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ECOLOGY AND INTEGRATED PEST MANAGEMENT OF MAJOR INSECT PESTS OF FIELD CORN

A dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy.
Entomology

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by
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May 2024

Accepted by:
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ABSTRACT

In the southeastern United States, the brown stink bug, *Euschistus servus* (Say), and the southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), are common economic pests of corn, Zea mays L. (Poaceae: Poales). Although commonly found, the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), is generally not an economic pest and is managed with transgenic corn hybrids expressing toxins produced by the *Bacillus thuringiensis* (Bt). Resistance to Bt toxins is a major thread to the sustainability of Bt crops. Understanding the ecology of stink bugs and corn earworms in field corn can help to effectively implement integrated pest management (IPM) and insecticide resistance management (IRM). We performed field studies to assess the spatial patterns of stink bugs, corn earworm, and their respective injuries in corn fields, understand the spatial interactions of these pests with plant phenology, monitor sublethal impacts of Bt toxins on corn earworm, and determine how corn stakeholders perceive the relative importance of these pests and implement management. We identified patterns of spatial aggregation of stink bugs and corn earworm. For stink bugs, spatial associations between bugs and ear injury were identified more commonly when stink bugs were sampled during the late vegetative stages of development. Both pests were also spatially associated with plant phenology that varied throughout a corn field. Corn earworm larval populations, in particular, were tightly linked with areas of the field that were more advanced through corn growth stages when sampled. We were the first to identify a competitive interaction between stink bugs and corn earworm in these sampling studies and in a follow up controlled trial. Stink bug ear injury and feeding resulted in a significant decline in corn earworm oviposition, larval density, and total larval feeding across four trials in two study years. In a study assessing the sublethal impacts of Bt toxins on corn earworm, we identified a continued decline in the impact of Bt corn expressing Cry1A.105 + Cry2Ab2 and Cry1Ab + Cry1F on pupal weight over nine years (2014-2023) due to resistance development. Finally, in a survey of corn stakeholders, we identified the most important insect pest considerations for growers, their reasonings for implementing various IPM and IRM strategies, and knowledge and use of IPM. Grower selections for hybrids and seed treatments were heavily dependent on perceived yield potential and industry recommendations rather than specific insect management concerns. Only 59% of growers surveyed indicated that they planned to plant non-Bt refuge, which is a major issue for IRM success. The results of these studies will aid in effective sampling and management of several major pest of field corn.

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Field corn growth and development

Field corn (*Zea mays* L.) is one of the most important cereal crops in the world. In the United States, 38 million hectares were planted in 2021, accounting for 10% of arable farmland (USDA NASS 2021). In 2020, field corn sales totaled US $51 billion (USDA NASS 2021). The primary use for field corn grain in the United States is for livestock feed, followed by alcohol production for industrial use, and food and industrial byproducts such as corn syrup, corn meal, and starch.

Corn growth stages and management concerns throughout the season are summarized from Ritchie et al. (1993). Corn develops through a number of vegetative growth stages and six reproductive growth stages (Hanway 1966). From emergence (VE), the vegetative stages of corn growth are defined by the number of fully opened leaves on the plant, which can be as many as 18 leaves. This number is dependent on hybrid and growing conditions. During germination, emergence, and early vegetative stages of development, corn is susceptible to injury by several groups of insect pests. Pests, including corn rootworms, *Diabrotica* spp. (Coleoptera: Chrysomelidae), wireworms, *Melanotus communis* Gyllenhal (Coleoptera: Elateridae), seed corn maggots, *Delia platura* Meigen (Diptera: Anthomyiidae), sugarcane beetles, *Eutheola humilis* Burmeister (Coleoptera: Scarabaeidae), and white grubs (Coleoptera: Scarabaeidae), feed on seed, roots, or shoot tissue below ground early in the season (Sappington et al. 2018). A complex of stink bugs (Hemiptera: Pentatomidae), billbugs (Coleoptera: Curculionidae), and cutworms (Lepidoptera: Noctuidae) feed on seedling plants above ground and can either clip plants or feed directly on the growth point, leading to stunting, growth deformities, or plant death (Sappington et al. 2018). Beyond the early stages of vegetative growth, defoliating insects, such as fall armyworm, *Spodoptera frugiperda* (J.E. Smith), can continue to feed on corn and have the potential to limit yield if feeding is severe enough (Marenco et al. 1992). Water and nutrient availability are critical during these early stages, and side dress applications of nitrogen are used in most management programs.

The final vegetative growth stage is tasseling (VT), which is defined as the last branch of the tassels being completely emerged from the top of the plant. Pollen is shed from the tassels during the first reproductive stage of corn, which results in pollination of the ear. The reproductive growth stages of corn are silking (R1), blister (R2), milk (R3), dough (R4), dent (R5), and physiological maturity (R6). At physiological maturity, each kernel will develop a thin black layer at the base. When the black layer forms, the corn is typically between 25% and 40% moisture content. Pollination is actively occurring during the VT and R1 stages when both the tassels and silks are fully emerged and tassels are opened. During reproductive stages, corn can be susceptible to damage from insect pests that feed directly on the developing ear. Common pests during these stages include a complex of stink bugs, western bean cutworm, *Striacosta albicosta* Smith (Lepidoptera: Noctuidae), fall armyworm, and corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae). Injury from ear-feeding pests can also impact the quality of grain by facilitating introduction of fungi and subsequent mycotoxin contamination (Ni et al.

2011, Ni et al. 2014, Opoku et al. 2019), in addition to allowing infestations of secondary insect pest species such as sap beetles. While there are a number of sporadic pests of corn that can cause damage during various stages of corn development under the right conditions (Sappington et al. 2018), the two most common insect pests of field corn in South Carolina are corn earworm and a complex of stink bug species (Cartwright 1939).

Corn earworm identification, life cycle, and damage

Corn earworm is the most common insect pest of corn in South Carolina (Cartwright 1939). Many species in this family (Lepidoptera: Noctuidae) are common pests of cultivated crops throughout the world, including tobacco budworm, *Heliothis virescens* (Fabricus), old world bollworm, *Helicoverpa armigera* (Hübner), black cutworm, *Agrotis ipsilon* (Hufnagel), and fall armyworm (Johnson 1979, Showers et al. 1983, Cunningham and Zalucki 2014, Baudron et al. 2019). Corn earworm successfully overwinters as a pupa in the soil at latitudes below 40 degrees (Hardwick 1965) and reaches the rest of its range in North America via annual migrations. Corn earworm adults are capable of flying at least 750 km, allowing them to infest corn throughout most of the United States (Hendrix et al. 1987, Lingren et al. 1994). The number of generations produced in a year varies in North America from one generation in Ontario, Canada, to seven generations in south Texas (Quaintance and Brues 1905). The lifecycle of the corn earworm from egg to mature adult can be completed in around 30 days (Hardwick 1965). Additional descriptions and life cycle information are summarized from Quaintance and Brues (1905) and Hardwick (1965).

Corn earworm undergoes a holometabolous life cycle that includes egg, larva, pupa, and adult stages. The eggs of corn earworm are dome-shaped and around 0.5 mm in height and 0.48 mm in width with a flat base. Eggs are pale white when freshly deposited, turning tan after the first day, and have around 14 rib-like ridges spanning the entire egg. Eggs are laid singly, often in early evening during peak moth flights. A single female moth can lay up to around 3,000 eggs, but fecundity is dictated by the quality of diet, and the number of eggs can vary significantly between moths (Gross and Young 1977). Eggs general hatch within 2-3 days; however, egg duration is highly dependent on temperature.

When larvae eclose from eggs they are referred to as neonates and are around one mm in length. Corn earworm larvae develop through six instars, and it takes 16 days on average to progress through all six stages. Larvae reach 42.2 mm on average at full growth. The main colors of larvae can vary widely from brown, green, yellow, or pink. The head capsules of larvae are generally orange to light brown and range in width from 0.29 mm in first instars to 3.1 mm for sixth instars. The pupa is similar to many other species in the family Noctuidae. They are shiny red to dark brown in color and 14 to 23 mm in length. The pupal stage lasts for around 12-14 days. Pupation occurs in the soil at an average depth of 46 mm, with depth likely a function of soil type, moisture content, and pupal size (Quaintance and Brues 1905). Adult moths average 18 mm in length and 40 mm in wingspan. Moths vary in color and wing markings, with forewings generally brown in color and often displaying a small dark spot in the center. The forewing also commonly has a darker colored band on the outer edge. The hind wings are white basally and dark brown to black distally, bearing the same dark spot as the forewing. Moths can live for 15 days on average.

Corn earworm can feed on vegetative stages of field corn, but this is rare (Quaintance and Brues 1905, Arends 2020). The primary feeding by corn earworm occurs on silks and ears. Most eggs are laid directly on the silks, and, after feeding on the egg shell (Quaintance and Brues 1905), larvae feed down the silk channel into the ear. Larvae can complete development by feeding on silks and the tip of the ear alone, without feeding on any harvestable kernels (Quaintance and Brues 1905, Hardwick 1965). The kernels on the tip of corn ears are generally not pollinated and do not contribute to the overall yield, meaning corn earworm feeding, which is commonly restricted to this area, does not impact yield in many cases (Reay-Jones and Reisig 2014, Bibb et al. 2018, Olivi et al. 2019). Yield is not impacted until between 40-60 kernels are damaged (Olivi et al. 2019).

Management of corn earworm

Chemical control. Foliar insecticide applications are often ineffective for managing corn earworm, as larvae are protected from insecticide contact by the husk of the corn ear (Widstrom et al. 1976). One study found that, even with an intensive weekly insecticide program, kernel injury can still occur as a result of corn earworm feeding (Reay-Jones and Reisig 2014). The timing of an insecticide application is critical for targeting the egg stage or larvae prior to entering into the ear, making it difficult and cost prohibitive for growers to employ. In sweet corn grown for the fresh market, there is very little tolerance for cosmetic injury caused by corn earworm which leads to a large number of insecticide applications targeting corn earworm eggs (Olmstead et al. 2016).

Cultural control. Host plant resistance to corn earworm via tolerance, non-preference, and antibiosis has been reported in corn (Wiseman and Davis 1990). Corn hybrids with tight husks and silk channels have been demonstrated to reduce corn earworm damage via reduced physical access for the corn earworm to kernels (Ni et al. 2007). Early planting can avoid peak corn earworm pressure in corn which typically occurs later in the season (Buntin et al. 2004). Additionally, deep tillage practices can reduce the number of overwintering pupae in the soil by either disrupting emergence ability or causing direct mortality (i.e. “pupae busting”) (Barber and Dicke 1937).

Biological control. Corn earworm eggs and larvae can be parasitized in several host crops, including corn (Puterka et al. 1985). In one study using field collected eggs and larvae, 28.3% of eggs and 9% of larvae were parasitized. *Trichogramma* and *Telenomous* species of parasitoids were the most commonly found parasitoids in a Texas study (Puterka et al. 1985). Nucleopolyhedroviruses (NPV) can also infect corn earworm and be applied as a biopesticide (Pingel and Lewis 1999, Black et al. 2022).

Transgenic Bt corn

Transgenic corn hybrids, expressing toxins produced by the naturally occurring soil bacterium *Bacillus thuringiensis* (Bt), are the most commonly employed management strategy for corn earworm and provide varying levels of control. In 2023, 85% of all corn planted in the United States expressed one or more of these Bt toxins (USDA 2023). *Bacillus thuringiensis* is characterized by its ability to produce crystalline and vegetative toxins during sporulation, many of which can be toxic to insects (Hofte and Whiteley 1989). These crystalline toxins work by embedding in the mid-gut of insect pests and causing it to rupture, leading to mortality (Gill et al. 1992). Insertion of DNA coding for Bt toxins into the corn genome allowed the development of hybrids which produce these toxins endogenously. Corn hybrids expressing Bt were first commercially introduced in 1996 to manage the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Noctuidae), and other stalk boring species (Koziel et al. 1993). Initially, these hybrids expressed only a single toxin (Cry1Ab) and provided excellent control for corn borers (Marcon et al. 1999). The widespread adoption of these hybrids and high level of efficacy for European corn borer, specifically, resulted in area-wide suppression of this pest across a range of cultivated crops (Hutchinson et al. 2010, Dively et al. 2018). The first commercial Bt hybrids expressing Cry1Ab or Cry1F have, for the most part, remained highly effective for corn borer, but resistance to these toxins was recently identified in Canada (Smith et al. 2019, Smith and Farhan 2023).

Early single toxin Bt corn traits (i.e., expressing Cry1Ab or Cry1F) had limited efficacy for corn earworm (Siegfried et al. 2000, Buntin et al. 2004). Since initial introduction of single toxin hybrids, more recent Bt products have been developed expressing multiple toxins, known as pyramided hybrids (Table 1). Multi-toxin hybrids now represent the majority of the market (Tabashnik et al. 2013). Some pyramided hybrids, such as Cry1A.105 + Cry2Ab2, initially provided better control for corn earworm (Reay-Jones et al. 2009, Siebert et al. 2012, Reay-Jones and Reisig 2014), while the combination of the early single toxins (i.e., Cry1F + Cry1Ab) only had moderately better efficacy than each individually (Reay-Jones and Reisig 2014, Reisig et al. 2015). The most recently introduced toxin in Bt corn, Vip3A20, is the only toxin to provide excellent control for corn earworm (Burkness et al. 2010).

Bt resistance management

The primary strategies for delaying the development of Bt resistance are the high dose/refuge (HDR) strategy and pyramiding of toxins (Gould 1998). The high-dose strategy theorizes that, if 95% of heterozygous resistant individuals are killed by the toxin, resistance can be significantly delayed. In corn, the refuge component involves planting a percentage of corn on a farm with non-Bt corn that can serve as a source of susceptible individuals which then can mate with potentially resistant individuals developed on Bt plants (Gould 1998). In the Corn Belt, refuge is planted via a percentage of non-Bt seed mixed into commercial bags of seed (i.e., blended refuge or “refuge in a bag”). In cotton growing regions and where the primary targets for Bt are ear-feeding pests, such as South Carolina, only a structured refuge can and should be used. The structured refuge must be planted in strips at least four rows wide within a Bt field, in larger blocks within a Bt field, or in a separate field within 0.8 km of the Bt field to be compliant with refuge requirements. In cotton growing regions, there is a minimum structured refuge requirement of 20% for corn expressing multiple toxins and 50% for corn expressing a single toxin.

The success of the HDR strategy is partially dependent on the genetics and ecology of the target species (Gould 1998, Siegfried and Hellmich 2012). The high-dose strategy relies on two assumptions; 1) resistance alleles are recessive and exceedingly rare in the population, and 2) Bt plants will express toxins in excess of 20-fold the dose to kill 99.9% of susceptible individuals (Gould 1998). The continued success of Bt hybrids for corn borer management is, at least in part, due to these assumptions holding true for these insects (Siegfried and Hellmich 2012), but that is likely not the case for corn earworm (Reisig et al. 2021). Many Cry toxins provided only moderate control for corn earworm at initial introduction (Buntin et al. 2004, Reay-Jones et al. 2009, Siebert et al. 2012, Reisig et al. 2015, Reay-Jones and Reisig 2014). The baseline frequency of non-recessive resistance alleles was also higher than expected for some toxins (Burd et al. 2003), and some alleles associated with Cry resistance have been found to be completely dominant (Yang et al. 2020). Use of Vip3A20 is closer to meeting the assumption of a HDR for corn earworm compared with Cry toxins (Burkness et al. 2010), but increasing frequencies of alleles conferring resistance to the newest trait have been detected at three different independent genetic loci (Yang et al. 2021, Yang et al. 2024). Another key assumption of the HDR strategy is that susceptible individuals that developed on the refuge plants will develop at the same rate as and mate with heterozygous resistant individuals from Bt plants (Gould 1998). Non-random mating patterns or differences in maturity times between susceptible and resistant individuals are potential challenges that could reduce the effectiveness of the refuge strategy (Gould 1998). The sublethal impacts of Bt exposure in resistant individuals resulting in delayed development time may indeed lead to assortative mating and reduce the effectiveness of refuge planting (Liu et al. 1999).

The success of the refuge strategy is also partially reliant on the willingness and ability of growers to comply with refuge requirements, particularly in areas where structured refuge is required. Surveys conducted by the Center for Science in the Public Interest found that 95% of growers were compliant with refuge requirements in 2004, but that dropped to 78% in 2008 (Jaffe 2009). A more recent survey of corn growers in North Carolina found that only 38.3-44.3% of growers indicating that they would plant refuge (Reisig 2017). Willingness to plant refuge is related to demographic factors, such as farm size and diversity of production (Reisig 2017). This survey also assessed potential strategies that would improve grower willingness to plant non-Bt refuge. Companies providing a rebate for non-Bt seed and providing non-Bt seed with competitive yields relative to Bt hybrids were the two most common strategies that growers indicated would improve compliance (Reisig 2017). It is important to note that ability is as important a factor in refuge adoption as willingness. The availability of non-Bt hybrids that yield competitively with Bt germplasm has steadily declined (Reisig and Kurtz 2018), and seed dealers are not incentivized to promote these products to producers. Because refuge compliance will prolong the efficacy of current Bt traits, continuing to assess growers' willingness to plant non-Bt corn and pushing industry towards more support for compliance are important considerations for tailoring Extension programming and policy aimed at preserving susceptibility to Bt traits.

While corn earworm is not an economic pest of corn, it is a major pest of cotton (*Gossypium hirustum* L.) (Luttrell and Jackson 2012). Corn earworm produces multiple generations per year in the southeastern United States, and moths that developed in field corn often disperse to cotton, amongst a number of other cultivated and wild hosts (Gould et al. 2002). Because Bt cotton varieties express many of the same or similar Bt toxins as Bt corn, selection pressure for resistance is increased by multiple exposures in a season. Continuing to monitor the development of resistance and implementing strategies to manage resistance are critical to prolonging the efficacy of Bt for managing corn earworm in cotton.

Status of Bt resistance in corn earworm

Deviations from the important assumptions of resistance management strategies for corn earworm and limited compliance with refuge planting have resulted in the development of Bt resistance to all available Cry toxins (Dively et al. 2016, Bilbo et al. 2019a, Reay-Jones et al. 2020, Yu et al. 2021). In diet overlay assays, resistance to Cry2Ab2 and Cry1A.105+Cry2Ab2 has been documented in field-collected populations in several studies (Bilbo et al. 2019a, Yu et al. 2021). In addition to lab studies comparing mortality dose responses, several studies have been conducted in the lab and the field to examine the sublethal effects of Bt toxins on corn earworm. Sublethal effects are changes in important life history traits of the insect as a result of insecticide exposure, including changes in size or weight, development time, flight and dispersal ability, or fecundity and egg viability. These sublethal effects may be associated with the toxicity of the toxin itself or with fitness costs of resistance.

A field study in North and South Carolina in 2012-2013 found no reduction in corn earworm pupal weight, pupal stadium, time to eclosion, or number of pupae in corn ears expressing Cry1Ab relative to non-Bt ears (Reisig and Reay-Jones 2015). This result was in contrast to a previous study which identified significant differences in these metrics between pupae from Cry1Ab and non-Bt corn (Storer et al. 2001), suggesting the evolution of corn earworm resistance to Cry1Ab over this period of time. Bilbo et al. (2018) found a significant reduction in pupal weight in pupae fed on Cry1F + Cry1Ab and Cry1A.105 + Cry2Ab2 corn relative to non-Bt corn, but this reduction decreased over the duration of the study. A follow-up study by Reay-Jones et al. (2020) incorporated data from Bilbo et al. (2018) with data from 2017-2020 and identified further degradation in these sublethal effects over time (Reay-Jones et al. 2020). Several studies have attempted to link pupal weight to life history traits of the subsequent generation. Bilbo et al. (2018) did not identify a link between pupal weight and egg viability or fecundity of the next generation. This was supported by Pezzini et al. (2023), which showed that pupal weight was unrelated to eclosion time or flight capacity of the subsequent generation. Continued work is needed to link sublethal effects to Bt exposure over time and determine implications for subsequent generations of corn earworm.

Vip3A20 remains the only highly efficacious toxin for corn earworm, but early warning signs for resistance have recently been reported in populations of corn earworm collected in Arkansas, Louisiana, Mississippi, Tennessee, and Texas (Yang et al. 2021). Several studies have also collected a small number of surviving larvae from ears expressing Vip3A20 (Bilbo et al. 2019b, Reay-Jones et al. 2020). Trends in increased injury and corn earworm infestations have also begun to emerge in sweet corn expressing Vip3A20 in a study involving plots in 16 states and 4 Canadian providences, further suggesting that resistance to Vip3A20 is beginning to develop (Dively et al. 2021). The resistance of corn earworm to pyramided Cry toxins and the potential for rapid development of resistance to Vip3A20 underpin the importance of continued resistance monitoring and management efforts.

Stink bugs as pests of corn

Several species of stink bugs are of economic importance in the United States, including brown stink bug, *Euschistus servus* (Say), green stink bug, *Chinavia hilaris* (Say), southern green stink bug, *Nezara viridula* (L.), and brown marmorated stink bug, *Halyomorpha halys* (Stal) (McPherson and McPherson 2000). Generally the two most common pest species in the southeastern United States are *E. servus* and *N. viridula* (Tillman 2010, Pilkay et al. 2015). Stink bugs are phytophagous pests that feed on a number of crop and non-crop hosts. In the southeastern landscape wheat (*Triticum aestivum* L.), cotton, soybean (*Glycine max* L.), sorghum (*Sorghum bicolor* L.), peanut (*Arachis hypogaea* L.), and corn are all suitable cultivated hosts (McPherson and McPherson 2000). Common mullein, *Verbascum thapsus* L., is reported to be both an overwintering as well as a suitable early season host for stink bugs and is readily available throughout most of North America (Rolston and Kendrick 1961, Munyaneza and McPherson 1994). Stink bugs have generally been considered a sporadic pest of corn in the past but have become more of a perennial issue for corn producers in recent years. Research on the factors driving these changes in the population dynamics of stink bugs in corn is limited.

Stink bug identification and life cycle

Brown stink bug. Stink bugs are hemimetabolous insects in the order Hemiptera and the family Pentatomidae. All species are generally oval or shield-shaped with a well-developed scutellum, piercing-sucking mouthparts, and five-segmented antennae. The brown stink bug occurs throughout most of the contiguous United States and parts of Canada. It is composed of two sub-species *Euschistus servus euschistoides*, occurring in the northern United States, and *Euschistus servus servus*, occurring in the southern United States. The brown stink bug produces two generations per year in most of North America (Munyaneza et al. 1994, MchPherson and McPherson 2000, Herbert and Toews 2012).

The adult brown stink bug is 11-15 mm in length and brown to light brown. The fourth and fifth antennal segments are darker in color (McPherson and McPherson 2000). The underside of the brown stink bug displays several different color morphs depending on the time of year and the physiological state of the insect (Babu et al. 2020). These colors range from reddish-brown, to yellow, to light or dark green. Generally, three population peaks in adult stink bugs are observed in farmscapes in the southeastern United States; one corresponding with overwintering emergence and one for each of the subsequent two generations (Munyaneza and McPherson 1994, Herbert and Toews 2012).

Brown stink bug eggs are cylindrical in shape and average 1.13 mm in height and 0.96 mm in width. Masses are arranged in rows of varying numbers of eggs making up a small circular mass. They are translucent yellow and develop a series of pinkish spots on the upper surface as they reach maturity. A single female can lay up to 18 egg masses, consisting of 8 to 41 eggs each and reported averages of between 17.6 and 19.1 per mass, depending on the study (Rolston and Kendrick 1961, Munyaneza and McPherson 1994). Eggs are generally laid on the host plant, allowing nymphs to disperse and feed after hatching. The incubation period for eggs ranges from 3 to 14 days, with averages of 4.8-5.8 days reported (Rolston and Kendrick 1961, Munyaneza and McPherson 1994).

Descriptions of the five nymphal stadia are briefly summarized from Munyaneza and McPherson (1994). The first instar averages 1.52 mm in length and 1.14 mm in width. First instars feed on the hatched egg shells prior to dispersing to feed on host plants. The head is yellowish brown to brown, with four-segmented antennae. Segments one through three are red, and the fourth segment is reddish brown to brown. The abdomen is a lighter brown color in comparison to the head and has several dark spots located centrally. Second instars average 2.42 mm in length and 1.53 mm in width and are similar in appearance to first instars. Third instars average 4.2 mm in length and 2.86 mm in width. Third instars have more numerous brown spots on the head, and a dark brown band develops along the posterior margin. The dark spots on the center of the abdomen appear reduced compared with the first and second instars. Fourth instars average 8.5 mm in length and 5.67 mm in width. The body of fourth instars is primarily pale green to yellowish in color, with a single patch of brown on the center of the abdomen and a yellowish margin on the abdomen and pronotum. Antennal segments one to three are reddish-brown in color, and the fourth segment is a dark brown. Fifth instars average 10.37 mm in length and 6.7 mm in width. Fifth instars are similar in appearance to fourth instars; however, the antennal segments lighten and become a more uniform reddish-brown color compared to fourth instars. The nymphal development length is highly dependent on temperature (Rolston and Kendrick 1961). Full development from egg hatch to a mature adult was found to be between 51.5 and 54.9 days in Virginia and an average of 44.3 days in laboratory reared colonies (Rolston and Kendrick 1961, Munyaneza and McPherson 1994).

Adult brown stink bugs overwinter in protected areas, such as ditch banks, weeds, field residues, or wooded areas. They generally prefer open overwintering sites over woody areas (Jones and Sullivan 1981, Munyaneza et al. 1994). Several studies conducted in South Carolina and Arkansas have demonstrated adult brown stink bugs successfully overwintering in crop residue, leaf litter, common mullein, wild radish, and other weeds at varying degrees of success (Rolston and Kendrick 1961, Jones and Sullivan 1981). The highest percent survival was found in cages with mullein and other weeds (Rolston and Kendrick 1961). In another study, overwintering adults become active in early April in Illinois (Munyaneza et al. 1994). A study in South Carolina found brown stink bugs emerging from late March through April (Jones and Sullivan 1981). The amount of time from spring emergence to oviposition can be quite long, ranging from 13 to 71 days in Arkansas and averaging 25.8 days (Rolston and Kendrick 1961).

Southern green stink bug. The southern green stink bug is a highly polyphagous invasive pest which feeds on over 150 species of cultivated and wild hosts (Panizzi et al. 2000). It is present throughout the world, including the southern United States and is thought to have originated in Ethiopia (Jones 1988). The species was first reported in the United States in Texas in 1880 but is thought to have been established in the United

States in California's Sacramento Valley in 1886. While they have a host preference for legumes, corn can serve as an early season host (Panizzi et al. 2000). Southern green stink bugs have three to five generations a year in the United States (Drake 1920, Todd 1989).

Adult southern green stink bugs are shield-shaped insects that are dull green in color. The dorsal surface is slightly darker than the ventral surface. They average 12-13 mm in length, and the female is generally slightly larger than males (Jones 1918). Small dark markings can be found along the sides of the abdomen. Similar to the brown stink bug, the southern green stink bug has five segmented antennae with the fourth and fifth antennal segments darker reddish-brown color compared with the first through third segments which match the pale green color of the rest of the insect. The green stink bug is very similar in appearance to the southern green stink bug but can be differentiated by examining the openings to scent glands on the ventral side of the insect between the second and third leg (Jones 1918, McPherson and McPherson 2000). The openings to the scent glands of the southern green stink bug are short and broad compared with the long and curved scent glands of the green stink bug. The green stink bug also has a large, pointed spine between the posterior most set of legs, which is absent or rounded in the southern green stink bug. Overwintering adults often display a darker purplish-brown color and quickly return to the pale green color upon emergence (Jones and Sullivan 1981, Musolin 2012).

Southern green stink bug eggs are pale yellow or cream colored when first laid. As they mature, eggs become a salmon color, and portions of nymphs are visible through the egg just before hatching (Jones 1918, Todd 1989). The cylindrical eggs are arranged in rows. The number of eggs per mass ranges from 60 to 90 (McPherson and McPherson 2000). The incubation period for eggs ranges from five days in the summer to as much as two to three weeks in early spring or late fall (Todd 1989).

Similar to many stink bug species, southern green stink bug develops through five nymphal stadia. There is considerable variation in the coloration and markings of nymphs even within the same growth stage (Jones 1918). First instars average 1.6 mm in length and 1.1 mm in width, have four antennal segments, and are red immediately after hatching and quickly turn black. First instars aggregate on the hatched egg mass and do not feed (Drake 1920, Todd 1989). Second through fifth instars average 3, 3.6, 6.2, and 10mm in length and 2, 2.6, 4.7, and 7mm in width, respectively (Jones 1918). Coloration of the second through fourth instars is fairly uniform, with the dorsal side being black with white spots (Rojas and Morales-Ramos 2014, Esquivel et al. 2018). The fourth and fifth instars can display two different color morphs, displaying either primarily a brownish-yellow or bright green color with white spots and red to pink margins on the abdomen (Jones 1918). The white spots on the dorsal surface of fifth instars can help to distinguish the southern green stink bug from the green stink bug.

Full development from egg to adult takes between 35 and 37 days and is highly dependent on temperature (Todd 1989, McPherson and McPherson 2000). Southern green stink bugs overwinter as adults. The southern green stink bug has been found to overwinter in leaf litter, behind bark of living and dead trees, and in structures, with a greater preference for above-ground overwintering sites relative to brown stink bugs

(Jones and Sullivan 1981). Spring emergence is stimulated by rising temperatures, and early season hosts include clover, small grains, early spring vegetables, weed hosts, and seedling corn (McPherson and McPherson 2000). Females can travel up to 1,000 meters to find suitable hosts and oviposition sites (Todd 1989).

Landscape-level movement of stink bugs

Adult stink bugs are highly mobile and will readily migrate between suitable crop and non-crop hosts after emergence. Dispersion is the greatest just after overwintering emergence and is least during the cropping season (Babu et al. 2020). Wheat is the preferred early season crop host for brown stink bugs over seedling corn, and the first generation can be completed in wheat before dispersing to corn (Reisig 2011, Reisig et al. 2013). Wheat harvest often leads to large-scale movement of brown stink bugs into adjacent corn fields during susceptible stages of corn development (Blinka 2008, Reisig 2011, Reisig et al. 2013). In the absence of a suitable early season crop hosts, stink bugs' preference for non-crop hosts is not well understood, with reports of preference for either more open weedy field borders or fallow fields (Jones and Sullivan 1981, Blinka 2008, Grabarczyk et al. 2021) or wooded habitats (Venugopal et al. 2014, Babu et al. 2019a).

During the growing season, population dynamics of stink bugs vary greatly depending on the surrounding landscape. Crop hosts are often planted near one another in southeastern farmscapes, which include wheat, corn, grain sorghum, cotton, soybeans, and peanuts. Soybeans often serve as the last crop host of the season, and populations often peak in soybean (Pilkay et al. 2015). As a result of soybean being a population sink at the end of the season, it has often been reported as an important factor in predicting population densities in the immediate area the following season (Olson et al. 2018). In peanut-cotton farmscapes, stink bugs that develop in peanut can disperse into cotton and aggregate on the interface between the two crops (Tillman et al. 2009). In several crops, there is a pronounced edge effect in stink bug populations (Tillman et al. 2009, Reay-Jones 2010, Reeves et al. 2010, Reisig et al. 2013, Venugopal et al. 2014). Further investigation of the aggregation patterns of stink bugs within a corn field could provide insight for effective and efficient sampling and management strategies.

Stink bug damage to corn

Stink bugs feed by inserting their piercing-sucking mouthparts into plant tissue and extracting fluids (McPherson and McPherson 2000). Plants are injured through the mechanical action as well as enzymes used by the insect to aid in digestion of the plant material. Stink bugs can be found in corn throughout the season, but corn is particularly susceptible to economic injury during a couple key phases of development. From emergence (VE) to around V6, feeding can cause plants to be stunted or deformed, ultimately reducing yield (Riley et al. 1987, Apriyanto et al. 1989a, Apriyanto et al. 1989b, Bryant et al. 2020, 2021). One easily identifiable symptom of stink bug injury is the development of tillers (i.e., multiple shoots from the same root system), which can lead to a delay in critical reproductive stages later in the season (Apriyanto et al. 1989a). Stink bug feeding during these early stages also results in a reduction in plant height, decreasing the overall photosynthetic potential of an injured plant (Apriyanto et al. 1989b, Bryant et al. 2020).

Corn is also susceptible to injury from stink bug feeding during the late vegetative stages of development (i.e., prior to tasseling) through early reproductive stages (i.e., R1) (Negrón and Riley 1987, Ni et al. 2010, Bryant et al. 2020). Symptoms of feeding during these reproductive stages results in crooked or banana-shaped ears, discolored, sunken, or aborted kernels, reduction in ear length, reduction in number of kernel rows or kernels per row, or reduction in kernel weight (Ni et al. 2010, Bryant et al. 2020). Feeding on developed ears can also result in kernel quality reduction through the introduction of fungal contamination and subsequent mycotoxin development (Ni et al. 2011, Opoku et al. 2019).

Stink bug management

Scouting. During seedling stages of corn growth, the majority of stink bugs are found at the base of the plant feeding directly on the growing point below the lowest leaf (Babu and Reisig 2018a). Stink bugs are found more frequently on higher parts of corn plants as they grow, with the majority found between two leaves above and three leaves below the ear during reproductive stages (Babu and Reisig 2018a). A subsequent study developed a sampling plan based on the within-plant distribution of stink bugs and found that partial plant sampling is equally effective to whole-plant sampling efforts (Babu and Reisig 2018b). A study conducted in North Carolina and Virginia in 2019 found economic injury levels of 7% (i.e., 7 stink bugs/100 plants) in seedling stages and 12% (i.e., 12 stink bugs/100 plants) from late vegetative stages through tasseling (Bryant et al. 2020). Infestations have to be present consistently for this entire period (i.e., 16 days from V10/V12-VT) to justify an insecticide application (Bryant et al. 2020). Based on the studies on within-plant distribution, sequential sampling, and economic injury levels, a sampling plan has been developed for brown stink bugs throughout the corn growing season (Table 2) (Reisig and Heinger 2020). As there are commonly observed edge effects, the edges of fields should be checked first, examining ten plants at several locations in a field.

Chemical control. Most commercially available corn seed is treated with an insecticidal coating (Douglas and Tooker 2015). These neonicotinoid seed treatments are systemic and can provide some control for early infestations of stink bugs at high rates (Bryant and Taylor 2021). Foliar applications of insecticides for stink bugs are commonly made at tasseling (VT) or silking (R1) via aerial applications. These applications are highly dependent on good coverage and penetration into the canopy and vary greatly in effectiveness (Reisig 2011). Timing of these applications is also critical, with several studies suggesting that damage occurs during late vegetative stages, as opposed to the first reproductive stages, when many growers apply a tank-mixed fungicide and insecticide (Negrón and Riley 1987, Ni et al. 2010, Bryant et al. 2020). Pyrethroids generally produce mortality when applied in corn, with bifenthrin being the most effective material (Babu and Reisig 2020). Acephate is also highly efficacious for stink bugs in late stages of corn (Babu and Reisig 2020); however, it is not labeled for use in the crop.

Biological control. Stink bug eggs are commonly parasitized in much of their natural range. One study found that 89.7% of field-collected *E. servus* egg masses were parasitized, with the most predominant parasitoid species being *Telenomus podisi*

(Koppel et al. 2009). A large-scale survey of southern green stink bug eggs found species in the genera *Trissolcus*, *Telenomus*, and *Gryon* are the most likely to establish (Jones 1988). While eggs are often parasitized, the parasites are unlikely to provide complete control, and insecticides used to target stink bugs are highly toxic to parasitoids (Koppel et al. 2011). Laboratory and field assays found that acephate, lambda-cyhalothrin, spinosad, and thiamethoxam were all ineffective in targeting stink bug egg masses; however, they all produced significant mortality in parasitoids (Koppel et al. 2011). This suggests that insecticide treatments targeting stink bug eggs specifically could negatively impact naturally occurring biological control in the field. One study in tomato also suggested that improving the availability of refuge host plants for natural enemies of stink bugs near a cultivated field can improve natural biological control (Haseeb et al. 2018).

Cultural control. It has been suggested that managing stink bugs in early season hosts can be effective while numbers are lower (Blinka 2008), although more research is needed. Wheat is a preferred early season host for stink bugs over seedling corn, but stink bugs can feed in the absence of wheat on a wide range of weed hosts, with common mullein being a common host available in southeastern landscapes (Jones and Sullivan 1981, Blinka 2008). Despite commonly found weeds around corn fields being suitable hosts, manipulating the presence of these weeds has not been found to limit numbers of stink bugs in the crop (Babu et al. 2019). Planting date could also play a role in the availability of susceptible hosts when stink bug populations are peaking. Late-planted corn can support higher populations of both brown and southern green stink bugs due to the availability of hosts suitable for nymphal development (Tillman 2010). Late-planted corn also sustained more damage during the seedling stages in one study (Bryant and Taylor 2021).

Spatial patterns of insect pests

Spatial patterns of insects in field crops have been extensively studied and provide valuable information which can strengthen the implementation of some integrated management strategies (Brenner et al. 2018). There are several methods for analyzing spatial data, and each method can provide distinct insight into the ecology of insects and implications for developing sampling plans. Taylor's power law, which describes the relationship between the mean and variance of populations, can be used to assess aggregation in a population and is unrelated to any kind of location data (Taylor 1961). Spatial analysis by distance indices (SADIE) uses count data and takes into account the location of each data point in two-dimensional space (Perry 1998). In SADIE analyses, cluster indices are assigned to each sampling location, with a positive cluster index above the mean indicating aggregation and a negative cluster index below the mean indicating a gap (Perry 1998). The results of SADIE analyses can then be interpolated using inverse distance weighted (IDW) methods in GIS software to generate maps of aggregations and gaps in populations throughout a field. Examining within-field aggregations of pests and their dispersal throughout a field over time can help improve sampling and management plans. Additionally, spatial distributions can be studied over time throughout an entire farmscape to assess the population dynamics and movement of pest species between multiple hosts (Pilkay et al, 2015). These methodologies have been used to describe the spatial patterns of both stink bugs and corn earworm.

Several studies have been conducted in Georgia farmscapes assessing both the aggregation and seasonal movement of stink bugs in a number of crops (Tillman et al. 2009, Grabarczyk et al. 2021). Strong aggregation patterns have been identified in stink bugs in pecan, cotton, corn, peanut, tobacco, and hay or fallow fields through pheromone trapping (Grabarczyk et al. 2021). A more specific study of the peanut-cotton system found that stink bugs can develop in peanut and disperse to cotton where they aggregate on the edge of the field (Tillman et al. 2009). A study in Maryland identified a similar aggregated pattern of stink bugs along corn and soybean interfaces, where aggregation on the edges of fields was also identified (Venugopal et al. 2014).

