Thresholds and Critical Growth Stages for Brown Stink Bug, *Euschistus servus* (Say), Management in Field Corn, *Zea mays*

Tim Bryant

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Sally Taylor
Aaron Gross
Roger Schürch

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Scientific Abstract

The brown stink bug, *Euchistus servus* (Say), is a polyphagous pest of multiple cultivated hosts in Virginia. It recently emerged as a potentially devastating pest of maize, *Zea mays* L. (Poaceae), in eastern Virginia where small grain (e.g., wheat, rye) production is common. In order to develop an integrated pest management (IPM) plan, research is needed to determine if brown stink bug feeding causes economic damage in maize at different growth stages and levels of infestations. Experiments were conducted in 2018 and 2019 to determine: 1) effectiveness of seed applied and in-furrow chemical control methods, 2) infestation levels in seedling and reproductive growth stages that cause economic damage, and 3) the effect, if any, of *E. servus* feeding on grain quality and mycotoxin contamination. Results of these experiments demonstrated that infestation levels (i.e., number of bugs divided by number of plants) of 11% and 15% in seedling and late vegetative maize, respectively, can cause measurable yield reduction at harvest. Seedling damage from *E. servus* is significantly mitigated by neonicotinoid seed treatments which are applied to nearly all commercial maize seed. Further, experiments indicated that maize quality can be affected by *E. servus* feeding in late reproductive stages of development. Results of these experiments will help to inform Virginia maize producers of the need to manage *E. servus* throughout the growing season.

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General Audience Abstract

The brown stink bug, *Euschistus servus*, has emerged as a potential economic pest of maize (commonly referred to as "corn" or "field corn") in Virginia following reduced broad-spectrum insecticide use and increased adoption of no-tillage or reduced-tillage crop production systems. Stink bug infestations in maize frequently occur at two times in the growing season: following cover crop termination and following small grain harvest. We need to determine the effects of brown stink bug infestations on maize yield and quality, as well as the effectiveness of chemical management options, to help minimize yield losses and input costs for maize producers in our region. Experiments were conducted to determine: 1) the control provided by insecticidal seed treatments and in-furrow insecticide applications, 2) the level of brown stink bug infestations that cause economic damage at different growth stages of maize, and 3) the effect of brown stink bug feeding and a *Fusarium* fungal pathogen on grain yield and quality. Results of these experiments determined economic injury levels in seedling corn and late vegetative stages. Additionally, we found that universally applied neonicotinoid seed treatments mitigated early damage. Further, stink bug feeding through reproductive stages of development can reduce grain quality. Our results will help Virginia maize producers to make informed pest management decisions throughout the season.

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Chapter 1: Review of Literature

Euschistus servus Biology and Ecology

Several species of stink bugs (Hemiptera: Pentatomidae) are economic pests of cultivated crops in the southeastern U.S. The brown stink bug, *Euchistus servus* (Say), green stink bug, *Acrosternum hilare* (Say), southern green stink bug, *Nezara viridula* (L.), rice stink bug, *Oebalus pugnax* (Fab), and brown marmorated stink bug, *Halyomorpha halys* (stal), are all pests of agronomic crops (McPherson and McPherson, 2000). The two dominant economic pest species within the stink bug complex in Virginia and North Carolina are *E. servus* and *A. hilare* (Blinka 2008, Reisig 2011).

Euchistus servus is a true bug in the order Hemiptera and family Pentatomidae. It is distributed across much of North America and has two subspecies; *Euschistus servus servus* (Say) and *Euschsitus servus euschistoides* (Voltenhoven). *Euschistus servus euschistoides* is distributed across Canada and parts of the northern U.S. and *E. servus servus* occurs in the southeastern U.S. extending west to California (McPherson and McPherson 2000). *Euschistus servus servus* is the primary subspecies in southeastern Virginia.

Adult *E. servus* are long, brownish-yellow, shield-shaped insects with piercing and sucking mouthparts. Mature adults measure 10-15 mm long and 7-9 mm wide. *Euschistus servus* has five nymphal instars, which are colorful, displaying orange, yellow, and green. Nymphs lack wings and typically have a vertical patch of brown along the abdomen. (Munyaneza and McPherson 1994, Kahan and Davern 1999, McPherson and McPherson 2000).

Stink bugs overwinter as adults in woody field borders, crop residues, or human built structures and begin emerging in early April and persist in the landscape through late October (Munyaneza and McPherson 1994). *Euschistus servus* prefer open overwintering sites, such as field residue, as opposed to woody field borders (Jones and Sullivan 1981). *Euschistus servus* produce two generations a year in Virginia. They mate in early May and again in early August and oviposit approximately 14 eggs (Munyaneza & McPherson, 1994; Tillman, 2010). Typically, the first generation of *E. servus* oviposit in a host such as winter wheat or rye, which *E. servus* causes no economic damage to, and the second generation disperses to other host crops such as maize (Blinka 2008, Reisig 2011, Reisig et al. 2013, Ni et al. 2016).

Euschistus servus move through different cropping systems in the southeastern United States within a single year (Reisig et al. 2013, Tillman et al. 2014, Venugopal et al. 2014). It is common for cotton, peanut, wheat, soybeans, and maize to be planted in relatively small fields and in close proximity to each other in this region. All of these crops can serve as a host to stink bug pests. A 2010 study in Georgia determined the density of *E. servus* populations in maize, cotton, peanut, and soybeans season-long (Herbert and Toews 2011). Results indicated that *E. servus* populations peak in maize in late June or early July, and the final generation peaks in soybeans in mid-October (Herbert and Toews 2011). There is evidence that *E. servus* moves from winter wheat to maize before and during wheat harvest (Reisig, 2011; Tillman, 2011a), which supports the timing of population peaks in maize. Winter wheat is often grown prior to planting soybeans and it can serve as a early-season host for *E. servus* populations and provide crop residue for overwintering habitat. Thus, soybean following small grains and maize planted in nearby fields provide year-round feeding, oviposition, and overwintering material for *E. servus* populations.

Maize Physiology

The vegetative stages of maize are abbreviated as VE (emergence), V1-V12 (indicating the number of visible leaf collars on the plant), and VT (tasseling). The reproductive stages of maize are denoted R1-R6 and are defined as follows; R1, silks emerges from husks; R2, blister stage; R3, milk stage; R4, dough stage; R5, dent stage; and R6, black layer. *Euschistus servus* can infest and injure all vegetative and reproductive stages of maize, however, some stages are more critical for management because economic damage can occur (Townsend and Sedlacek 1986, Sedlacek and Townsend 1988, Apriyanto et al. 1989a, Apriyanto et al. 1989b, Ni et al. 2010).

Maize seed germinates at 30% moisture, and upon germination, a radicle (root), followed by the coleoptile (shoot) and enclosed plumule (first leaves and growing point) emerge from the seed, this is known as VE. The growth point of the plant is 2.5-3.8 cm below the surface of the soil and will be covered by the soil for another three to four weeks. The most common method of staging vegetative maize is to count collared leaves including the first rounded leaf. During the V1-V2 stages, the roots of the first node begin elongating. During the V3-V5 stages, leaf and ear shoots are being initiated and the second node of roots are elongating. Maize is not thought to be susceptible to economic damage from stink bug feeding between V6 until V(n)/VT when grain begins to develop (Annan and Bergman 1988). Current maize hybrids typically begin tasseling after the V15 stage, but some hybrids can reach 18 leaves or higher before tasseling begins. The VT stage, or tasseling, is the last stage of vegetative growth in which the plant has reached its terminal height, the last branch of the tassel is visible, and pollen shed has begun. Critical periods of vegetative growth, and their implication for further development are summarized in the Table 1 (Berglund et al. 1999, Kahan and Davern 1999).

Reproductive stages of maize begin with R1, or silking. This stage is when pollination occurs and stress during this period can cause large reductions in grain yield. The R2 stage is known as blister and is defined by starch accumulation in the kernels. In the milk (R3) stage, kernels expand due to cell expansion and starch accumulation. There is very minimal root growth following R3. During R4, or dough stage, kernels have accumulated 50% of their dry weight and are approximately 70% moisture. The R5, or dent stage, occurs when the milk or starch line becomes visible across kernels. The kernel is at about 55% moisture at this stage. The R6 stage is physiological maturity and all kernels have reached their maximum dry weight and 30-35% moisture (Berglund et al. 1999). To safely store grain, 15% moisture or below is recommended. Maize harvest is frequently delayed after physiological maturity to achieve a desired moisture level. After R6, maize will dry 0.25 - 0.75% of its moisture content daily (Berglund et al. 1999).

Euschistus servus damage to maize

Stink bugs are polyphagous insects and, in recent years, have emerged as major pests of many different cultivated crops including maize (McPherson and

McPherson 2000, Tillman 2010, Reisig et al. 2013). Due to an increase in the use of transgenic maize and cotton, broad-spectrum broadcast insecticide usage has decreased (Greene et al. 2001). Transgenic maize is genetically modified to express agriculturally desirable traits such as herbicide resistance and/or insecticidal toxins. *Bacillus thuringiensis* (Bt) is a naturally occurring soil bacterium that produces proteins (Cry and Cyt) that are toxic to specific groups of maize pests. Advancements in molecular biotechnology methods have engineered maize to produce these toxins, thereby selectively targeting insect pests. Since transgenic traits in maize have significantly reduced or eliminated feeding of many lepidopteran and coleopteran insect pests, insecticide applications that coincidentally controlled a secondary pests in maize have decreased (McPherson and McPherson 2000, Greene et al. 2001).

Conservation or reduced tillage practices (e.g., strip tillage, vertical tillage, no tillage) promote accumulation of crop residues that provide stink bugs with overwintering material and may foster suitable weed hosts (Panizzi 1997, Flessner and Cahoon 2018). All stages of *E. servus* can be found in maize (Herbert and Toews 2011). Females will oviposit at a certain time of year regardless of the host crop growth stage; thus, egg density in maize is highest in mid to late May and again in mid June, while peak adult abundance occurs in late-May to mid-June (Tillman, 2010). Higher numbers of stink bugs are found throughout the season in late-planted maize (Tillman, 2010). Early-planted maize may reach physiological maturity before the second generation of eggs are laid, which limits food supply for developing insects (Tillman, 2010). Maize variety is not known to have an impact on *E. servus* abundance (Tillman, 2010).

Early in vegetative growth stages, maize plants are susceptible to producing tillers when fed upon by *E. servus* (Townsend and Sedlacek 1986, Sedlacek and Townsend 1988, Apriyanto et al. 1989a, Apriyanto et al. 1989b). Tillers are multiple shoots extending from the same root system and can cause a decrease in mean extended leaf height, mean grain weight per ear, and delayed silking (Apriyanto et al., 1989a). Tillering, as well as other stink bug injury, has been found to increase with stink bug density and duration of infestation (Annan and Bergman 1988, Apriyanto et al. 1989a). A single day of infestation between VE and V4 may result in yield loss (Annan and Bergman 1988). Emergence (VE) through V2 are the most susceptible stages for damage from onespotted stink bug, *Euschistus variolarious*, although damage can still occur at V4 (Annan and Bergman 1988). Damage from feeding at V6 only persisted for three days and had no impact on yield (Annan and Bergman 1988). From this study, potential for injury from stink bug feeding is likely the highest at emergence and decreases through V6, beyond which no damage will likely occur until reproductive development.

Reduced and no tillage systems promote crop residues that can interfere with the ability of the press wheels on a planter to close the seed trench or slot. When the seed slot remains open, the growing point is accessible to above-ground insect feeding. Stunted growth, growth deformities, or plant mortality may occur when *E. servus* feeds directly on the growth point (Annan and Bergman 1988, Sappington et al. 2018).

Experiments in Georgia demonstrated that maize is most susceptible to economic damage from *E. servus* during early stages of ear formation and kernel development (i.e., VT and R1) (Ni et al. 2010). Grain yield was affected by growth stage at the time of infestation, but yield was not affected by the infestation level (Ni et al., 2010). The southern green stink bug was found to be most damaging at V15 (Negrón and Riley 1987). In Virginia and North Carolina, late vegetative and early reproductive stages of maize often coincide with harvest of wheat fields, and *E. servus* population peak in maize during this time (Blinka 2008, Reisig et al. 2013). *Euschistus servus* can produce a generation in wheat, and nymphs and adults can migrate from wheat into maize following wheat harvest, but this varies with weather, crop phenology, and other available hosts (Reisig 2011).

Scouting and Management of *E. servus* in maize

Currently, there are no research-based guidelines for *E. servus* management in Virginia maize. Existing studies on economic injury levels in different maize growth stages may not be appropriate for mid-Atlantic cropping systems where growing seasons are shorter and yield potential, in many cases, is lower. Further, changes in crop genetics and availability of commercial hybrids may affect how plants compensate for stink bug feeding injury.

Virginia Cooperative Extension recommends scouting 10-20 consecutive plants for adult and nymph *E. servus* in multiple locations in the field. In reproductive maize, *E. servus* frequently aggregate on the edges of fields, but they can infest an entire field. Effective scouting should include rows from the edge to 20 rows in (Reisig et al. 2013, Tillman et al. 2014, Venugopal et al. 2014, Flessner and

Cahoon 2018). When scouting whole plants, 28 plants need to be sampled between V2 and V6, and eight plants between R1 and R4 to reliably estimate the infestation level (Babu and Reisig 2018a). In reproductive stages of development (i.e., R1-R4), most *E. servus* are found in the area surrounding the developing ear and in the leaf collar, and these areas should be the focus of scouting efforts (Blinka 2008, Babu and Reisig 2018b). Adjacent habitat has a strong influence on the abundance of *E. servus* found in maize, with the highest populations found on the edges of field bordering wood lines or other suitable crop hosts (Venugopal et al. 2014).

Virginia Cooperative Extension currently recommends that producers in Virginia apply insecticides for *E. servus* when there are one or more bugs per four plants (25%) during ear formation and one or more bugs per two plants (50%) from pollen shed to blister stage (Flessner and Cahoon 2018). North Carolina Cooperative Extension recommends insecticide applications at levels of 11% between VE and V6, 26% between V14 and VT, and 43% between R1 and R4 (Reisig 2018). There are no established seedling maize thresholds and grain development thresholds need to be validated for this region. Further, insecticides that are frequently applied at planting may control of *E. servus* and, if so, modify action thresholds.

Seed-applied insecticides or in-furrow applications may provide protection for seedling maize during the critical early period for *E. servus* injury. Pyrethroids, organophosphates, and neonicotinoids have all been shown to cause mortality in stink bugs in different cropping systems and using different application methods (Reisig 2011, Kuhar et al. 2012, Little 2014). Research is needed to determine the efficacy and rates of these insecticides for *E. servus* control in maize.

Neonicotinoid insecticides, namely clothianidin and thiamethoxam, are applied to almost all commercially-available maize seed (Douglas and Tooker 2015). Neonicotinoids are widely used in agriculture because of the variety of formulations available, efficacy against piercing-sucking insects, and low mammalian toxicity (Tomizawa and Casida 2003). Neonicotinoids are xylem mobile, meaning that they are translocated within the plant tissue. Detectable levels of clothianidin have been measured in maize roots, stems, and leaves for up to 47 days after planting when a seed treatment is used at the maximum commercially available rate (clothianidin at 1.25 mg A.I./seed, Poncho®, BayerCrop Science, RTP, NC) (Alford and Krupke 2017). Clothianidin is labeled for use against the southern green stink bug in maize. While clothianidin is not labeled for *E. servus,* it may provide protection against early feeding injury (Van Duyn et al. 2005). Clothianidin (Belay®, Syngenta, Greensboro, NC, USA) is labeled for foliar stink bug control and is highly effective against some stink bug species in vegetables (Kuhar et al. 2012). Given that a relatively low proportion of the active ingredient in systemic seed treatments gets translocated into shoot tissue, and the relatively short window of activity (Alford and Krupke 2017), it is not known if this insecticide affects *E. servus* feeding in seedling maize.

Organophosphate insecticides (e.g., terbufos, acephate, and dicrotophos) are not labeled for *E. servus* control in maize; however, some products are labeled for an in-furrow application to control maize billbug, *Sphenophorus maidis*, a species that feeds on aboveground plant tissue. Granular organophosphate insecticides can be applied in the furrow, or seed slot, at the time of planting.

Multiple pyrethroid insecticides (e.g., bifenthrin, lamda-cyhalothrin, zeta-cypermethrin, esfenvalerate) are labeled for broadcast applications on maize for *E. servus* control. Pyrethroids are not systemic insecticides and thus, the active ingredient is not translocated inside the plant tissue. Insects must come into contact or feed on material coated with residues in order to be intoxicated. It is unlikely that an in-furrow application of a pyrethroid would provide control of an aboveground pest, but there is the potential for exposure of *E. servus* to soil residues. Currently, all insecticides labeled for foliar management of *E. servus* are pyrethroids. Foliar applications in maize are typically applied aerially and vary in effectiveness (Flessner and Cahoon 2018). A large scale field experiment in North Carolina determined that aerial pyrethroid applications were not effective for *E. servus* management, perhaps because of low residual active material or low penetration of the insecticide into lower levels of vegetation in fields (Reisig 2011). Pyrethroid insecticides, in general, do not persist in the environment (Mueller-Beilschmidt 1990). First generation pyrethroid insecticides were designed to be more photostable than natural pyrethrins. Second generation pyrethroids are used more commonly in agriculture today and their residues are generally not found in high environmental concentrations. Thus, aerial applications are not expected to remain toxic to stink bugs for multiple days and weeks following an application.

Euschsitus servus eggs are parasitized throughout its natural distribution. A 2005-2006 survey observed that four species of wasp parasitoids in the family Scelionidae parasitize approximately 50% of *E. servus* eggs (Koppel et al., 2009). A 2011 survey observed seven species parasitizing *E. servus* in maize (Tillman, 2011b). The most prominent species of parasitoid of stink bugs eggs in southeastern farmscapes is *Telenomus podisi* in the family Platygastridae (Koppel et al. 2009). Exercising constraint in applying broad-spectrum insecticides to control sub-economic *E. servus* infestations will conserve valuable biological control agents in maize and subsequent host crops (Koppel et al. 2009).

Fusarium **Rot in Maize**

Grain yield loss from *E. servus* has been documented in early reproductive stages of maize growth (Ni et al. 2010). There is also potential for reduced grain from *E. servus* feeding on developing kernels. Insect feeding on crop plants can promote development of opportunistic fungi (Avantaggiato et al. 2003, Ni et al. 2011, Opoku et al. 2019). Insect feeding has the potential to increase the level of fungal growth in maize that, in turn, increases levels of mycotoxin contamination (Opoku et al. 2019). Mycotoxins are byproducts of fungal disease agents that are toxic to humans and animals (i.e., livestock) (Weaver et al. 1978, Fink-Gremmels 1999, Zain 2011). Mycotoxin contamination in livestock feed can result in a loss of productivity, reduced weight gain, and immunosuppression (Zain 2011). The Food and Drug Administration (FDA) sets mycotoxin limits at a maximum of four parts per million (ppm) for dry milled products for human consumption, and 20 ppm and 30 ppm for swine and cattle, respectively (USFDA 2001).

Fusarium fungi are the most common disease agents in maize worldwide and are causal agents of ear rot in maize. *Fusarium verticullioides* (Sacc.), *Fusarium proliferatum* (Matsush.), and *Fusarium graminearum* (Schwabe) have been observed at frequencies of 90% or higher in maize (Bacon and Nelson 1994). *Fusarium spp.*

are present and thrive in many environmental conditions where maize is grown. Specifically, warm and humid areas, like southeastern Virginia, favor *Fusarium* growth almost ubiquitously (Haggag 2013, Stumpf et al. 2013). *Fusarium* are opportunistic fungi (i.e., they are almost always present at some level), and mechanical injury, such as insect feeding, can provide an opening for infestation into the plant (Miller 2001, Avantaggiato et al. 2003). Feeding by the brown marmorated stink bug, *Halyomorpha halys*, has been shown to increase the incidence of *Fusarium* rot and fumonisin contamination in maize (Opoku et al. 2019). The potential for *E. servus* and maize weevils to introduce fungal contamination has also been demonstrated (Ni et al. in 2011).

Rationale

In 2018, 47 million bushels of maize were produced in Virginia at a value of approximately 185 million dollars (2017 State Agriculture Overview, USDA). Maize has the greatest total value of any crop grown in the U.S. and is the dominant use of arable land in Virginia and worldwide. Due to the extensive nature of maize production, and the potential damage from *E. servus* throughout multiple growth stages of the plant, effective management of this pest will help to ensure farm profitability. When commodity values are low and profit margins narrow, it is essential to identify scenarios when it is profitable to apply insecticides. There are currently no thresholds for management of *E. servus* in Virginia maize and *E. servus* can be observed infesting maize fields from emergence to harvest. Most peer refereed research on this pest comes from Georgia, a different climate and longer growing season compared to the Mid-Atlantic. Current maize hybrids have more vigorous growth compared to those grown several decades ago and, since their introduction in 1996, neonicotinoid seed coatings usage has dramatically increased. Changing weather patterns, such as increased instances of intense rainfall in spring and autumn and tropical storms, are affecting the timeliness of planting and grain harvest. Fluctuations in commodity markets have favored on farm storage of maize and other grains. Virginia producers need a dynamic threshold for different growth stages of maize and knowledge of the effect of stink bug feeding on quality of harvested grain.

Tables and Figures

Table 1. Critical growth stages of maize and their impact on grain development, modified from Berglund et al., 1999.

Growth Stage	Days after emergence	Growth Event	Importance
V3	9-12	Seminal root system and ear shoots initiated	Seedling vigor seen, ears are established
V4 – V5	14-21	Ear shoot initiation is complete	Number of kernel rows determined
V6	21-25	Nodal root system established	Plants ability to take up moisture and nutrients established
V12 – V14	42-49	Number of ovules determined	Number of kernels per row determined
R1	63-68	Pollen Shed begins	Kernel fertilization, Support ear weight, kernel fill

References

Alford, A., and C. H. Krupke. 2017. Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS One. 12: 1–19.

Annan, I. B., and M. K. Bergman. 1988. Effects of the Onespotted Stink Bug (Hemiptera : Pentatomidae) on Growth and Yield of Corn. J. Econ. Entomol. 81: 649–653.

Apriyanto, D., J. D. Sedlacek, and L. H. Townsend. 1989a. Feeding Activity of Euschistus servus and E. variolarius (Heteroptera : Pentatomidae) and Damage to an Early Growth Stage of Corn. J. Kansas Entomol. Soc. 62: 392–399.

Apriyanto, D., L. H. Townsend, and J. D. Sedlacek. 1989b. Yield Reduction from Feeding by Euschistus servus and E . variolar ius (Heteroptera : Pentatomidae) on Stage V2 Field Corn. J. Econ. Entomol. 82: 445–448.

Avantaggiato, G., F. Quaranta, E. Desiderio, and A. Visconti. 2003. Fumonisin contamination of maize hybrids visibly damaged by Sesamia. J. Sci. Food Agric. 83: 13–18.

Babu, A., and D. D. Reisig. 2018a. Developing a sampling plan for brown stink bug (hemiptera: Pentatomidae) in field corn. J. Econ. Entomol. 111: 1915–1926.

Babu, A., and D. D. Reisig. 2018b. Within-plant distribution of adult brown stink bug (hemiptera: Pentatomidae) in corn and its implications on stink bug sampling and management in corn. J. Econ. Entomol. 111: 1927–1939.

Bacon, C. W., and P. E. Nelson. 1994. Fumonisin Production in Corn by Toxigenic Strains of Fusarium moniliforme and Fusarium proliferatum. J. Food Prot. 57:

514–521.

Berglund, D. R., G. J. Endres, and D. A. McWilliams. 1999. Corn Growth and Management Quick Guide. (https://www.ag.ndsu.edu/pubs/plantsci/crops/a1173.pdf).

Blinka, E. L. 2008. Biological and ecological studies on green stink bug, Acrosternum hilare, and brown stink bug, Euschistus servus (Hemiptera: Pentatomidae), in Eastern North Carolina cropping systems.

Douglas, M. R., and J. F. Tooker. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. Field crops. Environ. Sci. Technol. 49: 5088–5097.

Fink-Gremmels, J. 1999. Mycotoxins: Their implications for human and animal health. Vet. Q. 21: 115–120.

Flessner, M., and C. W. Cahoon. 2018. Pest Management Guide: Field Crops. Virginia Coop. Ext. (www.ext.vt.edu).

Greene, J. K., S. G. Turnipseed, M. J. Sullivan, and O. L. May. 2001. Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. J. Econ. Entomol. 94: 403–9.

Haggag, W. M. 2013. Corn Diseases and Management. J. Appl. Sci. Res. 9: 39–43.

Herbert, J. J., and M. D. Toews. 2011. Seasonal Abundance and Population Structure of Brown Stink Bug (Hemiptera: Pentatomidae) in Farmscapes Containing Corn, Cotton, Peanut, and Soybean. Ann. Entomol. Soc. Am. 104: 909–918.

Jones, W. A., and M. J. Sullivan. 1981. Overwintering habitats, spring emergence patterns, and winter mortality of some South Carolina Hemiptera. Environ. Entomol. 10: 409–414.

Kahan, A., and D. Davern. 1999. Handbook of Corn Insects. The Entomological Society of America.

Koppel, A. L., D. A. Herbert, T. P. Kuhar, and A. K. Kamminga. 2009. Survey of Stink Bug (Hemiptera: Pentatomidae) Egg Parasitoids in Wheat, Soybean, and Vegetable Crops in Southeast Virginia. Environ. Entomol. 38: 375–379.

Kuhar, T., H. Doughty, K. Kamminga, C. Philips, J. Aigner, A. Wallingford, A. Wimer, L. Lilliston, L. Nottingham, A. Lohr, E. Fread, J. Jenrette. 2012. Performance of Insecticides on Brown Marmorated Stink Bug on Vegetables. (www.ext.vt.edu).

Little, B. A. 2014. Efficacy and Non-Lethal Effects of Clothianidin for the Management of Stink Bugs (Hemiptera: Pentatomidae) in Cotton.

McPherson, J. E., and R. M. McPherson. 2000. Stink Bugs of Economic Importance in America North of Mexico. CRC Press.

Miller, J. D. 2001. Factors that affect the occurrence of fumonisin. Environ. Health Perspect. 109: 321–324.

Mueller-Beilschmidt, D. 1990. Toxicology and environmental fate of synthetic pyrethroids. J. Pestic. Reform. 10: 32–37.

Munyaneza, J., and J. E. McPherson. 1994. Comparative study of life histories, lab rearing, and immature stages of Euschistus servus and Euschistus variolarius (Hemiptera: Pentatomidae). Gt. Lakes Entomol. 26: 263–275.

Negrón, J. F., and T. J. Riley. 1987. Southern green stink bug, Nezara viridula

(Heteroptera: Pentatomidae), feeding in corn. J. Econ. Entomol. 80: 666–669.

Ni, X., T. E. Cottrell, M. D. Toews, P. G. Tillman, and G. D. Buntin. 2016. Diurnal Activities of the Brown Stink Bug (Hemiptera : Pentatomidae) in and near Tasseling Corn Fields. J. Entomol. Sci. 51: 226–237.

Ni, X., K. Da, G. D. Buntin, T. E. Cottrell, P. G. Tillman, D. M. Olson, R. Powell, R. D. Lee, J. P. Wilson, and B. T. Scully. 2010. Impact of Brown Stink Bug (Heteroptera: Pentatomidae) Feeding on Corn Grain Yield Components and Quality. J. Econ. Entomol. 103: 2072–2079.

