frass may also taint the berries and give an unpleasant taste or odor, making the affected fruits unmarketable (Rice et al., 2014). A study in northern Italy, where rice is cultivated, has presented the first sign of BMSB feeding on panicles of rice (*Oryza sativa* L.), a crop that has not previously been labeled as a host crop of BMSB (Lupi et al., 2017). There have also been several documentations of BMSB damaging various ornamentals in Switzerland (Wermelinger et al., 2008). A study in Japan has shown that Japanese bird cherry trees, *Prunus grayana*, are a suitable host for the BMSB. The study also concludes that Japanese bird cherry trees are adequate for the BMSB to reproduce and the early instars are able to feed and survive on this plant, however, the damage has not been assessed or quantified (Funayama, 2007). Forest trees are included among host plants for the BMSB, however, damage assessment to forest trees has not been recorded in the U.S. In Japan, the BMSB is known to feed on parts of coniferous trees and is considered a cedar and cypress pest for farms that produce conifer seeds (Funayama, 2005).

1.4 Spatial Ecology

Geospatial analysis indicates that the BMSB has a tendency to cluster and aggregate as the fruits mature later in the season within the orchard (Leskey et al., 2012; Hahn et al., 2017). On apple, economic damage is higher where stink bugs tend to aggregate around orchard edges as opposed to orchard centers (Nielsen & Hamilton, 2009; Leskey et al., 2012). Joseph et al. (2014) designed an experiment encompassing not only spatial distribution within an orchard, but on each individual tree as well. Their results showed that apples from the upper canopy of border zone trees had the highest probability of experiencing both external and internal fruit injury. Basnet et al. (2015) discovered a similar theme within various vineyards in Virginia. The BMSB showed a tendency to aggregate along the edges of the vineyards. By using immunomarking techniques, Blaauw et al. (2016) tracked BMSB distribution and movement within peach orchards. They found that the BMSB primarily aggregates around the perimeters of the orchard, and this trend is primarily driven by the distribution of male insects. In 2015, Venugopal et al.

explored the spatiotemporal dynamics of BMSB in and between adjacent corn and soybean fields. They found that the highest densities of BMSB in both corn and soybean coincided with R2-R4 stages of corn and R5-R6 stages in soybean. In each field they examined, they found extremely low densities of BMSB beyond 25 m from the field edge, indicating that the BMSB clusters and aggregates around perimeters of the fields. This study indicates that reducing boundary areas shared between corn and soybean fields could reduce dispersal into soybean.

It has been suggested that BMSB tends to aggregate and remain along or near overwintering sites on fruit crops. In 2015, Bakken et al. found that, in corn and soybean fields, BMSB remain primarily around the edges and in the edge rows. In 2015, Venugopal et al examined BMSB abundance on plants in tree nurseries as an indicator of distance from field edges. They found that BMSB nymphs and adults were at much greater densities in field edges than in the core of the fields. They sampled tree nurseries adjoining different habitats, such as corn fields, soybean fields, residential areas, and production sod. They found that the proximity to soybean fields were associated with high nymphal and adult abundance. The level of fruit maturity has a distinct effect on the distribution of BMSB on the plant. Through experimental study, it was found that adult BMSB spend more time on the fruits of the plant when the fruits are mature, while egg masses and nymphal stages spend most of their time on the leaves, however, late instars do occasionally feed on the fruit. If fruits are absent, the adults and late instars will spend the majority of their time on the bark of the tree, while early instars and egg masses will be located on the leaves. When immature fruits are present, eggs, nymphal stages, and adults will all spend the majority of their time on the leaves of the plant, however the adults do occasionally feed upon the immature fruits (Shrewsbury et al., 2015). A New Jersey study has indicated through spatial analysis, geostatistics, and Bayesian linear regression, that BMSB tend to stay near urban areas (overwintering sites) and railroads (areas of introduction). They also determined that high densities of BMSB are typically related to agricultural areas (Wallner et al., 2014). Yearly BMSB density rate is highly variable in many different locations across the globe, Nielson et al. (2016) found that temperature and photoperiod influence yearly population dynamics of the BMSB. Throughout this three-year study, temperature was found to be the primary influencer on BMSB population dynamics. Knowledge on movement and spatiotemporal patterns of insect pest populations within landscapes and among preferred hosts can aide in the creation of effective and efficient pest management strategies and contribute to integrated pest management programs. By knowing that BMSB aggregate mainly in and near field edges, pesticides and other control measures may be applied only to field or orchard edges in order to save time and money and to reduce contributions to environmental pollution.

1.5 Cherry Production

Sweet cherry (*Prunus avium*) is a deciduous tree and perennial fruit crop that originated between the Caspian Sea and Black Sea in the Asia Minor area. Sweet cherry is a member of the Rosaceae family and is grown in 65 countries and on over 364,217 hectares worldwide. The worldwide average yield for this crop is over 5043 kg/ha (Rieger, 2012). The number one sweet cherry producing country is Iran. The United States rank number 3 in cherry production and Italy ranks number 4 in cherry production (FAO, 2004). In the United States, 253,854 tons are produced annually, with an industrial value of $436 million. The market price for sweet cherry averages around $2.09/kg. Per capita consumption of cherry is estimated to be 0.86 kg/yr (USDA, 2002).

Sweet cherry's ideal growing conditions are in areas that have cool and dry climates. The chilling requirement for sweet cherry is 1000-1500 hrs. The plant blooms from April to May and the fruit reaches maturity in July and August. Sweet cherry is primarily insect pollinated and is typically planted in a pollinizer ratio of 8-9:1. Brown rot is one of the largest economic and disease management problems in sweet cherry (Rieger, 2012). The possibility that the BMSB may transmit diseases or create superficial injuries that allow fungal entry makes the invasion threat of this pest in sweet cherry growing regions more serious.

1.6 Kiwifruit Production

Kiwifruit, *Actinidia deliciosa*, is a perennial fruit crop native to southeastern China (Ferguson, 1984). The fruit's common name was originally Chinese gooseberry because of the fruits origin and shape, however, the name was later changed to kiwifruit in order to make the fruit more appealing to consumers. Plants are insect pollinated and are typically planted in a ratio of 8 pistillates to 1 staminate in order to ensure adequate cross pollination and fruit development. The crop is a long season fruit and is typically hand harvested. Kiwifruit has been grown in Italy for more than 80 years, although most of the early plantings were experimentation or were used for ornamental purposes. The first kiwifruit orchards used for fruit production were planted in the 1960s. Most kiwifruit vines remain productive until the plant reaches an age of 20 years. Hayward is the most common cultivar grown around the world because it outperforms other cultivars in terms of fruit size. Another variety of kiwifruit commonly grown in Italy is the yellow kiwifruit (*Actinidia chinensis*) (Beutel, 1990).

In 2010, global production of kiwifruit was reported as 1.35 million tons. China leads in global production, producing an annual average of 480,000 metric tons from 2009 to 2012. Italy is the second largest producer of kiwifruit worldwide, producing an annual average of 450,000 metric tons from 2009 to 2012. New Zealand leads in global exports of kiwifruit, exporting around 90% of its production. Italy is second in exports, exporting around 70% of its produced fruits. In 2007, Italy reportedly had 26,700 ha of kiwifruit orchards grown within its borders (Testolin & Ferguson, 2009). The plant's production range is limited due to its temperature and solar requirements. Kiwifruit must have winter temperatures that fall below 7ºC for 600 to 700 hours in order to meet the chilling requirements to produce fruits. A time period of 225 to 240 days without frost is needed for kiwifruit production since the vines leaf out in March, flower in May, and are harvested in late October or early November. Frost that falls below -1ºC in the spring will kill the new shoots and fruit production will be minimal (Beutel, 1990). Due to growing conditions, most of Italy's kiwi orchards are grown on the main peninsula between latitudes 46º and 39ºN (Testolin & Ferguson, 2009). In the United States, kiwifruit production is minimal, with most of its production occurring in the state of California. Kiwifruit production is low compared to other fruits, making up only 0.22% of the global fruit market; however, kiwifruit production is growing. From 1997 to 2007, global production of kiwifruit increased by 50% (Ward & Courtney, 2013). Production costs are high for kiwifruit farmers, and profit margins are low. For the first 4 years of production, the average costs per acre to grow these fruits are $8,300 to $11,800. After the first 4 years, the average cost of production is $2000 per acre, although these costs vary significantly for each producer depending on management decisions. Average yield of kiwifruit in the United States is 8000 lb/acre, and the cost to grow these fruits are approximately 30 cents per pound. (Beutel, 1990). In the past 20 years, kiwifruit market price has fluctuated from 30¢ to $2 per pound (Testolin & Ferguson, 2009). On years where price is lower, new pest outbreaks, such as BSMB, could be devastating to kiwifruit farmers.

1.7 Management

The need for control measures for BMSB is increasing as its host range and habitat is expanding. The damage potential of this insect is high on many crops, and if not properly managed, can result in up to 100% yield losses (Bernon, 2004). In order to reduce environmental degradation through pesticide pollution and reductions in populations of beneficial insects, an integrated approach to control is needed. Monitoring is an important part of insect management in any agricultural setting. There are many ways to monitor for the BMSB. One effective way to monitor is by the use of pheromone traps. Using traps that mimic the BMSB aggregation pheromone is an effective way to attract insects in order to get an idea of pest abundance. Visual estimation is also an effective way to monitor fields or orchards. The usage of beat sheets or drop cloths and sweep nets are also effective ways to monitor for BMSB abundance and distribution in a given area (Quarles, 2011).

One option for BMSB control is through the use of chemical insecticides. Pyrethroids have been used to manage BMSB in some crops, however efficacy is limited. It has been reported that 33% of BMSB may recover from pyrethroid application (Leskey et al., 2012d). Some other insecticides that have been used include: endosulfuron, methomyl, and neonicotenoids (Nielson et al., 2008b; Leskey et al., 2012d). Biopesticides have the tendency to be less damaging to the environment and equally as effective. Neem (azadirachtin) has been one of the more successful biopesticides used, especially when paired with pyrethrin, which inhibits feeding activity. Chitin synthesis inhibitors also show some effect against nymphal stages of BMSB. There are also a few microbial biopesticides that have displayed control activity (Jacobs, 2014).

Some cultural control options also exist. The use of trunk barriers containing adhesive to stop motile forms of insects from climbing the trunks of tree crops has shown some effect. The use of pheromone baited traps such as pyramid traps and sticky traps has also been proven to capture or kill insects. The usage of insect proof mesh netting over the fruits, trees, or entire orchard is also a possibility to prevent damage from occurring (Lee et al., 2013; Jacobs, 2013). Repellants are another option for BMSB control. Essential oils such as clove, spearmint, ylang ylang oils, lemongrass, wintergreen, geranium, and rosemary have shown 60-100% repellant activity (Zhang et al., 2013).

Biological control for BMSB is also possible in some locations. Some species of entomopathogenic fungi, such as *Beauveria bussiana* and *Metarhizium anisolpliae,* have shown up to 100% BMSB mortality rates in laboratory settings (Gouli et al., 2012). For both the United States and for Europe, classical biological control is seemingly the most viable option. An Asian parasitoid species *Trissolcus halyomorphae* has proven to be a very effective egg parasitoid, with a parasitism rate of 50% (Yang et al., 2009). Many species of birds also feed on the BMSB, despite the emittance of defense chemicals (Ingels & Varela, 2014). Many generalist predators also feed on BMSB, including lacewings, ladybugs, pirate bugs, spiders, and big-eyed bugs (Leskey et al., 2012a). There are many ways to monitor and control for the BMSB, but in order to control the insect effectively without excess environmental harm, using an integrated approach is suggested.

Preventative and active control measures in the urban setting to manage BMSB as an urban pest that invades homes has been assessed. Watanabe et al. (1994) found that adding repellant, such as DEET, to window frames and other potential points of entry has an effect on the number of BMSB that enter the home. They also found that the use of “slit traps” was quite effective in capturing and preventing the insects from entering homes. Covering the home in a cyphenothrin-treated net proved to be effective in preventing BMSB entry as well. Laying cyphenothrin-treated polyethylene sheets in window frames and other possible points of entry aided in the prevention of BMSB entry and may be a possible solution to home invasions (Watanabe et al., 1994).

2. CHARACTERIZING DAMAGE POTENTIAL OF BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS*, IN CHERRY ORCHARDS

2.1 Introduction.

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), brown marmorated stink bug, is native to Asia and an economically important invasive pest to North America and Europe. It is known to cause damage on many different agricultural crops such as fruits (Haye et al., 2015; Joseph et al., 2014; Leskey et al., 2012a; Leskey et al., 2012b; Nielsen & Hamilton, 2009; Walton et al., 2016; Wiman et al., 2015; Acebes-Doria et al., 2016), tree nuts (Hedstrom et al., 2014; Bosco et al., 2018), row crops (Bakken et al., 2015; Kamminga et al., 2014; Koch and Rich, 2015; Nielsen et al., 2011; Owens et al., 2013; Venugopal et al., 2014), ornamentals (Skillman & Lee, 2017; Martinson et al., 2013; Shrewsbury et al., 2011; Wermelinger et al., 2008), and vegetables (Cissel et al., 2015; Haye et al., 2015; Kuhar et al., 2012; Zobel et al., 2016). *H. halys* is a highly polyphagous insect that has more than 120 known host plants, with new host plants being identified annually (Haye et al., 2015). In addition to being a major agricultural pest, *H. halys* is a major nuisance pest due to its tendency to enter man-made structures in search of suitable overwintering sites (Funayama, 2012; Inkley, 2012). *H. halys* was first identified in the United States in Allentown, Pennsylvania in 1996 and has since spread to 44 states and 4 Canadian provinces (Hoebeke & Carter, 2003: Leskey et al., 2015: Updates at www.stopbmsb.org). The first recorded presence of *H. halys* in Europe was in Switzerland in 2004 (Haye et al, 2014; Morrison et al., 2017). *H. halys* was first detected in Italy in the Emilia Romagna region in 2012 and was later detected in the Veneto region in 2015 (Maistrello et al., 2014; Bariselli et al., 2016). In Italy, severe damage to pear has been recorded and *H. halys* damage was also reported on many other crops such as persimmon, tomato, apple, apricot, peach, hazelnut and plum (Bariselli et al., 2016; Bosco et al., 2018).

H. halys damage on fruits is similar to that of other Pentatomidae. *H. halys* has piercing and sucking mouthparts that penetrate leaves, stems, buds, blossoms, seeds, and fruits in order to access the fluids within the plant tissue. The mouthparts are comprised of two outer mandibular stylets and two inner maxillary stylets. Saliva is pumped down one of the maxillary stylets while liquid food travels up the other maxillary stylet. The penetrating mouthparts produce both stylet saliva and watery saliva (Peiffer & Felton, 2014). When the mouthparts are inserted into the plant tissue, the saliva forms a sheath around them to break down plant tissue and aide in substrate acquisition (Mitchell, 2004; Peiffer & Felton, 2014). When the mouthparts are removed, the hardened saliva leaves a visible “stylet sheath” that may be used to quantify *H. halys* feeding events (Peiffer & Felton, 2014). *H. halys* salivary components are largely responsible for the damage that is caused to the plant tissue. The saliva affects plant maturity and development, which may alter the timing of harvest (Mitchell, 2004; Zalom et al., 1997). The penetration of the fruits with the piercing/sucking mouthparts creates access points for secondary fungal or bacterial infections. *H. halys* has not been known to transmit diseases to fruit crops; however, presence of fungal spores on the mouthparts or immediate environment may lead to inoculation with plant pathogenic fungi. Fungal or bacterial invasions due to penetration wounds left by hemipteran insects can cause significant yield loss (Mitchell, 2004; Zalom et al., 1997). The objective of this study was to evaluate and characterize *H. halys* damage and feeding intensity on cherry fruits.

2.2 Materials and Methods

2.2.1 Experimental establishment. The experiment took place during the 2017 cherry growing season in a cherry orchard (variety ISF 123) in Grezzana, Italy (45.5429, 11.0248). The experiment began by creating 1-meter long mesh cages from a 150-micron mesh material that is impenetrable to *H. halys*. On 31 May, adult *H. halys* specimens were collected using a beat sheet on various plants near pheromone traps that were located on the University of Padova campus in Legnaro, Italy (45.3451, 11.9576). On 1 June, four cherry (*Prunus avium*) trees were selected from the orchard in Grezzana and the mesh cages were placed on the trees with approximately 10 cherries per cage. In some cases, thinning of the fruit was necessary in order to achieve 10 fruits per cage. Prior to caging, the cherries were examined in order to ensure that they were undamaged before installing the cages. The cages were tightly sealed on both ends using wire ties. There were two different infestation duration time periods (7-day and 14-day) and five repetitions with four different *H. halys* density groups per repetition for each infestation time along with control group for the entire experiment. There was a total of 45 cages for this experiment. Once the cages were installed, each cage was assigned a different density of *H. halys* to be put inside for either a 7-day or 14-day period of infestation. The different densities of *H. halys* assigned to the cages were 0.1, 0.2, 0.5, or 1 adult female *H. halys* per cherry. Only *H. halys* females, 90 in total, were used in the experiment. The control group cages remained sealed without *H. halys* specimens for the entire 14-day duration. During this 14-day duration, the temperature at the experimental site ranged from 12.4ºC to 32.7 °C (mean: 22.91°C; SE: 0.52 °C: source ARPAV – Regione Veneto).

2.2.2 Preharvest evaluation. After 7 days, the stink bugs and cherries in the cages labeled for the 7-day time period were examined and the stink bugs were removed from within and the cages were re-sealed until harvest. Response variables included the number of stink bugs that died, number of egg/nymphal clusters present, and number of dislodged fruits. On the same day, the stink bugs and cherries in the 14-day cages were examined for any dead *H. halys* specimens and the deceased individuals were replaced with live ones. On 15 June, 14 days after the experiment was initiated, the same response variables were measured in the cages labelled for a 14-day infestation interval. All cherries from within the cages were hand harvested and placed into separate labeled containers and stored inside of a cooler with ice packs. The harvested cherries were then transported to the laboratory and held at 3-4ºC to be examined for damage the next day.

2.2.3 Postharvest evaluation. On 16 June, the cages were removed and the cherries were harvested, distinguishing between fruits attached to the plant and fruits that were prematurely dislodged. At harvest, some fruits (both attached and dislodged) showed symptoms of brown rot, *Monilia laxa* (Aderh. & Ruhland) infection. Fruits were then categorized with or without fungal symptoms. Fruits without fungal symptoms and attached to the plant at harvest were considered potentially marketable fruits. Fruit were moved to the laboratory and the potentially marketable cherries were weighed on a calibrated scale. Then, each individual cherry was placed under a dissecting microscope and examined, counting the number of stylet sheaths that were present on the exterior of each fruit and stem. Only on cherries without fungal symptoms was it possible to count the stylet sheaths. On fruits expressing signs of fungal infestation, the surface was completely covered by fungal mycelium rending it impossible to quantify the stylet sheaths. The exteriors of the fruits were also examined in order to quantify the percentage of the fruit that had visible catfacing or any other visible deformation. Lastly, the fruits that were not overtaken by fungal mycelium were tasted by two different subjects in order to determine the presence of off flavors caused by stink bug feeding.

2.2.4 Data analysis. Response variables were evaluated as a function of stink bug density and duration of infestation and using a Generalized Linear Mixed Model Analyses (PROC GLIMMIX, SAS 9.4, SAS Institute, Cary, NC). Categorical data (i.e., cherries with fungal symptoms; cherries prematurely dislodged; deformed cherries; potentially marketable cherries) were analyzed assuming a binomial distribution and logit link function, while the quantitative responses (i.e., number of stylet sheaths; cherry weight; yield) were modeled using a normal distribution with an identity link function. The experimental unit for this experiment was a caged cluster of cherries with a known number of stink bugs for a known amount of time. Experimental design was a randomized complete block with period of infestation time (i.e., 7 or 14 days) as a fixed effect and density as a numerically related effect that was analyzed using trend analyses; observations were blocked by tree, which was modelled as a random effect. Wald χ^2 and F tests were applied to test fixed effects (density, duration of infestation, and their interaction) in the analysis of categorical and continuous data respectively. Degrees of freedom were estimated using the Kenward and Roger method. Coefficients for the trend analyses by duration of infestation were generated using PROC IML (SAS Institute). For purposes of determining statistically significant differences, interactions were evaluated with an α set at 0.10 while main effects were evaluated at $\alpha = 0.05$. Pearson residual plots showed that model fit of cherry weight and yield was improved by applying square root transformation. Untransformed variables were used in data presentation.

2.3 Results

All *H. halys* individuals in this experiment survived and eggs were laid within the cages (data not shown). The number of eggs produced per female was similar among treatments (mean: 0.2±0.04 egg masses per female; df=7, 26.2; F = 1.01; P = 0.448). At the end of the experiment, a number of cherries showed signs of infection by brown rot. The proportion of fruits with fungal symptoms increased with *H. halys* density and duration of infestation (Table 2.1; Figure 2.2). No significant duration of infestation by density interaction emerged: the response to *H. halys* density in terms of proportion of fruits with fungal symptoms showed a quadratic trend for both infestation time periods (Table 2.1; Figure 2.2). A number of fruits prematurely dislodged in *H. halys* infested treatments (Figure 2.3). The number of stylet sheaths per cherry was proportional to infestation density. No significant main effects were detected considering the percentage of dislodged cherries while the duration of infestation by density interaction was significant (Table 2.1; Figure 2.3), suggesting that there were differences in the response to density by infestation time. The percent cherries dislodged as a function of infestation density exhibited an interaction between stink bug density and duration of infestation. Further scrutiny indicated that trend analysis of the 7 days of infestation data showed no significant trend, while a cubic trend was evident at 14 days of infestation (Figure 2.3), where a strong increase in percentage of dropped cherries were observed at highest infestation levels. It should be noted that all dislodged fruits showed signs of brown rot.

At harvest, the number of potentially marketable cherries (i.e., those that remained attached to the plants and without fungal symptoms), was influenced by *H. halys* infestation density (Figure 2.4). The percentage of this type of cherry decreased in response to increasing *H. halys* density and duration of infestation; the interaction of infestation density by duration was not significant (Table 2.1; Figure 2.4). Independent of duration of infestation, quadratic trends were observed (Figure 2.4). On infested fruits, we detected the presence of stylet sheaths (Figure 2.5) that was positively associated with *H. halys* infestation density, while no effect of duration and density by duration interaction was found (Table 2.1). The response to infestation density in terms of number of stylet sheaths on fruits showed a linear trend (Figure 2.5). A number of cherries also showed deformities and the proportion of deformed cherries were correlated to *H. halys* density (Table 2.1; Figure 2.6). The interaction of infestation density by duration interaction was significant (Table 2.1; Figure 2.6) because of different trends in response to density by duration of infestation. At time = 7, the response followed a linear trend, while no clear response emerged at time = 14 (Figure 2.6).

H. halys infestation resulted in a decrease of cherry weight as response to *H. halys* infestation density and duration, however their interaction was not significant (Table 2.1; Figure 2.7). Independently of duration of infestation, the trend was linear (Figure 2.7). As a result of *H. halys* infestation, the overall yield decreased with a significant response to *H. halys* density and duration of infestation and interaction of infestation density by duration (Table 2.1; Figure 2.8). Yield was lost linearly in both 7-day and 14-day infestation trials. Finally, the taste of each potentially marketable cherry was assessed and stink bug taint was completely undetectable to the taste subjects (df = 4,32; $\chi 2 = 0.00$; P = 1.000). The conclusion is that there were no distinguishable differences in the taste of the fruits with different exposure times or *H. halys* densities.