A spatial analysis of major pests of corn in Brazil, including corn earworm, identified significant aggregation of earworm and no major differences in spatial aggregation patterns between Bt and non-Bt corn (Silva et al. 2018). An aggregated pattern of corn earworm kernel injury was also identified in trials in Bt and non-Bt corn in North and South Carolina (Reay-Jones et al. 2018). These data were used to determine the number of samples needed to accurately assess corn earworm kernel injury in Bt and non-Bt corn.

The spatial patterns of several insect species or their damage can also be compared with one another to determine if there is any association between their spatial patterns (Perry and Dixon 2002, Reay-Jones et al. 2010, Ni et al. 2011, Reay-Jones 2012). A study on the spatial patterns of aflatoxin levels in corn found that the aggregation of aflatoxin contamination was highly correlated with the aggregation of stink bugs and maize weevils and had a strong edge effect. The aflatoxin contamination was not correlated with the amount of ear feeding caterpillar damage (Ni et al. 2011). A similar study in cotton comparing the spatial distributions of stink bugs with their associated boll injury found that these variables were not always positively correlated (Reay-Jones et al. 2010). The spatial association of stink bugs and corn earworm and their respective injury to corn has not been explored and could provide valuable insight on the interaction of these two common pests.

Ecological interactions of insects

Insect pests rarely exist in a field setting in isolation and more often occur as a complex of insects, which may be pests of the same plant species, serve as natural biological control of pests, or occur incidentally in that setting. Competition between individuals of a species or between two species is a commonly described ecological concept which can play an important role in driving population dynamics at various spatial scales. This competitive interaction is commonly reported amongst phytophagous insects across a wide range of insects and plant systems. Competition is thought to have a larger influence on insect populations which are of the same feeding guild, but this interaction can also be driven by changes in host plant physiology as a result of herbivory, alterations in natural enemy populations, or abiotic factors like temperature and weather (Kaplan and Denno 2007).

Herbivore-induced changes in host-plant defense and volatile emissions can both play a role in competitive interactions between insects. For example, a series of studies on birch trees found that feeding by sawflies (Hymenoptera: Symphyta) early in the season reduced the quality of those trees for subsequent herbivores and altered their development rate (Haukioia and Niemala 1979, Hanhimaki 1989). This interaction was likely due to an induced plant defense response. Herbivore-induced changes in plant volatiles can also send a signal to attract predators and parasitoids and increase the impact of biological control on several herbivore species, particularly herbivores that occur later in the season (Dicke et al. 1990, Oppenheim and Gould 2002).

In a field setting, no prior study has examined the relationship between stink bugs and corn earworm, two common and important pests of field corn. Competition between Heliothine caterpillars and stink bugs has been identified in cotton, where feeding by bollworm larvae on cotton bolls reduced the growth rates of stink bugs feeding on those bolls (Zeilinger et al. 2011) and caused stink bugs to avoid those plants for oviposition (Zeilinger et al. 2015). Understanding the interaction of these two pests in field corn could have important implications for implementation of pest and insecticide resistance management.

Integrated pest management

Integrated Pest management (IPM) is a holistic approach to pest management with an emphasis on using multiple control strategies. Kogan (1998) provides the following definition: “IPM is a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment.” The term integrated management was coined in 1959 as the integration of biological and chemical control tactics (Stern et al. 1959). This development was in response to a period of time, often referred to as the “dark ages of integrated insect control,” beginning in the early 1940s after the development of organosynthetic insecticides, when research on pest biology and non-insecticidal control methods was lacking (Newsom 1980). The four main tenants of integrated control, as initially proposed, included recognition of the effect of one management strategy on the entire ecosystem, use of population sampling and prediction, augmentation of natural enemies, and the use of selective insecticides (Stern et al. 1959). The term ‘integrated pest management’ and the associated acronym ‘IPM’ were officially accepted by the scientific community in 1972, after a message from the United States president to congress included the term, and it was subsequently published in a report by the Council on Environmental Quality (Council on Environmental Quality 1972). Since its formal definition, a large amount of research and Extension work has been done to identify important management strategies that fit in an IPM framework, both broadly and specifically to individual pests, to improve producers access to critical information and to evaluate the outcomes and benefits of IPM.

The core principles of IPM, as we know them today, are summarized by Barzman et al. (2015). These include 1) prevention and suppression of pest populations, 2) monitoring populations levels, 3) decision-making based on knowledge gained from monitoring, 4) the use of non-chemical control measures, 5) the selective use of pesticides, carefully chosen to be as target specific as possible, 6) reduced pesticide use through the implementation of economic thresholds designed to identify the point at which pesticide usage is economically beneficial to the producer, 7) implementing strategies to manage the development of pesticide resistance, and 8) evaluation of management program successes and failures (Barzman et al. 2015). The ability for producers to implement all these steps is highly dependent on knowledge of IPM strategies and requires access to a wide range of information for each specific target pest. The roles of research and Extension are critical in improving the rate of producer success in implementing IPM programs (Allen and Rajotte 1990).

Biological, cultural, mechanical, and behavior controls are all options which have been extensively studied as non-chemical control strategies (Rebek et al. 2012, Barzman et al. 2015). Biological control involves the preservation or augmentation of naturally occurring populations of beneficial arthropods, or the introduction of exotic biological control agents (Stern et al. 1959, Rebek et al. 2012). Cultural controls include mostly abiotic management strategies, such as altering planting date, rotating crops, or using host-plant resistance (Rebek et al. 2012, Barzman et al. 2015). Mechanical control involves physical prevention of a pest species from accessing the plant and can consist of trapping pests, netting plants to prevent access, or destroying a certain insect life stage via tillage (Rebek et al. 2012, Barzman et al. 2015). Behavioral control seeks to alter pest behaviors, such as mating, aggregation, or host identification through the use of highly species-specific pheromones or semiochemicals.

There are different levels of integration in IPM programs, with each successive level incorporating considerations from the prior level and expanding the scope. Level one integration is a plan specifically targeting an individual pest species or species complexes using all available strategies synergistically, including biological, cultural, and chemical control. Level two integration expands that management plan to incorporate the entire community of pest species (i.e., insects, weeds, and diseases). Level three integration expands the focus of management to consider the entire cropping and surrounding ecosystem. Level three integration may take into consideration the environmental fate of pesticides or the impact of management strategies on pollinator or wildlife health. The fourth level of IPM integration considers management plans in the context of the entire farming community and includes social and economic components for both the producer and the community (Kogan 1998, Bottrell and Schoenly 2018).

IPM adoption

The extent to which producers both understand and implement IPM strategies on farm is critical for shaping future research and Extension programs. Surveys of producers are often used to assess the level of IPM adoption in addition to the benefits of those management schemes (Napit et al. 1988, Olson et al. 2008, Jasinski and Haley 2014, Creissen et al. 2019). For key pests that receive a large amount of attention in research and Extension programs, growers' knowledge about the pest and the important considerations for fully integrated management can be extensive. One example of this involves management of soybean aphid, *Aphis glycines* (Hemiptera: Aphidae), in soybeans in the Upper Midwest, where a survey found that 80% of growers were aware of the biology of the aphid species, the economic injury level, and information on susceptibility of various plant stages (Olson et al. 2008). A survey of 407 sweet corn growers in the Great Lakes region of the United States found that only 26 were classified as high-level adopters of IPM, with 251 respondents classified as moderate IPM adopters. The categorization in this survey was based on growers' efforts in continuing education, record keeping, preplant, at-plant, in-season, and post-harvest management considerations, as well as scouting (Jasinski and Haley 2014). A survey conducted in the United Kingdom found that all cereal producers surveyed used some level of IPM; however, only 5.8% of producers used 85% or more of the available strategies (Creissen et al. 2019). The level of IPM adoption in this study was calculated as a function of growers' use of the eight principles of IPM as described above, with several questions from each principle specific to cereal production. While adoption of higher level IPM is still limited in some cases, one study found higher yields and less variable returns for growers using higher levels of IPM in corn, cotton, soybeans, peanut, and apples (Napit et al. 1988).

Implementation of IPM is not without its challenges and limitations. Stoner et al. (1986) summarized some of the major challenges and barriers to deployment of IPM tactics. One of the major factors in production, particularly of fruits and vegetables for fresh market, that limits the use of IPM is the cosmetic standards of consumers for produce. Generally, IPM some tolerance for injury below an economic threshold is common with an IPM approach, but blemishes are often unacceptable for fresh market production. This limits the ability of producers to make management decisions based on economic thresholds. Another consideration is the amount of knowledge about each pest that is required to implement an effective IPM program. While certain management strategies can be implemented individually, the true benefit of IPM comes from a holistic approach which requires a large amount of research and knowledge. For some less common pests, this research may also be unavailable (Stoner et al. 1986). There can also be numerous technological or socio-economic constraints driving hesitancy to adopt IPM (Rossi et al. 2019). A producer's perception of a new technology or decision-making tool in regard to its ease of use, cost effectiveness, efficiency, relevance to their production system, and agreement with social demands of conformity all play a part in willingness to adopt new tools (Rossi et al. 2019).

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Table 1.1 Single and pyramided Bt events and corresponding toxins expressed for above ground pests of corn

Bt Events	**Bt Toxin**
Bt11	Cry1Ab
Bt11, MIR162	Cry1Ab, Vip3A
Bt11, MIR162, TC1507	Cry1Ab, VIP3A, Cry1F
TC1507	Cry1F
TC1507, MON810	Cry1F, Cry1Ab
TC1507, MON810, MIR162	Cry1F, Cry1Ab, Vip3A
MON810	Cry1Ab
MON89034	Cry1A.105, Cry2Ab2
MON89034, TC1507	Cry1A.105, Cry2Ab2, Cry1F
MON89034, TC1507, MIR162	Cry1A.105, Cry2Ab2, Vip3A

Table 1.2 Sequential sampling plan and action thresholds for brown stink bug in corn throughout the season (Reisig and Heinger 2020)

Growth Stage	Area to Sample	Do not treat	Take more samples	Treat
V1 to V6	Base of the stalk below the lowest green leaf	≤ 6	7 to 12	≥ 13
V14 to VT	Stalk form the first leaf above and below the primary ear	≤ 4	5 to 9	≥ 10
R1 to R2	Stalk at one leaf above and two leaves below the primary ear	≤ 14	15 to 27	≥ 28

CHAPTER TWO

WITHIN-FIELD SPATIAL PATTERNS OF *EUSCHISTUS SERVUS* AND *NEZARA VIRIDULA* (HEMIPTERA: PENTATOMIDAE) IN FIELD CORN[1]

Introduction

Field corn, *Zea mays* L., is the most important cereal crop in the U.S., accounting for around 13 million hectares of farmland in 2022 (USDA NASS 2022). Insect pests have the potential to be yield limiting in corn, and a complex of stink bugs are the most common and damaging pests of concern to growers in the southeastern U.S. (Reisig et al. 2021). Of this complex of species, the brown stink bug, *Euschistus servus* (Say), and southern green stink bug, *Nezara viridula* (L.), are the most common in corn (Tillman 2010).

Stink bugs can be present in field corn throughout the season, but corn is most susceptible to injury and subsequent yield loss from feeding during the early vegetative stages (VE-V6) and the late vegetative stages through early reproductive stages (V10-R1) (Clower 1958, Negrón and Riley 1987, Apriyanto et al. 1989a, Bryant et al. 2020). During early vegetative stages, stink bug feeding leads to a number of different growth deformities, including stunting, leaf holes, or tillering, and can cause plant death if feeding is severe (Apriyanto et al. 1989a, Apriyanto et al. 1989b, Bryant et al. 2020, 2021). Corn ear initiation begins around V6, and the maximum yield potential is fully determined prior to tasseling (i.e., VT) (Lejeune and Bernier 1996). Around V10, ear

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shoots are protected by a single layer of leaf sheath and can be directly fed upon by stink bugs. Feeding on young ear shoots often results in ear deformity (Negrón and Riley 1987, Ni et al. 2010, Bryant et al. 2020). One of the most identifiable symptoms of this type of injury is the formation of a crooked or "banana-shaped" ear (Clower 1958), which can directly reduce the grain yield of the ear as well as expose it to secondary insect pests and pathogens. During early stages of reproductive corn development, direct feeding on ears can result in discolored, sunken, or aborted kernels and a reduction in overall kernel weight (Ni et al. 2010, Bryant et al. 2020).

Stink bugs are bivoltine in the southeastern U.S. and generally complete their first generation in a wild or cultivated host, such as winter wheat, prior to moving into field corn (McPherson and McPherson 2000, Blinka 2008, Reisig 2011, Reisig et al. 2013, Babu et al. 2019a). In the southeastern U.S., crop landscapes are highly fragmented with many small fields planted in close proximity to each other in addition to unmanaged habitats. The most commonly rotated crops are winter wheat, field corn, cotton, peanut, and soybeans, all of which are suitable hosts for stink bugs (Tillman et al. 2009, Pilkay et al. 2015, Grabarczyk et al. 2021). Stink bugs are also highly mobile species and will readily move between crop hosts based on their suitability throughout the season (Tillman 2011). These conditions can allow stink bug populations to persist perennially and, in some cases, reach damaging levels in field corn.

Aggregation patterns have been identified in populations of *Chinavia hilaris* (Say), *E. servus*, and *N. viridula* in a number of studies at farmscape scales, including in field corn (Venugopal et al. 2014, Cottrell and Tillman 2015, Pilkay et al. 2015, Grabarczyk et al. 2021). Aggregations on field margins have been reported adjacent to recently harvested winter wheat (Reisig 2011, Reisig et al. 2013) and in corn fields adjacent to wooded habitats (Venugopal et al. 2014, Babu et al. 2019). The spatial patterns of stink bug injury directly to kernels (i.e., discoloration) have also been found to be aggregated and located primarily on field margins (Ni et al. 2011). Developing our understanding of how stink bugs colonize corn fields throughout the season can help to improve sampling plans. This is particularly salient for a key pest group like stink bugs, for which sampling is laborious and costly (Babu and Reisig 2018a).

There are a number of quantitative and geospatial methods for analyzing the spatial patterns of species in the environment (Perry et al. 2002). Spatial analysis by distance indices (SADIE) can be used to describe spatial patterns of insects and it allows for hypothesis testing on the spatial randomness of populations (Perry and Dixon 2002). SADIE can be used to identify clusters of either high-density counts or low-density gaps. Spatial patterns of stink bugs and their injury have been studied using SADIE in cotton (Reay-Jones et al. 2010), wheat (Reay-Jones 2010), and farmscapes composed of commonly rotated crops in the southeastern U.S. (Tillman et al. 2009, Pilkay et al. 2015, Grabarczyk et al. 2021). Spatial associations between densities of different life stages of stink bugs, between different stink bug species, or between stink bugs and their injury have also been studied using SADIE methodology (Tillman et al. 2009, Reay-Jones et al. 2010, 2016, Reisig et al. 2013).

The within-field spatial distributions of common stink bugs in field corn have not been extensively studied in the southeastern U.S., particularly for the key pest species *E.*

servus. For instance, prior studies have examined spatial patterns using transects (Venugopal et al. 2014, Babu et al. 2019), rather than whole-field grid sampling, as addressed in the current study, or have focused on other stink bug species in corn (*C. hilaris*; Cottrell and Tillman 2015). Additionally, the spatial patterns of stink bug injury have been sparsely described, and their association with sampled populations at various corn phenological stages has not been reported. The objective of the current study was to describe the spatial patterns of stink bugs, their injury in field corn, and their spatial association using SADIE to help improve sampling plans and our understanding of stink bug dynamics in corn.

Materials and methods

Stink bugs and associated injury were sampled in 20 fields (10 fields in 2021 and 10 in 2022), which included 15 commercial fields and five fields at the Clemson University Pee Dee Research and Education Center in Florence, SC (Table 2.1). Several fields were sprayed with commercial formulations of bifenthrin (110 g/ha a.i.), bifenthrin (97 g/ha a.i.) and chlorantraniliprole (64 g/ha a.i.), or *lambda*-cyhalothrin at a rate of 30 g/ha a.i. In 2021, bifenthrin was sprayed on 3 (field 1) and 25 May (field 5 and 6), and bifenthrin and chlorantraniliprole was sprayed on field 3 on 10 June. In 2022, bifenthrin was sprayed on 20 (fields 14 and 15) and 22 (field 18 and 19) June, and *lambda*-cyhalothrin was sprayed on field 20 on 12 May.

A sampling grid was established in each field, with one sampling point for every 0.37 ha and each point separated by 61 m. Each point was marked with a 38 cm wire flag to identify the sampling location throughout the study. GPS coordinates were recorded at each point using an iPhone XR (Apple, Cupertino, CA). Sampling was conducted biweekly from V2/V4 through VT/R1 in each field, with one additional sample taken at R2.

At each sampling point, 25 plants were visually examined for stink bugs, stink bug eggs, and characteristic signs of stink bug damage. Stink bugs were summed by species and life stage (adult or nymph) across the 25-plant sample. Signs of stink bug damage during vegetative stages of corn included “window-pane”-like parallel holes in leaves, tillers, and stunted plants (Apriyanto et al. 1989a, Bryant et al. 2021). This type of injury is similar to and can also commonly be attributed to that of either billbugs or slugs (Morgan and Beckham 1960, Byers and Calvin 1994). These pests were not detected in any of the fields in our study. Once ears developed, the main symptom of stink bug injury assessed in this study was a crooked or banana-shaped ear (Clower 1958). Injury was expressed as the number of injured plants per 25 plant sample.

In 2021, an average growth stage for each field (i.e., > 50% of the field at a given stage) was recorded. In 2022, the growth stage was recorded at each individual sampling point to measure variability in plant phenology within a field. Additionally, the heights of five plants at each sampling point were measured in 2022 from the soil to the tip of the tallest extended leaf and averaged for each sampling point. Plant heights were then rounded to the nearest integer for analyses. Plant height measurements were taken from the first sampling date through V10/VT in each field.

Data Analysis

All variables were summed across the 25 plants at each sampling point prior to analysis. Spatial analysis by distances indices (SADIE) was used to analyze stink bug numbers by species and life stage, stink bug totals (summed across all life stages and species), stink bug eggs, plant height, stink bug injury to vegetative stages, and stink bug injury to ears for each week of sampling, in addition to the year-end totals. SADIE analysis was conducted using the "epiphy" package (Gigot 2018) in R version 3.6.3 (R Core Team 2020). The SADIE analyses provide local indices of dispersion at each sampling point, with either a positive cluster index (V_i) for counts above the average or a negative gap index (V_j) for counts below the average. A random spatial pattern has indices $V_i = -V_j = 1$. The overall index of dispersion (I_a) indicates either aggregation ($I_a > 1$), random ($I_a = 1$), or uniform spatial distribution ($I_a < 1$). The null hypothesis of spatial randomness is rejected when $P < 0.025$ for aggregation or $P > 0.975$ for uniformity. Each analysis consisted of 5,967 randomizations. In addition, linear regression models were used separately for each stink bug species and life stage with aggregation indices as the dependent variable and total stink bug counts as the independent variable using the package "lme4" (Bates et al. 2015) to determine if aggregation patterns were density dependent.

SADIE association analysis was then used to spatially associate aggregation indices between two datasets sharing the same sampling locations (Perry and Dixon 2002). An overall index of association (X) was determined between two paired datasets, with $X > 0$ ($P < 0.025$) representing a positive association and $X < 0$ ($P > 0.975$) representing a negative association. A positive association indicates either a gap or a cluster in both datasets at the same location, and a negative association indicates a gap in one dataset and a cluster in the other dataset at the same location. Association analyses were conducted between stink bug counts at a given sampling date and stink bug injury recorded at the vegetative and at R2 stages. Additional association analyses were conducted between stink bug densities and corn plant height measurements for each field in 2022. The local aggregation or association indices were spatially interpolated using the inverse distance weighted (IDW) method in ArcGIS Pro version 2.9 (Esri 2020).

Results

Stink Bug and Crop Variables

The brown stink bug, *E. servus*, and southern green stink bug, *N. viridula*, were the most common species identified across all fields sampled in both years of the study. A small number of green stink bugs, *C. hilaris*, and rice stink bugs, *Oebalus pugnax* (F.), were also found (<1% of total) and were excluded from analyses. In 2021, a total of 1,268 stink bugs were found, ranging from 12 to 636 total stink bugs per field. In 2022, a total of 516 stink bugs were found, ranging from 5 to 111 total stink bugs per field. *Euschistus servus* accounted for 48 (89%) of the total stink bugs, *N. viridula* accounted for 52 (11%) of the total stink bugs in 2021 and 2022, respectively. In 2021, 543 (43%) of the *E. servus* were adults, and 59 (5%) were nymphs. In 2022, 396 (77%) of *E. servus* were adults and 61 (12%) were nymphs. In 2021, 251 (20%) of *N. viridula* were adults, and 415 (32%) were nymphs. In 2022, 47 (9%) of *N. viridula* were adults, and 12 (2%) were nymphs.

Populations of stink bugs peaked in both years in late-June to early-July, depending on the planting date of the field. Peak densities of stink bugs generally coincided with the late vegetative or early reproductive stages of corn development (i.e., V10/V12 – R2). Peak numbers of adults for *E. servus* per sampling point ranged from 0.14 ± 0.08 [SEM] to 2.60 ± 0.49 in 2021 and from 0.08 ± 0.04 to 1.79 ± 0.45 in 2022. For *N. viridula*, peak populations of adults per sampling point ranged from 0.02 ± 0.02 to 1.43 ± 0.43 in 2021 and from 0.03 ± 0.03 to 0.47 ± 0.47 in 2022 (Figs. 2.1 and 2.2).

A total of 1,365 plants with symptoms of vegetative stink bug injury were identified. In 2021, season total plants injured by stink bugs ranged from six to 195 in a field, averaging between 0.16 ± 0.13 and 8.06 ± 2.32 per sampling point. In 2022, the season total of injured plants ranged from 35 to 151, averaging between 0.78 ± 0.23 and 5.96 ± 1.26 per sampling point. A total of 353 stink bug injured ears (i.e., “banana ears”) were identified across both years of the study at R2. In 2021, the total number of injured ears ranged from one to 196 for an individual field with an average of 0.03 ± 0.03 to 4.66 ± 0.94 ears per sampling point. In 2022, the total number of injured ears ranged from two to 23 with an average of 0.05 ± 0.05 to 0.79 ± 0.59 ears per sampling point.

Plant height and corn growth stage varied greatly, depending on field conditions. Variability in plant height and growth stage was generally greater as the season progressed, with stunted sampling points primarily located on field margins and adjacent to wooded areas. In field 11, where plant height was aggregated, plant growth stages varied within the field by up to seven leaves and 142 cm in plant height. Additionally, plant growth stage within field 20 varied by up to five leaves, and plant height varied by up to 88 cm.

Aggregation Analysis

Out of 400 aggregation analyses, 36 (9%) had significant overall aggregation indices, indicating a pattern of spatial aggregation ($\alpha = 0.025$). Aggregation patterns in populations of eggs or nymphs of both *E. servus* and *N. viridula* were rarely identified across both years of the study. Only one of 36 (3%) analyses was significant for *E. servus* nymphs, zero of 33 analyses were significant for *N. viridula* nymphs, and zero of 27 analyses were significant for stink bug eggs. Adults of *N. viridula* also rarely had significant aggregation patterns, with three of 51 analyses (6%) yielding significant patterns of aggregation. Interpolation maps of local aggregation indices therefore focus only on *E. servus* (Fig. 2.3). Aggregation patterns were most commonly identified in adult populations of *E. servus*, with 12 of 88 significant analyses (14%), with overall aggregation indices ranging from 1.513 to 1.891 across both years. Of the 12 significant aggregation analyses, six were for season totals, and six were for individual sampling dates. All spatial aggregations of *E. servus* adults occurred at growth stages beyond V8 across both years of the study, with the majority occurring in early reproductive stages of corn development (i.e., VT-R2; Table 2.2). Spatial aggregation was identified at least once in the season or when analyzing season totals in seven of the 20 fields sampled. Using regression models, aggregation indices were not associated with total numbers of *E. servus* adults ($P = 0.24$, $R^2 = 0.02$), *E. servus* nymphs ($P = 0.83$, $R^2 = 0.001$), or *N. viridula* nymphs ($P = 0.54$, $R^2 = 0.01$), but had a positive association with *N. viridula* adults (*P* = 0.001, R^2 = 0.22 [y = 1.026 + 0.004x]). Aggregation indices were also positively associated with the total number of stink bugs combined across life stage and species (*P* = 0.03, R^2 = 0.01 [y = 1.102 + 0.0005x]). When stink bug counts were summed over species and life stages, 10 out of 67 (15%) SADIE aggregation indices were significant. Two of these analyses were for season totals, and the remaining eight were for individual sampling dates. Spatial interpolation of local aggregation indices for *E. servus* season totals revealed aggregations on the field margins in fields 4, 5, 11, 13, 16, and 18 (Fig. 2.3). Aggregations of adult *E. servus* occurred in more interior sections of the field at individual sampling dates in fields 4, 5, and 13 (Fig. 2.3).

Of 70 analyses conducted on vegetative symptoms of stink bug injury, spatial aggregation was identified in nine (13%) analyses, with overall aggregation indices ranging from 1.371 to 1.826 (Table 2.2). At R2, spatial aggregation was not identified in ears injured by stink bugs, with zero of 22 analyses yielding a significant aggregation pattern. Plant height was aggregated in three of 38 (8%) total analyses. Two of these significant analyses were on 3 and 18 May in field 11 with overall aggregation indices of 2.45 and 2.24, respectively. One additional analysis was significant on 11 May in field 20 with an overall aggregation index of 1.78. Significant aggregations in plant height were not identified beyond V7 in either field, but, generally, stunted plants on the edges of fields did not grow to plant heights comparable with plants located in the interior of fields.

Association Analysis

Of 61 association analyses between *E. servus* adults at a given sampling date and plants with vegetative symptoms of stink bug injury, 17 (28%) were significant, with overall indices of association ranging from -0.643 to 0.713. For *N. viridula* adults, 15 of 26 (58%) yielded significant associations with stink bug injured plants, with indices ranging from -0.731 to 1.000. For *E. servus* adults, 11 analyses yielded positive associations, and six analyses yielded negative associations with stink bug injured plants. For *N. viridula* adults, 12 analyses were positive associations, and three were negative associations. Of 28 analyses between *E. servus* nymphs and vegetative injury, 10 (36%) were significant, with overall aggregation indices ranging from -0.603 to 0.559. Of 21 analyses between *N. viridula* nymphs and vegetative injury, eight (38%) were significant, with overall aggregation indices ranging from -0.517 to 0.620. For *E. servus* nymphs, six analyses yielded positive associations, and four analyses yielded negative associations with stink bug injured plants. For *N. viridula* adults, seven analyses yielded positive associations, and one yielded a negative association.

Of 31 analyses between *E. servus* adults at a given sampling date and stink bug damaged ears at R2, 10 (32%) were positively associated and three (10%) were negatively associated, with overall indices of association ranging from 0.364 to 0.759 for positive associations and from -0.770 to -0.416 for negative associations. Associations were more frequently significant at V10-V12 growth stages than at earlier or later stages (Fig. 4). Seasonal total counts of *E. servus* and stink bug damaged ears at R2 were also significantly associated in one of five (20%) analyses, with an association index of 0.338. For *N. viridula* adults at individual sampling dates, eight of 23 analyses (35%) yielded significant positive associations with stink bug injured ears, with overall association indices ranging from 0.515 to 0.848. Significant negative associations were identified between *N. viridula* adults and damaged ears in two of 23 analyses (9%), with overall association indices of -0.377 and -0.555. Season total counts of *N. viridula* and stink bug damaged ears at R2 were not associated in any analyses. Similar to *E. servus* adults, associations of *N. viridula* and ear injury at R2 were more frequently significant for *N. viridula* sampled at V10, V12, and VT than at earlier or later stages of corn development (Fig. 2.4). At a given sample date, nymphs of *E. servus* and *N. viridula* were associated with injured ears in five of 16 (31%) and two of seven (29%) analyses, respectively. Season totals of *E. servus* nymphs were associated with ear injury at R2 in four of six analyses (66%), with association indices ranging from 0.463 to 0.889. Season totals of *N. viridula* nymphs were not associated with ear injury in any analyses. Interpolation of local association indices showed that *E. servus* and *N. viridula* adults were positively associated with ear injury on the margin of field 4 (Fig. 2.5). In field 15, aggregations were not identified in *E. servus* adults, but significant associations were identified between *E. servus* adults and ear injury on the southwest margin of the field (Fig. 2.5). In field 5, *N. viridula* was significantly associated with ear injury, but *E. servus* adults were not (Fig. 2.5).

In 2022, plant height was negatively associated with stink bug densities in 37 (12%) and positively associated in 30 (10%) of 290 analyses, with overall association indices ranging from -0.689 to 0.659. *E. servus* adults and nymphs accounted for 26 and 17 significant analyses, respectively, and *N. viridula* adults and nymphs accounted for 15 and eight significant analyses, respectively. When comparing season total stink bug densities with the last recorded plant height (i.e., V10/VT) for each field, seven of 26 (27%) total analyses had significant negative associations. Spatial interpolation of local association indices between stink bug populations and plant height revealed significant negative associations in fields 16, 18, and 19, where clusters of stunted plants often on the field margins had gaps in stink bug populations (Fig. 2.6). In field 11, positive associations were identified between plant height and *E. servus* nymphs at sampling some points along the edge of the field.

Discussion

Patterns of spatial aggregation were identified in populations of stink bugs and their injury across both years of the present study. Numerous previous studies have examined spatial patterns of stink bug populations in corn primarily at crop interfaces (Tillman 2010, Reisig 2011, Venugopal et al. 2014), but this study provides valuable insight on within-field spatial patterns of stink bugs in the crop. Individual sampling dates throughout the season had inconsistent indices of aggregation, but season totals often revealed a pattern of aggregation on the field margins (Fig. 2.3). Stink bug injury during vegetative and reproductive stages was also found to have patterns of aggregation at a much lower rate than stink bug populations. These patterns of injury and stink bugs were spatially associated in some cases, but the timing of sampling was critical for identifying these associations. Plant phenology, crop history, field conditions at planting, and surrounding landscape can play important roles in the risk of stink bug infestation in corn (Reisig et al. 2013, Tillman et al. 2014, Olson et al. 2018).

Stink bug populations peaked in late June to early July in both years of the study, with later-planted fields often having later peaks in populations (e.g., fields 5, 16, and 20). Previous studies have shown similar seasonal patterns of stink bugs in corn in the southeastern U.S. (Tillman 2010). Previous reports have shown that corn is most susceptible to direct ear injury during the late stages of vegetative growth (Negrón and Riley 1987, Bryant et al. 2020), which often coincided with peak populations of stink bugs in this study. Our analysis showed that association indices between stink bug populations and stink bug damaged ears varied depending on the corn growth stage when stink bugs were sampled (Fig. 2.4), with late vegetative stages most commonly having significant associations. This result indicates that late vegetative stages (i.e., V10/V12 for many hybrids) during ear development are indeed the most critical for management of stink bugs. Applications of insecticide targeting stink bugs are often applied at layby or tank mixed with fungicide and applied aerially at tasseling or R1 (Bryant unpublished data), which misses the maximum injury potential of stink bugs. In field 5, an insecticide application targeting stink bugs was made in late May around the V8 growth stage, which was likely too early for optimal control of stink bugs; a large population of stink bugs subsequently invaded the field most likely from the adjacent harvested wheat, a common occurrence in southeastern landscapes (Reisig et al. 2013). This illustrates the importance of properly timing insecticide applications to maximize their value and limit injury potential.

Corn is also susceptible to injury or even plant death if high enough densities occur at early vegetative stages (i.e., VE-V4/V6) (Bryant et al. 2020), but significant early infestations only occurred in one field (i.e., field 5) out of the 20 sampled in this study. While stink bugs were often not found until later stages of corn growth, typical symptoms of stink bug injury to early stages of corn were found in 18 of the 20 sampled fields. The identification of injury symptoms prior to our detection of stink bugs suggests that sampling inefficiencies for stink bugs during early stages of corn development may lead to an underestimation of actual population densities. Previous research has shown that stink bugs are primarily found below the lowest green leaf during early stages of corn growth (Babu and Reisig 2018a). In several fields in the current study, corn was planted into heavy residue from a winter cover crop, which potentially limited the ability to accurately sample stink bugs near the soil line. Additionally, stink bugs that were identified prior to symptoms of injury were only spatially associated with that injury in 13 instances, including five negative associations. More research is needed to optimize sampling efforts for stink bugs during early vegetative stages of corn.

Interpolation of local aggregation indices of season total *E. servus* adult populations illustrated aggregation patterns in several fields (Fig. 2.3). Edge effects have been reported for stink bugs in field corn in a number of previous studies (e.g., Tillman 2010, Venugopal et al. 2014) and were also identified in the present study. In addition to these observed edge effects in season totals, clusters of high stink bug counts were sometimes present away from the edge in the interior of some fields at individual sampling dates (e.g., Fig 2.3., field 13). The presence of non-crop and crop hosts directly adjacent to corn has been reported to be one of the primary factors in facilitating invasions of stink bugs into corn (Tillman et al. 2009, Olson et al. 2012, Reisig et al.

2013). In several fields in the present study, stink bugs likely moved into corn from adjacent wheat and may not have dispersed into the field beyond the sampling points on the edge. These infestations primarily occurred later in the season around V10 or VT, when corn phenology was more suitable for stink bugs (Tillman 2011) and also more susceptible to injury. In field five, where a significant early season infestation occurred, it was adjacent to a wooded area, which can be a source of stink bug infestations in corn (Olson et al. 2012). This field was also planted with soybeans in the previous year, which can serve as a population sink late in the season for stink bugs that can then overwinter in the immediate vicinity or in the field. *Euschistus servus* have been reported to overwinter successfully in soybean stubble in the field, but *N. viridula* prefers elevated overwintering sites such as woods (Jones and Sullivan 1981). The early season populations in this field were primarily *E. servus* adult and nymphs (65%), which may have overwintered in the field in soybean residue, while the *N. viridula* population more likely originated from surrounding woods.

Populations of stink bugs and their injury to crops can also be influenced by landscape level factors (Rice et al. 2017, Olson et al. 2018, Grabarczyk et al. 2021). For instance, the percentage of soybean acreage in the landscape had the most impact on *E. servus* populations in corn, peanut, cotton, and soybean within a suite of 16 landscape factors (Olson et al. 2018). The highest densities of stink bugs were found in our study in 2021 in field 5, which had a high percentage of soybeans in the surrounding landscape and was planted to soybeans in the previous year (NASS CropScape Layer 2022). Winter wheat was then planted adjacent to field 5, which can be a significant source of stink bugs migrating into corn (Reisig et al. 2013). In field 4 in 2021, where the density of stink bugs was also high, a large percentage of the landscape in the previous year was also planted to soybeans. Because soybeans are generally the latest cultivated crop hosts for stink bugs in the landscape of southeastern field crops (Pilkay et al. 2015), the relative abundance of soybean likely plays an important role in infestation levels in the subsequent year. While our highest population fields supported this hypothesis, examination of the area in and around the remainder of our study fields revealed that a large percentage of soybeans in the landscape in the previous year did not always facilitate large populations in the sampled corn fields (e.g., fields 7 and 17), and a number of other environmental factors need to be considered to fully assess the risk of large infestations in corn.

Association analysis revealed both positive and negative associations between plant height and stink bug densities in several fields, indicating that plant phenology may play an important role in the invasion and subsequent movement of stink bugs in corn fields. In cases where negative associations were identified between stink bug densities of both species and plant height (i.e., fields 16 and 19), local indices revealed that areas of delayed plant growth, primarily on the field margins, had a higher density of stink bugs. Crop growth and yield are frequently hindered by proximity to natural landscape elements such as trees due to competition for light (Raatz et al. 2019), and shorter corn plants are often found along field edges adjacent to woody habitats. Since dispersal of stink bugs into a crop can decrease with crop height (Tillman et al. 2014), aggregation of stink bugs along the edge of corn fields may occur due to a barrier effect of taller corn plants away from the field edge. In other cases (e.g., field 11), only positive associations were identified between stink bug densities and plant height. These positive associations were identified in *E. servus* nymph populations, which may have a decreased ability to select their host based on phenology due to the lack of wings and a lesser dispersal ability compared to adult stink bugs.

Aggregation patterns for stink bugs were more frequent for adults than for nymphs in this study, even when a large number of nymphs were found on a given sampling date. Additionally, our data suggest that aggregation patterns were not density dependent across both years of the study for adults of *E. servus* and nymphs of *N. viridula* and *E. servus*. For adults of *N. viridula*, a significant positive relationship was found between densities and SADIE aggregation indices, indicating a density-dependence response, as shown for other arthropods (Park and Tollefson 2006). While factors that are intrinsic to a given species set boundaries to its aggregation characteristics, spatial distributions of an arthropod species can range from uniform, to random, to aggregated as densities increase (Wilson 1985). Aggregation of stink bugs can occur through a number of mechanisms, including the oviposition of eggs in masses (Hokyo and Kiritani 1962), limited dispersal of early instars (Bastola and Davis 2018), the attraction of males to females (Nakasuji et al. 1965), and aggregation pheromones produced by males (Tillman et al. 2010). Limited aggregation of nymphal stages reported here may be a result of their movement into the field, as compared with nymphs which hatch and develop in the crop. Aggregations of nymphs in a host such as wheat (Reay-Jones 2010), where reproduction occurs more commonly, could occur more frequently than in corn.

Yield loss potential from stink bug feeding is the greatest during the late vegetative stages, where feeding can lead to ear deformation (Bryant et. al 2020, Negron and Riley 1987). Feeding by stink bugs during reproductive growth stages has less yield limiting potential, but yield loss and grain quality reduction can still occur at high densities. Discoloration of kernels has been reported to be one of the best indicators of stink bug injury at these later stages on corn ears (Ni et al. 2010, Bryant et al. 2020), which was not evaluated in this study. Future studies could be conducted to assess the spatial patterns of additional injury symptoms or corn yield and their association with sampled stink bug populations, to further understand the population dynamics and injury to corn throughout the season. It should also be noted that overall population levels in 2022 were low compared with 2021. Additionally, several sampling dates in both years had only a few stink bugs limited to a couple sampling points, which could limit the interpretation of aggregation pattern results.

Aggregation patterns of stink bugs and their injury were identified in this study in fields with a range of surrounding habitats. Plant phenology, particularly on the field margins, along with field history and surrounding landscape, were identified as potential factors affecting the invasion and movement of stink bugs in a corn field. Stink bug injury was spatially associated with sampled stink bug populations in most cases, but the timing of sampling is critical, and early season sampling can be complicated by cover crop residue. Based on these results, we recommend that growers focus sampling on field margins, with particular emphasis on fields adjacent to wheat or with a recent of history of soybean in the field or the surrounding landscape. Early season sampling may not accurately estimate population levels, especially in fields with heavy crop residue, but preventative seed treatments that are almost universally applied to commercial corn seed may provide some protection (Bryant and Taylor 2021). Sampling is recommended two weeks prior to tasseling to make management decisions for late-season applications of insecticide. Insecticide applications made at or beyond pollination will likely be too late to prevent stink bugs from injuring ears. This study provided valuable insight into the spatial dynamics of stink bugs in corn, which will help to refine sampling recommendations for decision making within integrated pest management programs.