Ni, X., J. P. Wilson, G. David Buntin, B. Guo, M. D. Krakowsky, R. Dewey Lee, T. E. Cottrell, B. T. Scully, A. Huffaker, and E. A. Schmelz. 2011. Spatial patterns of aflatoxin levels in relation to ear-feeding insect damage in pre-harvest corn. Toxins (Basel). 3: 920–931.

Opoku, J., N. M. Kleczewski, K. A. Hamby, D. A. Herbert, S. Malone, and H. L. Mehl. 2019. Relationship between invasive brown marmorated stink bug (halyomorpha halys) and fumonisin contamination of field corn in the Mid Atlantic U.S. Plant Dis. 103: 1189–1195.

Panizzi, A. R. 1997. Wild Hosts of Pentatomids: Ecological Significance and Role in Their Pest Status on Crops. Annu. Rev. Entomol. 42: 99–122.

Reisig, D. D. 2011. Insecticidal management and movement of the brown stink bug, Euschistus servus, in corn. J. Insect Sci. 11: 168.

Reisig, D. D. 2018. New Stink Bug Thresholds in Corn. NC State Ext. (go.ncsu.edu/readext?522534).

Reisig, D. D., M. Roe, and A. Dhammi. 2013. Dispersal Pattern and Dispersion of

Adult and Nymph Stink Bugs (Hemiptera: Pentatomidae) in Wheat and Corn. Environ. Entomol. 42: 1184–1192.

Sappington, T., L. Hesler, K. C. Allen, R. Luttrell, and S. Papiernik. 2018. Prevalence of Sporadic Insect Pests of Seedling Corn and Factors Affecting Risk of Infestation. J. Integr. Pest Manag. 9: 1–27.

Sedlacek, J. D., and L. H. Townsend. 1988. Impact of Euschistus servus and E. variolarius (Heteroptera : Pentatomidae) Feeding on Early Growth Stages of Corn. J. Econ. Entomol. 81: 840–844.

Stumpf, R., J. dos Santos, L. B. Gomes, C. N. Silva, D. J. Tessmann, F. D. Ferreira, M. Machinski, and E. M. Del Ponte. 2013. Fusarium species and fumonisins associated with maize kernels produced in Rio Grande do Sul State for the 2008/09 and 2009/10 growing seasons. Brazilian J. Microbiol. 44: 89–95.

Tillman, P. G. 2010. Composition and Abundance of Stink Bugs (Heteroptera: Pentatomidae) in Corn. Environ. Entomol. 39: 1765–1774.

Tillman, P. G. 2011a. Influence of Corn on Stink Bugs (Heteroptera: Pentatomidae) in Subsequent Crops. Environ. Entomol. 40: 1159–1176.

Tillman, P. G. 2011b. Natural Biological Control of Stink Bug (Heteroptera: Pentatomidae) Eggs in Corn, Peanut, and Cotton Farmscapes in Georgia. Environ. Entomol. 40: 303–314.

Tillman, P. G., T. E. Cottrell, R. F. Mizell, and E. Kramer. 2014. Effect of field edges on dispersal and distribution of colonizing stink bugs across farmscapes of the Southeast USA. Bull. Entomol. Res. 104: 56–64.

Tomizawa, M., and J. E. Casida. 2003. Selective Toxicity of Neonicitinoids

Attributable to Specificity of Insect and Mammalian Nicotinic Receptors. Annu. Rev. Entomol. 48: 339–364.

Townsend, L. H., and J. D. Sedlacek. 1986. Damage to corn caused by Euschistus servus, E. variolarius, and Acrosternum hilare (Heteroptera: Pentatomidae) under greenhouse conditions. J. Econ. Entomol. 79: 1254–1258.

USFDA. 2001. Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds. (http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulaoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/uc109231.htm)

Van Duyn, J. W., R. W. Heiniger, S. R. Koenning, A. C. York, and S. J. Toth. 2005. Crop Profile for Field Corn in North Carolina. Crop Profile/PMSP database, USDA NIFA.

Venugopal, P. D., P. L. Coffey, G. P. Dively, and W. O. Lamp. 2014. Adjacent habitat influence on stink bug (Hemiptera: Pentatomidae) densities and the associated damage at field corn and soybean edges. PLoS One. 9.

Weaver, G. A., H. J. Kurtz, C. J. Mirocha, F. Y. Bates, J. C. Behrens, T. S. Robinson, and W. F. Gipp. 1978. Mycotoxin-induced abortions in swine. Can. Vet. J. 19: 72–74.

Zain, M. E. 2011. Impact of mycotoxins on humans and animals. J. Saudi Chem. Soc.

Chapter 2: Efficacy of seed-applied and in-furrow insecticides for early season control of *E. servus*.

Introduction:

Several species of stink bugs (Hemiptera: Pentatomidae) are economic pests of a wide range of cultivated crops grown in southeastern U.S. farmscapes. The brown stink bug, *Euchistus servus* (Say), green stink bug, *Acrosternum hilare* (Say), southern green stink bug, *Nezara viridula* (L.), rice stink bug, *Oebalus pugnax* (Fab), and brown marmorated stink bug, *Halyomorpha halys* (stal), are all pests of agronomic crops, including maize (McPherson and McPherson, 2000). *Euschistus servus* is the primary stink bug pest species of maize in the southeastern United States. *Euchistus servus* pest populations are an increasing due to a variety of factors, including decreased use of broadcast insecticides and shifting farm management practices (Annan and Bergman 1988, McPherson and McPherson 2000, Greene et al. 2001). Widespread planting of transgenic Bt maize hybrids for lepidopteran pests decreased use of broad-spectrum insecticides that coincidentally targeted stink bug pests (McPherson and McPherson 2000, Greene et al. 2001). High residue cropping practices (e.g., reduced or no tillage, cover crops) that are favored for soil health and reduced erosion also provide stink bugs with undisturbed habitat and overwintering shelter (Annan and Bergman 1988).

Seedling maize is susceptible to injury and subsequent yield reduction when fed upon by *E. servus* (Townsend and Sedlacek 1986, Annan and Bergman 1988, Sedlacek and Townsend 1988, Apriyanto et al. 1989a, Apriyanto et al. 1989b). Stink bug feeding can delay maturity; cause growth deformities, such as tillering or multiple shoots extending from the same root system; and decrease mean extended leaf height and mean grain weight per ear (Apriyanto et al. 1989a). Damage from stink bug feeding increases with the infestation level and duration of infestation; however, yield can be reduced by a single day of feeding (Annan and Bergman 1988, Sedlacek and Townsend 1988, Apriyanto et al. 1989b). The most susceptible stages of maize development to stink bug injury are VE (emergence) and V2, and susceptibility decreases through V6, beyond which no yield impact likely occurs (Annan and Bergman 1988, Sedlacek and Townsend 1988).

Plant injury as a result of *E. servus* feeding often develops several weeks after feeding occurs and scouting for stink bug pests is complicated by their mobility and low visibility in crop residues. Thus, in some high-risk scenarios, preventative measures may be needed to effectively mitigate injury. Insecticides used currently on maize seeds and or that are applied during planting to target other maize pests may additionally control *E. servus* without unnecessarily increasing current production costs or environmental residues. Neonicotinoid insecticides, namely clothianidin and thiamethoxam, are applied to almost all commercially-available maize seed (Douglas and Tooker 2015). Neonicotinoids are detectable in maize roots, stems, and leaves for up to 47 days after planting when applied at the maximum commercially available rate (1.25 mg A.I./seed) (Alford and Krupke 2017). These residues may prevent *E. servus* damage early in the season (Van Duyn et al. 2005). Organophosphate insecticides (e.g., terbufos, acephate, and dicrotophos) can be applied in the seed furrow at planting to control some soilborne pests. Pyrethroid insecticides (e.g., bifenthrin, lamda-cyhalothrin) are similarly used and are the only labeled class of insecticides for foliar management of *E. servus* in maize. Although organophosphates and pyrethroids are targeting below ground pests when applied in the seed-furrow, *E. servus* could potentially encounter toxic residues in or on top of the soil.

Seed-applied and or in-furrow insecticide applications may provide protection for maize until plants mature out of the critical period for *E. servus* injury. Pyrethroids, organophosphates, and neonicotinoids have all been shown to cause mortality in stink bugs in different cropping systems and using different application methods (Reisig 2011, Kuhar et al. 2012, Little 2014). Our current study seeks to assess the impact of *E. servus* feeding in the presence of various early season insecticide treatments. The impact on early plant growth and injury, and the ultimate impact on grain yield, were assessed.

Materials and Methods:

Insects

Adult *E. servus* were collected using sweep nets (38 cm diameter) from wheat and rye fields in May and June, and by hand from maize in late June – August in Suffolk and Southampton County, Virginia, USA. Insects were stored in mesh enclosures (30 cm x 30 cm x 30 cm) for transfer from the field. Approximately 20-30 individuals were placed in plastic containers, and maintained in environmental chambers (Percival I-36VL, Percival Scientific, Perry, IA, USA) at a photoperiod of 16:8 and at 25±2 based on rearing protocols from several previous studies (Munyaneza and McPherson 1994, Koppel et al. 2009) until use in experiments.

Each container was lined with a paper towel and contained a cotton ball soaked in distilled water and surface sterilized green beans (*Phaseolus vulgaris*). Green beans were sterilized by rinsing with 10% bleach solution for one minute and rinsed with distilled water three times.

Field experiments

Experiments were conducted in 2018 and 2019 at the Tidewater Agricultural Research and Extension Center in Suffolk, Virginia, USA to determine if insecticides applied at planting provide early-season protection from *E. servus* injury. Untreated seed was compared to seed treated with different insecticides applied either on the seed coating or in the seed furrow at the time of planting.

Treatments in 2018 included; 1) untreated seed, 2) clothianidin applied in the seed coating at 1.25 mg a.i./seed (Poncho 1250, Bayer CropScience, RTP, NC, USA), 3) an in-furrow application of terbufos at the rate of 170.1 g per 304.8 m (Counter 20G, AMVAC, Los Angeles, CA, USA), and 4) clothianidin applied on the seed coating at 1.25 mg a.i./seed (Poncho 1250, Bayer CropScience) with an in-furrow application of bifenthrin at the rate of 0.714 liter per hectare (Ethos XB, FMC Corporation, Philadelphia, PA, USA). In 2019, treatments included; 1) fungicide-only coated seed, 2) clothianidin applied in the seed coating at 0.25 mg a.i./seed (Poncho 250, BASF), 3) clothianidin applied on the seed coating at 0.50 mg a.i./seed (Poncho 500, BASF), 4) clothianidin applied on the seed coating at 1.25 mg a.i./seed (Poncho 1250, BASF), 5) an in-furrow application of terbufos at the rate of 170.1 g per 304.8 m (Counter 20G, AMVAC), and 6) an in-furrow application of bifenthrin at the rate of 0.714 liter per hectare (Ethos XB, FMC Corporation, Philadelphia, PA). Seed were provided and seed coatings were applied by the manufacturer. In-furrow treatments were applied at the time of planting directly into the seed slot. Plots consisted of four 10.66 m rows spaced 0.91 m apart with 0.152 m seed spacing. Treatments were arranged in a randomized complete block design. In 2018, treatments were replicated three times and a single planting date was used (n = 3). In 2019, treatments were replicated four times and two planting dates were used (n = 8).

In 2018, hybrid 'KSC6815' with the VT Triple Pro insect trait package (Cry1A, Cry2Ab2, and Cry3Bb1) was planted on April 30. In 2019, the hybrid 'KSC6614' with the VT Double Pro insect trait package (Cry1A and Cry2Ab2) was planted on April 17 and April 30. Both hybrids were glyphosate tolerant (Roundup Ready 2).

In 2018, when seedlings were in the V2 growth stage (approximately 14 days after planting), mesh enclosures (1.83 m x 1.83 m x 1.83 m) were used to cover twenty plants per plot. Twenty field-collected adult *E. servus* were introduced per enclosure (1 *E. servus*/plant). In 2019, when seedlings were in the V2 growth stage (approximately 14 days after planting), three plants were enclosed in a 1.22 m x 0.61 m mesh sleeve supported by two fiberglass poles at either end and secured with binder clips. Each plot contained three enclosures placed near the approximate center of the plot. Two enclosures per plot had three *E. servus* introduced in each. The third enclosure served as a control and no *E. servus* were introduced. Both male and female adult *E. servus* were used randomly because previous studies indicated that the impact of their feeding did not differ (Sedlacek and Townsend 1988). Thus, the ratio of male and female used was representative of the field populations collected. In both years, once per day, the number of *E. servus* observed on plants was recorded for each enclosure to best approximate how many insects were actively feeding. The number of dead or missing insects were also recorded and subsequently replaced. Insects were considered dead if they displayed no movement or response to prodding. Enclosures and insects were removed after seven days. In both years, a foliar application of bifenthrin at the rate of 0.714 l per hectare was applied after the enclosures were removed to minimize effects from natural populations of *E. servus* and other early season pests. After enclosures were removed, all enclosed plants were marked with flags for later identification.

Data Collection

Plants were measured from the soil level to the tip of the tallest extended leaf (extended leaf height) immediately following removal of enclosures. Leaf injury, stunted plants, and number of tillers were also recorded. In 2019, extended leaf height was measured weekly for four weeks following enclosure removal, and again at plant maturity.

Once the plants reached physiological maturity, as determined by kernel black layer and 15-20% moisture content, primary ears (top ears) were hand harvested. The harvested ears were stored in plastic containers with dichlorvos strips (Hot Shot No-pest Strips, Spectrum Brands, Madison, WI, USA) to minimize stored grain pests until processing. Each ear was assessed for four components: 1) number of kernel rows per ear, 2) number of kernels per row, 3) average kernel weight (as determined by a 100 kernel weight sample), and 4) kernel moisture %. Moisture readings were taken for each plot (Dickey-john mini GAC 2500, DICKEY-john, Auburn, IL, USA). Yield was calculated using the formula:

Yield (kg/ha) = number of plants per hectare * number of ears per plant * number of kernel rows per ear * number of kernels per row * average kernel weight

Yields were standardized to 15.5 percent moisture. One enclosure was considered an experimental unit, and the responses for all early season measurements as well as yield components were averaged for analysis.

Statistical analysis

The relationship between insecticide treatment and the number of insects observed feeding (summed for the entire week), and insecticide treatment and the number of deceased insects (summed for the entire week), were analyzed using Pearson's chi-squared test with base statistics in R (R Core Team 2019).

Early symptoms of plant injury as a result of stink bug feeding (leaf injury, stunting, and tillering) were analyzed using generalized linear mixed effect models fitted to a binomial distribution using the lme4 (Bates et al. 2015) package in R. Models were optimized using the package optimx (Nash and Varadhan 2011). Effects of each model term on the odds of plant injury were then estimated as odds ratios and confidence intervals. Odds ratios were used to indicate the effect of insecticide treatment on plant injury and whether odds of plant injury changed throughout the season. Insecticide treatment was a fixed effect and the replication a random effect in both years. Weeks after infestation was also a fixed effect in 2019. Maximum likelihood ratio tests were used to determine the significance of fixed model effects.

In 2018, extended leaf height and yield were analyzed using a linear mixed effect model using lme4 in R. Insecticide treatment was considered a fixed effect and the replication a random effect. As a substitute for extended leaf height in 2019, the difference in extended leaf height was calculated between plants in enclosures with and without *E. servus* introduced (mean extended leaf height of plants in enclosures with insects – mean extended leaf height of plants in enclosures without insects) for each treatment. As a substitute for yield, the differences in yield (kg/ha) were calculated between plants in enclosures with and without *E. servus* (yield of plants in enclosures with insects – yield of plants in enclosures without insects) for each treatment. This was done because insecticidal treatments, below ground pests, and enclosure effects can potentially impact plant growth. For plant height, insecticide treatment and observation week (i.e., number of weeks after infestation) were fixed effects and the replication was a random effect. For differences in yield, insecticide treatment was the fixed effect and the replication was a random effect.

Visual inspection of residual plots for all listed models did not reveal any deviations from the assumptions of linear models. For all analyses in 2019, planting dates were analyzed separately. In both years, for model terms having a significant effect on the response, estimated marginal means were determined using the package emmeans (Lenth 2019), and Tukey's adjusted pairwise comparisons were made at $\alpha = 0.05$. Figures were generated using means from the raw data sets with ggplot2 (Gómez-Rubio 2017).

Results:

***E. servus* feeding and mortality**

In 2018, there was a relationship between insecticide treatment and *E. servus* feeding on plants ($X^2 = 27.05$; df = 3; $p < 0.001$), and a relationship between insecticide treatment and the number of *E. servus* mortalities ($X^2 = 27.05$; df = 3; $p < 0.001$). The number observed feeding on plants was highest in the highest rate of clothianidin combined with bifenthrin and this treatment also had the highest number of mortalities (Fig. 1 and Fig. 2).

In early planted maize in 2019, there was no relationship between the insecticide treatment and the number of *E. servus* feeding on plants ($X^2 = 3.94$; df = 5; $p = 0.558$) (Fig. 3). There was a relationship between insecticide treatment the number of *E. servus* mortalities ($X^2 = 35.32$; df = 5; $p < 0.001$). The number of mortalities were highest in the low (0.25 mg a.i./seed) and high (1.25 mg a.i./seed) rates of clothianidin (Fig. 4).

In late planted maize in 2019, there was a relationship between the insecticide treatment and the number of *E. servus* feeding on plants ($X^2 = 15.67$; df = 5; $p = 0.008$), and a relationship between the insecticide treatment and the number of *E. servus* mortalities ($X^2 = 44.76$; df = 5; $p < 0.001$). The number of insects feeding on plants was lowest in the low (0.25 mg a.i./seed) and high (1.25 mg a.i./seed) rates of clothianidin (Fig. 5). Mortality was also highest in the low (0.25 mg a.i./seed) and high (1.25 mg a.i./seed) rates of clothianidin (Fig. 6).

Plant Injury

In 2018, insecticide treatment affected leaf injury ($X^2 = 11.06$; df = 3; p = 0.011), but it did not affect the number of stunted plants ($X^2 = 2.14$; df = 3; p = 0.545) (Table 1).

In early planted maize in 2019, insecticide treatment ($X^2 = 75.83$; df = 5; p < 0.001), observation week ($X^2 = 195.64$; df = 4; p < 0.001), and the interaction of these two factors ($X^2 = 33.27$; df = 20; p = 0.046) affected leaf injury. Insecticide treatment ($X^2 = 94.22$; df = 5; p < 0.001), observation week ($X^2 = 150.09$; df = 4; p < 0.001), and the interaction of these two factors ($X^2 = 44.91$; df = 20; p = 0.001) affected the number of stunted plants (Table 2).

In late planted maize in 2019, insecticide treatment ($X^2 = 216.86$; df = 5; p < 0.001), observation week ($X^2 = 25.57$; df = 4; p < 0.001), and the interaction of these two factors ($X^2 = 82.14$; df = 20; p < 0.001) affected leaf injury. Insecticide treatment ($X^2 = 20.49$; df = 5; p = 0.001) and observation week ($X^2 = 28.02$; df = 4; p < 0.001) affected the number of stunted plants. There was no effect from the interaction of these two factors ($X^2 = 16.33$; df = 20; p = 0.696) (Table 3).

No variable affected the number of tillered plants in both years and tillering was determined to be a poor indicator of early *E. servus* injury (data not shown).

Plant height

In 2018, insecticide treatment (F = 5.25; df = 3,5.13; p = 0.051) affected plant height (Fig. 7). In early planted maize in 2019, observation week (F = 4.09; df = 4,192; p = 0.003) affected plant height. The insecticide treatment (F = 0.87; df = 5,15; p = 0.523) and the interaction of observation week and insecticide treatment

(F = 0.77; df = 20,192; p = 0.750) did not affect plant height (Table 4). In late planted maize in 2019, insecticide treatment (F = 5.12; df = 5,15; p = 0.006) and observation week (F = 4.37; df = 4,192; p = 0.002) affected plant height. The interaction of observation week and insecticide treatment (F = 1.44; df = 20,192; p = 0.109) did not affect plant height (Table 5).

Yield

In 2018, insecticide treatment did not affect grain yield (F = 21.13; df = 3,5.17; p = 0.212) (Fig. 8). In early planted maize in 2019, insecticide treatment did not affect ear weight (F = 0.71; df = 5,39; p = 0.617) or grain yield (F = 1.27; df = 5,39; p = 0.297) (Fig. 9). In the late planted maize in 2019, insecticide treatment affected ear weight (F = 2.46; df = 5,39; p = 0.049) and grain yield (F = 2.90; df = 5,39; p = 0.025). The overall model indicated that insecticide treatment had a significant impact on ear weight, but Tukey's adjusted pairwise comparisons did not separate any of the insecticide treatment means (Fig. 10). Thus, we concluded that there was no effect from insecticide treatment on overall ear weight.

Discussion:

We observed effects of *E. servus* feeding on seedling maize similar to previous studies including decreased plant height, increased leaf injury, and increased number of stunted plants (Townsend and Sedlacek 1986, Sedlacek and Townsend 1988, Apriyanto et al. 1989a, Apriyanto et al. 1989b). It is important to report that tillering did not occur as a result of feeding in our experiment. Tillering, a commonly reported symptom of *E. servus* feeding, has been documented in all the previously listed studies. Plant response could have differed as a result of multiple variables including stink bug species, the timing of infestation, maize hybrid used, and field conditions (e.g., weather, nutrient availability). For example, tillering resulted from onespotted stink bug, *Euschistus variolarius*, feeding when infestations were timed closer to emergence (VE) (Annan and Bergman 1988). Our study applied infestations later in plant development (V4).

The impact of *E. servus* feeding on plant height developed one week after stink bugs were removed and persisted until plant maturity. In contrast, other symptoms of stink bug injury (i.e., leaf injury, the number of stunted plants) were observed one to three weeks after infestation and were not observable at maturity. It is likely that severely stunted plants do not fully recover over the course of the season, but that plants can compensate for some level of injury. The level of injury from which maize can recover throughout the season is not known, and is likely dependent on seasonal variations in temperature and water availability. Symptoms of early injury were not always present at plant maturity, and differences in plant height were inconsistent. This is likely a result of the nature of growth in modern maize hybrids and compensatory growth by plants surrounding stunted plants. In our study, we observed that plants within a single enclosure may exhibit an uneven response to early stink bug feeding. This is consistent with a field setting where uninjured plants that are adjacent to severely stunted plants receive more sunlight and better compete for nutrients throughout the season and net a greater grain yield that compensates for losses from its stunted neighbor.

In 2018 and in late planted maize in 2019, insecticide treatments affected plant height. Maize plants treated with medium (0.5 mg a.i./seed) and high (1.25 mg a.i./seed) rates of clothianidin were taller than untreated maize throughout the entire season. Across both years, the highest rate of clothianidin prevented virtually all reduction in plant height and visible plant injury. Some published Cooperative Extension recommendations have suggested that a high rate of clothianidin can prevent early *E. servus* injury (Van Duyn et al. 2005, Flessner and Cahoon 2018), which is supported here. Although the highest rate of clothianidin provided almost complete control, there is evidence that lower rates can be just as effective. The window of protection provided by different rates of clothianidin is likely affected by seasonal variations (Alford and Krupke 2017). Neonicotinoid residues are found in shoot tissue, in low levels, for up to three weeks after planting (Alford and Krupke 2017). Our results suggest that the amount of insecticide active ingredient in plant tissue at V4 is sufficient to reduce or negate the impact of *E. servus* infestations. We also demonstrated that in-furrow applications of organophosphate and pyrethroid insecticides did not affect stink bug injury in plants. The toxicity of bifenthrin and terbufos is primarily dependent on contact with the insect cuticle, which likely did not occur in this experiment.

The insecticide treatment did not impact plant height in early planted maize in 2019. The number of insects observed actively feeding throughout the early planted experiment was relatively low and not related to the insecticide treatment. These results suggest that *E. servus* is not feeding as actively during the window when maize is planted early and thus, later planted maize may be more susceptible to injury. *Euschistus servus* have also been found in higher numbers in later planted maize (Tillman 2010). In areas where *E. servus* management is a annual concern, earlier planting and higher rates of systemic seed treatments could eliminate the potential for yield loss from stink bug feeding.

There was a relationship between insect mortality and insecticide treatment in both years and both planting dates in 2019. There was also a relationship between the number of insects feeding on plants and the insecticide treatment in 2018 and in the late planted maize in 2019. The connection between the mortality and feeding activity of *E. servus* and insecticide treatment provides further evidence that preventative insecticide treatments can mitigate the impacts of early infestations. The effectiveness of clothianidin in preventing plant injury may be a result of a combination of anti-feeding activity and intoxicating effects on *E. servus*. The exact mechanism of control that these insecticide treatments provide requires further examination. Grain yield was only significantly impacted by insecticide treatment in late planted maize in 2019, providing evidence of greater susceptibility in later planted maize. Maize treated with the median rate (0.5 mg a.i./seed) of clothianidin had higher yields compared to the untreated seed. The vast majority (80-100%) of maize planted in the U.S. is pre-treated with a systemic insecticide (Douglas and Tooker 2015). The standard treatment may be sufficient to mitigate early season *E. servus* concerns without adding additional inputs or increasing seed costs. In high pressure scenarios (i.e., one or more *E. servus* per plant), a higher rate of clothianidin may be needed for complete control.

It has been suggested that neonicitinoids have the potential to increase plant growth and yield (North et al. 2018), which could confound the results of this experiment. However, empirical studies have demonstrated that neonicitinoid seed treatments did not impact plant growth in the absence of insect feeding (Cox et al. 2007, Wilde et al. 2007). In 2019, we used the difference in plant height and yield between plants with and without *E. servus* exposure within each insecticide treatment. Our goal was to examine only the impact of *E. servus* feeding and minimize potential confounding factors such as other soil pests and benefits of the insecticide formulations on plant growth and physiology.

Results of our study demonstrate that systemic insecticides have the potential to mitigate early season impact plant injury and yield loss as a result of *E. servus* feeding. These insecticides are pre-applied to almost all commercial maize seed. There was no benefit to adding an in-furrow granular or liquid insecticide and only a slight benefit, if any, of higher rates of seed applied insecticides. Farmers can save money and reduce environmental residues without accruing financial losses.

Tables and Figures

Figure 1. The number of *E. servus* observed on maize plants in artificial enclosures in the presence of various at plant insecticide treatments, Suffolk, Virginia 2018 ($X^2 = 27.05$; df = 3; $p < 0.001$).