2.4 Discussion

The intention of this study was to characterize the impact of *H. halys* on cherry production. The results suggest that *H. halys* has the potential to become a severe pest in cherry orchards. Cherry is reported to be a host plant of *H. halys* in native and invaded areas (Lee et al., 2013; Banken et al., 2015). In temperate climatic areas, cherry may be one of the first fruits available with a high potential risk of infestation, especially by overwintered insects. Infestations by *H. halys* were observed in commercial cherry orchards early in the season and these insects were observed feeding on cherries both in the field and in the lab. The timing of cherry infestation was close to harvest, beginning at the onset of fruit ripening (Moore et al., unpublished). Here we proved that a *H. halys* attack to cherry can induce serious damage to this crop and we also characterized the symptoms that were induced by this pest. *H. halys* feed on fruit by inserting its mouthparts and extracting the liquids within the fruit. Inserting its stylets into the fruit, *H. halys* creates puncture wounds and secretes watery saliva into these wounds in order to break down the plant tissue. The hardened saliva leaves visible sheaths, which were used to quantify feeding intensity (Peiffer & Felton, 2014). This watery saliva can cause necrosis of the fruit tissue. This necrosis may lead to dehydration of the fruit, resulting in further reduction in fruit weight and fruit quality. This watery saliva may also distort the growth of the fruit, resulting in fruit deformation and catfacing. Puncture wounds may allow entry of different fungi or bacteria because the fruit's protective epidermal layer has been penetrated. *H. halys* is known to transmit a phytoplasma disease to *Paulownia* spp. trees in its native range (Hiruki, 1999). Though the transmission of diseases by *H. halys* is possible, it is likely that fungal spores or bacterial cells are present on the stylets of the insects and may be transmitted upon feeding like many other hemipterans (Mitchell, 2004). There have been several other connections identified between fungal invasion and the presence of pentatomids on various crops (Fennell et al., 1975; Hollay et al., 1987; Kennedy et al., 1983; Lee et al., 1993; Medrano et al., 2009; Mitchell, 2004). Presence of the pathogens in the environment and on the insect, itself may play a role in the spread of fungal infestation.

All of these aspects seem to be involved in the damage induced by *H. halys* on cherry. Yield losses were associated with increasing *H. halys* infestation density and duration of infestation. The decrease in yield associated to *H. halys* density was higher in the 14-day infestation compared to the 7-day infestation. According to our results, a 50% reduction in yield can be expected with an infestation of 0.5 *H. halys* per fruit for 7 days, while the same level of reduction can be induced by a lower density (0.1-0.2 *H. halys* per fruit) in 14 days. Most of the losses are associated with the increase of *M. laxa* on infected fruits. *H. halys* infestations were associated with higher number of fruits bearing symptoms of brown rot. The number of fruits with fungal damage increased with increasing infestation densities and duration of infestation. It is noteworthy that the experiment was performed in a commercial orchard where common plant disease management is applied. Following a quadratic trend, the proportion of cherries bearing fungal symptoms increased at low infestation density and a tendency to plateau with a density higher than 0.5 *H. halys* per fruit was observed. Around 80% of the fruits showed fungal symptoms with 1 *H. halys* per fruit for 14 days. Most of these fruits dislodged prematurely. The association between the increase of fungal or bacterial fruit infection and *H. halys* is not a new concept (Leskey et al. 2012b; Kamminga et al. 2014; Rice et al. 2014; Dobson et al. 2016). *H. halys* has the capability to transmit the yeast *Eremothecium coryli* to different fruit crops (Brust & Rane, 2011). A more recent study using a proteomic approach found no bacterial or yeast proteins in the watery saliva or salivary sheaths of the *H. halys* (Peiffer & Felton 2014). Here, we quantified the fungal infection as damage induced by the *H. halys* and we found a correlation with pest density and duration of infestation. This is probably connected with probing that penetrates the protective epidermal layer which can allow fungal infections, but these aspects deserve further investigation. It should be noted that the environmental conditions found during the experiment were within the appropriate range for high injury frequency (Wiman et al., 2014).

Consequentially, the number of potentially marketable fruits were also reduced in response to increasing *H. halys* infestation density and duration of infestation. For 7 days of infestation, a maximum reduction of 40% was reached at the highest density, while this amount of reduction was observed with 0.2 *H. halys* per fruit for 14 days. A single *H. halys* per fruit for 14 days can reduce the number of marketable fruits by 80%. On these fruits, stylet sheaths were observed and their number increased in correlation with increased infestation density. A relationship between fruit deformation and *H. halys* density was observed in the 7-day infestation, where the proportion of deformed fruits was linearly related with infestation density. With 14-day infestation, the proportion of deformed fruits was higher in infested cages compared to the control, but no clear trend was found. This could be correlated with the fact that, with 14 days of infestation, most of the attached fruits were infected by *M. laxa*, therefore these fruits were not considered potentially marketable and were discarded before deformation assessment.

Stink bug taint may be detected upon the tasting of different fruits that have been exposed to *H. halys*. Stink bug taint has been recorded primarily in grapes and wine and has been recorded as negatively affecting wine quality. The stink bug taint found in wine samples comes from the physical crushing of grapes and stink bugs that may be hiding in the clusters at harvest. The taste of stink bug taint is undesirable to the consumer and may lead to the rejection of the fruit sale (Mohekar, 2016; Mohekar et al., 2017). The cherries in this experiment were treated as though they would be sold as fresh produce instead of crushing for juice. No taste alteration was detected on the potentially marketable fruits, but a reduction of the fruit weight was associated with *H. halys*.

Environmental conditions in cherry growing areas contain temperatures suitable for *H. halys* feeding in the days prior to cherry harvest. According to Wiman et al. (2014), the active feeding temperature range of *H. halys* is between 3.5ºC and 29.6ºC, and the preferred temperature is between 16ºC and 17ºC. This temperature range is fully compatible with temperatures occurring in the ripening period of fruits in cherry growing areas. The actual global distribution of *H. halys* includes some of the most important countries for cherry production (i.e., United States, Italy, Spain, Chile, Romania, Russian Federation, Greece). Other important countries for cherry production (i.e., Turkey, Iran) are included in potential distribution areas of *H. halys* (Zhu et al., 2012).

The result obtained here and in other damage characterization studies are important because they provide information on correct identification of insect damage symptomology and on the potential impact of this polyphagous pest on different crops (i.e., Kamminga et al., 2014; Smith et al., 2014; Wiman et al., 2015; Zobel et al., 2016; Lara et al., 2017). These aspects are the key for designing Integrated Pest Management strategies (Pedigo et al., 1986; Radcliffe et al., 2009). We can conclude that *H. halys* can potentially cause high damage to cherry even at low pest densities. The results that we have produced here can be used for the selection of the most appropriate management tactics after an economic evaluation of their applications. We should also consider that, since *H. halys* is primarily a fruit feeding insect, cherry is one of the first fruits available in temperate areas and the only fruit available for *H. halys* emerging from overwintering sites in many areas. For this reason, the risk of *H. halys* invasion is high for cherry production. In Southern Europe and in much of the U.S., *H. halys* is bivoltine (Lara 2016; Nielsen et al., 2016; Costi et al., 2017; Ingels and Daane, 2018). Overwintered *H. halys* lay 2 to 15 egg masses per female with a median number of 28 eggs (Haye et al., 2014; Costi et al., 2017). If left unmanaged, *H. halys* can lay eggs and increase in number rapidly in and around cherry orchards. We can speculate that proper management of overwintered populations may potentially contribute to the control of this pest on other crops that are colonized later in the season (Leskey and Nielsen, 2018), but these aspects merit further investigation.

Table 2.1: Generalized linear mixed model statistics for the experiment examining the influence of *H. halys* density and duration of infestation (fixed) effects on cherry production. An F test was used on continuous variables, while Wald χ^2 test was used for categorical ones. Main effects were considered significant with an α = 0.05, while an α = 0.10 was considered for the interactions. Cherry trees were considered a random effect. Degrees of freedom in all models were calculated using the Satterthwaite approximation.

Parameter	**Effect**	***df***	χ^2	***F***	***P***
% Cherries dislodged	***H. halys* density**	4; 32	4.93		0.1771
	Duration of infestation	1; 32	0.01		0.983
	***H. halys* density*Duration of infestation**	4; 32	11.94		0.008
Stylet sheaths per cherry	***H. halys* density**	4; 36		79.16	< 0.001
	Duration of infestation	1; 36		0.07	0.797
	***H. halys* density*Duration of infestation**	4; 36		0.97	0.434
% Cherries with fungal symptoms	***H. halys* density**	4; 32	89.02		< 0.001
	Duration of infestation	1; 32	9.55		0.002
	***H. halys* density*Duration of infestation**	4; 32	7.83		0.010
% Potentially marketable cherries	***H. halys* density**	4; 32	87.77		< 0.001
	Duration of infestation	1; 32	9.72		0.002
	***H. halys* density*Duration of infestation**	4; 32	7.63		0.106
Average weight per cherry	***H. halys* density**	4; 35.46		9.80	< 0.001
	Duration of infestation	1; 35.43		10.20	0.003
	***H. halys* density*Duration of infestation**	4; 35.46		1.38	0.260
Yield	***H. halys* density**	4; 35.48		25.87	< 0.001
	Duration of infestation	1; 35.46		15.50	0.001

Parameter	**Effect**	***df***	χ^2	***F***	***P***
	***H. halys* density*Duration of infestation**	4; 35.48		2.18	0.0909
% Deformed cherries	***H. halys* density**	4; 32	22.89		< 0.001
	Duration of infestation	1; 32	0.06		0.801
	***H. halys* density*Duration of infestation**	4; 32	2.84		0.023

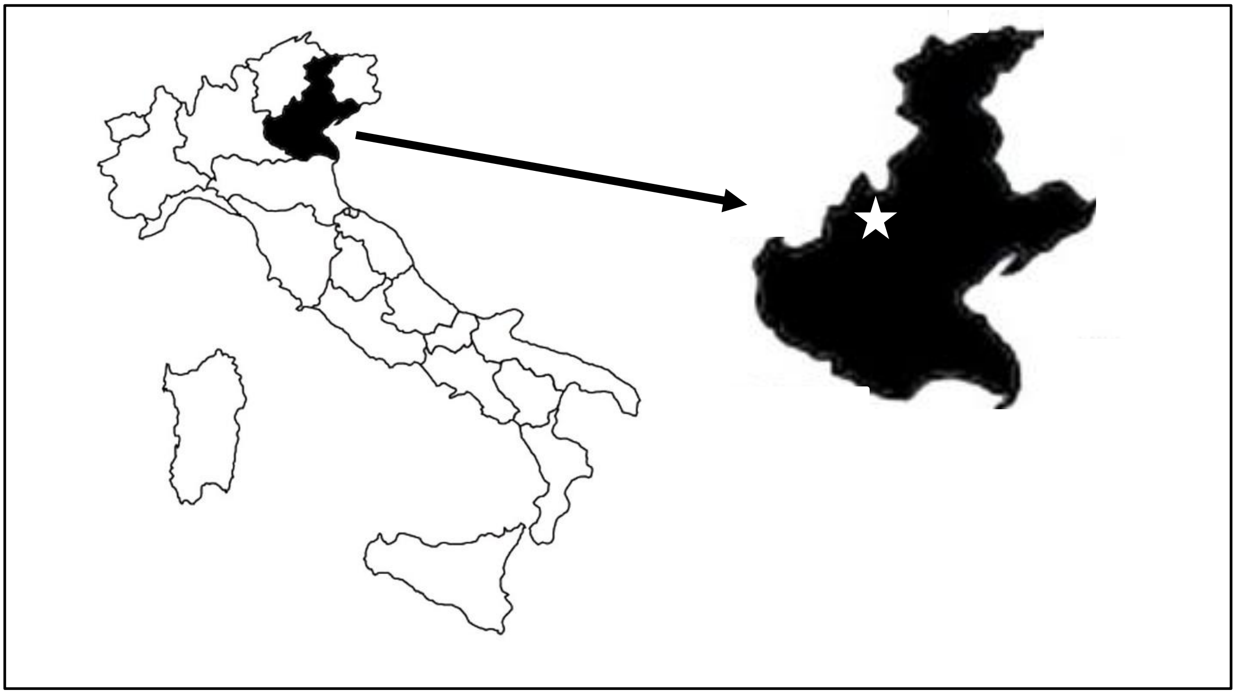

Figure 2.1. Map of Italy with the Veneto region highlighted. The enlarged outline of the Veneto region is depicted on the right, and the city of Grezzana, the location of the cherry orchard, is indicated by a white star.

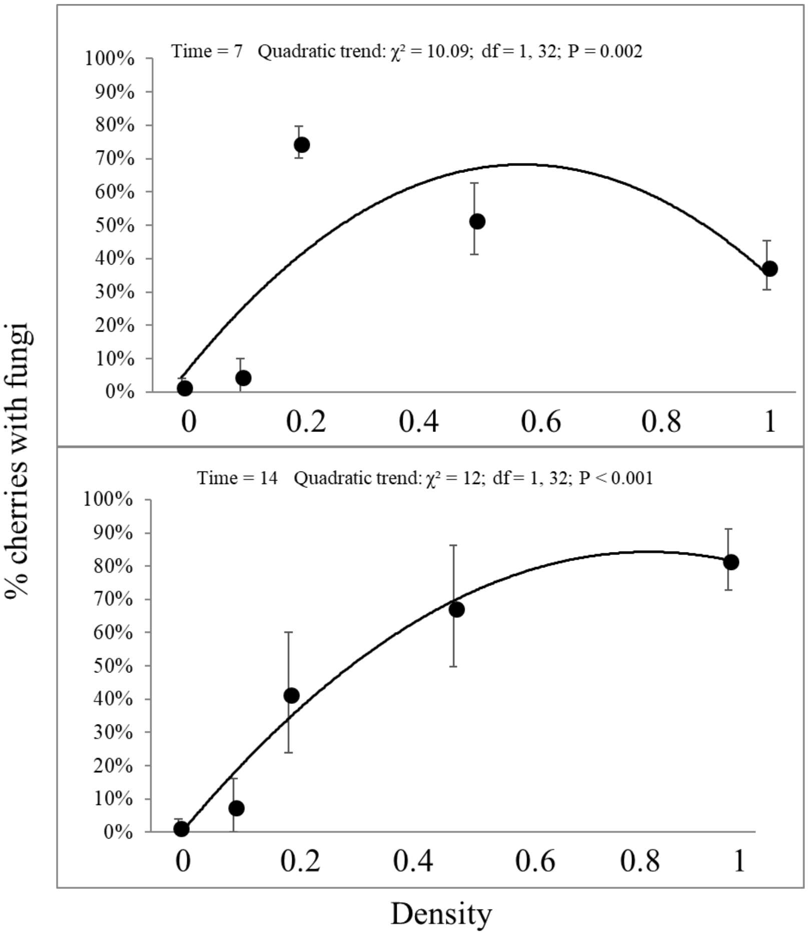

Figure 2.2. Mean ±SE percentage of cherries containing visible fungus that were exposed to different densities of feeding *H. halys* for 7-day (top) or 14-day (bottom) time durations.

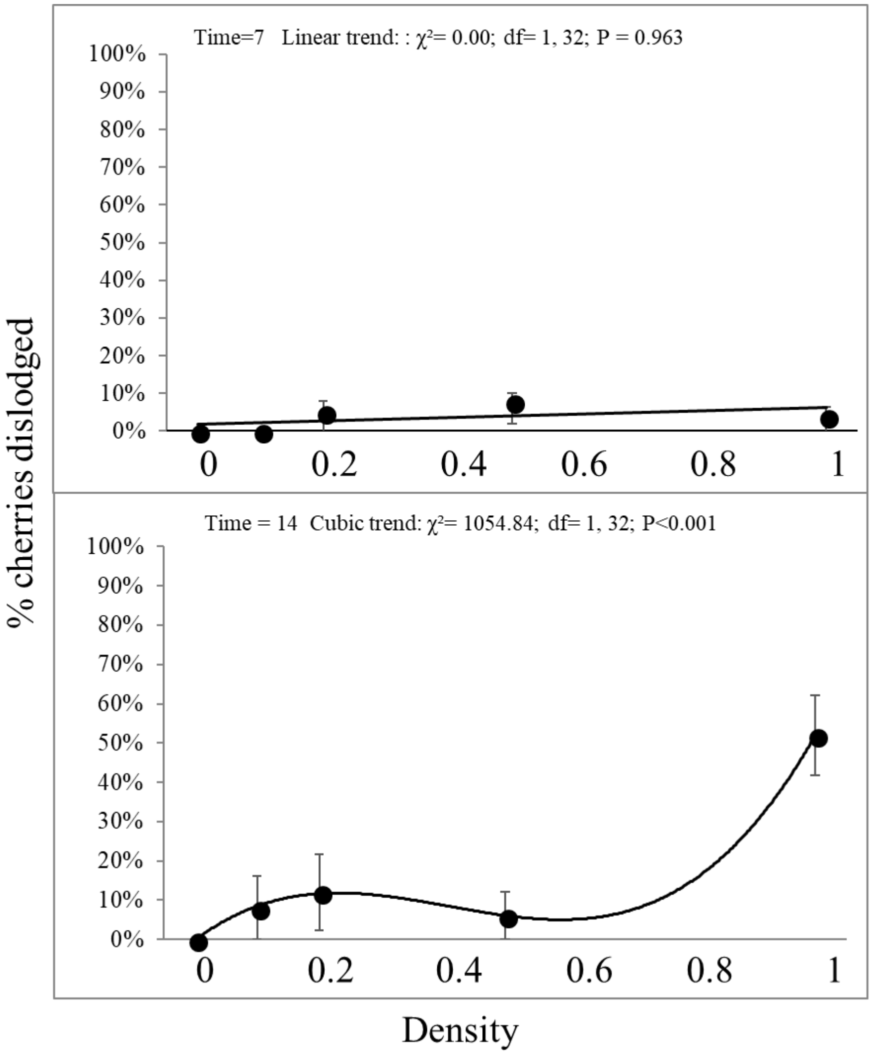

Figure 2.3. Mean ±SE percentage of prematurely dislodged fruits on treatments characterized by different infestation levels of *H. halys* for a 7-day (top) or 14-day (bottom) duration of time.

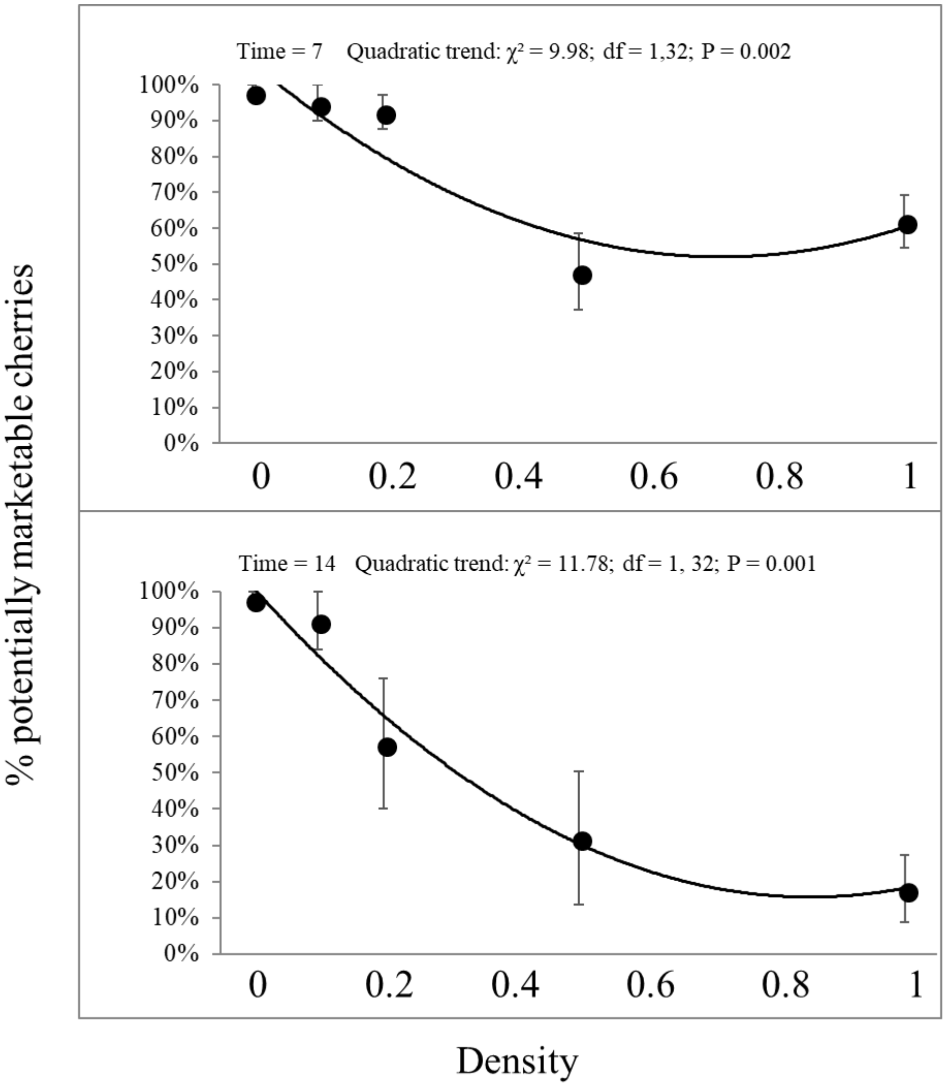

Figure 2.4. Mean ±SE percentage of potentially marketable fruits (without fungi and were not prematurely dislodged) for each treatment group after exposure to feeding *H. halys* for a 7-day (top) or 14-day (bottom) duration.

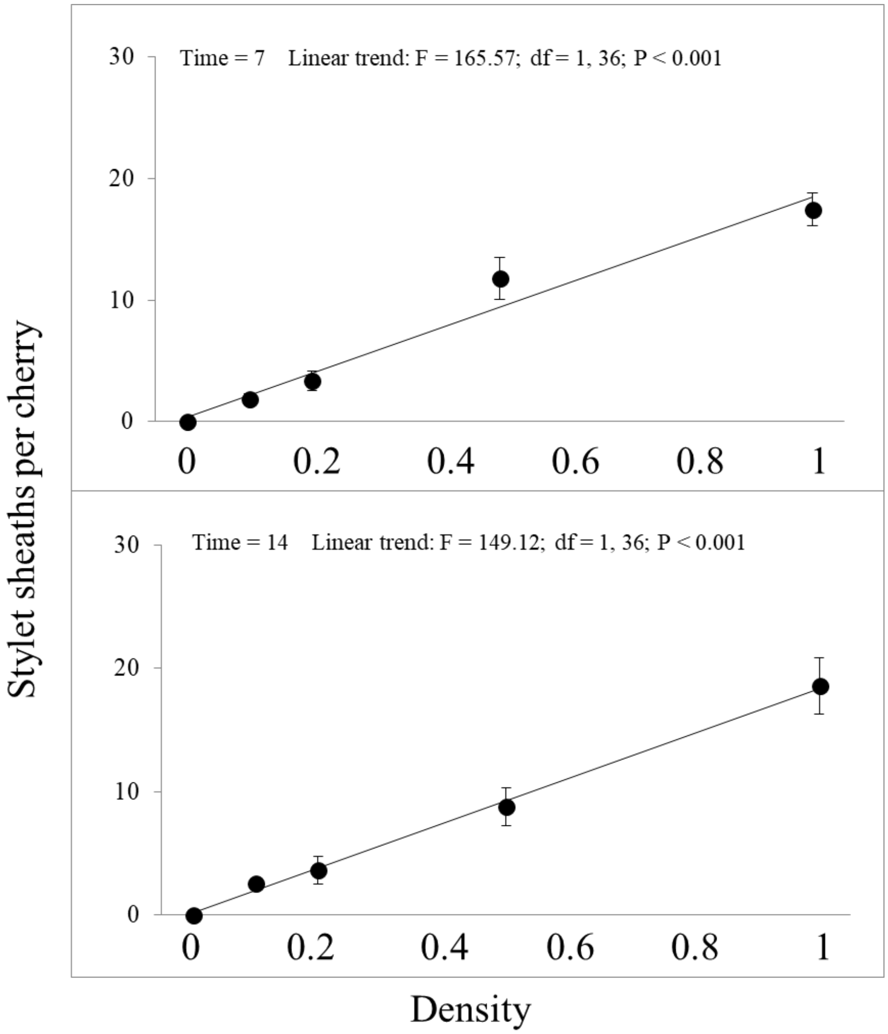

Figure 2.5. Mean ±SE number of stylet sheaths per cherry that were exposed to *H. halys* feeding for 7-day (top) or 14-day (bottom) time periods.