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Table 2.1 Field sites for sampling stink bugs in corn in South Carolina, 2021-2022.[a]

Year	Field	Coordinates	Field size (ha)	Planting date	Hybrid	Adjacent habitat/crops
2021	1 (Field 14)	34.30743, -79.74037	8.6	Apr 15	DKC 65-20	Woods
	2 (Field 45)	34.30116, -79.73548	8.6	Apr 1	Progeny 2025	Woods, corn
	3 (Marion 1)	34.04823, -79.32786	10.5	Mar 25	DKC 66-94	Woods, corn
	4 (Marion 2)	33.93533, -79.33918	9.2	Apr 10	DKC 66-94	Woods, fallow, corn
	5 (Horry 1)	34.15008, -78.94254	8.6	Apr 16	DKC 67-44, DKC 66-94 (refuge)	Woods, corn, wheat
	6 (Horry 2)	34.08015, -78.93060	12.0	April 13	Pioneer 1870YHR, 1870 (refuge)	Woods
	7 (Darlington 1)	34.44763, -79.80747	6.0	-	Pioneer 1464YHR	Woods
	8 (Darlington 2)	34.36047, -79.84740	6.6	-	DKC 65-20	Woods, fallow
	9 (Florence)	34.21310, -79.80230	19.8	Mar 30	DKC 69-16	Woods, commercial properties
	10 (Chesterfield)	34.51728, -79.88437	10.4	Apr 30	unspecified conventional hybrid	Woods
2022	11 (Field 29)	34.28346, -79.74825	6.1	Mar 28	DKC 68-69	Corn, cotton, wheat
	12 (Field 25)	34.28775, -79.74249	5.8	Apr 4	DKC 68-69	Corn, cotton, wheat

Year	**Field**	**Coordinates**	**Field size (ha)**	**Planting date**	**Hybrid**	**Adjacent habitat/crops**
2022	13 (Field 7)	34.30979, -79.74590	6.1	Apr 4	DKC 69-16	Woods, wheat, sorghum
	14 (Marion 1)	34.02245, -79.34458	6.3	Mar 28	DKC 66-94	Woods, corn
	15 (Marion 2)	33.93804, -79.34409	15.5	Apr 4	DKC 66-94	Woods, corn, residential
	16 (Horry 1)	34.16725, -78.95792	3.9	Apr 18	DKC 65-20, DKC 66-94 (refuge)	Corn, cotton, residential
	17 (Horry 2)	34.08417, -78.94881	9.4	Apr 21	DKC 65-20, DKC 66-94 (refuge)	Woods, corn
	18 (Darlington 1)	34.39850, -79.84109	10.5	Mar 25	DKC 65-20	Pasture, wheat, cotton
	19 (Darlington 2)	34.32496, -79.92639	8.7	Apr 6	DKC 65-20	Solar field, cotton, corn
	20 (Florence)	34.23757, -79.78246	7.4	Apr 21	DKC 69-16, speedway (refuge)	Woods

[a] All fields, with the exception of non-Bt fields (i.e., 3, 4, 10, 14, 15) and refuge plantings, were planted with corn with Bt events MON89034 (Cry1A.105 and Cry2Ab2), TC1507 and MON810 (Cry1F and Cry1Ab), or MON89034 and TC1507 (Cry1A.105, Cry2Ab2, and Cry1F) for above-ground insect pests.

Table 2.2 Overall aggregation indices for adults of *Euschistus servus* and stink bug injury in 2021 and 2022. For stink bug injury, aggregation analyses for growth stages V2-VT are for vegetative symptoms of stink bug injury, and aggregation analyses for growth stages R1-R2 are for damaged ears (banana ears), unless otherwise noted.

		Corn growth stage								**ST**
Variable	**Field**	**V2-V3**	**V4-V5**	**V6-V7**	**V8-V9**	**V10-V11**	**V12-VT**	**R1**	**R2**	
2021 *E. servus* adults	1	-	-	-	-	0.938	-	-	1.335	1.008
	2	-	-	-	-	0.892	-	0.995	1.141	0.975
	3	-	-	-	-	-	1.135	0.977	0.999	1.187
	4	-	-	-	1.244	1.341	-	1.283	**1.466***	**1.533***
	5	-	-	-	1.15	0.999	1.049	1.190	0.836	1.107
	6	-	-	-	0.945	-	1.082	-	1.125	1.275
	7	-	-	-	-	0.767	-	1.165	1.252	1.139
	8	-	-	-	0.896		**1.866***	-	0.973	**1.625***
	9	-	-	-	**1.556***	-	-	0.913	**1.891***	**1.823***
	10	-	-	-	-	**1.603***	-	0.902	-	1.434
2021 SB injury	1	1.184	-	0.878	-	-	-	-	-	-
	2	-	-	1.120	-	1.387	-	-	-	-
	3	-	-	-	-	-	-	-	-	-
	4	-	-	1.068	1.302	1.083	-	**1.438***	1.298	-
	5	-	**1.650***	-	1.385	**1.644***	-	1.133	1.029	-
	6	-	**1.573***	0.818	0.952	-	-	-	-	-
	7	**1.443***	0.916	-	-	-	-	0.964	-	-
	8	-	-	-	-	-	-	-	1.250	-
	9	-	1.091	0.994	-	-	-	-	-	-
	10^	-	-	-	1.424	1.437	-	1.135	-	-

Variable	Field	Corn growth stage								ST
		V2-V3	V4-V5	V6-V7	V8-V9	V10-V11	V12-VT	R1	R2	
2022 *E. servus* adults	11	-	-	0.782	-	1.199	-	**1.590***	0.902	**1.715***
	12	-	0.756	-	-	-	-	1.118	1.086	0.843
	13	-	-	-	1.211	-	1.266	-	0.983	1.496
	14	-	-	-	-	-	1.431	-	0.841	1.350
	15	-	-	-	0.876	-	1.232	-	1.344	1.107
	16	-	-	-	1.375	0.865	-	1.847	1.080	**1.878***
	17	-	-	-	-	-	0.762	-	0.704	0.762
	18	-	-	-	1.334	1.200	-	-	1.006	**1.514***
	19	-	-	-	1.116	0.954	-	0.893	1.085	1.163
	20	-	-	-	0.969	-	1.029	-	0.986	1.118
2022 SB injury	11^	0.936	**1.733***	0.896	-	0.769	-	1.497	1.056	**-**
	12^	0.949	1.276	0.959	1.100	-	-	1.029	-	**-**
	13	1.047	0.749	0.987	1.022	-	0.821	-	0.946	**-**
	14	-	1.094	0.873	1.269	-	1.123	-	-	**-**
	15^	0.867	0.896	-	**1.467***	-	0.923	-	**1.826***	**-**
	16^	1.170	1.069	-	0.896	1.064	-	1.153	0.963	**-**
	17^	0.945	1.074	-	**1.558***	-	**1.527***	1.094	0.892	**-**
	18	1.101	-	1.250	0.890	-	1.342	-	0.993	**-**
	19^	-	1.010	-	0.870	0.811	-	**1.371***	0.982	**-**
	20	1.099	-	1.432	0.749	-	1.384	-	1.215	**-**

* $p < 0.025$

^ 2021: Chesterfield SB injury aggregation indices are all for damaged plants

^ 2022: Darlington 2, Field 25, and Horry 1; R1 aggregation indices are for damaged plants. Field 29 and Horry 2; R1 and R2 aggregation indices are for damaged plants. Marion 2; R2 aggregation index is for damaged plants.

Figure 2.1 Densities of stink bugs per sampling point (± SE) by sampling date in each field in 2021.

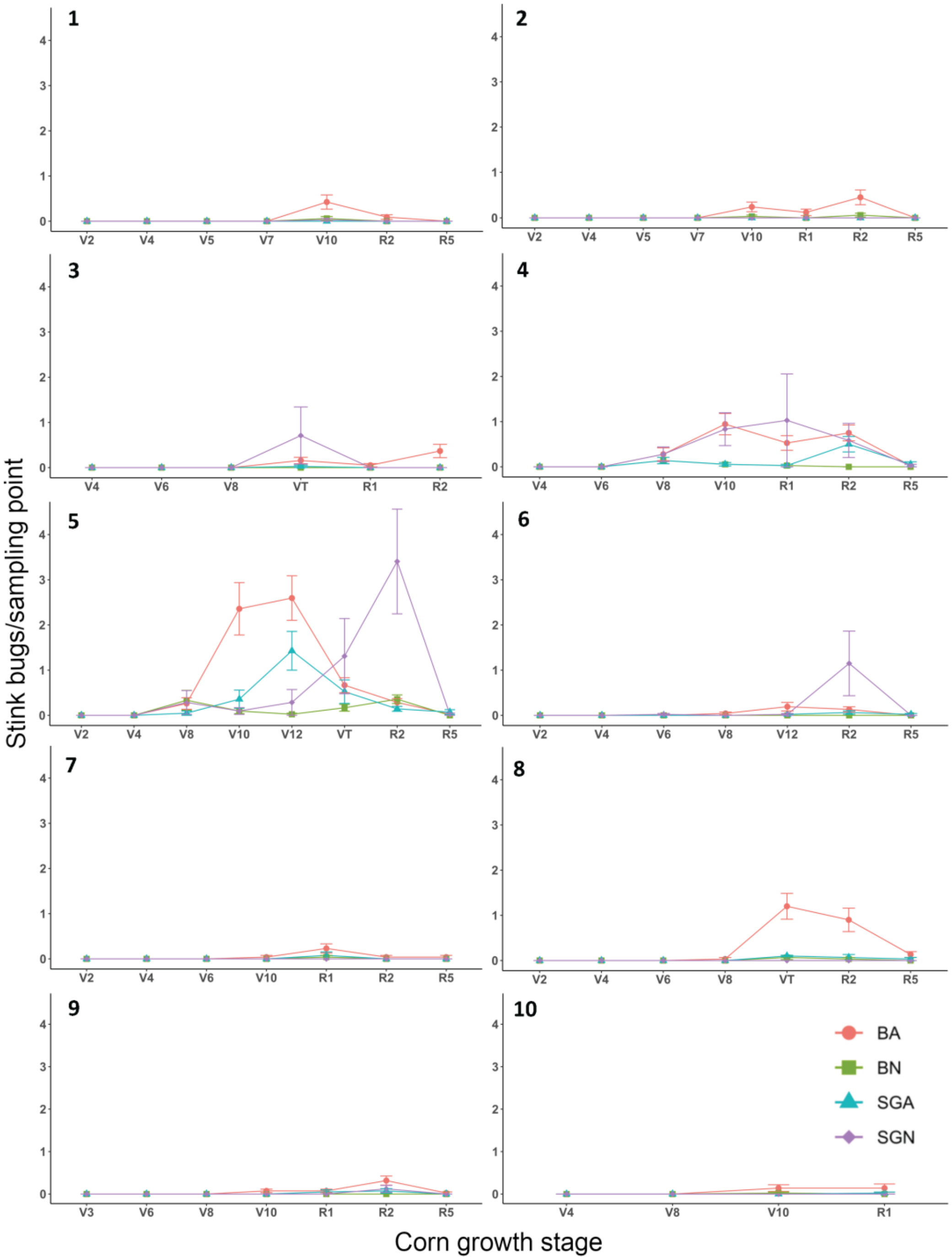


Figure 2.2 Densities of stink bugs per sampling point (± SE) by sampling date in each field in 2022.

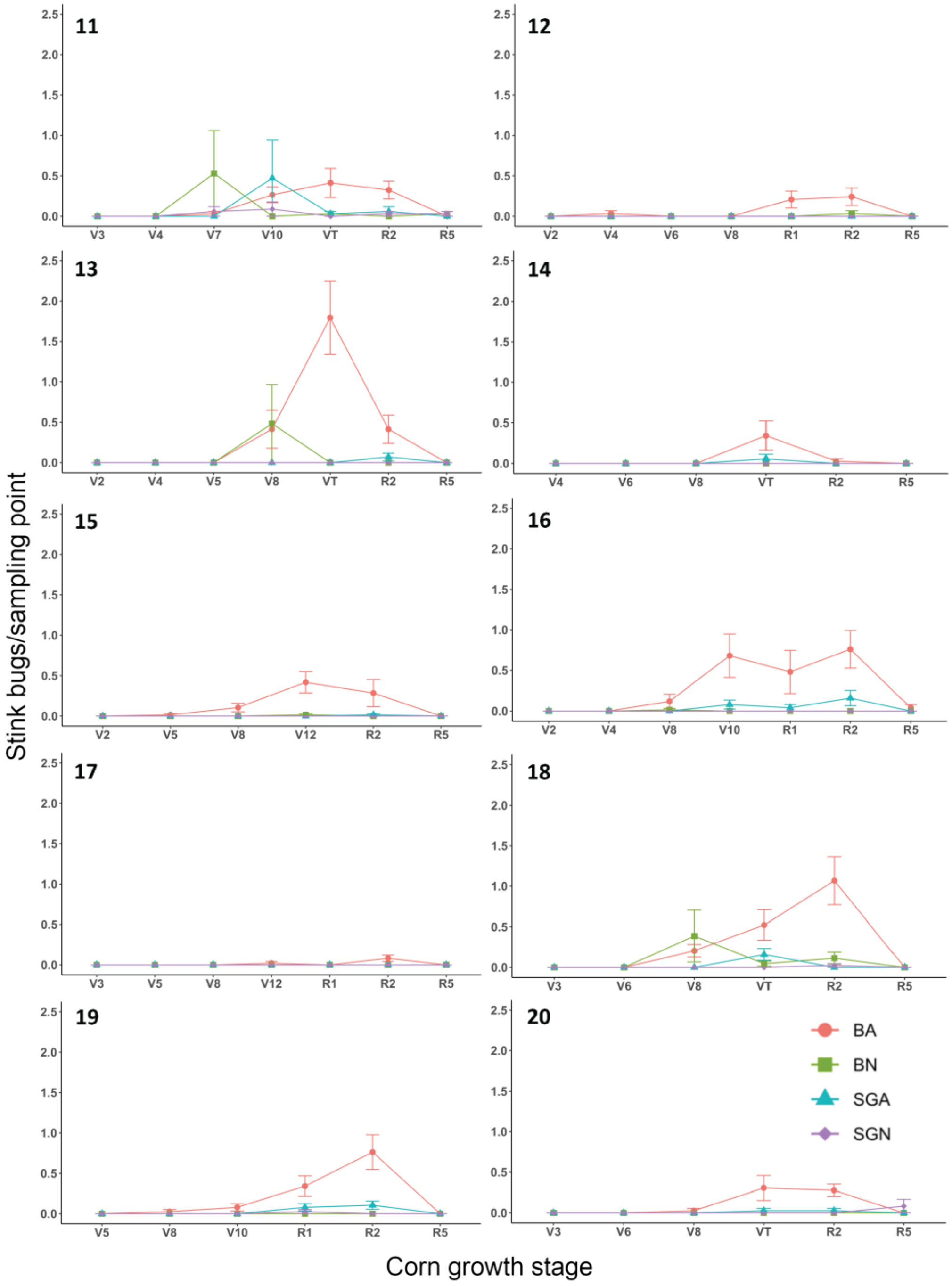


Figure 2.3 Inverse distance weighted spatial interpolation of local aggregation indices for adults of *Euschistus servus* at three individual sampling dates and season totals for fields 4 and 5 in 2021 and fields 11, 13, 16, and 18 in 2022. Black dots represent each sampling point in the field. Clusters are indicated by excluding aggregation indices between -1.5 and 1.5, with patches indicated in black ($V_i > 1.5$) and gaps indicated in grey ($V_j < -1.5$). Significant aggregation indices are indicated by asterisks (*$P < 0.025$). Relevant surrounding field conditions are indicated on the first map for each field. Wh = wheat, W = woods, C = corn, Co = cotton, and F = fallow. In the first map for each field, scale bar equals 150m.

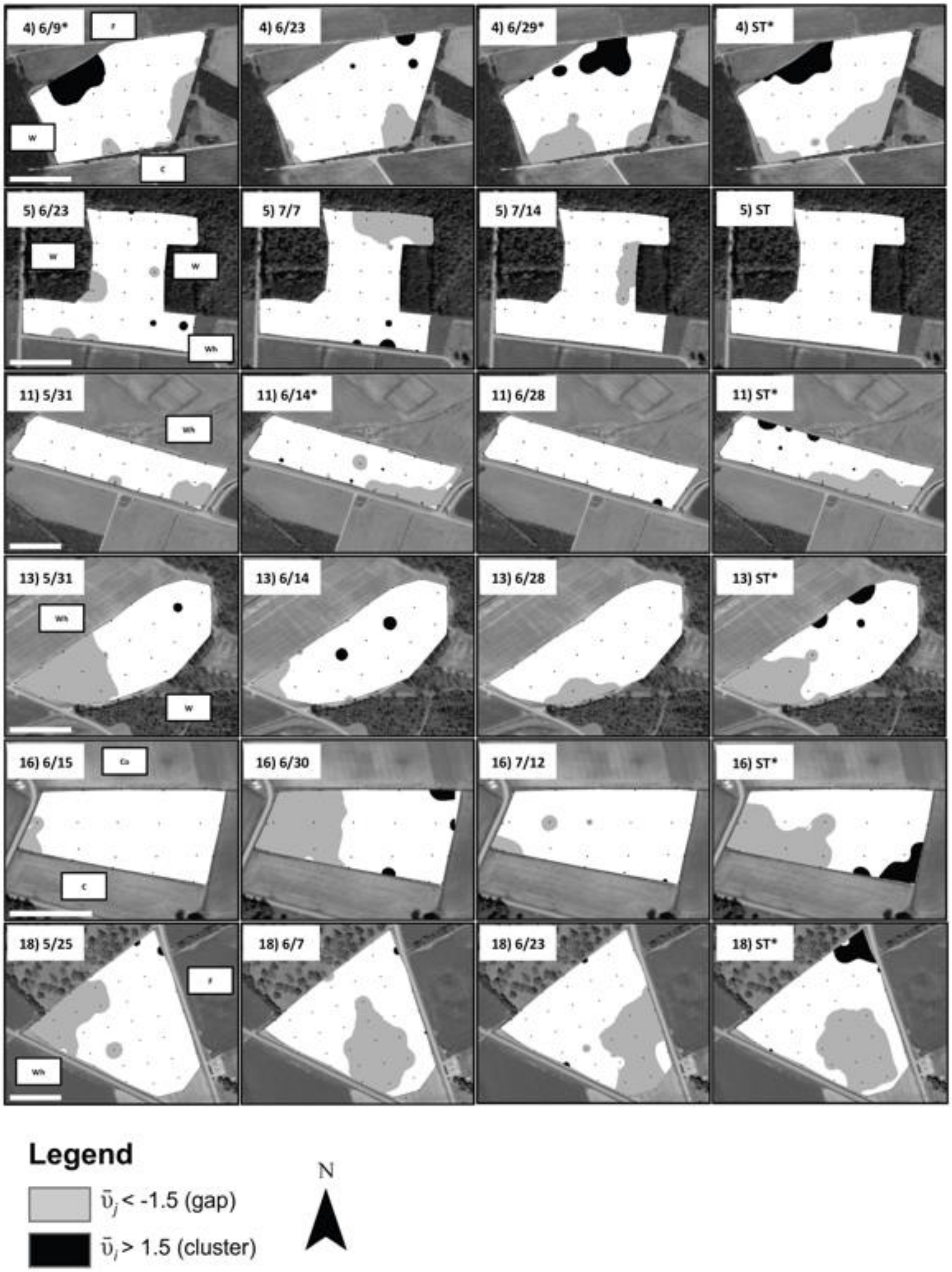

4) 6/9*
F
W
C
4) 6/23
4) 6/29*
4) ST*
5) 6/23
W
W
Wh
5) 7/7
5) 7/14
5) ST
11) 5/31
Wh
11) 6/14*
11) 6/28
11) ST*
13) 5/31
Wh
W
13) 6/14
13) 6/28
13) ST*
16) 6/15
Co
C
16) 6/30
16) 7/12
16) ST*
18) 5/25
F
Wh
18) 6/7
18) 6/23
18) ST*
Legend
$\bar{\upsilon}_j < -1.5$ (gap)
$\bar{\upsilon}_i > 1.5$ (cluster)
N

Figure 2.4 Box plots of SADIE association analyses between adults of *Euschistus servus* or *Nezara viridula* and stink bug ear injury at R2 by corn growth stage.

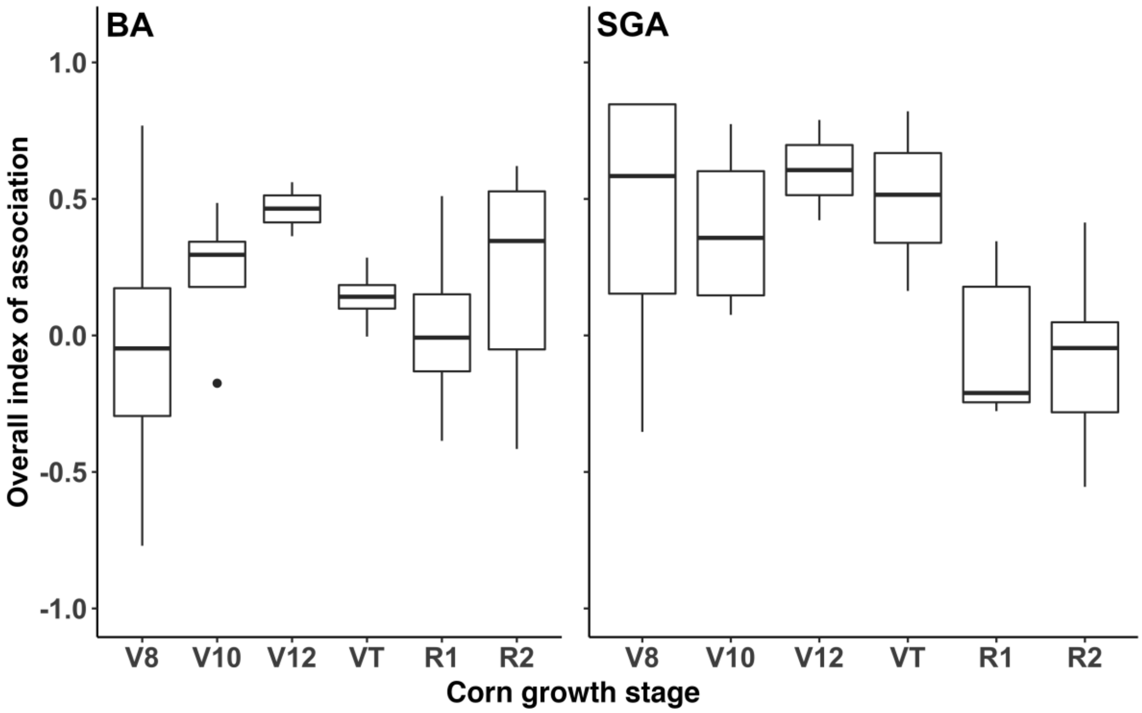
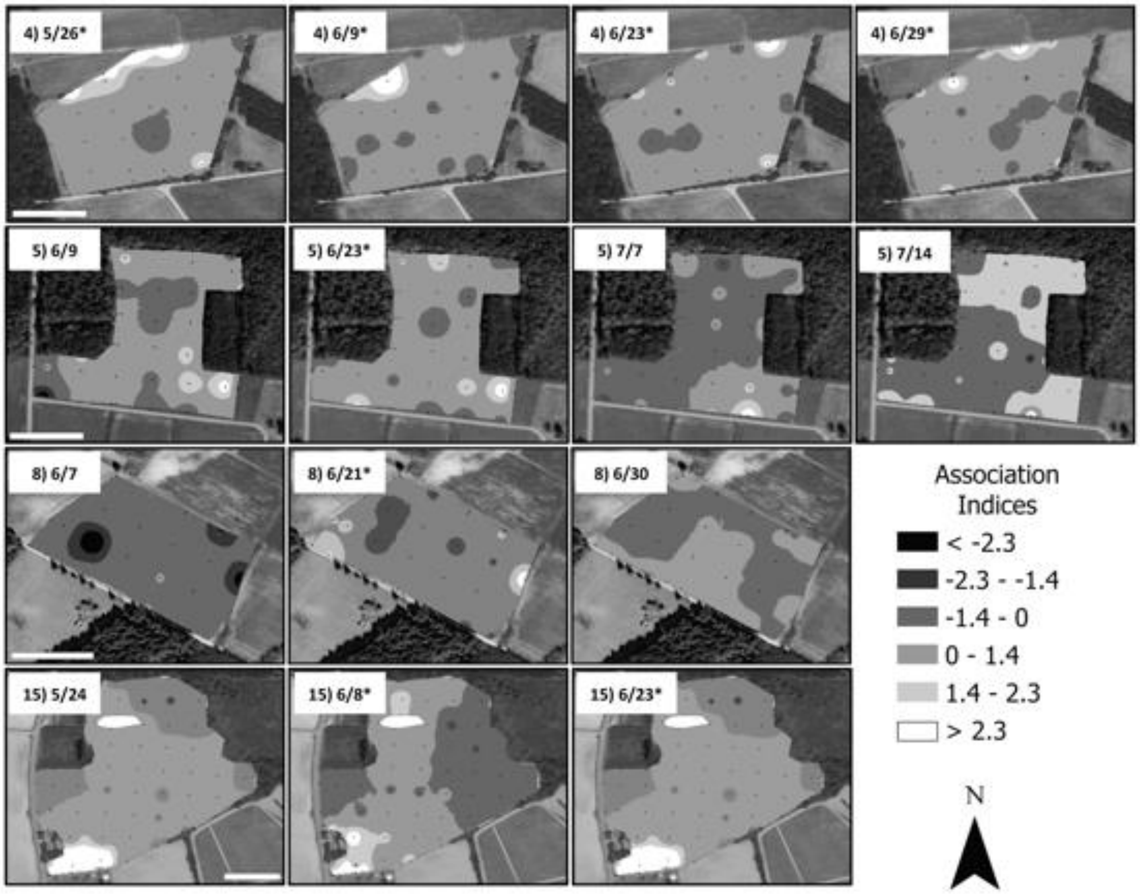


Figure 2.5 Inverse distance weighted spatial interpolation of local association indices between adult densities of *Euschistus servus* and *Nezara viridula* at various sampling dates and injured ears at R2. Row 1-4 are associations for adults of *E. servus* and row 5-7 are associations for adults of *N. viridula*. Black dots represent each sampling point in the field. Field numbers listed in table 1 are provided on each map. Significant association indices are indicated by asterisks (*$P < 0.025$). In the first map for each field, scale bar equals 150m.


4) 5/26*
4) 6/9*
4) 6/23*
4) 6/29*
5) 6/9
5) 6/23*
5) 7/7
5) 7/14
8) 6/7
8) 6/21*
8) 6/30
15) 5/24
15) 6/8*
15) 6/23*
Association Indices
■ < -2.3
■ -2.3 - -1.4
■ -1.4 - 0
■ 0 - 1.4
■ 1.4 - 2.3
□ > 2.3
N

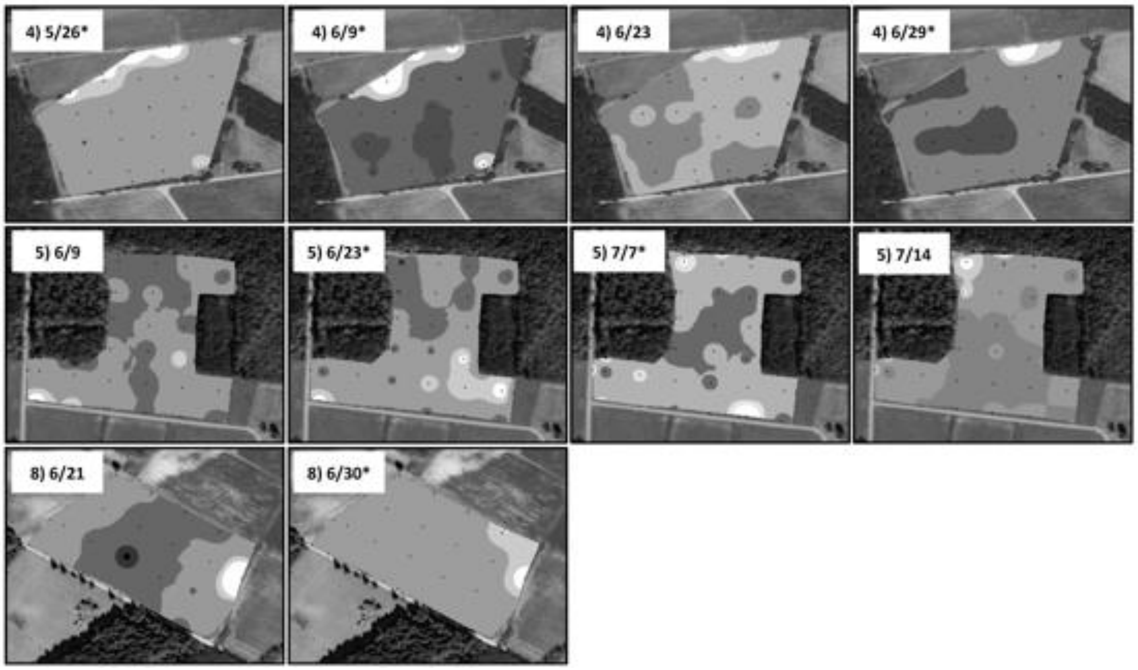
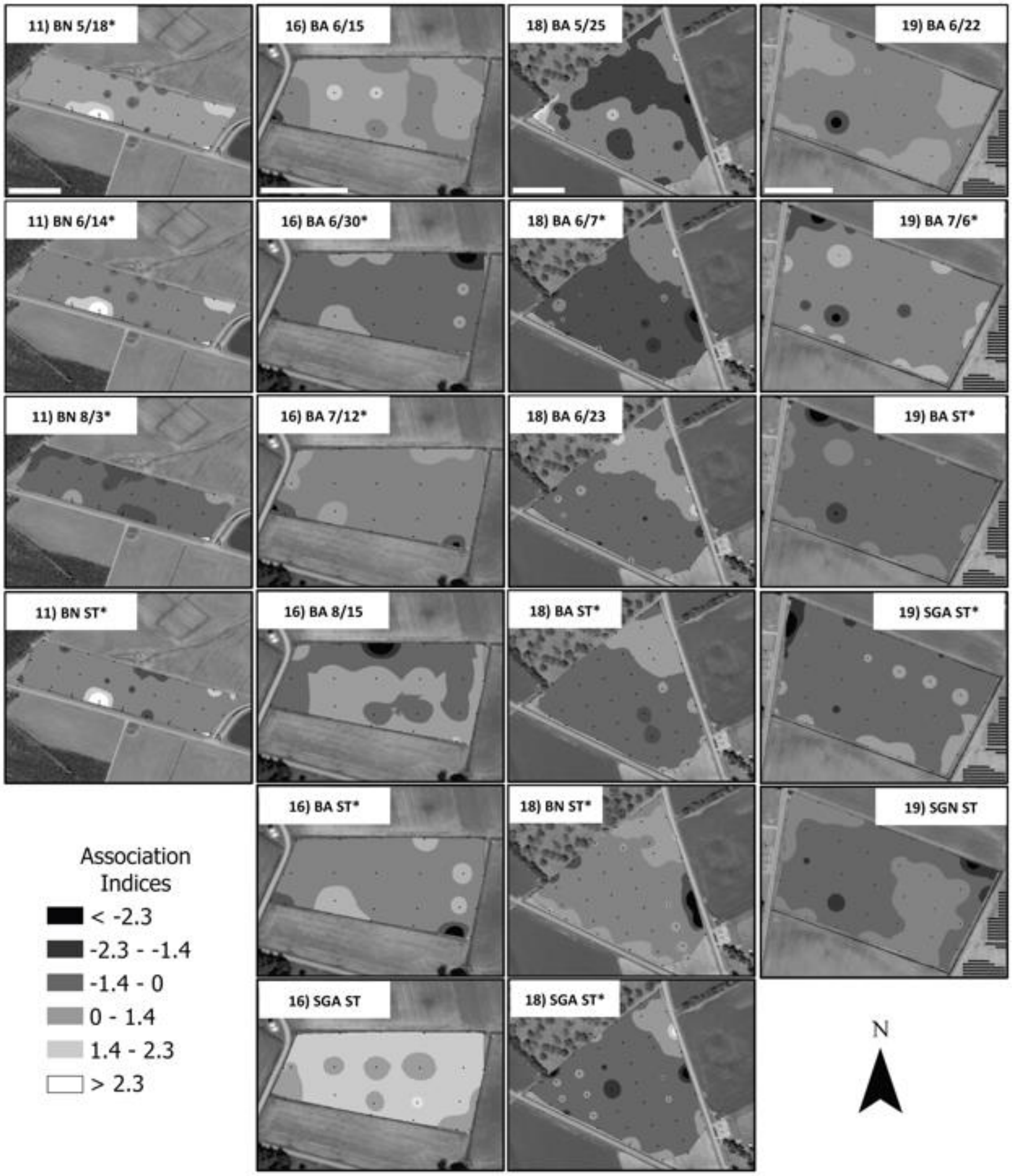

4) 5/26*
4) 6/9*
4) 6/23
4) 6/29*
5) 6/9
5) 6/23*
5) 7/7*
5) 7/14
8) 6/21
8) 6/30*

Figure 2.6 Inverse distance weighted spatial interpolation of local association indices between densities of stink bug adults and nymphs at various sampling dates or season totals and plant height. Black dots represent each sampling point in the field. Field numbers listed in table 1 are provided on each map. Significant association indices are indicated by asterisks (**P* < 0.025). Field 11 plant height date is 5/18, field 16 plant height date is 6/15, field 18 plant height date is 5/25, and field 19 plant height date is 6/22. BA or BN = brown stink bug (*Euschistus servus*) adult or nymph, respectively, SGA or SGN = southern green stink bug (*Nezara viridula*) adult or nymph, respectively. In the first map for each field, scale bar equals 150m.


11) BN 5/18*
16) BA 6/15
18) BA 5/25
19) BA 6/22
11) BN 6/14*
16) BA 6/30*
18) BA 6/7*
19) BA 7/6*
11) BN 8/3*
16) BA 7/12*
18) BA 6/23
19) BA ST*
11) BN ST*
16) BA 8/15
18) BA ST*
19) SGA ST*
16) BA ST*
18) BN ST*
19) SGN ST
16) SGA ST
18) SGA ST*
Association Indices
< -2.3
-2.3 - -1.4
-1.4 - 0
0 - 1.4
1.4 - 2.3
> 2.3
N

CHAPTER THREE

WITHIN-FIELD SPATIAL PATTERNS OF *HELICOVERPA ZEA* (LEPIDOPTERA: NOCTUIDAE) AND SPATIAL ASSOCIATIONS WITH STINK BUGS AND THEIR INJURY IN FIELD CORN[2]

Introduction

The corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), is a polyphagous and cosmopolitan pest in the southeastern United States field crop landscape. It infests and injures cotton, *Gossypium hirsutum* L., soybean, *Glycine max* (L.) Merr., sorghum, *Sorghum bicolor* (L.) Moench, field corn, *Zea mays* L., and numerous other crop and non-crop hosts (Neunzig 1969). Field corn is a major mid-season host in the landscape for *H. zea* reproduction and subsequent dispersal into other crops or wild flowering hosts (Roltsch and Mayse 1984, Bradley 1993). In field corn, *H. zea* eggs are generally laid directly on the recently emerged silks, and larvae move down the silk channel into the ear to feed on developing kernels (Wiseman et al. 1978). In the southern United States, below 40 degree latitude, *H. zea* overwinters in the soil as a pupa and emerges in the spring (Hardwick 1965). At more northern latitudes, *H. zea* reaches corn and other hosts via migration, which can occur over large distances. Analyzing the presence of pollen on *H. zea*, migration of at least 750 km was shown to occur from Texas to Arkansas (Hendrix et al. 1987). A similar study in Oklahoma indicated migration from Florida, the Caribbean, Mexico, or Central America (Lingren et al. 1994). The ability of *H. zea* to

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disperse over large spatial scales plays a major role in their widespread pest status (Jones et al. 2019).

Compared with long distance migration, short-range movement of *H. zea* has been less studied, and likely plays an important role in spatial patterns on within fields and across landscapes. A mark-recapture study conducted in South Carolina indicated local movement occurred mainly downwind, with crop patterns having no impact (Culin 1995). A more recent mark-recapture study showed a local dispersal capacity of 150-1,600 m for *H. zea* (Tavares et al. 2019). The spatial heterogeneity of insect distributions is caused by a number of factors, including the ability of a species to disperse over long and short distances (Tscharntke and Brandl 2004). Given the primary focus of the literature on spatial patterns of *H. zea* regarding long-range migration, the within-field spatial and dispersal patterns of *H. zea* in field corn and other crops have not been extensively studied.

Patterns of aggregation in *Helicoverpa* spp. larvae have been found in corn ears, but not consistently (Ni et al. 2014, Silva et al. 2018). In tomato fields, infestations of *Helicoverpa armigera* (Hübner) were shown to initially concentrate on field margins and slowly moved to more interior areas as weather conditions favored dispersal (Moral García 2006). A similar pattern of edge concentration was identified in the tomato leafminer, *Phthorimaea absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Martins et al. 2018). In field corn, spatial patterns of *Spodoptera frugiperda* (J.E. Smith) were initially aggregated and then moved increasingly towards spatial randomness as populations developed, with a strong association in spatial patterns between plant phenology and infestations (Farias et al. 2008, Hernández-Mendoza et al. 2008). The spatial patterns of H. zea in corn may be similarly dependent on the age of the population in the field and the plant phenology as seen in similar noctuid pests.