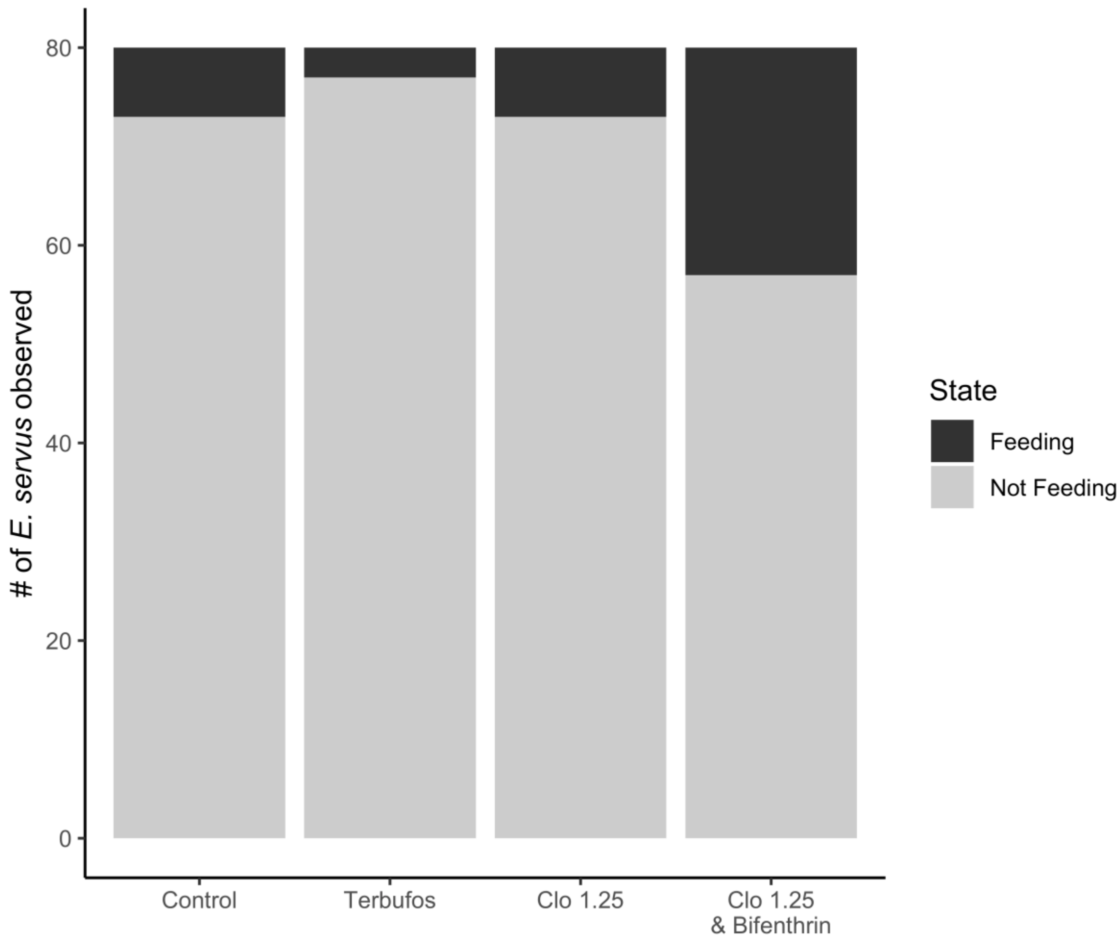


Figure 2. The number of dead *E. servus* observed in artificial enclosures in the presence of maize plants treated with various at plant insecticide treatments, Suffolk, Virginia 2018 ($X^2 = 27.05$; df = 3; $p < 0.001$).

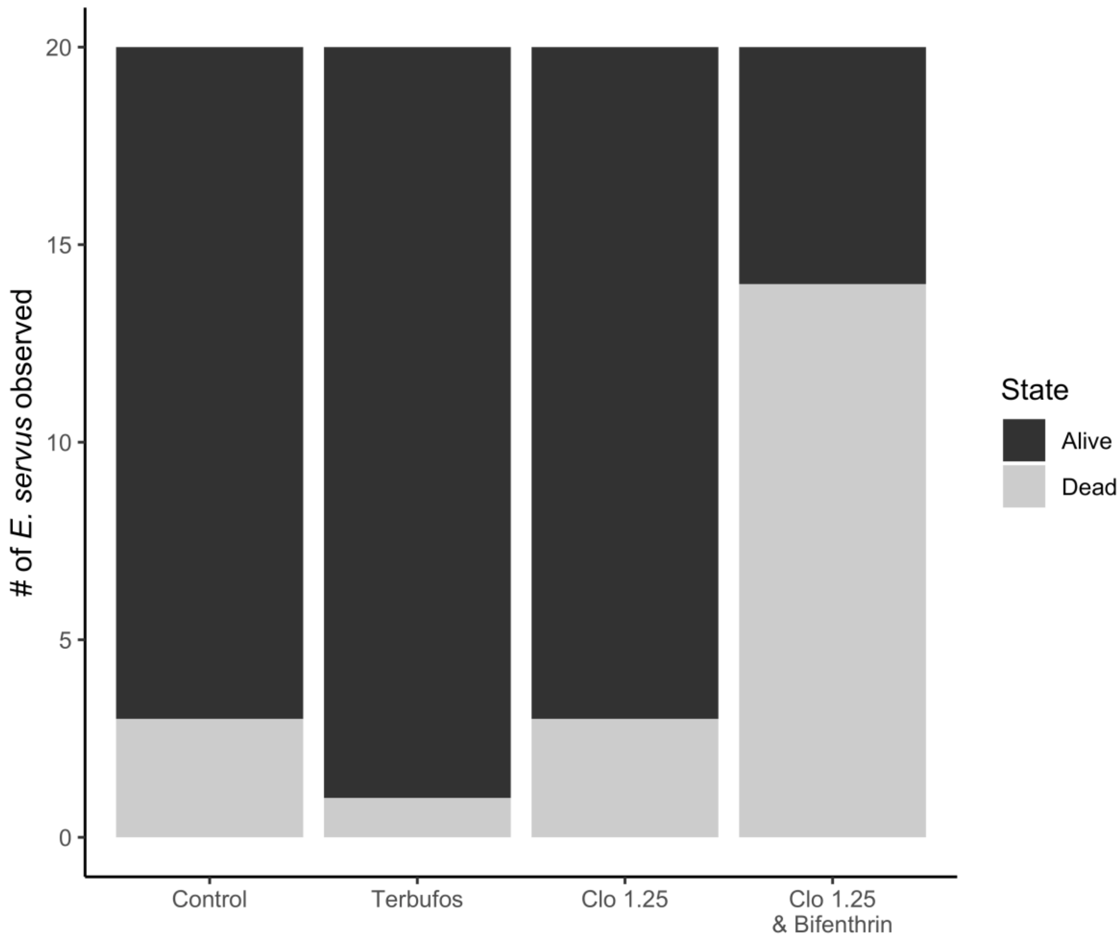


Figure 3. The number of *E. servus* observed on early planted maize plants in artificial enclosures in the presence of various at plant insecticide treatments, Suffolk, Virginia 2019 ($X^2 = 3.94$; df = 5; p = 0.558).

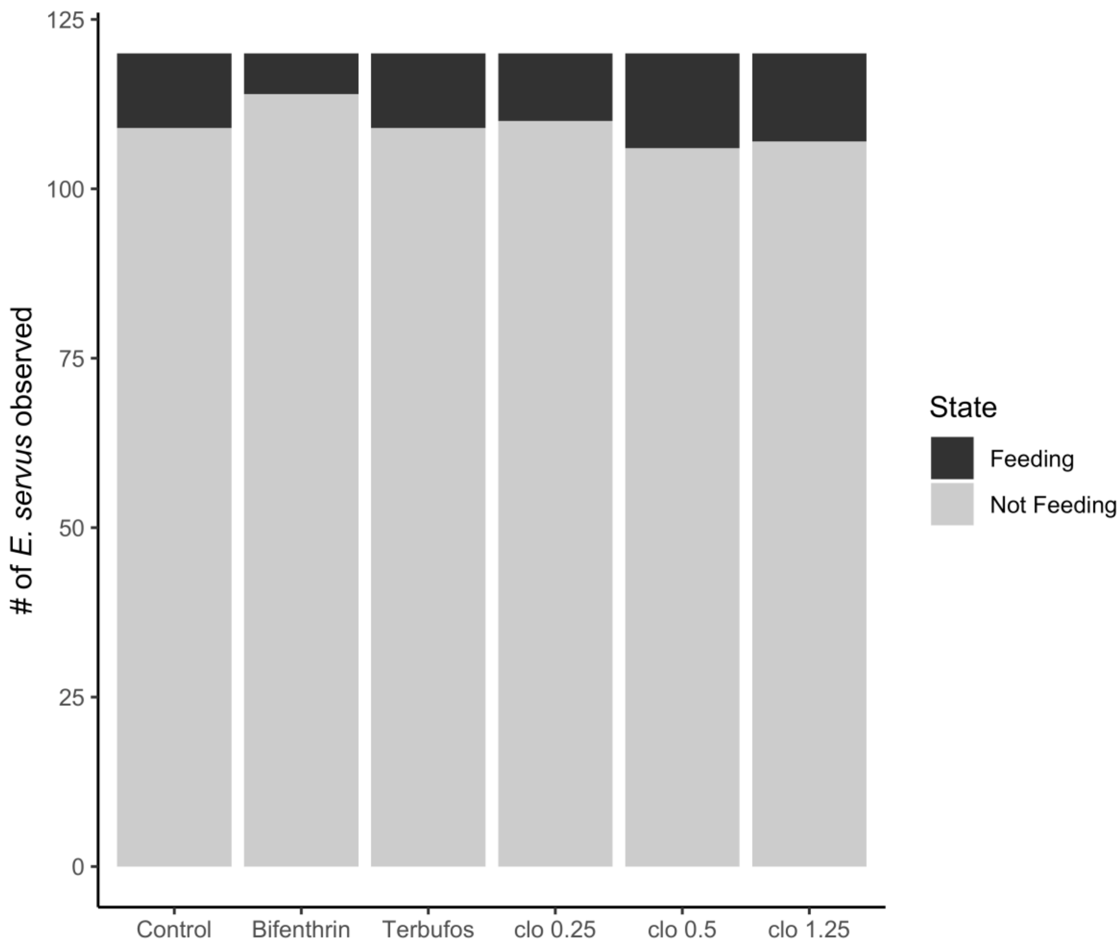


Figure 4. The number of dead *E. servus* observed in artificial enclosures in the presence of maize plants treated with different at plant insecticide treatments, Suffolk, Virginia 2019 ($X^2 = 35.32$; df = 5; $p < 0.001$).

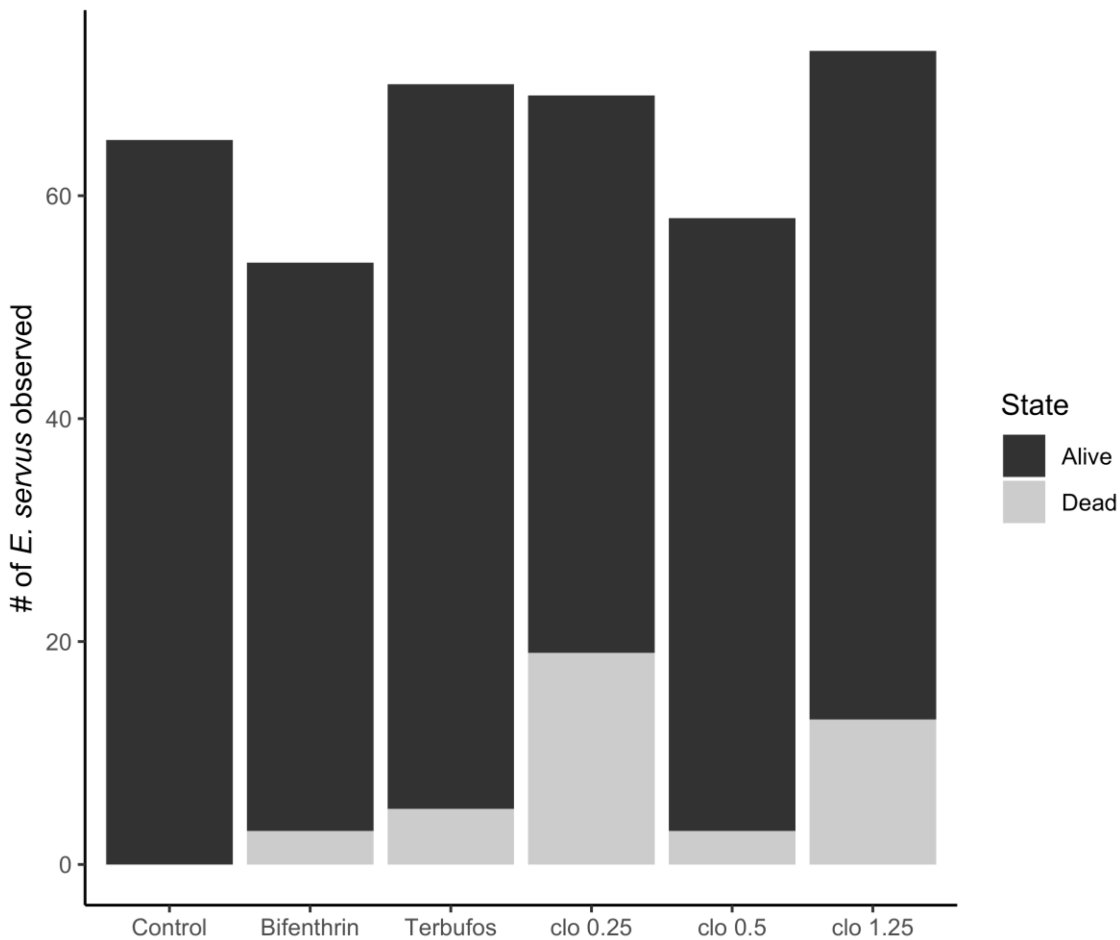


Figure 5. The number of *E. servus* observed on late planted maize plants in artificial enclosures in the presence of various at plant insecticide treatments, Suffolk, Virginia 2019 ($X^2 = 15.67$; df = 5; $p = 0.008$).

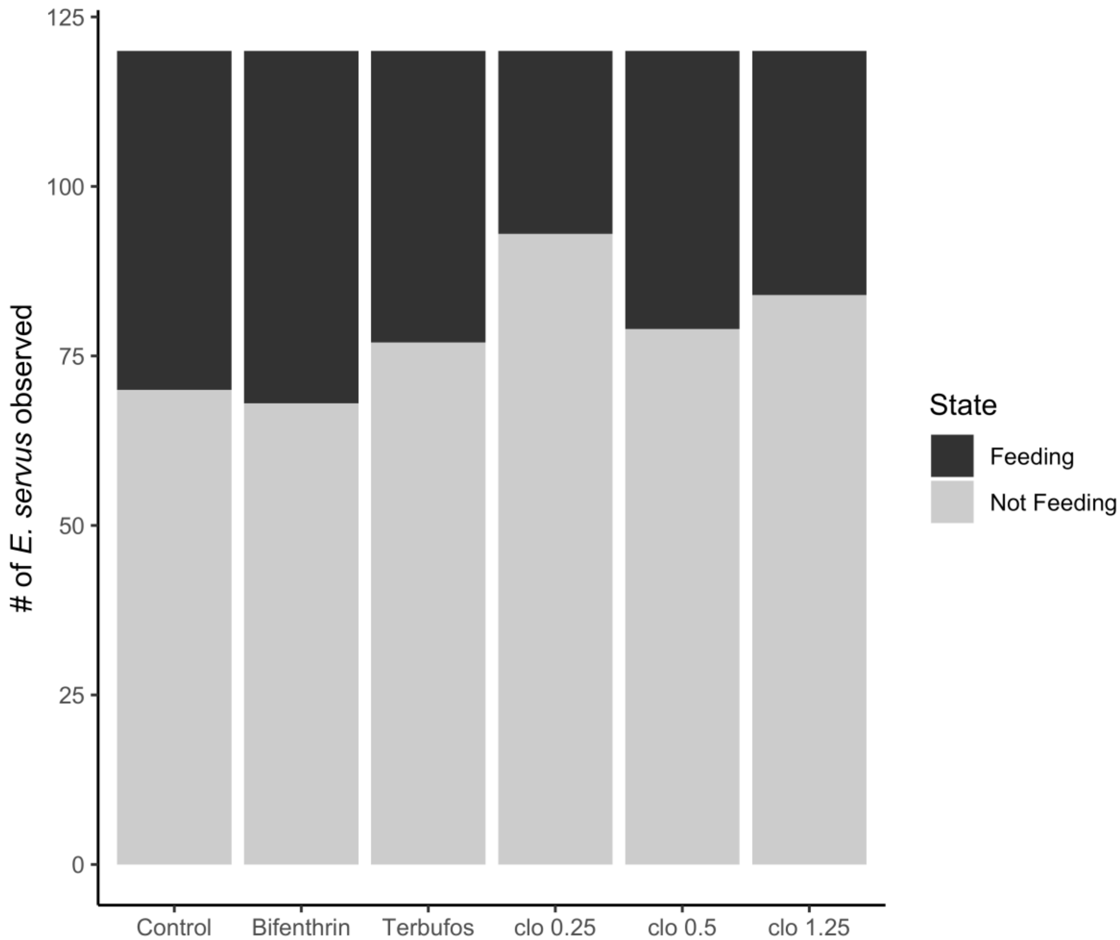


Figure 6. The number of dead *E. servus* observed in artificial enclosures in the presence of maize plants treated with different at plant insecticide treatments, Suffolk, Virginia 2019 ($X^2 = 44.76$; df = 5; $p < 0.001$).

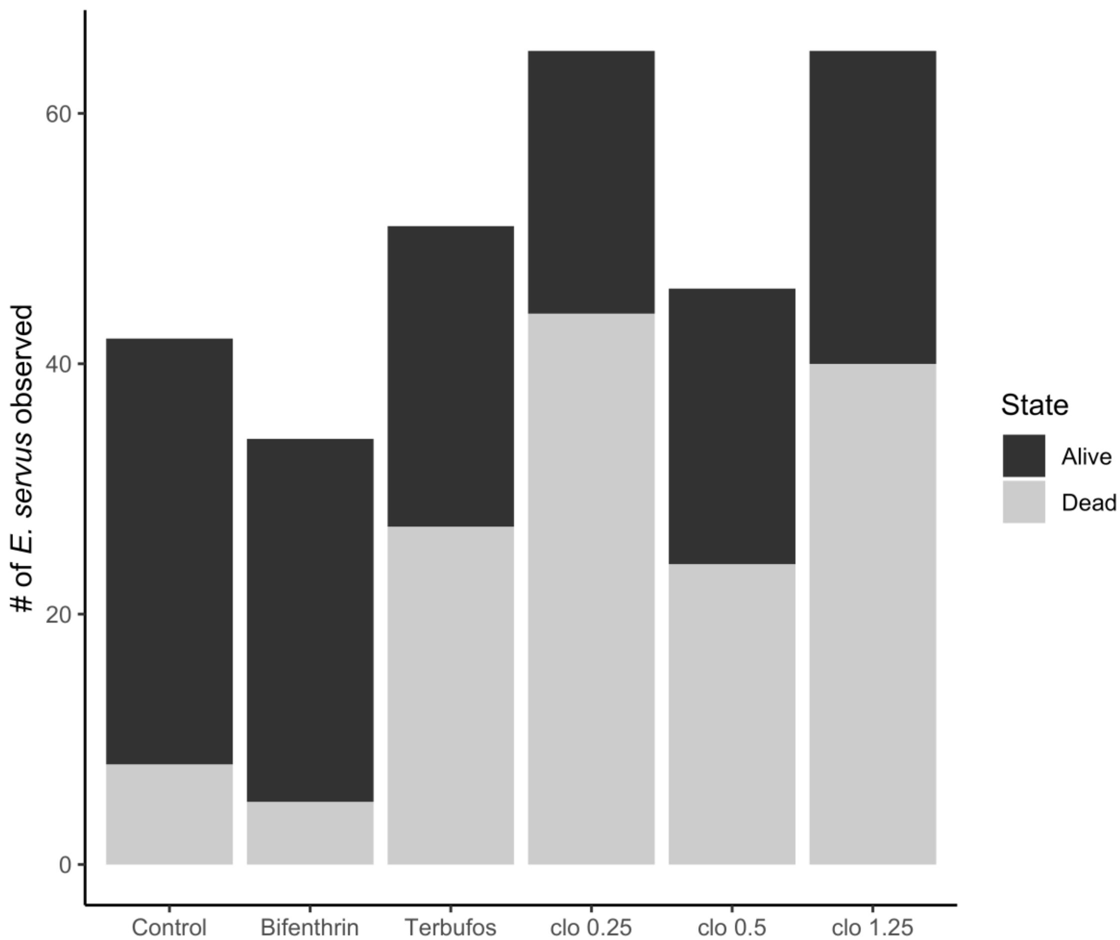


Table 1. Odds ratios (95% CI) and p-values of *E. servus* feeding causing leaf injury and stunting of plants in the presence of different insecticide treatment, in reference to the untreated control in 2018.

Insecticide Treatment (rate)	Leaf Injury		Stunting	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Terbufos (170.1 g/304.8 m)	0.76 (0.33 to 1.76)	0.522	1.00 (0.06 to 17.75)	1.000
Clothianidin (1.25 mg a.i./seed)	0.14 (0.03 to 0.67)	0.014	0.00 (0.00 to inf)	0.999
Clothianidin (1.25 mg a.i./seed) and bifenthrin (0.714 l/ha)	0.36 (0.14 to 0.96)	0.042	2.04 (0.17 to 24.77)	0.576

Table 2. Odds ratios (95% CI) and p-values of *E. servus* feeding causing leaf injury and stunting of plants in the presence of different insecticide treatments and throughout the season after infestation in early planted maize, 2019. Odds ratios for insecticide treatments are in reference to untreated control, and weeks after infestation odds ratios are in reference to the week enclosures were removed.

	Leaf Injury		Stunting	
Insecticide Treatment	Odds Ratios (95% CI)	p-value	Odds Ratio (95% CI)	p-value
Terbufos (170.1 g/304.8 m)	0.60 (0.19 to 1.89)	0.383	0.48 (0.15 to 1.58)	0.229
Bifenthrin (0.714 l/ha)	1.43 (0.44 to 4.66)	0.549	1.72 (0.53 to 5.56)	0.368
Clothianidin (0.25 mg a.i./seed)	0.14 (0.04 to 0.54)	0.004	0.04 (0.00 to 0.35)	0.004
Clothianidin (0.5 mg a.i./seed)	0.51 (0.16 to 1.60)	0.247	0.39 (0.12 to 1.32)	0.999
Clothianidin (1.25 mg a.i./seed)	0.03 (0.00 to 0.26)	0.002	0.00 (0.00 to inf)	0.998
Weeks after infestation				
1	1.19 (0.37 to 3.81)	0.766	0.58 (0.18 to 1.89)	0.368
2	1.19 (0.37 to 3.81)	0.766	0.04 (0.00 to 0.35)	0.004
3	0.84 (0.27 to 2.65)	0.769	0.04 (0.00 to 0.35)	0.004
11	0.03 (0.00 to 0.26)	0.002	0.00 (0.00 to inf)	0.998

Table 3. Odds ratios (95% CI) of *E. servus* feeding causing leaf injury and stunting of plants in the presence of different insecticide treatments and throughout the season after infestation in late planted maize, 2019. Odds ratios for insecticide treatments are in reference to untreated control, and weeks after infestation odds ratios are in reference to the week enclosures were removed.

		Leaf Injury		Stunting
Insecticide Treatment	Odds Ratios (95% CI)	p-value	Odds Ratio (95% CI)	p-value
Terbufos (170.1 g/304.8 m)	0.71 (0.22 to 2.24)	0.559	0.72 (0.05 to 10.55)	0.809
Bifenthrin (0.714 l/ha)	0.49 (0.15 to 1.60)	0.236	0.00 (0.00 to inf)	0.999
Clothianidin (0.25 mg a.i./seed)	0.11 (0.02 to 0.56)	0.008	0.31 (0.01 to 7.36)	0.469
Clothianidin (0.5 mg a.i./seed)	0.00 (0.00 to inf)	0.997	0.00 (0.00 to inf)	0.999
Clothianidin (1.25 mg a.i./seed)	0.11 (0.02 to 0.56)	0.008	0.00 (0.00 to inf)	0.999
Weeks after infestation				
1	13.00 (2.48 to 68.05)	0.002	11.26 (2.01 to 62.89)	0.006
2	13.00 (2.48 to 68.05)	0.002	3.09 (0.51 to 18.86)	0.221
3	8.27 (1.94 to 35.34)	0.004	2.30 (0.36 to 14.77)	0.380
11	1.00 (0.32 to 3.11)	1.000	7.66 (1.36 to 43.15)	0.021

Figure 7. Extended leaf height (cm) of maize plants planted with and without insecticide treatments and exposed to *E. servus* for seven days in artificial enclosures, Suffolk, Virginia, 2018. Standard error bars with different letters above them were statistically different at alpha =0.05.

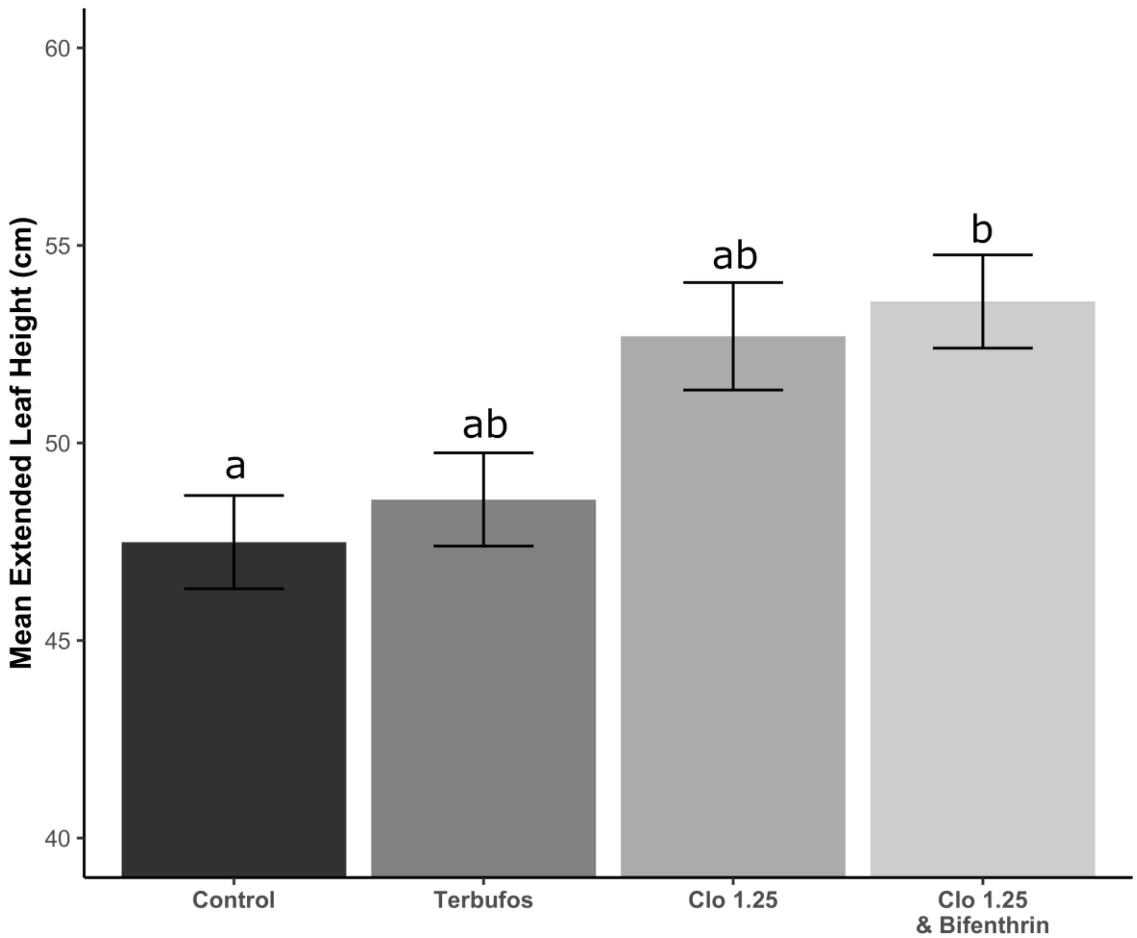


Table 4. Difference in extended leaf height (cm) (SEM) of early planted maize with different insecticide treatments and exposed to *E. servus* adults for seven days in artificial field enclosures, Suffolk, Virginia, 2019. Maize plants in the same treatment were caged with and without *E. servus* adults. Values represent the difference in extended leaf height of maize plants in enclosures with *E. servus* subtracted from the extended leaf height of maize plants in enclosures without *E. servus*. Heights were measured for four weeks following removal of enclosures and at plant maturity. There was no effect on plant height resulting from *E. servus* exposure at alpha = 0.05.