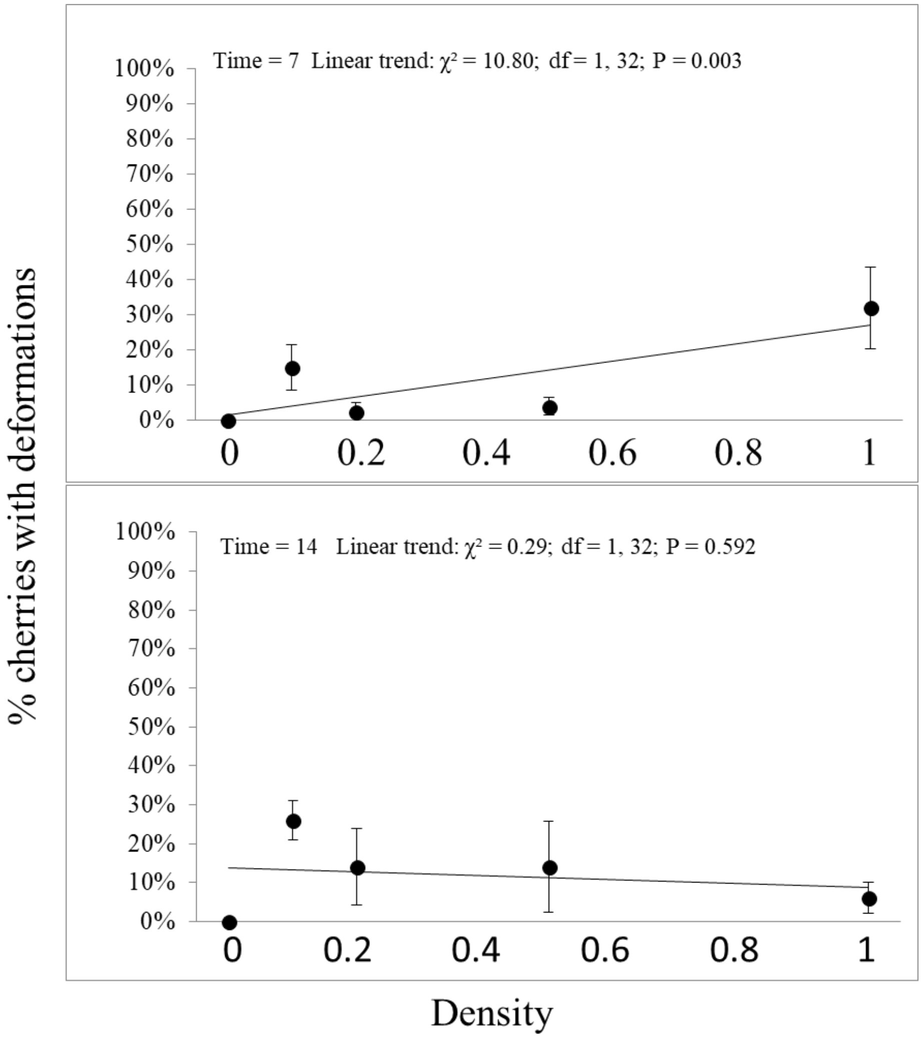

Figure 2.6. Mean ±SE percentage of cherries containing deformation/catfacing that were exposed to differing densities of feeding *H. halys* for 7-day (top) or 14-day (bottom) durations.

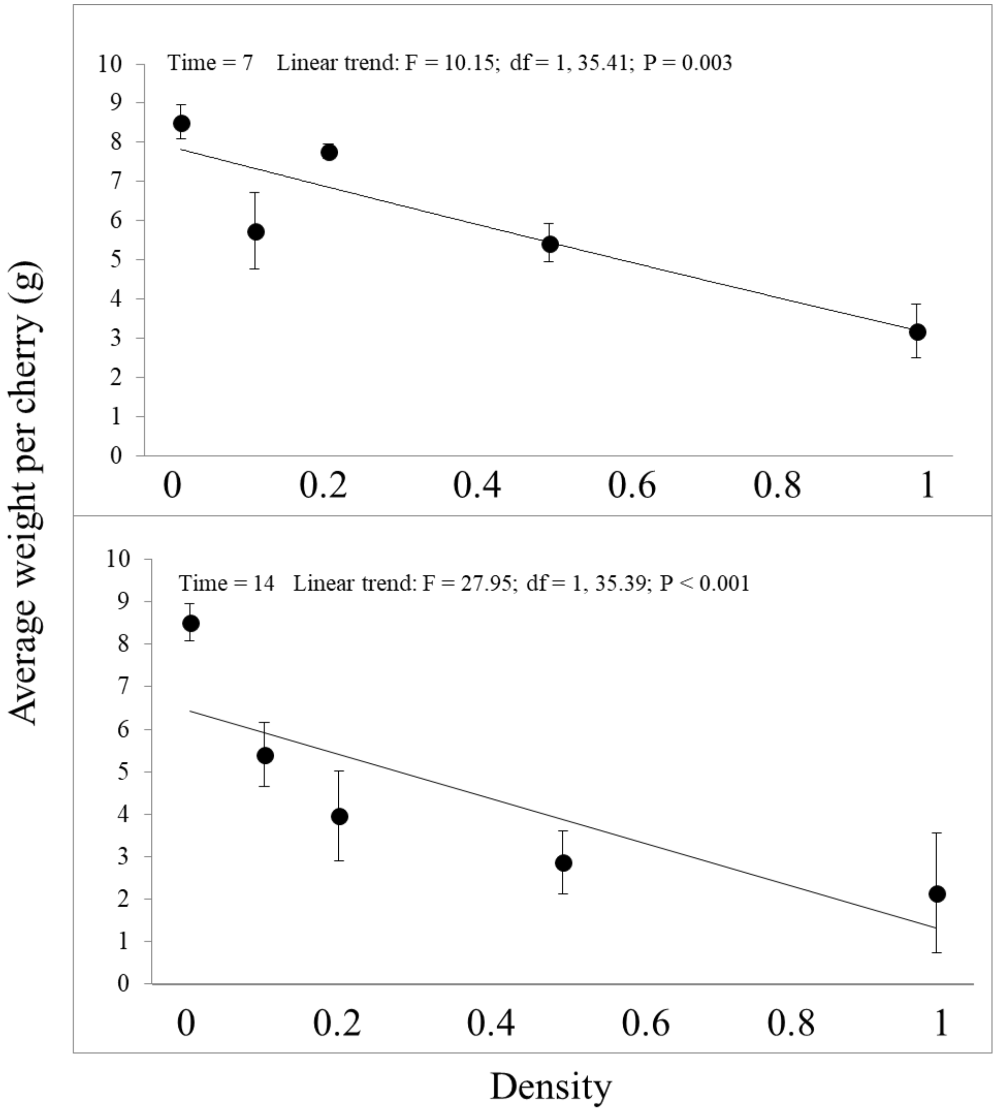

Figure 2.7. Mean ±SE average weight per cherry for each treatment group after exposure to feeding *H. halys* for a 7-day (top) or 14-day (bottom) duration.

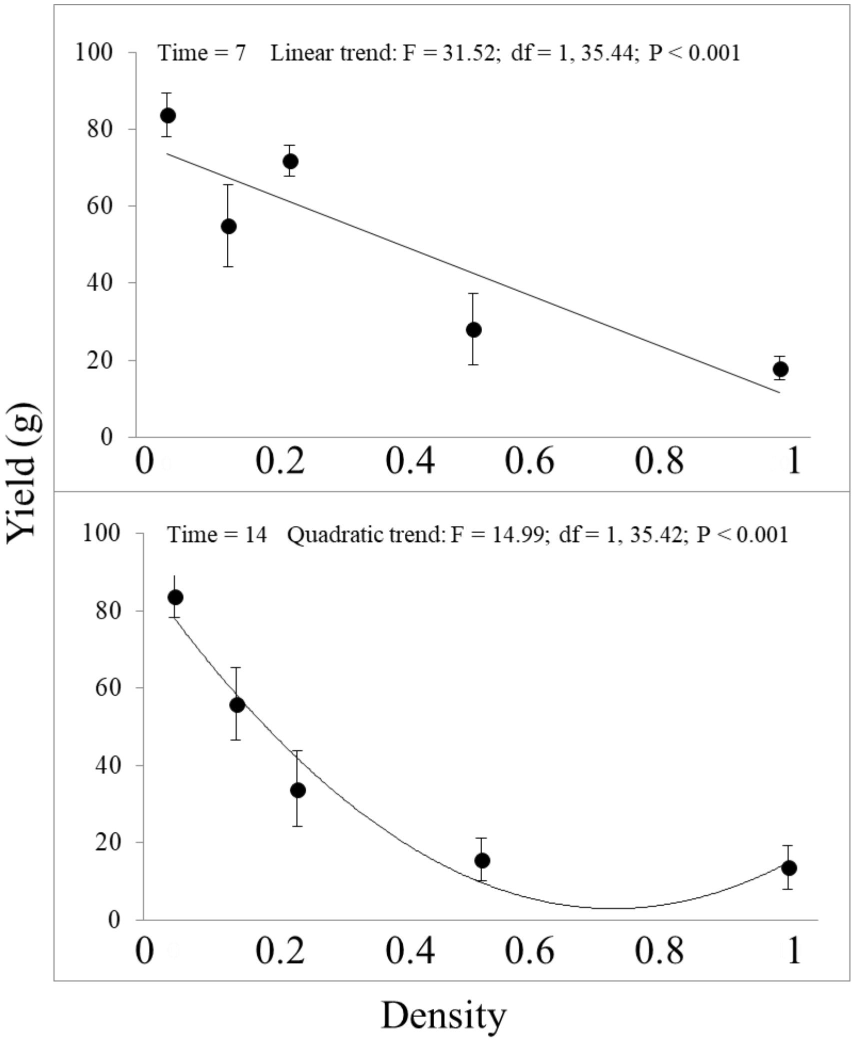

Figure 2.8. Mean ±SE total yield of cherries (without damage) that were exposed to feeding *H. halys* for a duration of 7 days (top) or 14 days (bottom).

3. CHARACTERIZING DAMAGE POTENTIAL OF BROWN MARMORATED STINK BUG, *HALYOMORPHA HALYS*, IN KIWIFRUIT ORCHARDS

3.1 Introduction

The brown marmorated stink bug, *Halyomorpha halys*, is in the order Hemiptera and family Pentatomidae (Hoebeke & Carter, 2003). *H. halys* and other Pentatomid pests are known to cause severe crop damage to various crops in many countries across the world. *H. halys* is a relatively new invasive pest insect to both the United States and Europe (Leskey et al., 2012b). It is considered both an urban nuisance and an agricultural pest that has been recorded feeding on fruits (Haye et al., 2015; Joseph et al., 2014; Leskey et al., 2012a; Leskey et al., 2012b; Nielsen et al., 2009; Walton et al., 2016; Wiman et al., 2015; Acebes et al., 2016), vegetables (Cissel et al., 2015; Haye et al., 2015; Kuhar et al., 2012), row crops (Bakken et al., 2015; Kamminga et al., 2014; Koch and Rich, 2015; Nielsen et al., 2011; Owens et al., 2013; Venugopal et al., 2014), tree nuts (Hedstrom et al., 2014), and ornamentals (Shrewsbury et al., 2011; Wermelinger et al., 2008). In 2010, in the mid-Atlantic region of the United States, *H. halys* caused more than $37 million in damage to apple production alone (Leskey et al., 2012a). Recently, *H. halys* was also found in the Veneto and Friuli regions of Italy (Maistrello et al., 2014; Bariselli et al. 2016), where it causes damage to several crops and is a nuisance in urban areas, as also reported by the local media. Due to human activities and the natural progressive dispersal behavior, Italian populations were found to have been derived from Switzerland, Asia, and/or North America, due to multiple introduction events (Gariepy et al., 2014; Cesari et al., 2015; Morrison et al., 2017).

In Europe, the host plant list is growing following the expansion of the *H. halys* into new areas (Haye et al., 2014; Maistrello et al. 2016).

In its native range, *H. halys* is reported to feed on 106 host plant species belonging to 45 different families (Lee et al., 2013). Kiwifruit *Actinidia spp.,* Liang & Ferguson is reported as a host plant of *H. halys* in Asia (Lee et al., 2013). Although it is a globally important crop, little is known about the impact of this pest on kiwifruit (Ferguson, 2015).

In 2010, worldwide production of kiwifruit was recorded as 1.35 million metric tons. China is the leader in worldwide kiwifruit production, providing an average of 480,000 metric tons of kiwifruit per year from 2009 to 2012. Italy is second in terms of global kiwifruit production, producing an average of 450,000 metric tons per year from 2009 to 2012. New Zealand leads in worldwide export of kiwifruit, distributing around 90% of its production, and Italy is second in exports, distributing approximately 70% of its fruits. In 2007, Italy reported having 26,700 ha of kiwifruit orchards grown within its borders (Testolin & Ferguson, 2009). The U.S. also produces kiwifruit, grown primarily in California (Beutel et al., 1976). Kiwifruit is a perennial fruit crop native to Southeastern China, same native range as *H. halys* (Ferguson, 1984). *H. halys* is present in many kiwifruit-growing areas and is considered as one of the top pests of concern and a biosecurity to New Zealand agriculture (Lara et al., 2018). Examination of past Chinese publications revealed that *H. halys* has been reported as a serious pest in kiwifruit production in China for many years; however, its damage is reduced by the presence of natural enemies that are not yet present in other parts of the world (Yang et al., 2009; Ferguson, 2015; Teulon & Xu, 2017).

Pentatomid insects have piercing and sucking mouthparts that they use to penetrate the external surface of plant tissue in order to access the internal fluids of the plant. The insect's watery saliva further liquefies the plant tissue to facilitate feeding success. This watery saliva causes tissue degradation, resulting in corky tissue and sometimes leading to necrosis (Peiffer & Felton, 2014). In addition to the damage that the saliva causes, the puncture wounds that the insects leave sometimes allow entry of harmful pathogens or lead to fruit dehydration (Mitchell, 2004). The diverse feeding strategies of *H. halys* allow them to obtain nutrition from many different plant structures including the leaves, stems, fruits, pods and seeds (Panizzi et al., 2000). Damage to kiwifruit in a laboratory setting by *H. halys* has been briefly described by Lara et al. (2018). They described the internal damage to kiwifruit as white corking of the pulp (Lara et al., 2018).

The objective of this project was to characterize the damage potential of *H. halys* on kiwifruit in the Veneto region of Italy with different densities of insects and at different stages of fruit development. Concerns about this pest are increasing among the kiwifruit growers in this region. Characterizing the damage of this pest will allow insight into correct identification of the damage so that farmers may recognize the presence of this pest and manage the issue accordingly. Understanding when the fruits are most susceptible to damage is important so that growers may monitor or manage the pest at that particular time instead of throughout the entire season.

3.2 Materials and Methods

3.2.1 Experimental Establishment. All field experiments were conducted between 8 June 2017 and 19 October 2017 on a privately-owned kiwifruit orchard near the town of

Cittadella in the Veneto region of Italy (45.6488,11.7836) (Figure 3.1). In this orchard, 35 kiwifruit plants (Hayward variety) were selected within 5 plots (7 plants per plot) and utilized for this experiment. Two hundred 1-meter long insect proof mesh cages were created and placed onto limbs of these 35 kiwifruit plants containing 5 kiwifruits per cage at the beginning of the experiment on 8 June 2017. All 200 cages were placed on kiwifruit plants before the experiment began in order to prevent insect damage from occurring on the fruits used for this experiment throughout the growing season. Sections of limbs were first examined closely to ensure that none of the fruits were already damaged and that there were no harmful insects pests present. The cages were sealed on both ends with wire ties so that no insects could enter or exit the cages throughout the experiment. The treatment groups were different densities of mixed age adult *H. halys* that were placed in each individual cage. The cages contained 1 (1 female), 5 (3 females and 2 males), or 10 (5 females and 5 males) experimentally naïve *H. halys* specimens for approximately 10-day time periods. New *H. halys* specimens were field collected near pheromone traps located close to the orchards. The control cages contained no *H. halys* specimens. After 8-12 days had passed, insects were removed from the cages and the cages were re-sealed. The same densities of *H. halys* were then placed into different cages for another 10-day period. This pattern continued until kiwi harvest on 19 October 2017. Each of the resulting treatments of infestation density and time of infestation was replicated five times (one replication per plot). The preharvest variables that this experiment examined were *H. halys* density and fruit stage (time) at exposure.

3.2.2 Preharvest Evaluation. Immediately following each 10-day period, the *H. halys* specimens were removed from the cages. The interior of each cage and the plant parts within the cages were visually examined for egg masses or nymphal clusters, and each insect or egg mass was removed in order to prevent further damage. The number of dead insects within each cage was recorded at the end of each 10-day period.

3.2.3 Postharvest Evaluation. On 19 October 2017 all of the cages were removed and the kiwifruit were hand harvested and placed into their associated cages. The fruits that dislodged prematurely was recorded. The kiwifruits harvested from the plants were then transported to the University of Padova and placed in a refrigerator at 3-4ºC. On 25 October, all kiwifruits were analyzed by evaluating the following parameters. Fruits presenting darker pigmentation of the fruit's skin due to frass deposit were recorded and the severity of this type of damage was assessed. The dark pigmentation was categorized using a 0 to 4 grading scale; 0 (no damage), 1 (1-24% damage), 2 (25-49% damage), 3 (50-74% damage), and 4 (75-100% damage) (Figure 3.2). On fruit without external damage a portion of each fruit's skin was then removed from opposing sides of the fruit, and the firmness of each fruit was tested on both sides on the skinless sections using a handheld penetrometer (FT 30, Wagner Instruments, Greenwich, CT). Next, the sugar content of each fruit was assessed using a handheld optical brix refractometer (RHB-18ATC, Lumen Optical Instrument Co., Ltd, Fuzhou, China). A drop of each fruit's juice was placed on the brix refractometer, and the sugar content was recorded once for each fruit. The fruits were then laterally sliced using a knife and the yellowing/browning of the pulp and presence of corky tissue due to *H. halys* feeding was recorded using a 0-4 scale: 0 (no damage), 1 (1-24% damage), 2 (25-49% damage), 3 (50-74% damage), and 4 (75-100% damage) (Figure 3.2). The kiwifruits from each cage were then placed into an industrial sized blender and were blended for 20 seconds, or until the sample became uniform. A sample of each solution was weighed after blending. The samples were then placed into a drying oven at 102˚C and were dried for at least 24 hours. Once the samples were completely dry, they were weighed again and the difference between the fresh weight and dry weight was taken in order to calculate the percentage of dry matter for each sample.

3.2.4 Data Analysis. Response variables were evaluated as a function of insect density and initiation time of infestation by using a Generalized Linear Mixed Model Analyses (PROC GLIMMIX, SAS 9.4, SAS Institute, Cary, NC). In this experiment, the experimental unit was a caged kiwifruit vine segment with a known number of kiwifruits, a known number of *H. halys* individuals, at a known time of infestation within the kiwifruit growing season. Experimental design was a randomized complete block with time of infestation (i.e., 8 June; 15 June; 22 June; 30 June; 10 July; 19 July; 31 July; 9 August; 21 August; 31 August; 13 September; 21 September; 2 October) as a fixed effect and insect density (i.e., 0; 0.2; 1; 2 insects per fruit) as a numerically related effect that was interpolated by utilizing trend analyses (linear, quadratic and cubic effects). Observations were blocked by plant and plot, which were modelled as random effects. F tests ($\alpha = 0.05$) were used to test the fixed effects (density, time of infestation) and their interaction in the interpolation of response variables. Degrees of freedom (df) were projected using the Kenward and Roger method. Coefficients for the trend analyses were produced through the utilization of PROC IML (SAS 9.4, SAS Institute, Cary, NC). Interactions were evaluated with $\alpha = 0.05$ in order to find significant differences. Orthogonal contrasts using an F test ($\alpha = 0.05$) were designed to perform pairwise comparisons in case of significant effects of time of infestation.

Prior to the analysis, response variables that were expressed as proportions or percentages (i.e., insect mortality; percentage kiwifruit prematurely dislodged; percentage of fruits with internal damage; percentage of fruits with external damage; dry matter content) were arcsine transformed, while square root or log x +1 transformation was applied to the other variables (i.e., number of egg masses; external damage severity; internal damage severity; yield; fruit firmness; sugar content), when the transformation improved model fit as assessed using Pearson residual plots. Untransformed variables were used in data presentation.

3.3 Results

During the experiment, mean temperatures fluctuated at values higher than 20°C from mid-June until the end of August, when temperature decreased. The highest temperatures (37°C) were reached in August, and the lowest temperatures were recorded in October (4°C) (Figure 3.3).

Some *H. halys* specimens did not survive throughout this experiment and significantly higher insect mortality rates were found from June to August compared to other times of infestation (Table 3.1; Figure 3.4). Throughout this experiment, a number of kiwifruit were prematurely dislodged from the plants. We found a significant positive correlation between increased insect density and instances of prematurely dislodged fruits (Table 3.1; Figure 3.5). We also found a significant variation in the proportion of dislodged fruits and the time of infestation (Table 3.1; Figure 3.6). It was found that higher percentages of dislodged fruits occurred in July and August and the lowest were observed in the earlier and later parts of the season (Table 3.1; Figure 3.6). Infestation between 30 June and 21 August resulted in a linear response to *H. halys* density in terms of dislodged fruits (Table 3.3; Figure 3.7).

The external fruit injury that was found on the fruits that were exposed to *H. halys* was found primarily around the stem end of the fruit. Feeding damage resulted in black or brown tissue found on the epicarp of the fruit or the blackening of large epidermal sections of the epicarp. Upon removal of the blackened epicarp, it was found that necrosis or necrotic spots were the underlying cause of the discoloration. Occasionally the presence of frass staining was found around the injured tissue and may also be considered evidence of *H. halys* feeding. Fruits bearing this type of damage are considered unmarketable by the growers. We found that the number of fruits with external damage was related to insect density and varied according to time of infestation (Table 3.1; Figure 3.8, 3.9). The relationship between the overall proportion of fruits with external damage and infestation density followed a cubic trend (Figure 3.8). The highest percentage (i.e., > 60%) of fruits with visible external feeding injury resulted between 30 June and 21 August, and the lowest values were observed from the end of August onwards (Figure 3.9). A significant effect of the interaction between infestation density and time of infestation was observed on the proportion of fruit with external damage (Table 3.1; Figure 3.10). This was determined by the different trends observed in the proportion of fruits with external damage in relation to infestation density (Figure 3.10). The severity of external damage increased with increasing *H. halys* densities following a cubic trend (Table 3.1; Figure 3.11). There was also a significant effect of time of infestation on external fruit damage severity: fruits infested between June and the beginning of August showed high external damage severity, while those infested at the end of August and September resulted with the lowest levels (Table 3.1; Figure 3.12).

The early stages of the internal injury of kiwifruits caused by the feeding of *H. halys* can be described as corky spots in the mesocarp caused by the penetration of the epidermal layer of the fruits. These small areas of corky tissue can be described as small darkened spots just beneath the epicarp of the fruit. These small corky spots lead to necrosis of the fruit tissue and yellowing/browning of the pulp. We found a significant relationship between the number of fruits containing internal injury and insect density that followed a cubic trend (Table 3.1; Figure

3.13). A significant effect of time of infestation was observed on fruit with internal damage (Table 3.1; Figure 3.14). The highest proportions (i.e., > 85%) of fruit with internal damage were induced by infestation occurring from 15 June to 21 August, while the lowest were observed on fruit infested in September (Figure 3.14). It was also found that there was a positive cubic relationship between insect density and increased internal damage severity (Table 3.1; Figure 3.15). There was a significant effect on internal fruit injury severity and time of infestation (Table 3.1; Figure 3.16) with the highest levels observed on fruit infested in June and July and the lowest on fruit infested in September (Figure 3.16).

The overall yield was calculated as the weight of the fruits that were still attached to the plants at harvest and did not exhibit external damage within each cage. It was found that, with increasing infestation density, the overall yield decreased significantly following a linear trend (Table 3.1; Figure 3.17). It was also found that the time of infestation had a significant effect on the yield (Table 3.1; Figure 3.18). The lowest yields were induced by infestations occurring in July-August (Figure 3.18).

No effect of *H. halys* infestation was observed on fruit firmness or sugar content (Table 3.2). In regards to dry matter content, we did not find any significant correlations between dry matter content and insect density or from a density by time of infestation interaction (Table 3.2). However, we did find a significant effect of time of infestation (Table 3.2; Figure 3.19). Fruit infested in June-July tend to have a low dry matter content (Figure 3.19).