In addition to broadening our understanding of pest ecology, examining within field distributions of *H. zea* can help to refine insect resistance management strategies (Jeger 1999, Downes et al. 2017). The majority of corn grown in the United States has been genetically engineered to express toxins produced by the soil-borne bacterium *Bacillus thuringiensis* (Bt). Transgenic corn expressing Bt toxins can provide varying levels of control of *H. zea*, with resistance reported to all Cry toxins, and Vip3A currently being the only Bt toxin providing very good levels of control (Bilbo et al. 2019a, Reay-Jones et al. 2020, Yang et al. 2021, Reisig et al. 2023). While *H. zea* is not considered an economic pest in corn (Reay-Jones and Reisig 2014, Bibb et al. 2018, Olivi et al. 2019), it is a major pest of cotton, a crop that expresses many of the same or similar Bt toxins found in corn (Kanel et al. 2016). Cotton is one of the major cultivated hosts for *H. zea* dispersing from field corn, and natural refuge is widely available at that point in in the season (Gould et al. 2002, Head et al. 2010). As such, resistance management is primarily implemented in corn via non-Bt refuge planting (Bates et al. 2005). Refuge is a percentage of corn acreage planted with non-Bt corn hybrids in strips or blocks in or near Bt fields. These blocks or strips of non-Bt corn allow individuals that are susceptible to Bt toxins to successfully develop and reproduce, thus maintaining some level of susceptible individuals in the population. Pest ecology, dispersal ability, and host selection behaviors linked to plant phenology can play a major role in the success of these spatially based refuge strategies (Parry et al. 2017). Quantifying within-field spatial patterns of *H. zea* may help to better understand infestation dynamics in both Bt and non-Bt refuge corn and improve the implementation of resistance management.

In addition to *H. zea*, a complex of stink bugs (Hemiptera: Pentatomidae), namely *Euschistus servus* (Say) and *Nezara viridula* (L.), are common and important economic pest species in field corn in the southeastern United States (Reisig et al. 2021). Stink bug feeding on field corn during the late vegetative stages of development (i.e., V10-VT) leads to the development of misshapen or “banana-shaped” ears and a reduction in yield (Clower 1958, Bryant et al. 2020). These damaged ears are likely exposed to secondary pests and pathogens, which would normally not be able to access the ear inside the husk. Additionally, stink bug damaged ears may be less attractive or suitable as a host for *H. zea* based on ear quality or exposure to natural enemies, although such interactions have yet to be studied. Interspecific competition between *Heliothine* larvae and stink bugs has been identified in controlled studies in cotton (Zeilinger et al. 2011, Zeilinger et al. 2015a,b), but these studies explicitly examined the impact of larval feeding on stink bug feeding and host seeking behavior.

Understanding the spatial ecology of *H. zea* larvae and injury in the southeastern landscape in relation to plant phenology, as well as potential spatial interactions with other major pests, may provide foundational ecological information for future improvements to IPM programs (Jeger 1999). The goals of this study were to 1) assess the spatial patterns of *H. zea* larvae and their injury at two different sampling timings in corn, and 2) assess the spatial associations among *H. zea* larvae, injury, plant phenology, and stink bug populations and their injury.

Materials and Methods

Fifteen commercial and five research fields at the Clemson University Pee Dee Research and Education Center in Florence, SC, were selected in 2021 and 2022, for a total of ten fields per year, to sample *H. zea* populations and their injury (Table 3.1). A previous study reported stink bug spatial patterns from the same set of corn fields (Bryant et al. 2023). Applications of bifenthrin (110 g a.i./ha), bifenthrin (97 g/ha a.i.) and chlorantraniliprole (64 g a.i./ha), or *lambda*-cyhalothrin at a rate of 30 g a.i./ha were made in some fields, as reported in Bryant et al. (2023). In 2021, bifenthrin was applied on 3 (field 1) and 25 (field 5 and 6) May, and bifenthrin and chlorantraniliprole were applied on field 3 on 10 June. In 2022, bifenthrin was applied on 20 (fields 14 and 15) and 22 (field 18 and 19) June, and *lambda*-cyhalothrin was sprayed on field 20 on 12 May. These insecticide applications were made by growers to target stink bugs. Corn earworm is very difficult to manage even using properly timed foliar insecticide applications (Reay-Jones and Reisig 2014), and all applications in our study were made prior to silking when *H. zea* moths are attracted to corn for oviposition. As such, these applications likely had no impact on the population densities and dynamics of *H. zea* in this study. Various hybrids were planted in our study fields, including some fields with hybrids expressing Bt toxins that have the potential to impact *H. zea* (Table 3.1).

A sampling grid was used in each field, with a distance of 61 m between sampling points or one sampling point for every 0.37 ha. This distance among sampling points was chosen based on prior sampling studies conducted in row crops (e.g., Reay-Jones et al. 2010; Reay-Jones 2014). Additional sampling points were placed on the edge of the field at the end of each grid line to determine potential edge effects. A 38 cm wire stake flag was placed at each sampling point. GPS coordinates were recorded at each point using an iPhone XR (Apple, Cupertino, CA). Sampling was conducted in each field at corn growth stages R2 to assess active *H. zea* larval populations and early injury and at R5 to assess end of season injury and residual larval populations.

For *H. zea* sampling, 25 ears were opened at each sampling point and examined for larvae categorized by size (small, medium, and large) and injury in cm^2. Small larvae were categorized as first and second instars, medium larvae were categorized as third and fourth instars, and large larvae were categorized as fifth and sixth instars. Injury was categorized as either injury to the tip of the ear, where pollination failed and no kernels developed, or injury to kernels (Bilbo et al. 2019b). Samples were taken on the row of the sampling point at R2 and on the adjacent row at R5.

In addition to sampling *H. zea* larvae and injury described above, stink bugs and stink bug ear injury (i.e., banana ears) were counted and summed over 25 plants at each sampling point at R5. Stink bug samples taken from emergence to R2 were reported in Bryant et al. (2023), and one additional stink bug density and injury sample taken at R5, previously unpublished, is reported here. Stink bugs were identified to species (i.e., *E. servus* or *N. viridula*) and life stage (i.e., adult or nymph). The primary symptom of stink bug injury to ears used in analyses was a “banana-shaped” ear, as described in Clower (1958). As also reported in Bryant et al. (2023), the heights of five plants at each sampling point were measured in 2022 from the soil to the tip of the tallest extended leaf and averaged for each sampling point. Plant heights were then rounded to the nearest integer for analyses. Plant height measurements were taken from the first sampling date through V10/VT in each field. Only seasonal totals of stink bugs and plant heights from Bryant et al. (2023) were used in the data analysis of the current study, with all *H. zea* data and stink bug data at R5 being new and previously unpublished.

Statistical Analyses

Variables were first summed across the 25 plants at each sampling point. Spatial analysis by distances indices (SADIE) was used to analyze *H. zea* larvae and injury metrics. SADIE analyses were conducted using the "epiphy" package (Gigot 2018) in R version 3.6.3 (R Core Team 2020). The SADIE analyses generate local indices of dispersion at each sampling point, with either a positive cluster index (V_i) for counts above the average or a negative gap index (V_j) for counts below the average. A random spatial pattern has indices $V_i = -V_j = 1$. The overall index of dispersion (I_a) indicates either aggregation ($I_a > 1$), random ($I_a = 1$), or uniform spatial distribution ($I_a < 1$). The null hypothesis of spatial randomness is rejected when $P < 0.025$ for aggregation or $P > 0.975$ for uniformity. Each analysis consisted of 5,967 randomizations.

SADIE association analysis was then used to spatially associate aggregation indices between two datasets sharing the same sampling locations (Perry and Dixon 2002). An overall index of association (X) was determined between two paired datasets, with $X > 0$ ($P < 0.025$) for a positive association and $X < 0$ ($P > 0.975$) for a negative association. A positive association specifies either a gap or a cluster in both datasets at the same location, and a negative association specifies a gap in one dataset and a cluster in the other dataset at the same location. Association analyses were conducted between *H. zea* larval densities and injury at a given sampling date and stink bug season totals (Bryant et al. 2023) and stink bug ear injury at R5. Additional association analyses were conducted between *H. zea* and corn plant height (Bryant et al. 2023) taken at each sampling date through V10/VT for each field in 2022. The local aggregation or association indices were spatially interpolated using the inverse distance weighted (IDW) method in ArcGIS Pro version 2.9 (Esri 2020). The effect of Bt hybrid on number of *H. zea* larvae and total injury (i.e., the sum of tip and kernel injury from *H. zea* feeding) was analyzed using a linear model in the package "lme4" (Bates et al. 2015). Additionally, the effect of *H. zea* larval size, corn growth stage, and the interaction was analyzed for each year of the study using linear models.

Results

***Helicoverpa zea* Variables**

Larvae and injury were present in every field regardless of corn hybrid. Bt hybrid did not have a significant impact on the total number of *H. zea* larvae at R2 (F = 1.177; df = 3, 16; *P* = 0.350) or at R5 (F = 0.482; df = 3, 14; *P* = 0.700). Generally, larvae of *H. zea* did not exceed one per ear, with the exception of several sampling points in fields 10, 15, and 18 (with maximums of 1.52, 1.20, and 2.04 larvae/ear at a given sampling point for each field, respectively). Average total *H. zea* larvae (i.e., small, medium, and large) per sampling point ranged from 0.003 to 0.473 per ear at R2 and from zero to 0.103 per ear at R5. At R2, small larvae accounted for a large percentage of larvae in both years

(53% in 2021 and 44% in 2022). Medium and large larvae accounted for 24 and 23% of the larvae in 2021 and 25 and 30% of larvae in 2022 at R2, respectively (Fig. 3.1). At R5, few larvae were found in both years, with the percentage distribution of larval stage being generally uniform at this stage (Fig. 3.1). Average total *H. zea* injury ranged from 0.04 to 4.69 cm^2 per ear at R2 and from 0.10 to 3.44 cm^2 per ear at R5. There was no significant difference between the number of larvae per ear in each size category in 2021 (F = 1.927; df = 2,48; $P = 0.1567$) or 2022 (F = 0.432; df = 2,54; $P = 0.432$) (Fig. 1). There were significantly more larvae at R2 than R5 in both years of the study (2021; F = 11.409; df = 1,48; $P = 0.001$, 2022; F = 29.436; df = 1,54; $P < 0.001$). The interaction of corn growth stage and larval size was not significant (2021; F = 2.195; df = 2,48; $P = 0.122$, 2022; F = 0.991; df = 2,54; $P = 0.377$). Bt hybrid did not have a significant impact on total ear injury (i.e., kernel and tip) at R2 (F = 0.362; df = 3, 16; $P = 0.782$) or at R5 (F = 1.483; df = 3, 14; $P = 0.262$). Of the total *H. zea* injury, the percentage of this injury categorized as kernel injury ranged from 38 to 78% and the percentage of total injury categorized as ear tip injury ranged from 22 to 62% depending on the field. Injury levels varied among fields, growth stages, and hybrids (Fig. 3.2).

Aggregation Analyses

Out of a total of 250 analyses of *H. zea* larvae and their injury, 35 (14%) indices indicated significant aggregation ($P < 0.025$). Of these 35 analyses, 18 were at growth stage R2 and the remaining 17 were at growth stage R5 (Tables 3.2 and 3.3). At R2, where larval densities were higher, aggregation analyses were more commonly significant for *H. zea* larvae than injury metrics. At R5, aggregation analyses were more commonly significant for injury metrics than for the larval populations remaining in the field at R5. Where significant aggregation patterns were identified, interpolation of local aggregation indices for *H. zea* larvae at R2 revealed that clusters were primarily at interior sampling points, reaching the field margin in some cases (Fig. 3.3). Interpolation of local aggregation indices for injury at R5 revealed both large clusters of increased *H. zea* injury and gaps of reduced injury within some of the sampled fields (Fig. 3.4). Compared with interpolation of *H. zea* larvae, interpolation of injury data at R5 displayed larger gaps and clusters, emphasizing the uneven infestation and injury potential of *H. zea* in the field (Fig. 3.4). Stink bug ear injury was observed at R5 in 11 of the 20 sampled fields across both years, with significant aggregation found in only one (Field 18; $I_a = 1.716$, $P = 0.002$) (8%) of 11 analyses.

Association Analyses

Association analyses between plant height measurements taken throughout the season and *H. zea* larvae and injury yielded significant positive associations and significant negative associations in 145 (31%) and 14 (3%) analyses, respectively, out of 460 total analyses. The last plant height measurement in each field (i.e., V10/VT) was used for subsequent interpolation mapping. Of a total of 119 analyses associating the last plant height measurement with *H. zea* larvae and injury at R2 and R5, 42 (35%) positive and three (3%) negative associations were identified, respectively. Fifteen and 23 significant positive associations were identified between the last plant height measurement and *H. zea* larvae and injury, respectively, at R2 (Table 3.4). At R5, two positive associations were identified between the last plant height measurement and *H.*

zea larvae (Table 3.4). Four positive and three negative associations were identified between plant height and *H. zea* injury at R5 (Table 3.4). Interpolation of local association indices showed that field margins, where plants were reduced in height and delayed in reproductive development, had gaps in *H. zea* larvae at R2 in some fields (Fig. 3.5). More interior sampling points where plants were often better developed relative to the field margin had increased densities of *H. zea* larvae at R2. In some cases, where a negative association between plant height and *H. zea* injury was identified at R5 (Table 3.4), an increased level of injury was clustered on field margins where ears developed later.

Out of 727 spatial association analyses between stink bug season totals by species and life stage with *H. zea* larvae and injury at R2 and R5, 106 (15%) and 56 (7%) were positively and negatively associated, respectively. A total of 380 of these association analyses were with *H. zea* at R2, and 347 were with *H. zea* at R5. At R2, *H. zea* larvae and injury were positively and negatively associated in 72 (19%) and 22 (6%) analyses, respectively. Associations were less frequent at R5, with 34 (10%) positive and 34 negative associations. Of 61 association analyses between stink bug ear injury (i.e., "banana-shaped" ears) at R5 and *H. zea*, seven were negatively associated and one was positively associated (Table 3.5). Interpolation of local association indices, particularly in Field 4, showed large patches of stink bug injured ears on the margin of the field, where *H. zea* larval populations and subsequent injury were highly reduced (Fig. 3.6).

Discussion

Spatial aggregation patterns of *H. zea* populations and their injury were identified in corn fields with and without Bt hybrids. To our knowledge, our spatial association analyses are the first to show that clusters or gaps in *H. zea* spatial patterns can be related to plant phenology, which usually varies to some degree throughout a field, particularly given the highly variable soils of the southeastern Coastal Plain (Duffera et al. 2007). Additionally, spatial patterns of stink bug injury were negatively associated with *H. zea* larvae and injury in a small number of cases, indicating some type of competition between these two pests in corn or differences in host selection behaviors relative to plant phenology. The spatial patterns of *H. zea* in field corn have only been sparsely described (Ni et al. 2011, 2014), and this is the first study that demonstrates spatial relationships between *H. zea* larvae and injury and plant phenology, as well as stink bugs, which are the most important pest group in corn in the southeastern United States (Reisig et al. 2021).

Lepidopteran pests of field corn have generally been shown to be randomly or uniformly distributed in corn fields, although published data are limited (Farias et al. 2008, Hernández-Mendoza et al. 2008, Ni et al. 2014, Silva et al. 2018). In our study, *H. zea* larval populations and injury were aggregated in a number of fields at R2. At R5, the total number of ears injured by *H. zea* and the amount of injury were aggregated far more commonly than for populations of *H. zea* larvae remaining in the field at that stage. Differences in Bt hybrids used in our fields did not have a significant impact on the number of larvae or the level of injury. This finding is unsurprising, given that resistance to Cry toxins expressed in Bt hybrids planted in our study has been widely reported in *H.*

zea in the southeastern United States (Bilbo et al. 2019a, Yu et al. 2021, Reisig et al. 2023). The spatial patterns described herein were likely not influenced by differences in Bt toxins expressed and more dependent on other factors discussed herein, including pest ecology, natural enemies, host seeking behavior, plant phenology, or presence of other herbivores.

Aggregation of *H. zea* larvae was less common at R5 than at R2, which may result from differences in the age of larvae between the two corn growth stages. The degree of clumping (i.e., spatial aggregation) has been found to decrease as populations of *Helicoverpa* spp. Age, which was attributed to egg or early larval mortality (Wilson and Room 1983). In our study, the proportion of larvae categorized as large was higher at R5 where fewer aggregation patterns were identified relative to R2 (Fig. 3.2). This may support the hypothesis that the degree of aggregation is in part related to the age of the population. Additionally, the number of larvae per ear on average did not exceed one in the present study, with only a few exceptions. If multiple eggs are laid on a single ear, cannibalism commonly occurs in *H. zea* (Dial and Adler 1990), contributing to movement towards a more random or uniform distribution. *Helicoverpa zea*, as well as other lepidopteran pest species, commonly prefer to oviposit on previously uncolonized fruit or plants (Thiery et al. 1995, Silva et al. 2018), which may also contribute to more uniform spatial distributions.

Where random distributions were detected at R2 or R5 it may have been related to a number of factors, including *H. zea* abundance, greater uniformity in plant phenology, or the local and long-distance dispersal capability of *H. zea*. For example, distributions of

S. frugiperda have been shown to become more uniform as densities increase (Farias et al. 2001, Hernandez-Mendoza et al. 2008). A similar result was found in some of the fields in our study with the highest total densities of larvae at R2 (i.e., Fields 11 and 18), which had random spatial distributions. In contrast to this result, Fields 13 and 15 had similar population levels to those observed in Fields 11 and 18, but had aggregated patterns of total larval populations at R2. More research is needed to better understand the role of intraspecific competition in *H. zea* spatial patterns, but it may be a mechanism in driving spatial uniformity as populations increase.

The long-range and local dispersal ability of *H. zea* may facilitate random or uniform infestations in a field. Short-range movement either to find a suitable developmental host or mediated by weather conditions can commonly lead to uniform patterns of distribution after initial infestations in many lepidopteran pests (Hernández-Mendoza et al. 2008, Martins et al. 2018, Silva et al. 2018). Volatiles emitted by corn silks have a significant impact on the mating and ovipositional behaviors of *H. zea* moths (Raina et al. 1992), suggesting that, where patterns of aggregation were identified in these studies, it may have been linked to plant phenology, as opposed to dispersal ability. Association analyses with plant height supported this hypothesis. At R2, when oviposition was peaking (Lopez et al. 1978), many plants, particularly on the margins of fields, were stunted and delayed in reproductive maturity, which is common in fields close to natural landscape elements, such as trees (Raatz et al. 2019). These gaps in plant height were positively associated with gaps of *H. zea* larvae, indicating that ovipositing *H. zea* moths may avoid areas with reduced plant height and delayed reproductive maturity. Negative associations were then identified between plant height and *H. zea* larvae in several fields at R5. As the delayed plants finally reached reproductive maturity, they likely became more attractive for *H. zea* oviposition relative to other parts of the field that had become less attractive; this is supported by clusters of larvae that were negatively associated with plant height at this stage (i.e., shorter plants with more larvae). Positive associations were also identified between larvae at R5 and plant height. In some cases, the stunted plants on field margins never developed ears, which may have contributed to the occurrence of these positive associations. Spatial patterns of fall armyworm have been shown to be dependent on plant phenology (Hernández-Mendoza et al. 2008), but these studies examined infestations occurring during vegetative stages of corn development. Several studies have also shown *H. zea* having ovipositional preference for cotton with increased irrigation and nitrogen application rates, leading to better fruit set and host quality (Braswell et al. 2019a, 2019b). Infestations of *H. zea* can occur occasionally during corn vegetative stages (Quaintance and Brues 1905; Arends 2020), but their preference for a fairly narrow window during reproductive stages of corn when green silks are available likely increases non-uniform spatial patterns in fields where variability in plant phenology is high.

In most cases, no significant associations were detected between stink bugs and *H. zea* for both injury and densities. However, a small number of negative spatial associations between stink bug ear injury and *H. zea* larvae and injury were identified, indicating some potential spatial interaction between the two pest complexes and their injury. Several potential mechanisms could lead to this observation. Corn ears and silks release a number of plant volatiles which likely play an important role in host-seeking behavior for *H. zea* (Potter et al. 2015). The complex of emitted volatiles can be modified or induced by herbivorous insect feeding, in this case stink bug feeding, and can alter the attractiveness of the host for lepidopteran pests (von Mérey et al. 2013, Zeilinger et al. 2015a), have a negative impact on the development of larvae (von Mérey et al. 2013), or attract natural biological control agents (Turlings et al. 2008). The kernels of stink bug injured ears are also often exposed from the husk, which normally protects developing *H. zea* larvae from predators and parasitoids (Manley et al. 1991). The combination of increased exposure on stink bug injured ears and plant volatile attraction of natural enemies may limit *H. zea* infestations in these areas of the field. Stink bug populations are often aggregated on the edge of fields, dispersing into a crop from adjacent crops or habitats (Venugopal et al. 2014, Bryant et al. 2023). If *H. zea* avoids fields margins and stink bugs colonize these areas of the field, it may also result in the observed negative associations seen here.

Stink bug densities summed over the season were negatively associated with *H. zea* in 7% of analyses, providing some further support for the spatial interactions of these two species as reported above. However, stink bug densities were also positively associated with *H. zea* in 15% of analyses, which may result from the influence of plant phenology acting equally on the spatial dynamics of stink bugs and *H. zea*. In some cases, the numbers of stink bugs found were likely not significant enough or were not synchronous with plant phenology to induce the ear injury described here. In this case, the presence of stink bugs may not have had an impact on *H. zea* oviposition preference or injury. This is the first study to examine the potential impact of stink bug feeding on *H. zea* infestations in corn. Results indicate that the two pests interact in the field corn landscape, but further studies are needed to better understand the extent of competition between these two pests in the field under various environmental conditions.

Given that Bt resistance is widespread in *H. zea* (e.g., Bilbo et al. 2019a, Reisig et al. 2023), which is supported by infestations levels in Bt corn presented here, resistance management is critical for prolonging the use of an important management tool. *Helicoverpa zea* resistance to Bt toxins is primarily managed in corn using non-Bt refuge plantings (Bates et al. 2005). These refuge plantings are either in blocks in or near the Bt field or in strips within a Bt field. Given that refuge plantings may be within, on the edge of, or nearby Bt fields, the spatial patterns of *H. zea* within a field may have an impact on the percentage of susceptible moths that develop in the refuge and successfully mate with heterozygous resistant individuals from the nearby Bt plants. Additionally, asynchronous development of *H. zea* on Bt and non-Bt crops can lead to assortative mating, which may accelerate resistance evolution due to an increased number of homozygous resistant individuals (Liu et al. 1999). This asynchronicity in development between Bt and refuge fields may be further compounded by the spatial variability in plant phenology and its association with *H. zea* spatial patterns as reported here. The spatial aggregation patterns of *H. zea* described here and the differences in larval development linked to plant phenology provide insight that may help improve future recommendations on refuge planting requirements.

This study provides the first comprehensive look at the spatial patterns of *H. zea* within corn fields. Patterns of spatial aggregation were identified in a number of fields and were linked with plant phenology. While *H. zea* is not managed in field corn beyond the use of Bt hybrids, understanding the local dispersal and population dynamics of *H. zea* provides valuable insight that can be used to further improve integrated pest management and insect resistance management programs for this major pest (Jeger 1999, Downes et al. 2017). This study also showed spatial interactions between stink bugs and *H. zea*, which was previously unreported in field corn. The results of these studies provide valuable information on the ecology of a major pest of the southeastern field crop landscape and can provide foundational knowledge to further improve future management strategies.

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Table 3.1 Field sites for sampling stink bugs in corn in South Carolina, 2021-2022.[a]

Year	Field	Coordinates	Field size (ha)	Planting date	Hybrid	Adjacent habitat/crops
2021	1 (Field 14)	34.30743, -79.74037	8.6	Apr 15	DKC 65-20	Woods
	2 (Field 45)	34.30116, -79.73548	8.6	Apr 1	Progeny 2025	Woods, corn
	3 (Marion 1)	34.04823, -79.32786	10.5	Mar 25	DKC 66-94	Woods, corn
	4 (Marion 2)	33.93533, -79.33918	9.2	Apr 10	DKC 66-94	Woods, fallow, corn
	5 (Horry 1)	34.15008, -78.94254	8.6	Apr 16	DKC 67-44, DKC 66-94 (refuge)	Woods, corn, wheat
	6 (Horry 2)	34.08015, -78.93060	12.0	April 13	Pioneer 1870YHR, 1870 (refuge)	Woods
	7 (Darlington 1)	34.44763, -79.80747	6.0	-	Pioneer 1464YHR	Woods
	8 (Darlington 2)	34.36047, -79.84740	6.6	-	DKC 65-20	Woods, fallow
	9 (Florence)	34.21310, -79.80230	19.8	Mar 30	DKC 69-16	Woods, commercial properties
	10 (Chesterfield)	34.51728, -79.88437	10.4	Apr 30	unspecified conventional hybrid	Woods
2022	11 (Field 29)	34.28346, -79.74825	6.1	Mar 28	DKC 68-69	Corn, cotton, wheat
	12 (Field 25)	34.28775, -79.74249	5.8	Apr 4	DKC 68-69	Corn, cotton, wheat

Year	Field	Coordinates	Field size (ha)	Planting date	Hybrid	Adjacent habitat/crops
	13 (Field 7)	34.30979, -79.74590	6.1	Apr 4	DKC 69-16	Woods, wheat, sorghum
2022	14 (Marion 1)	34.02245, -79.34458	6.3	Mar 28	DKC 66-94	Woods, corn
	15 (Marion 2)	33.93804, -79.34409	15.5	Apr 4	DKC 66-94	Woods, corn, residential
	16 (Horry 1)	34.16725, -78.95792	3.9	Apr 18	DKC 65-20, DKC 66-94 (refuge)	Corn, cotton, residential
	17 (Horry 2)	34.08417, -78.94881	9.4	Apr 21	DKC 65-20, DKC 66-94 (refuge)	Woods, corn
	18 (Darlington 1)	34.39850, -79.84109	10.5	Mar 25	DKC 65-20	Pasture, wheat, cotton
	19 (Darlington 2)	34.32496, -79.92639	8.7	Apr 6	DKC 65-20	Solar field, cotton, corn
	20 (Florence)	34.23757, -79.78246	7.4	Apr 21	DKC 69-16, speedway (refuge)	Woods

[a] Fields were also sampled for stink bugs in Bryant et al. (2023).

Table 3.2 Overall aggregation indices for larvae of and injury from *Helicoverpa zea* on corn at R2 in South Carolina. CEW S = small (need instars here), CEW M = medium (need instars here), and CEW L = large (need instars here) of *H. zea*; CEW total = all larvae; total injured ears = total number of ears at a sampling point with any *H. zea* injury; tip dam = *H. zea* injury to non-kernel areas on the tip of the ear; kernel dam = *H. zea* injury to kernels; total injury = tip and kernel damage. Significant aggregations are indicated by bold and * ($P < 0.025$).

Year	**Field**	**CEW S**	**CEW M**	**CEW L**	**CEW Total**	**Total injured ears**	**Tip Dam**	**Kernel Dam**	**Total Injury**
2021	1	1.2611	0.8209	0.8928	1.0628	1.2120	-	-	0.9599
	2	1.0405	0.9310	1.0644	0.9428	0.9354	-	-	0.9961
	3	1.1287	1.3269	1.1988	1.3079	1.3339	-	-	1.1696
	4	1.1945	0.9353	0.9724	1.0321	1.0980	-	-	0.9404
	5	0.8743	0.9991	1.0154	0.8946	1.0055	-	-	1.1801
	6	1.1296	1.0358	1.2962	1.1379	1.1558	-	-	1.2744
	7	0.9340	0.8443	0.9958	0.8452	0.8233	-	-	0.8839
	8	1.3572	1.3144	-	**1.5322***	**1.5176***	-	-	**1.8450***
	9	0.8085	0.9604	1.2556	1.0186	0.9121	-	-	1.2750
	10	0.8339	1.3457	0.8703	0.8377	0.8151	-	-	0.9191
2022	11	1.0146	1.0629	**1.7774***	0.9614	**1.9467***	**1.9245***	1.5726	**2.1286***
	12	0.8702	0.9681	0.7753	0.8992	1.3660	1.1748	1.7223	1.4028
	13	**1.8860***	1.2404	1.2347	**1.5313***	1.5024	1.2592	1.1725	1.2321

Field	CEW S	CEW M	CEW L	CEW Total	Total injured ears	Tip Dam	Kernel Dam	Total Injury
14	1.2372	1.3962	1.3862	1.375	1.2840	1.0325	1.3277	1.1841
15	**1.6132***	1.2173	0.9348	**1.5391***	**1.4849***	1.2981	1.3957	1.1721
16	1.0033	0.9173	0.9955	1.0035	1.0119	1.0535	1.2927	1.0080
17	0.9912	0.7064	-	0.8870	0.8622	0.9308	0.9638	0.8971
18	**1.6282***	0.8000	1.2651	1.2862	1.2456	0.9144	0.8426	0.8605
19	1.2474	0.9814	1.3988	1.1102	1.2876	**1.6237***	1.349	**1.7099***
20	1.3535	0.7548	1.2375	1.4668	1.3409	1.2008	1.2589	1.2559

* $p < 0.025$

Table 3.3 Overall aggregation indices for larvae of and injury from *Helicoverpa zea* on corn at R5 in South Carolina. CEW S = small (need instars here), CEW M = medium (need instars here), and CEW L = large (need instars here) of *H. zea*; CEW total = all larvae; total injured ears = total number of ears at a sampling point with any *H. zea* injury; tip dam = *H. zea* injury to non-kernel areas on the tip of the ear; kernel dam = *H. zea* injury to kernels; total injury = tip and kernel damage. Significant aggregations are indicated by bold and * ($P < 0.025$).

Year	Field	CEW S	CEW M	CEW L	CEW Total	Total injured ears	Tip Dam	Kernel Dam	Total Injury
2021	1	0.9307	0.9505	0.9552	0.9062	1.0231	1.1686	0.9864	1.0129
	2	0.8392	**1.3029***	0.9021	1.0277	1.2298	1.0560	1.4097	**1.3553***
	4	-	1.1952	0.9020	1.0408	**1.3852**	1.3392	0.9614	0.8413
	5	1.3811	**1.5732****	1.3794	1.3605	**1.9915****	**1.5352***	1.4346	**1.8174***
	6	0.8898	0.8278	0.8506	0.9412	0.8087	1.1989	0.8626	0.7688
	7	-	-	-	-	1.1315	1.3225	1.2466	1.2836
	8	1.4172	1.0140	1.0595	1.3314	**1.8727***	**1.7355***	**2.1213***	**2.4163****
	9	1.1438	0.9990	0.8183	1.3011	0.8462	1.1017	1.4083	1.0060
2022	11	-	-	-	-	1.1776	1.0510	0.7763	0.7197
	12	-	1.0151	1.4230	1.3167	**1.8073***	0.7793	**1.7202***	1.2786
	13	-	1.1042	0.9100	0.9916	**1.8924***	**1.9970***	**1.4682**	**1.9198***
	14	-	-	-	-	1.3853	0.9198	1.5389	1.3129

Field	CEW S	CEW M	CEW L	CEW Total	Total injured ears	Tip Dam	Kernel Dam	Total Injury
15	0.8452	**1.5514***	0.8426	1.3226	**1.5647***	1.1874	**1.6213***	**1.5288***
16	-	-	-	-	1.2072	1.5128	1.1753	1.2353
17	-	-	-	-	0.9898	1.0123	0.9100	1.0469
18	-	-	-	-	1.1370	**1.6084***	1.3789	1.3551
19	-	-	-	-	1.0546	0.9940	1.1018	0.9781
20	-	-	0.8881	0.8871	1.2135	1.0090	0.9874	1.0156

* $p < 0.025$ ** $p < 0.001$

Table 3.4 Association analysis between plant height at tasseling and *H. zea* larvae and injury sampled in corn at R2 and R5 in South Carolina. CEW S = small (need instars here), CEW M = medium (need instars here), and CEW L = large (need instars here) of *H. zea*; CEW total = all larvae; total injured ears = total number of ears at a sampling point with any *H. zea* injury; tip dam = *H. zea* injury to non-kernel areas on the tip of the ear; kernel dam = *H. zea* injury to kernels; total injury = tip and kernel damage. Significant aggregations are indicated by bold and * (*P* < 0.025).

Growth stage	**Field**	**CEW S**	**CEW M**	**CEW L**	**CEW Total**	**Total injured ears**	**Tip Dam**	**Kernel Dam**	**Total Injury**
R2	11	0.0565	0.0778	0.2531	**0.3987***	**0.5568***	**0.5146***	0.1765	0.2899
	12	0.3476	**0.4568***	0.2237	**0.4183***	**0.4507***	0.3839	0.5125	**0.5113***
	13	0.1665	**0.6371****	**0.6486****	**0.6026***	**0.6466****	**0.7435****	**0.7628****	**0.7490****
	14	-0.2899	0.1196	**0.4339***	0.1120	**0.3485***	**0.5537****	**0.5375***	**0.5169***
	15	**0.4097***	**0.4117***	**0.3952***	**0.3979***	**0.4164***	**0.3071***	**0.2990***	**0.4721***
	16	0.0340	0.0290	0.3321	0.1644	0.2105	**0.5736***	**0.5282***	**0.5225***
	17	-0.0303	0.2062	-	0.1406	-0.0433	-0.0397	-0.0266	-0.0392
	18	0.1278	0.3018	0.1927	0.2075	0.2770	0.2604	0.0317	0.1847
	19	0.3460	-0.0670	-0.0431	0.1935	0.1450	0.0168	-0.0406	-0.0707
	20	0.3461	0.1125	**0.5018***	**0.5886****	**0.5779***	**0.4924***	**0.5914***	**0.5614***
R5	11	-	-	-	-0.1858	-0.0135	0.2311	0.1173	0.0685
	12	-	-0.0651	-0.1584	-0.2501	0.3628	-0.1286	0.4032	0.2641

Field	CEW S	CEW M	CEW L	CEW Total	Total injured ears	Tip Dam	Kernel Dam	Total Injury
13	-	0.0336	-0.1142	0.0717	**0.5097***	0.2352	**0.3823***	0.3565
14	-	-	-	-	0.3335	-0.0648	0.2921	0.0965
15	0.0284	-0.1089	-0.0547	-0.0277	**0.3321***	**0.3545***	0.1399	0.2575
16	-	-	-	-	-0.0163	-0.1518	-0.2769	-0.2109
17	-	-	-	-	-0.2596	**-0.3345***	**-0.3068***	-0.2700
18	-	-	-	-	-0.0129	-0.0277	0.0217	-0.1648
19	-	-	-	-	-0.2070	-0.0803	-0.0360	-0.0919
20	-	-	**0.4256***	**0.4342***	-0.0783	-0.1733	**-0.3767***	-0.3255

$p < 0.025$ or $p > 0.975$ *
$p < 0.001$ or $p > 0.999$ **

Table 3.5 Association analysis between stink bug damaged ears (i.e., banana shaped ears) and *H. zea* larvae and injury in corn assessed at R5 in South Carolina. CEW S = small (need instars here), CEW M = medium (need instars here), and CEW L = large (need instars here) of *H. zea*; CEW total = all larvae; total injured ears = total number of ears at a sampling point with any *H. zea* injury; tip dam = *H. zea* injury to non-kernel areas on the tip of the ear; kernel dam = *H. zea* injury to kernels; total injury = tip and kernel damage. Significant aggregations are indicated by bold and * ($P < 0.025$).

Year	**Field**	**CEW S**	**CEW M**	**CEW L**	**CEW Total**	**Total injured ears**	**Tip Dam**	**Kernel Dam**	**Total Injury**
2021	4	-	**-0.3299***	-0.2975	-0.3003	**-0.4227***	**-0.4396***	-0.0088	-0.3338
	5	0.0867	0.1274	-0.0944	-0.0318	0.0892	0.0169	0.2306	0.2463
	6	-0.1752	0.0691	0.1327	0.0849	-0.0665	**-0.4717***	0.1435	-0.1079
	8	-0.1259	-0.0861	-0.0464	-0.1027	0.1653	-0.1001	0.2718	0.2013
2022	11				0.0961	0.1054	0.1046	0.2061	0.2071
	14	-	-	-	-	**-0.4646***	-0.2898	**-0.4203***	-0.4146
	16	-	-	-	-	-0.1981	0.2308	-0.0657	0.0008
	17	-	-	-	-	-0.1146	-0.2576	-0.1407	-0.1534
	18	-	-	-	-	-0.1562	0.2530	**-0.5433***	-0.0302
	19	-	-	-	-	0.0330	0.0335	-0.0700	-0.0269
	20				-0.0971	**0.3635***	0.2661	0.0962	0.1616

$p < 0.025$ or $p > 0.975$ *

Figure 3.1 Larvae of *Helicoverpa zea* per corn ear (± SE) at each sampled growth stage in a) 2021 and b) 2022 in South Carolina. Larvae were categorized by size as either small (S, instars here), medium (M, instars here), or large (L, instars here). Significance letters indicate significance between growth stages within each year ($P < 0.05$). No significant difference was found between sizes of larvae ($P > 0.05$).

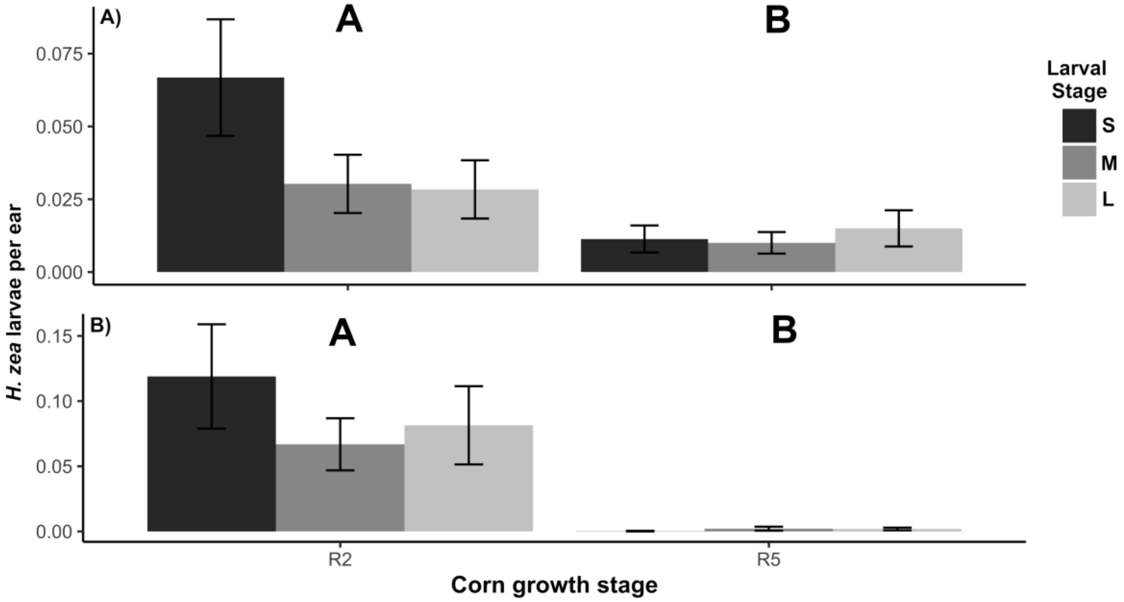


Figure 3.2 Total injury per corn ear from *Helicoverpa zea* at R2 and R5 in 2021 and 2022 in South Carolina. Fields were grouped by commercial Bt traits. Non-Bt = no Bt toxin; SmartStax = Cry1A.105, Cry2Ab2, Cry1F; Intrasect = Cry1F, Cry1Ab; VT2P = Cry1A.105, Cry2Ab2. Fields 3 and 10 were not sampled at R5 in 2021 due to growers harvesting prior to sampling. Box and whisker plots represent the median, interquartile range, and the highest and lowest values. Black dots represent outliers for total injury within each field.