Treatment	Observation Week				
	1	2	3	4	12
Fungicide only	-4.56 (0.46)	-14.5 (3.86)	-8.73 (3.90)	-3.58 (5.33)	3.39 (7.10)
Bifentrhin (0.714 l/ha)	-6.21 (1.18)	-12.86 (4.80)	-16.67 (2.92)	-10.59 (4.90)	-14.46 (7.46)
Terbufos (170.1 g/304.8 m)	-3.81 (1.68)	-8.31 (4.21)	-3.98 (2.43)	-3.71 (2.16)	-5.25 (3.24)
Clothianidin (0.25 mg a.i./seed)	-1.77 (1.08)	-7.99 (3.14)	-7.79 (3.65)	-5.00 (4.33)	-0.85 (5.45)
Clothianidin (0.5 mg a.i./seed)	-3.73 (1.88)	-6.51 (3.62)	-6.77 (4.59)	-5.33 (6.01)	-4.23 (6.63)
Clothianidin (1.25 mg a.i./seed)	-0.11 (1.52)	-10.48 (5.21)	-0.52 (3.44)	-1.63 (4.89)	0.80 (4.78)

Table 5. Difference in mean extended leaf height (cm) (±SEM) by observation week between late planted maize with and without exposure to *E. servus* adults for seven days in artificial field enclosures, Suffolk, Virginia, 2019. Values represent the mean difference in extended leaf height of maize plants in enclosures with *E. servus* subtracted from the extended leaf height of maize plants in enclosures without *E. servus*. Maize was planted with and without different insecticide treatments. Heights were measured for four weeks following removal of enclosures and at plant maturity. Week one represents the date that enclosures were removed. Values within each column having the same letter are not significantly different at alpha = 0.05.

Treatment	Observation Week				
	1	2	3	4	12
Fungicide only	-6.50a (2.05)	-24.21a (5.19)	-24.83a (6.86)	-15.83a (7.34)	-18.15a (6.81)
Bifentrhin (0.714 l/ha)	-5.04a (0.84)	-15.6b (3.15)	-14.04ab (4.50)	-7.37ab (2.83)	-5.40ab (1.58)
Terbufos (170.1 g per 304.8 m)	-5.11a (1.70)	-13.65ab (3.96)	-5.63ab (3.37)	-1.67ab (4.60)	-10.95ab (5.71)
Clothianidin (0.25 mg a.i./seed)	-3.13a (1.09)	-3.21b (2.04)	-1.71b (2.90)	-2.88ab (3.95)	4.76b (4.22)
Clothianidin (0.5 mg a.i./seed)	-0.64a (1.76)	-2.96b (3.24)	-2.54b (4.72)	0.75ab (3.58)	3.86b (4.02)
Clothianidin (1.25 mg a.i./seed)	1.69a (1.70)	3.15b (2.44)	4.39b (3.09)	8.33b (3.44)	2.38b (4.15)

Figure 8. Mean yield (kg/ha) of maize planted with and without different insecticide treatments and exposed to *E. servus* adults for seven days in artificial field enclosures, Suffolk, Virginia, 2018. Error bars represent standard error of means. There were no differences in yield resulting from *E. servus* exposure at α = 0.05 (F = 21.13; df = 3,5.17; p = 0.212).

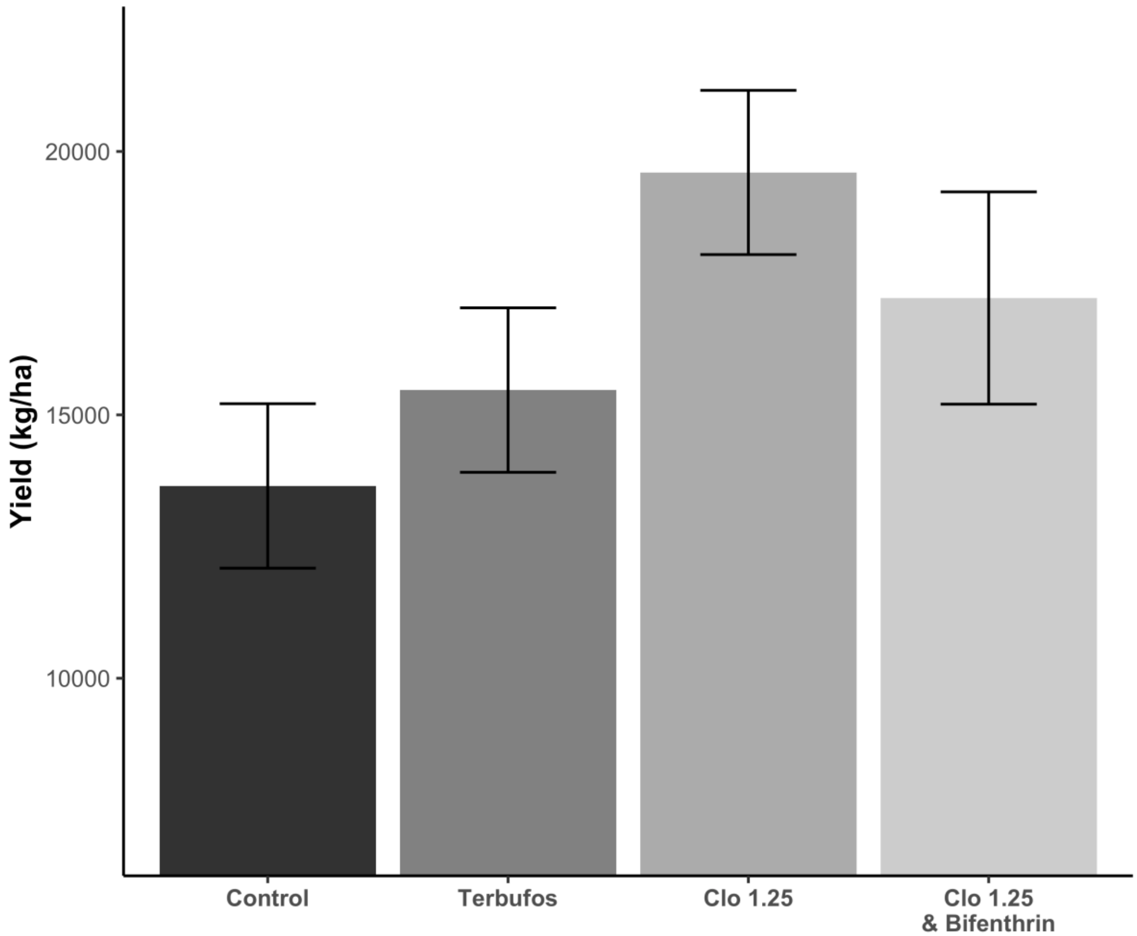


Figure 9. Difference in mean ear weight (g) between early and late planted maize with and without exposure to *E. servus* adults for seven days in artificial field enclosures, Suffolk, Virginia, 2019. Values represent the mean difference in ear weight of maize plants in enclosures with *E. servus* subtracted from the ear weight of maize plants in enclosures without *E. servus*. Maize was planted with and without different insecticide treatments. Weights were measured at plant maturity. Error bars represent standard error of means. Pairwise comparisons revealed no significant differences in ear weight resulting from *E. servus* exposure at alpha = 0.05 ($F = 0.71$; $df = 5,39$; $p = 0.617$).

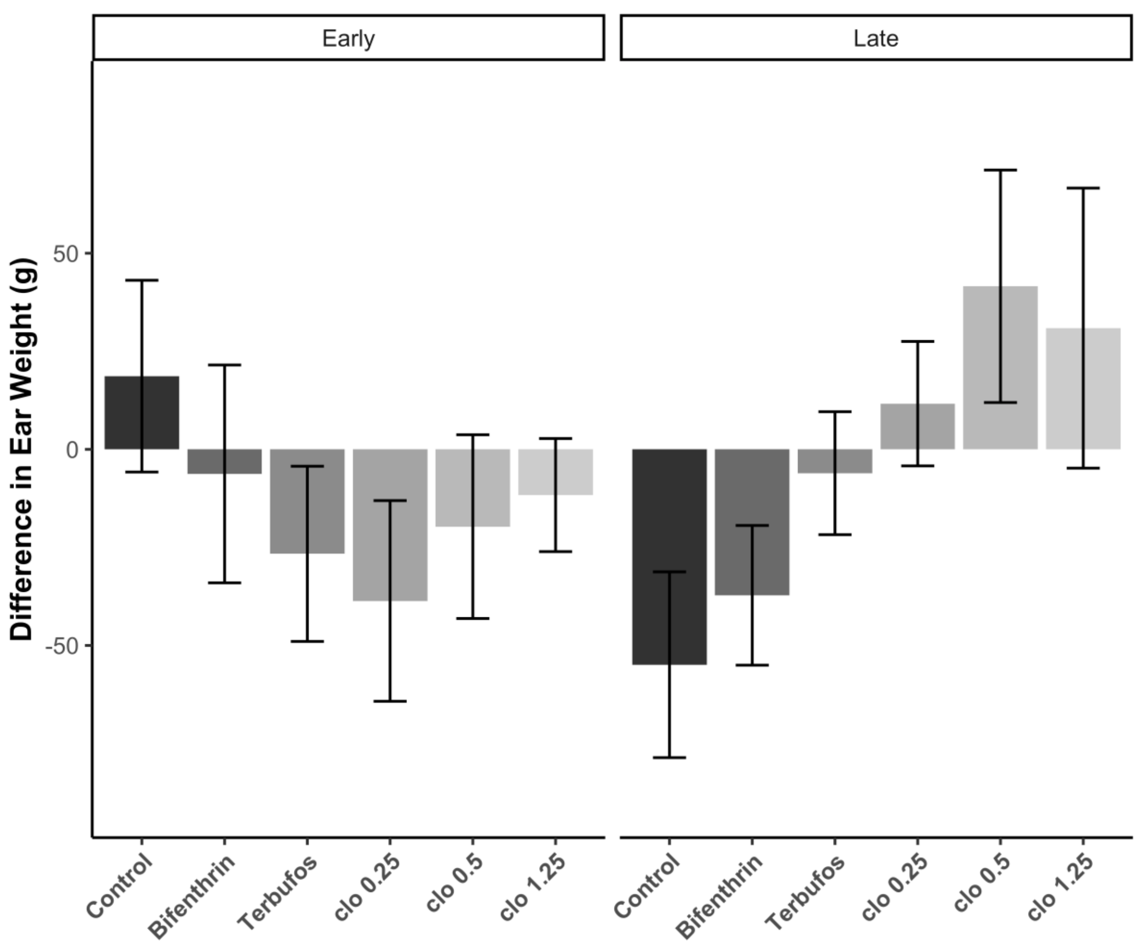
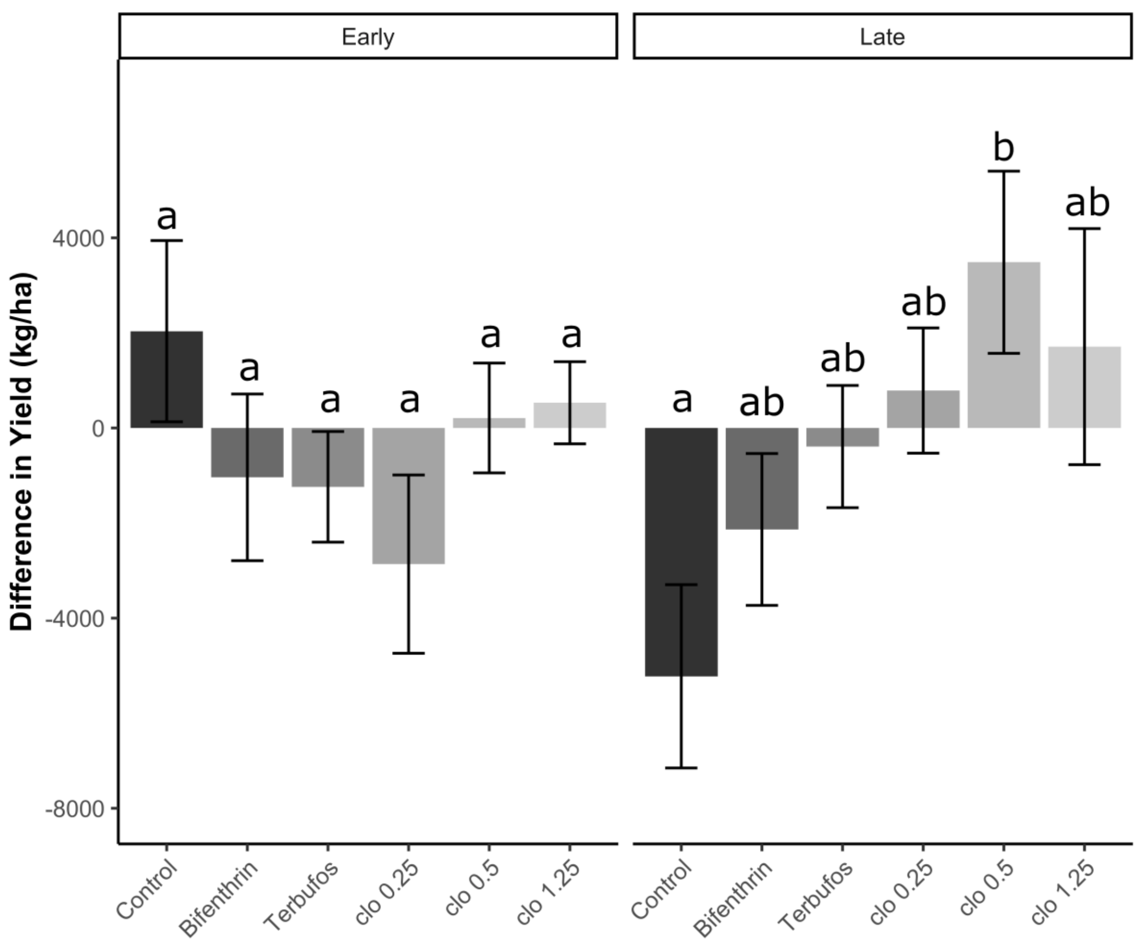


Figure 10. Difference in yield (kg/h) between early and late planted maize with and without exposure to *E. servus* adults for seven days in artificial field enclosures, Suffolk, Virginia, 2019. Values represent the mean difference in yield of maize plants in enclosures with *E. servus* subtracted from the yield of maize plants in enclosures without *E. servus*. Maize was planted with and without different insecticide treatments. Yields were measured at plant maturity. Error bars represent standard error of means. Error bars with different letters above them were statistically different within each planting date at α = 0.05.



References

Alford, A., and C. H. Krupke. 2017. Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS One. 12: 1–19.

Annan, I. B., and M. K. Bergman. 1988. Effects of the Onespotted Stink Bug (Hemiptera : Pentatomidae) on Growth and Yield of Corn. J. Econ. Entomol. 81: 649–653.

Apriyanto, D., J. D. Sedlacek, and L. H. Townsend. 1989a. Feeding Activity of Euschistus servus and E. variolarius (Heteroptera : Pentatomidae) and Damage to an Early Growth Stage of Corn. J. Kansas Entomol. Soc. 62: 392-399.

Apriyanto, D., L. H. Townsend, and J. D. Sedlacek. 1989b. Yield Reduction from Feeding by Euschistus servus and E . variolar ius (Heteroptera : Pentatomidae) on Stage V2 Field Corn. J. Econ. Entomol. 82: 445–448.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed effects models using lme4. J. Stat. Softw. 67.

Cox, W. J., E. Shields, and J. H. Cherney. 2007. The effect of clothianidin seed treatments on corn growth following soybean. Crop Sci. 47: 2482–2485.

Douglas, M. R., and J. F. Tooker. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. Field crops. Environ. Sci. Technol. 49: 5088–5097.

Flessner, M., and C. W. Cahoon. 2018. Pest Management Guide: Field Crops. Virginia Coop. Ext. (www.ext.vt.edu).

Gómez-Rubio, V. 2017. ggplot2 - Elegant Graphics for Data Analysis (2nd Edition) .

J. Stat. Softw. 77: 2–5.

Greene, J. K., S. G. Turnipseed, M. J. Sullivan, and O. L. May. 2001. Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. J. Econ. Entomol. 94: 403–9.

Koppel, A. L., D. A. Herbert, T. P. Kuhar, and A. K. Kamminga. 2009. Survey of Stink Bug (Hemiptera: Pentatomidae) Egg Parasitoids in Wheat, Soybean, and Vegetable Crops in Southeast Virginia. Environ. Entomol. 38: 375–379.

Kuhar, T., H. Doughty, K. Kamminga, C. Philips, J. Aigner, A. Wallingford, A. Wimer, L. Lilliston, L. Nottingham, A. Lohr, E. Fread, J. Jenrette. 2012. Performance of Insecticides on Brown Marmorated Stink Bug on Vegetables. (www.ext.vt.edu).

Lenth, R. 2019. Estimated Marginal Means, aka Least-Square Means. R package version 1.3.5.1. https://CRAN.R-project.org/package=emmeans

Little, B. A. 2014. Efficacy and Non-Lethal Effects of Clothianidin for the Management of Stink Bugs (Hemiptera: Pentatomidae) in Cotton.

McPherson, J. E., and R. M. McPherson. 2000. Stink Bugs of Economic Importance in America North of Mexico. CRC Press.

Munyaneza, J., and J. E. McPherson. 1994. Comparative study of life histories, lab rearing, and immature stages of Euschistus servus and Euschistus variolarius (Hemiptera: Pentatomidae). Gt. Lakes Entomol. 26: 263–275.

Nash, J. C., and R. Varadhan. 2011. Unifying Optimization Algorithms to Aid Software System Users: optimx for R. J. Stat. Softw. 43.

North, J. H., J. Gore, A. L. Catchot, S. D. Stewart, G. M. Lorenz, F. R. Musser, D. R.

Cook, D. L. Kerns, B. R. Leonard, and D. M. Dodds. 2018. Value of neonicotinoid insecticide seed treatments in mid-south corn (Zea mays) production systems. J. Econ. Entomol. 111: 187–192.

Reisig, D. D. 2011. Insecticidal management and movement of the brown stink bug, Euschistus servus, in corn. J. Insect Sci. 11: 168.

Sedlacek, J. D., and L. H. Townsend. 1988. Impact of Euschistus servus and E. variolarius (Heteroptera : Pentatomidae) Feeding on Early Growth Stages of Corn. J. Econ. Entomol. 81: 840–844.

Tillman, P. G. 2010. Composition and Abundance of Stink Bugs (Heteroptera: Pentatomidae) in Corn. Environ. Entomol. 39: 1765–1774.

Townsend, L. H., and J. D. Sedlacek. 1986. Damage to corn caused by Euschistus servus, E. variolarius, and Acrosternum hilare (Heteroptera: Pentatomidae) under greenhouse conditions. J. Econ. Entomol. 79: 1254–1258.

Van Duyn, J. W., R. W. Heiniger, S. R. Koenning, A. C. York, and S. J. Toth. 2005. Crop Profile for Field Corn in North Carolina. Crop Profile/PMSP database, USDA NIFA.

Wilde, A. G., K. Roozeboom, A. Aqeel, B. Gordon, G. Wilde, K. Roozeboom, A. Ahmad, M. Claassen, B. Gordon, W. Heer, L. Maddux, V. Martin, and P. Evans. 2007. Seed Treatment Effects on Early-Season Pests of Corn and on Corn Growth and Yield in the Absence of Insect Pests. J. Agric. Urban Entomol. 24: 177–193.

Chapter 3: Determining action thresholds for *Euschistus servus* management at different maize growth stages in Virginia.

Introduction:

Stink bugs are polyphagous insects that feed on a wide range of crop hosts including cotton, soybeans, and maize (McPherson and McPherson 2000). The two primary species of stink bug pests in Virginia and North Carolina are the brown stink bug, *Euschistus servus*, and the green stink bug, *Chinavia hilaris* (Blinka 2008). Extensive planting of transgenic maize and cotton for lepidopteran pest control, and its associated reduction in broad-spectrum insecticide use, has favored stink bug pest outbreaks (McPherson and McPherson 2000, Greene et al. 2001). In Virginia, crop landscapes can have multiple suitable hosts for stink bugs (e.g., wheat, soybeans, maize, cotton) grown in close proximity to each other within a single year. Further, high adoption of reduced or no tillage practices increased the amount of residue in fields and created undisturbed overwintering habitat. Increased crop residue can increase populations of stink bugs, as they prefer open overwintering sites (Jones and Sullivan 1981, Tillman 2011). The brown stink bug, *E. servus,* is the primary stink bug pest in Virginia maize and the focus of the current study.

Euschistus servus can infest maize throughout the growing season and cause injury at different stages of plant development. Early in vegetative growth stages (i.e., VE to V6), seedling maize plants are susceptible to injury and subsequent yield reduction when fed upon by *E. servus.* Damage results from mechanical and chemical injury to the terminal meristem from feeding early in plant development.

All feeding stages of *E. servus* are capable of causing injury, but adults have the largest impact and are most likely to be found in early maize (Sedlacek and Townsend 1988, Tillman 2010). *Euschistus servus* feeding can cause a decrease in mean extended leaf height and delay silking, resulting in a decrease in overall grain yield (Apriyanto et al. 1989a). The level of infestation, duration of infestation, and growth stage at the time of infestation are critical factors in the economic impact of *E. servus* feeding (Townsend and Sedlacek 1986, Annan and Bergman 1988, Sedlacek and Townsend 1988, Apriyanto et al. 1989b). Emergence (VE) and V2 are most susceptible to injury, V4 exhibits some injury, and at V6, plants typically compensate for injury (Annan and Bergman 1988, Sedlacek and Townsend 1988). One day of stink bug feeding can reduce yield by 50% when feeding is on recently emerged plants (Annan and Bergman 1988). Tillering, or multiple shoots extending from the same root system, is a commonly reported symptom of stink bug feeding in maize and contributes to reduced plant growth and grain yield (Townsend and Sedlacek 1986, Apriyanto et al. 1989a).

Grain yield and quality is further susceptible to damage from *E. servus* infestations during late vegetative (VT) and early reproductive stages (R1-R2) of development (Ni et al. 2010). In Virginia, small grain harvest often overlaps with the late vegetative and early reproductive stages of maize. Further, maize is often planted bordering winter wheat, where *E. servus* can overwinter and complete a full generation (Blinka 2008). Subsequent movement of *E. servus* into maize after wheat harvest is well documented and can result in large populations of *E. servus* during susceptible stages of maize development (Reisig 2011, Reisig et al. 2013). *Euschistus* *servus* infestation of three bugs per plant has been found to significantly reduce kernel weight and increase the percentage of discolored kernels at tassel (VT) (Ni et al. 2010). Economic levels of infestation have been identified as 0.5 *E. servus* per plant at VT and fewer at R1, suggesting that thresholds differ by growth stage. In contrast, Negron and Riley (1987) identified V15 as the most significant stage for southern green stink bug management. The growth stages of maize most susceptible to damage from stink bug injury, as well as the infestation levels capable of causing that damage, require further study in the mid-Atlantic US and modern maize hybrids.

There are currently no established economic injury levels for *E. servus* infestation in seedling maize in Virginia. North Carolina recommends that insecticides be applied when infestations reach 26% between VE and V6 (Reisig 2018). Nearly all maize seed sold in the US is coated with a systemic neonicotinoid insecticide, namely clothianidin and thiamethoxam (Douglas and Tooker 2015). Residues of these materials are present in shoot tissue, in low levels, up to three weeks post planting (Alford and Krupke 2017). The presence of these insecticides has the potential to mitigate early injury from *E. servus* (Van Duyn et al. 2005). Thus, existing prophylactic applications could eliminate the need for additional broadcast insecticides. The *E. servus* infestation levels capable of causing economic damage in the presence of seed applied insecticides is largely unexplored.

Virginia Cooperative Extension recommends that maize producers in Virginia treat for *E. servus* when one bug is found per four plants (25%) during ear formation and one bug per two plants (50%) from pollen shed to blister stage

(Flessner and Cahoon 2018). North Carolina has similar recommendations for action thresholds: 26% infestation between V14 and VT and 43% between R1 and R4 (Reisig 2018). Later stages of maize require aerial applications of insecticides, which can be costly and ineffective if there is low penetration and residual activity (Reisig 2011). Developing research-based action thresholds and determining the most critical growth stages for management are critical to avoid unnecessary production costs in the mid-Atlantic US.

The objectives of our study are to 1) determine the level of stink bug infestation that will cause economic impact on maize in seedling stages with and without seed-applied insecticides, and 2) determine the growth stage and infestation level that has the greatest economic impact on maize in late vegetative and early reproductive stages of development. Our findings will inform management strategies for *E. servus* in mid-Atlantic US maize at different times in the season.

Materials and Methods:

Insects

Adult *E. servus* were collected using sweep nets (38 cm diameter) from wheat and rye fields in May and June, and by hand from maize in late June – August in Suffolk and Southampton County, Virginia, USA. Insects were stored in mesh enclosures (30 cm x 30 cm x 30 cm) for transfer from the field. Approximately 20-30 individuals were placed in plastic containers, and maintained in environmental chambers (Percival I-36VL, Percival Scientific, Perry, IA, USA) at a photoperiod of 16:8 and at 25±2 based on rearing protocols from several previous studies (Munyaneza and McPherson 1994, Koppel et al. 2009) until use in experiments.

Each container was lined with a paper towel and contained a cotton ball soaked in distilled water and surface sterilized green beans (*Phaseolus vulgaris*). Green beans were sterilized by rinsing with 10% bleach solution for one minute and rinsed with distilled water three times.

Seedling Threshold

Field experiments were conducted at the Tidewater Agricultural Research and Extension Center in Suffolk, Virginia, USA in 2018 and 2019 to determine the economic impact of varying levels of *E. servus* infestations in seedling maize planted with and without seed-coated insecticides.

Treatments included the number of *E. servus* adults per three plants: 1) zero (control or 0% infestation), 2) one (33% infestation), 3) two (66% infestation), and 4) three (100% infestation). In 2018, treatments were arranged in a randomized complete block design and all seed was coated with a clothianidin seed treatment applied at 0.25 mg a.i./seed (Poncho 250, BASF, Research Triangle, NC, USA). In 2019, treatments were arranged in a split plot design. The presence or absence of a clothianidin seed treatment at 0.25 mg a.i./seed (Poncho 250, BASF) was the main plot effect. Infestation level was the sub-plot factor. In 2018, each infestation level was replicated five times and in 2019, each infestation level and seed treatment combination were replicated four times.