3.4 Discussion

The purpose of this study was to characterize the effect of *H. halys* on kiwifruit production. In Italy, infestation of kiwifruit by *H. halys* has been observed throughout the season, but little is known on the effect of this pest on this crop (Pozzebon et al., unpublished). By using a cage experiment, we simulated *H. halys* infestation. The temperature during this study fluctuated between 4.6˚C and 36.8 ˚C, with the lowest temperatures occurring in October and the highest temperatures occurring in August (Figure 3.3). This is likely to have influenced the survival of the pest, since the high mortality rates observed in this period follow the high temperatures that were registered. Exposure to a high temperature regime for certain time periods can reduce the survival of this pest (Scaccini et al., unpublished). Feeding of *H. halys* on kiwifruit resulted in prematurely dislodged fruits, severe internal and external fruit injury, and decreased dry matter content of the fruit. Feeding *H. halys* have been observed feeding on kiwifruit both in the field and in the lab (Pozzebon et al., unpublished). Damage that results from *H. halys* feeding relates to the insect's mouthparts and how the insect inserts them into the fruit, piercing the epicarp and retrieving liquids from the mesocarp. This piercing and sucking action causes the mesocarp to lose moisture and the watery saliva that the insect excretes causes necrosis within the mesocarp tissue, resulting in corky tissue spots, similar to the damage *H. halys* causes on other fruits (Acebes-Doria et al., 2016; Basnet et al., 2014; Leskey et al., 2012a; Nielsen & Hamilton, 2009; Smith et al., 2014; Wiman et al., 2015). Low levels of internal damage may be accepted by the market, while high severity of this type of damage can be considered as unacceptable. This necrosis becomes visible from the exterior, causing the epicarp around the damaged areas to darken at high damage severity. The premature dislodging of the fruits is likely caused by the kiwifruit plant's reaction to high feeding intensity, resulting in the abortion of highly damaged fruits. It is likely that the decreased dry matter content was a direct effect of the puncture wounds caused from *H. halys* feeding. The corking of the pulp tissue most likely results in stunted growth and the development of smaller fruits. It should be noted that the majority of the observed feeding and damage occurred on the stem end of the fruits, and this may degrade vascular tissue by reducing phloem transport within the fruits. In addition to direct fruit damage, the insect may also produce secondary effects, such as the excrement of frass that stains the exterior of the fruit. Internal damage cannot be detected from the exterior of the fruits; however, the presence of secondary external damage may be used as criteria for fruit selection to discard the damaged fruits. It was evident that internal damage of the kiwifruits was much more prominent and severe than external damage in this experiment. On many occasions, the kiwifruits that did not exhibit external damage were found to contain severe internal damage upon further inspection. This could cause issues at the market level when customers purchase kiwifruit that look healthy, but are, in fact, heavily damaged on the interior. This implies that new ways of detecting internal damage from the exterior of the fruit need to be developed.

On the different parameters considered in this study, we also found a significant effect of time of infestation. Particularly high impact of *H. halys,* was observed on fruits infested in mid-summer. The time of infestation that resulted in the highest yield loss was the central part of the season (July to August), while lower impact was observed for late season infestation. This is most likely due to the fruits becoming softer, the sugar content increasing, and the time period's temperature being within the preferred feeding range. According to Wiman et al. (2014), the active feeding temperature range of *H. halys* is between 3.5ºC and 29.6ºC, and the favored temperature with the highest feeding intensity and labial dabbing activity is between 16ºC and 17ºC, which is more likely to occur in early and mid-season. As argued by Wiman et al. (2014) it must be noted that temperatures experienced by *H. halys* feeding within a canopy can potentially be much cooler than air temperatures due to interaction of factors such as evapotranspiration, radiation absorption, and other processes. This is completely compatible with our observation and the temperature regimes that occurred in mid-season during our experiment.

The average storage life of kiwifruits in controlled conditions is approximately 6 months. Fruits exposed to feeding *H. halys* may negatively affect the storage life of fruits. Quantifying the number of stylet sheaths on the exterior of fruits has proven to be an effective method for measuring feeding intensity, however the presence of pubescence on the exterior of kiwifruits in this experiment made the stylet sheaths difficult to locate or quantify. Perhaps future studies using kiwifruit varieties that contain less superficial pubescence, such as the golden pulp varieties, will give better insight on *H. halys* feeding intensity for this crop in the form of stylet sheath quantification. Knowledge on the appearance of *H. halys* damage to kiwifruit and time *H. halys* inflicts the most damage during the season will aide in knowing which management tools to select and when their application would be economically justified. Understanding how different densities of *H. halys* effect kiwifruits will help aide in creating economic thresholds for this pest in kiwifruit orchards and will help in understanding the potential for *H. halys* associated economic losses. According to our study, the premature dislodging of fruit seems to be induced if infestation density reaches greater than 1 insect per fruit occurring in July. Both internal and external damage can be induced by relatively low infestation levels of *H. halys*. As a result, the yield is also reduced starting from low infestation levels. This information may be important for economic threshold development.

Kiwifruit is a long season crop, with fruits that grow for a period of 8 to 9 months, until maturity. Hayward is the dominant kiwifruit variety, accounting for a high percentage of global kiwifruit production. In Italy, onset of Hayward bud break is typically between 10 March and 30 March. Flowering of Hayward (50% of flowers opened) typically begins from 8 May to 30 May.

Depending on latitude, 160 to 180 days after flowering, the fruits reach maturity and are ready for harvest. In the Veneto region, harvest of the fruits typically begins from the end of October to mid-November. The fruits are harvested when the sugar content (brix) reaches at least 6.2% (Testolin & Ferguson, 2009). Unlike other stink bug species, *H. halys* was found to be a season long pest of peaches, nectarines, apples, and pears (Nielsen & Hamilton, 2009; Leskey et al., 2012a; Leskey et al., 2012b; Rice et al., 2014). Some farmers have experienced nearly 100% crop losses since its introduction in the United States (Leskey et al., 2012b). From our study, we conclude that *H. halys* also has the potential to be more damaging in the central part of the season (July –August). However, in a previous study, we found that there is a definite correlation between *H. halys* infestation and brown rot in cherry production (Moore et al., unpublished). Although we did not find any brown rot in this study, it is possible for the correlation between *H. halys* and brown rot to appear in kiwifruit production in the future. There is an existing correlation between *H. halys* and the increase of bacterial or fungal fruit infection (Leskey et al., 2012b; Kamminga et al., 2014; Rice et al., 2014; Dobson et al., 2016). This aspect should be further evaluated to clearly understand the overall impact of this pest on kiwifruit.

Table 3.1. Generalized linear mixed model statistics for this experiment examining the influence of *H. halys* density and duration of infestation (fixed) effects on kiwifruit production. Individual kiwifruit plants were considered a random effect for this experiment. Degrees of freedom for every model were derived by using the Satterthwaite approximation.

Parameter	**Effect**	***Df***	***F***	***P***
Insect mortality	***H. halys* density**	3, 155.2	131.25	<0.0001
	Time of infestation	12, 158.7	10.08	<0.0001
	Density * Time	36, 163.4	4.39	<0.0001
Dislodged fruits	***H. halys* density**	3, 221	15.32	<0.0001
	Time of infestation	12, 221	4.88	<0.0001
	Density * Time	36, 221	2.05	0.0009
% Fruits with external damage	***H. halys* density**	3, 221	89.5	<0.0001
	Time of infestation	12, 221	3.6	<0.0001
	Density * Time	36, 221	1.52	0.0369
External damage severity	***H. halys* density**	3, 156.9	16.51	<0.0001
	Time of infestation	12, 157	3.21	0.0004
	Density * Time	36, 157	1.91	0.0036
% Fruits with internal damage	***H. halys* density**	3, 221	438.95	<0.0001
	Time of infestation	12, 221	3.01	0.0006
	Density * Time	36, 221	1.08	0.3519
Internal damage severity	***H. halys* density**	3, 156.9	68.74	<0.0001
	Time of infestation	12, 157.2	5.69	<0.0001
	Density * Time	36, 157.4	2.46	<0.0001
% Fruits with damage (internal and external)	***H. halys* density**	3, 221	475.15	<0.0001
	Time of infestation	12, 221	3.13	0.0004
	Density * Time	36, 221	1.06	0.3787
Yield	***H. halys* density**	3, 154.2	17.05	<0.0001
	Time of infestation	12, 155.5	2.88	0.0013
	Density * Time	36, 156.4	1.59	0.0288

Table 3.2. Generalized linear mixed model statistics for this experiment examining the influence of *H. halys* density and duration of infestation (fixed) effects on kiwifruit production. Individual kiwifruit plants were considered a random effect for this experiment. Degrees of freedom for every model were derived by using the Satterthwaite approximation.

Parameter	**Effect**	***df***	***F***	***P***
Fruit firmness	***H. halys* density**	3, 156.6	0.33	0.8067
	Time of infestation	12, 157.4	1.33	0.2053
	Density * Time	36, 158	1.00	0.4829
Sugar content	***H. halys* density**	3, 155.6	1.48	0.2231
	Time of infestation	12, 158.5	1.52	0.1225
	Density * Time	36, 160.9	0.80	0.7770
% Dry matter	***H. halys* density**	3, 153.8	1.94	0.12514
	Time of infestation	12, 154.2	2.52	0.0047
	Density * Time	36, 154.7	1.43	0.0721

Table 3.3. Generalized linear mixed model statistics for the proportion of prematurely dislodged cherries by time of infestation (fixed) effects on kiwifruit production. Individual kiwifruit plants were considered a random effect for this experiment. Degrees of freedom for every model were derived by using the Satterthwaite approximation.

Time	**Trend**	***df***	***F***	***P***
June 8		1; 221	0.00	1.000
June 15		1; 221	0.00	1.000
June 22		1; 221	0.00	1.000
June 30	**Linear**	1; 221	4.49	0.035
July 10		1; 221	2.91	0.090
July 19	**Linear**	1; 221	9.33	0.003
July 31	**Linear**	1; 221	24.46	<.0001
Aug 9	**Linear**	1; 221	60.69	<.0001
Aug 21		1; 221	2.63	0.107
Aug 31		1; 221	2.63	0.107
Sep 13		1; 221	0.00	1.000
Sep 21		1; 221	0.00	1.000
Oct 2		1; 221	2.06	0.153

Table 3.4. Generalized linear mixed model statistics for the proportion of fruits with external damage by time of infestation (fixed) effects on kiwifruit production. Individual kiwifruit plants were considered a random effect for this experiment. Degrees of freedom for every model were derived by using the Satterthwaite approximation.

Time	**Trend**	***df***	***F***	***P***
June 8	**Cubic**	1; 221	5.37	0.021
June 15	**Cubic**	1; 221	6.39	0.012
June 22	**Quadratic**	1; 221	8.90	0.003
June 30	**Quadratic**	1; 221	7.36	0.007
July 10	**Cubic**	1; 221	5.72	0.018
July 19	**Cubic**	1; 221	6.31	0.013
July 31	**Cubic**	1; 221	9.00	0.003
Aug 9	**Linear**	1; 221	34.24	< 0.001
Aug 21	**Quadratic**	1; 221	4.38	0.037
Aug 31	**Linear**	1; 221	15.63	0.000
Sep 13	**Quadratic**	1; 221	5.39	0.021
Sep 21	**Linear**	1; 221	21.01	< 0.001
Oct 2	**Linear**	1; 221	22.22	< 0.001

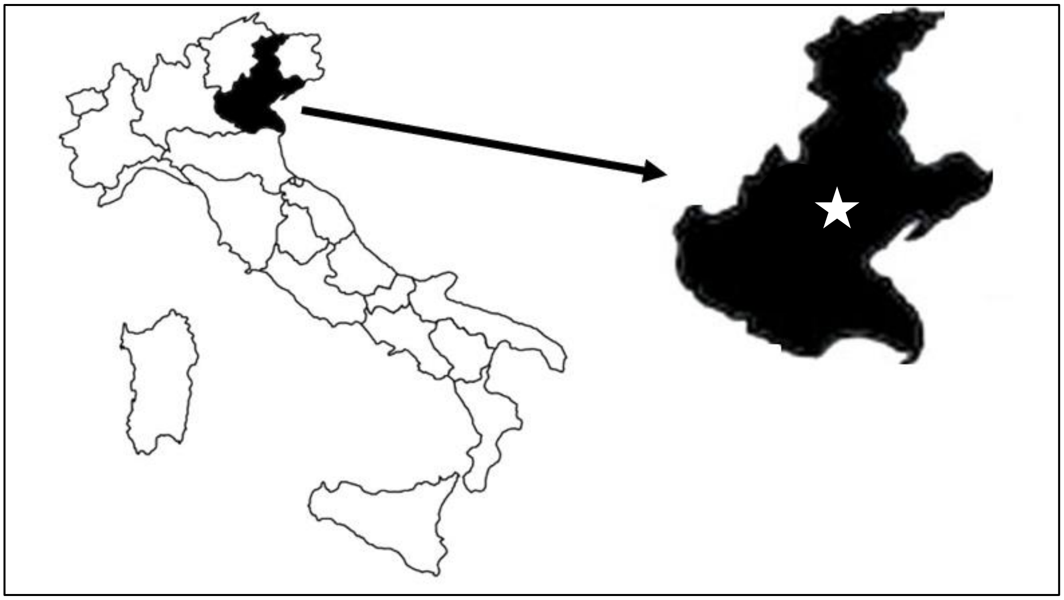

Figure 3.1. Map of Italy with the Veneto region highlighted. The enlarged outline of the Veneto region is depicted on the right, and the city of Cittadella, the location of the kiwifruit orchard, is indicated by a white star.

Figure 3.2. Representation of the different internal and external damage severity scores. Observed damage was caused by feeding *H. halys*. 0 = no damage, 1 = 1-24% damage, 2 = 25-49% damage, 3 = 50-74% damage, and 4 = 75-100% damage.

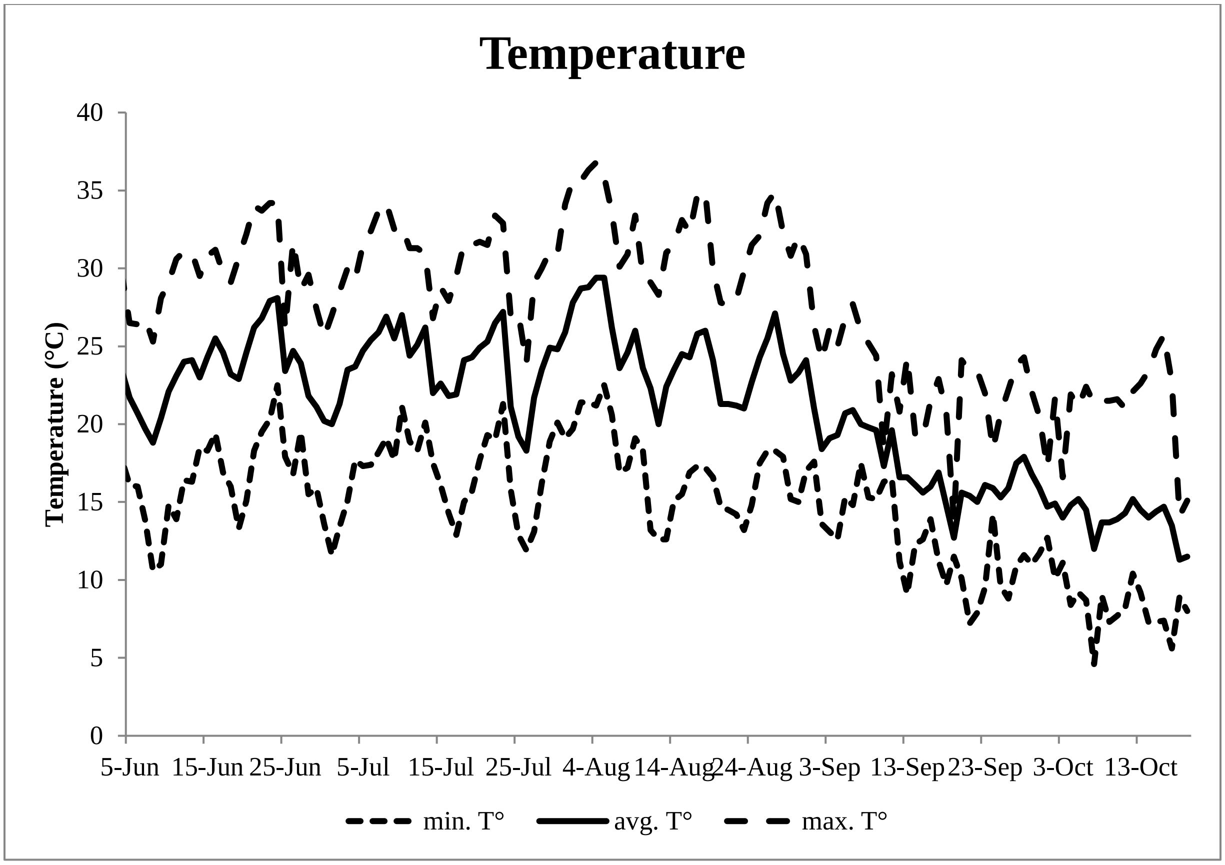

Figure 3.3. Minimum, average, and maximum temperature (°C) at the experimental site throughout the duration of this experiment. Data was obtained through ARPAV (2018).

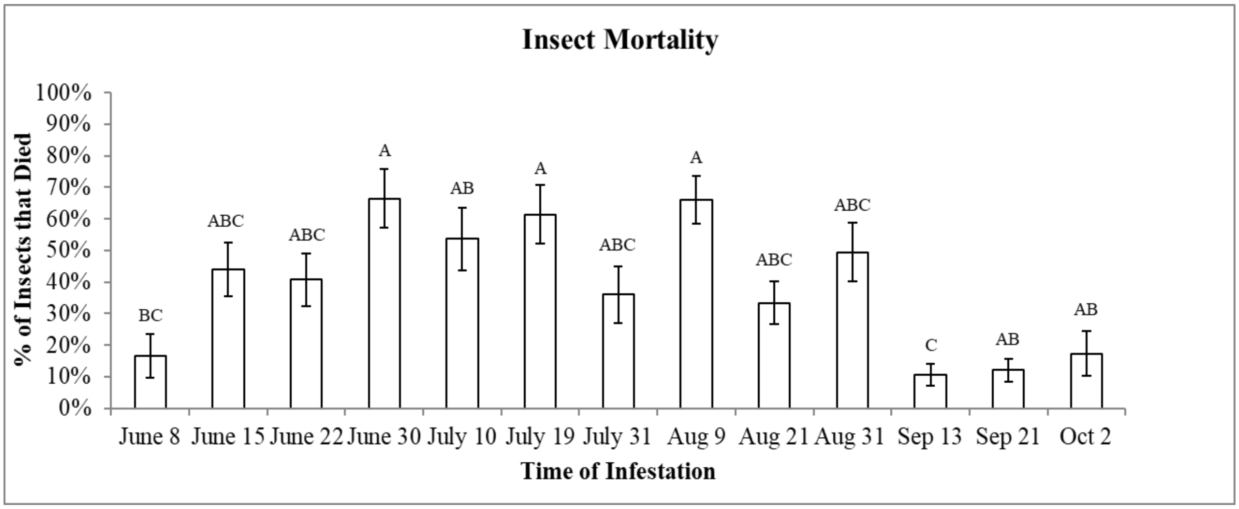

Figure 3.4. Percent mortality (mean ±std. err.) of insects within the cages (excluding control) in relation to time of infestation. The dates on the x-axis represent the dates that the insects were introduced to the cages. Each period lasted for approximately 10 days. Different letters represent significant differences at pairwise comparisons using contrast (p = 0.05).

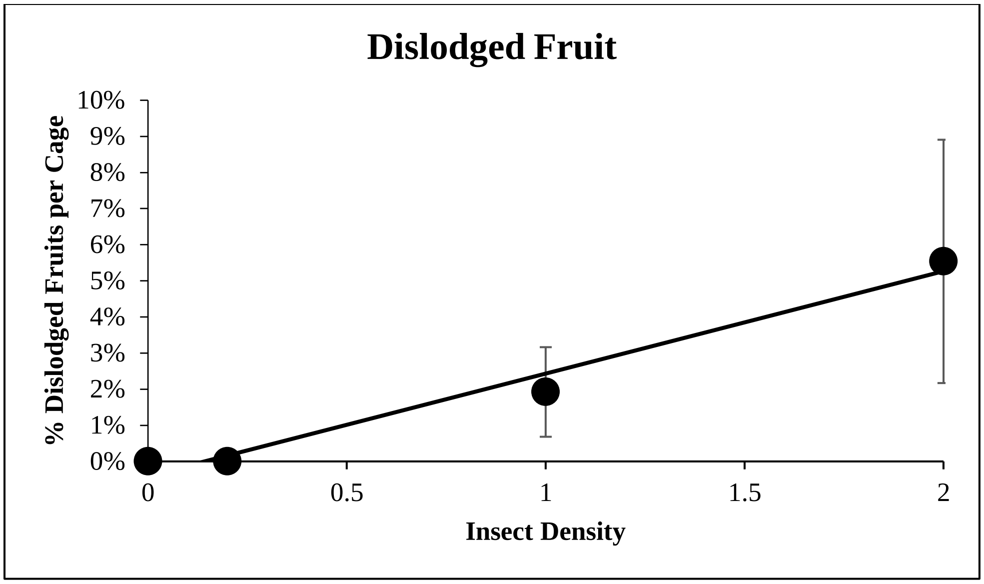

Figure 3.5. Relationship between the percentage of prematurely dislodged kiwifruit (mean ±std. err.) and the density of *H. halys* within each cage. (Linear trend: df = 1, 221; F = 45.39; $P < 0.001$)

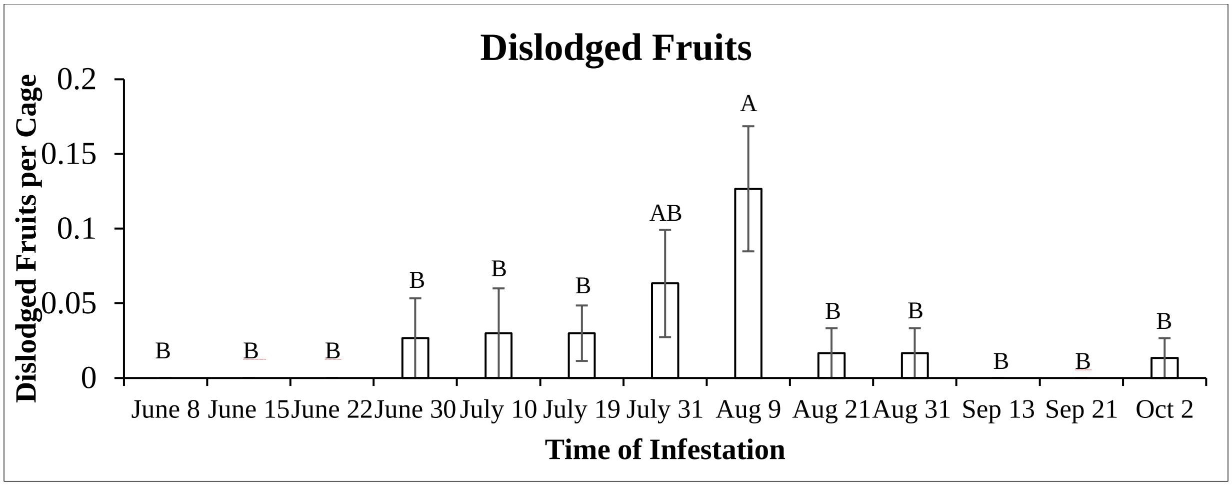

Figure 3.6. Prematurely dislodged kiwifruit in *H. halys* infested treatments at different times of infestation. The dates listed on the x-axis indicate the day that the insects were introduced to the fruits. Each period lasted for approximately 10 days. Different letters indicate significant differences at pairwise comparisons using contrast (p = 0.05).

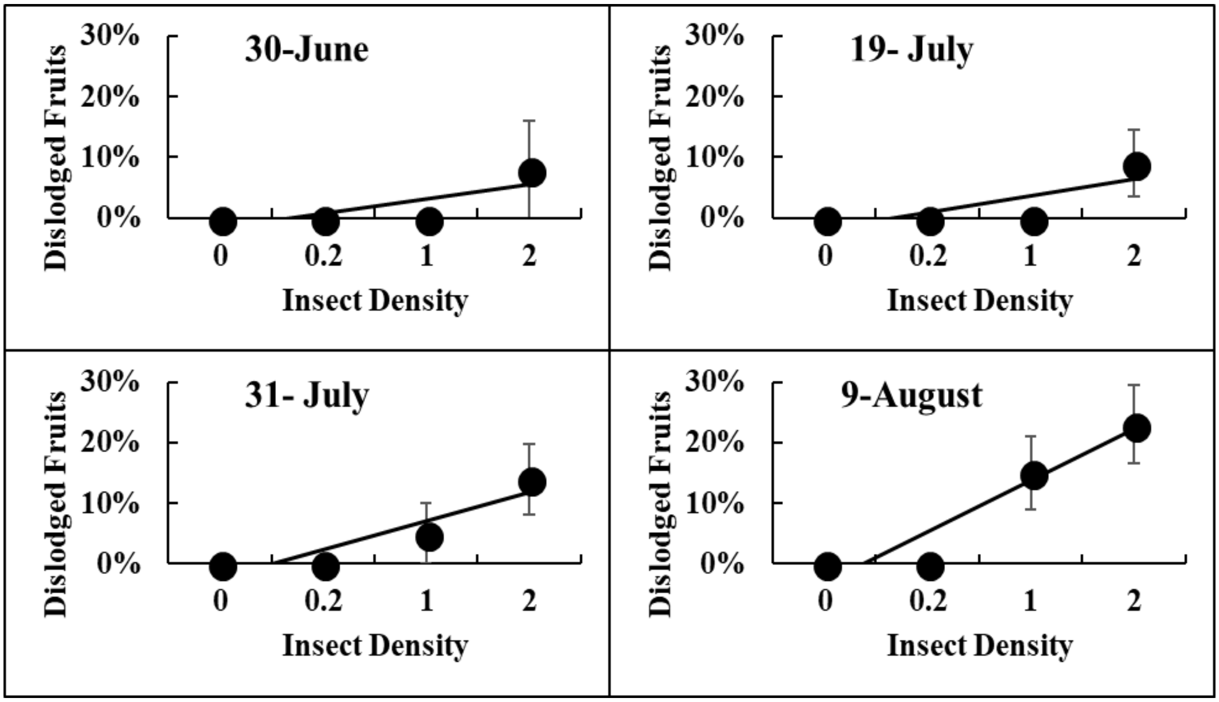

Figure 3.7. Relationship between the density of *H. halys* that was introduced to each cage, the number of prematurely dislodged fruits (mean ±std. err.), and the time of infestation of the most highly effected periods. The dates represented atop each graph indicate the date that *H. halys* were introduced to the cages and were left within the cages for approximately 10-day periods.