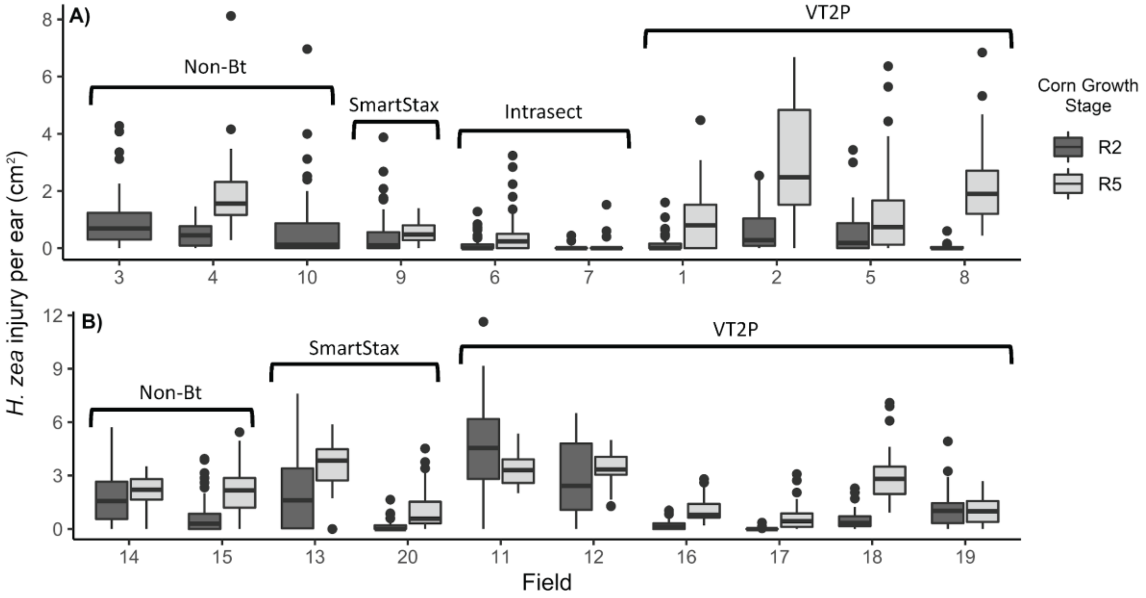


Figure 3.3 Inverse distance weighted spatial interpolation of local aggregation indices for larvae of *Helicoverpa zea* at R2, categorized by size (S = small, instars here; M = medium, instars here; and L = large, instars here) and total (T, all instars) in corn in South Carolina. Black dots represent each sampling point in the field. Field numbers listed in Table 1 are provided on each map. Clusters are indicated by excluding aggregation indices between -1.5 and 1.5, with patches indicated in black ($V_i > 1.5$) and gaps indicated in grey ($V_j < -1.5$). Significant aggregation indices are indicated by asterisks ($*P < 0.025$). In the first map for each field, scale bar equals 150m.

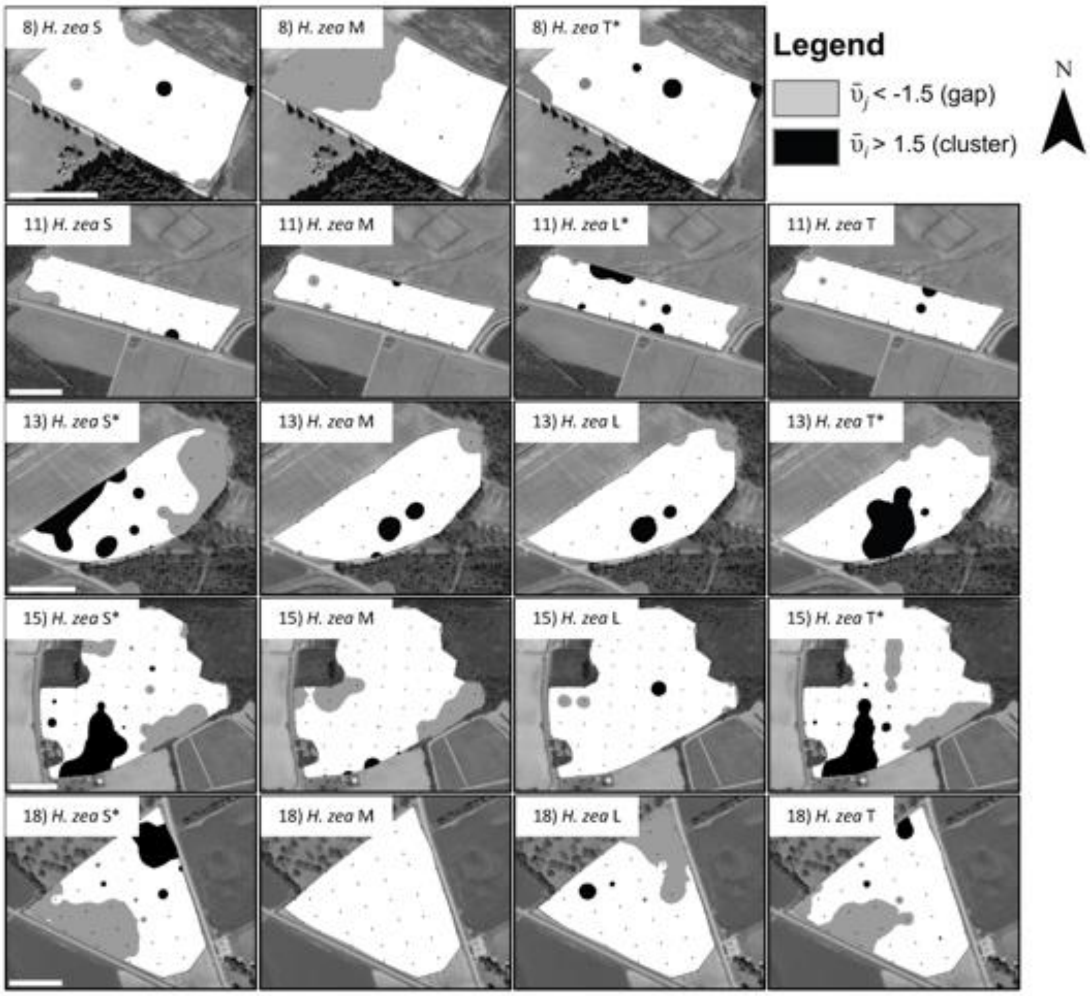


Figure 3.4 Inverse distance weighted spatial interpolation of local aggregation indices for *H. zea* injury to corn ear tips, kernels, and total injury from *Helicoverpa zea* at R5 in South Carolina. Black dots represent each sampling point in the field. Field numbers listed in Table 1 are provided on each map. Clusters are indicated by excluding aggregation indices between -1.5 and 1.5, with patches indicated in black ($V_i > 1.5$) and gaps indicated in grey ($V_j < -1.5$). Significant aggregation indices are indicated by asterisks (*$P < 0.025$). In the first map for each field, scale bar equals 150m.

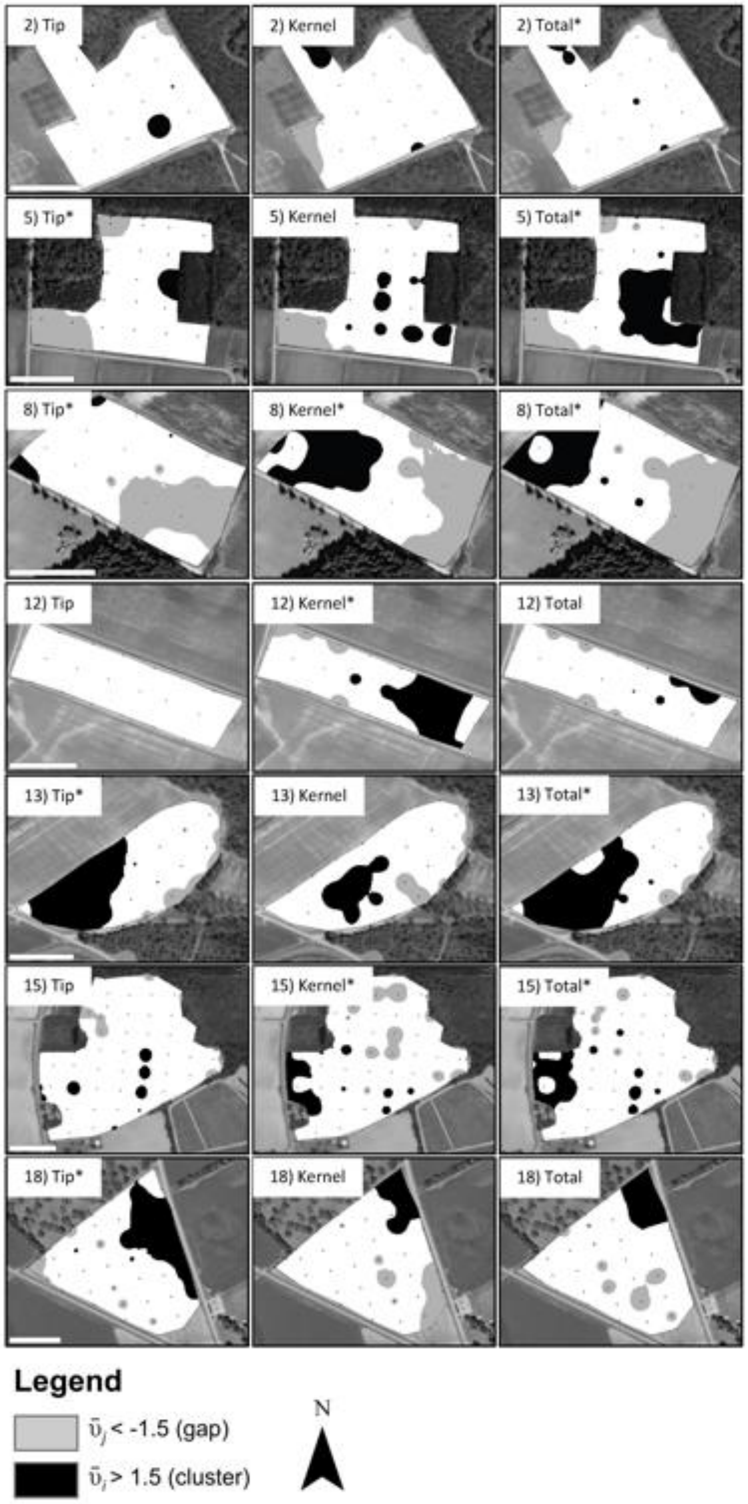

2) Tip
2) Kernel
2) Total*
5) Tip*
5) Kernel
5) Total*
8) Tip*
8) Kernel*
8) Total*
12) Tip
12) Kernel*
12) Total
13) Tip*
13) Kernel
13) Total*
15) Tip
15) Kernel*
15) Total*
18) Tip*
18) Kernel
18) Total
Legend
$\bar{\upsilon}_j$ < -1.5 (gap)
$\bar{\upsilon}_i$ > 1.5 (cluster)
N

Figure 3.5 Inverse distance weighted spatial interpolation of local association indices between larvae of *Helicoverpa zea*, sampled in corn at R2, categorized by size (S = small, instars here; M = medium, instars here; and L = large, instars here; and T= total, all instars) and plant height taken at V12/VT in South Carolina. Black dots represent each sampling point in the field. Field numbers listed in table 1 are provided on each map. Significant association indices are indicated by asterisks (**P* < 0.025). In the first map for each field, scale bar equals 150m.

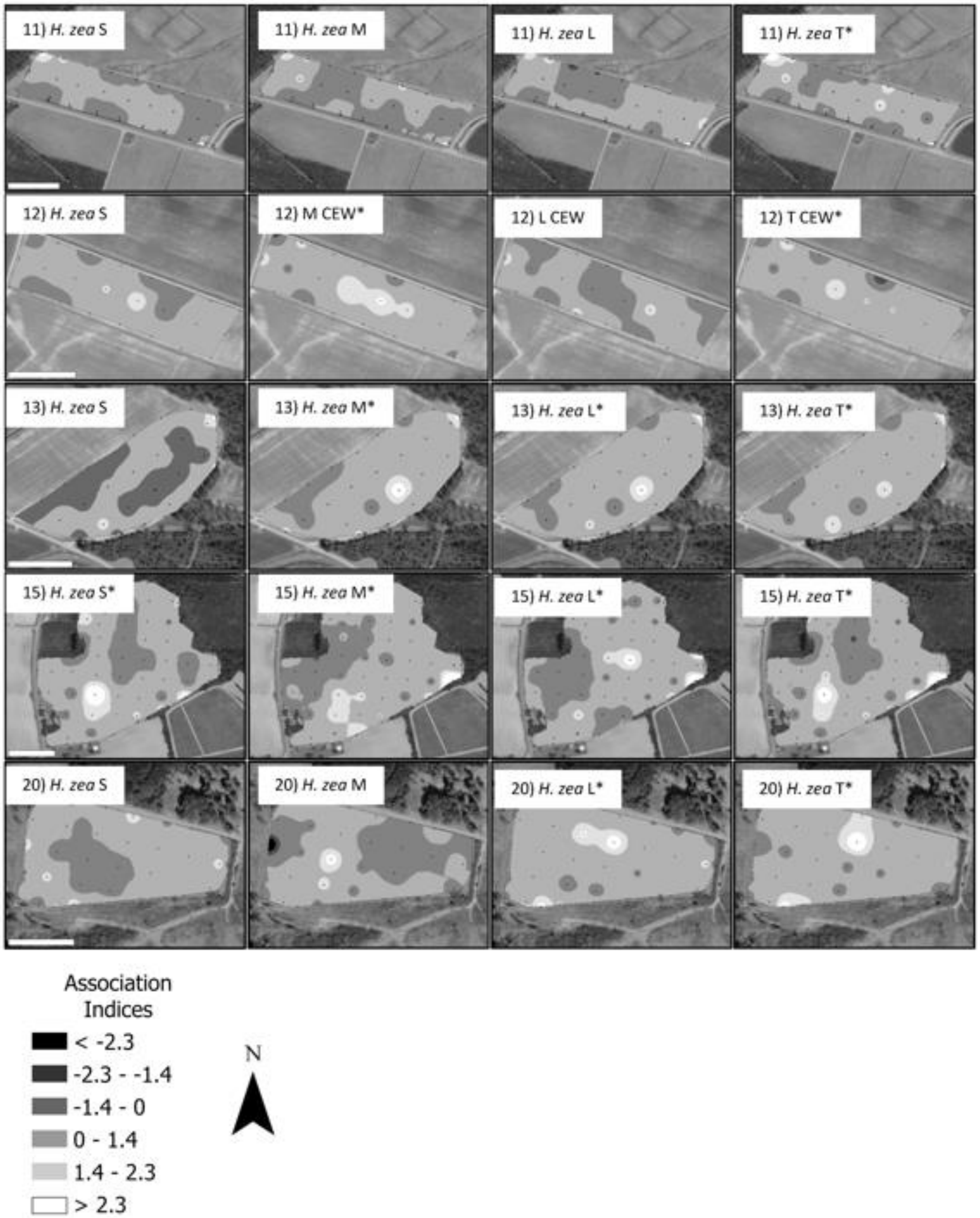
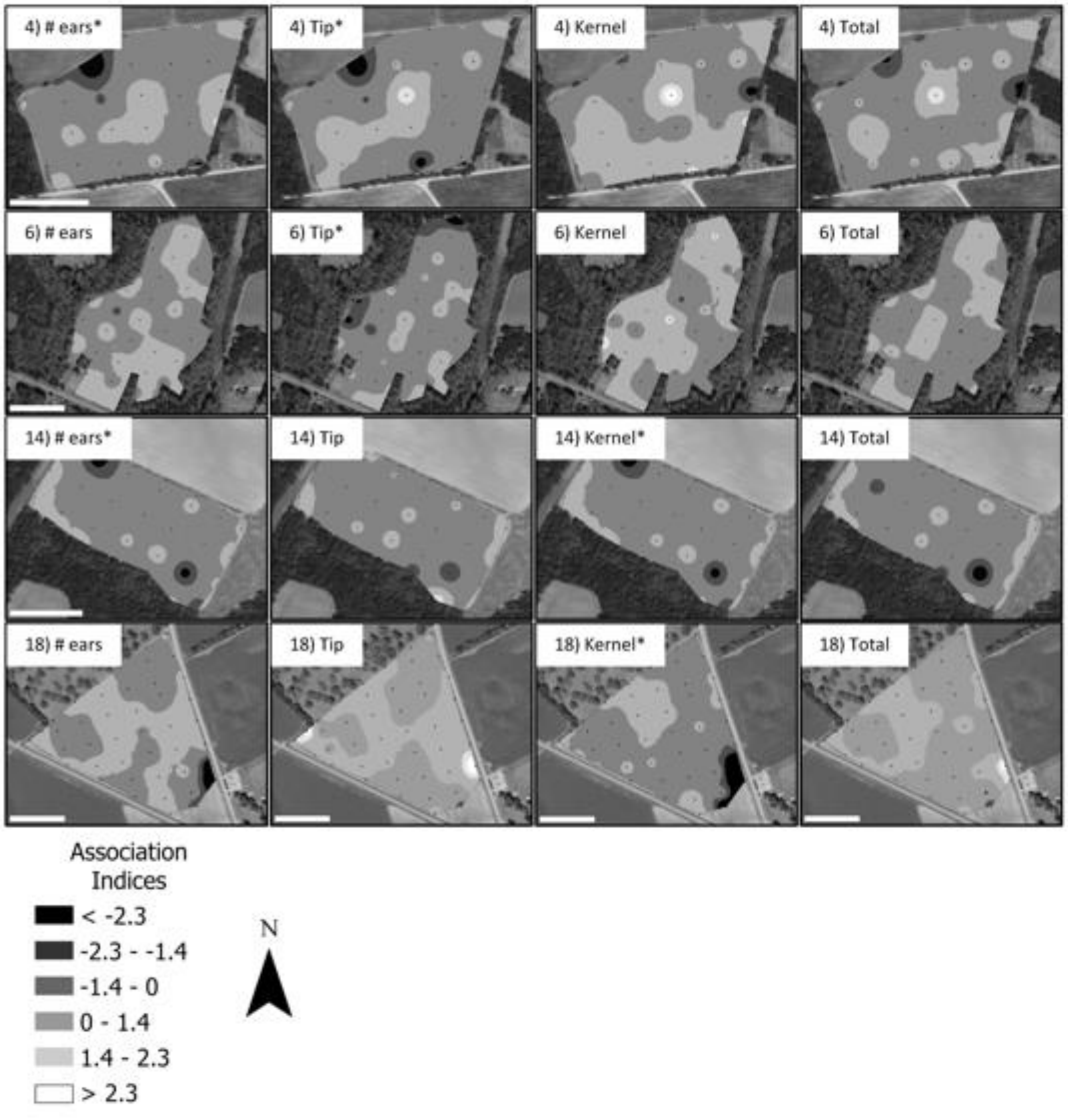

11) H. zea S
11) H. zea M
11) H. zea L
11) H. zea T*
12) H. zea S
12) M CEW*
12) L CEW
12) T CEW*
13) H. zea S
13) H. zea M*
13) H. zea L*
13) H. zea T*
15) H. zea S*
15) H. zea M*
15) H. zea L*
15) H. zea T*
20) H. zea S
20) H. zea M
20) H. zea L*
20) H. zea T*
Association Indices
< -2.3
-2.3 - -1.4
-1.4 - 0
0 - 1.4
1.4 - 2.3
> 2.3
N

Figure 3.6 Inverse distance weighted spatial interpolation of local association indices between injury from *Helicoverpa zea*, sampled in corn at R5, to the ear tip, kernels, and total and stink bug injured ears (i.e., banana-shaped ears) in South Carolina. Black dots represent each sampling point in the field. Field numbers listed in Table 1 are provided on each map. Significant association indices are indicated by asterisks (*$P < 0.025$). In the first map for each field, scale bar equals 150m.



CHAPTER FOUR

COMPETION BETWEEN BROWN STINK BUG (HEMIPTERA: PENTATOMIDAE) AND CORN EARWORM (LEPDIOPTERA: NOCTUIDAE) IN FIELD CORN

Introduction

Interspecific competition refers to the interaction of two species which are attempting to exploit the same resources, such as space or food. More specifically, competition most often refers to a density-dependent relationship, wherein individuals in competition with one another may undergo alterations in their population structure as a result of another species exploiting resources more efficiently or effectively (Crombie 1947). Competition can therefore result in alterations to population structures which potentially can change the impact of those populations on the exploited resource. Amongst phytophagous insects, interspecific competition has been widely reported across many different species complexes (Kaplan and Denno 2007).

Traditionally, competition is thought to occur more frequently or be most severe between two insect species if they are closely related or exploit the same portions of a host plants' anatomy (Crombie 1947, Denno et al. 1995). More recent meta-analyses have argued that competition is agnostic to feeding guild or species classification and is mediated by other factors, including the host plant response to feeding, alterations in populations of natural enemies, or environmental factors, such as weather or temperature (Denno et al. 1995, Kaplan and Denno 2007). Insect feeding can alter plant physiology which may negatively impact the success of another herbivore feeding later in the season or on different plant parts. For example, in birch trees, early season feeding by sawflies

(Hymenoptera: Symphyta) reduces the quality of the host for subsequent insect herbivores, decreasing their development rate (Haukioja and Niemela 1979, Hanhimaki 1989). Additionally, spittlebugs (Hemiptera: Cercopidae) and plume moths (Lepidoptera: Pterophrodiae) co-occurring on ornamental plants exhibit asynchronous competition resulting in population shifts across multi-year temporal scales (Karban 1986). Herbivory by one insect may also induce plant defense responses or change volatile emissions which attract predator or parasitoid arthropods, reducing the success of later or co-occurring insect populations regardless of the portion of the plant exploited or the higher classifications of the species (Dicke et al. 1990, Oppenheim and Gould 2002).

Field corn, *Zea mays* L. (Poales: Poaceae), in the southeastern United States is susceptible to insect feeding throughout the season, and a range of insect pests target different portions of the plant at different growth stages. Of the insect pests impacting field corn production, a complex of stink bugs (Hemiptera: Pentatomidae) and the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) are the two most commonly occurring in the southeastern United States (Reisig et al. 2023). Among stink bugs, the brown stink bug, *Euschistus servus* (Say), is a key pest species in corn (Tillman 2010, Bryant et al. 2023a). Stink bugs can be present throughout the season but commonly peak during the late vegetative stages of corn production while the ear is in early stages of development (Tillman 2010, Pilkay et al. 2015, Bryant et al. 2023a). This type of feeding leads to growth deformities in the ear during later stages of reproductive development (Clower 1958), and, ultimately, a reduction in grain yield and quality. These deformities also often expose portions of the ear that would normally be tightly protected by the ear husk. Corn earworm typically oviposits in corn during the early reproductive stages of development when fresh silks are available (McColloch et al. 1920). If damaging populations of stink bugs are present prior to corn earworm oviposition, deformed ears may be less suitable and/or expose larvae to pressures normally excluded by the corn husk, although this has yet to be examined empirically.

Corn earworm is generally not considered to be an economic pest of field corn in the southeastern United States (Reay-Jones and Reisig 2014, Bibb et al 2018). However, the insect remains a target of transgenic corn hybrids expressing toxins produced by the soil bacterium *Bacillus thuringiensis* (Bt), in addition to a number of other above and below-ground pests. Corn earworm is a major economic pest of cotton, which expresses many of the same or similar Bt toxins that populations are exposed to in corn (Gould et al. 2002, Von Kanel et al. 2016). Widespread selection pressure in Bt corn has led to corn earworm populations developing resistance to most Bt toxins used in corn and cotton (Dively et al. 2016, Bilbo et al. 2019, Yang et al. 2022). The primary mechanism for delaying development of resistance to Bt corn is the planting of non-Bt corn refuge plants in or nearby Bt corn fields. Because the success of Bt refuge can be spatially dependent (Vacher et al. 2003) and competition can in part drive spatial patterns of insects (Kaplan and Denno 2007), understanding the interactions among major pests in the field may play an important role in modeling resistance evolution and implementing effective resistance management strategies.

Competition between Heliothine larvae and stink bugs has been identified previously in cotton (Zeilinger et al. 2011, 2015). These studies found that feeding by

Heliothine larvae significantly reduced the growth rates of several different stink bug species (Zeilenger et al 2011), and that stink bugs avoided oviposition on plants damaged by larvae (Zeilenger et al. 2015). While competition between Heliothine larvae and stink bugs has not been studied in corn, a negative spatial association between stink bug injury and corn earworm larvae and their injury was identified in a previous sampling study in field corn (Bryant et al. 2023b), suggesting that competition between these pests may occur in corn fields. Therefore, the goal of the present study was to determine if late-vegetative feeding and ear injury by the brown stink bug influences oviposition and larval success of corn earworm.

Materials and Methods

Insects

Adult and nymph brown stink bugs (*E. servus*) were collected using sweep nets from winter wheat, *Triticum aestivum* (L.), at the Clemson University Pee Dee Research and Education Center (REC) in Florence, SC and from commercial fields in Darlington, Marion, and Horry Counties in the state from April through June of 2022 and 2023. After wheat harvest, additional stink bugs were collected by hand from field corn, as needed. Field-collected stink bugs were then transferred to the laboratory at the Clemson University Pee Dee REC and reared following protocols from Munyaneza et al. (1994) and Koppel et al. (2009). In short, approximately 30 individuals were stored in each round plastic container (11 cm height, 16.5 cm diameter) in an environmental chamber held at a photoperiod of 16:8 (L:D) and 25°C. Containers were lined with filter paper and contained a cotton ball soaked in distilled water, surface sterilized green beans as food, and mesh or cheese cloth for oviposition. Green beans were sterilized using a 10% bleach solution for one minute and then rinsed with distilled water at least three times.

Field Experiments

The corn hybrid DKC 67-70 was planted in plots at the Clemson University Pee Dee REC on 12 April and 23 June in 2022 and 13 April and 15 May in 2023. The same corn hybrid was used for each planting date in all trials and was chosen as it does not express Bt toxins that would impact *H. zea*. Each trial consisted of a randomized complete block design with four replications, with two rows per plot and a row length of 15.2 m. Around 7-10 days prior to tasseling, corn plants were artificially infested with adult brown stink bugs by using a mesh sleeve around the node containing the developing primary ear (Bryant et al. 2020). The timing of infestation was determined by the presence of the ear sheath tip starting to emerge from the above leaf collar. Previous studies have shown that infestations of two stink bugs per ear for a period of 16 days from V10-VT/R1 caused a reduction in yield (Bryant et al. 2020). Therefore, infestations were applied at a rate of zero (control), two, four, and eight stink bugs per plant. In the late-planted trial in 2022, only infestation rates of zero and four bugs per plant were used due to limitations in the number of healthy adult stink bugs remaining in the colony. In each plot, 10 mesh sleeves were placed over randomly selected plants representing uniform phenology. During the infestation period, enclosures were checked every other day and dead or missing stink bugs were replaced as needed. As silks began to emerge at R1, stink bug enclosures were removed to allow for naturally occurring corn earworm oviposition to occur.

In 2023, two to four days after stink bug enclosures were removed, fresh silks were examined for enumeration of corn earworm eggs on five ears per plot. Egg assessments were conducted at night using a UV flashlight to aid in the identification of eggs. In both years of the study at R2, five of the ten ears with mesh sleeves were assessed for corn earworm larval populations and stink bug injury. Corn earworm larvae were categorized by growth stage (i.e., small, medium, or large). First and second instar larvae were categorized as small, third and fourth instar larvae were categorized as medium, and fifth and sixth instar larvae were categorized as large. Corn ears were classified as either uninjured from stink bug feeding or injured with visible signs of ear deformation resulting in a crooked or banana-shaped ear (Clower 1958). At R5 near crop maturity, the remaining five ears in each plot that were not examined at R2 were assessed for both end of season corn earworm injury and stink bug injury. Corn earworm injury was recorded in square centimeters of feeding area separately for the tip of the ear and for kernels (Bilbo et al. 2019). Stink bug injury was recorded as previously described.

At 15-20% moisture, ears were hand harvested and assessed for yield. The number of kernel rows, the average number of kernels per row, and the average kernel weight (determined for a 100-kernel sample) were measured (Bryant et al. 2020). Yield calculations used the following formula and corrected to 15.5% moisture content:

Yield (kg/ha) = # of plants/hectare * # kernel rows * # of kernels/row * average weight per kernel

Yield was averaged for each set of five ears assessed at R5, as the early corn earworm assessment at R2 left those ears exposed, potentially impacting yield relative to ears assessed at R5.

Statistical Analysis

All statistical analysis was conducted using R version 4.3 (R core team 2022). To meet the assumptions of ANOVA, the proportion of stink bug injured ears in a plot was arcsine square root transformed, and count data for corn earworm larvae and eggs were log + 1 transformed. Linear mixed effect models were used in the package *lme4* (Bates et al. 2015). The package *emmeans* (Lenth et al. 2023) was used to perform Tukey's adjusted means separations where applicable. No significant difference was identified in the proportion of stink bug injured ears between ears sampled at R2 or R5, so all ten ears in a plot were combined for analysis (10 subsamples per plot). Corn earworm egg and larvae analyses were conducted using only ears assessed at R1 or R2 (five subsamples per plot). Corn earworm injury was analyzed using ears assessed at R5 (five subsamples per plot). Model assumptions were verified by visual examination of residual plots. Linear regression models were used to assess corn earworm larvae and injury with the proportion of stink bug injured ears as the independent variable. In addition to the described statistical analyses, corn earworm injury data were pooled by presence or absence of stink bug injury in each trial for visualization.

Results

Stink bug density had a significant impact on the proportion of stink bug injured ears (i.e., banana ears) in both years and planting dates of the study (Table 4.1). No stink bug-injured ears were found in control plots (i.e., zero bugs/plant), with the exception of a single ear in the early planted trial in 2022. There were no significant differences in stink bug-injured ears among stink bug densities above zero in 2022, which were all significantly greater than the control. In the early planted study in 2023, the proportion of injured ears was significantly higher at the eight-bug density than at two or four (Fig. 4.1). In the late planted trial in 2023, stink bug densities of four and eight had a significantly higher proportion of injured ears than densities of two bugs (Fig. 4.1). Corn yield significantly declined with stink bug density, with the exception of the late planted trial in 2022 (Table 4.1). Yield reduction ranged from 87% to 92% in the early planted trial in 2022 (Fig. 4.2). Overall, there was less yield reduction as a result of stink bug feeding in 2023, ranging from 19% to 62% in the early planting, and from 17% to 60% in the late planting (Fig. 4.2).

Stink bug density had a significant impact on the number of corn earworm eggs in the early planted trial in 2023, but not in the late planted trial (Table 4.1). The stink bug density of four bugs/plant had significantly fewer eggs than zero bugs/plant, but not two or eight in the early planted trial (Fig. 4.3). While there was no significant difference in the late planted trial ($P = 0.0801$), there was a numerical trend in reduced numbers of eggs proportionate to the increase in stink bug density (Fig. 4.3). The number of corn earworm larvae at R2 was significantly reduced compared to the control at stink bug densities of four and eight in all trials, except the late planted trial in 2022 (Table 4.1). There was no significant difference in the number of larvae among any of the stink bug densities above zero in all trials (Fig. 4.4A). The total numbers of larvae averaged across stink bug densities was eight-fold and ten-fold greater in late planted trials compared to early planted trials in 2022 and 2023, respectively. Compared to control ears, ears caged with stink bugs had between 29% and 92% less total larvae on average, depending on stink bug density. The percent reduction in the number of larvae was generally less in late planted trials than in early planted trials. Regression analyses showed that the total number of corn earworm larvae decreased with the proportion of stink bug injured ears in all but the late planted trial in 2022 (Table 4.2; Fig. 4.5A).

Total corn earworm injury was significantly impacted by stink bug density in all trials (Table 4.1). Similar to the trends with corn earworm larvae, there was no significant difference in total corn earworm injury among stink bug densities above zero (Fig. 4.4B), with significantly greater injury in the control compared to all other densities except the four-stink bug density in the early trial in 2022. Overall, total corn earworm feeding was reduced by between 46% and 85% as stink bug density increased. The total corn earworm injury decreased with the proportion of stink bug injured ears in all trials with the exception of the late planted trial in 2022 (Table 4.2; Fig. 4.5B). Additionally, when all ears across treatments were categorized by stink bug injured vs uninjured, there was a clear reduction in overall corn earworm injury across all trials (Fig. 4.6).

Discussion

While previous studies in cotton identified a negative impact of Heliothine larval feeding on subsequent stink bug oviposition and development (Zeilenger et al. 2011, 2015), this is the first study demonstrating the inverse of this relationship in field corn. Across four trials in two study years, densities of corn earworm eggs and larvae, and corn earworm injury were consistently reduced proportionately with increases in adult brown stink bug densities or the proportion of stink bug injured ears. As expected, increasing stink bug densities during the late-vegetative corn stages (i.e., V12-VT) produced deformed or “banana-shaped” ears and reduced grain yield. Additionally, we found that corn earworm density was generally higher in later-planted trials, as supported in other studies showing greater pheromone trap captures of corn earworm as the season progresses (Latheef et al. 1991, Campbell et al. 1992) and greater larval densities in corn later in the season (Buntin et al. 2004). Several co-occurring mechanisms may explain the negative association between stink bug densities and corn earworm infestations described here: 1) corn earworm ovipositional preference as evidenced by the reduced number of eggs on stink bug injured ears, 2) reduced host plant quality and corn earworm larval survival on stink bug injury ears, and/or 3) increased impact of natural enemies on corn earworm larvae on stink bug injury ears. While these mechanisms of competition have been described for other systems and insects (Kaplan and Denno 2007), more research is needed to identify which factors are driving the competitive interactions between stink bugs and corn earworm in corn.

Ovipositional behaviors of lepidopteran insects are driven by a number of different biotic and abiotic factors. Visual cues, such as shape and color of host plant parts, are often reported to be the most important in host searching (Renwick and Chew 1994). It is unlikely that visual cues played a role in the results here, as the stink bug injury described (i.e., banana ears) often does not fully develop until later stages of reproductive development, after peak earworm oviposition. Plant volatile emissions are another important factor in host seeking behaviors (Renwick and Chew 1994). It is widely reported that herbivore feeding can alter the emission of green leaf volatiles, in particular, which could alter the attractiveness of suitability of certain plants for oviposition (Frago et al. 2022). Corn earworm is also particularly attracted to volatile emissions from corn silks (Cantelo and Jacobson 1979, Shaver et al. 1998). Because stink bug feeding impacts ear development at an early stage, silk production and volatile emissions may also be altered, impacting host seeking behavior by corn earworm.

Herbivore feeding has the potential to change the quality of the host plant for subsequent herbivores (Wratten et al. 1988, Denno et al. 2000, Silva et al. 2021). For example, feeding by one species of plant hopper, *Prokelisia marginata* Van Duzee (Hemiptera: Delphacidae), on smooth cordgrass, *Spartina alterniflora* Loisel, resulted in decreased amino acid concentration in the plant and caused it to be a poor developmental host for another species, *Prokelisia dolus* Wilson (Denno et al. 2000). Interestingly, this interaction only occurs when one species feeds prior to the other, but not if these two species exist contemporaneously on the host plant. Competition is commonly thought to be more severe between two species that feed on the same portion of a plant or have the same feeding type (i.e., chewing or piercing-sucking) (Crombie 1947). However, reductions in host plant quality as a result of herbivory can allow temporally asynchronous competition to occur, which is likely the case here where stink bugs feeding occurred prior to corn earworm oviposition. The preference-performance hypothesis indicates that female insects will maximize their fitness by laying eggs on the most suitable host plant for the success of their offspring (Gripenberg et al. 2010). For polyphagous insects, such as corn earworm, the effect of preference on host plant selection may not be as strong relative to insects with narrower host ranges (Gripenberg et al. 2010). However, female corn earworm moths may indeed select corn plants that have not been injured by stink bugs, as a mechanism to avoid direct induced plant defense responses (Chen 2008). Alternatively, it is possible for induced plant defense responses to have no impact on herbivore preference or performance and instead simply make the plant more tolerant of subsequent feeding (Karban and Myers 1989). Cleary, more work is needed to untangle the mechanisms underlying the interactions between stink bugs and corn earworm in field corn.

Corn earworm eggs are subject to parasitism by *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) and *Telenomus* spp. (Hymenoptera: Scelionidae) in the field (Puterka et al. 1985, Kauffman and Kennedy 1989), but larvae are protected from parasitism and predation by the corn husk (Manley et al. 1991). Herbivore-induced changes in plant volatile emissions or defenses which reduce ovipositional preference and influence larval success may also increase the number of predator or parasitoid insects attracted to those plants (Dicke et al. 1990, Turlings et al. 1990, Whitman and Eller 1990). For example, feeding by the brown marmorated stink bug, *Halyomorpha halys* (Stål), was shown to induce changes in volatile emissions associated with natural enemy attraction from two tree species (Peterson et al. 2022). Compounded with the changes in husk tightness around the ear as a result of stink bug injury, corn earworm larvae on stink bug injured ears may be more susceptible to parasitism and predation than those on uninjured ears. This is supported by previous studies identifying links between ear husk tightness and the rate of predation on earworm by birds (Dicke and Barber 1944) or by other insects (Rhino et al. 2014).

Our results conform with several previous studies indicating that the late vegetative stages of corn development are susceptible to grain yield loss due to stink bug feeding (Ni et al. 2010, Bryant et al. 2020, 2023a). This emphasizes the need for effective sampling during these stages and the use of thresholds to prevent economic losses. The density of stink bugs that led to a significant reduction in grain yield varied among trials. Significant reductions in grain yield relative to the control occurred at densities of two stink bugs per plant in 2022 and four stink bugs in 2023, although trends for reduced yields at the two stink bugs per plant were observed in 2023. These results mirror the significant increase in the number of deformed ears observed with increasing densities in these trials. This suggests that these deformities can be a primary marker for potential yield loss in future studies on stink bug impacts on corn yields. It is important to note that the densities tested here are significantly higher than the recommended economic threshold level for brown stink bugs in field corn, which is 12 stink bugs per 100 plants at the late stages of vegetative development (i.e., 0.12 stink bugs per plant; Bryant et al. 2020). Further study is needed to determine the frequency of the observed interactions between stink bugs and corn earworm in commercial fields.

