Identifying Integrated Pest Management Strategies for corn earworm, *Helicoverpa zea*, in Hemp

by

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Abstract

Corn earworm, *Helicoverpa zea* (Lepidotera: Noctuidae), is a polyphagous insect species that feeds on the foliar, fruiting, and flowering structures of their host plants. In hemp (*Cannabis sativa* L.) corn earworm feeds on the flower buds which is the marketable portion of the plant. Managing this pest in hemp is challenging due to limited effective pest management tools and the complex regulatory measures surrounding chemical control. To address this issue, field trials and laboratory bioassays were conducted to evaluate the efficacy of insecticides against corn earworm. The field trials involved the application of biological insecticides registered for hemp and conventional standards used in other crops. Damage ratings, caterpillar counts, and harvest data were collected. Results from the field trials showed no significant difference in the effectiveness of insecticide treatments, but conventional insecticides were more effective than biological insecticides. In the laboratory bioassays, conventional insecticides were effective one day after insecticidal exposure, while the biological insecticides took on average four days to provide effective control.

Two field experiments were conducted in 2022 to evaluate alternative control strategies. The first experiment utilized a sweet corn trap crop to reduce corn earworm damage in hemp. Different sweet corn planting dates were tested, and the trap crop showed potential for reducing damage to hemp. The second experiment assessed varietal preferences of corn earworm in four hemp varieties. Significant differences were observed in plant measurements, cannabinoid concentrations, caterpillar numbers, and damage ratings. These findings highlight the importance of considering cultural control strategies and varietal selection to manage corn earworm in hemp effectively.

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ADAI	Alabama Department of Agriculture & Industries
BT	*Bacillus thuringiensis*
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBDV	Cannabidivirin
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBN	Cannabinol
DAT	Day after treatment
Delta 8-THC	Delta-8-Tetrahydrocannabinol
EPA	Environmental Protection Agency
FIRFA	Federal Insecticide, Fungicide, and Rodenticide Act
IPM	Integrated Pest Management
IRAC	Insecticide Resistance Action Committee
NPV	Nucleopolyhedrovirus
THC	Tetrahydrocannabinol
THCA	Tetrahydrocannabinol acid
THCV	Tetrahydrocannabivarin
USDA	United States Department of Agriculture

Chapter 1

Introduction and Literature Review

The corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae), is an economically important pest in a variety of crops and agricultural systems (Olmstead et al. 2016). It is a holometabolous insect species, whose larval stage is widely known to cause damage to its host plants (Hardwick 1965). Given the economic impacts of corn earworm, extensive research has been conducted over the past 100 years to understand how to control this pest (Olmstead et al. 2016). Control strategies such as foliar insecticide applications and plant-incorporated protectants using genetically modified varieties with *Bacillus thuringiensis* have been widely used over the past several decades (Shelton et al. 2013).

Industrial hemp, *Cannabis sativa* L., is now being grown extensively in the United States since its legalization with the 2014 Farm Bill that allowed hemp production in pilot programs (Bouloc 2013, U.S. H.R.2642 - Agricultural Act of 2014 113th Congress [113-333]) and 2018 Farm Bill which legalized commercial hemp production (U.S. H.R. 2 –115th Congress [15-334]).

Although this crop may create new income opportunities, little is known about basic agronomic practices, and further, how to control insect pests in this crop (Britt et al. 2021). Corn earworm is the most damaging pest in outdoor hemp by creating damage in the buds, which are the marketable portions of the plant (Cranshaw et al. 2019). Exploring integrated pest management strategies and understanding corn earworm biology are key to creating better management strategies against this pest (Cranshaw et al. 2019).

Cannabis sativa L.

Cannabis sativa L. has been grown for thousands of years, being one of agriculture's oldest crops (Small 2015). *Cannabis* cultivation is distributed throughout the world, ranging from Africa, to America, Asia, and Europe (Piluzza et al. 2013). Taxonomic differences in *Cannabis* species is an ongoing challenge for differentiation between biotypes (Small 2015).