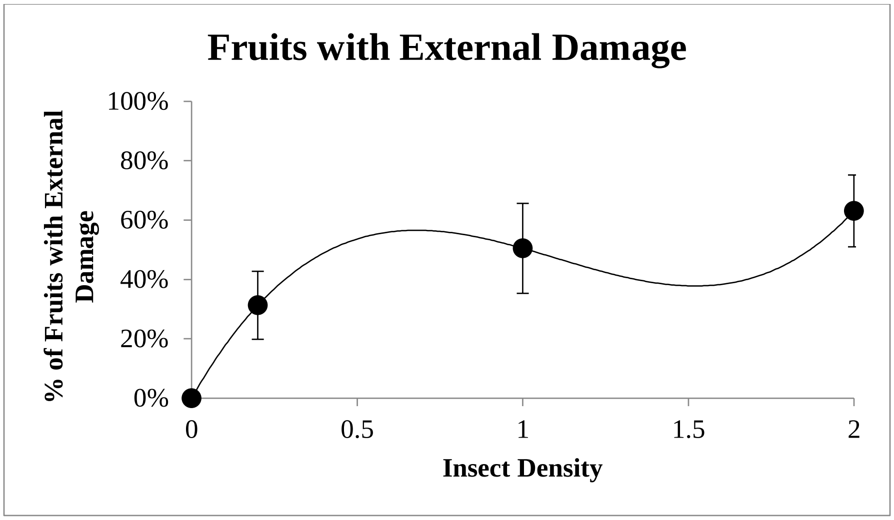

Figure 3.8. The relationship between the percentage of fruits that contained external feeding injury (mean ±std. err.) and the density of *H. halys* within each cage. (Cubic trend: df = 1, 221; F = 27.12; P < 0.001)

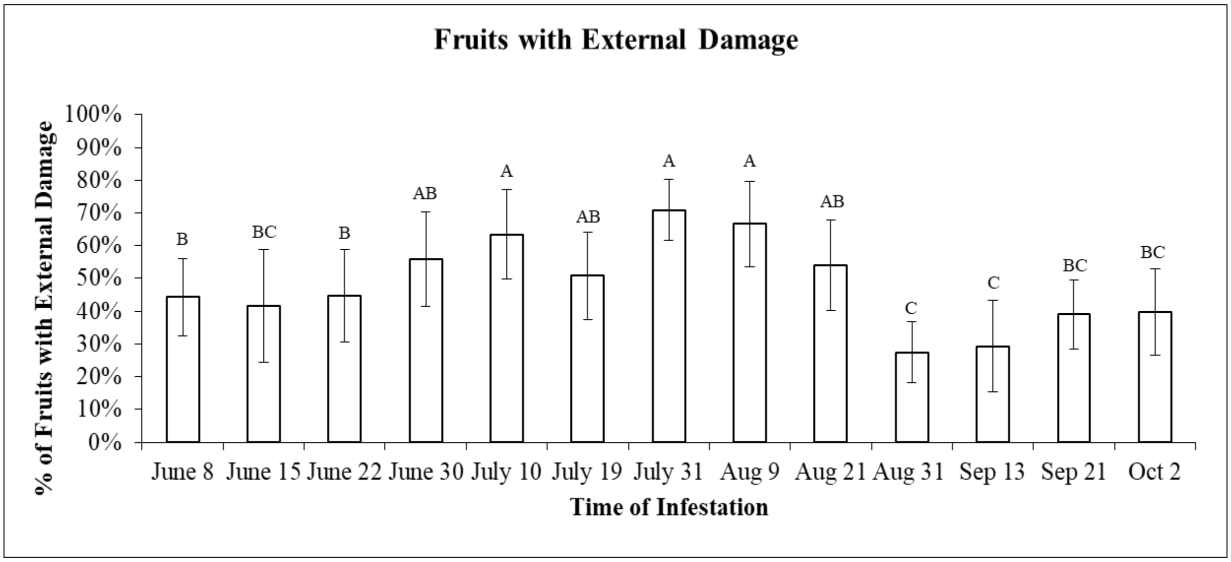

Figure 3.9. Percentage of fruits that contained external feeding injury and the time of *H. halys* infestation. The dates on the x-axis represent the date that *H. halys* were introduced to the kiwifruit within the cages. Each period of time was approximately 10 days in length. Different letters indicate significant differences through pairwise comparisons using contrast (p = 0.05).

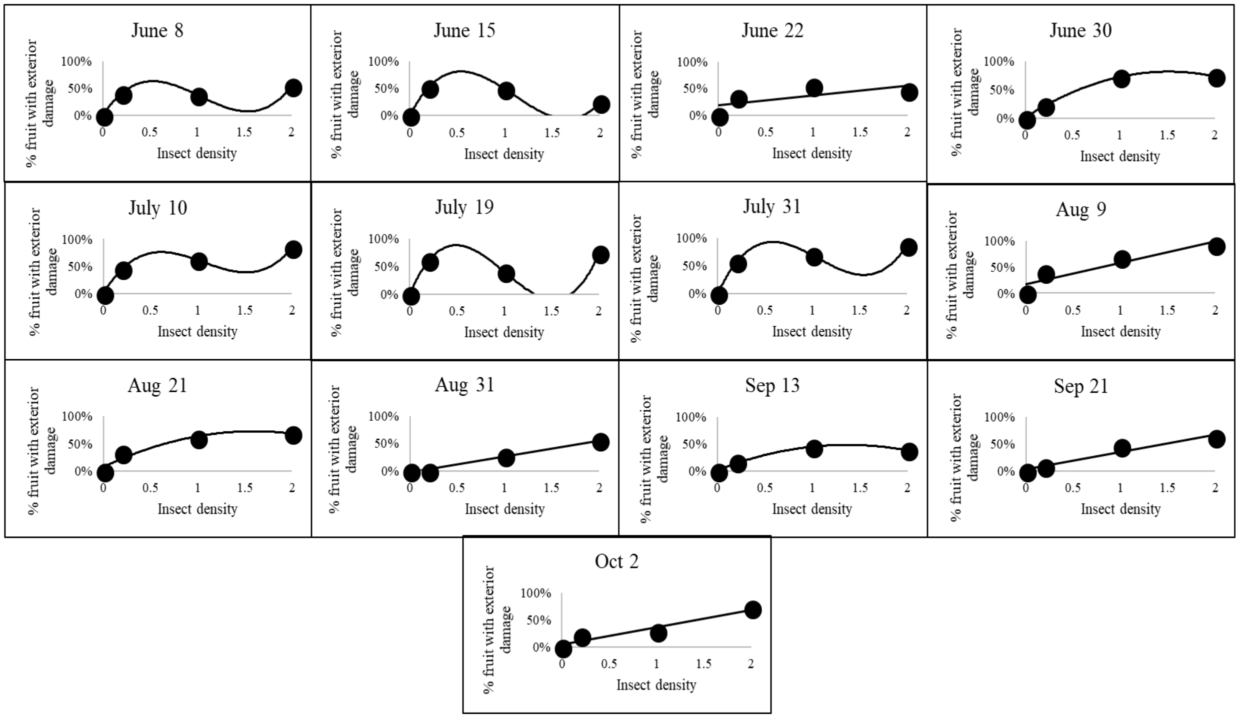
June 8
June 15
June 22
June 30
July 10
July 19
July 31
Aug 9
Aug 21
Aug 31
Sep 13
Sep 21
Oct 2

Figure 3.10. The relationship between the time of infestation, density of feeding *H. halys*, and the percentage of fruits with external damage. The dates on each graph represent the beginning of a period of time that the cages were exposed to feeding *H. halys* (approximately 10 days).

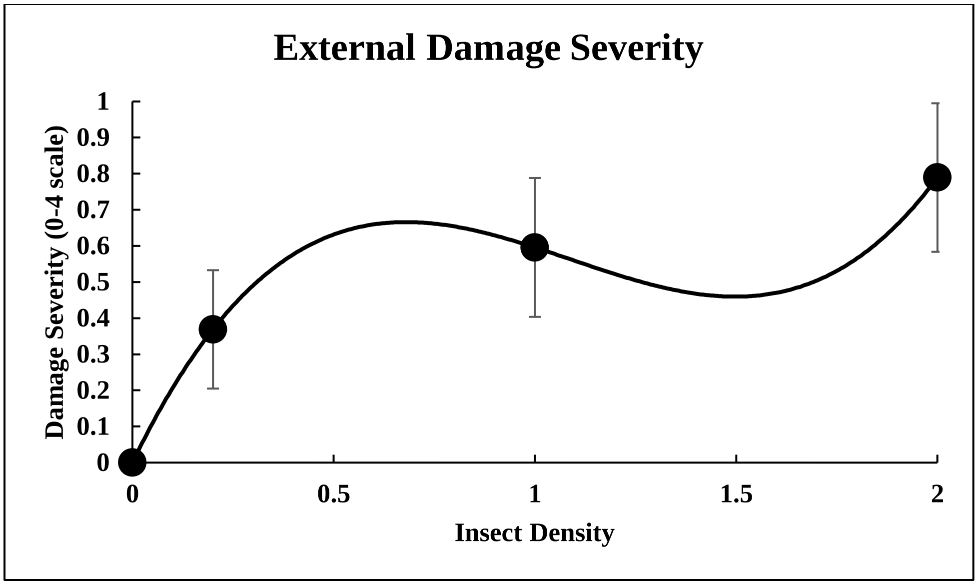

Figure 3.11. The relationship between external damage severity of kiwifruit (mean ±std. err.) in relation to *H. halys* density within each cage. The damage severity was based on a 0-4 scale, with 0 meaning no damage and 4 meaning 75-100% damage. (Cubic trend: df = 1, 156.9; F = 6.33; P = 0.013)

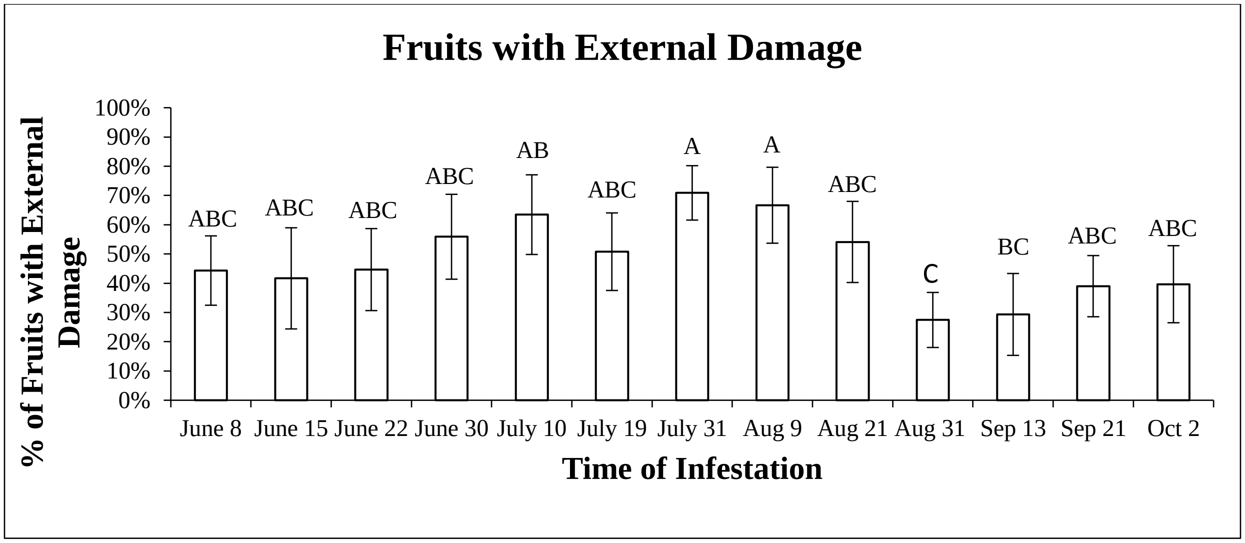

Figure 3.12. External damage severity and the time of *H. halys* infestation. The dates on the x-axis represent the date that *H. halys* were introduced to the kiwifruit within the cages. Each period of time was approximately 10 days in length. Different letters represent statistical differences through pairwise comparisons using contrast (p=0.05)

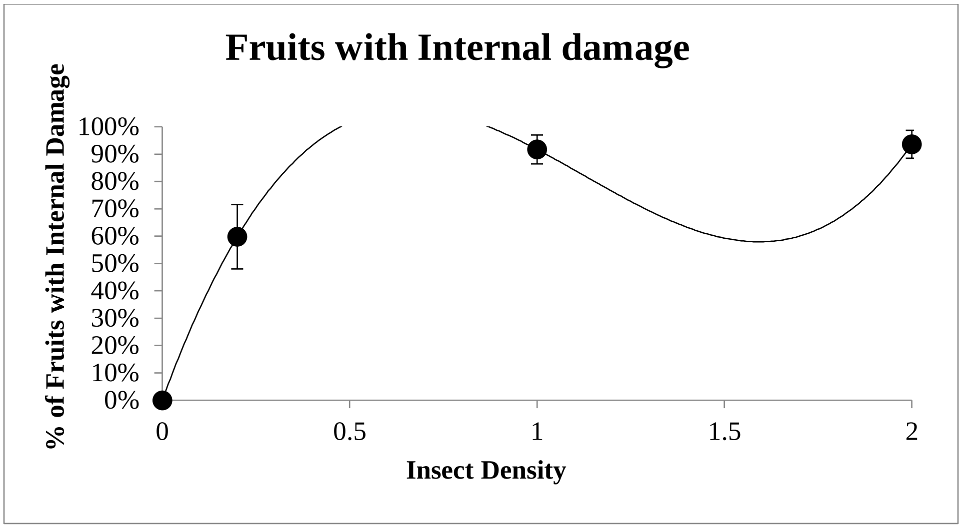

Figure 3.13. The relationship between the percentage of fruits containing internal feeding damage (mean ±std. err.) and the density of feeding *H. halys* that were introduced to each cage (Cubic trend: df = 1,221; F = 158.05; P < 0.001).

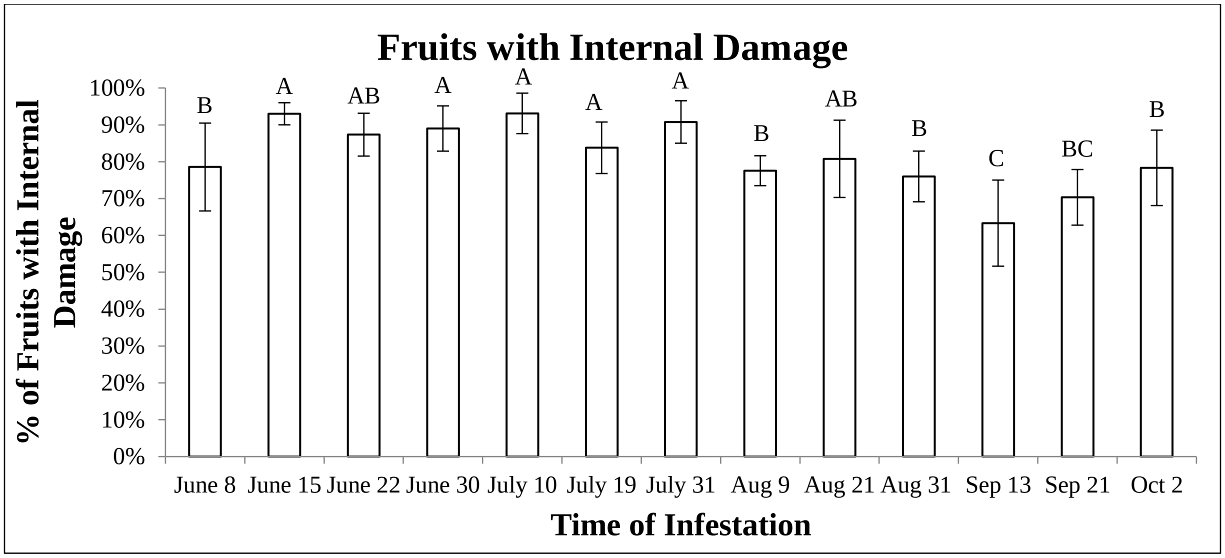

Figure 3.14. Number of fruits exhibiting internal feeding damage and the time of *H. halys* infestation. The dates on the x-axis represent the date that *H. halys* were introduced to the kiwifruit within the cages. Each period of time was approximately 10 days in length. Different letters represent statistical differences through pairwise comparisons using contrast (p=0.05).

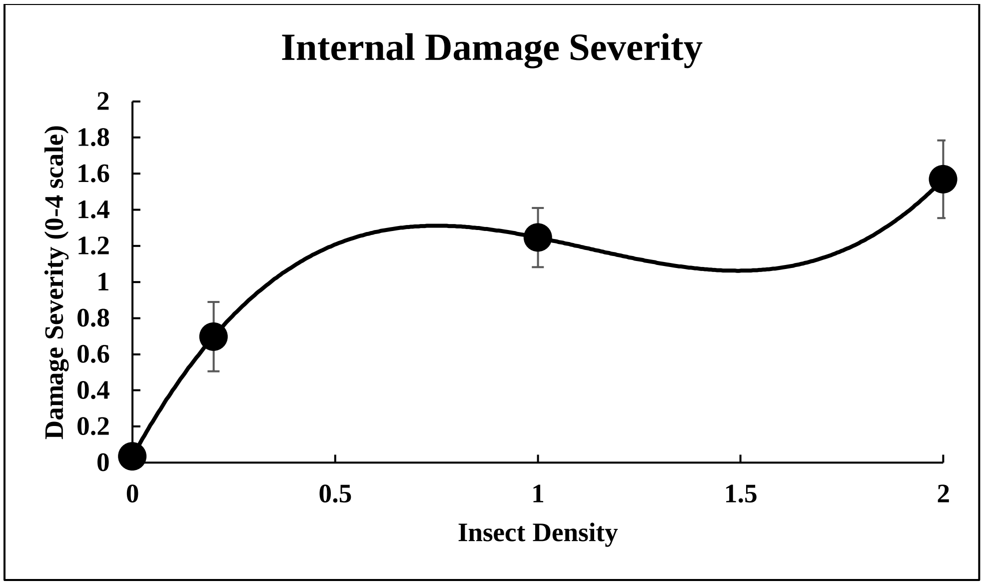

Figure 3.15. The relationship between internal damage severity (mean ±std. err.) and the density of *H. halys* that was introduced to each cage. The damage severity was based on a 0-4 scale, with 0 meaning no damage and 4 meaning 75-100% damage. (Cubic trend: df = 1, 156.6; F = 30.9; P < 0.001)

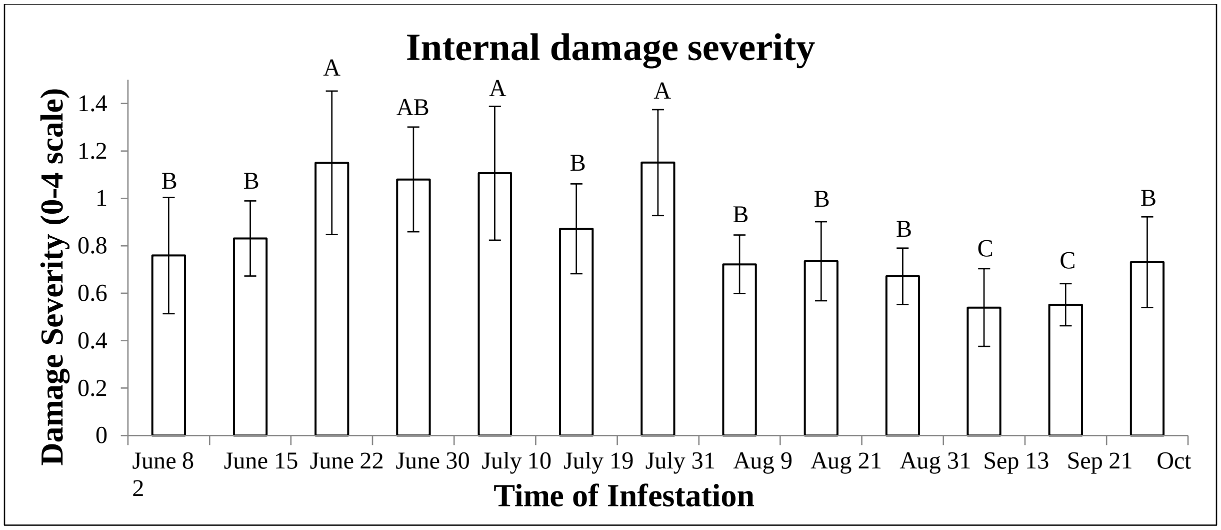

Figure 3.16. Internal damage severity and the time of *H. halys* infestation. The dates on the x-axis represent the date that *H. halys* were introduced to the kiwifruit within the cages. Each period of time was approximately 10 days in length. Different letters represent statistical differences through pairwise comparison using contrast (p = 0.05).

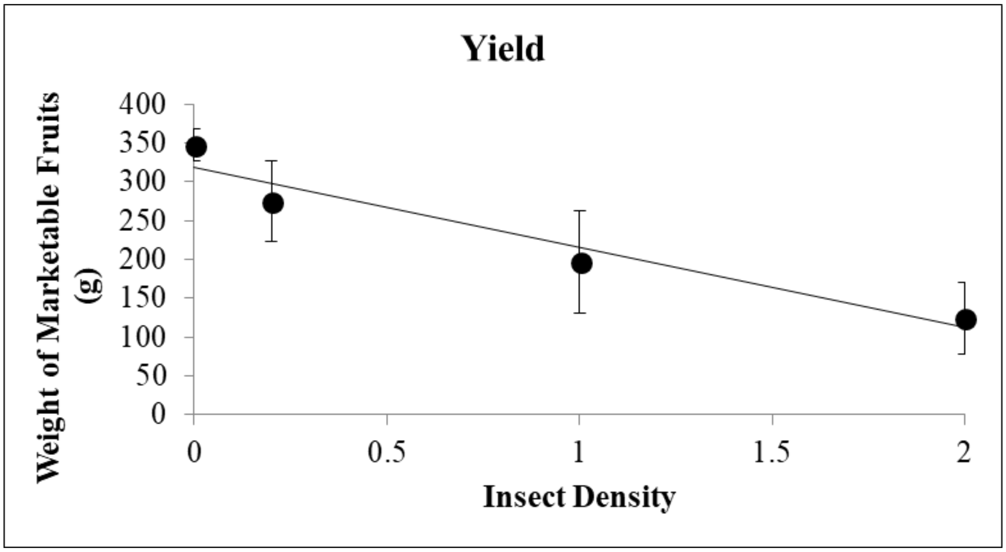

Figure 3.17. The relationship between the yield of the samples (mean ±std. err.) (the weight of the fruits that were attached to the plant at the end of the experiment and did not contain any external feeding injury) and the density of *H. halys* within each cage. (Linear trend: df = 1, 153.1; F = 27.78; P < 0.001)

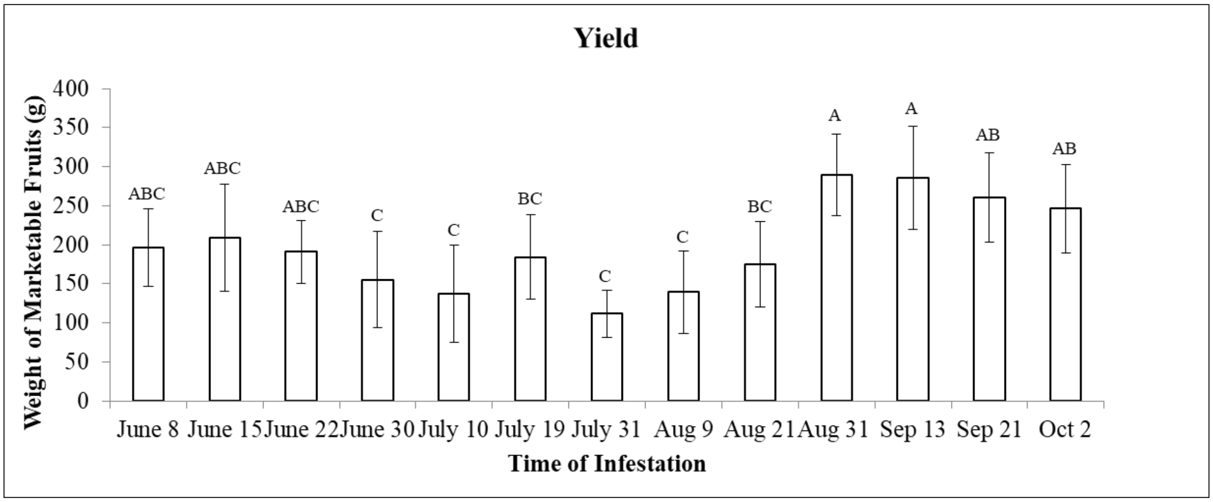

Figure 3.18. The relationship between the yield of the samples and the time of *H. halys* infestation. The dates on the x-axis represent the date that *H. halys* were introduced to the kiwifruit within the cages. Each period of time was approximately 10 days in length. The yield was calculated as the weight of the fruits that were attached to the plant at the end of the experiment and did not contain any external feeding injury. Different letters indicate significant differences through pairwise comparison using contrast ($p = 0.05$).