Bt resistance management is implemented in field corn by planting non-Bt refuge in blocks in or nearby a Bt field or in strips of at least four rows. A number of previous studies have shown that stink bugs and their injury are often aggregated on the margins of field corn (Reisig 2011, Ni et al. 2011, Reisig et al. 2013, Bryant et al. 2023a). If non-Bt refuge is also planted on field margins, there may be higher potential for the competitive interaction identified here to decrease the attractiveness of refuge plants for earworm oviposition or the survival of corn earworm larvae on refuge plants. The percentages of non-Bt refuge required by the EPA are based on complex modeling involving assumptions about pest ecology. While stink bugs are generally a sporadic pest in field corn (Sappington et al. 2018, Bryant et al. 2023a), this interaction could nonetheless play a role in the effectiveness of structured refuge and the durability of Bt traits. Given that resistance to Bt toxins is a major threat to the sustainability of Bt crops (Tabashnik et al. 2023), optimizing the deployment of non-Bt refuges by understanding the competitive interactions between these two key pests may help to delay resistance evolution.

Plant-mediated indirect interactions can occur between herbivores on a given crop plant, with implications for pest management (Wielgoss et al. 2012). Our results indicate that there is an indirect competitive interaction between stink bugs and corn earworm in field corn. When various infestation levels of stink bugs were artificially confined around developing corn ears, there was a subsequent reduction in corn earworm oviposition, number of larvae, and total larval feeding relative to the control. Even where a reduction in oviposition was not identified, fewer corn earworm larvae were found, suggesting some reduction in host plant quality as a result of stink bug feeding. This competitive interaction is likely mediated by changes in host plant physiology and volatile emission and predator/parasitoid interactions, but further study is needed to identify specific mechanisms. Understanding the interaction of these two species could play an important role in implementation and success of both integrated pest management and insect resistance management.

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Table 4.1 ANOVA statistics for the effect of stink bug densities on stink bug (SB) injured ears, corn earworm (CEW) egg oviposition, corn earworm larvae at R2, and corn earworm injury at R5 in early and late planted corn trials, 2022-2023.

Year	**Planting date**	**F-statistics**				
		SB Injured ears	CEW eggs	CEW larvae at R2	CEW injury at R5	Corn yield (kg/ha)
2022	Early[a]	**30.64****	**-**	**7.27****	**4.67***	**8.74****
	Late[b]	**182.48****	**-**	2.68	**21.94****	5.51
2023	Early[a]	**58.36*****	**4.14***	**9.50****	**7.00****	**9.78****
	Late[a]	**92.10*****	3.13	**8.27****	**14.83****	**16.42****

[a] df = 3,9 [b] df= 1,3
* $p < 0.05$
** $p < 0.025$
*** $p < 0.0001$

Table 4.2. Regression statistics for corn earworm larvae and injury as dependent variables and proportion of stink bug injured ears as independent variable. Bolded *P* values are significant at $P < 0.05$.

Year	**Planting date**	**CEW larvae**				**CEW injury**			
		Intercept (±SE)	Slope (±SE)	R^2	P	Intercept (±SE)	Slope (±SE)	R^2	P
2022	Early[a]	1.49 (±0.30)	-1.49 (±0.45)	0.43	**0.005**	26.23 (±2.24)	-19.03 (±2.94)	0.74	**<0.001**
	Late[b]	7.20 (±1.78)	-2.06 (±4.40)	0.03	0.654	23.35 (±3.88)	-18.21 (±10.08)	0.35	0.121
2023	Early[a]	3.17 (±0.41)	-3.15 (±0.61)	0.65	**<0.001**	11.73 (±11.79)	-11.79 (±2.74)	0.56	**<0.001**
	Late[a]	18.01 (±1.67)	-8.95 (±3.28)	0.34	**0.016**	19.57 (±1.56)	-15.54 (±2.49)	0.73	**<0.001**

[a] df = 3,9 [b] df= 1,3

Figure 4.1 Proportion of stink bug injured ears (± SE) (i.e., banana shaped) by stink bug density in 2022 and 2023. Proportion of stink bug injured ears is the number of deformed ears divided by the total number of stink bug treated ears in each plot. Different letters within a year and planting date represent statistically different values at alpha = 0.05 based on Tukey's HSD. In the late planted trial in 2022, stink bug densities of two and eight bugs/plant were not tested (N/A).

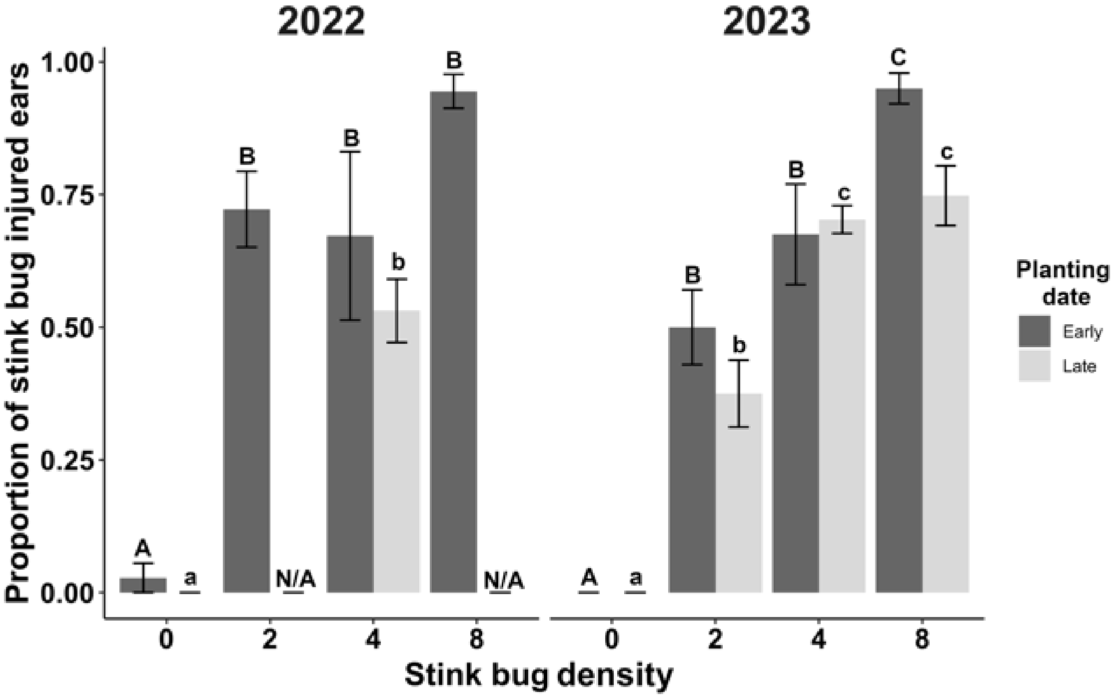


Figure 4.2 Corn yield (kg/ha) (± SE) by stink bug density in 2022 and 2023. Different letters within a year and planting date represent statistically different values at alpha = 0.05 based on Tukey's HSD. In the late planted trial in 2022, stink bug densities of two and eight bugs/plant were not tested (N/A).

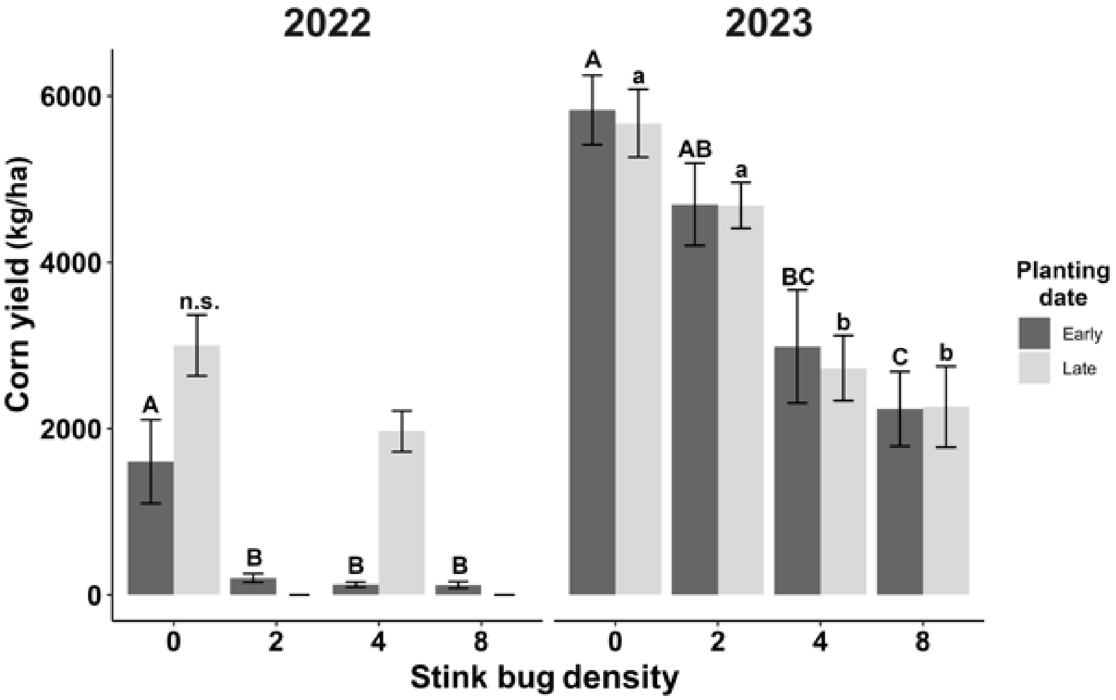


Figure 4.3 Corn earworm (CEW) eggs/ear by stink bug density in 2023 in early and late planted field corn. Different letters within a planting date represent statistically different values at alpha = 0.05 based on Tukey's HSD.

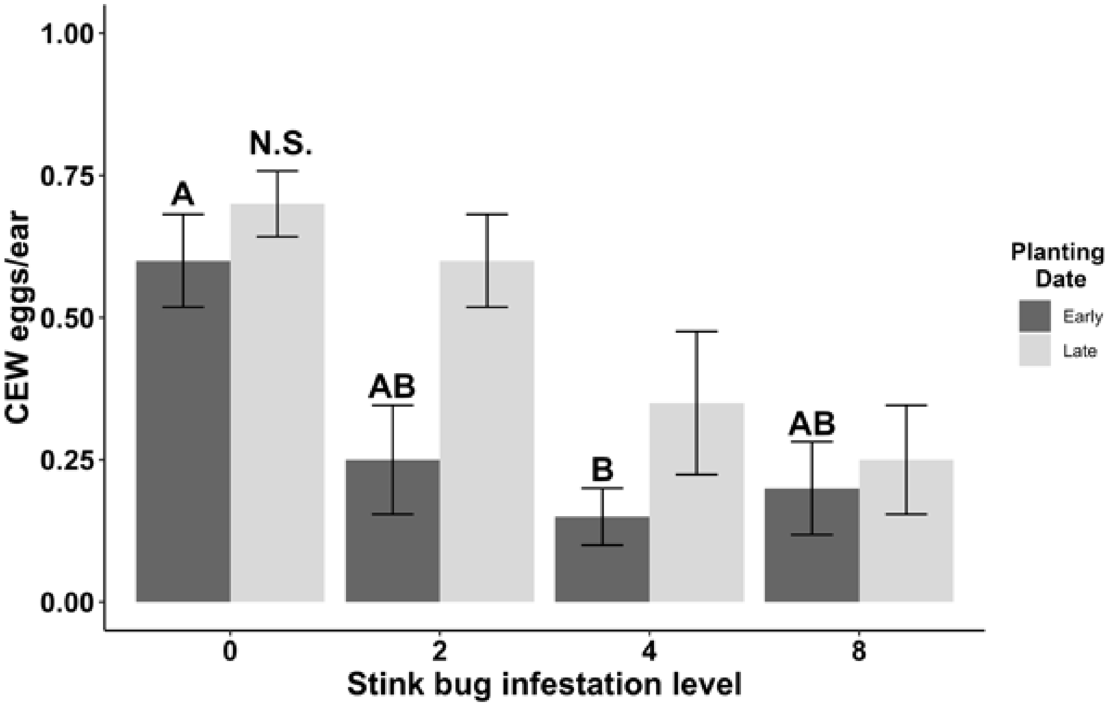


Figure 4.4 A) Corn earworm (CEW) larvae/ear at R2 and B) total CEW injury/ear at R5 by stink bug density in 2022 and 2023. Different letters within a year and planting date represent statistically different values at alpha = 0.05 based on Tukey's HSD. In the late planted trial in 2022, stink bug densities of two and eight bugs/plant were not tested (N/A).

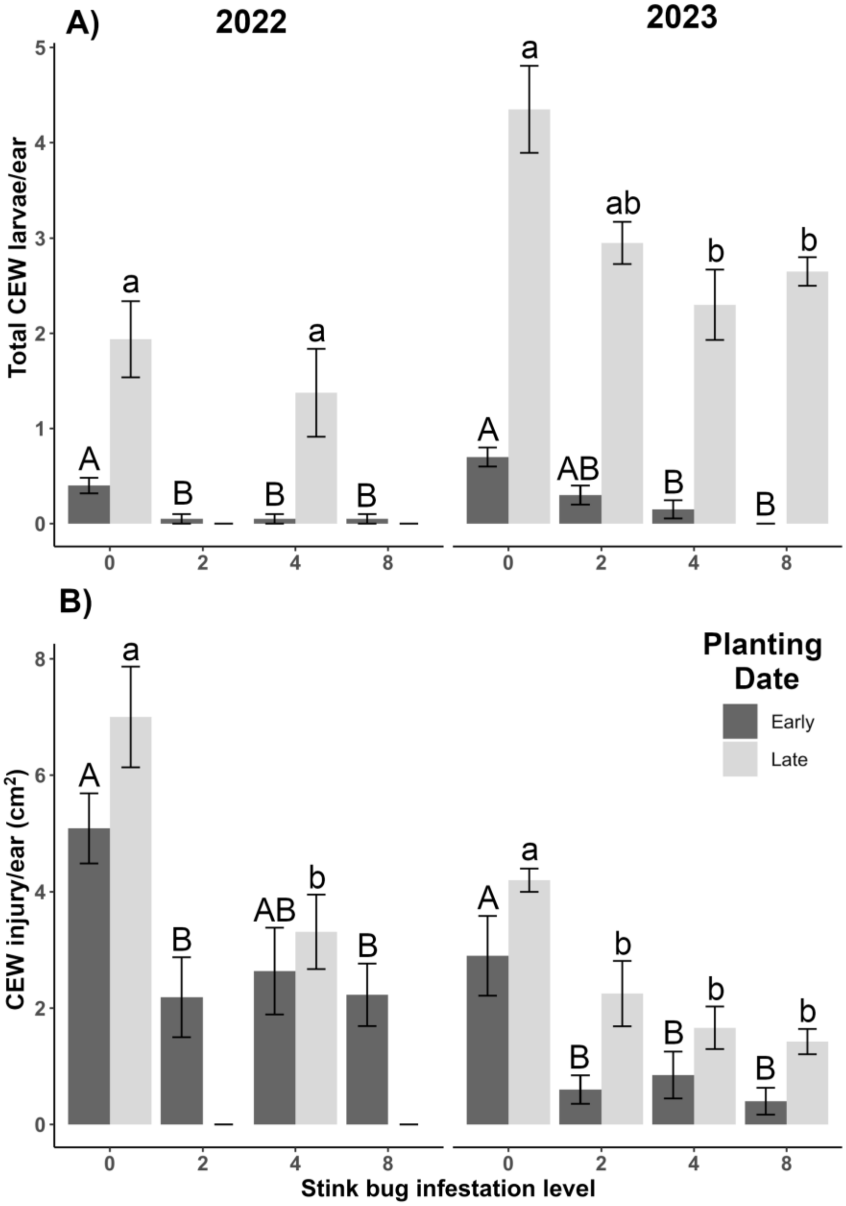


Figure 4.5 Linear regression of A) corn earworm (CEW) larvae/ear at R2 and B) total CEW injury (cm^2)/ear at R5 by proportion of stink bug injured ears in a plot in early and late planted trials in 2022 and 2023. Proportion of stink bug injured ears represents the number of deformed ears divided by the total number of stink bug treated ears in each plot.

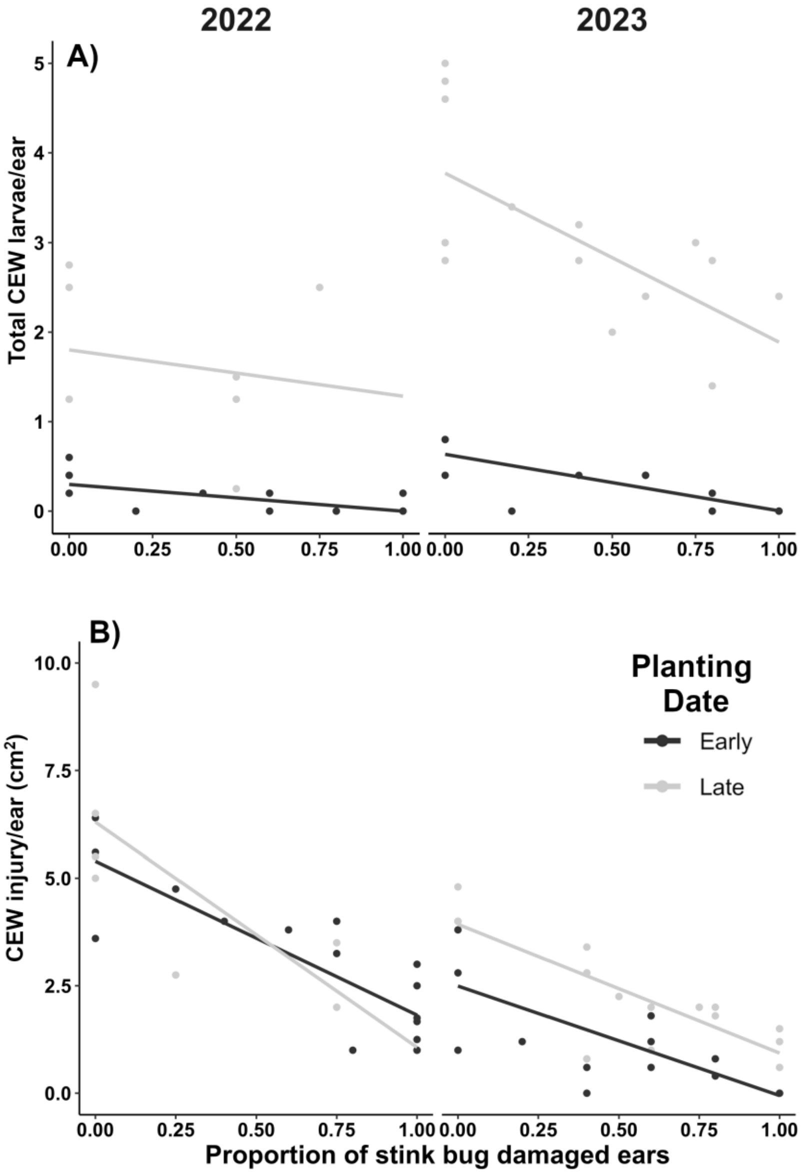
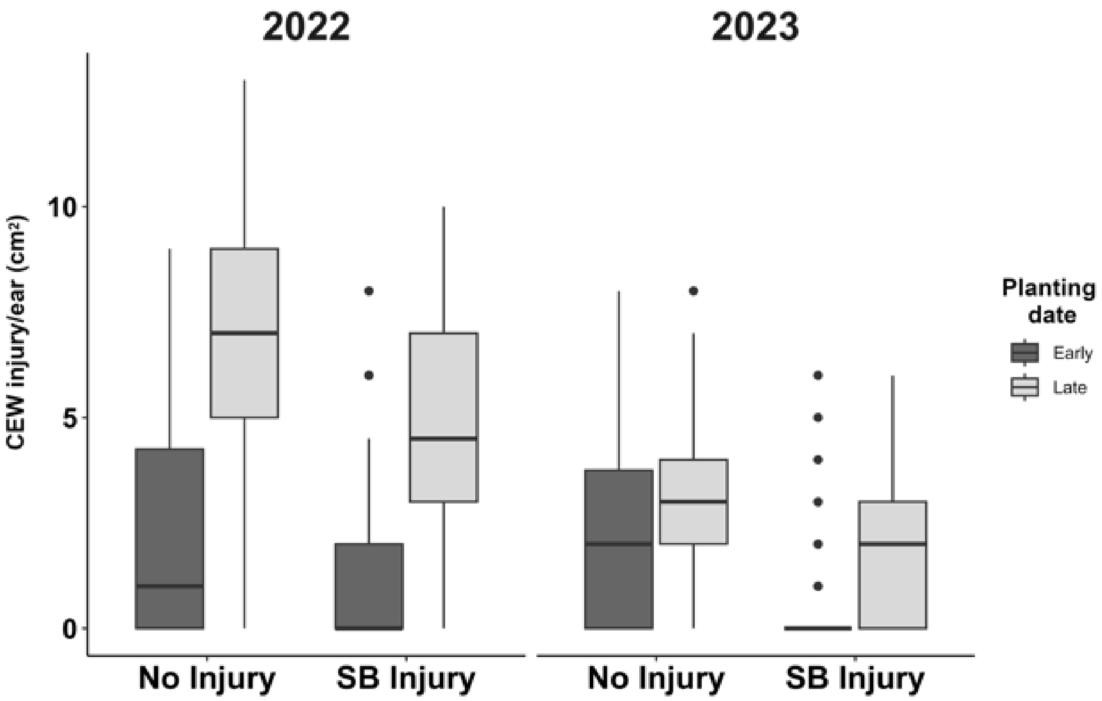


Figure 4.6 Box plots of total corn earworm (CEW) injury in stink bug (SB) injured vs. uninjured corn ears across early and late planted trials in 2022 and 2023.



CHAPTER FIVE

CONITNUED DECLINE IN SUBLETHAL EFFECTS OF BT TOXINS ON *HELICOVERPA ZEA* (LEPIDOPTERA: NOCTUIDAE) IN FIELD CORN

Introduction

Field corn hybrids expressing *Bacillus thuringiensis* (Bt) toxins are widespread in the row crop landscape. An estimated 85% of the corn planted in the United States expressed one or more Bt toxins in 2023 (USDA 2023). Bt corn hybrids were initially introduced in 1996 to target European corn borer, *Ostrinia nubalis* (Hubner) (Lepidoptera: Noctuidae), in addition to a number of other stalk boring pests (Siegfried and Hellmich 2012). Initial hybrids expressed only a single Bt toxin, Cry1Ab and Cry1F, which had excellent efficacy for stalk boring species (Koziel et al. 1993). Since the introduction of single toxin hybrids, a number of different Bt toxins have been introduced in pyramided hybrids expressing two or more toxins including Cry2Ab2, Cry1A.105, and Vip3A. These multi-toxin hybrids now represent the majority of transgenic corn acreage (Tabashnik et al. 2013).

In addition to stalk borers, Bt toxins also have efficacy for ear feeding pests including the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), which is one of the major targets for these toxins in the southeastern United States. The first commercial hybrids expressing only a single Bt toxin (Cry1Ab or Cry1F), had only poor to fair efficacy for corn earworm (Buntin et al. 2004, Reay-Jones et al. 2009). Pyramided hybrids which were first introduced 14 years after initial single gene hybrids improved control, however efficacy varied among traits. Corn hybrids expressing Cry2Ab2 +

Cry1A.105 (Genuity VT Double Pro, Bayer Crop Science) initially provided up to 97% control for earworm in North and South Carolina (Reay-Jones and Reisig 2014), while hybrids expressing Cry1Ab + Cry1F (Optimum Intrasect, Pioneer), provided only 55% control (Reay-Jones and Reisig 2014). The Bt toxin, Vip3A, is the most recently commercialized, and is only toxin that has provided excellent control for corn earworm (Burkness et al. 2010, Dively et al. 2023). Corn earworm is not considered to be an economic pest of field corn in the southeastern United States under most scenarios (Reay-Jones and Reisig 2014, Bibb et al. 2018). It can be a major pest of cotton however, where it is known as bollworm (Luttrell and Jackson 2012). After corn earworms feed, develop, pupate, and complete a generation on corn, adult females fly to and oviposit on cotton, as one of the next major cultivated hosts, where it is known as bollworm (Gould et al. 2002). Given that 89% of cotton is planted with Bt toxins (USDA 2023) and that the same or similar Bt toxins are expressed in both corn and cotton, movement of moths from Bt corn to Bt cotton increases selection pressure for resistance to Bt toxins.

Bt resistance is managed via the high-dose/refuge strategy. Bt toxins expressed at a rate high enough to kill 95% of the heterozygotes with a resistance allele meet the high-dose component of this strategy (Gould 1998). For the refuge component, field corn producers are required to plant a percentage of their Bt corn acreage with non-Bt corn hybrids. In the Corn Belt, producers can plant “refuge-in-a-bag” (RIB), where the correct percentage of non-Bt seed are pre-mixed into the bag with the Bt hybrid. In the southeastern United States where ear feeding pests are the primary target, producers must plant a structured refuge, as variable levels of Bt expression which can occur in a refuge plant as a result of cross pollination with surrounding Bt plants, potentially accelerating resistance (Yang et al. 2022a). There is abundant natural refuge available in the landscape later in the season during cotton production (Gould et al. 2002, Jackson et al. 2008, Head et al. 2010), meaning non-Bt corn refuge is the primary mechanism of resistance management that is actionable for producers. Due to the low levels of refuge adoption, and failure of many available toxins to meet the important initial assumption of high-dose (Storer et al. 2001, Reisig and Reay-Jones 2015), widespread practical Bt resistance in corn earworm has been identified to all Cry toxins (Dively et al. 2016, 2023, Reisig et al. 2018, Bilbo et al. 2019a, 2019b, Yang et al. 2022b). Vip3A remains the only highly effective toxin for corn earworm, but genetic markers for resistance have been identified in several populations (Yang et al. 2024) and reports of increased feeding on Vip3A expressing corn and cotton are increasingly common (Yang et al. 2019, Yang et al. 2021, Yang et al. 2022b, Dively et al. 2023).

Toxic effects of Bt hybrids can include a variety of sublethal impacts on life history traits of target insects. Larval or pupal development time, pupal weight, and fecundity have all been reported as sublethal impacts of Bt toxins on corn earworm (Storer et al. 2001, Horner et al. 2003). A lack of these sublethal impacts has also been attributed to the development of Bt resistance in a number of previous field trials (Reisig and Reay-Jones 2015, Dively et al. 2016). Pupal weight of lepidopteran insects in particular, can play an important role in the success of many different important physiological processes, such as diapause and overwintering (Liu et al. 2010). Pupal weight has been found to be significantly reduced by Bt toxins relative to non-Bt toxins

(Bilbo et al. 2018), although this reduction has been reduced over time (Reay-Jones et al. 2020) as a result of Bt resistance development. The sublethal impacts of Bt toxins on life history traits of corn earworm may have important implications for resistance management, as the refuge strategy relies heavily on synchronous development of susceptible and potentially resistant individuals (Liu et al. 1999).

Bt resistance is major threat to the sustainability of corn and cotton production in the United States, and continuing to monitor sublethal impacts of these toxins in field trials is an important part of overall resistance monitoring efforts. Changes in sublethal impacts can provide evidence of resistance development prior to field failures. The goal of the present study was to continue assessing the sublethal impacts of two different Bt corn hybrid families on corn earworm. Pyramided hybrids expressing Cry1F + Cry1Ab, Cry1F + Cry1Ab+Vip3A, and Cry2Ab2+Cry1A.105 were compared to non-Bt near isolines to assess these effects. Additionally, data from two previous studies by Bilbo et al. (2018) and Reay-Jones et al. (2020) were included with four years of unpublished data in this study to examine the reduction in these sublethal effects over a period of nine years (2014-2023). Data from this study can be used to help improve resistance modeling efforts and understand how these sublethal impacts may impact continued efforts to improve resistance management.

Materials and Methods

Field trials were conducted at the Clemson University Pee Dee Research and Education Center in Florence, South Carolina and the North Carolina State University Vernon G. James Research Center in Plymouth, North Carolina in 2020, 2021, 2022, and

2023 (Table 5.1). Two trials representing an early and a late planting date were used in each year of the study. Each trial consisted of five corn hybrids from Dekalb and Pioneer arranged in a randomized complete block design with four replications. Pioneer Bt hybrids expressed either Cry1F + Cry1Ab (P1366YHR, Optimum Intrasect, Dow AgroSciences, Indianapolis, IN; events TC1507 and MON810) or Cry1F + Cry1Ab + Vip3Aa20 (P1366VYHR, Optimum Leptra; events TC1507, MON810, MIR162), and the Deklab Bt hybrid expressed Cry1A.105 + Cry2Ab2 (DKC 67-72 VT2P, Genuity VT Double Pro, Bayer Crop Science, St Louis, MO; event MON89034). The non-Bt hybrid was DKC 67-70 for the DeKalb family and P1366R for the Pioneer family. All standard recommended agronomic practices were used for plant spacing, fertilization, herbicide applications, and irrigation.

Once plots reached the second reproductive stage of development (R2), sampling began for late-instar corn earworm larvae. Once fourth and fifth instar larvae were identified, 50 ears from each plot were hand harvested. To maximize the number of corn earworm larvae in the harvested ears, the silks of each ear were lightly pulled. If the silks are easily removed, it generally indicates the presence of late instar larvae. In plots where the larval density was low (i.e., less than 50 ears had injured silks), the remainder of the 50 ears was randomly harvested from the plot at the appropriate growth stage. Harvested ears were immediately placed vertically in plastic containers containing sifted soil (Bilbo et al. 2018, Reay-Jones et al. 2020). The orientation of ears in the plastic container mimicked the position on a corn plant and allowed the larvae to complete their development on harvested ears, emerge, and pupate in the soil within each container. The containers were placed under an outdoor shelter to prevent rain from impacting pupation and pupal survival. Twice per week, ears were removed from each container and the soil was carefully sifted to collect pupae. Sifting of the soil continued until there were no more pupae recovered from any hybrid. Collected pupae were rinsed with water and dried in the laboratory, then weighed and sexed.

Statistical Analysis

Weight was averaged for all pupae collected from each plot prior to analysis. A previous study by Reay-Jones et al. (2020) following the same protocol did not identify a statistical difference in corn earworm pupal weight between sexes, so male and female pupae were combined for analysis in this study. Linear mixed effect models were used for analysis of pupal weight in the package *lme4* (Bates et al. 2015) in R version 4.3 (R Core team 2022). Corn hybrid, trial, and the interaction of hybrid and trial were fixed effects, and block nested within trial was the random effect. A separate analysis was conducted for each hybrid family and state. Tukey-adjusted mean separation tests were then conducted as needed using the package *emmeans* (Lenth et al. 2023). A total of 20 and zero larvae were collected in North and South Carolina, respectively, from hybrids expressing Vip3Aa20 and these pupae were excluded from analysis. Additionally, only eight total pupae were recovered from the early planted trial in North Carolina in 2023, which were also excluded from overall analyses.

The percentage reduction in pupal weight between non-Bt and Bt hybrids within each hybrid family were calculated for each trial. Linear regressions were then performed examining the change in this percent reduction in pupal weight over time. These regression analyses included data from 2014 to 2016 previously published in Bilbo et al. (2018) and from 2017 to 2019 previously published in Reay-Jones et al. (2020). The percent reduction in pupal weight was the dependent variable and the trial was the independent variable for linear regression analyses.

Results

A total of 5,856 pupae were recovered across all trials, with 3,122 from North Carolina and 2,734 from South Carolina (Table 5.2). The number of pupae recovered varied based on planting date and corn hybrid. Across all trials and hybrids, there was a 55% and 88% increase in the number of pupae recovered in late planted trials relative to early planted trials in North and South Carolina, respectively. In North Carolina, there were 23% and 42% less pupae recovered from hybrids expressing Cry1F + Cry1Ab (847 pupae) and CryA105 + Cry2Ab2 (418 pupae), respectively, relative to each non-Bt near isoline hybrids (1098 and 722 pupae). A similar trend was observed in South Carolina, with a 16% and 39% decrease in pupae recovered from hybrids expressing Cry1F + Cry1Ab (704 pupae) and CryA105 + Cry2Ab2 (543 pupae), respectively, relative to each non-Bt near isoline (592 and 895 pupae). Only 20 pupae were recovered from hybrids expressing Vip3A across all trials and were all in North Carolina, with four in early and 16 in late plated trials.

Corn hybrid had a significant impact on pupal weight in North and South Carolina in both hybrid families (Table 5.3). Averaged across trials, Cry1A.105 + Cry2Ab2 hybrids reduced pupal weight on average by 6% and 9% in North and South Carolina, respectively, relative to the non-Bt near isoline (Fig. 5.1A). Cry1F + Cry1Ab hybrids reduced pupal weight on average by 3% and 8% in North and South Carolina, respectively, relative to the non-Bt near isoline (Fig. 5.1B). Trial (i.e., year and planting date) also had a significant impact on pupal weight in both hybrid families and states (data not shown). The interaction of hybrid and trial was significant for both hybrid families and states. The impact of the Bt hybrids on pupal weight varied between trials, and in a number of cases there was no significant difference between Bt hybrids and the non-Bt near isoline in a given trial (Fig. 5.2).

The reduction in pupal weight using data from 2014-2016 (Bilbo et al. 2018), from 2017-2019 (Reay-Jones et al. 2020), and from the present study significantly decline over time (Table 5.4, Fig 5.3). This negative trend over time was significant for both hybrid families and in both states. When regression analyses were run using average pupal weight as the independent variable, rather than percent reduction between Bt and non-Bt hybrids, only pupae from hybrids expressing Cry1A.105 + Cry2Ab2 in South Carolina significantly increased in weight over time ($P = 0.001$; data not shown).

Discussion

Results of this study illustrate a continued degradation of the sublethal impacts of pyramided Bt hybrids expressing Cry1 and Cry2 toxins on corn earworm. While we still observed a significant impact of Bt on pupal weight averaged across trials from 2020-2023, the effect of Cry toxins on pupal weight relative to non-Bt near isolines has significantly declined. Regression models indicate that corn earworm pupal weight in Bt corn are increasing relative to non-Bt corn, which is linked with continued development of resistance to Cry1Ab + Cry1F and Cry1A.105 + Cry2Ab2 over the duration of the present and two previous studies (2014-2023) (Bilbo et al. 2018, Reay-Jones et al. 2020). This result conforms with many reports of resistance to these toxins both using bioassays in laboratory with field derived populations (Tabashnik et al. 2008, Bilbo et al. 2019b, Yang et al. 2022b) and field trials (Dively et al. 2016, 2023). Continued decline in efficacy of Cry toxins can potentially increase selection pressure on Vip3A, the only remaining effective toxin for corn earworm, emphasizing the importance of continued efforts to monitor and manage resistance.

Pupal weight can be impacted by a number of different biotic and abiotic factors. Temperature (Huang et al. 2018), host plant and quality (Bessin and Reagan 1990), and insecticide exposure (Bilbo et al. 2018, Reay-Jones et al. 2020, Pezzini et al. 2023) can all impact pupal weight of Lepidopteran insects. Pupal weight of corn earworm, specifically, has been used in a number previous studies to quantify the sublethal impacts of Bt toxin exposure. Corn earworm feeding on Cry1Ab corn had reduced pupal weight compared to a non-Bt corn hybrid in 1996–1997 in North Carolina (Storer et al. 2001). In contrast, Reisig and Reay-Jones (2015) did not identify a difference in pupal weight between corn hybrids expressing Cry1Ab alone and non-Bt hybrids, which was attributed to resistance development. Bilbo et al. (2018) found a significant reduction in pupal weight from a range of different Bt toxins in corn relative to non-Bt corn, which was supported by a follow up study by Reay-Jones et al. (2020). These results conform with the overall reduction in pupal weight identified in the present study across all trials.

The success of the Bt resistance management strategy of refuge planting relies on several key assumptions; 1) individuals from Bt plants and non-Bt refuge have synchronous development, and 2) the impacts of Bt on dispersal ability do not affect random mating (Gould 1998). Deviations from these assumptions as a result of the sublethal effects of Bt exposure could result in asynchronous development or assortative mating and limit the efficacy of refuge plantings (Tabashnik 1998, Liu et al. 1999). Because pupal weight can be associated with a number of life history factors in the subsequent generation, including facultative diapause (Liu et al. 2010), development time (Anilkumar et al. 2008, Gassmann et al. 2009), and fecundity (Honek 1993, Bessin and Reagan 1990), it may have a direct impact on the success of resistance management. For corn earworm in Bt and non-Bt corn, several studies have attempted to associate pupal weight with various subsequent life history traits. Bilbo et al. (2018) did not identify any impact of pupal weight on egg viability or fecundity of the subsequent generation. Pezzini et al. (2023) did not identify a link between pupal weight and flight capacity or eclosion time. While these previous studies did not identify impacts of reduced pupal weight in corn earworm, more work is needed to identify determine how these sublethal effects on pupal weight may influence the biology of corn earworm, Bt resistance development, and management in corn.

It is unclear if the reduction in pupal weight observed here results from Bt toxicity or fitness costs of resistance. Fitness costs are generally recessive, and can also vary depending on ecological conditions (Gassmann et al. 2009). Fitness costs are generally considered to limit the development of resistance evolution, particularly if they are not recessive (Gould 1998, Gassmann et al. 2009). Further understanding the physiological mechanisms of the trends seen in the present study could play an important role in modeling the evolution of resistance moving forward. The EPA uses simulation models to forecast the development of resistance and inform regulatory action (FIFRA Scientific Advisory Panel 2001). These models are based on a number of different types of empirical datum, including ecological and life history data described here, as impacted by Bt toxicity (Matten et al. 2012).

Reports of increased incidence and severity of corn earworm feeding on Vip3A expressing corn and cotton have been more common in recent years (Yang et al. 2022b, Dively et al. 2023). The mechanisms of this resistance found in several corn earworm strains were primarily related to binding sites and associated with three different independent genetic loci (Yang et al. 2024). In the current study, only 20 pupae were recovered from Vip3A expressing hybrids across the entire four-year study. Tissue concentrations of Bt toxins can vary significantly spatially and temporally within a plant (Dutton et al. 2004, Bilbo et al. 2019), and it is possible that these pupae were from larvae which did not feed on tissue which fully expressed the Vip3A toxin. Ears were not sampled for larval feeding in this study, so it is also possible that some low levels of feeding occurred from larvae that did not successfully pupate. Regardless, it appears that Vip3A is still highly efficacious for corn earworm in a field corn setting in North and South Carolina. This result underpins the importance of continued efforts to preserve this technology through the planting of non-Bt refuge.