In 2018, the maize hybrid DKC 67-44 (Dekalb, Bayer CropScience, St. Louis, MO, USA) was planted on April 23. In 2019, KSC6614 (Kitchen Seed Company, Inc., Arthur, IL, USAz) was planted on April 23. In both hybrids, trait packages included

VTDoublePRO (Cry1A and Cry2Ab2) and Roundup Ready2 (glyphosate resistance). Seed was planted on 0.91 m rows with 0.15 m seed spacing.

At the V2 stage (approximately 14 days after planting), three adjacent maize plants were enclosed in a single 1.22 m x 0.61 m mesh sleeve supported with two fiberglass poles and secured at the top with binder clips. The bottom of the enclosure was covered with soil. *Euschistus servus* adults were added to enclosures at different levels of infestation (0, 1, 2, 3 per enclosure). Each plot contained one enclosure in 2018. Each plot contained two enclosures in 2019. Each enclosure was observed daily for *E. servus* mortality and feeding. Enclosures were observed for one minute. Dead and missing insects were replaced as needed. The number of insects found actively feeding (as determined by the number observed on the plant) was recorded. After seven days, enclosures were removed and flags were placed on either side of the three plants for later identification.

Reproductive Threshold

Experiments were conducted at the Tidewater Agricultural Research and Extension Center in 2018 and 2019 and at the Vernon G. James Research Station in Plymouth, North Carolina, USA in 2019. Experiments were designed to determine economic impact, if any, of varying levels of *E. servus* infestations in late vegetative and early reproductive maize growth stages. In Suffolk in 2018 and 2019, maize hybrid DKC 67-44 (Dekalb Seeds, St. Louis, MO, USA) was planted April 23 and April 17, respectively. In Plymouth in 2019, DKC 67-70 was planted on April 26. Both maize hybrids included the VTDoublePRO (Cry 1A and Cry2Ab2) and Round Up

Ready 2 trait packages. Seeds were planted on 0.91 m rows with 0.15 m seed spacing at both locations.

Infestation levels were evaluated at silking (R1) in 2018. In 2019, infestation levels were tested at different stages of late vegetative maize (V14 or the last vegetative stage, two to three days prior to tasseling, VT) and across the entire testing period (V14-VT). In 2018, treatments included the number of *E. servus* adults per plant (infestation level): zero (0% or control), one (100%), two (200%), and three (300%). In 2019, treatments included the number of *E. servus* applied per four plants: zero (0% or control), one (25%), two (50%), four (100%), and eight (200%). Insects were caged on plants using 20.32 cm x 40.64 cm mesh harvest bags (Midco Enterprises, Inc., Kirkwood, MO, USA). The sealed end of the harvest bag was cut to allow the mesh to slide over the entire plant to cover the area of the developing ear. The bags were secured at both ends with cotton string.

In 2018, treatments were arranged in a randomized complete block design. Each plot consisted of a single row of maize. Four plants were randomly chosen in each plot and plots were replicated four times. In 2019, treatments were arranged in a randomized split block design. The main plot factor was infestation level and the split plot factor was plant growth stage at the time of infestation. A single plot consisted of four rows. Four plants were randomly chosen in each row. All plants within the same plot had the same infestation rate and each row was infested at a different growth stage. Each plot was replicated four times. In the first three rows of each plot, infestations were applied for eight days beginning at the designated growth stage. In the fourth row, infestations were applied for 16 days spanning the entirety of tested growth stages. For infestation rates of less than one *E. servus* per plant (i.e., one and two *E. servus* per four plants), *E. servus* were moved between plants to simulate infestation of multiple plants by the same insect. For the 25% infestation (one *E. servus* per four plants), a single *E. servus* was placed in an enclosure on the first plant at the start of the infestation period. Every two days, it was moved to one of the remaining three enclosures of the same row. For 50% infestation (two *E. servus* per four plants), a single *E. servus* was placed into each of the first two enclosures. Following four days, they were moved to the remaining two enclosures. All enclosures were checked daily for mortality and dead *E. servus* were replaced. No *E. servus* escaped during the experiment at either location. All *E. servus* were removed at the end of the designated infestation period. Enclosures remained on the plant until all growth stages had been completed. Upon the removal of *E. servus* in the final treatment, enclosures were removed and plants were marked with flagging tape for later harvest.

Data Collection

Seedling threshold

Once enclosures were removed, each plant was measured from the soil level to the tip of the tallest extended leaf (extended leaf height). Leaf injury characteristic of *E. servus* feeding (holes), the number of stunted plants, and number of tillers were recorded. In 2019, extended leaf height, leaf injury, stunted plants, and number of tillers were recorded following enclosure removal (week 1), every seven days for the three weeks following enclosure removal (weeks 2-4), and at terminal plant height (week 12 or maturity) to determine when and if plants compensated for injury.

Once plants reached physiological maturity, as determined by kernel black layer and 15-20% moisture content, primary ears (i.e., the top ear on each plant) were hand harvested. Ears were stored in plastic containers with dichlorvos strips (Hot Shot No-pest Strips, Spectrum Brands, Madison, WI, USA) to minimize grain pests until processing. Four measurements were taken for each ear: 1) number of kernel rows, 2) number of kernels per row, 3) average kernel weight (as determined by a 100 kernel weight sample), and 4) kernel moisture % (Dickey-john mini GAC 2500, DICKEY-john, Auburn, IL, USA). Yield was calculated using the formula:

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

Yields were standardized to 15.5% moisture. Ear weight and number of discolored kernels were recorded in the reproductive threshold experiment in 2019. For the seedling experiment, one enclosure (three adjacent plants) was considered an experimental unit, and the responses for all early season measurements as well as yield components were averaged for analysis.

Statistical analysis

Seedling Threshold

Mean extended leaf height (cm) and yield (kg/h) were analyzed using separate linear mixed effect models using the package lme4 (Bates et al. 2015) in R

(R Core Team, 2017). Additionally, yield was analyzed using a separate linear model to estimate an action threshold.

Plant injury as a result of stink bug feeding (leaf holes, stunted plants, and tillering) was analyzed using generalized linear mixed effect models fitted to a binomial distribution using the lme4 package in R. Models were optimized using the package optimx (Nash and Varadhan 2011). In 2018, loglikelihood ratio tests were used to determine the significance of infestation level. In 2019, a full factorial model for fixed effects was used. A stepwise regression procedure was used to determine significance of model terms based on logliklihood ratio tests. Effects of the remaining terms on the odds of plant injury were estimated as odds ratios with 95% confidence intervals. Odds ratios indicated the effect of infestation level and insecticide treatment on plant injury and how plant injury changed throughout the season.

In 2018, the fixed effect was infestation level. In 2019, the fixed effects were infestation level, insecticidal seed coating, observation week (i.e., the time since enclosures were removed), and all interactions. The random effect in both years was blocking factor. Visual inspection of residual plots for models did not reveal deviation from model assumptions. When fixed effects were significant, estimated marginal means were determined using the package emmeans (Lenth 2019) and Tukey adjusted pairwise comparisons were made at $\alpha = 0.05$. Comparisons were made within each year of the experiment. Data from both years were combined to estimate economic threshold levels.

Reproductive Threshold

Yields were analyzed with linear mixed effect models using the package lme4 (Bates et al. 2015) in R (R Core Team, 2017). Yield was additionally analyzed using a separate linear model at each growth stage to estimate an economic threshold. Percentage of discolored kernels was analyzed using a beta regression in the package betareg (Cribari-Neto and Zeileis 2010) and a stepwise regression was performed to determine which, if any, fixed effects were significant based on logliklihood ratio tests. In 2018, the fixed effect was infestation level and the random effect was blocking factor. In 2019, the fixed effects were growth stage, infestation level, and their interaction. Random effects in 2019 were blocking factor and location (VA or NC). Visual inspection of residual plots did not reveal deviations from model assumptions. When fixed effects were significant, estimated marginal means were determined using the package emmeans, and Tukey adjusted pairwise comparisons were made at $\alpha = 0.05$. Figures were generated using back-transformed means in ggplot2 (Gómez-Rubio 2017).

Results:

Seedling Threshold

Plant Height

In 2018, infestation level did not affect plant height ($F = 1.00$; $df = 3,12$; $p = 0.425$) (Fig. 1). In 2019, infestation level ($F = 22.03$; $df= 3,277$, $p < 0.001$), insecticide treatment ($F = 9.81$; $df = 1,277$, $p = 0.002$), observation week ($F = 4199.56$; $df= 4, 277$, $p < 0.001$), and the interaction of infestation level and insecticide treatment ($F= 3.60$, $df = 3,277$, $p = 0.014$) affected plant height. The interaction of infestation level and observation week ($F = 1.07$; $df = 12,277$; $p = 0.386$), insecticide treatment and observation week ($F = 0.30$; $df = 4,277$; $p = 0.876$), and the interaction of all three ($F = 0.19$; $df = 12,277$; $p = 0.999$) did not affect plant height (Table 1 and 2).

Plant Injury

In 2018, infestation level affected leaf injury ($X^2 = 13.21$, $df = 3$, $p = 0.004$) and number of stunted plants ($X^2 = 9.68$, $df = 3$, $p = 0.022$).

In 2019, infestation level ($X^2 = 126.48$, $df= 3$, $p < 0.001$), sampling week ($X^2 = 181.83$, $df= 4$, $p < 0.001$), and insecticide treatment ($X^2 = 81.50$, $df= 1$, $p < 0.001$) affected leaf injury (Table 2). Only sampling week ($X^2 = 25.31$, $df= 4$, $p < 0.001$) affected the number of stunted plants (Table 3).

No variable affected the number of tillered plants in both years and tillering was determined to be a poor indicator of early *E. servus* injury (data not shown).

Yield

In 2018, infestation level did not affect yield ($F = 1.44$; $df = 3,12$; $p = 0.280$) (Fig 2). In 2019, infestation level ($F = 1.38$; $df = 3,56$; $p = 0.277$) and insecticide treatment ($F = 0.14$; $df = 1,56$; $p = 0.715$) did not affect grain yield, but the interaction of infestation level and insecticide treatment ($F = 3.42$; $df = 3,56$; $p = 0.036$) had an effect (Fig. 3).

Threshold estimation

The regression of infestation level and grain yield was assessed for both years separately and combined (Fig 4). Insecticide treatment alone did not affect grain yield in 2019, so data with and without insecticide treatments were combined from both years. In 2019, grain yield was not correlated with the *E. servus* infestation level (r^2 = 0.04 and p = 0.059). In 2018 and in both years combined, grain yield was correlated with infestation level (2018, y = 19419 – 5939x [r^2 = 0.15, n = 18, p = 0.048]; 2018 and 2019, y = 17188 – 3069x [r^2 = 0.08, n = 82, p = 0.006]). The regression for 2018 and 2019 combined, although weakly correlated, indicated that seedling stages of maize are critical for *E. servus* management. Thus, results from the combined data are used for subsequent discussion.

Reproductive Threshold

Yield

In 2018, infestation level (F = 0.97; df = 3,9; p = 0.448) did not affect grain yield (fig 5). In 2019, infestation level (F = 5.61; df = 4,136; $p < 0.001$) and growth stage (F = 2.83; df = 3,136; p = 0.041) affected grain yield and the interaction of infestation level and growth stage (F= 1.16; df = 12,136; p = 0.316) did not. The same trend was observed in ear weight: infestation level (F = 7.58; df = 4,136; $p < 0.001$) and growth stage (F=3.58; df = 3,136; p = 0.016) affected ear weight, but their interaction did not (F = 0.64, df = 12,136; p =0.801). The 200% infestation level, when applied across all the tested growth stages, resulted in a significant reduction in yield and ear weight (4,998 kg/ha and 48 g, respectively) (Table 5 and 6). The 100% infestation level and lower did not reduce yield or ear weight at any tested growth stage or stages.

Discolored Kernels

In 2019, the percentage of discolored and sunken kernels was affected by infestation level (X^2 = 15.53; df = 4; p = 0.004). Growth stage (X^2 = 5.67; df = 3; p = 0.129) and the interaction of the growth stage and infestation level (X^2 = 15.94; df =

12; $p = 0.019$) did not affect the percentage of discolored kernels. Overall, the percentage of discolored or sunken kernels was relatively low ($\leq$ 1%). Pairwise comparisons were made between infestation levels and averaged across all growth stages (Fig. 6).

Threshold estimation

In 2018, grain yield was not correlated with the infestation level (Fig. 7). In 2019, grain yield was not correlated with the infestation level at V10/V12 ($r^2 < 0.05$; $p = 0.211$), pre-tasseling ($r^2 < 0.05$; $p = 0.273$), or VT ($r^2 < 0.05$; $p = 0.3125$). The grain yield was correlated with infestation level when the infestation was applied across all the tested growth stages ($y = 15864 – 2328x$ [$r^2 = 0.35$, $n = 38$, $p < 0.001$]) (fig. 8).

Discussion:

Seedling Threshold

Our study confirmed findings documented in several previous studies that *E. servus* can affect maize grain yield when it infests seedling stages (Townsend and Sedlacek 1986, Sedlacek and Townsend 1988, Apriyanto et al. 1989a, Apriyanto et al. 1989b). It is important to note that several studies identified tillering as characteristic of stink bug feeding (Townsend and Sedlacek 1986, Sedlacek and Townsend 1988, Apriyanto et al. 1989a, Apriyanto et al. 1989b). We observed no effect on tillering across all infestation levels with and without seed treatments. Yield reduction from early stink bug feeding has been related to the production of tillers and a subsequent delay in silking (Apriyanto et al. 1989a); however, tillering was not widespread or consistent across any infestation level and we observed a reduction in grain yield. Yield decreases in our study likely a resulted from decreased plant growth and not a developmental delay as previously described.

Plant height was not affected by infestation level in 2018, but in 2019, it was affected by the interaction of infestation level and insecticide treatment. It has been suggested that systemic seed treatments can increase early plant growth in the absence of insect pressure, perhaps as a result of other ingredients applied in the seed coating. Few datasets are available on maize seed treatments that include fungicides applied with and without insecticides. We concluded that the effect on plant height we observed is likely due to *E. servus* feeding because the effect of neonicotinoids on plant growth is inconclusive and several studies have suggested nonexistent (Cox et al. 2007, Wilde et al. 2007).

Infestation level affected plant injury in both years, with infestations of 100% (1 *E. servus*/plant) resulting in up to 1400% higher odds of leaf injury. When comparing maize planted with and without insecticide seed treatments in 2019, the odds of leaf injury were reduced by 83% in the presence of insecticidal seed coating. This result suggests that a low rate of clothianidin does mitigate the impact of early feeding to an extent. Although the incidence of plant injury was significantly reduced by the insecticide treatment, plant height was still reduced by insect feeding. Thus, the severity of feeding was reduced by insecticides, but not eliminated.

The interaction of insecticide treatment and the infestation level had a significant impact on the grain yield in 2019. The same infestation levels in the did not have a significant impact on the yield in 2018, but we did not test untreated seed. This suggests that the impact of infestation level in seedling maize is dependent on the presence of insecticides. Yet, yield was reduced in plants with insecticide and not in plants without insecticide, which was not our expected result. This may have resulted from inconsistent feeding behavior from field-collected populations of *E. servus* or differences in plant response between treated and untreated plants. Although results were not consistent with our expectations, they suggest that the insecticidal seed treatment affects the damage caused by *E. servus* feeding and further study is necessary to understand how neonicotinoids impact both *E. servus* feeding and plant response.

Yield regressions indicated that, in the presence of the minimum rate of the most commonly applied seed treatment (0.25 mg a.i./seed clothianidin), a 10% increase in infestation level during the V2 growth stage results in a yield loss of 307 kg/ha (4.89 bu/ac). Based on the combined data for 2018 and 2019, if an insecticide application costs $20 a hectare and the current (January 2020) grain price is $0.15/kg ($3.87/bu), the economic injury level is an 11% infestation level (11 bugs/100 plants). It is important to note that the price of insecticide applications and the price of grain fluctuate over time. Our results provide support for seedling management recommendations for *E. servus* made in North Carolina (Reisig 2018). The cost of the seed treatment is not included in this calculation because most commercial maize seed is cannot be purchased without at least this rate of insecticidal coating. Since clothianidin is offered at higher rates (up to 1.25 mg a.i./seed), further studies could examine how insecticide rate influences *E. servus* thresholds in seedling maize. In areas where *E. servus* are a perennial pest, higher rates of seed treatments could be an alternative to applying foliar insecticides.

Management of *E. servus* in seedling maize is complicated by the difficulty in scouting for them. Current recommendations include observing 100 whole plants between emergence and V6 to determine the infestation level (Van Duyn et al. 2005, Flessner and Cahoon 2018). In high residue fields, *E. servus* can shelter at the base of plants and in field residues. *Euschistus servus* have been found to be more active early in the day in the evening (Ni et al. 2016) and are likely sheltering in residues during mid-day. How *E. servus* activity changes throughout the day can aid scouting efforts and thus, management decisions.

Our study provides evidence that *E. servus* feeding can impact early plant growth and subsequent grain yield in the mid-Atlantic U.S. We also provide research-based thresholds for Virginia grain producers to make informed management decisions for *E. servus* during seedling stages of maize development.

Reproductive Threshold

Our study confirmed findings documented in several previous studies that *E. servus* can affect maize grain yield (Negrón and Riley 1987, Ni et al. 2010). The maize growth stages that we determined to be most susceptible to *E. servus* were earlier than previously documented. Ni et al. (2010) reported an economic threshold of 0.5 bugs/ear at VT and fewer at R1. Our study indicated that there was no impact on grain yield from *E. servus* infestation levels at R1. This is supported by the lack of correlation between infestation level and grain yield at this stage. Thus, in Virginia, the R1 growth stage is not susceptible to economic injury as previously documented in Georgia. The difference in findings between the two regions may be related to differences in *E. servus* feeding activity and when it overlaps with different growth stages.

Based on our results at the R1 growth stage, and previous documentation of stink bugs effecting grain yield when feeding on earlier growth stages of maize (Negrón and Riley 1987), our 2019 experiment was designed to determine the effects of *E. servus* infestations at late-vegetative growth stages. We observed that grain yield was only impacted when infestations of *E. servus* span V12 (or last vegetative growth stage) through VT. The same trend was observed in ear weight. The percentage of discolored kernels was unaffected by the growth stage, indicating that the injury affected overall size of the ear, but not the quality of the individual kernels. Since we could not determine the most critical stage for injury during this period, it is likely the cumulative effect of continued feeding that decreases overall grain yield. This suggests that extended periods of infestation are required in relatively high densities to cause economic injury.

Our regression between yield and infestation level indicated that a 10% increase in infestation level for a prolonged period from late vegetative stages through pollination, resulted in a loss of 232.8 kg/ha (3.71 bu/ac). At an application cost of $22/ha and at the current (January 2020) price of grain ($0.15 per kg or $3.87 per bushel), the economic injury level for these growth stages would be around 15% (15 bugs/100 plants). This is not a fixed economic threshold, as the price of insecticide application and grain vary from year to year. This action threshold provides research based support for management recommendations made in North Carolina (Reisig 2018)

Effective scouting for *E. servus* in reproductive stages of maize involves examining plants at several different places in the field. Scouting can often be confined to one leaf above and below the developing ear (Blinka 2008), which is more difficult in late vegetative stages when the developing ear is not yet visible or obvious. There is a pronounced edge effect commonly seen with *E. servus* infestations (Reisig et al. 2013, Ni et al. 2016). Based on the results of the current study, scouting should begin around V10 and continue through pollination. Persistent infestations for several weeks prior to the last vegetative stages of maize may require insecticidal management.

The only application method for late stages of maize development are aerial. This method can be ineffective due to minimal penetration into the canopy, as well as low residual activity of labeled products (Reisig 2011). The minimal effectiveness of insecticide application at these stages emphasizes the need for scouting to avoid potentially ineffective applications for sub-economic levels of infestation.

Consideration should be made for the effect of insecticides on natural enemies of stink bugs when making management decisions. Up to 90% of *E. servus* egg masses can be parasitized in maize (Koppel et al. 2009). Products labeled for foliar management of *E. servus*, specifically insecticides in the pyrethroid class, are highly toxic to natural enemies. Depending on the timing of planting and availability of other hosts, susceptible reproductive stages of maize could be fed on by *E. servus* adults prior to oviposition (Tillman 2010) and parasitism and predation of egg masses could reduce the infestation levels below economic levels.

Our study documented yield loss in growth stages of maize that have not been previously identified. Determining when to manage pests is as critical as identifying the damaging infestation levels. Our study, conducted in two states in 2019, provides mid-Atlantic grain producers with information on how to make management decisions on managing *E. servus* in late stages of maize development.

Tables and Figures

Figure 1. Mean extended leaf height (cm) of maize plants exposed to *E. servus* adults at different levels of infestation, Suffolk, 2018. Three maize plants were enclosed at growth stage V2 for one week with zero, one, two, or three adult *E. servus*. Extended leaf height was measured following cage removal. There was no significant effect of infestation level on mean leaf height at $\alpha = 0.05$ ($F = 1.00$; $df = 3,12$; $p = 0.425$).

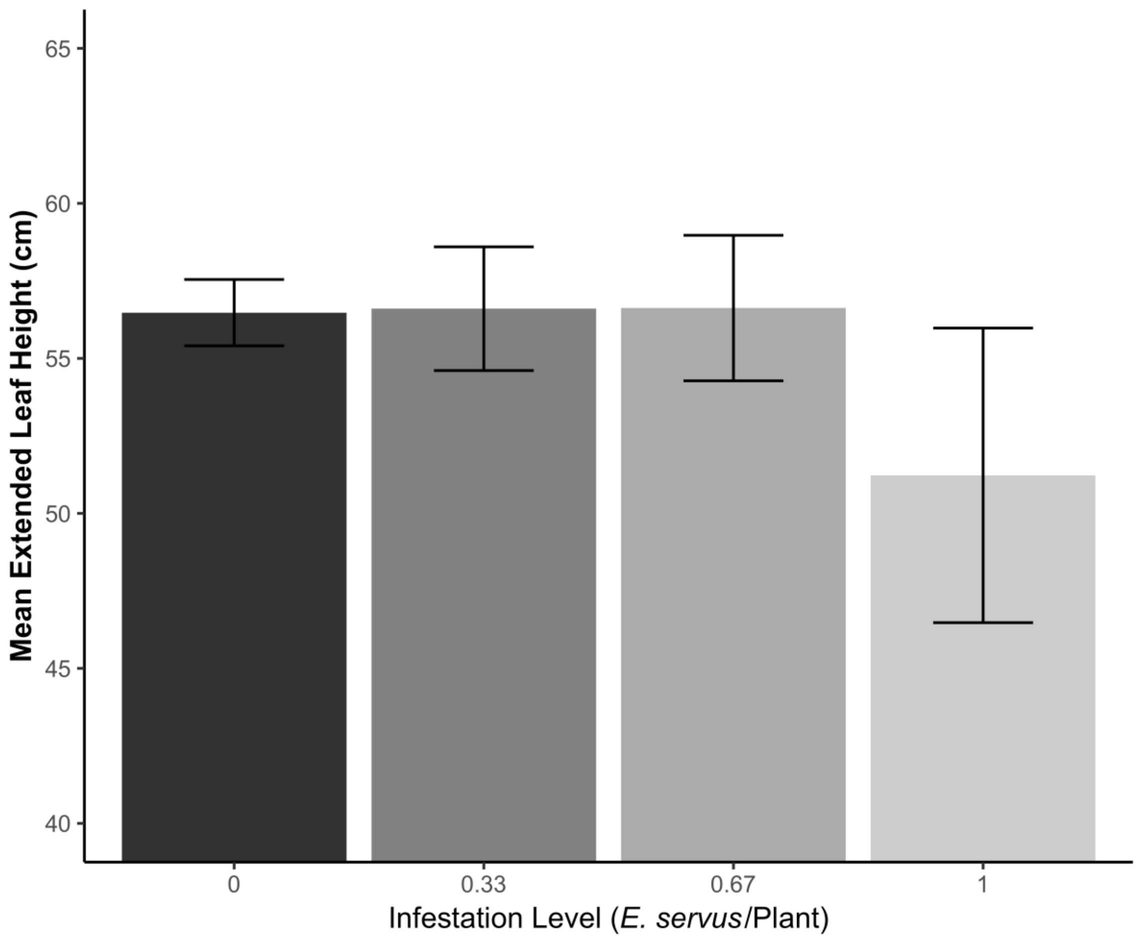


Table 1. Effect of infestation level (number of *E. servus* per plant), insecticide seed treatment, observation week (i.e., weeks after infestation), and interactions of these variables on the mean extended leaf height of maize plants exposed to E servus adults for one week at growth stage V2, Suffolk 2019. Bold values are significant at alpha = 0.05.

	F - value	df	p-value
Infestation level	22.03	3, 277	**< 0.001**
Insecticide treatment	9.81	1, 277	**0.002**
Observation week	4199.56	4, 277	**< 0.001**
Infestation Level * insecticide treatment	3.60	3, 277	**0.014**
Infestation Level * observation week	1.07	12, 277	0.386
Insecticide Treatment * observation week	0.30	4, 277	0.876
Infestation Level * observation week * insecticide Treatment	0.19	12, 277	0.999

Table 2. Mean extended leaf height (cm) (± SE) of maize planted with and without a clothianidin seed treatment applied at 0.25 mg/a.i. per seed (Poncho 250, BASF, RTP, North Carolina) and exposed to *E. servus* adults at different infestation levels (i.e., number of bugs per plant) by observation week (i.e., weeks after infestation), Suffolk 2019. Three maize plants were enclosed using mesh cages at the V2 growth stage and exposed to *E. servus* for one week at the given infestation level. Week 1 represents the height of plants immediately following removal of enclosures. Means followed by different letters within the same week of measurement are different at α = 0.05.

Seed treatment	Infestation level	Mean extended leaf height (cm) (±SE) by sampling week				
		1	2	3	4	5
Yes	0%	53.3a (0.9)	86.2a (1.1)	116.6a (1.8)	152.7a (1.8)	252.2a (1.8)
	33%	52.3a (1.2)	79.8ab (3.2)	111.6ab (92.2)	148.3ab (3.1)	251.4a (4)
	67%	48.0a (2.1)	73.3abc (3.6)	103.9ab (4.7)	139.4ab (5.4)	248.4ab (5.1)
	100%	46.4a (2.0)	69.8bc (4.5)	101.0b (4.0)	138.4ab (3.9)	239.9ab (3.9)
No	0%	48.7a (1.4)	79.7ab (2.0)	109.1ab (1.7)	147.9ab (1.7)	248.0ab (2.4)
	33%	45.3a (0.8)	68.9bc (2.3)	103.4ab (3.0	142.7ab (4.3)	249.0ab (3.3)
	67%	48.6a (1.5)	75.7abc (1.7)	107.3ab (2.8)	143.2ab (3.8)	250.1a (2.9)
	100%	45.9a (1.9)	63.1c (3.9)	98.1b (4.8)	136.5b (6.0)	234.7b (9.2)

Table 3. Odds ratios (± 95% CI) of leaf injury at different *E. servus* infestation levels (i.e., number of bugs per plant) and observation week (i.e., weeks after infestation), on maize planted with and without clothianidin applied on the seed coating at 0.25 mg a.i./seed (Poncho 250, BASF, RTP, North Carolina), Suffolk, 2019. Three maize plants at the V2 growth stage were enclosed with mesh cages and exposed to *E. servus* adults for one week. Odds ratios are in reference to maize with no *E. servus* exposure and no insecticide treatment applied that were measured at time enclosures were removed.