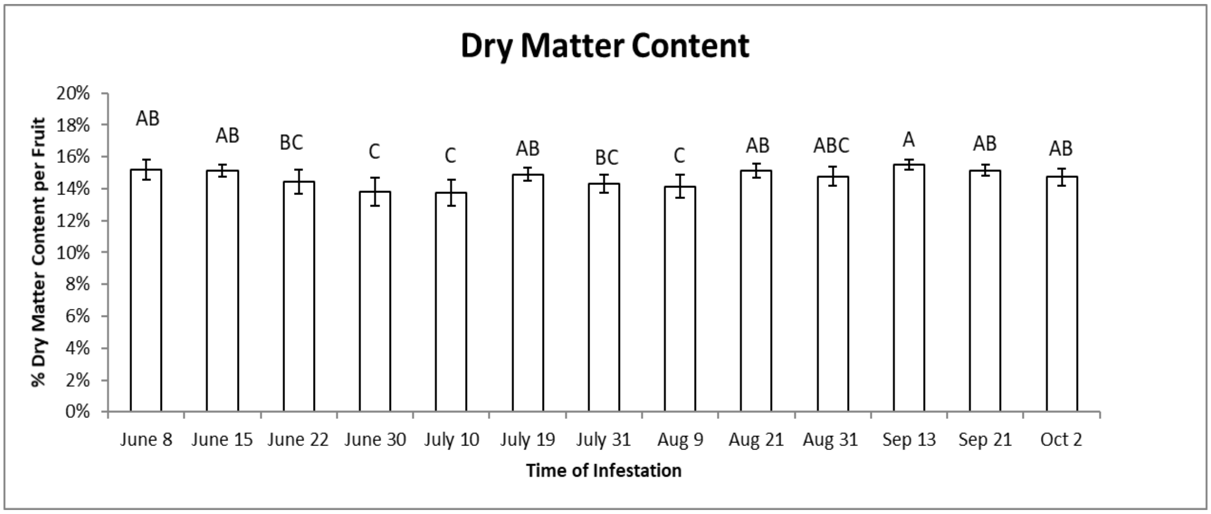

Figure 3.19. The relationship between the percentage of dry matter per fruit and the time of *H. halys* infestation. The dates on the x-axis represent the date that *H. halys* were introduced to the kiwifruit within the cages. Each period of time was approximately 10 days in length. Different letters indicate statistical differences through pairwise comparison using contrast (p = 0.05).

4. SPATIAL AND TEMPORAL PATTERNS OF THE BROWN MARMORATED STINK BUG IN KIWIFRUIT ORCHARDS

4.1 Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stal), is a highly polyphagous invasive pentatomid pest to both Europe and North America. It was first reported in the United States in 1996 in Allentown, Pennsylvania due to its tendency to overwinter in manmade structures (Hoebeke & Carter, 2003). Currently, as of December 2017 (www.stopbmsb.org for updates), *H. halys* has been reported in 44 U.S. states, 4 Canadian provinces, and in many of the southern European countries such as Switzerland, France, Greece, Hungary, Italy, Germany, and Liechtenstein, and is becoming an increasing threat to various crops worldwide (Arnold, 2009; Heckmann, 2012; Callot & Brua, 2013; Pansa et al., 2013; Maistrello et al., 2014; Vetek et al., 2014; Milonas & Partsinevelos, 2014; Cesari et al., 2014; Haye et al., 2015). In Italy, *H. halys* was first detected in 2012 in the Emilia Romagna region and was later found in the Veneto region (location of this experiment) in 2015 (Maistrello et al., 2014; Bariselli et al., 2016). Similar to other pests in the family Hemiptera, *H. halys* has piercing and sucking mouthparts that penetrate the external surface of fruits, foliage, and stems of many important crops (Panizzi et al., 2000; Peiffer & Felton, 2014). *H. halys* has reportedly been observed feeding on many fruits (Haye et al., 2015; Joseph et al., 2014; Leskey et al., 2012a; Leskey et al., 2012b; Nielsen et al., 2009; Walton et al., 2016; Wiman et al., 2015), vegetables (Cissel et al., 2015; Haye et al., 2015; Kuhar et al., 2012), tree nuts (Hedstrom et al., 2014; Lara et al., 2017), ornamentals (Shrewsbury et al., 2011; Wermelinger et al., 2008; Bergmann et al, 2016), row crops (Bakken et al., 2015;

Kamminga et al., 2014; Koch and Rich, 2015; Nielsen et al., 2011; Owens et al., 2013; Venugopal et al., 2014), and native plants both in its native and introduced regions (Bakken et al., 2015; Leskey et al., 2012b). *H. halys* has inflicted severe economic damage in tree fruits, row crops, and ornamentals since 2010 in the mid-Atlantic region of the United States (Rice et al., 2014; Leskey et al., 2012a). In 2010, *H. halys* damage resulted in $37 million (USD) of losses in apple production in the mid-Atlantic region of the U.S. alone (Rice et al, 2014). In addition to being an agricultural pest, *H. halys* is also considered an urban nuisance due to its overwintering habits (Schulz, 2018; Funayama, 2012; Inkley, 2012; Nielsen & Hamilton, 2009, Saulich & Musolin, 2014). *H. halys* invades homes in the late summer and early fall months in search of overwintering shelters. Although overwintering does not cause immense damage to homes, sometimes the frass may stain surfaces and the unpleasant smell that *H. halys* produces may disturb the property holders (Inkley, 2012).

Like *H. halys*, kiwifruit, *Actinidia deliciosa*, is of Asian origin (Hoebeke & Carter, 2003; Liang & Ferguson, 2011). *H. halys* has been reported feeding on kiwifruit both in its native range and now also in Italy (Teulon & Xu, 2017; Lara et al., 2018; Moore et al., unpublished). In 2010, the global production of kiwifruit reached 1.35 million tons. China is the number 1 producer of kiwifruit worldwide, producing an annual average of 480,000 metric tons from 2009 to 2012. Italy is second in terms of overall production, producing and annual average of 450,000 metric tons from 2009 to 2012. In a 2007 survey, Italy reportedly had 26,700 ha of kiwifruit orchards within its borders (Testolin & Ferguson, 2009). Kiwifruit farmers have high production costs and low profit margins. The introduction of a new serious pest has the potential to devastate kiwifruit production in areas where it is introduced. *H. halys* has been reported feeding on kiwifruit both in the field and in the lab and direct injury has been observed as a result (Moore et al., unpublished).

Previous studies about the spatial and temporal patterns of *H. halys* are somewhat limited at the landscape level. Stink bugs are a vagile species and readily disperse among various crop hosts when food sources become available at different stages in plant growth (McPherson & McPherson, 2000; Venugopal et al, 2015). Adults are equipped with wings and can travel long distances when searching for food sources. Lee and Leskey (2015) found that flight behavior increases immediately following diapause. Through the use of flight mills, it was found that the mean flight distances within a 22-h period were 2442 m and 2083 m for male and female *H. halys* respectively, although temperature had a significant effect on flight capacity (Lee & Leskey, 2015). Nymphs do not have fully developed wings but are still capable of travelling by walking and climbing. One study found that *H. halys* nymphs can easily travel up to 20 meters in a 12-hour time period (Lee et al., 2014a). Spatiotemporal patterns of *H. halys* at the field level have been assessed in several studies, but never on kiwifruit orchards. The consensus of the majority of the experiments are the same, suggesting that *H. halys* tend to aggregate around the edges and perimeters of crop fields and orchards as opposed to the orchard centers (Nielson & Hamilton, 2009; Leskey et al., 2012c; Basnet et al., 2015; Joseph et al., 2014; Blaauw et al., 2016; Venugopal et al., 2015a; Venugopal et al., 2015b). This concept is not new as it has been proven with many other stink bug species (Bundy & McPherson, 2000; Reeves et al., 2010; Tillman et al., 2009). It has also been determined that densities of *H. halys* populations are higher on field edges near other fields or orchards containing alternate hosts, such as soybeans or corn, indicating that outbreak potential is higher on edges with adjacent host plants that are at the desired phenological stage for feeding. It has been suggested that the surroundings of a field or orchard have a significant effect on stink bug population dynamics (Tillman et al., 2009; Venugopal et al., 2014; Venugopal et al., 2015b; Wallner et al., 2014). *H. halys* tends to aggregate and cluster later in the season as fruits mature within the orchard (Leskey et al., 2012c; Hahn et al., 2017). It has been suggested that the availability of food mitigated by the landscape structure influences pentatomid spatiotemporal patterns (Tillman et al., 2009). It has also been found that *H. halys* crop damage is significantly higher around the edges of the fields and orchards where the insects tend to aggregate (Nielson & Hamilton, 2009; Leskey et al., 2012c; Joseph et al., 2014; Basnet et al., 2015). Studies conducted regarding proximity to potential geographical stink bug reservoirs such as rivers and riparian buffers are limited. Through spatial analysis, geostatistics, and Bayesian linear regression, one study found that *H. halys* tend to stay near overwintering sites (urban areas) and areas of introduction (railroads). It was also determined that large densities of *H. halys* are typically correlated with agricultural areas (Wallner et al., 2014). Increasing information on the effects of surrounding areas at both the field and landscape level will help give insight into outbreak probability and timing of invasions. All spatial and temporal studies on *H. halys* have, so far, been conducted in the United States. This study was conducted in a kiwifruit growing area throughout the growing season in the Veneto region of Italy.

In this study, we recorded the population abundance of *H. halys* regarding kiwifruit orchard surroundings. We coupled this information with different time periods within the kiwifruit growing season to better understand infestation potential. We also took into consideration the orchard's proximity to the Brenta River that is a semi-natural area and can potentially constitute stink bug reservoirs in the region. The design of the experiment allows insight to 1) at what part of the growing season orchard are colonized by *H. halys*? 3) do *H. halys* tend to aggregate around the edges of kiwifruit orchards, similar to their behavior in other crops? 4) does proximity to a major river and the associated riparian buffer play a role in *H. halys* spatiotemporal dynamics? 5) is organic kiwifruit production more at risk of *H. halys* infestation than conventionally managed orchards? 6) do hail nets provide kiwifruit orchards with any form of protection against *H. halys*? We also considered interactive effect among the above listed factors. Understanding the spatial and temporal dynamics of *H. halys* in kiwifruit orchards will allow the implementation of more efficient control strategies that are both economically and environmentally improved.

4.2 Materials and Methods

The study was conducted on 12 different kiwifruit orchards near the city of Cittadella in the Veneto region of Italy during the 2017 growing season (Figure 4.1). Each orchard contained the Hayward variety of kiwifruit. The kiwifruit orchards varied in dimensions, age, location, management strategies, hail net coverage and proximity to the Brenta River. Proximity to the Brenta River and the associated riparian buffer was taken into consideration when the orchards were selected for the experiment. The orchards within 2 km of the Brenta River were considered in close proximity to the river and those that were a greater distance than 2 km were considered as far from the river. Beginning on 8 July, four samples were taken using a beating sheet on each edge and in the center of each orchard for a total of 20 samples per orchard per sampling date. The vines that contained fruit were shaken and the number of *H. halys* adult males, adult females, and immature specimens that fell into the beating sheet was recorded. Observational data was also collected for egg masses that were visible in the locations where the beating sheet samples were taken. In approximately 10-day intervals, this process was repeated until kiwifruit harvest that occurred on October 19th. At harvest, 10 fruits from each edge and middle (50 total per farm per sampling date) were selected from each farm and the number of fruit with external and internal damage caused by *H. halys* feeding was visually assessed and recorded. The internal damage was evaluated by laterally slicing the fruits.

Adhesive traps activated with the aggregation pheromone (PHEROCON® BMSB lure and STKY™, Tréce Inc., Adair, OK USA) were used to sample *H. halys* during the experiment on each farm. The traps were placed at least 20 meters away from any kiwifruit plants. These traps were checked every 10 days until harvest. The number of adult male, adult female, and immature *H. halys* that were attached to the trap or were within approximately 3 meters of the trap were recorded. The traps were replaced when their ability to capture insects declined and the pheromones were replaced every 40 days. All insects were removed from the traps each time data was collected.

4.2.1 Data Analysis. Data on the number of *H. halys* adults, nymphs (I instar and II to V instar) as well as the total number of the *H. halys* motile forms, were analyzed separately using a Restricted Maximum Likelihood (REML) repeated measures model with the Proc MIXED of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC). Proximity to river, orchard management, presence of anti-hail nets, position in the orchards (boarder vs. middle), time of sampling date, and their interactions were considered as sources of variation and tested using an F test ($\alpha = 0.05$). Number of eggs were not included in the analysis due to the low numbers observed. The average number of adults, nymphs and motile forms of the *H. halys* observed at each sampling site were considered as response variables with repeated measures made at different times, i.e. sampling dates. In the model, ‘fruit orchard’ was considered as a random effect (Littell et al., 2006). Differences among treatments were evaluated with a t-test ($\alpha = 0.05$) to least square means with the Bonferroni adjustment. All data was checked for normality assumption and thus the number of insects per trap was transformed in log x+1.

Data on external and internal damage assessment was analyzed separately using an ordinal logistic regression model with the Proc GLIMMIX of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC). In the analysis, the proportion of fruits containing damage was considered as the response variable. Proximity to river, orchard management, presence of anti-hail netting, position in the orchards (boarder vs. middle), and their interactions were considered as sources of variation and tested using an χ^2 test ($\alpha = 0.05$). In the model, 'fruit orchard' was considered as a random effect term (Littell et al. 2006). Differences among treatments were evaluated with a t-test ($\alpha = 0.05$) to least square means with the Bonferroni adjustment.

4.3 Results

On the adhesive traps, *H. halys* captures were higher at the beginning of the season and decreased thereafter (Figure 4.2). It was found that proximity to the Brenta River and the associated riparian buffer had a significant effect on the number of insects that were collected by the trap, with significantly greater numbers of *H. halys* collected on traps in orchards closer to the Brenta River than orchards farther away from the river (Table 4.1; Figure 4.2).

On plants, *H. halys* were observed from the beginning of the experiment and, in particular, for the nymphal abundance, an increase was observed until mid-September. The presence of insects on plants was observed until the end of October. A significant effect of proximity to the Brenta River was observed on infestation levels with the numbers of nymphal (Table 4.2; Figure 4.3), adult (Table 4.3; Figure 4.4), and motile forms (Table 4.4; Figure 4.5) of

H. halys. The effect was higher in orchards closer to the Brenta River as opposed to the orchards that were farther away from the river (Table 4.2-4.4; Figure 4.3-4.5).

Differing management strategies lead to differences of nymphal densities. It was found that organic kiwifruit orchards contained significantly higher numbers of *H. halys* nymphs than orchards that use conventional management strategies (Table 4.2; Figure 4.6). On nymphs, a significant interaction between proximity to the Brenta River and orchard management strategy was found (Table 4.2; Figure 4.7). The effect of the management strategy was significant in orchards closer to the river (t = 3.37: df = 44: P = 0.009; Figure 4.7), while no significant differences emerged in orchards far from the river (t = 0.86; df = 44; P = 1.00; Figure 4.7).

Infestation levels were influenced by anti-hail netting; for example, fewer adult (Table 4.3; Figure 4.8) and motile forms of *H. halys* (Table 4.4; Figure 4.9) were present within orchards that contained an anti-hail net. Analyses of nymphal *H. halys* demonstrated a significant second order interaction between proximity to the Brenta River, management strategy, and the presence of anti-hail netting, and this interaction was influenced by time (Table 4.2; Figure 4.10). A significant reduction in the number of nymphs due to anti-hail was observed in organic orchards close to the river (t = 4.45; df = 44; P < 0.001; Figure 4.10) but not in conventionally managed orchards (t = 1.41; df = 44; P = 1.0; Figure 4.10). Additionally, these effects were not consistently significant, with August and September displaying the highest densities of nymphal *H. halys* (Figure 4.10). These differences did not emerge in orchards far from the river (Figure 4.10).

The infestation was also influenced by position within the orchards, particularly with the number of adults (Table 4.3; Figure 4.11), and motile forms of *H. halys* (Table 4.4; Figure 4.12) in the borders of kiwifruit orchards compared to the orchard middles.

Proportion of damaged fruit was different among orchards, being higher in organic orchards as compared to conventional ones (Table 4.5; Figure 4.13 & 4.14), and higher in orchards without anti-hail netting than those with the netting (Table 4.5; Figure 4.15 & 4.16).

4.4 Discussion

Here, we found how insect abundance in kiwi fruit orchards is influenced by different spatial scale effects. We found a landscape effect on *H. halys* infestations. Our results suggest that *H. halys* distribution within the landscape is affected by major rivers and associated riparian buffers. We found that kiwifruit orchards closer in proximity to the Brenta River contained higher densities of *H. halys,* compared to the orchards that were not close to the river. This association may be linked to the riparian buffer and different hosts that surrounds the Brenta River. This riparian buffer provides a viable habitat with plentiful overwintering sites and alternative host plants for when the agricultural host crops are not available. The suggestion that *H. halys* distribution within the landscape is affected by the presence of different geological or man-made structures is not a new concept. It has been found that greater densities of *H. halys* were associated with proximity to urban areas, rail roads, and agricultural areas (Wallner et al., 2014; Rice et al., 2016). Landscape structure may influence the establishment, dispersal, and severity of pest infestation in other insect species (Saki et al., 2001; With, 2002; Venugopal et al., 2014). Understanding *H. halys* distribution within the landscape helps provide insight as to which farms or locations may be more at risk of invasion, and preventative or active management tactics may be implemented as a result.

At orchard scale, *H. halys* abundance was influenced by management of the orchards. In particular, this effect emerged on nymphs. The differences between organic and conventional management are not only related to control tactics but also include differences, for instance, in type of fertilizers and weed control that can be used. However, in kiwifruit orchards the differences are mostly related to insecticides that can be used: only pesticides that are organically-certified are approved in organic orchards. Insecticides labelled for *H. halys* are limited for kiwifruit farmers in Italy, however the management options decrease significantly when moving to organic production, and the efficacy of organically approved insecticides is limited (Kuhar and Kamminga 2017). The fact that management effect was showed only on nymphs is not surprising because these are the most susceptible stages to insecticide treatment (Nielsen et al. 2008, Kuhar and Kamminga 2017). Adults are the most mobile stages that can easily recolonizing the orchards after insecticide application. It is well known that residual effect of insecticides is low on *H. halys* adults (Leskey et al. 2014). The limited efficacy in nymph management in organic orchards was also reflected on damage data. Our results highlight that the research for effective management options approved for organic management in kiwifruit orchards should be a priority.

In line with these needs, one option can be represented by insect exclusion using netting. The use of insect-proof netting for *H. halys* management has already been suggested in previous studies (Dobson 2015, Andian et al. 2018). Through our data, we have also demonstrated that anti-hail nets have the ability to reduce the number of *H. halys* that are able to enter the orchard. This is mainly because the net is impenetrable to *H. halys* adults, and, when the net is applied, *H.*

halys is unable to enter the orchard from above. To enter the orchard, the insects must come from the sides, which remain uncovered in most orchards. Insect-proof nets have proven to reduce *H. halys* infestation in other crops as well (Bariselli et al., 2016; Candian et al., 2018). Anti-hail nets have also proven to be effective in excluding other pests, such as *Cydia pomonella* (L.) (Lepidoptera Tortricidae) in apple orchards (Tasin et al., 2008; Pasqualini et al., 2013; Chouinard et al., 2017). According to our results, orchards with anti-hail netting can be considered at lower risk of *H. halys* infestation. Specific inset-proof nets are usually more expensive than anti-hail netting. We suggest that, in orchards with existing anti-hail netting, the application of nets along the side of the orchards is expected to increase the efficacy in *H. halys* control in a relatively cost-effective manner compared to the application of complete insect-proof netting systems.

Another aspect that can be considered in *H. halys* management is the edge-mediated accumulation of stink bugs on target crops. This is a direct result of their directional movement among an array of hosts in a particular area in response to the decrease in the suitability of the previously infested host plants and the increase in suitability of the target host plant (Venugopal et al., 2014; Todd, 1989). Our results suggest that *H. halys* tend to aggregate and infest kiwifruit orchards around the edges as opposed to orchard centers. Previous studies have found the same edge-mediated response of *H. halys* in several other crops (Blaaw et al., 2014; Blaaw et al., 2016; Basnet et al., 2015; Joseph et al., 2014; Leskey et al., 2012b; Nielson & Hamilton, 2009; Rice et al., 2014; Venugopal et al., 2014; Venugopal et al., 2015a; Venugopal., 2015b). This edge-mediated response is likely due to the insect’s tendency to utilize multiple hosts within a farmscape and move freely among different hosts according to the preferred phenological stages of each crop. Insects that move from adjacent host plants or overwintering sites to the kiwifruit orchard are more likely to inhabit the plants closer in proximity, which would be the closest edge rows available. Once the insects are on the edge plants of the orchard, they may travel further within the orchard and lay eggs, and the resulting nymphal instars are able walk to the center. There was not a high density of *H. halys* found in the orchard centers, however it was common to find a few. This aspect could be taken into consideration with IPM strategies in kiwifruit orchards. Our results suggest that control tactic applied at the orchard's boarders could potentially reduce *H. halys* infestations, as suggested by the IPM-CPR (Crop Perimeter Restructuring) (Blaauw et al. 2015). Additional studies should also elucidate if there are differences determined by the different types of crop surrounding orchards on infestation patterns.

The highest densities of *H. halys* adults were found within the kiwifruit orchards in the beginning of September, while the lowest densities were recorded in early June and late October (Figure 4.10). Kiwifruit is one of the first fruits to become available for insect feeding in Northern Italy and is a long season crop. The fruits grow for a period of 8 to 9 months until they are ready for harvest. Hayward is the primary kiwifruit variety grown throughout the world, and every orchard that was used in this experiment was of the variety Hayward. In Italy, Hayward flowering (50% of the flowers opened) typically initiates from 10 May and 30 May. Usually, depending on latitude, 160 to 180 days after flowering, the fruits approach maturity stage and become ready for harvest. In the Veneto region, kiwifruit harvest typically occurs between the end of October and mid-November (Testolin & Ferguson, 2009). Moore et al. (unpublished) found that *H. halys* has the capability of becoming a serious pest to kiwifruit. Using a no-choice feeding experiment, they demonstrated that *H. halys* feeds on and severely damages kiwifruit in the form of premature dislodging, external epicarp staining, internal pulp tissue corking, fruit weight loss, and decreased dry matter content. The highest yield losses were observed in the central part of the season (July to August), whereas the highest densities of insects found in this experiment occurred in early September. We conclude that the best time to apply management strategies for *H. halys* would be from July to mid-September in northern Italy (Moore et al., unpublished).