While it is generally not considered to be an economic pest of field corn (Bibb et al. 2018, Reay-Jones and Reisig 2014), corn earworm can be a major pest of a number of other crops, including cotton and sweet corn. Because both Bt cotton and sweet corn are grown in the United States, Bt resistance has major implications for management of an important pest in both of these crops. Public perception of transgenic technology has limited the adoption of Bt sweet corn hybrids, but the percentage of cotton planted with Bt toxins exceeds that of corn (USDA 2023). The widespread adoption of Bt cotton in the United States has led to a reduction corn earworm injury and insecticide usage, in addition to an increase in yield, based on a meta-analysis from data from 1996-2015 (Fleming et al. 2018). However, practical resistance to Bt cotton expressing two Cry toxins has developed in corn earworm populations in the United States, leading to increased injury and insecticide applications in cotton (Reisig et al. 2018). In the southeastern United States, where multiple generations of corn earworm occur, selection pressure is increased by exposure in both corn and cotton in the same season, underlying the need to comply with IRM practices in corn.

Some conclusions on planting date as it related to management in other crops may be drawn based on the current study. More pupae were recovered in later planted trials in both hybrid families, as shown in previous studies (Reay-Jones et al. 2020). Early planted trials in this study generally represent the recommended agronomic window for commercial production in North and South Carolina. While it was not explicitly studied in these trials, the stark contrast between these properly timed plantings and late planting dates emphasizes the importance of planting date for reducing overall corn earworm densities exposed to Bt toxins in corn. However, a recent study in cotton showed only a marginal impact of planting date on Heliothinae pressure (corn earworm and tobacco budworm, *Chloridea virescens* (F.)) due to the ability of cotton to compensate from injury and the longer reproductive window in earlier planting dates when pest pressure was higher (Reisig et al. 2023).

It is important to note that corn earworm is not the only target pest for Bt corn hybrids in the southeastern United States Single toxin hybrids (i.e., Cry1F, Cry1Ab) were initially introduced to target European corn borer, *Ostrinia nubalis* (Lepidoptera: Noctuidae). The widespread adoption of Bt corn expressing Cry toxins and excellent efficacy for stalk-boring pests resulted in area-wide suppression of corn borers, benefitting a range of cultivated host plants (Hutchinson et al. 2010, Dively et al. 2018). However, populations of European corn borer were recently identified in Canada with resistance to Cry1F and Cry1Ab (Smith et al. 2019, Smith and Farhan 2023). If this resistance continues to develop and spread, pyramided toxins may become increasingly important for this pest in particular, although Vip3A has no corn borer activity (Burkness et al. 2010). Restricting Bt hybrid usage has been suggested as one potential strategy for limiting the continued development of resistance in corn earworm (Reisig et al. 2022); however, Bt corn remains effective for a number of other target pests, providing a range of benefits to growers. This underpins the importance of IRM strategies in corn to protect the remaining Cry efficacy for corn earworm for cotton production, delay the development of Vip3A resistance, and control or suppress other important pests of field corn.

The present study demonstrates a continued decline in the sublethal impacts of pyramided Cry toxins on corn earworm pupae in the southeastern United States. When combined with previous efforts from Bilbo et al. (2018) and Reay-Jones et al. (2020), the percent difference in pupal weight between Bt hybrids and non-Bt near isolines has significantly decreased over a period of nine years (2014-2023). Corn hybrids expressing Vip3A remain highly effective for corn earworm based on the very small number of recovered pupae from these hybrids. The rate of adoption of non-Bt refuge planting has been reported to be as low as 38% in North Carolina in 2017 (Reisig 2017), and 59% in North and South Carolina in 2023 (Bryant et al. 2024). Willingness or ability to plant refuge is dictated by a number of factors including, farm size, producer knowledge on the benefits and reasoning for refuge, farm logistics, and availability of non-Bt hybrids (ABSTC 2016, Reisig 2017, Reisig and Kurtz 2018). Regulatory agencies rely on the use of forecasting models to implement policy to limit continued evolution of resistance to plant incorporated protectants like Bt. Data from this study could be used in conjunction with others to aid in that effort, and to support extension work aiming to improve the adoption of non-Bt refuge plantings.

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Table 5.1 Field trial locations and details, 2020-2023.

Year	Location	Planting Date	# Rows	Dates of ear sampling
2020	Florence, SC (early)	4/15	14	7/9-7/13
	Florence, SC (late)	6/23	14	8/28-9/1
	Plymouth, NC (early)	5/27	14	7/30
	Plymouth, NC (late)	6/18	14	8/24
2021	Florence, SC (early)	4/12	8	7/6-7/8
	Florence, SC (late)	6/28	8	8/30-9/1
	Plymouth, NC (early)	5/6	8	7/27
	Plymouth, NC (late)	6/28	8	8/10
2022	Florence, SC (early)	4/12	8	6/28-7/5
	Florence, SC (late)	6/23	8	8/26-9/8
	Plymouth, NC (Early)	5/6	8	7/26
	Plymouth, NC (Late)	6/1	8	8/10
2023	Florence, SC (early)	4/13	8	7/3-7/10
	Florence, SC (late)	6/27	8	8/24-8/30
	Plymouth, NC (Early)	5/10	8	7/27
	Plymouth, NC (Late)	6/1	8	8/7

Table 5.2 Total number of corn earworm pupae from each trial and hybrid, 2020-2023.

Year	Hybrid	Bt protein	Early-planted		Late-planted	
			SC	NC	SC	NC
2020	P1366R	-	60	276	82	130
	P1366YHR	Cry1F+Cry1Ab	59	216	60	139
	P1366VYHR	Cry1F+Cry1Ab+Vip3A	0	1	0	0
	DKC 67-70	-	83	233	93	90
	DKC 67-72	Cry1A.105+Cry2Ab2	72	97	38	90
2021	P1366R	-	24	107	108	74
	P1366YHR	Cry1F+Cry1Ab	22	28	79	25
	P1366VYHR	Cry1F+Cry1Ab+Vip3A	0	2	0	5
	DKC 67-70	-	28	53	125	54
	DKC 67-72	Cry1A.105+Cry2Ab2	31	11	64	51
2022	P1366R	-	86	66	156	159
	P1366YHR	Cry1F+Cry1Ab	76	85	132	132
	P1366VYHR	Cry1F+Cry1Ab+Vip3A	0	0	0	1
	DKC 67-70	-	109	23	172	89
	DKC 67-72	Cry1A.105+Cry2Ab2	81	7	87	68
2023	P1366R	-	33	2	171	284
	P1366YHR	Cry1F+Cry1Ab	39	0	125	225
	P1366VYHR	Cry1F+Cry1Ab+Vip3A	0	0	0	11
	DKC 67-70	-	112	5	173	177
	DKC 67-72	Cry1A.105+Cry2Ab2	40	1	130	94

Table 5.3. ANOVA statistics for corn earworm pupal weights collected in corn trials in Florence, SC and in Plymouth, NC, 2020–2023.

State	Family	Effect	Df	F	P
NC	Dekalb	Hybrid	1, 20.10	31.53	**< 0.0001**
		Trial	6, 16.91	5.96	**0.0017**
		Hybrid x Trial	6, 16.98	1.89	0.1341
	Pioneer	Hybrid	1, 20.05	20.28	**0.0002**
		Trial	6, 16.98	3.69	**0.0156**
		Hybrid x Trial	6, 19.23	8.83	**0.0001**
SC	Dekalb	Hybrid	1, 24	154.75	**< 0.0001**
		Trial	7, 21	25.38	**< 0.0001**
		Hybrid x Trial	7, 24	2.77	**0.029**
	Pioneer	Hybrid	1, 24	56.07	**<0.0001**
		Trial	7, 21	8.47	**0.0001**
		Hybrid x Trial	7, 24	3.62	**0.0083**

Table 5.4. Regression statistics for reduction in pupal weight between Bt and non-Bt near-isolines as the dependent variable and trial as the independent variable (Plymouth, NC, and Florence, SC, 2020–2023).

State	Family	Percent reduction in pupal weight (non-Bt to Bt)			
		Intercept (SE)	Slope (SE)	R^2	P
NC	Dekalb	0.1653 (0.0253)	-0.0071 (0.0030)	0.11	**0.0241**
	Pioneer	0.1657 (0.0247)	-0.0062 (0.0030)	0.07	**0.0494**
SC	Dekalb	0.2075 (0.0235)	-0.0092 (0.0023)	0.20	**0.0002**
	Pioneer	0.1697 (0.0206)	-0.0082 (0.0021)	0.19	**0.0002**

Figure 5.1 Effect of A) Cry1F + Cry1Ab and B) Cry1A.105 + Cry2Ab2 Bt toxins on pupal weight (±SE) relative to non-Bt near isolines averaged across all trials in North (NC) and South Carolina (SC), 2020-2023.

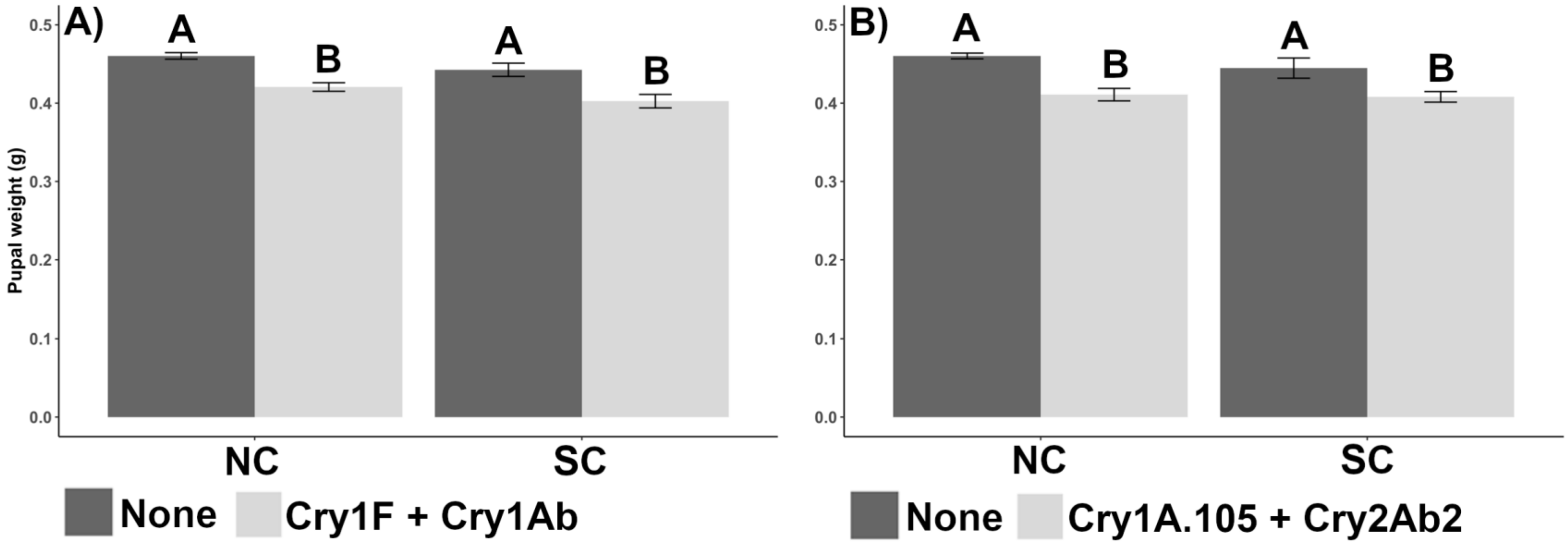


Figure 5.2 Interaction effect of trial and A) Cry1A.105 + Cry2Ab2 and B) Cry1F + Cry1Ab Bt toxins in South Carolina, and C) Cry1F _ Cry1Ab Bt toxins in North Carolina, on pupal weight (±SE), 2020-2023. Bars with different letters within each hyrbid family and state were significantly different at alpha = 0.05.

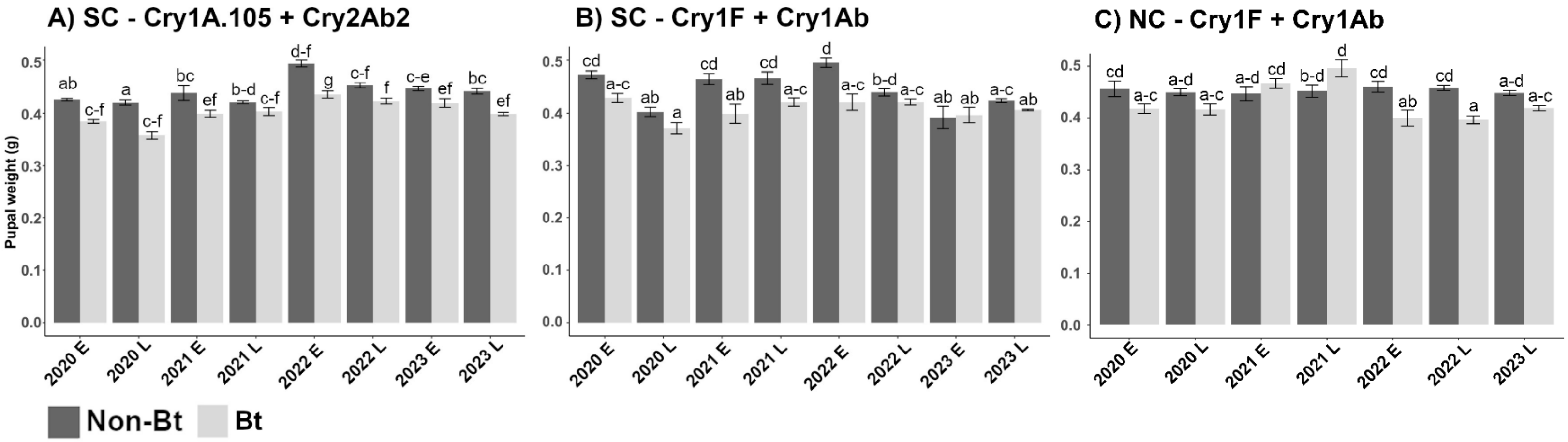
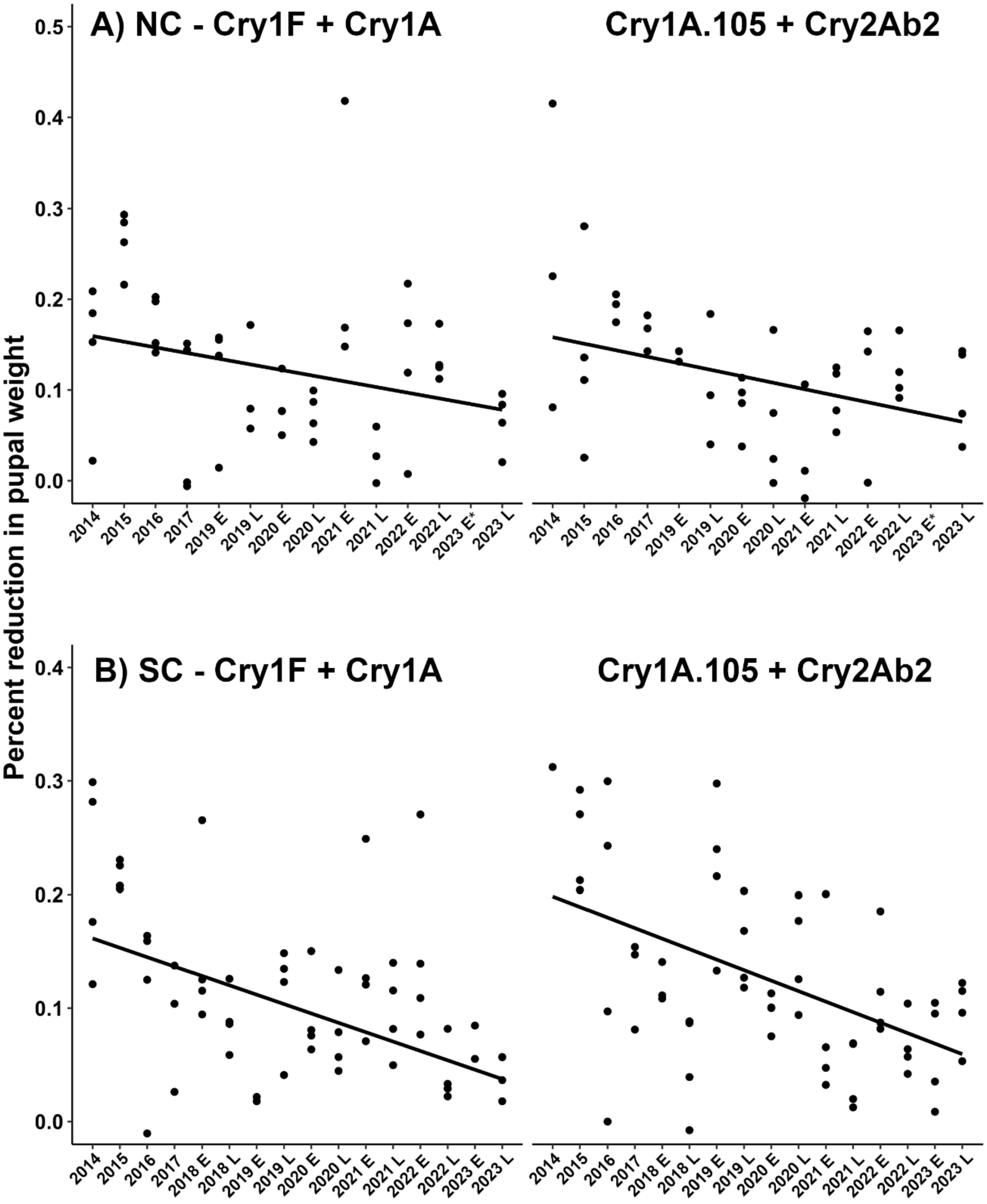


Figure 5.3. Linear regressions of reduction in pupal weight between Bt hybrids and non-Bt near isolines for A) Cry1F + Cry1A and Cry1A.105 + Cry2Ab2 in North Carolina and B) South Carolina, 2014-2023. Note there were not enough pupae in NC in 2023 early to calculate percent reduction.



CHAPTER SIX

GROWER PERCEPTIONS AND ADOPTION OF IPM AND NON-BT REFUGE IN FIELD CORN: A SURVEY IN NORTH AND SOUTH CAROLINA[3]

Field corn, *Zea mays* L. (Poales: Poacaea), is the most important cereal crop in the United States, accounting for over 35 million hectares of arable land use in 2022 at a value of over $91 billion USD (USDA Quick Stats 2023). Of the factors impacting the production of field corn, insect pests are commonly reported to be a yield-limiting factor, causing an estimated 7.3% loss in yield across the United States in 2022 (Reisig et al. 2022). In the southeastern United States, the two most common insect pests are corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (Reay-Jones 2019), and a complex of stink bugs (Hemiptera: Pentatomidae) (Reisig et al. 2022). In the stink bug complex, brown stink bug, *Euschistus servus* (Say) (Hemiptera: Pentatomidae), and southern green stink bug, *Nezara viridula* (Say) (Hemiptera: Pentatomidae), are the most common in the southeastern U.S. (Tillman 2010a, Bryant et al. 2023). However, grower perception of the relative impact of these insects on corn production is not well understood. Additionally, the use of transgenic corn expressing insecticidal toxins from *Bacillus thuringiensis* (Bt) (Berliner) and insecticidal seed treatments is widespread in field corn production in the United States (Douglas and Tooker 2015). Despite the extensive use of these control tactics, our understanding of how and why growers choose to implement these strategies, and for what pests, is limited. To address these knowledge gaps, grower surveys can be used to assess grower perceptions of pests, implementation of management strategies,

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and the barriers to use of various control tactics (Parsa et al. 2014, Jabbour and Noy 2017, Reisig 2017, Lane et al. 2023). More specifically, while the rates of adoption of resistance management strategies for Bt technology have been assessed using direct grower surveys (Wilson et al. 2005, Reisig 2017, Pezzini et al. 2023), these studies were either conducted outside the southeastern United States or were narrowly focused relative to the scope of this study.

Corn Earworm, Bt Corn, and Insect Resistance Management (IRM)

Corn earworm is present in field corn in most fields in most years in the southern and southeastern United States (Cartwright 1939, Reisig et al. 2015). The insect historically does not overwinter in the United States at latitudes north of 40° (Hardwick 1965) and only reaches those areas each year via migration, however its northern overwintering range has and will continue to increase under climate change (Lawton et al. 2022). While it has a cosmopolitan distribution in the southeastern United States, it is generally not an economic pest of field corn (Reay-Jones and Reisig 2014, Bibb et al. 2018). Despite not being an economic pest, corn earworm is managed in field corn using transgenic Bt corn hybrids. Corn expressing Bt toxins was primarily introduced for control of the European corn borer, *Ostrinia nubilalis* (Hübner), and other corn borer species (Siegfried and Hellmich 2012), but a range of insect species can be suppressed or controlled by Bt corn, including the corn earworm (Arends et al. 2021, 2022). Since the introduction of Bt corn hybrids expressing a single toxin (i.e., Cry1Ab followed by Cry1F), a number of different toxins have been used in Bt corn, with more recent Bt corn hybrids expressing two or more Bt toxins. The degree of efficacy for corn earworm control varies among toxins, and practical resistance to many of these toxins is now widespread (Dively et al. 2016, Bilbo et al. 2019, Yang et al. 2022). The Vip3Aa20 toxin is the only plant-incorporated protein (PIP) that remains highly efficacious for corn earworm, but early signs of resistance have been identified (Yang et al. 2021). Corn earworm or bollworm is also a major pest of cotton, which expresses many of the same or similar Bt toxins found in commercial corn hybrids (Kanel et al. 2016). Corn earworm has multiple generations per year in the southeastern United States, and cotton is often the next major cultivated host after corn (Gould et al. 2002). Movement of moths from corn to cotton expressing the same or similar Bt toxins increases the selection pressure and the risk of further resistance development to Bt toxins.

Transgenic crops expressing Bt toxins are important tools for management of bollworm in cotton, and because new traits take years to develop and commercialize, management of Bt resistance is critical for extending the efficacy of current Bt products. Resistance to Bt proteins is primarily managed via the high-dose refuge strategy. This approach involves using hybrids expressing a high concentration of Bt toxin and concurrent and proximate plantings of non-Bt refuge plants. Non-Bt plants in or nearby Bt corn fields allow homozygous susceptible individuals to successfully develop and mate with potentially heterozygous resistant individuals (assuming the genetic basis of resistance is recessive and located on a single allele) from the Bt crop, thus, delaying the evolution of resistance in the population. In non-cotton growing regions, corn growers are able to plant blended refuge or “refuge in a bag,” where non-Bt seed is mixed with Bt seed directly in the bag of seed (EPA 2022). In cotton growing regions, a structured non-

Bt corn refuge is required, which is more challenging to implement. The current refuge requirement in cotton-growing areas is 50% for single toxin corn hybrids and 20% for hybrids expressing multiple toxins (EPA 2022). Grower compliance with the required refuge in this region (North Carolina) has been reported to be as low as 38% (Reisig 2017). Factors potentially influencing the adoption rate of refuge planting include limited knowledge about which corn hybrids are non-Bt, availability of non-Bt hybrids, lack of understanding of the value of refuge, and perceived limited yield potential of non-Bt hybrids (Reisig 2017, Pezzini et al. 2023). Continuing to monitor the adoption rate of refuge plantings and understanding why growers select Bt hybrids is important for tailoring Extension programming to deficiencies in knowledge that can lead to lower levels of resistance management adoption. It is also important to note that the availability of non-Bt seed can be limited, so efforts should not solely focus on producers, but also on private ag industry to help improve the accessibility of this critical resistance management strategy.

Pest Status and Management of Stink Bugs

Stink bugs, primarily the brown stink bug and southern green stink bug, are the most important pests of corn in the southeastern United States (Reisig et al. 2022) and can feed on corn at several growth stages during the growing season. From emergence (VE) through around V6, stink bugs feed directly on the growth point of young plants, leading to delayed growth, leaf holes, development of tillers, and plant death in some cases (Apriyanto et al. 1989a, 1989b, Bryant et al. 2020, 2021). During late vegetative stages, around two weeks prior to tasseling, feeding by stink bugs causes deformed or “banana-shaped” ears, limiting overall yield potential (Clower 1958, Ni et al. 2010, Bryant et. al 2020). Finally, direct feeding on kernels by stink bugs during the first reproductive stages (i.e., R1 and R2) has the potential to introduce grain quality concerns (Ni et al. 2011).

For the early stages of corn development, high rates of systemic insecticide seed treatments provide some preventative control for stink bugs (Bryant and Taylor 2021, Reisig and Goldsworthy 2023), as well as a number of other soil pests. Foliar applications of insecticides for stink bug control in corn throughout the season can be effective, although efficacy is heavily dependent on scouting and timely applications at economic thresholds, as well as a number of other factors related to stink bug behavior and spray coverage (Reisig 2011). Assessing what tactics growers implement for stink bug management as well as grower perception of their pest status are important for shaping future research and Extension programs.

Insecticide Seed Treatments in Corn

Insecticidal seed treatments, primarily in the neonicotinoid class of insecticides, are applied to the vast majority (87% in 2011) of commercially available field corn seed (Douglas and Tooker 2015). It is virtually impossible today to source corn seed without an insecticidal seed treatment. While these seed treatments were developed to target below-ground pests (e.g., rootworms, wireworms) and early season vegetative pests (e.g., cutworms, stink bugs, chinch bugs), these sporadic pests often occur at very low densities in many of the areas where seed treatments are deployed (Sappington et al. 2018). Preventative seed treatments can be considered an IPM strategy, provided a rescue treatment is not an option or will be ineffective (Sappington et al. 2018). In the case of early season stink bug infestations, scouting can sometimes underestimate population levels (Bryant et al. 2023), and the window for economically valuable foliar applications is narrow (Babu and Reisig 2018, Bryant et al. 2020). Understanding the reasons why growers select seed treatments and what pests they hope to target may reveal deficiencies in producer knowledge about the use of seed treatments.

Integrated Pest Management (IPM) Adoption in Field Corn

Integrated pest management (IPM) is a sustainable approach to managing pests by combining biological, cultural, physical and chemical tools in a way that minimizes economic, health, and environmental risks (EPA 2022). Integrated pest management plans ideally put emphasis on the use of cultural, physical, or biological control to prevent pests from reaching damaging levels, only using reactive biological or chemical control if pests reach economic thresholds. Historically, IPM has not been extensively implemented in field corn production (Meissle et al. 2011, Blanco et al. 2014) because of a number of suggested limitations, including a lack of knowledge of pest ecology (Parsa et al. 2014), a lack of awareness about the basic principles of IPM (Lane et al. 2023), and cost of implementation relative to conventional chemical management programs (Blake et al. 2007, Parsa et al. 2014, Lane et al. 2023). In field corn in particular, the ease of chemical management and widely available preventative tactics such as Bt hybrids and preventative seed treatments can reduce the perceived value of IPM to growers (Meissle et al. 2011, Blanco et al. 2014). Profitability has often been reported to be the number one factor influencing corn growers’ willingness to adopt IPM strategies (Lane et al. 2023).

Survey Objectives

The goals of this survey conducted with corn growers and corn production stakeholders in North and South Carolina were to assess 1) perception of insect pest status in corn, 2) management strategies implemented for those pests, 3) justification for management implementations, 4) Bt refuge compliance levels, and 5) the understanding and implementation of IPM.

Survey Development and Distribution

Corn producers and stakeholders from North and South Carolina were surveyed in 2022 and 2023. The survey was developed in collaboration with field-crop entomologists from North and South Carolina. The survey consisted of 16 questions and was separated into several categories of questions related to critical aspects of managing insect pests of corn (supplemental Fig. S.6.1). The categories included general respondent information, status of insect pests in corn, use of insecticide seed treatments, use of Bt corn, planting of non-Bt refuge, management of stink bugs, and implementation of IPM. Each question had between three and nine multiple choice responses and required either a single answer or multiple answers, depending on the question.

Surveys were primarily distributed through pre-existing Extension programming targeted towards growers, including field days and grower meetings. At in-person meetings, both paper surveys and a virtual survey using a QR code were provided to respondents. Online surveys were provided using Qualtrics online surveying tool (Qualtrics, Provo, UT). Some additional mechanisms of distribution included a QR code in Clemson University’s IPM Newsletter and on North Carolina State University’s

Extension webpage. Online distributions accounted for a small percentage (<1%) of total respondents compared with in-person distributions of paper and virtual surveys. This survey is a component of a USDA NIFA Crop Protection and Pest Management Extension Implementation grant (No. 2021-70006-35508), which was submitted to the Clemson University Office of Research Compliance for consideration and approval, and was declared exempt from review (IRB# 2021-0169).

While the survey was distributed at meetings attended by ag industry representatives, university personnel, and crop consultants, and was open to all attendees, some questions about implementation of management strategies were targeted for producers only. Results were grouped based on respondents' response to question one of the survey (supplemental Fig. 1). Categories of respondents included 1) growers/producers, 2) ag industry personnel, 3) university personnel (Extension agents or specialists/researchers), and 3) crop consultants. A small percentage of respondents identified as "other." Only grower responses for questions on the use of seed treatments, stink bug management, and Bt use and refuge planting (Figs. 2-4) are reported, as these questions are not directly relevant to any other category of respondent. It is also important to note that a small number of surveys were incomplete, with respondents either skipping questions or terminating their response before the last question. Results were reported as proportion of respondents as the number of responses varied slightly among questions due to incomplete survey responses. Pearson's chi-squared contingency tests were conducted using R version 3.6.3 (R Core Team 2020) to determine whether or not producer knowledge or perceptions were associated with their implemented management or intention to plant non-Bt refuge. A p-value of less than 0.05 in these tests indicated that the responses to the given questions were not independent.

Key Findings

General Survey Questions

A total of 210 paper and online surveys were completed in 2022 and 2023. The majority of survey respondents were growers (136 respondents). Representatives from ag industry were the second highest proportion of respondents (35 respondents). The remaining respondents identified as crop consultants (12), university personnel (18), or other (9). The respondents who identified as “other” included students and combinations of both growers and Extension agents or ag industry personnel. Of grower respondents, the primary source of insect management information varied relatively equally between direct interactions with university Extension personnel (15%), Extension publications (28%), their farming community (24%), and from ag industry personnel (33%).

A large majority of all categories of respondents indicated that they considered insects to be a yield-limiting factor in producing corn, including 87% of grower respondents (Fig. 6.1A). A larger percentage of crop consultants did not consider insects to be a yield-limiting factor relative to other respondents (Fig. 6.1A). Stink bugs and corn earworm were identified as the most common and second most common pests managed in corn, respectively, across all respondents (Fig. 6.1B).

Insecticide Seed Treatments

Growers’ decisions on the rate and type of insecticide seed treatment were most commonly based on Extension recommendations (29%), seed company selection (29%), specific insect concerns (28%), and yield potential or cost (19%) (Fig. 6.2A). Only a small percentage of growers reported having no option to select their rate and type of insecticide seed treatment (3%) or not using any insecticide seed treatment (1%). Of growers who had specific insect management concerns when selecting a seed treatment, they were most commonly targeting early season stink bug infestations (42%), followed by soil pests (34%), billbugs (14%), and other (10%) (Fig. 6.2B).

Bt Corn Use and Refuge Implementation

The majority of growers (80%) indicated that they planted Bt corn. The remaining grower respondents either did not plant Bt corn (14%) or did not know whether they planted Bt corn or not (6%). Grower perception of insects being a yield-limiting factor was independent of whether or not they planted Bt corn ($X^2 = 0.010$; df = 1; $P = 0.919$). The most common factor influencing grower selection of Bt hybrid was yield potential (43%) followed by recommendations from ag industry or their seed retailer (34%) (Fig. 6.3A). Insect control efficacy was less commonly reported as a factor (11%), along with hybrid price (10%), Extension recommendations (8%), and Bt resistance management concerns (6%) (Fig. 6.3A).

Most growers (71%) indicated that they were familiar with which hybrids were non-Bt and could be used for refuge plantings (Fig. 6.3B). Around 59% of growers indicated that they would plant non-Bt refuge corn in both 2022 and 2023 (Fig. 6.3C and 6.3D). A higher percentage of growers were uncertain about if they would plant refuge in 2023 at the time of surveying (Fig. 6.3D). Growers' plans to plant non-Bt refuge in 2022

($X^2 = 22.93$; df = 1; $P < 0.001$) and 2023 ($X^2 = 17.67$; df = 1; $P < 0.001$) were associated with their knowledge of which hybrids were non-Bt.

Stink Bug Management

The majority of grower respondents (52%) identified ear development as the most critical growth stage for managing stink bug populations in corn (Fig. 6.4A). Tasseling and the early vegetative stages were less commonly reported as critical stages for management, and a small percentage of growers responded that they did not know (Fig. 6.4A). The most commonly reported management strategy used for stink bugs in corn was applying an insecticide at the economic threshold level (28%). Growers reported scouting for stink bugs during early vegetative stages of corn (26%) more commonly than at late vegetative or reproductive stages of development (16%). An application of insecticide mixed with fungicide at tasseling was used by 12% of grower respondents (Fig. 6.4B). Stink bugs were not managed in corn by 21% of grower respondents, despite the large percentage (62%) of growers identifying stink bugs as the most common pest species. Whether or not a grower chose to implement some type of management for stink bugs was dependent on if they identified stink bugs as the most important pest ($X^2 = 23.05$; df = 1; $P < 0.001$).

IPM Implementation

Across all categories of respondents, the majority (65%) identified as somewhat confident in their ability to address pest management concerns in corn (Fig. 6.5A). Crop consultants had a higher percentage of respondents (63%) who felt very confident in their ability to address pest management concerns (Fig. 6.5A). Grower confidence in addressing insect pest management concerns in corn was found to be independent of whether or not they implemented IPM ($X^2 = 4.20$; df = 4; $P = 0.379$). The majority of respondents indicated that they understood the concepts of IPM well, with crop consultants and university personnel having a higher percentage relative to growers or ag industry personnel (Fig. 6.5B). A similar percentage of respondents indicated that they also implement IPM in corn production (Fig. 6.5C).

Discussion

This survey provides valuable information on stakeholder perceptions of insect pests and a range of pest management-related decisions in field corn in the southeastern United States. The most common insect pests, commonly implemented management strategies for those pests, Bt corn use and resistance management, the justification for the use of seed treatments, and producer knowledge and implementation of IPM were all assessed in this study. Transgenic Bt technology is a critical management tool for corn borers, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), and other species, but, over time, widespread use of Bt traits has resulted in reduced efficacy for corn earworm due to the evolution of resistance. Therefore, continued monitoring of resistance management implementation (i.e., non-Bt refuge) was of particular importance in this survey. Refuge adoption was found to be ~20% higher amongst respondents in the current survey compared with responses on a similar survey of corn growers conducted in North Carolina in 2017 (Reisig 2017). While this may be interpreted as a positive trend for resistance management, it is important to note the inherent bias that exists in surveying efforts. A large percentage of surveys were completed by producers willing to attend an Extension meeting and voluntarily take the time to complete a survey, many through the use of a QR code. This likely represents a population of producers who readily adopt and implement new technologies and conform to regulations, as previously suggested by Pezinni et al. 2023. Additionally, farms in North and South Carolina are generally small compared to the national average farm size at 74 and 77 hectares, respectively (USDA NASS 2017), and farm income is lower as a result. Grower age, farm size, income, and education all have been shown to play a role in resistance management or IPM adoption (Feder et al. 1985, Shennan et al. 2001, Reisig 2017) and may have played a role in our survey results, but were not explicitly studied. Due to the regulatory pressure to comply with non-Bt refuge, self-reported rates of compliance might also be inflated. Grower justification for Bt and seed treatment use was also still found to be primarily based on recommendations from ag industry and yield potential, rather than specific insect pest management concerns. The current survey indicates a continued need for extending the reach of Extension work to a wide range of producers and to ag industry to improve the rate of adoption of IPM and resistance management strategies in corn production.

Insect Pest Status

The overwhelming majority (86%) of survey respondents identified insects as a yield-limiting factor in field corn. Stink bugs and corn earworm were the two most common insect pests reportedly managed by growers, with stink bugs being the dominant response. Stink bugs are consistently reported to be the most important pest of corn in the southeastern United States (Reisig et al. 2022), but corn earworm is not considered to be an economic pest of timely planted field corn, despite its cosmopolitan nature (Reay-Jones and Reisig 2014, Bibb et al. 2018, Olivi et al. 2019). While stink bugs are more sporadic in field corn compared with corn earworm, growers are evidently aware that stink bugs have more potential to reduce yield if they reach damaging levels and need to be managed. A large percentage (80%) of growers still identified corn earworm as their most managed pest, despite widespread use of Bt corn hybrids and their limited potential for economic injury. This result is likely due to the majority of growers planting Bt corn hybrids which have some efficacy for corn earworm. Therefore, many survey respondents reported corn earworm as their most managed pest despite its low yield-limiting potential. Additionally, some level of conspicuous earworm feeding still occurs on many Bt hybrids due to Bt resistance, which may further contribute to the perception of them as a major pest. In comparison to nationwide insect losses (Reisig et al. 2022), soil pests, and corn rootworms in particular, were not a major concern for the majority of respondents, which is expected given the limited occurrence of corn rootworms in the region.

Use of Bt Corn and Non-Bt Refuge

Regardless of grower perception of how important corn earworm is as a pest, the most common grower response for how a Bt hybrid is selected was yield potential, rather than specific insect management concerns. A recent survey of corn producers in Brazil reported a similar, albeit slightly lower, level of growers selecting Bt hybrids for yield potential (Pezzini et al. 2023). In contrast to the prevailing perception among growers, research has shown that corn yields in southern and southeastern United States are not increased by the use of a Bt hybrid in the absence of an economic pest (Reay-Jones and Reisig 2014, Bibb et al. 2018). Restricting Bt hybrids or specific Bt traits in areas where it provides little to no benefit to growers has been suggested as a best management practice for IRM in corn (Reisig et al. 2021). Currently, Vip3A is the only toxin that provides excellent control of corn earworm. If the use of hybrids expressing Vip3A is restricted in field corn, the subsequent reduced selection pressure on this trait may benefit the durability of cotton hybrids expressing the same toxin. Restricting the use of only Vip3A would also allow corn growers to plant hybrids expressing older Cry toxins to control insect pests that remain susceptible to these Cry toxins. This strategy assumes that non-Bt hybrids and hybrids expressing older Cry toxins are readily available and have similar or better yield potential than more modern hybrids, which has been reported as a limiting factor in the adoption of refuge plantings (Reisig 2017). While the adoption of corn expressing Vip3A remains low, it has been increasing in recent years, with a reported 14.3% across 12 cotton producing states in 2020 (Yang et al. 2021). However, the majority of the Bt corn planted in the southern United States currently expresses either Cry1A.105 + Cry2Ab2 or Cry1Ab + Cry1F, which both only provide moderate levels of corn earworm control (Reay-Jones 2019, Yang et al. 2022).