Infestation level	Odds ratio (95% CI)	p-value
33%	0.08 (0.03 to 0.22)	**< 0.001**
67%	18.75 (5.71 to 61.61)	**< 0.001**
100%	14.33 (4.35 to 47.27)	**< 0.001**
Observation week		
1	2.11 (1.26 to 3.51)	**0.008**
2	1.51 (0.91 to 2.51)	0.140
3	0.97 (0.58 to 1.61)	0.900
11	0.00 (0.00 to inf)	0.989
Insecticide Treatment		
Yes	0.17 (0.08 to 0.36)	**< 0.001**

Table 4. Odds ratios (± 95% CI) of stunted plants at different *E. servus* infestation levels (i.e., number of bugs per plant) and observation week (i.e., weeks after infestation), on maize planted with and without clothianidin applied on the seed coating at 0.25 mg a.i./seed (Poncho 250, BASF, RTP, North Carolina), Suffolk, 2019. Three maize plants at the V2 growth stage were enclosed with mesh cages and exposed to *E. servus* adults for one week. Odds ratios are in reference to maize with no *E. servus* exposure and no insecticide treatment applied that were measured at time enclosures were removed.

Infestation Level	Odds Ratio (95% CI)	P Value
33%	5.03 (0.18 to 137.77)	0.339
67%	3.79 (0.13 to 113.44)	0.442
100%	18.04 (0.76 to 427.11)	0.073
Weeks after infestation		
1	4.00 (1.13 to 14.12)	**0.032**
2	0.48 (0.07 to 3.08)	0.437
3	0.00 (0.00 to inf)	0.997
11	0.48 (0.07 to 3.08)	0.437
Insecticide Treatment		
Yes	0.24 (0.04 to 1.52)	0.129

Figure 2. Mean grain yield of maize infested with different levels of *E. servus,* Suffolk, Virginia, 2018. Three maize plants at the V2 growth stage were enclosed with mesh cages and exposed to *E. servus* adults for one week. Error bars represent standard error. There were no differences between the infestation levels at $\alpha = 0.05$ ($F = 1.44$; $df = 3,12$; $p = 0.280$).

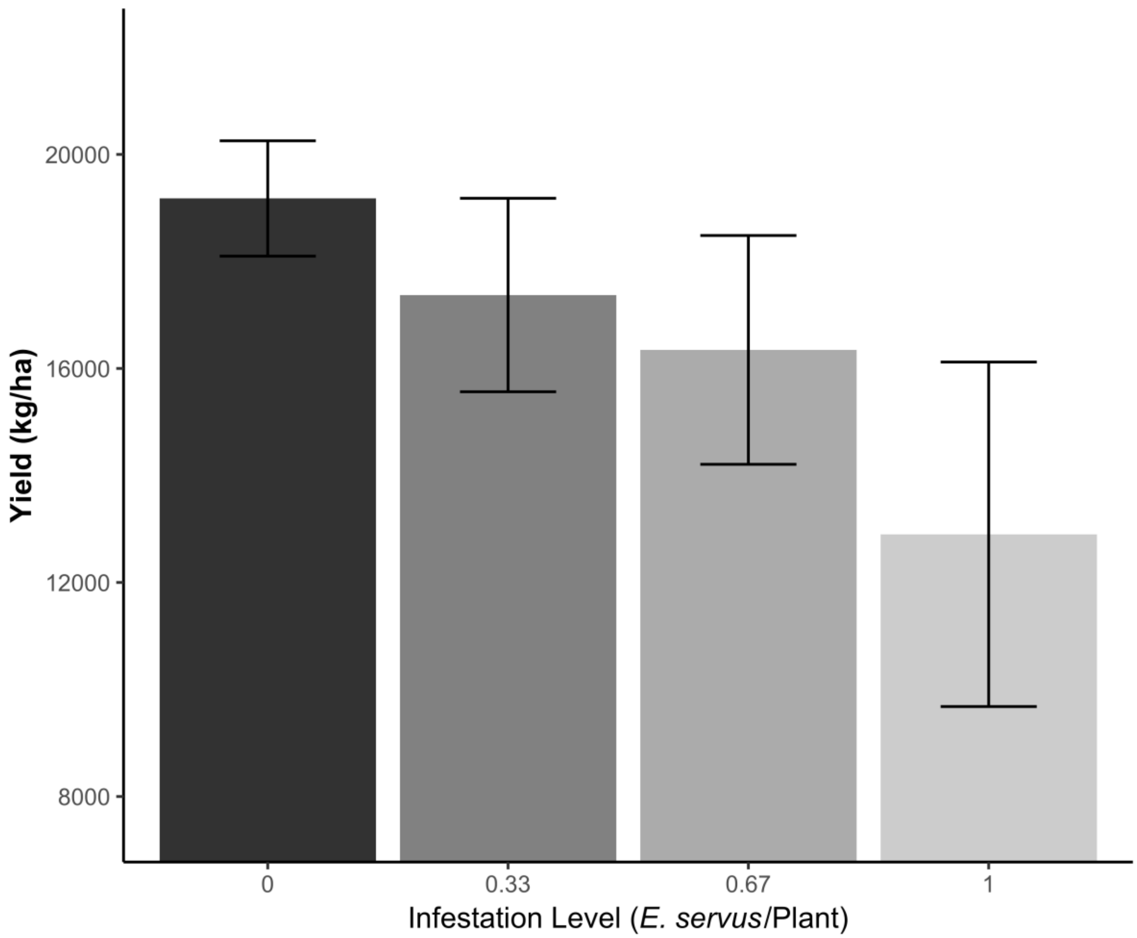


Figure 3. Mean grain yield of maize planted with and without clothianidin applied in the seed coating at 0.25 mg a.i./seed (Poncho 250, BASF, RTP, North Carolina) at different *E. servus* infestation levels (i.e., number of bugs per plant), Suffolk, Virginia, 2019. Three maize plants at the V2 growth stage were enclosed with mesh cages and exposed to *E. servus* adults for one week. Error bars represent standard error. Error bars with different letters above them within each insecticide treatment (yes or no) are different at $\alpha = 0.05$.

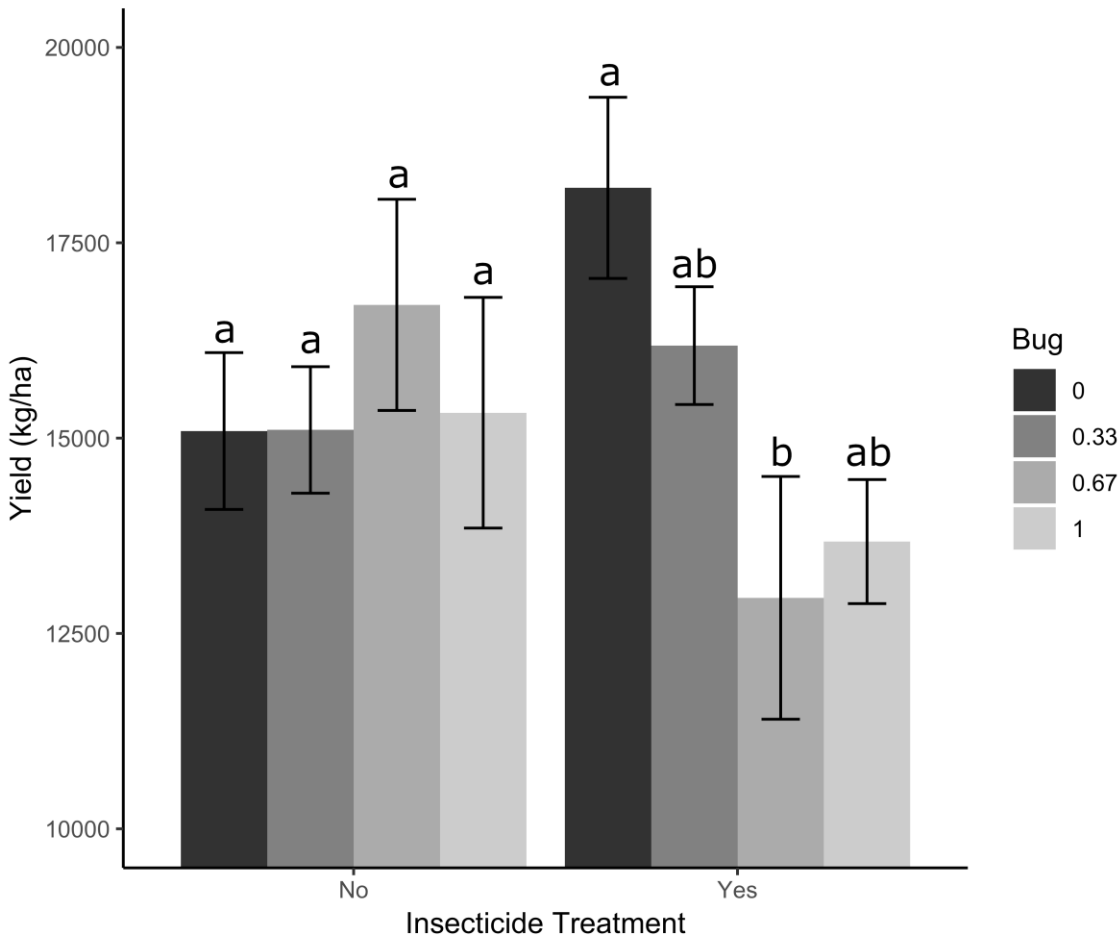


Figure 4. Relationship between grain yield (kg/ha) and *E. servus* infestation level (i.e., number of bugs per plant), Suffolk, Virginia, 2018 and 2019. Data are from seed treated with clothianidin at the rate of 0.25 mg a.i./seed (Poncho 250, Bayer CropScience, RTP, North Carolina) in 2018, and treated and untreated seed combined in 2019. Three maize plants at the V2 growth stage were enclosed with mesh cages and exposed to *E. servus* adults for one week. Summary statistics are given for the combined data set (2018/2019).

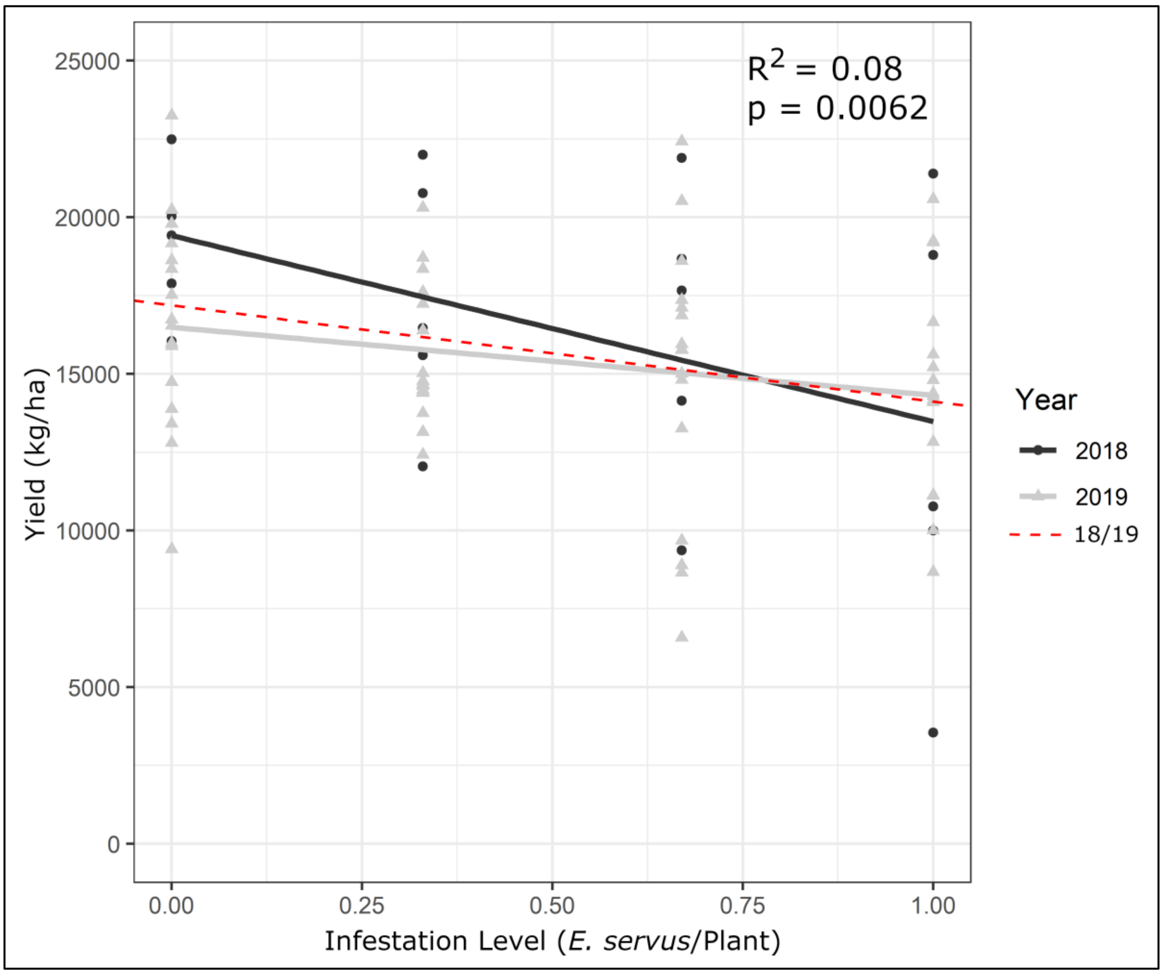


Figure 5. Mean grain yield of maize infested with different levels of *E. servus*, Suffolk, Virginia, 2018. Adult *E. servus* were caged on maize plants in the region of the developing ear for one week beginning at growth stage R1. Error bars represent standard error. There were no differences between the infestation levels at $\alpha = 0.05$ ($F = 0.97$; $df = 3,9$; $p = 0.448$).

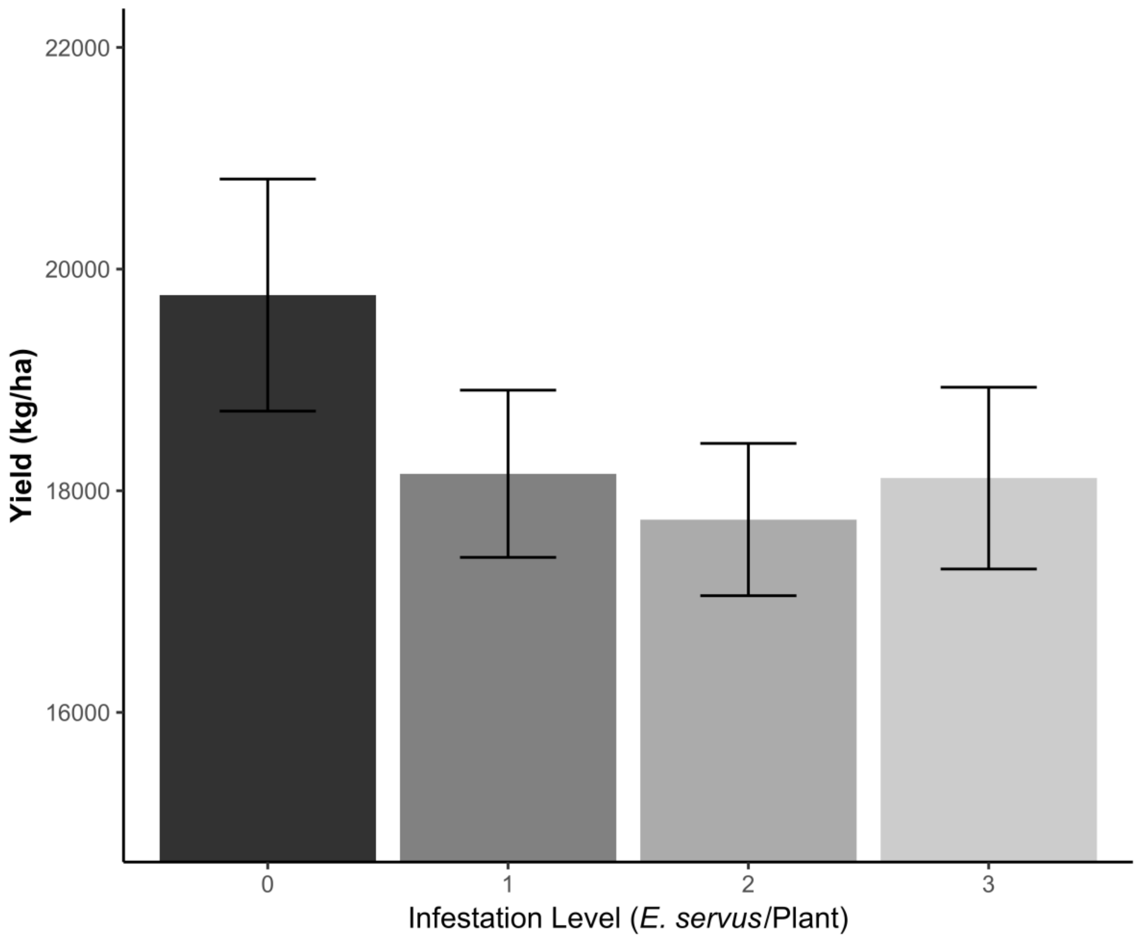


Table 5. Mean grain yield (kg/ha) (±SEM) of maize exposed to varying levels of *E. servus* infestation at different growth stages. Data were pooled from Plymouth, North Carolina and Suffolk, Virginia in 2019. Adult *E. servus* were caged on maize plants in the region of the developing ear for one week beginning at the growth stage indicated. Means followed by the same letter within each growth stage are different at α = 0.05.

Growth Stage					Infestation Level
	0%	25%	50%	100%	200%
V10/V12	14804.9a (1136.5)	14207.9a (881.1)	15214.4a (912.5)	13358.2a (786.0)	13458.3a (940.8)
Pre-Tasseling	13546.1a (1311.3)	14967.8a (885.6)	15507.9a (648.0)	15045.5a (696.5)	12752.5a (1343.6)
VT	15463.8a (1056.4)	15237.7a (799.9)	16241.0a (960.8)	15816.7a (457.6)	14418.3a (665.7)
All Stages	16079.2a (1055.1)	14691.5a (759.4)	14900.0a (704.7)	13837.7ab (716.8)	11081.3b (915.7)

Table 6. Mean ear Weight (g) (±SEM) of maize exposed to varying levels of *E. servus* infestation at different growth stages. Data were pooled from Plymouth, North Carolina and Suffolk, Virginia in 2019. Adult *E. servus* were caged on maize plants in the region of the developing ear for one week beginning at the growth stage indicated. Means followed by the same letter within each growth stage are different at α = 0.05.

Growth Stage	Infestation Level				
	0%	25%	50%	100%	200%
V10/V12	230.8a (9.6)	221.7a (8.5)	214.9a (9.4)	210.2a (11.0)	197.0a (10.0)
Pre-Tasseling	218.6a (10.0)	236.1a (4.7)	226.1a (9.2)	226.2a (6.3)	201.2a (16.3)
VT	222.9a (8.3)	235.1a (6.5)	242.9a (12.9)	239.6a (4.0)	208.1a (14.3)
All Stages	218.3a (8.3)	226.0a (8.5)	218.4a (10.3)	214.6ab (8.5)	178.2b (10.9)

Figure 6. Mean percentage of discolored kernels resulting from different *E. servus* infestation levels (i.e., number of bugs per plant), averaged across all tested growth stages, in Plymouth, North Carolina and Suffolk, Virginia in 2019. Error bars represent standard error. Bars with the same letter above them are not different at α = 0.05.

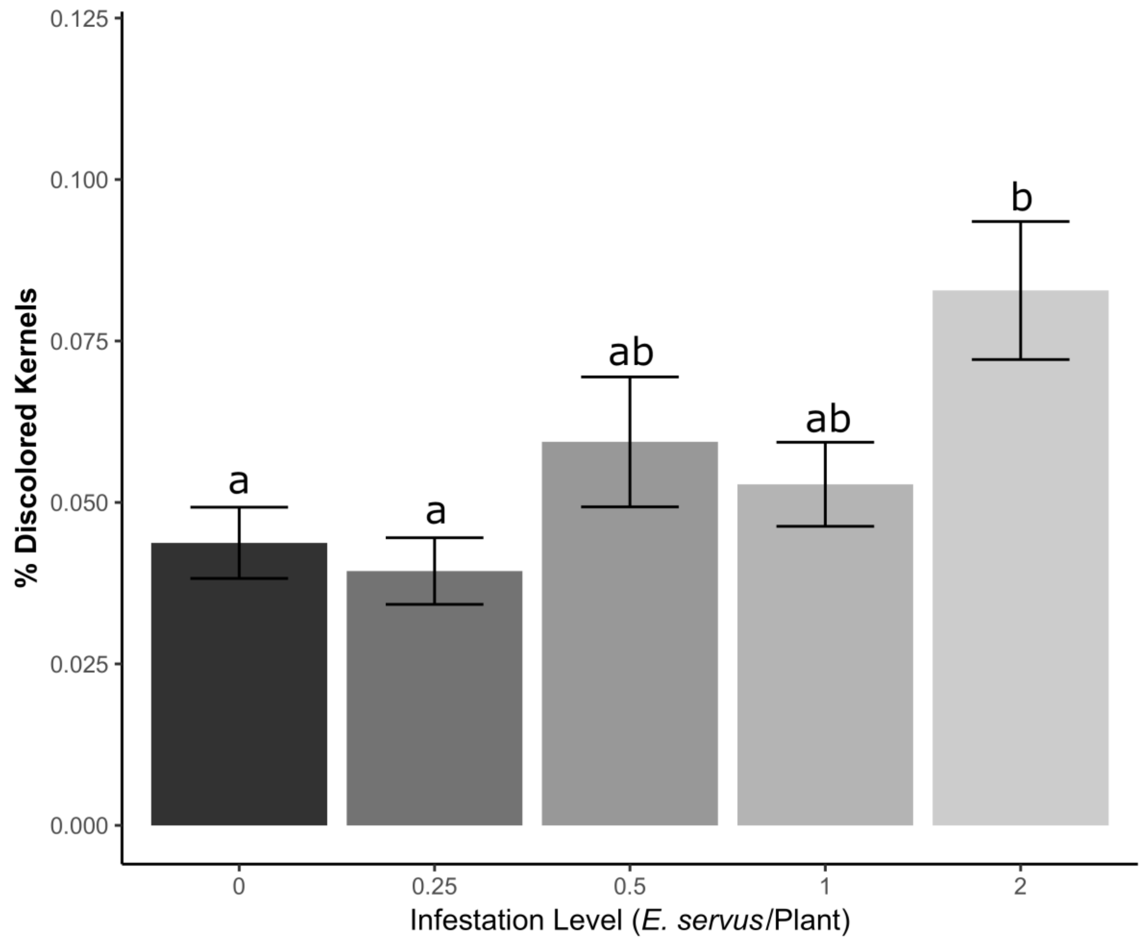


Figure 7. Relationship between grain yield (kg/ha) and *E. servus* infestation level (i.e., number of bugs per plant), Suffolk, Virginia, 2018. Adult *E. servus* were caged in the region of the developing ear on maize plants in the R1 stage for one week.

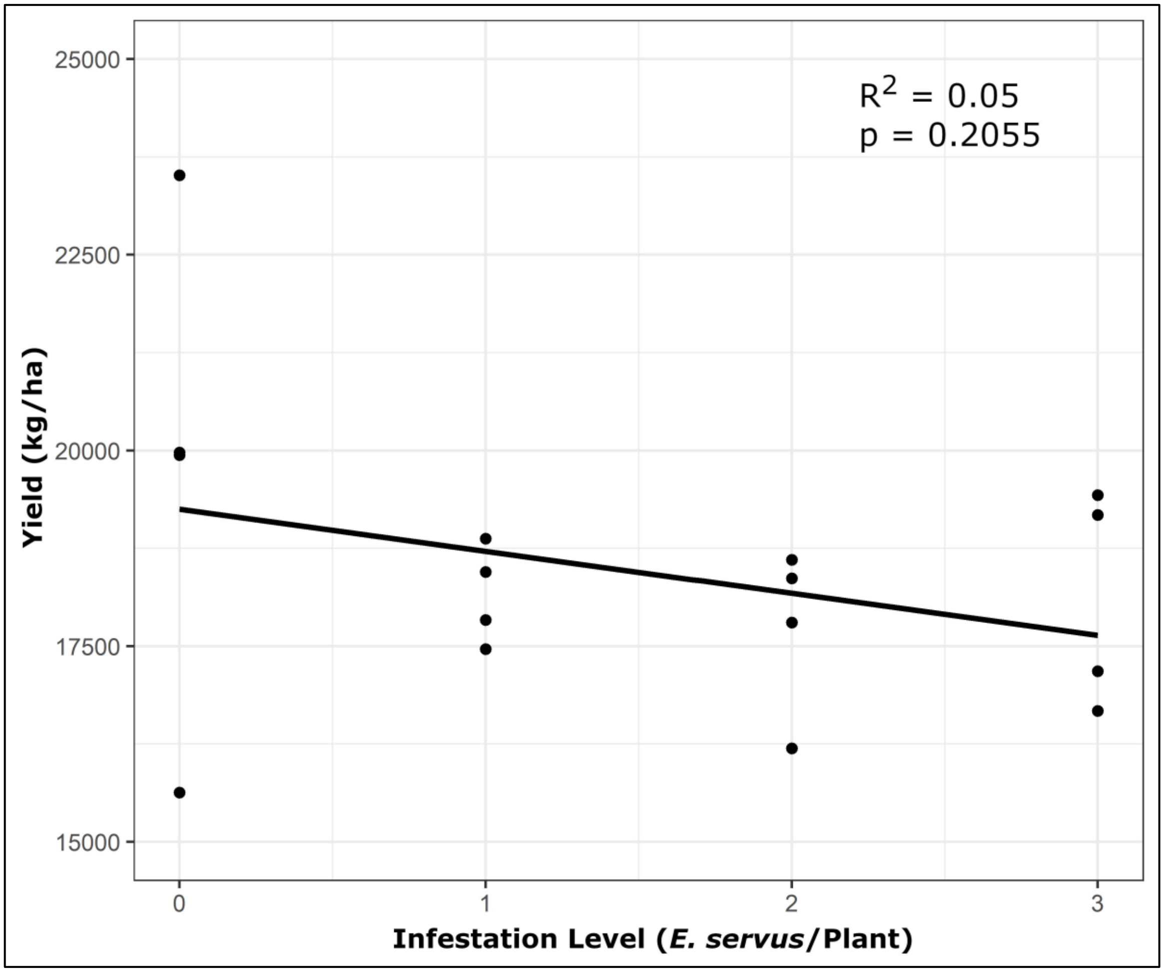
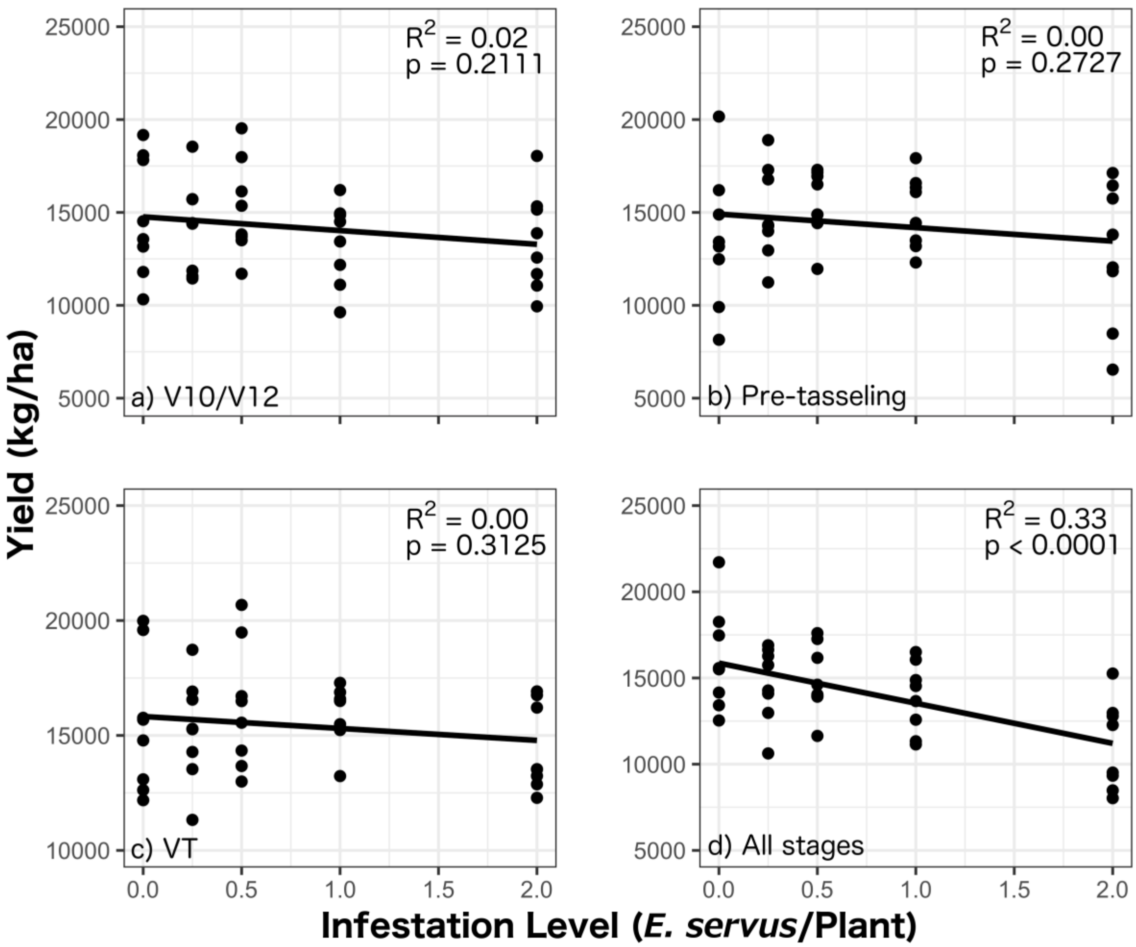


Figure 8. Relationship between grain yield (kg/ha) and *E. servus* infestation level (i.e., number of bugs per plant), Plymouth, North Carolina and Suffolk, Virginia in 2019. Adult *E. servus* were caged in the region of the developing ear on maize plants for one week beginning at the indicated growth stage. Growth stages included (1a) At terminal vegetative growth, (1b) just prior to tasseling and pollination, (1c) at full tassel, and (1d) across all the tested growth stages (terminal vegetative growth – full tassel).