Modern taxonomists classify *Cannabis* into a single species of *Cannabis sativa* divided in two subspecies, *C. sativa* and *C. indica* (Small and Cronquist 1976). *Cannabis* has also been classified by others into three species, *C. sativa, C. indica, and C. ruderalis* (Zhukovskii 1971). *C. sativa* is morphologically characterized by a light to medium green color with a tall structure. *C. indica* varieties have narrow leaves with loose and elongated inflorescence (Pollio 2016). *Cannabis sativa* is further divided into industrial hemp and marijuana (Bouloc 2013, Small 2015). Hemp is grown primarily for its cannabinoids, the most prominent one being cannabidiol (CBD) (Piluzza et al. 2013). Differentiation between hemp and marijuana is based on the concentration of the psychoactive phytocannabinoid Δ 9 -tetrahydrocannabinol (THC) (Cherney and Small 2016). U.S. federal law classifies hemp as *Cannabis sativa* produced with 0.3% or less THC content (FDA 2021). While the average THC content in marijuana plants' flowers and leaves is around 10%, certain strains can exhibit significantly higher concentrations, with levels ranging from 20% to 30% or even greater (Johnson 2014).

Cannabis sativa is a medium to tall erect herb belonging to the Cannabaceae family (Cherney and Small 2016). It is an annual plant with flowering induced by photoperiod (Small 2015). In *Cannabis*, photoperiodism is a key factor to trigger florigen, the hormone that initiates the flowering process (Adesina et al. 2020). *Cannabis sativa* is a dioecious plant; male flowers are characterized by their long, multibranched panicles while female plants are packed together (Clarke 1999). Pollination between males and females depends on wind patterns (Clarke 1999). Morphological traits differentiate male and female plants. Male plants are usually taller, with small leaves surrounding the flowers (Clarke 1999). Male flowers are distinguished by their yellow to green color petals. These petals hang down and stamens emerge consisting of pollen sacs (McPartland et al. 2008). The male plant dies as soon as the pollen sac is shed (Clarke 1999). Female flowers have stigmas stretching from their membranous bract. These bracts are covered with trichomes, measuring from 2 to 8 mm in length, and containing the ovule (Clarke 1999). When matured, stigmas become darker, and the ovule produces achene fruit (commonly known as seed) covered by a thin perianth (seed coat) (Moliterni et al. 2004).

Industrial hemp is a multipurpose crop that has several applications in various industries (Adesina et al. 2020). Types of industrial hemp vary depending on its end product and growing conditions, this includes fiber production, seed production, cannabidiol (CBD) production, and dual purpose plants (Bouloc 2013).

History

Hemp, a crop with a long history of cultivation, originated in the Yunnan Province of China and spread throughout Asia (Bouloc 2013). Over 2,000 to 4,000 years ago, hemp cultivation was introduced to Europe, and it was later brought to the New World during Spanish colonization. The first recorded instance of hemp planting in the New World was in Chile in 1545 (Bouloc 2013), and it was likely introduced to North America in 1606 (Cherney and Small 2016). Hemp production was initially popular in Missouri and Illinois during the 1840's and 1850's, but after the Civil War, Kentucky emerged as a major growing region for hemp (Cherney and Small 2016). As cordage production was in decline, Kentucky became a leading producer of hemp (Fike 2016).

In the 20th century, hemp production relied on labor-intense practices (Fike 2019). The outdated technologies in hemp created a disadvantage compared to other field crops (Fike 2019). Researchers recognized the need for new technologies to aid in the efficiency of hemp production. By the 1920's, these efforts had led to significant advancements, with the

Department of Agriculture of Canada and the United States Department of Agriculture actively researching and promoting the use of fiber hemp (Cherney and Small 2016).

Despite the promising growth and potential of the hemp industry, its course shifted with the introduction of the Marijuana Tax Act of 1937 (Cherney and Small 2016). This legislation was passed due to the growing concerns of recreational use of psychoactive strains of drug type marijuana (Fike 2019). Hemp production came under the control of the U.S Treasury Department, which required hemp growers to register and obtain licenses from the federal government (Fike 2019). Hemp production gradually declined and was further restricted with the passing of the Controlled Substances Act in 1970, classifying hemp in the Schedule drug type 1 (Fike 2019).