While Bt corn may not provide economic value as it pertains to corn earworm, Bt technology was originally introduced to target corn borers, namely the European corn borer, *Ostrinia nuablis* (Hubner) (Lepidoptera: Noctuidae) (Koziel et al. 1993), and to a lesser extent the southwestern corn borer, *Diatraea grandiosella* (Dyar) (Lepidoptera: Crambidae). European corn borer is difficult to control using foliar insecticides and was responsible for an estimated $1 billion (USD) in losses prior to widespread Bt use (Hyde et al. 2000). Single toxin Bt hybrids (i.e., expressing Cry1Ab or Cry1F) have, for the most part, remained effective for European corn borer (Siegfried and Hellmich 2012), and their use has led to area-wide suppression of this pest (Hutchinson et al. 2010). Despite the success of Bt technology for European corn borer management, resistance to Cry1F was recently identified in populations in Canada (Smith et al. 2019, Smith and Farhan 2023). If the resistance to Bt toxins in the European corn borer continues to expand, this may further increase the value of pyramided hybrids for this pest. Corn hybrids expressing Bt proteins also control at least 16 other pests (Reisig et al. 2021), including fall armyworm (Bilbo et al. 2020, Moscardini et al. 2020). In the southeastern United States, populations of fall armyworm increase later in the season due to the migratory behavior of the species, often leading to increased damage in late-planted corn (Pair et al. 1986). Additionally, excessive ear feeding by corn earworm or fall armyworm has the potential to introduce grain quality concerns in the form of fungi and mycotoxin contamination (Widstrom 1979, Ni et al. 2011). While the widespread use of Bt hybrids puts additional selection pressure on these technologies for corn earworm and other lepidopteran pests, Bt corn still has value to producers to prevent damage from a number of potential pests.

Among the 80% of respondents who planted Bt corn, the rate of planting non-Bt refuge was ~20% higher than reported in North Carolina in 2017 (Reisig 2017). Grower knowledge about which hybrids are non-Bt and the lack of availability of non-Bt hybrids have been reported as factors limiting the practice of planting non-Bt refuge (Reisig and

Kurtz 2018). In the present study, contingency tests indicated that growers' intentions to plant non-Bt refuge in both 2022 and 2023 was dependent on their knowledge of which hybrids were non-Bt, supporting this hypothesis. Promisingly, recent efforts have been made in South Carolina to specify which hybrids are non-Bt in statewide official variety trials. Moreover, in North Carolina, a few non-Bt hybrids were included in the 2022 and 2023 statewide official variety trials for the first time in many years. Notably, these entries were driven by North Carolina Cooperative Extension and not the seed companies. Because official variety trial data are used by growers to select corn hybrids, having non-Bt entries in formal trials might increase the number of growers choosing a non-Bt corn hybrid either as an alternative to a Bt hybrid or to comply with refuge requirements. The vast majority of growers (71%) indicated that they were familiar with which corn hybrids were non-Bt, which may account for some of the relatively high levels of refuge adoption observed. However, increasing grower knowledge on non-Bt refuge does not necessarily increase grower intention to plant refuge (Reisig 2017). External factors, such as farm size, income, grower age, and influence of ag industry, affect decision-making processes and can account for the limited adoption of non-Bt corn refuge.

Resistance management also plays a key role in prolonging Bt efficacy for all pests targeted by Bt corn, including fall armyworm and European corn borer. Practical resistance to Cry1F was recently identified in European corn borer populations in Canada (Smith et al. 2019, Smith and Farhan 2023), and fall armyworm evolved resistance to corn expressing a single toxin rapidly upon their introduction into corn production systems in Central and South America (Storer 2010, Farias et al. 2014, Chandrasena et al. 2017). In countries with high fall armyworm pressure and without access to Bt corn hybrids, as many as 12 insecticide applications may be needed for effective control of the pest (Blanco et al. 2016). As climate change favors expanded distribution (i.e., successful overwintering pushed northward and potential longer growing seasons) for fall armyworm in North America (Ramasamy et al. 2022), maintaining Bt efficacy through resistance management (i.e., refuge planting) could become more important for producers.

Insecticide Seed Treatments

Specific concerns about managing insect pests were more commonly reported as a selection factor for insecticidal seed treatments than for Bt corn hybrid selection. Growers also reported Extension recommendations and seed company offerings as common selection factors for using seed treatments. Of the growers who targeted specific pests with seed treatments, stink bugs were the most reported, followed by soil pests (i.e., wireworms, white grubs, rootworms), and billbugs. All of these pests or pest complexes are generally considered to be sporadic during early vegetative stages of field corn (Sappington et al. 2018, Bryant et al. 2023). The compatibility of a preventative insecticide seed treatment with an IPM program relies on two factors: 1) rescue treatments are not an option or are ineffective and 2) the pest is likely to cause economic injury (Douglas and Tooker 2015). Several species of soil pests often occur simultaneously, yet economic thresholds usually only apply to a single pest (Pedigo et al. 1986). Additionally, much of the injury that occurs because of feeding by soil pests may not be seen until it is too late to apply a rescue treatment, in addition to requiring extensive sampling to make a control decision. These two factors suggest that the use of insecticide seed treatments is compatible with IPM in fields with historically high densities of soil pests. It is important to note, that to determine changes in soil pest pressures over time, producers need to sample for soil pests during the off-season; however, to our knowledge, widespread and consistent sampling for early season pests of corn is rare in the southeastern United States.

Insecticidal seed treatments have also been shown to limit stink bug injury, but mainly at the highest commercial rates and only during early vegetative stages (Bryant and Taylor 2021, Reisig and Goldsworthy 2023). In contrast to soil pests, stink bugs can be managed effectively with rescue treatments, if sampling is conducted during early vegetative stages of corn when seed treatments would provide control. The main limiting factor in this case is sampling inefficiencies which can occur for early season populations of stink bugs, particularly in fields with high crop residue (Bryant et al. 2023). In addition to the potential for rescue treatments, corn is capable of recovering from early stink bug injury in many cases (Bryant et al. 2022). This suggests that insecticide seed treatments applied specifically to target stink bugs may be less compatible with IPM, in the absence of other soil pests.

Many growers reported selecting a seed treatment based on yield potential and cost, rather than a specific insect pest management concern. However, several studies in the southeastern United States have shown that neonicotinoid insecticide seed treatments do not increase corn yields in high-yielding fields (Del Pozo-Valdivia et al. 2018) and in the absence of insect pests (Wilde et al. 2007). Given this, emphasis should be placed on identifying the risk of soil and early season pest pressure in each field to decide on the need to use an insecticide seed treatment and at what rate. Some growers also reported that they did not feel like they had any option to select a rate and type of insecticide seed treatment, which may limit the ability to even make this type of management decision. A small percentage of growers (1%) indicated that they did not use seed treatments, which speaks to the inherent error in these self-reported data, as untreated seed are not commercially available. These growers were either unaware of what is applied to their seed, or not informed by seed dealers.

The widespread use of neonicotinoid seed treatments has presented a number of different environmental and regulatory concerns. In some states in the United States, parts of Canada, and in the European Union, their use has been restricted or banned entirely (Stokstad 2018). These bans are due in part to the long-term environmental fate (Thompson et al. 2020) of these materials and their potential impact on pollinator health (Lu et al. 2020). Overuse of these materials could potentially lead to regulatory action on their use and/or the development of pesticide resistance in key target pests. Therefore, more latitude should be provided to producers to make field-level decisions on seed treatment use to protect both their ability to use them and their efficacy for situations where the risk of soil and early season pests is high.

Stink Bug Management

A large percentage of growers indicated that they scouted for stink bugs either during early or late vegetative stages of field corn and used economic thresholds when applying insecticides. Only a small percentage of growers indicated that they applied a fungicide tank mixed with a pyrethroid at tasseling or used a calendar-based application. Many growers also indicated that they do not apply any insecticides for stink bugs in corn. This result speaks to the sporadic nature of stink bugs as pests of corn. In many cases in the southeastern United States, particularly in early planted corn, stink bugs do not reach damaging levels at any point in the season (Tillman 2010a, Bryant et al. 2023). Limited research has been done on the dynamics of stink bug populations in more northern areas, but stink bugs generally do not reach damaging levels in seedling corn stages in these areas (Koch and Pahs 2015). The sporadic nature of stink bugs as a pest and the risk of damaging populations aligning with susceptible stages of corn phenology emphasizes the importance of adequate sampling strategies (Babu et al. 2018). Sampling and economic thresholds were used by many of the growers surveyed in our study. Studies have shown field edges, where higher densities of stink bugs often occur, should be a primary target for scouting (Tillman 2010a, Reisig et al. 2013, Bryant et al. 2023), particularly along the interface of corn and a small grain crop. More research is needed to identify additional landscape-level risk factors leading to damaging stink bug levels in corn.

Data on additional IPM strategies for stink bugs in corn are either limited or the practices investigated are challenging to implement. Both predators and parasitoids of stink bug eggs have been found to be prevalent in corn in the southeastern United States (Tillman 2010b, Tillman 2011). These beneficial insects are also highly susceptible to the insecticides that are frequently applied to manage stink bugs (Koppel et al. 2011). The susceptibility of natural enemies to insecticides further emphasizes the need for only applying insecticides at economic thresholds, which many growers reported doing in the current study. The surrounding landscape also has the potential to influence both the populations of biological control agents and stink bugs themselves in corn (Ademokoya et al. 2022). Some growers reportedly manipulated the surrounding landscape to limit the risk of damaging populations of stink bugs, as stink bugs are highly mobile insects that can move between host crops during the season (Tillman et al. 2009, Pilkay et al. 2015). Wheat is an important early season host that often serves as a source of damaging populations in corn (Reay-Jones 2010, Tillman 2011, Reisig et al. 2013). Limiting the interface of corn and wheat is a cultural practice that fits within an IPM program for stink bugs and is implemented by some growers in the southeastern United States. Host plant resistance has not been extensively explored for stink bugs in corn. An on-farm sampling study in Georgia reported no difference in stink bug densities among several corn hybrids (Tillman 2010a), but several other studies explicitly studying host plant resistance have shown that corn hybrid can have an impact on feeding preference and development of stink bugs (Ni et al. 2007, Bueno et al. 2021). While timely applications of insecticides can be highly effective for managing stink bugs, research is needed to develop IPM strategies to limit the impact of this pest group on corn production and reduce chemical inputs.

IPM Implementation and Sources of Information

The majority of respondents indicated that they understood the concepts of IPM well and implemented IPM in field corn. This is further evidenced by the high percentage of grower respondents using scouting and economic threshold levels for stink bug management, both of which are fundamental components of IPM programs. The percentage of respondents implementing IPM in corn contrasts with previous reports of limited adoption or producer hesitancy (Lane et al. 2023). It is important to note that this survey included university Extension personnel and consultants which likely shifted the results in favor of higher IPM adoption overall. Even still, when isolating grower responses, 50% reported using IPM in corn production. A high percentage of Extension personnel and consultants using IPM likely results in a trickle-down effect, resulting in more growers understanding and implementing these practices. Furthermore, recent research on stink bugs in corn in the region has resulted in a big push from Extension personnel, including the authors, resulting in more IPM adoption. While it is promising to see these rates of IPM knowledge and implementation amongst growers, more work is needed to increase the adoption of a more holistic approach to IPM that would consider, for instance, multiple pest species simultaneously or optimize the use of preventative management strategies such as seed treatments and Bt hybrids only for high-risk situations.

In the present study, 24% of growers indicated that they received management information directly from their farming community, a similar rate to previous reports from the United States and Brazil (Hahn 1976, Turpin and Maxwell 1976, Pezzini et al. 2023). In addition to their farming community, many growers reported that their primary source of management information was ag industry personnel (i.e., seed or chemical dealers). The reliance of some growers on recommendations from ag industry representatives was also evident in responses to questions on how growers selected both Bt hybrids and insecticide seed treatments (Figs. 3a and 4a). In both cases, a larger percentage of growers reported using a Bt hybrid or insecticide seed treatment that was suggested by their seed dealer rather than for specific pest management concerns. Given the large percentage of growers reporting corn earworm as one of the most common pests, it is interesting that control efficacy was not a bigger factor in selecting a Bt hybrid. The reliance on ag industry also may play a role in the adoption of resistance management strategies. Extension work focused directly on industry, as opposed to on growers, may therefore have a greater potential for improving adoption rates.

The question remains to what extent growers can truly implement an IPM approach in corn given the widespread use of modern Bt hybrids and insecticide seed treatments. The compatibility of these strategies with IPM ultimately depends on the frequency and density of target pests and the risk of those pests causing economic injury in the absence of these management strategies. If it is assumed that all of these pests are present ubiquitously, the use of Bt and seed treatments can significantly lower use of broad-spectrum insecticides, reducing both environmental and non-target impacts. Unfortunately, many of the pests targeted by these technologies either have limited capacity for economic injury or occur sporadically in the southeastern United States. Additionally, the results of the present survey suggest that the current use of these management strategies is more influenced by external factors unrelated to pest management. For growers to move up the IPM continuum (i.e., from chemically intensive to biologically based management practices), a great deal more understanding about the ecology of pest species and the ability to effectively predict infestation risk and subsequent damage is required. Predictive power would allow growers to more selectively implement prophylactic management without risking losses from insect pests. In addition to increased predictive power for insect pests of corn, incentivizing growers to implement IPM in a way that mitigates the perceived short-term financial risk may allow for more growers to start adopting these strategies. Given that many growers rely on their community for pest management information, the rate of IPM adoption could potentially increase exponentially with each new producer using IPM.

Conclusion

This study provides a current assessment of the perceptions of insect pests in field corn in the southeastern United States. Additionally, the reasoning for implementing various IPM or IRM strategies were shown. The results of this survey illustrates the importance of both ag industry and the farming community in grower decision-making processes, emphasizing the role of widespread Extension work and ag industry in IPM and IRM. Finally, we showed that a large portion of growers felt confident in their understanding of IPM and its use in corn. Based on the results of this survey, more work is needed to emphasize the importance of non-Bt refuge plantings. The combination of pesticide seed treatments and transgenic insect resistance or herbicide tolerance into an easily adoptable crop protection package has been referred to as ‘Shock and Awe Pest Management" (Hurley and Sun 2019). The widespread adoption of this approach appears to be incompatible with IPM. Given concerns with resistance among pests to transgenic traits and seed treatments, a greater emphasis on basic principles of IPM will likely be warranted to maintain the sustainability of corn production in the future.

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Figure 6.1 Proportion of survey responses to the questions; A) "Do you consider insects to be a yield limiting factor in corn?" and B) "What is the most common insect pest you manage in corn?". Responses are categorized by the respondents self-identified classification as a grower, ag industry representative, university personnel, or a consultant. Proportions are based on a total of 136 grower, 35 ag industry, 18 university, and 12 consultant respondents for both questions.

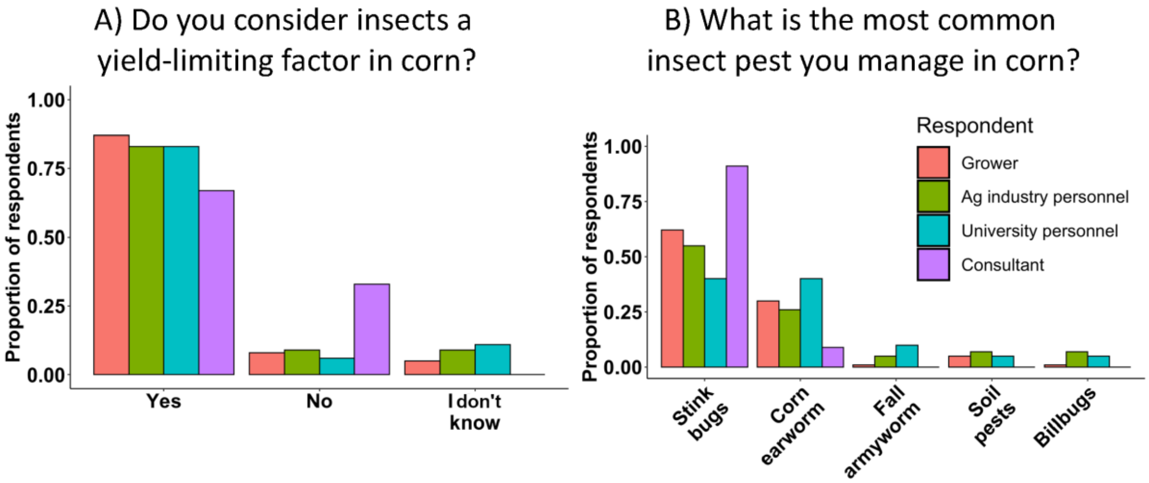


Figure 6.2 Proportion of grower responses to the questions; A) "How do you decide on rate and type of insecticide seed treatments?" and B) "What pests do you hope to target with insecticide seed treatments?". Only those who selected "specific insect concerns" in question A provided an answer for question B. Proportions for question A are based on a total of 133 grower respondents. Proportion for question B is based on 37 growers who selected "specific insect concerns" in question A. For both questions, respondents were able to select all applicable answers.

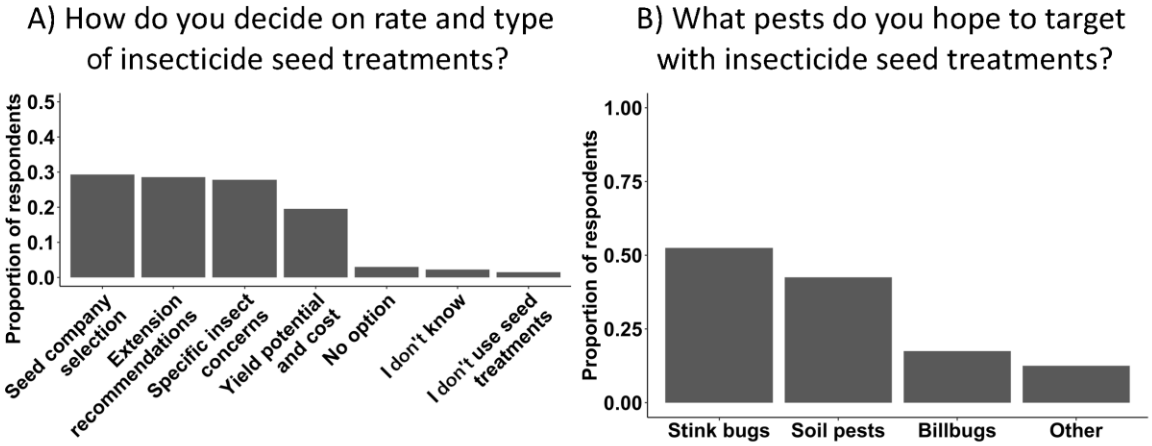


Fig 6.3 Proportion of grower responses to the questions; A) “How you select what hybrid and Bt traits to plant?”, B) “Are you familiar with which hybrids are non-Bt and can be planted as refuge?”, C) “Will you plant non-Bt refuge this year? (2022)”, and D) “Will you plant non-Bt refuge next year? (2023)”. For question A, respondents were able to select all applicable answers. Proportions are based on a total of A) 136, B) 121, C) 121, and D) 122 grower respondents.

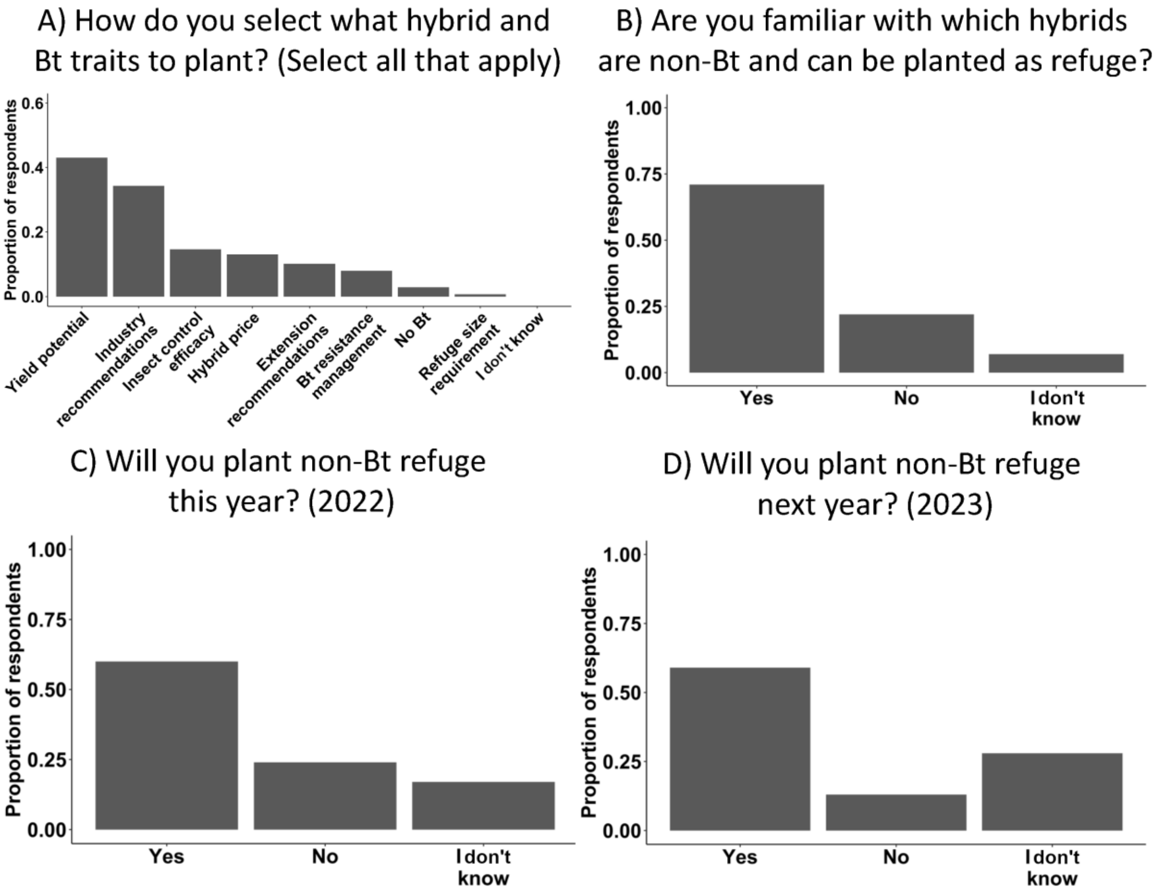


Fig 6.4. Proportion of grower responses to questions; A) “At what point in the growing season do you think stink bugs pose the most risk to corn yield?” and B) “What management strategies do you use for stink bugs?”. For question B, respondents were able to select all applicable strategies. Proportions are based on a total of A) 122 and B) 137 respondents.

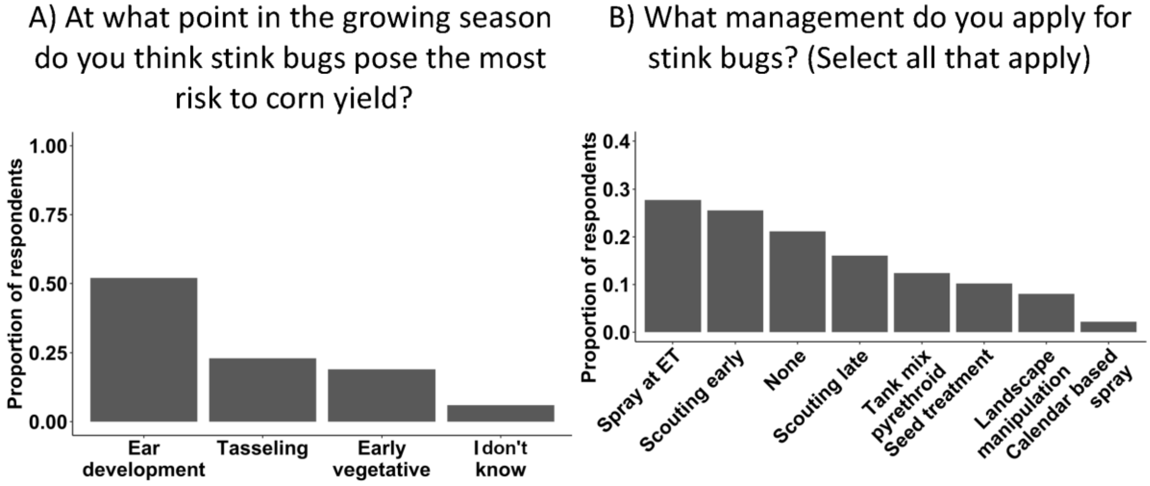
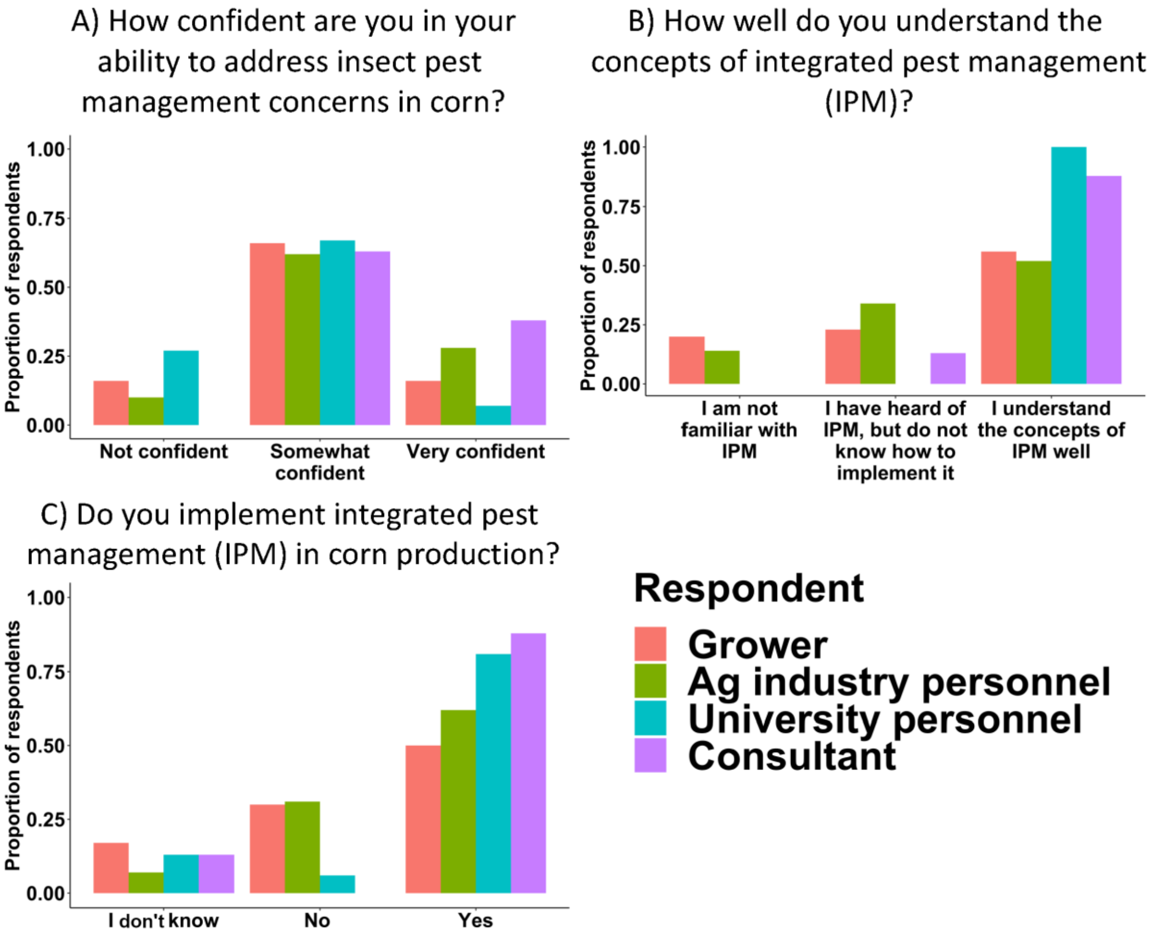


Fig 6.5. Proportion of survey responses to questions; A) “How confident are you in your ability to address insect pest management concerns in corn?”, B) “How well do you understand the concepts of integrated pest management (IPM)?”, and C) “Do you implement integrated pest management (IPM) in corn production?”. Responses are categorized by the respondents self-identified classification as a grower, ag industry representative, university personnel, or a consultant. Proportions are based on a total of 119 grower, 29 ag industry, 15 university, and 8 consultant respondents for question A; 121 grower, 29 ag industry, 16 university, and 8 consultant respondents for question B; and 119 grower, 29 ag industry, 16 university, and 8 consultant respondents for question C.


A) How confident are you in your ability to address insect pest management concerns in corn?
Proportion of respondents
1.00
0.75
0.50
0.25
0.00
Not confident
Somewhat confident
Very confident
B) How well do you understand the concepts of integrated pest management (IPM)?
Proportion of respondents
1.00
0.75
0.50
0.25
0.00
I am not familiar with IPM
I have heard of IPM, but do not know how to implement it
I understand the concepts of IPM well
C) Do you implement integrated pest management (IPM) in corn production?
Proportion of respondents
1.00
0.75
0.50
0.25
0.00
I don't know
No
Yes
Respondent
Grower
Ag industry personnel
University personnel
Consultant

CHAPTER SEVEN

CONCLUSIONS AND FUTURE WORK

Field corn is one of the most important commodities produced in the United States. There are a number of important insect pests of field corn which either directly impact yield or are relevant on a landscape level. A complex of stink bugs, including the brown stink bug, *Euschistus servus*, and southern green stink bug, *Nezara viridula*, are direct pests of field corn and can cause economic injury at various points throughout the season. Understanding the ecology of these insects and their association with other variables, like plant phenology or other pests, can help improve sampling efforts and implementation of integrated pest management. Corn earworm, *Helicoverpa zea*, is not a direct yield limiting pest in corn in most cases, but it can be a major pest of cotton which is often the next major cultivated host after corn in the southeastern United States. Because the majority of corn hybrids and cotton varieties express the same or similar Bt toxins, and resistance to Bt is widespread in corn earworm, understanding the ecology of this pest in field corn could provide valuable insight on the successful implementation of resistance management. Continuing to monitor the evolution of Bt resistance in a field setting is also a critical element of informing resistance models used by regulatory agencies to make policy on transgenic technology. Additionally, understanding grower perception about each of these pests, how they manage them, and if they use insecticide resistance management or integrated pest management can help improve research and Extension efforts. The goals of our studies were to 1) describe the spatial patterns of these major insects in field corn, 2) identify spatial associations of these pests with their injury, plant phenology, or each other, 3) determine if there is any competitive interaction between these major pests, 4) continue to monitor the sublethal impacts of various Bt toxins on corn earworm in the field, and 5) assess corn growers’ perceptions of major pests and implementation of insecticide resistance and integrated pest management.

Our research identified patterns of aggregation amongst two important species of stink bug and corn earworm in field corn. Stink bugs have often been reported to be aggregated on field margins which was supported in some fields in our study, but these patterns varied with the density of insects and the surrounding habitat. We also identified patterns of aggregation in populations of corn earworm larvae. The spatial patterns of these pests were commonly associated with differences in plant phenology naturally occurring throughout a field. This effect was particularly apparent for corn earworm. We also found a negative spatial association between stink bug injury and corn earworm larvae in several fields. After identifying this relationship between the two pests, we conducted a follow up study to further examine the interaction. In a controlled trial, we found that increasing stink bug density and ear injury resulted in reduced corn earworm oviposition and larval success consistently across four trials in two study years. This was the first study to illustrate this interaction between the two most important pests of field corn in the southeastern United States. Understanding the spatial patterns and ecological interactions of these major pests plays an important role in efficient sampling, management, and potentially insecticide resistance management.

While the results of these spatial and ecological studies can aid in effective sampling for stink bugs, further study is needed to effectively predict population densities of stink bugs in space and time. Using the data collected for our sampling study and additional data from fields with a range of surrounding habitats, a follow up analysis could be done to understand what landscape level factors drive populations. Using USDA CropScape, climate, and landscape fragmentation data sets, we could analyze potential factors that may influence the risk of damaging densities of stink bugs occurring in corn. Additionally, understanding the physiological response of corn plants because of stink bug feeding may provide insight on the mechanisms of the competitive interaction between stink bugs and corn earworm described here. Such studies may include an assessment of herbivore-induced plant volatiles emitted by corn after stink bug feeding, choice and no-choice assays using corn earworm moths and healthy and stink bug injured corn ears, and an assessment of biological control rates for corn earworm on deformed and healthy corn ears.

Development of resistance to Bt traits is a major threat to the sustainability of corn and cotton production. As part of a long-term effort monitoring the sublethal impacts of Bt toxins, we collected corn earworm pupae from various Bt corn hybrids and non-Bt near isolines. Feeding on corn expressing Cry1F+Cry1Ab or Cry1A.105+Cry2Ab2 resulted in a reduction in pupal weight overall, but this effect varied by trial, and, when combined with data from 2014-2019, the percent reduction in pupal weight has significantly reduced over time. Only a small number of pupae were recovered from corn hybrids expressing Vip3A20 hybrids across all these studies, which emphasizes the importance of resistance management strategies to preserve this technology for as long as possible, given that resistance to all other Bt toxins is widespread in corn earworm. Continued efforts to monitor resistance development over time can help to keep producers informed when control failures occur, which may require supplemental management, and can also inform resistance modeling efforts by regulatory agencies.

The final study we conducted was a large-scale survey of corn stakeholders in both North and South Carolina. Through this effort we were able to identify what insect pests producers viewed as the most important, how they implemented management for these pests, including modern technologies like insecticidal seed treatments and Bt hybrids, the rate of insecticide resistance management adoption, and understanding and adoption of IPM strategies. One important finding in these surveys was that 59% of growers indicated that they planned to plant non-Bt corn refuge. While this represented a slight increase from previous reports, survey data are associated with some inherent bias in responses, and this rate of refuge adoption is still relatively low for effective resistance management. Many producers also indicated that they were familiar with IPM concepts and implemented them in corn production. A follow-up survey to better understand what growers classify as IPM and the extent to which it is truly implemented would aid in our understanding of how these concepts can be used in corn. Continued efforts to understand the challenges or perceived barriers to non-Bt refuge planting would also help to guide research and Extension efforts to overcome those challenges.

These studies provide valuable insight on the ecology and management of several major pests of field corn in the southeastern United States. Given the relative importance of stink bugs and corn earworm to corn producers and their relevance in a number of other crops in the agricultural landscape, these ecological studies can facilitate more effective implementation of integrated pest management and insecticide resistance management in the future.

APPENDICES

Appendix A

List of Publications

The following chapters have been previously published in refereed journals. These works have been reproduced exactly as they appear in print with the permission of the publisher, with the exception of formatting adjustments to conform to the dissertation guidelines of Clemson University.

Chapter II

Bryant TB, Greene JK, Reay-Jones FPF. 2023. Within-field spatial patterns of *Euschistus servus* and *Nezara viridula* (Hemiptera: Pentatomidae) in field corn. Environmental Entomology. 52: 709-721.

Chapter III

Bryant TB, Greene JK, Reay-Jones FPF. 2023. Within-field spatial patterns of *Helicoverpa zea* (Lepidoptera: Noctuidae) and spatial association with stink bugs and their injury in field corn. Journal of Economic Entomology. 116: 1649-1661.

Chapter VI

Bryant TB, Greene JK, Reisig DD, Reay-Jones FPF. 2024. Grower perceptions and adoption of IPM and non-Bt refuge in field corn: a survey in North and South Carolina. Journal of Integrated Pest Management. 15:1-12.

Appendix B

Voucher specimens for all major study species (i.e. *Euschistus servus*, *Nezara viridula*, *Helicoverpa zea*) deposited in the Clemson University Arthropod Collection in May 2024.

<u>Appendix C</u>

Supplementary data for chapter six (S.6.1). Full survey of corn stakeholders.

1. I am a (select only one):
    a. Grower
    b. Extension agent
    c. Crop consultant
    d. University researcher
    e. Ag industry personnel
    f. Other _______
2. Where do you **<u>primarily</u>** get management information and recommendations?
    a. Directly from Extension personnel
    b. University Extension publications (variety trials, Extension websites, etc.)
    c. Farming community (neighbors, grower associations, trade journals, etc.)
    d. Ag industry personnel
3. Do you consider insects to be a yield-limiting factor in corn?
    a. Yes
    b. No
    c. I don't know
4. What are the most common insect pests you manage in corn? (select only one)
    a. Corn earworm
    b. Fall armyworm
    c. Stink bugs
    d. Soil pests (white grubs, wireworms, rootworms)
    e. Billbugs
    f. Other ______
5. How do you decide on rate and type of insecticidal seed treatments? (select all that apply)
    a. Specific insect pest management concerns
    b. Extension recommendations
    c. I use whatever the seed company applies to my chosen hybrid
    d. Yield potential and cost
    e. I don't know
    f. I don't use insecticidal seed treatments in corn
    g. I don't feel like I have an option for seed treatments
6. If option "a" was selected above, what pests do you hope to target with insecticidal seed treatments? (select all that apply)
    a. Stink bugs
    b. Billbugs
    c. Soil pests (wireworm, white grubs, rootworms)
    d. Other
    e. Did not select 'a'
7. Do you plant Bt corn?

    a. Yes
    b. No
    c. I don’t know
8. How do you select what hybrid and Bt traits to plant? (select all that apply)
    a. Insect control efficacy
    b. Smaller non-Bt refuge size (i.e., 20% for multiple Bt toxins vs. 50% non-Bt for single Bt toxin)
    c. Bt resistance management concerns
    d. Extension personnel recommendations
    e. Seed industry recommendations
    f. Hybrid price
    g. Yield potential
    h. I don’t know
    i. I don’t plant Bt corn
9. Are you familiar with which hybrids are non-Bt and can be planted as refuge?
    a. Yes
    b. No
    c. I don’t know
10. Will you plant non-Bt (refuge) corn this year (2022)?
    a. Yes, I plant non-Bt refuge
    b. No
    c. I don’t know
11. Will you be planting non-Bt (refuge) corn next year (2023)?
    a. Yes
    b. No
    c. I don’t know
12. At what point in the growing season do you think stink bugs pose the most risk to corn yield?
    a. Early vegetative
    b. Tasseling
    c. Ear development
    d. I don’t know
13. What management strategies do you use for stink bugs in corn? (select all that apply)
    a. Selecting a seed treatment insecticide rate directed at stink bug control
    b. Scouting during early vegetative corn stages
    c. Applying insecticide based on economic thresholds
    d. Managing surrounding landscape for potential stink bug hosts that serve as a source for infestations in corn
    e. Scouting during late vegetative and reproductive stages of corn
    f. Calendar or growth stage based insecticide applications
    g. Tank mix pyrethroid with a calendar based fungicide application
    h. I do not spray corn for stink bugs

14. How confident are you in your ability to address insect pest management concerns in corn year to year?
    a. Very confident
    b. Somewhat confident
    c. Not confident
15. How well do you understand the concepts of integrated pest management (IPM)?
    a. I understand the concepts of IPM well
    b. I have heard of IPM, but don’t know how to implement it
    c. I am not familiar with IPM
16. Do you implement integrated pest management (IPM) in corn production?
    a. Yes
    b. No
    c. I don’t know