References

Alford, A., and C. H. Krupke. 2017. Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS One. 12: 1–19.

Annan, I. B., and M. K. Bergman. 1988. Effects of the Onespotted Stink Bug (Hemiptera : Pentatomidae) on Growth and Yield of Corn. J. Econ. Entomol. 81: 649–653.

Apriyanto, D., J. D. Sedlacek, and L. H. Townsend. 1989a. Feeding Activity of Euschistus servus and E. variolarius (Heteroptera : Pentatomidae) and Damage to an Early Growth Stage of Corn. J. Kansas Entomol. Soc. 62: 392-399.

Apriyanto, D., L. H. Townsend, and J. D. Sedlacek. 1989b. Yield Reduction from Feeding by Euschistus servus and E . variolar ius (Heteroptera : Pentatomidae) on Stage V2 Field Corn. J. Econ. Entomol. 82: 445–448.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed effects models using lme4. J. Stat. Softw. 67.

Blinka, E. L. 2008. Biological and ecological studies on green stink bug, Acrosternum hilare, and brown stink bug, Euschistus servus (Hemiptera: Pentatomidae), in Eastern North Carolina cropping systems.

Cox, W. J., E. Shields, and J. H. Cherney. 2007. The effect of clothianidin seed treatments on corn growth following soybean. Crop Sci. 47: 2482–2485.

Cribari-Neto, F., and A. Zeileis. 2010. Beta Regression in R. J. Stat. Softw. 34: 1–24.

Douglas, M. R., and J. F. Tooker. 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. Field crops. Environ. Sci. Technol. 49: 5088–5097.

Flessner, M., and C. W. Cahoon. 2018. Pest Management Guide: Field Crops. Virginia Coop. Ext. (www.ext.vt.edu).

Gómez-Rubio, V. 2017. ggplot2 - Elegant Graphics for Data Analysis (2nd Edition) . J. Stat. Softw. 77: 2–5.

Greene, J. K., S. G. Turnipseed, M. J. Sullivan, and O. L. May. 2001. Treatment thresholds for stink bugs (Hemiptera: Pentatomidae) in cotton. J. Econ. Entomol. 94: 403–9.

Jones, W. A., and M. J. Sullivan. 1981. Overwintering habitats, spring emergence patterns, and winter mortality of some South Carolina Hemiptera. Environ. Entomol. 10: 409–414.

Koppel, A. L., D. A. Herbert, T. P. Kuhar, and A. K. Kamminga. 2009. Survey of Stink Bug (Hemiptera: Pentatomidae) Egg Parasitoids in Wheat, Soybean, and Vegetable Crops in Southeast Virginia. Environ. Entomol. 38: 375–379.

Lenth, R. 2019. Estimated Marginal Means, aka Least-Square Means. R package version 1.3.5.1. https://CRAN.R-project.org/package=emmeans

McPherson, J. E., and R. M. McPherson. 2000. Stink Bugs of Economic Importance in America North of Mexico. CRC Press.

Munyaneza, J., and J. E. McPherson. 1994. Comparative study of life histories, lab rearing, and immature stages of Euschistus servus and Euschistus variolarius (Hemiptera: Pentatomidae). Gt. Lakes Entomol. 26: 263–275.

Nash, J. C., and R. Varadhan. 2011. Unifying Optimization Algorithms to Aid Software System Users: optimx for R. J. Stat. Softw. 43.

Negrón, J. F., and T. J. Riley. 1987. Southern green stink bug, Nezara viridula (Heteroptera: Pentatomidae), feeding in corn. J. Econ. Entomol. 80: 666–669.

Ni, X., T. E. Cottrell, M. D. Toews, P. G. Tillman, and G. D. Buntin. 2016. Diurnal Activities of the Brown Stink Bug (Hemiptera : Pentatomidae) in and near Tasseling Corn Fields. J. Entomol. Sci. 51: 226–237.

Ni, X., K. Da, G. D. Buntin, T. E. Cottrell, P. G. Tillman, D. M. Olson, R. Powell, R. D. Lee, J. P. Wilson, and B. T. Scully. 2010. Impact of Brown Stink Bug (Heteroptera: Pentatomidae) Feeding on Corn Grain Yield Components and Quality. J. Econ. Entomol. 103: 2072–2079.

Reisig, D. D. 2011. Insecticidal management and movement of the brown stink bug, Euschistus servus, in corn. J. Insect Sci. 11: 168.

Reisig, D. D. 2018. New Stink Bug Thresholds in Corn. NC State Ext. (go.ncsu.edu/readext?522534).

Reisig, D. D., M. Roe, and A. Dhammi. 2013. Dispersal Pattern and Dispersion of Adult and Nymph Stink Bugs (Hemiptera: Pentatomidae) in Wheat and Corn. Environ. Entomol. 42: 1184–1192.

Sedlacek, J. D., and L. H. Townsend. 1988. Impact of Euschistus servus and E. variolarius (Heteroptera : Pentatomidae) Feeding on Early Growth Stages of Corn. J. Econ. Entomol. 81: 840–844.

Tillman, P. G. 2010. Composition and Abundance of Stink Bugs (Heteroptera: Pentatomidae) in Corn. Environ. Entomol. 39: 1765–1774.

Tillman, P. G. 2011. Influence of Corn on Stink Bugs (Heteroptera: Pentatomidae) in Subsequent Crops. Environ. Entomol. 40: 1159–1176.

Townsend, L. H., and J. D. Sedlacek. 1986. Damage to corn caused by Euschistus servus, E. variolarius, and Acrosternum hilare (Heteroptera: Pentatomidae) under greenhouse conditions. J. Econ. Entomol. 79: 1254–1258.

Van Duyn, J. W., R. W. Heiniger, S. R. Koenning, A. C. York, and S. J. Toth. 2005. Crop Profile for Field Corn in North Carolina. Crop Profile/PMSP database, USDA NIFA.

Wilde, A. G., K. Roozeboom, A. Aqeel, B. Gordon, G. Wilde, K. Roozeboom, A. Ahmad, M. Claassen, B. Gordon, W. Heer, L. Maddux, V. Martin, and P. Evans. 2007. Seed Treatment Effects on Early-Season Pests of Corn and on Corn Growth and Yield in the Absence of Insect Pests. J. Agric. Urban Entomol. 24: 177–193.

Chapter 4: Effect of *E. servus* feeding, with and without the presence of *Fusarium verticculioides,* on maize grain yield and quality

Introduction:

The brown stink bug, *Euschistus servus* (Hemiptera: Pentatomidae), is a highly polyphagous pest of many cultivated hosts. Extensive planting of transgenic maize [*Zea mays* L. (Poaceae)] and cotton (Gossypium hirsutum L.) for lepidopteran pests, and the associated decrease in broad-spectrum insecticide use, has increased stink bug pest outbreaks (McPherson and McPherson 2000). In Virginia, multiple suitable hosts for stink bugs (e.g., small grains, soybeans, maize, cotton) are grown in close proximity to each other, facilitating movement of pests between cropping systems. *Euschistus servus* infests maize in the greatest numbers in mid to late July during the early reproductive stages of maize development (Blinka 2008, Tillman 2010, Reisig et al. 2013). Harvest of wheat often coincides with late vegetative and early reproductive stages of maize in Virginia. *Euschistus servus* can overwinter and complete a full generation in wheat (Blinka 2008) and its subsequent movement into maize after wheat harvest is well documented (Reisig 2011, Reisig et al. 2013).

Maize grain yield and quality is susceptible to damage from *E. servus* during late vegetative and early reproductive growth stages. Studies have measured the impact of stink bug feeding on grain yield and identified V15, VT, and R1 as the most critical stages for management (Negrón and Riley 1987, Ni et al. 2010). Stink bug feeding can introduce fungal rots beyond these growth stages, potentially extending the period of management (Ni et al. 2011, Opoku et al. 2019).

Fusarium fungi are the most common disease agents in maize worldwide. *Fusarium spp*. are present and thrive in environmental conditions common to maize growing areas, specifically warm and dry areas like southeastern Virginia (Miller 2001, Stumpf et al. 2013). *Fusarium* fungi; *Fusarium verticullioides* (Sacc.), *Fusarium proliferatum* (Matsush.), and *Fusarium graminearum* (Schwabe) are common causal agents of ear rot in maize, which can have frequencies of 90% or higher (Bacon and Nelson 1994). Although *Fusarium* are ubiquitous in the environment, mechanical injury, such as insect feeding, is often needed for them to economically damaging (Miller 2001, Avantaggiato et al. 2003). Mycotoxins are byproducts of fungal disease agents that have detrimental effects on humans and other animals when they are consumed (Weaver et al. 1978, Fink-Gremmels 1999, Zain 2011). Mycotoxin contamination in livestock feed can result in lost productivity, reduced weight, and immunosuppression. Specifically, fumonisin is a mycotoxin by-product of *Fusarium spp*. Currently, the FDA recommends a maximum of four parts per million (ppm) for dry milled products for human consumption and 20 ppm and 30 ppm for swine and cattle, respectively (USFDA 2001).

The relationship between *Fusarium* ear rots, fumonisin, and insect populations has been documented. Brown marmorated stink bug feeding has been positively correlated with fumonisin contamination and the incidence of *Fusarium* rot in maize (Opoku et al. 2019). *Euschistus servus* feeding has been correlated with an increase in aflatoxin contamination in maize (Ni et al. 2011). The timing of inoculation for *Fusarium* rots in maize have also been reported. The highest level of fungal growth was recovered when inoculum was applied close to silking (Schaafsma et al. 1993). This suggests that maize may be most susceptible to fungal infection early in reproductive development; however, the relationship of insect pressure, maize growth stage, and fungal infection has not been fully explored.

Infestations of *E. servus* can cause yield loss (Negrón and Riley 1987, Ni et al. 2010) and the association of insect feeding and mycotoxin contamination has been documented in other regions of the corn belt and with other insect pests (Miller 2001, Avantaggiato et al. 2003, Ni et al. 2011, Opoku et al. 2019). Research is needed on the relationship between *E. servus* feeding, *Fusarium* rot, and fumonisin contamination in the mid-Atlantic US because of its unique climate where multiple adverse weather events can occur within a single maize production season (e.g., drought, tropical systems). Maize growth stage has not been comprehensibly studied for its effect on insect-pathogen relationships. In our study, *E. servus* and *Fusarium* inoculum were applied to maize plants at different growth stages to determine the interaction of these two factors and their effect, if any, on grain yield and quality. The goal of this study was to 1) determine if *E. servus* feeding increases *Fusarium* rot and fumonisin contamination and 2) determine if growth stage plays a role in the insect pathogen relationship.

Materials and Methods:

Insects

Adult *E. servus* were collected using sweep nets (38 cm diameter) from wheat and rye fields in May and June, and by hand from maize in late June – August in

Suffolk and Southampton County, Virginia, USA. Insects were stored in mesh enclosures (30 cm x 30 cm x 30 cm) for transfer from the field. Approximately 20-30 individuals were placed in plastic containers, and maintained in environmental chambers (Percival I-36VL, Percival Scientific, Perry, IA, USA) at a photoperiod of 16:8 and at 25±2 based on rearing protocols from several previous studies (Munyaneza and McPherson 1994, Koppel et al. 2009) until use in experiments. Each container was lined with a paper towel and contained a cotton ball soaked in distilled water and surface sterilized green beans (*Phaseolus vulgaris*). Green beans were sterilized by rinsing with 10% bleach solution for one minute and rinsed with distilled water three times.

Fusarium suspension

Pure cultures of *Fusarium verticillioides* were isolated and stored on filter paper. Spores were taken from these cultures and grown on ¼ strength Potato dextrose agar (PDA) at room temperature for five to seven days. After sufficient growth, cultures were scraped into 500 ml of sterile distilled water. The spore suspension was shaken vigorously to mix the evenly throughout. Concentration was determined using a hemocetometer and diluted to 3×10^5 conidia/ml. A spray bottle sterilized with 10% bleach and rinsed with distilled water three times was filled with the spore suspension for experimental applications.

Experimental design

Experiments were conducted in 2018 and 2019 to determine the impact of *E. servus* feeding on the incidence of *Fusarium* growth, fumonisin contamination, and grain yield in maize.

Treatments included the presence *E. servus* (either zero or two adult *E. servus* per ear), *Fusarium* inoculum (no inoculum or Inoculum at 3 x 10^5 conidia/ml), and all combinations of these factors. Maize plants were tested at three different growth stages: R1 (silking), R2 (blister), and R3 (milk).

Seed was planted on 0.91 m rows with 0.15 m spacing. Plots were 40 m long and contained four rows. Treatments were arranged in a randomized split block design. *Euschistus servus* infestation and *Fusarium* inoculation were the whole plot factor and growth stage was the subplot factor. Three rows of each plot were selected to receive *E. servus* and inoculum applied at the appropriate growth stage. The fourth row served as a border. Five plants were randomly chosen to receive treatments in each row. Plots were replicated four times (total of 20 plants per treatment and growth stage). Hybrid 'Dekalb DKC 67-44' with the VT2PRO (Cry1A and Cry2Ab2) and glyphosate resistance trait packages was planted on April 20 in 2018 and April 17 in 2019.

Euschistus servus adults and *Fusarium* inoculum were applied to individual ears using modified 20.32 cm x 40.64 cm mesh harvest bags (Midco Enterprises, Inc., Kirkwood, MO, USA). The sealed end of the harvest bag was cut to allow the mesh to slide over the entire plant to the area of the developing ear. Both ends of the bag were secured with cotton string. *Fusarium* inoculum was applied by spraying the surface of the ear six times (approximately 2.4 ml total) with a spray bottle containing the prepared spore suspension. Enclosures were checked daily for *E. servus* mortality and dead insects were replaced as necessary. After seven days, *E.*

servus and enclosures were removed. Treated plants were marked with flagging tape for later harvest.

Data collection

Once the plants reached physiological maturity, as determined by kernel black layer and 15-20% moisture content, primary ears (i.e., the top ear on each plant) were hand harvested. Harvested ears were stored in plastic containers with dichlorvos strips (Hot Shot No-pest Strips, Spectrum Brands, Madison, WI, USA) to minimize stored grain pests until processing. Four measurements were taken on each ear: 1) number of kernel rows, 2) number of kernels per row, 3) average kernel weight (as determined by a 100-kernel weight sample), and 4) kernel moisture %. Moisture readings were taken for each plot (Dickey-john mini GAC 2500, DICKEY-john, Auburn, IL). Yield was calculated using the formula:

Yield (kg/ha) = number of plants per hectare x number of ears per plant x number of kernel rows x number of kernels per row x average kernel weight

Yields were standardized to 15.5 percent moisture. Additionally, the number of discolored kernels was recorded for each ear. The number of discolored kernels was divided by the total number of kernels to determine the percentage of discolored kernels per ear. Samples (100 g) from each treated row of each plot were saved and stored for further testing.

Frequency of infection and fumonisin contamination methods were based on Opoku et al., 2019. Frequency of fungal infection was determined using 20 randomly selected kernels from each sample. Kernels were surface sterilized with 10% commercial bleach solution and air dried. Kernels were then plated (5/plate) on ¼ strength PDA (PDA, 9.75 g/liter) (Fisher Scientific, Ottawa, Canada) amended with 100 mg/L chloramphenicol and 100 mg/L chlorentetracycline. Plated samples were maintained at room temperature for five to seven days. The number of kernels observed visually with fungal growth were recorded and fungus was identified to genus based on morphology (Opoku et al. 2019).

Fumonisin contamination levels were determined for each sample using Neogen Reveal Q+ for fumonisin testing strips (Neogen Corporation, Lansing, MI, USA). Twenty grams of kernels from each sample were ground using a coffee grinder (F203, Krups, Solingen, Germany). To extract fumonisin, 50 ml of 65% ethanol was added to a 10g sample of ground corn and shaken vigorously for three minutes. Fumonisin contamination level was then quantified following manufacturers protocols using the Reveal Q+ test strips and Accuscan Pro reader. The detection range for fumonisin was 0.3 to 6 ppm. If the sample exceeded the detection range, the solution was diluted 1:2 with the provided dilatant and quantified.

Statistical analysis

Fumonisin concentration (ppm) was log transformed to meet the assumptions of a linear model. Grain yield (kg/ha) and fumonisin concentration (ppm) were analyzed using linear mixed effect models with the lme4 package (Bates et al. 2015) in R (R Core Team 2019). Percentage of discolored kernels and frequency of *Fusarium spp.* development were analyzed using a beta regression in the package betareg (Cribari-Neto and Zeileis 2010). A stepwise regression procedure was used to determine significance based on logliklihood ratio tests. Linear regressions were used to determine correlation between discolored kernel number from *E. servus* feeding, fumonisin concentration, and *Fusarium* infection frequency.

Growth stage, *Fusarium* inoculation, and *E. servus* presence, along with all interactions between the three were fixed effects. Blocking factor was a random effect. Visual inspection of residual plots did not reveal any deviations from model assumptions. Estimated marginal means were determined using the package emmeans (Lenth 2019) and Tukey adjusted pairwise comparisons were made at $\alpha = 0.05$. All figures were generated using back-transformed means in ggplot2 (Gómez-Rubio 2017). 2018 and 2019 data were analyzed separately.

Results:

Yield

In 2018, grain yield was reduced by *E. servus* ($F = 17.53$; df = 1,33; $p < 0.001$), but not by *Fusarium* inoculum ($F = 0.00$; df = 1,33; $p = 0.994$), growth stage ($F = 1.10$; df = 2,33; $p = 0.345$), or any interaction terms (*E. servus**Fusarium*, $F = 0.01$; df = 1,33; $p = 0.916$; *E. servus**growth stage, $F = 1.59$; df = 2,33; $p = 0.219$; *Fusarium**growth stage, $F = 1.52$; df = 2,33; $p = 0.233$; *E. servus* * *Fusarium* * growth stage, $F = 1.00$; df = 2,33; $p = 0.379$; Table 1). Pairwise comparisons between *E. servus* and *Fusarium* treatments indicated yield was reduced by approximately 2000 kg/ha by *E. servus* alone when averaged over all tested growth stages (Fig. 1). In

2019, grain yield was not affected by any treatments or interaction of treatments (F = 7.623; df = 11; p = 0.747) (Table 1).

Percentage of Discolored Kernels

In 2018, the percentage of discolored kernels increased by *E. servus* presence (X^2 = 7.52; df = 1; p = 0.006). Growth stage of the maize did not affect the percentage of discolored kernels (X^2 = 5.80; df = 2; p = 0.055). In 2019, percentage of discolored kernels was not affected by any treatments or interaction of treatments (X^2 = 16.42; df = 11; p = 0.126).

Fumonisin Concentration

In 2018, fumonisin concentration increased in the presence of *E. servus* (F = 15.27; df = 1,33; p < 0.001) and *Fusarium* inoculum (F = 9.39; df = 1,33; p = 0.004), but was not affected by the growth stage of maize (F = 0.19; df = 2,33; p = 0.825), or any interactions (*E. servus***Fusarium*, F = 3.08; df = 1,33; p = 0.089; *E. servus**growth stage, F = 0.06; df = 2,33; p = 0.942; *Fusarium**growth stage, F = 0.389; df = 2,33; p = 0.681; *E. servus* * *Fusarium* * growth stage, F = 0.18; df = 2,33; p = 0.834; Table 2). The presence of *E. servus* alone increased the fumonisin concentration by approximately 13 ppm when compared to the control. The *Fusarium* inoculum treatments also had higher fumonisin concentrations than treatments without inoculum (t = -3.06; df = 33; p = 0.004). The interaction of *E. servus* and *Fusarium* inoculum did not increase fumonisin concentration when compared to the inoculum alone (Fig. 3).

In 2019, fumonisin concentration increased in the presence of *Fusarium* inoculum (F = 21.80; df = 1,33; p < 0.001) and by the interaction of the *Fusarium* inoculum and *E. servus* presence (F = 6.24; df = 1,33; p = 0.018), but was not affected by the presence of *E. servus* alone (F = 0.89; df = 1,33; p = 0.352), the growth stage (F = 2.27; df = 2,33), or any of the other interactions (*E. servus**growth stage, F = 0.49; df = 2,33; p = 0.615; *Fusarium**growth stage, F = 0.83; df = 2,33; p = 0.447; *E. servus* * *Fusarium* * growth stage, F = 0.39; df = 2,33; p = 0.678; Table 2). The *Fusarium* inoculum treatments had higher fumonisin concentrations than treatments without inoculum (t = -4.67; df = 33; p < 0.001) (Fig. 4).

Fumonisin concentration from *E. servus* and *Fusarium* inoculum combined was 24.65 ppm and 22.46 ppm in 2018 and 2019, respectively, exceeding FDA regulation levels for human and some animal consumption. Fumonisin concentrations were positively correlated with the percentage of discolored kernels from *E. servus* feeding in 2018 (y = 5.976 + 88.299x [r^2 = 0.23, df = 1, 46, p < 0.001]) and 2019 (y = -7.01 + 272.61x [r^2 = 0.27, df = 1, 46, p < 0.001]).

Frequency of Infection

Frequency of infection was increased by *Fusarium* inoculum in both years (2018, X^2 = 12.49; df = 1; p < 0.001; 2019, X^2 = 44.43; df = 1; p < 0.001). It increased by 20% and 40% in 2018 and 2019, respectively (Fig. 5 and 6). Growth stage also affected frequency in both years (2018, X^2 = 9.43; df = 1; p = 0.009; 2019, X^2 = 20.72; df = 2; p < 0.001). The interaction of the inoculum and growth stage affected frequency of infection in 2019 (X^2 = 6.04; df = 2; p = 0.049). The frequency was higher across all treatments in R2 and R3 than in R1 in 2018 (Fig. 5); however, the frequency was higher in R1 and R2 than in R3 in 2019 (Fig. 6).

Frequency of infection was not correlated with discolored kernels from *E. servus* feeding in 2018 (r^2 = 0.01, df = 1, 46, p = 0.487), but it was positively correlated in 2019 (y = 0.3325 + 1.9330x [r^2 = 0.09, df = 1, 46 p = 0.034]).

Discussion:

Studies have documented the relationship between stink bug feeding and development of different mycotoxins (Ni et al. 2011, Opoku et al. 2019), as well as impact on grain yield (Ni et al. 2010). Our study is the first to examine the relationship of *E. servus* feeding, *Fusarium* rot, and subsequent fumonisin contamination at different and potentially susceptible growth stages of maize.

Percentage of discolored kernels was affected by *E. servus* as well and growth stage in 2018, but not in 2019. The percentage of discolored kernels was also unaffected by the *Fusarium* inoculum or the interaction of *E. servus* and inoculum in both years. Discolored kernels indicated the amount of insect feeding and gave a visual identifier of *Fusarium* development. Although results were inconsistent between the years, 2018 results suggest that *E. servus* can penetrate the husk of ears and feed on kernels more effectively at R1 than during later growth stages. Grain yield was impacted by the presence of *E. servus* alone in 2018, but not in 2019. Previous studies have documented reduced grain yield and quality at R1 (Ni et al. 2010).

Fumonisin concentration increased with *E. servus* feeding in 2018. The fumonisin concentration was also impacted by the *Fusarium* inoculum in both years, and the interaction of the inoculum and *E. servus* in 2019. We expected that the fumonisin level would increase with the introduction of an inoculum; however, the concentration also increased with the introduction of *E. servus* alone in both years. Insect feeding can increase the subsequent development of mycotoxin from *Fusarium* ear rots (Miller 2001, Avantaggiato et al. 2003, Opoku et al. 2019), which was illustrated here. The frequency of *Fusarium* rot on kernels was affected by the *Fusarium* inoculation and growth stage of maize in both years. As expected, the frequency of infection was higher when inoculum was applied at any growth stage, and it is unclear if some growth stages are more susceptible than others. In 2018, there was more rot in R2 and R3, but in 2019 there was more rot at stage R1. Likely, fungal growth depends on seasonal and climatic factors in addition to our tested variables.