Political views shifted in the 21st century regarding hemp production (Fike 2019). After several years of illegalization of hemp production, the 2014 Farm Bill (Section 7606) allowed its resurgence for research purposes (U.S. H.R. 2462 – 113th Congress [113-333]). In addition, this farm bill determined the difference between drug type marijuana and hemp (Federal Register 2016). The 2018 Farm Bill (U.S. H.R. 2 –115th Congress [15-334])) removed hemp from the Controlled Substances Act, allowing hemp to be commercially produced and establishing the domestic hemp production program (Abernethy 2019).

The 2018 Farm Bill has paved the way for hemp production in the United States, allowing U.S states and Indian tribes to engage in the production and commercialization of this crop (Abernethy 2019). In 2017, there were seven states (Colorado, Kentucky, Oregon, North Dakota, Minnesota, New York, North Carolina) that grew industrial hemp (Ajayi and Samuel-Foo 2021). By 2018, 38 states have passed laws that define industrial hemp as a distinct crop from marijuana. Since its re-introduction, the Alabama Hemp Act 2016-393 authorized the

Department of Agriculture and Industries (ADAI) to develop an industrial hemp research program (Alabama Department of Agriculture & Industries 2021). Barriers and stigmas that had limited hemp production were dismantled and the hemp industry across United States increased (Ajayi and Samuel-Foo 2021).

Hemp popularity grew globally, with 30 nations actively cultivating hemp by 2018 (Sunoj Valiaparambil Sebastian et al. 2023). Hemp seed oil production has shown a positive increase reaching 5449 tons by 2020, with Russia being the largest distributor followed by Chile and Ukraine (Sunoj Valiaparambil Sebastian et al. 2023). Europe, China, Korea and Russia are leaders in hemp production (Johnson 2014). Europe leads hemp production across the world according to the Food and Agriculture Organization (FAO) (Johnson 2014). European countries such as France, Netherlands, Lithuania, and Romania excel in the production of hurds, seeds, fibers, and pharmaceuticals (Johnson 2014). Additionally, China contributes to the production of hemp textiles (Crini et al. 2020).

In 2022, the value of hemp production was $238 million with a 71% decrease from 2021 which was $824 million (USDA 2023). In the United States, floral hemp production remains the strongest, followed by fiber and grain production (USDA 2023). The estimated value of floral hemp was $179 million in 2022, $28.3 million for fiber hemp, and $3.63 million for grain hemp (USDA 2023).

Following the enactment of the 2018 Farm Bill, hemp production in the United States has experienced a downward trend (Singular 2022). According to data from New Frontier, the number of acres licensed for hemp cultivation decreased from 511,442 in 2019 to 336,655 in 2020, and further declined to 107,702 in 2021 and 51,016 in 2022 (Singular 2022). The downward of hemp production is driven by its unstable market, growers unable to meet the federal THC standards, as well as the lack of pest management strategies in this crop (Singular 2022).

Hemp

Hemp, *Cannabis sativa* L., is a diecious, obligate cross-pollinated crop with diploid genome (Fike 2016, Rupasinghe et al. 2020). Hemp is a multi-purpose crop that has several uses (e.g., fiber, seed, and cannabidiol [CBD]) (Fike 2016). The different types of industrial hemp have unique characteristics and qualities that make them well-suited for specific uses.

The growing conditions of hemp are directly linked to the desired product. For instance, fiber hemp is grown primarily for its strong and durable fibers. Hemp seed is cultivated for its nutritious content, and floral hemp is grown for its essential oils. Each type has unique agronomic practices to minimize pest losses and maximize yield.