Table 4.1. Statistical analysis outputs for the numbers of *H. halys* found attached to the adhesive trap. The following abbreviations were used in this table: "Man" for management strategy (organic or conventional), "Hail" for the presence of hail netting, "River" for proximity to river, and "Time" for the time within the season.

Effect	***df***	***F***	***P***
Management strategy	1,40	0.12	0.7328
Proximity to river	1,40	4.44	0.0413
River*Man	1,40	0.18	0.6757
Presence of anti-hail netting	1,40	0.33	0.5668
Man*Hail	1,40	2.07	0.1575
River*Hail	1,40	1.03	0.3172
River*Man*Hail	1,40	1.35	0.2529
Time within season	9,40	2.79	0.0122
Time*Man	9,40	0.66	0.7425
Time*River	9,40	0.43	0.9131
Time*River*Man	9,40	0.54	0.8373
Time*Hail	9,40	0.66	0.7417
Time*Man*Hail	9,40	0.71	0.6975
Time*River*Hail	9,40	0.5	0.8688
Time*River*Man*Hail	8,40	0.33	0.9479

Table 4.2. Statistical analysis outputs for the nymphal forms of *H. halys*. The following abbreviations were used in this table: "Man" for management strategy (organic or conventional), "Hail" for the presence of hail netting, "River" for proximity to river, "Position" for position within the orchard (border or center), and "Time" for the time within the season.

Effect	***df***	***F***	***P***
Management strategy	1,44	6.28	0.016
Proximity to river	1,44	19.86	<.0001
River*Man	1,44	5.1	0.029
Position within orchard	1,44	3.79	0.0579
Man*Position	1,44	1.71	0.1972
River*Position	1,44	0.82	0.3707
River*Man* Position	1,44	1.08	0.3046
Presence of anti-hail netting	1,44	1.15	0.2887
Man*Hail	1,44	3.2	0.0804
River*Hail	1,44	0.87	0.3572
River*Man* Hail	1,44	12.05	0.0012
Hail*Position	1,44	1.05	0.3115
Man*Hail* Position	1,44	0.01	0.9216
River*Hail*Position	1,44	1.3	0.2611
River*Man* Hail*Position	1,44	0	0.98
Time within season	9,396	4.58	<.0001
Time*Man	9,396	0.78	0.637
Time*River	9,396	1.17	0.3154
Time*River*Man	9,396	0.73	0.6806
Time* Position	9,396	0.32	0.9685
Time*Man* Position	9,396	0.29	0.9787
Time*River*Position	9,396	0.69	0.7202
Time*River*Man*Position	9,396	0.21	0.9932
Time*Hail	9,396	1.11	0.3552
Time*Man*Hail	9,396	0.46	0.8981
Time*River*Hail	9,396	0.82	0.5979
Time*River*Man*Hail	9,396	2.37	0.0127
Time*Hail*Position	9,396	0.49	0.8798
Time*Man*Hail*Position	9,396	0.59	0.8095
Time*River*Hail*Position	9,396	0.3	0.9746
Time*River*Man*Hail*Position	9,396	0.27	0.9819

Table 4.3. Statistical analysis outputs for adult *H. halys*. The following abbreviations were used in this table: “Man” for management strategy (organic or conventional), “Hail” for the presence of hail netting, “River” for proximity to river, “Position” for position within the orchard (border or center), and “Time” for the time within the season.

Effect	***df***	***F***	***P***
Management strategy	1,44	0.45	0.5059
Proximity to river	1,44	5.1	0.0289
River*Man	1,44	0.2	0.6551
Position within orchard	1,44	9.31	0.0038
Man*Position	1,44	1.19	0.2818
River*Position	1,44	0.05	0.8267
River*Man* Position	1,44	0.16	0.6868
Presence of anti-hail netting	1,44	5.93	0.019
Man*Hail	1,44	0.56	0.4568
River*Hail	1,44	0.13	0.7168
River*Man* Hail	1,44	0.37	0.5445
Hail*Position	1,44	0.52	0.4731
Man*Hail* Position	1,44	1.28	0.2633
River*Hail*Position	1,44	0.75	0.3925
River*Man* Hail*Position	1,44	0.7	0.4075
Time within season	9,396	2.19	0.0222
Time*Man	9,396	0.37	0.9468
Time*River	9,396	0.98	0.4531
Time*River*Man	9,396	0.31	0.97
Time* Position	9,396	0.74	0.6708
Time*Man* Position	9,396	0.34	0.9619
Time*River*Position	9,396	0.99	0.4501
Time*River*Man*Position	9,396	0.32	0.9678
Time*Hail	9,396	0.74	0.6682
Time*Man*Hail	9,396	0.93	0.4987
Time*River*Hail	9,396	1.26	0.2586
Time*River*Man*Hail	9,396	0.66	0.7409
Time*Hail*Position	9,396	0.26	0.9856
Time*Man*Hail*Position	9,396	0.68	0.728
Time*River*Hail*Position	9,396	0.61	0.786
Time*River*Man*Hail*Position	9,396	0.55	0.8387

Table 4.4. Statistical analysis outputs for the motile forms of *H. halys*. The following abbreviations were used in this table: “Man” for management strategy (organic or conventional), “Hail” for the presence of hail netting, “River” for proximity to river, “Position” for position within the orchard (border or center), and “Time” for the time within the season.

Effect	***df***	***F***	***P***
Management strategy	1,44	2.24	0.1419
Proximity to river	1,44	21.65	<.0001
River*Man	1,44	3.09	0.0859
Position within orchard	1,44	9.3	0.0039
Man*Position	1,44	2.2	0.1455
River*Position	1,44	0.33	0.5687
River*Man* Position	1,44	0.87	0.3573
Presence of anti-hail netting	1,44	5.34	0.0256
Man*Hail	1,44	0.89	0.3509
River*Hail	1,44	1.19	0.282
River*Man* Hail	1,44	1.19	0.282
Hail*Position	1,44	0.31	0.5776
Man*Hail* Position	1,44	0.03	0.8747
River*Hail*Position	1,44	1.76	0.1915
River*Man* Hail*Position	1,44	0.13	0.7166
Time within season	9,396	4.52	<.0001
Time*Man	9,396	0.86	0.5594
Time*River	9,396	1.12	0.3448
Time*River*Man	9,396	0.51	0.8674
Time* Position	9,396	0.39	0.9387
Time*Man* Position	9,396	0.36	0.9542
Time*River*Position	9,396	1.24	0.2677
Time*River*Man*Position	9,396	0.31	0.971
Time*Hail	9,396	0.65	0.7583
Time*Man*Hail	9,396	0.64	0.7618
Time*River*Hail	9,396	1.12	0.3442
Time*River*Man*Hail	9,396	1.3	0.2368
Time*Hail*Position	9,396	0.26	0.9854
Time*Man*Hail*Position	9,396	0.57	0.8193
Time*River*Hail*Position	9,396	0.55	0.8337
Time*River*Man*Hail*Position	9,396	0.28	0.9807

Table 4.5. Statistical analysis outputs for the internal and external feeding injury to the kiwifruits assessed in this experiment. The following abbreviations were used in this table: “Man” for management strategy (organic or conventional), “Hail” for the presence of hail netting, “River” for proximity to river, and “Position” for position within the orchard (border or center).

Type	**Effect**	***df***	χ^2	***P***
External	**Management strategy**	1,8	9.22	0.0024
External	**Proximity to river**	1,8	0.71	0.3979
External	**River*Man**	1,8	1.11	0.292
External	**Position within orchard**	1,8	1.45	0.2292
External	**Man*Position**	1,8	2.02	0.1548
External	**River*Position**	1,8	0.16	0.6872
External	**River*Man*Position**	1,8	1.05	0.3051
External	**Presence of anti-hail netting**	1,8	12.73	0.0004
External	**Man*Hail**	1,8	0.15	0.7003
External	**River*Hail**	1,8	0.07	0.795
External	**River*Man*Hail**	1,8	0.31	0.5785
External	**Hail*Position**	1,8	0.17	0.6843
External	**Man*Hail*Position**	1,8	1.28	0.258
External	**River*Hail*Position**	1,8	1.1	0.2938
External	**River*Man*Hail*Position**	1,8	0.09	0.7585
Internal	**Man**	1,8	7.09	0.0077
Internal	**River**	1,8	0.63	0.4274
Internal	**River*Man**	1,8	0.45	0.5019
Internal	**Position**	1,8	0.48	0.4884
Internal	**Man*Position**	1,8	0.29	0.5884
Internal	**River*Position**	1,8	0	0.9671
Internal	**River*Man*Position**	1,8	0.28	0.5982
Internal	**Hail**	1,8	5.91	0.015
Internal	**Man*Hail**	1,8	1.04	0.3088
Internal	**River*Hail**	1,8	0.29	0.5871
Internal	**River*Man*Hail**	1,8	2.53	0.1117
Internal	**Hail*Position**	1,8	0.11	0.7419
Internal	**Man*Hail*Position**	1,8	2.58	0.1082
Internal	**River*Hail*Position**	1,8	1.75	0.1855
Internal	**River*Man*Hail*Position**	1,8	0.01	0.9306

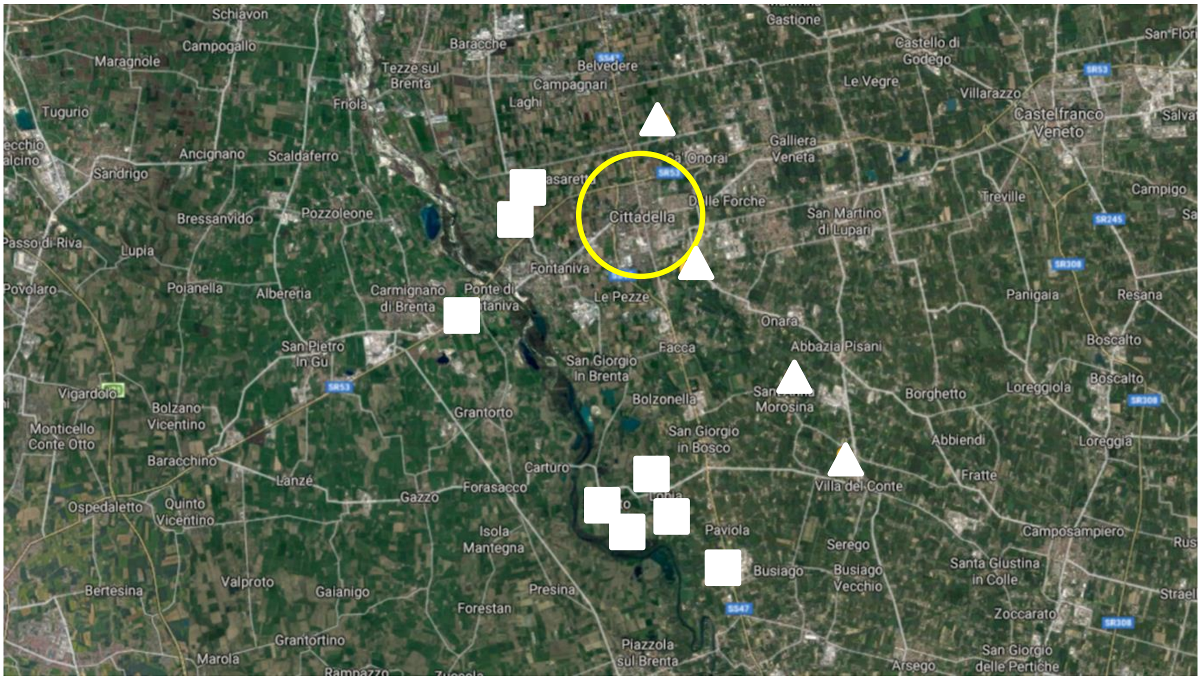

Figure 4.1. The 12 kiwifruit orchards near the city of Cittadella. The yellow circle represents the city of Cittadella. The squares indicate orchards that are near the Brenta River and the triangles indicate orchards that are far from the river.

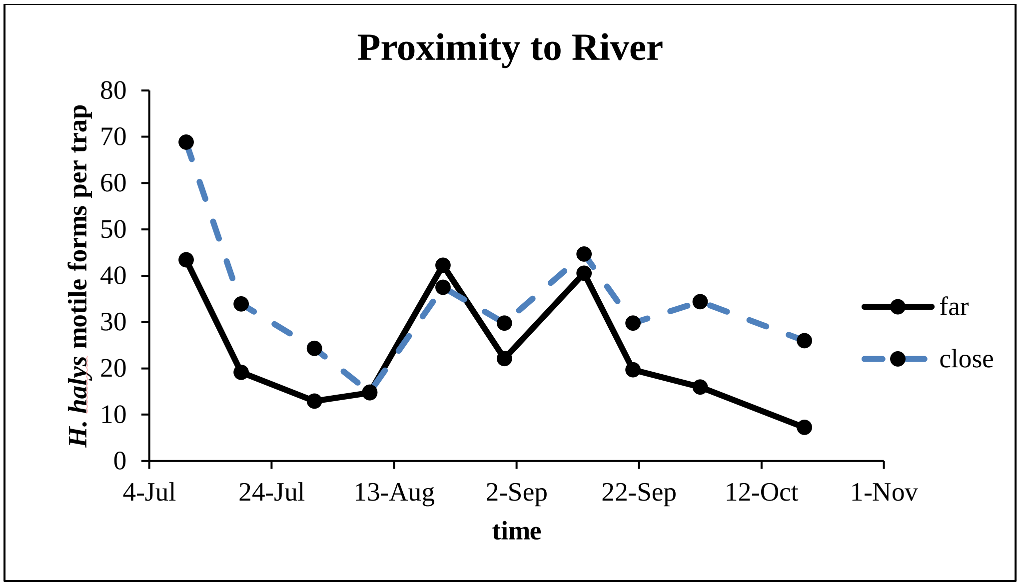

Figure 4.2. Density of *H. halys* motile forms captured on traps during the study in orchard close and far from the Brenta river. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of *H. halys* motile forms collected per adhesive pheromone trap is displayed on the y-axis.

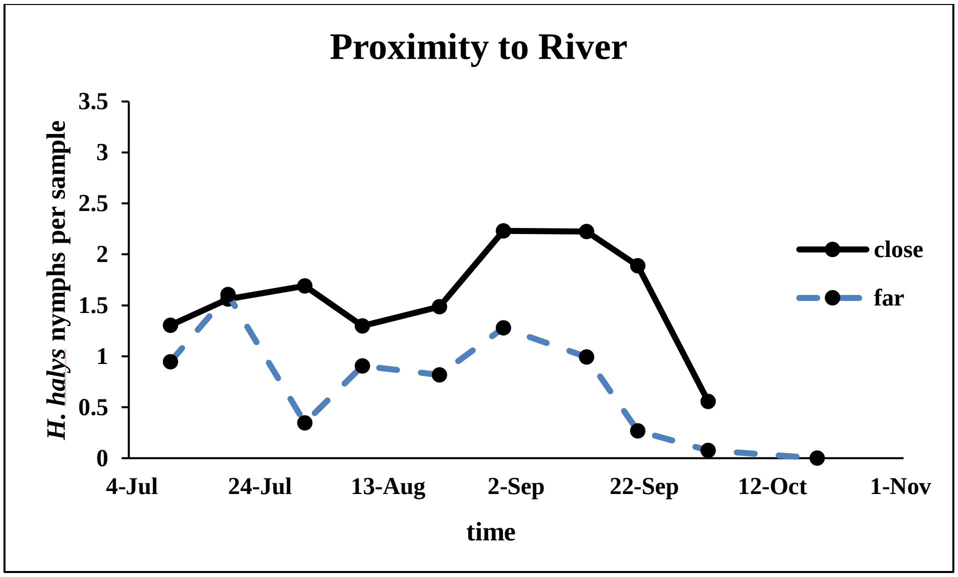

Figure 4.3. Density of *H. halys* nymphs observed on plants during the study in orchard close and far from the Brenta river. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of nymphal *H. halys* collected per beating sheet sample is displayed on the y-axis.

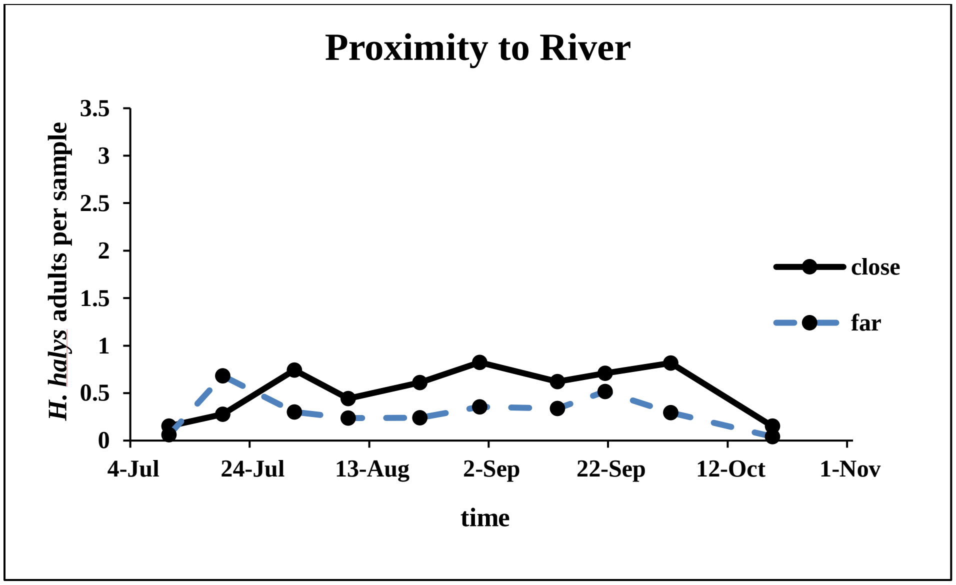

Figure 4.4. Density of *H. halys* adults observed on plants during the study in orchard close and far from the Brenta river. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of adult *H. halys* collected per beating sheet sample is displayed on the y-axis.

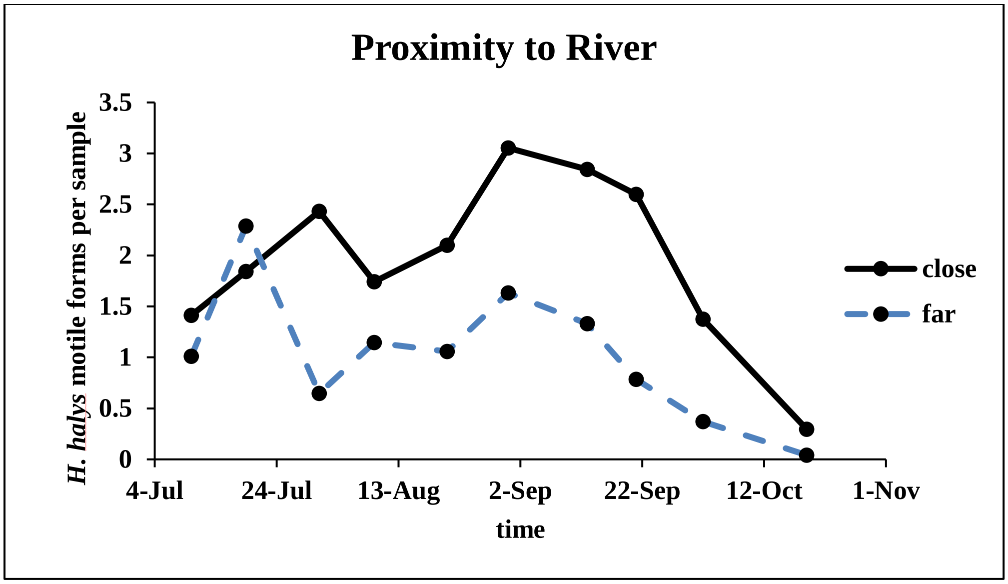

Figure 4.5. Density of *H. halys* motile forms observed on plants during the study in orchard close and far from the Brenta river. Proximity to the Brenta River effect on *H. halys* densities. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of *H. halys* motile forms collected per beating sheet sample is displayed on the y-axis.

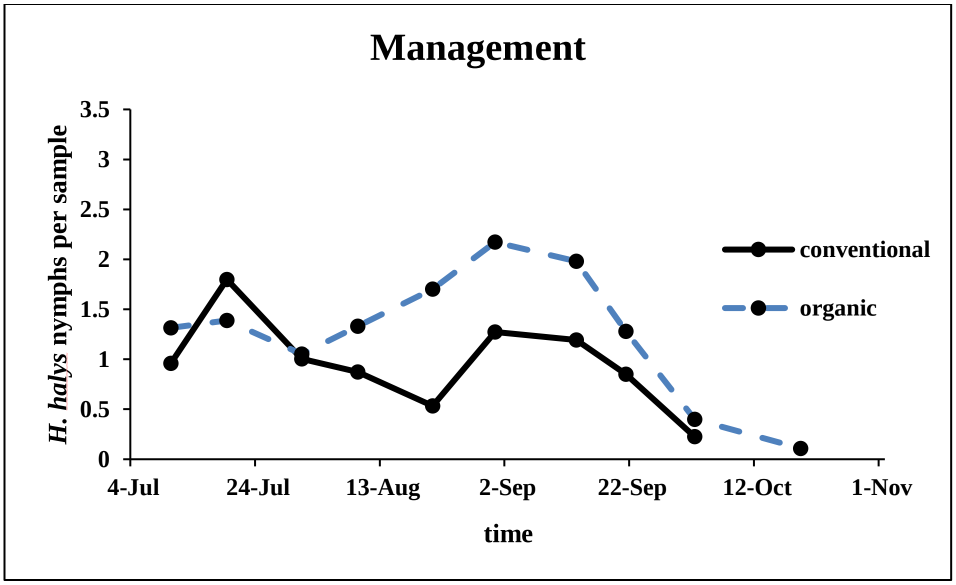

Figure 4.6. Density of *H. halys* nymphs observed on plants during the study in organic and conventional orchards. Effect of the orchard management strategy (organic vs. conventional) on *H. halys* nymphal population density. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of nymphal *H. halys* collected per beating sheet sample is displayed on the y-axis.

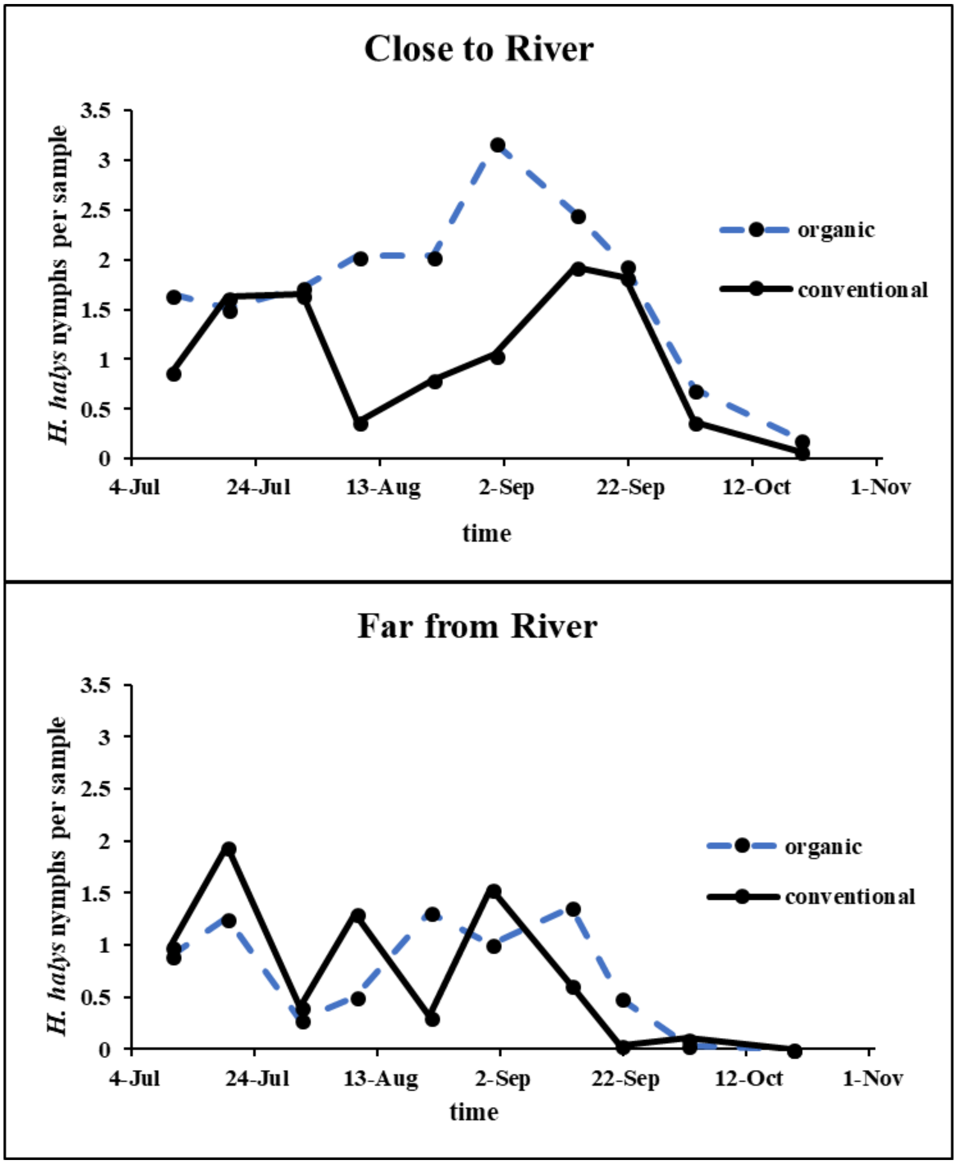

Figure 4.7. Density of *H. halys* motile forms observed on plants during the study in organic and conventional orchards located at different distance to Brenta river. The period within the kiwifruit growing season that each sample was taken is represented on the x-axis of each graph and the average number of *H. halys* motile forms collected per beating sheet sample is represented on the y-axis of each graph. The top graph represents the effect on orchards that were in close proximity to the Brenta River while the bottom graph represents the orchards that were not in close proximity to the Brenta River.