The frequency of infection was relatively high across all treatments and was not impacted by *E. servus* feeding. Previous studies have suggested that *Fusarium* inoculation causes more frequent rot when fresh silks are present (Munkvold et al. 1997, Clements et al. 2003), which could have played an important role in the development of *Fusarium* rot. The fumonisin concentration was affected by *E. servus* feeding, which suggests that *Fusarium* rot itself was not impacted by *E. servus*, but that feeding increased the subsequent development of fumonisin. The impact of the growth stage was observed in the frequency of fungal contamination and discolored kernels, but this affect was inconsistent between years suggesting that any of the early reproductive stages of maize development are susceptible to *Fusarium* rot.

There were two large rain events and significant precipitation with lower temperatures in both years, which could have significantly mitigated the development of *Fusarium* and subsequently, the amount of fumonisin concentration.

Fusarium verticulioides thrives in warm, dry conditions (Miller 2001). Precipitation events could have also affected the ability of *E. servus* to actively feed throughout the duration of plant exposure.

We did not explore the potential for *E. servus* to move *Fusarium* inoculum via feeding or on its body. All insects used in this experiment were field collected and maintained in growth chambers for up to a week before being used in the experiment. Initial contamination nor the potential for spread of inoculum between individuals during storage was not explored explicitly in this study; however, the presence of the insects alone did not significantly increase the frequency of rot.

Based on our results, the impact of *E. servus* feeding on the development of *Fusarium* ear rot and fumonisin concentration is variable. Results from individual years suggest that the growth stage of maize can impact *Fusarium*; however, these data are inconsistent. Feeding increased the fumonisin concentration level in our study, but not the level of *Fusarium* rot. Further experiments are needed to determine if the number of insects affects this relationship and how *E. servus* distributes fungal inoculum. A better understanding of the relationship between fungal pathogens, *E. servus* feeding, and maize development will be critical in making management decisions when considering all potential impacts of this increasingly relevant pest.

Tables and Figures

Table 1. Effect of *E. servus*, *Fusarium* inoculum, growth stage of maize, and the interaction of these terms on grain yield in 2018 and 2019, in Suffolk, Virginia. Terms with bold p-values had an effect at α = 0.05.

Year	Model Term	F-value	d.f.	P-value
2018	*E. servus*	17.53	1,33	**< 0.001**
	Fusarium Inoculum	0.00	1,33	0.994
	Growth Stage	1.10	2,33	0.345
	E. servus * inoculum	0.01	1,33	0.916
	Growth stage * *E. servus*	1.59	2,33	0.219
	Growth Stage * Inoculum	1.52	2,33	0.233
	E. servus * Inoculum * Growth Stage	1.00	2,33	0.379
2019	*E. servus*	1.35	1,33	0.254
	Fusarium Inoculum	0.45	1,33	0.509
	Growth Stage	0.19	2,33	0.829
	E. servus * inoculum	0.13	1,33	0.722
	Growth stage * *E. servus*	0.32	2,33	0.727
	Growth Stage * Inoculum	0.71	2,33	0.499
	E. servus * Inoculum * Growth Stage	0.94	2,33	0.401

Figure 1. Grain yield (kg/ha) from plants exposed to *E. servus* and *Fusarium* inoculum averaged over all tested growth stages, Suffolk, Virginia, 2018. The bars (± SE bars) with different letters within inoculum and no inoculum were different at α = 0.05.

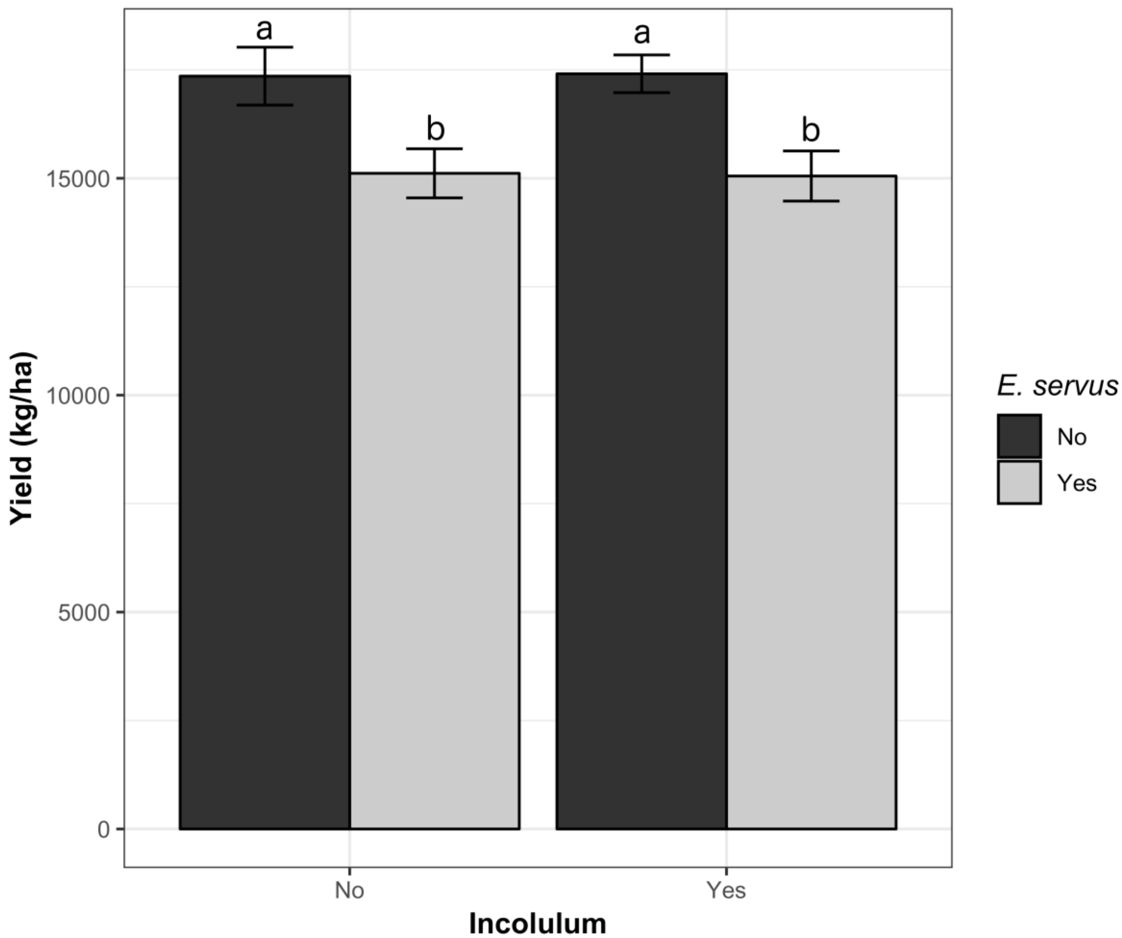


Table 2. Effect of *E. servus*, *Fusarium* inoculum, growth stage of maize, and the interaction of these terms on the fumonisin concentration in 2018 and 2019, in Suffolk, Virginia. Terms with bold p-values had an effect at alpha = 0.05.

Year	Model Term	F-value	d.f.	P-value
2018	*E. servus*	15.27	1,33	**< 0.001**
	Fusarium Inoculum	9.39	1,33	**0.004**
	Growth Stage	0.19	2,33	0.345
	E. servus * inoculum	3.07	1,33	0.089
	Growth stage * *E. servus*	0.06	2,33	0.942
	Growth Stage * Inoculum	0.39	2,33	0.680
	E. servus * Inoculum * Growth Stage	0.18	2,33	0.834
2019	*E. servus*	0.89	1,33	0.352
	Fusarium Inoculum	21.80	1,33	**< 0.001**
	Growth Stage	2.27	2,33	0.119
	E. servus * inoculum	6.24	1,33	**0.018**
	Growth stage * *E. servus*	0.49	2,33	0.615
	Growth Stage * Inoculum	0.83	2,33	0.447
	E. servus * Inoculum * Growth Stage	0.39	2,33	0.678

Figure 2. Fumonisin concentration levels (ppm), averaged across all tested growth stages, in 2018 from plants exposed to *E. servus* and *Fusarium* inoculum. The bars (± SE bars) with different letters above them were different at α = 0.05.

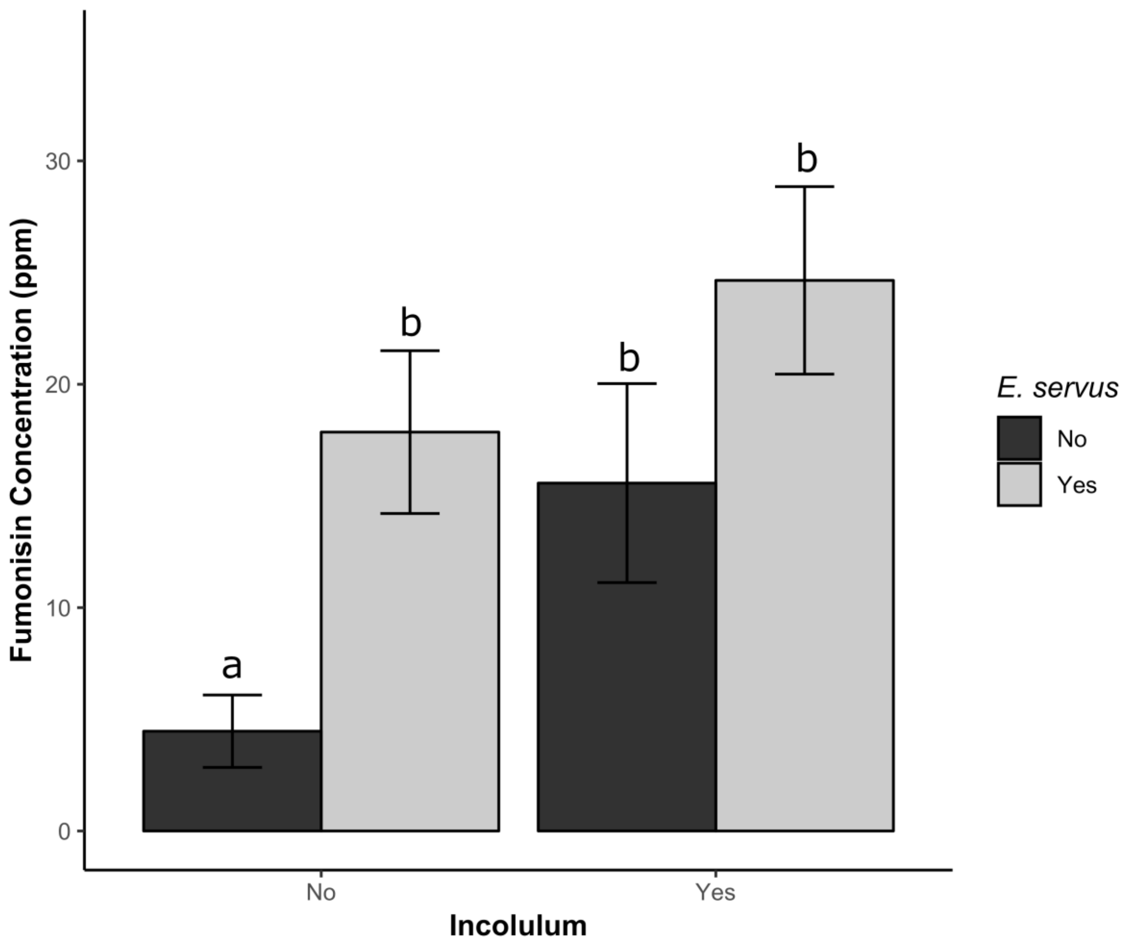


Figure 3. Fumonisin concentration levels (ppm), averaged across all tested growth stages, in 2019 from plants exposed to *E. servus* and *Fusarium* inoculum. The bars (± SE bars) with different letters above them were different at α = 0.05.

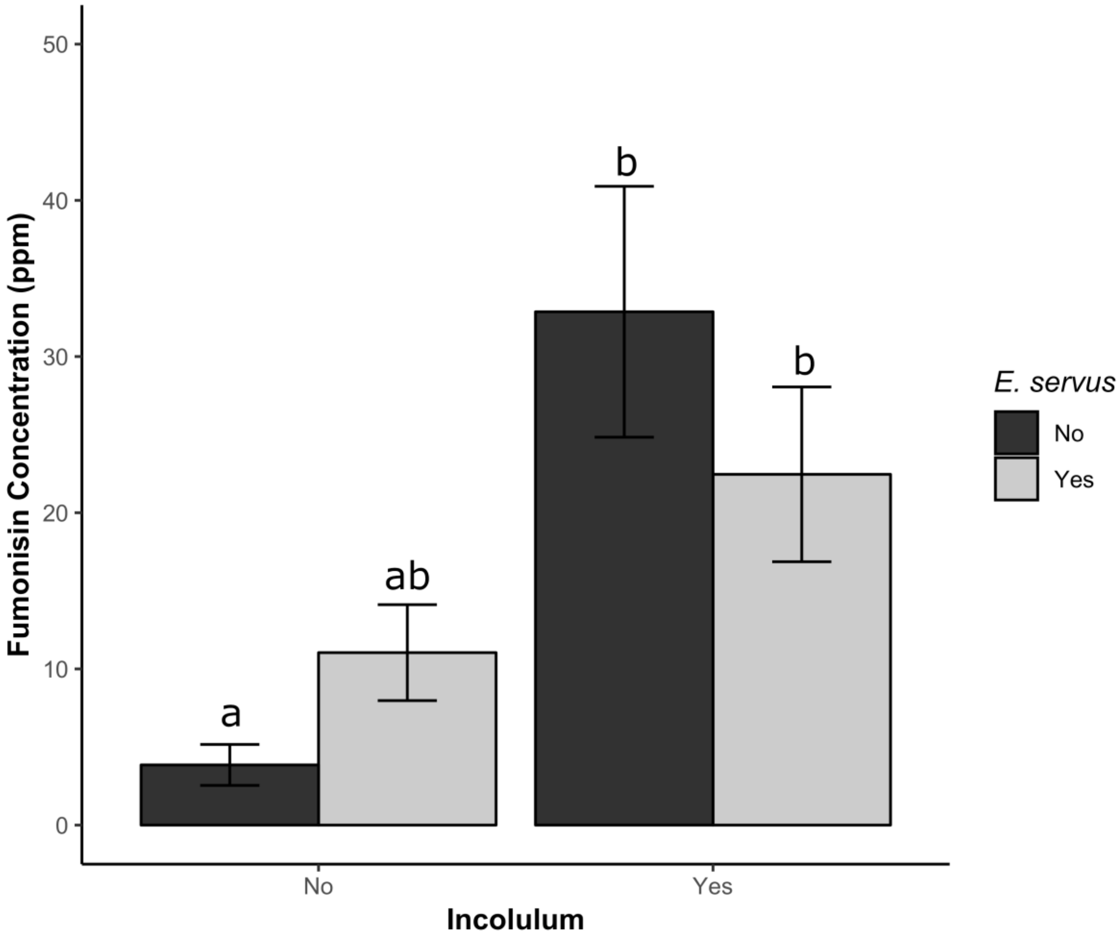


Figure 4. Frequency of *Fusarium* infection in maize plants exposed to *E. servus* and *Fusarium* inoculum at different growth stages of maize, Suffolk, Virginia, 2018. Bars (± SE) with different letters are different within each growth stage at α = 0.05. Growth stages with different letters next to them are different averaged across all other treatments within each growth stage at α = 0.05.

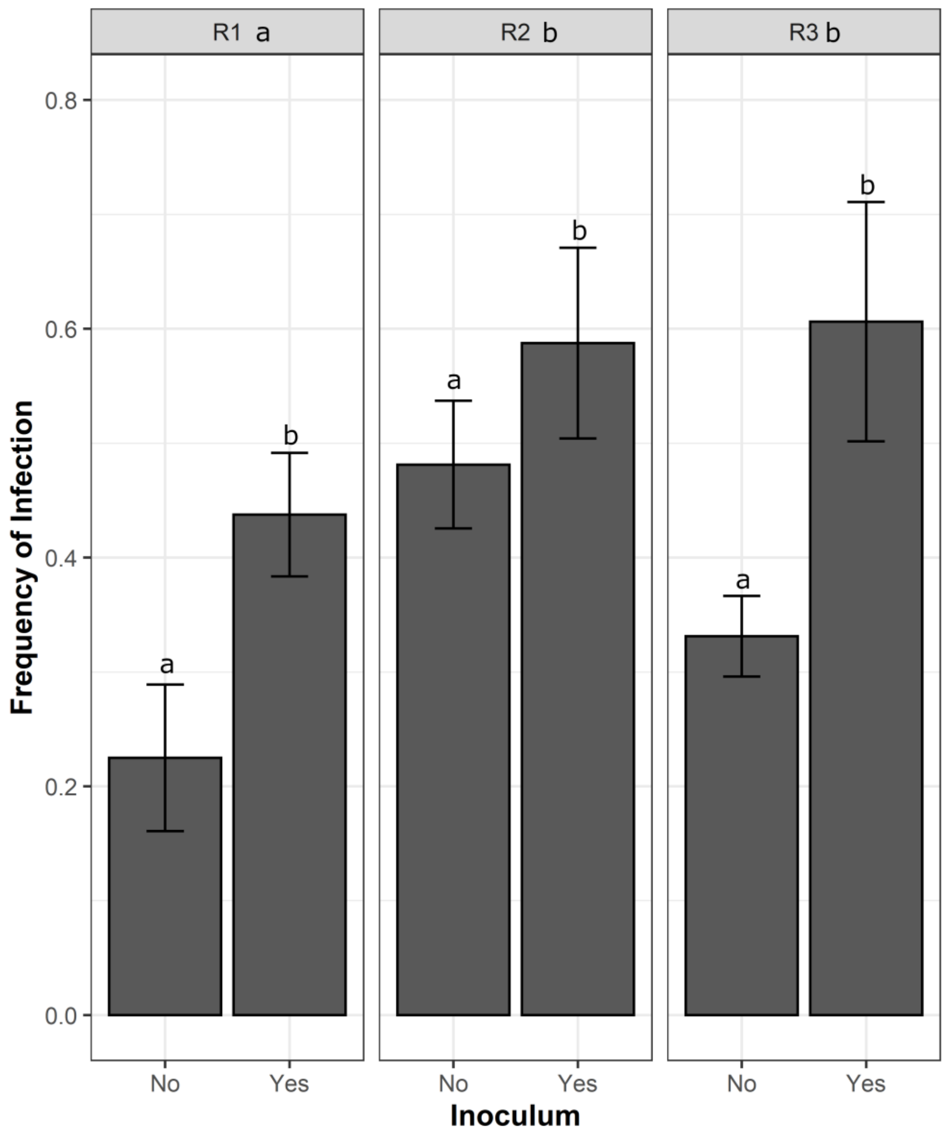
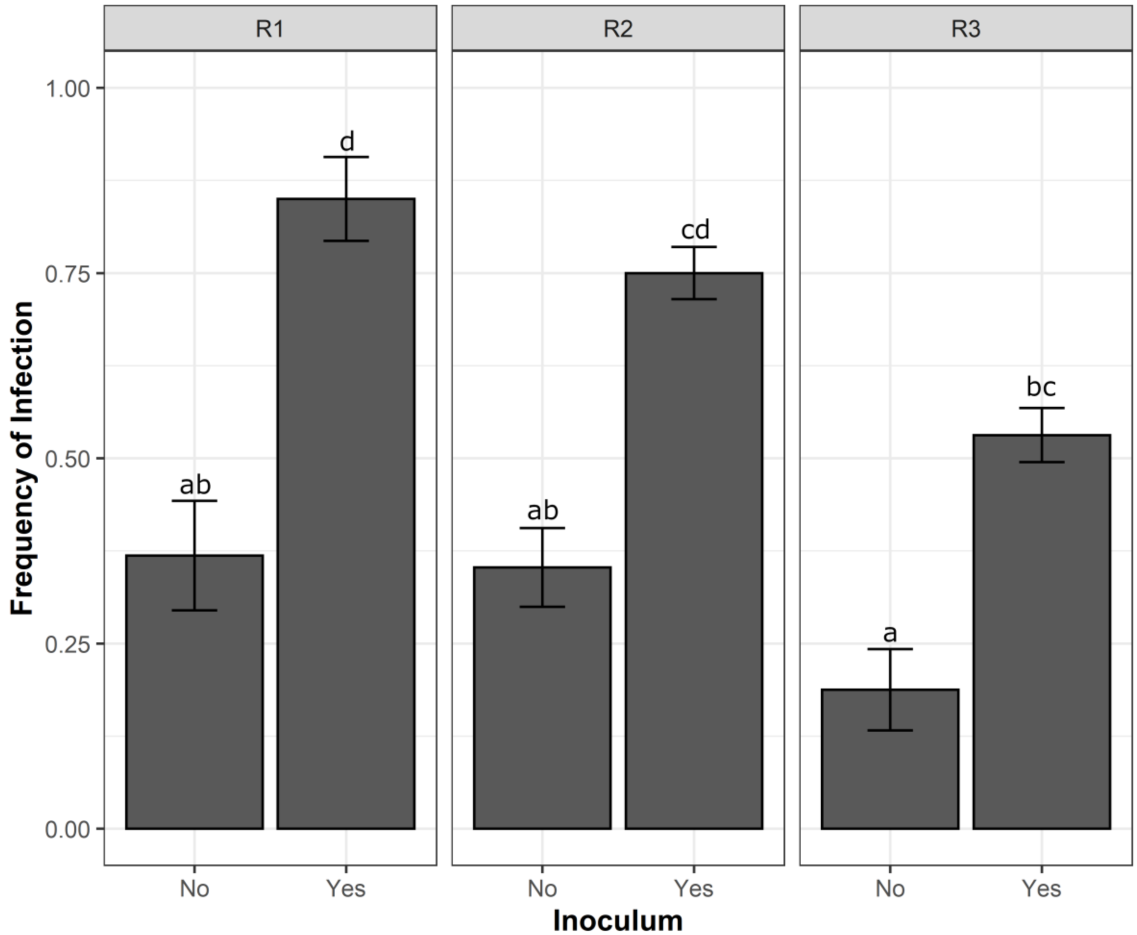


Figure 5. Frequency of *Fusarium* infection in maize plants exposed to *E. servus* and *Fusarium* inoculum at different growth stages, Suffolk, Virginia, 2018. Bars (± SE) with different letters are different at $\alpha = 0.05$.



References

Avantaggiato, G., F. Quaranta, E. Desiderio, and A. Visconti. 2003. Fumonisin contamination of maize hybrids visibly damaged by Sesamia. J. Sci. Food Agric. 83: 13–18.

Bacon, C. W., and P. E. Nelson. 1994. Fumonisin Production in Corn by Toxigenic Strains of Fusarium moniliforme and Fusarium proliferatum. J. Food Prot. 57: 514–521.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed effects models using lme4. J. Stat. Softw. 67.

Blinka, E. L. 2008. Biological and ecological studies on green stink bug, Acrosternum hilare, and brown stink bug, Euschistus servus (Hemiptera: Pentatomidae), in Eastern North Carolina cropping systems.

Clements, M. J., C. E. Kleinschmidt, C. M. Maragos, J. K. Pataky, and D. G. White. 2003. Evaluation of inoculation techniques for fusarium ear rot and fumonisin contamination of corn. Plant Dis. 87: 147–153.

Cribari-Neto, F., and A. Zeileis. 2010. Beta Regression in R. J. Stat. Softw. 34: 1–24.

Fink-Gremmels, J. 1999. Mycotoxins: Their implications for human and animal health. Vet. Q. 21: 115–120.

Gómez-Rubio, V. 2017. ggplot2 - Elegant Graphics for Data Analysis (2nd Edition) . J. Stat. Softw. 77: 2–5.

Koppel, A. L., D. A. Herbert, T. P. Kuhar, and A. K. Kamminga. 2009. Survey of Stink Bug (Hemiptera: Pentatomidae) Egg Parasitoids in Wheat, Soybean, and Vegetable Crops in Southeast Virginia. Environ. Entomol. 38: 375–379.

Lenth, R. 2019. Estimated Marginal Means, aka Least-Square Means. R package version 1.3.5.1. https://CRAN.R-project.org/package=emmeans

McPherson, J. E., and R. M. McPherson. 2000. Stink Bugs of Economic Importance in America North of Mexico. CRC Press.

Miller, J. D. 2001. Factors that affect the occurrence of fumonisin. Environ. Health Perspect. 109: 321–324.

Munkvold, G. P., D. C. McGee, and W. M. Carlton. 1997. Importance of different pathways for maize kernel infection by Fusarium moniliforme. Phytopathology. 87: 209–217.

Munyaneza, J., and J. E. McPherson. 1994. Comparative study of life histories, lab rearing, and immature stages of Euschistus servus and Euschistus variolarius (Hemiptera: Pentatomidae). Gt. Lakes Entomol. 26: 263–275.

Negrón, J. F., and T. J. Riley. 1987. Southern green stink bug, Nezara viridula (Heteroptera: Pentatomidae), feeding in corn. J. Econ. Entomol. 80: 666–669.

Ni, X., K. Da, G. D. Buntin, T. E. Cottrell, P. G. Tillman, D. M. Olson, R. Powell, R. D. Lee, J. P. Wilson, and B. T. Scully. 2010. Impact of Brown Stink Bug (Heteroptera: Pentatomidae) Feeding on Corn Grain Yield Components and Quality. J. Econ. Entomol. 103: 2072–2079.

Ni, X., J. P. Wilson, G. David Buntin, B. Guo, M. D. Krakowsky, R. Dewey Lee, T. E. Cottrell, B. T. Scully, A. Huffaker, and E. A. Schmelz. 2011. Spatial patterns of aflatoxin levels in relation to ear-feeding insect damage in pre-harvest corn. Toxins (Basel). 3: 920–931.

Opoku, J., N. M. Kleczewski, K. A. Hamby, D. A. Herbert, S. Malone, and H. L.

Mehl. 2019. Relationship between invasive brown marmorated stink bug (halyomorpha halys) and fumonisin contamination of field corn in the Mid Atlantic U.S. Plant Dis. 103: 1189–1195.

Reisig, D. D. 2011. Insecticidal management and movement of the brown stink bug, Euschistus servus, in corn. J. Insect Sci. 11: 168.

Reisig, D. D., M. Roe, and A. Dhammi. 2013. Dispersal Pattern and Dispersion of Adult and Nymph Stink Bugs (Hemiptera: Pentatomidae) in Wheat and Corn. Environ. Entomol. 42: 1184–1192.

Schaafsma, A. W., J. D. Miller, M. E. Savard, and R. J. Ewing. 1993. Ear rot development and mycotoxin production in corn in relation to inoculation method, corn hybrid, and species of fusarium. Can. J. Plant Pathol. 15: 185-192.

Stumpf, R., J. dos Santos, L. B. Gomes, C. N. Silva, D. J. Tessmann, F. D. Ferreira, M. Machinski, and E. M. Del Ponte. 2013. Fusarium species and fumonisins associated with maize kernels produced in Rio Grande do Sul State for the 2008/09 and 2009/10 growing seasons. Brazilian J. Microbiol. 44: 89–95.

Tillman, P. G. 2010. Composition and Abundance of Stink Bugs (Heteroptera: Pentatomidae) in Corn. Environ. Entomol. 39: 1765–1774.

USFDA. 2001. Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds. (http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulaoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/uc109231.htm)

Weaver, G. A., H. J. Kurtz, C. J. Mirocha, F. Y. Bates, J. C. Behrens, T. S. Robinson,

and W. F. Gipp. 1978. Mycotoxin-induced abortions in swine. Can. Vet. J. 19: 72–74.

Zain, M. E. 2011. Impact of mycotoxins on humans and animals. J. Saudi Chem. Soc.