Fiber hemp

Hemp fibers are classified in bast fibers and hurds (Kaiser et al. 2015). Bast fibers make up 20-30% of the stalk, while hurds make up 70-80% (Adesina et al. 2020). Hurds are the waste product produced from fibers and have been used in the production of animal beddings, and light concentrates (i.e., wall construction, insulation, and underfloor) (Crini et al. 2020). It can also be used for paper production, providing high quality compared to wood paper (van der Werf et al. 1994). Bast fibers can also be used in hemp-based materials to build houses, including as wood, walls, pipes, and paint (Brzyski et al. 2017). Depending on whether it is bast fiber or hurd, different construction products can be developed (Crini et al. 2020). Bast fibers have been used to manufacture textiles, fabrics, ropes, yarns, rugs, clothing, and canvas (Crini et al. 2020). This fiber is widely known for its resistance to water damage, flexibility, and strength compared to other fibers such as cotton (Crini et al. 2020). Harvesting occurs at the flowering stage during seed formation, which ensures higher yields and better fiber quality (Adesina et al. 2020).

Grain hemp

Hemp seed is a versatile and nutritious ingredient that is used to create various food and beverage products for both human and animal consumption (Johnson 2014). End products from hemp seed have been commercialized by several countries such as Canada, United States, United Kingdom, Germany and France (Crini et al. 2020). Popularity in hemp seed consumption is due to the rich source of protein and essential fatty acids, specially ω-6/ω-3 fatty acids which are commonly known to aid in human health development (Adesina et al. 2020, Leonard et al. 2020). Additionally, hemp seed contains all nine essential amino acids that the human body requires (Leonard et al. 2020).

All hemp food items are derived from hemp seeds; this includes the seeds themselves and several products being protein powder, flour, oil, meal, and bioactive compounds (Crini et al. 2020). By converting hemp seed into flour, it can be used as a protein source and provide a healthier option for baking (Adesina et al. 2020). Seed oil is the principal product originated from hemp seed (Crini et al. 2020).

In addition, hemp seed includes production of body-care products, industrial oils, cosmetics, and pharmaceuticals (Johnson 2014). In China, hemp seed oil has been used to help with stress, anxiety, muscular pain, digestion, and sleep deprivation (Rehman et al. 2021). Several methods have been created to extract hemp seed oil such as cold pressing, solvent extraction, percolation, Soxhlet, and supercritical fluid extraction (Devi and Khanam 2019).

Floral hemp

Hemp grown to produce cannabidiol (CBD) has received the greatest interest by growers (Bouloc 2013). Hemp presents a great range of chemical compounds, with more than 100 phytocannabinoid identified (Hanuš 2009). CBD is the most important phytocannabinoid in hemp; it is a terpenophenolic compound with non-physoactive components (Adesina et al. 2020).

Since the extraction of CBD in late 1930, it is thought that this plant may reduce inflammation and anxiety (Adesina et al. 2020). CBD has antioxidant and neuroprotective agents, and as a result, may reduce of migraines and stress, and help with bowel conditions (Abernethy 2019).

Growing conditions

Hemp is affected by photoperiod, influencing the shift from vegetative to reproductive stages (Adesina et al. 2020). This plant adapts to a variety of soil types and can grow in temperatures ranging from 13 to 22°C (Rehman et al. 2021). Hemp cultivars thrive in soils that are deep and well aerated, with a pH of 6 (Ranalli 1999). High moisture is key for the first six weeks to optimize hemp production (Rehman et al. 2021). Studies demonstrate that hemp requires 500-7000 mm of water to achieve plant health and optimize yield (Sunoj Valiaparambil Sebastian et al. 2023). Plant spacing varies depending on the type of hemp cultivated (i.e., fiber, CBD, seed).

Fiber hemp

Fiber hemp should be planted close together to develop stalk extension (Adesina et al. 2020). Drills are the preferred planting method for fiber production with a row spacing from 7.6 to 17.8 cm (Amaducci et al. 2002). There are significant variations in plant density when it comes to fiber production ranging from 50 to 750 plants per square meter (Ranalli 1999). To obtain quality yield it was found that the suitable plant spacing was 120 plants per square meter with an inter-row spacing of one-half meter (Adesina et al. 2020).

Grain hemp

Hemp grown for seed are also grown further apart and planted using drills (Amaducci et al. 2002, Adesina et al. 2020). To achieve maximum yield, the optimal density for grain production is 30 plants per square meter (Ranalli 1999).