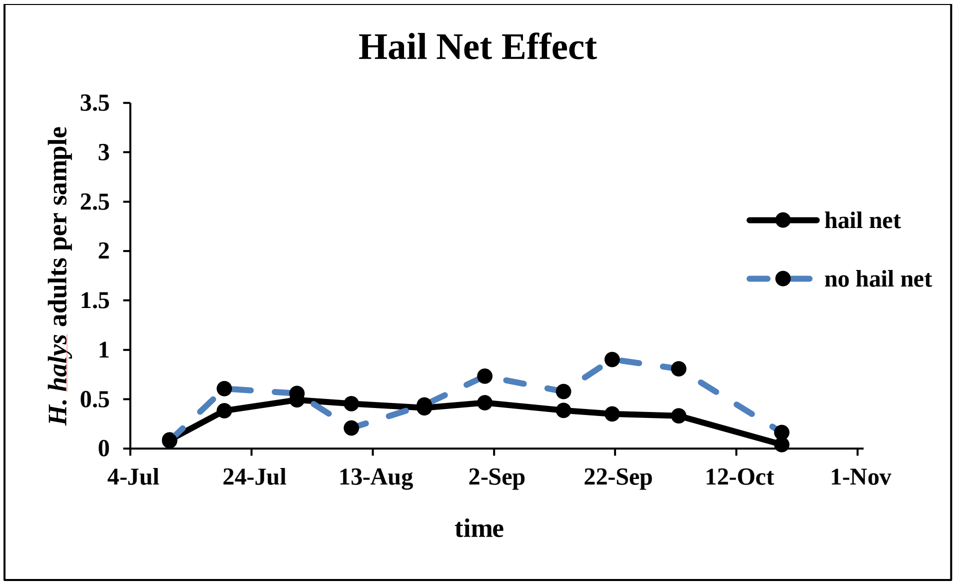

Figure 4.8. Density of *H. halys* adults observed on plants during the study in orchards with or without anti-hail net. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of adult *H. halys* collected per beating sheet sample is displayed on the y-axis.

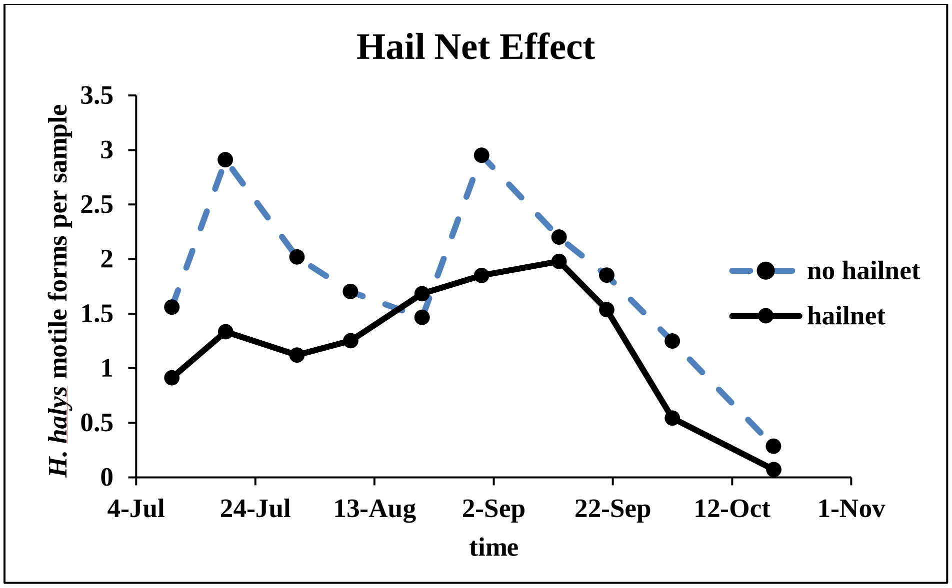

Figure 4.9. Density of *H. halys* motile forms observed on plants during the study in orchards with or without anti-hail net. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of *H. halys* motile forms collected per beating sheet sample is displayed on the y-axis.

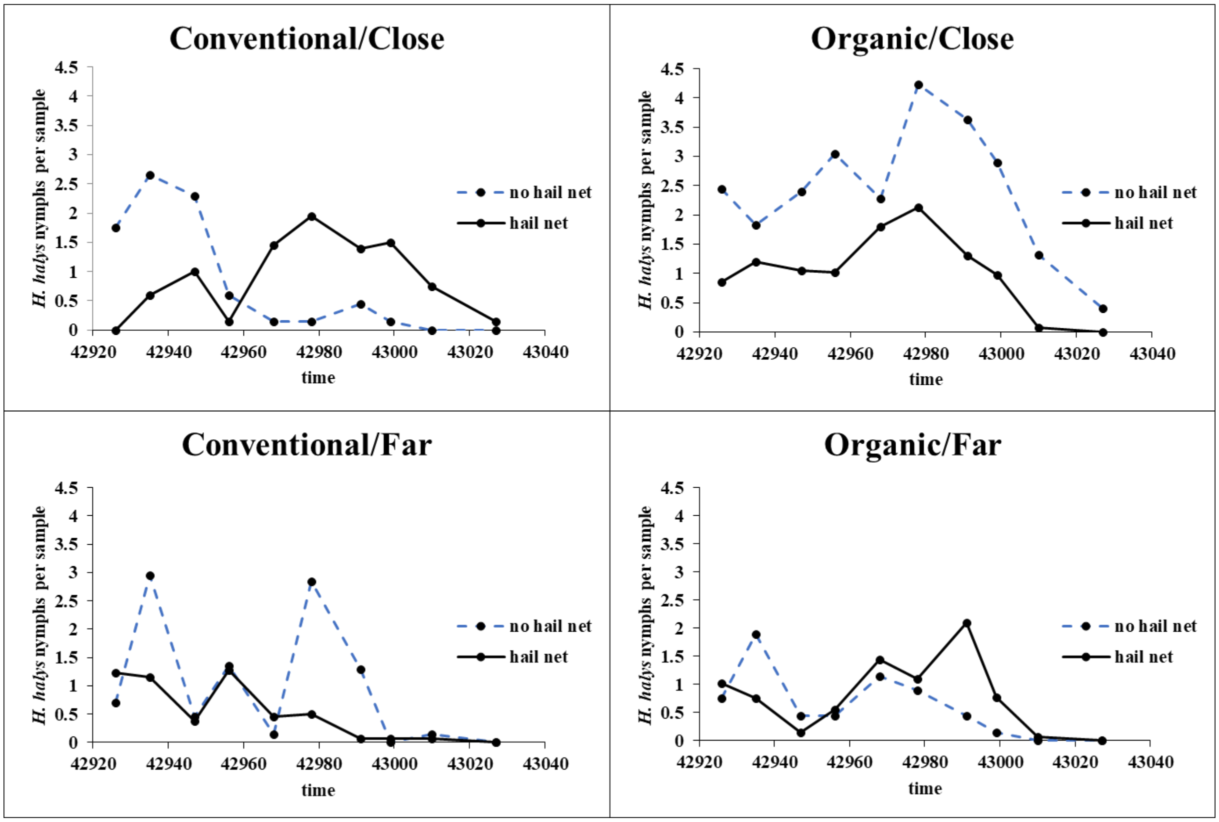

Figure 4.10. Density of *H. halys* nymphs observed on plants during the study in orchards with or without anti-hail net and at different position form the Brenta River. The interaction between the proximity to the Brenta River, the management strategy (organic or conventional), the presence of anti-hail netting, and time of the kiwifruit growing season in relation to the number of nymphal *H. halys* captured per sample.

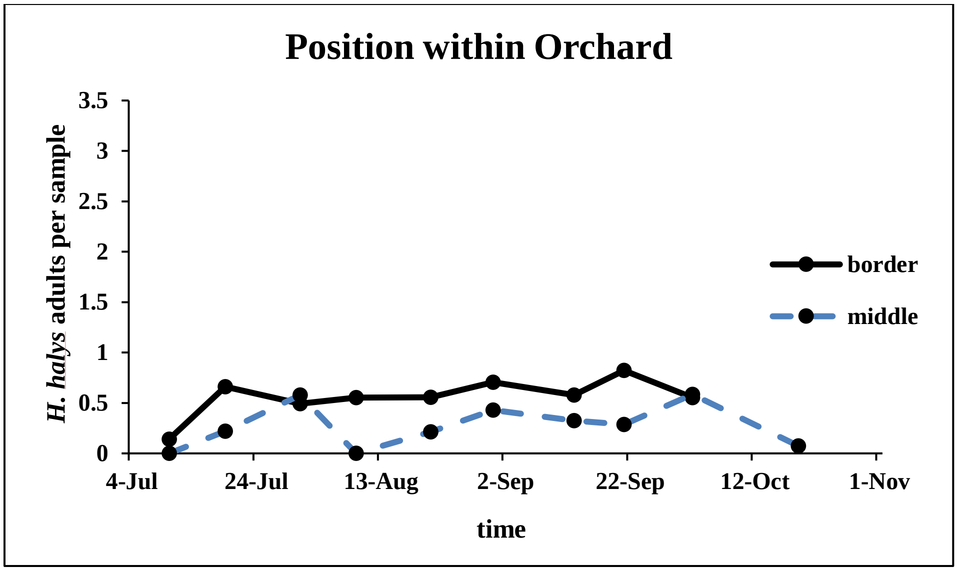

Figure 4.11. Density of *H. halys* adults observed on plants during the study at the border or center of the orchards. Effect of location within kiwifruit orchards (orchard border vs. orchard middle) on *H. halys* densities. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of adult *H. halys* collected per beating sheet sample is displayed on the y-axis.

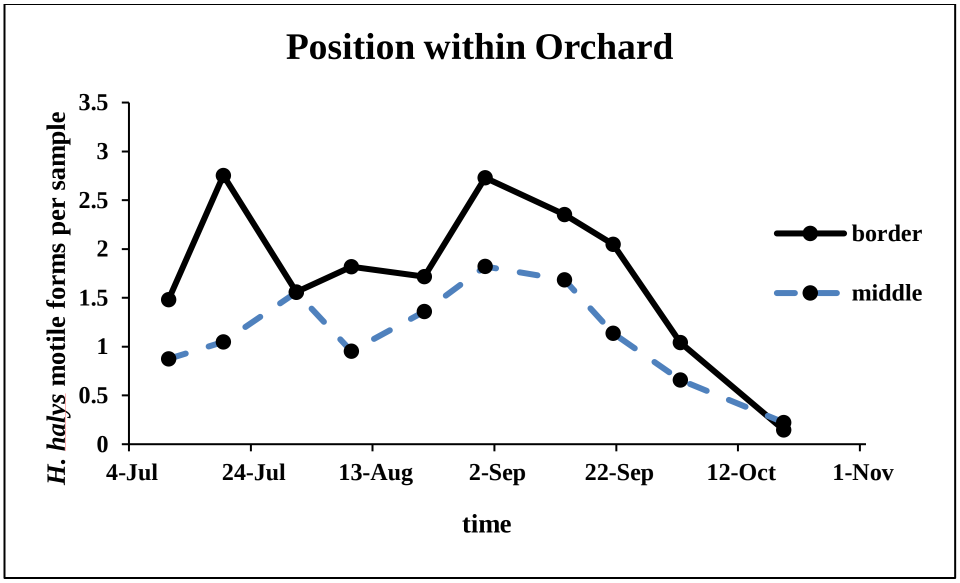

Figure 4.12. Density of *H. halys* motile forms observed on plants during the study at the border or center of the orchards. The period within the kiwifruit growing season that the samples were collected is displayed on the x-axis and the average number of *H. halys* motile forms collected per beating sheet sample is displayed on the y-axis.

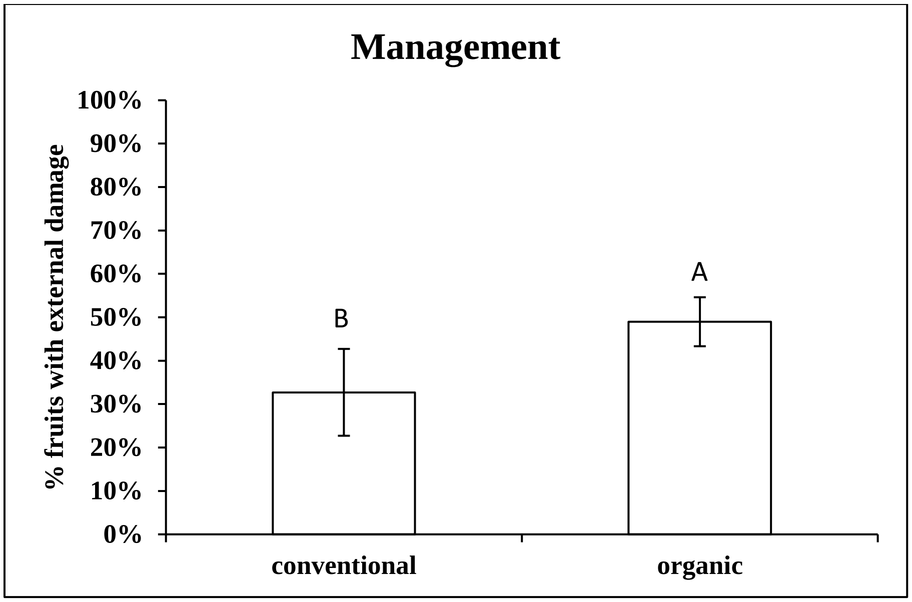

Figure 4.13. Proportion of externally damaged fruit in conventional and organic orchards. The management strategy is displayed on the x-axis while the average external fruit damage severity per fruit is displayed on the y-axis. Different letters indicate significant differences at t-test with Bonferroni adjustment on least-square means (P = 0.05)

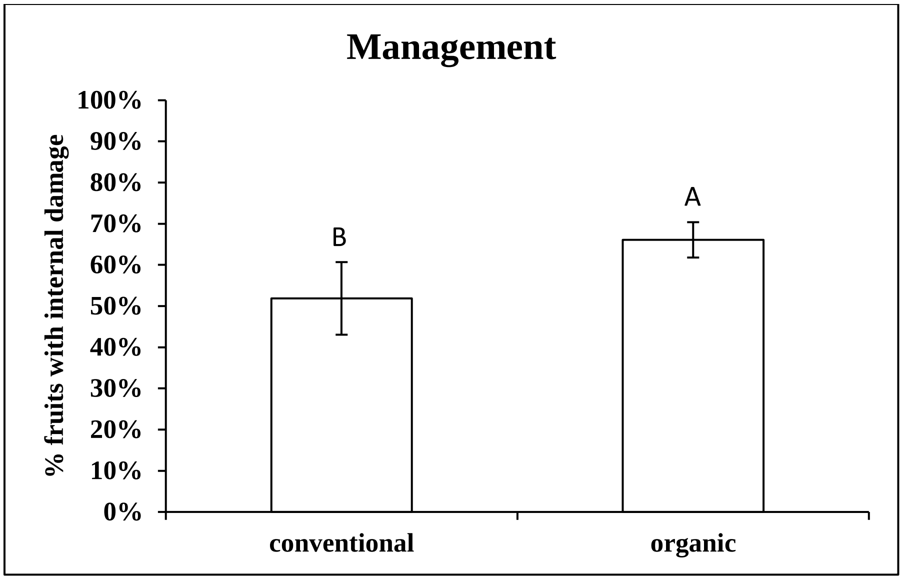

Figure 4.14. Proportion of internally damaged fruit in conventional and organic orchards. The management strategy is displayed on the x-axis while the average internal fruit damage severity per fruit is displayed on the y-axis. Different letters indicate significant differences at t-test with Bonferroni adjustment on least-square means (P = 0.05)

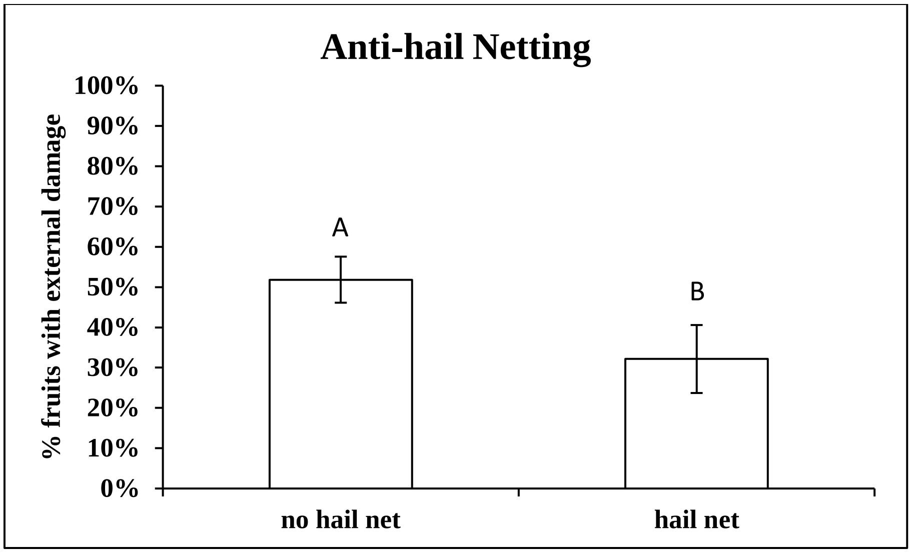

Figure 4.15. Effect of anti-hail netting on *H. halys* inflicted external fruit damage within the kiwifruit orchards. The presence or absence of anti-hail netting is displayed on the x-axis while the average external fruit damage severity per fruit is displayed on the y-axis. Different letters indicate significant differences at t-test with Bonferroni adjustment on least-square means (P = 0.05).

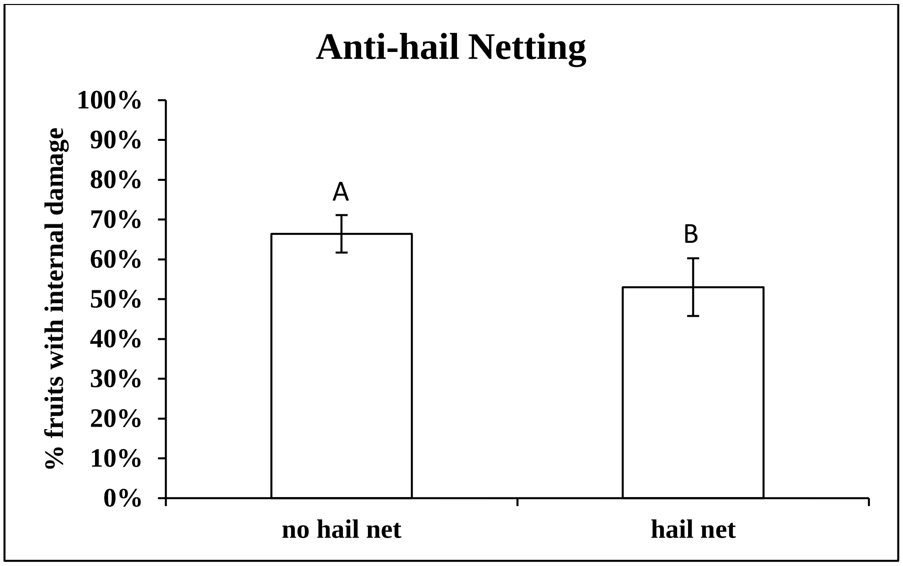

Figure 4.16. Effect of anti-hail netting on *H. halys* inflicted internal fruit damage within the kiwifruit orchards. The presence or absence of anti-hail netting is displayed on the x-axis while the average internal fruit damage severity per fruit is displayed on the y-axis. Different letters indicate significant differences at t-test with Bonferroni adjustment on least-square means (P = 0.05).

5. GENERAL CONCLUSIONS

Halyomorpha halys is an invasive pest insect that is rapidly spreading across the world. Its polyphagous feeding habits are putting various crops across the world in danger, for some of which the damage potential of this insect has not yet been assessed. By conducting a cage study in the field, we were able to assess the damage that *H. halys* causes to cherry fruits. *H. halys* feeding injury has been observed and described on many crops in the past, however this is the first evidence and description of the damage on cherry. Our trial showed strong evidence that *H. halys* has the ability to cause significant damage to cherry fruits in the form of fruit dislodging, decreased fruit weight, catfacing, as well as having an association with the fungal disease *Monilia laxa*. We speculate that, if infestation density is high enough and if time of exposure is long enough, then severe crop damage could take place and economic losses may occur if proper management actions are not taken.

We also evaluated the potential of *H. halys* to cause damage to kiwifruit and were among the first to describe the observed damage. According to our results, it is evident that *H. halys* has the ability to cause significant damage to kiwifruit in the form of premature fruit dislodging, internal corking of the pulp, external blackening of the epicarp, and decreased dry matter content. If high densities of *H. halys* are present during the middle of the kiwifruit growing season, then severe economic losses could occur. Our data could contribute to an analysis of economic thresholds in the future. Proper management practices may be necessary in orchards exposed to feeding *H. halys*.

It has been established that kiwifruit is considered a crop that may be subject to *H. halys* feeding, however the spatial and temporal dynamics of this insect within kiwifruit orchards and within the landscape in regards to kiwifruit orchard proximity to major river riparian buffers has not yet been assessed prior to this experiment. Our results show that the semi-natural habitats within the riparian buffers have a significant effect on *H. halys* population densities within nearby kiwifruit orchards. We also found that *H. halys* tends to aggregate around the edges of kiwifruit orchards rather than moving towards the middles of the orchards. One of the results that we found indicate that organic kiwifruit orchards are more at risk of *H. halys* invasion than conventionally managed orchards. The lack of control options for organic kiwifruit growers indicate that further research on more effective control options for organic farmers is necessary. We found that there were less insects within orchards that are equipped with anti-hail netting, indicating that anti-hail netting and netting in general may be a useful tool for *H. halys* management. Understanding the spatiotemporal patterns of this insect in regards to the landscape can aide in infestation predictions. The edge mediated effect that we found could be used in IPM programs. Spraying only the edges of orchards will lessen the likelihood of resistance to insecticides as well as benefit the farmer economically by using less resources and contributing less to environmental degradation.

The identification of this insect pest within cherry and kiwifruit orchards is very important and severe fruit damage may result from its presence. Cage studies provide a useful tool for *H. halys* damage assessment on various crops. Kiwifruit and cherry growers need to be aware of this insect’s ability to cause damage and preventative or combative measures may be necessary to protect from economic losses. The information presented will allow insight to *H. halys* behaviors in and around the orchards as well as allowing growers to identify damage caused by this insect.

Growers may use this knowledge to target this insect with precision, which could benefit the grower financially as well as benefitting the environment by using pesticides more conservatively.

Potential future research topics may be focused on developing economic thresholds for *H. halys* on kiwifruit and on cherry as well as investigating various integrated pest management strategies for these two crops. The potential impact of *H. halys* to kiwifruit orchards in areas where the insect is not yet present, such as New Zealand, should be assessed and invasions should be monitored for and prevented. Variety susceptibility of kiwifruit and cherry to *H. halys* should also be investigated to understand which varieties may be most at risk of invasion. Investigation of alternative host plants present within the semi-natural areas correlated with riparian buffers should also be a topic of future research. The information obtained from these experiments provide an excellent foundation for future studies relating to *H. halys* effects on kiwifruit and cherry.

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