Floral hemp

In hemp grown for CBD plants usually are sown as seeds in a greenhouse and hand transplanted two to four weeks after sowing (Cockson et al. 2019). CBD hemp can be grown indoors in high tunnels or greenhouses, as well as outdoors using raised beds or on bare ground (Cockson et al. 2019). CBD hemp that is grown in the field is planted on a 0.6 to 1.8 meter grid (Arnall et al. 2019). CBD hemp is planted further apart than other types of hemp to ensure quality yield in flower material (Adesina et al. 2020).

Challenges in hemp

Due to the restrictive laws surrounding hemp production in the last century, little research has been conducted on best management practices for this crop. Therefore, growers are struggling to effectively grow hemp in today's environment (Adesina et al. 2020). One of the major issues in hemp production is the presence of narcotic THC in hemp paired with the 0.3% legal THC limit (Sunoj Valiaparambil Sebastian et al. 2023). Different factors such as soil type and conditions, cultural control practices, and abiotic factors can affect the CBD:THC ratio in hemp production as well as the total THC content (Trancoso et al. 2022).

Nutrient requirements and the relationship between nitrogen and CBD and THC in hemp production is still not quite understood (Trancoso et al. 2022). Nitrogen, a crucial nutrient in hemp cultivation, has been found to influence cannabinoid production, but its effect on pest pressure cannot be ignored. Higher levels of nitrogen can increase pest populations, causing damage and yield loss as shown in crops such as corn, wheat, and rice (Folina et al. 2021). Growers across the United States have difficulty balancing the use of nitrogen fertilizers to increase CBD levels while still managing insect pests (Thweatt et al., unpublished).

Another major challenge for hemp producers is identifying proper management techniques to control insect pests, diseases, and weeds (Punja 2021).

Diseases in hemp

Since hemp's reintroduction, emerging diseases have caused yield losses for growers across the U.S. (Punja 2021). The current method to best identify diseases in hemp is morphological criteria and molecular markers (Punja 2021). Different pathogens affect different plant components. For example, roots are affected by *Fusarium oxysporum, Fusarium solani, Fusarium brachygibbosum, Pythium dissotocum, Pythium myriotylum,* and *Pythium aphanidermatum* (Punja et al. 2019). Roots may express several symptoms such as stunting, discoloration, and in extreme cases, plant death (Punja et al. 2019).

The most common foliar disease is powdery mildew which is caused by *Golovinomyces (Erysiphe) cichoracearum* (Punja 2018). Affected leaves may show white, gray or tan fungal growth (Gauthier et al. 2019). Diseases affecting flower buds are penicillium bud rot caused by *Penicillium olsonii* and *Penicillium copticola*, botrytis bud rot caused by *Botrytis cinerea,* and fusarium bud rot caused by *F. solani, F. oxysporum* (Punja et al. 2019). The term bud rot is commonly used in the cannabis industry referring to several pathogens affecting the flower buds (Punja and Ni 2021). Plants infected with *Fusarium* spp. may have decay of the flower buds, spore production producing grey mold, and death (Punja and Ni 2021).

Weeds in hemp

Weeds coexist with hemp in the field; some examples are crabgrass *(Digitaria sanguinalis)*, goosegrass (*Eleusine indica*), barnyardgrass (*Echinochloa crus-galli*), foxtails (*Setaria* spp.), pigweeds (*Amaranthus* spp.), common lambsquarters (*Chenopodium album*), smartweeds (*Polygonum* spp.), jimsonweed (*Datura stramonium*), and morning glories (*Ipomoea* spp.) (Britt et al. 2020, Velez, personal observation).

Few herbicides are registered for hemp, therefore the use of different strategies such as tillage and use of nonselective insecticides before transplanting is necessary (Gage 2022). Other strategies are using cover crops, and crop rotation other than corn and soybean (Gage 2022).

To suppress weeds in grain and fiber cultivars, cultural control methods used by farmers include increasing seed rates and optimizing soil fertility as well as using suitable varieties (Britt et al. 2020). However, weed suppression strategies for hemp grown for CBD follow different guidelines. In CBD hemp, weeds are suppressed using beds covered with mulch. Additionally, physical methods such as hand pulling are commonly used (Britt et al. 2020).

Insects in hemp

Hemp, as compared to other agricultural crops, does not possess a long history of pest management strategies. The newness of this crop creates challenges in modern cultivations regarding control of arthropod pests in indoor or outdoor hemp (Cranshaw et al. 2019). In the United States, numerous mites and insects reside in hemp, and can result in different types of damage. The type of damage will vary depending on the mouth part the insect has, feeding preference, and if it is an outdoor or indoor pest. Insect pests include leaf defoliators, piercing-sucking pests, those that damage leaves and flower buds, stem and stalk borers, and root feeders (Ajayi and Samuel-Foo 2021).

Defoliators that feed on the foliage of the plant are southern corn rootworm/spotted cucumber beetle, *Diabrotica undecimpunctata howardi* (Barber), Japanese beetle, *Popillia japonica* (Newman), differential grasshopper, *Melanoplus differentialis* (Thomas), and yellowstriped armyworm, *Spodoptera ornithogalli* (Guenée) (Cranshaw et al. 2019).

Piercing-sucking insects include true bugs and mites, causing damage to the leaves, flower buds, and stem (Ajayi and Samuel-Foo 2021). Hemp pests include two-spotted spider mite, *Tetranychus urticae*, hemp russet mite, *Aculops cannabicola* (Farkas), whitefly, *Trialeurodes vaporariorum* and cannabis aphid, *Phorodon cannabis* (Passerini). These insect pests are more predominant in indoor hemp, causing damage by sucking fluids resulting in stunted growth and death (McPartland et al. 2000, Britt et al. 2020).

Root feeders in hemp are understudied, but common insect species identified are white grubs, larvae of *Phyllophaga tristis (Fabr.),* pavement ant, *Tetramorium caespitum (L.),* and rice root aphid *Rhopalosiphum abdominalis* (Sasaki) (Cranshaw et al. 2019, Britt et al. 2020).

Stem and stalk borers feed on the stems resulting in breakage (Cranshaw et al. 2019). Some examples of stem and stalk borers in hemp are European corn borer, *Ostrinia nubilalis* (Hübner), and Eurasian hemp borer, *Grapholita delineana* (Walker).

Chewing insects that have been reported in hemp are Eurasian hemp borer, *Grapholita delineana* (Walker), which burrows through the stems and produce wounds that damage terminal buds (McPartland 2002). While limited to the southeastern United States, fire ants are a problem in outdoor hemp causing damage by burrowing in the stem, cutting off nutrients, and stunted growth (Hirsch and Kesheimer 2021). The most damaging pest in hemp is corn earworm, *Helicoverpa zea,* which uses its chewing mouth parts on flower buds that create open wounds and may lead to flower bud death (Cranshaw et al. 2019)

Corn earworm

Corn earworm, *Helicoverpa zea* (Lepidotera: Noctuidae), is a polyphagous insect species that feeds on the foliar, fruiting, and flowering structures of their host plants (Cranshaw et al. 2019, Britt et al. 2021). This insect species has multiple common names (e.g, tomato fruitworm, soybean podworm, and cotton bollworm) due to the variety of host that it feeds on (Bibb et al. 2018, Reay-Jones 2019). Throughout this thesis, *H. zea* will be referred to as corn earworm.

Corn earworm is a multivoltine insect species capable of having up to five generations per year, and their populations are influenced by various abiotic factors such as relative humidity, day length, and moisture (Reay-Jones 2019). Adult corn earworm moths are highly mobile and capable of long-distance flights. There are three types of corn earworm movement: short movements within habitats, migratory movements throughout states, and long-distance movements between different habitats (Fitt 1989). The southern United States has a significant population of corn earworms in various crops, which can cause damage and yield loss (Jackson et al. 2008).

Population dynamics of corn earworm larvae can differ between different southern states and crops. Jackson et al. (2008) summarized the population dynamics of corn earworms on various crops, including corn, cotton, sorghum, soybean, and peanut, in Arkansas, Georgia, Louisiana, Mississippi, and North Carolina. Overall, high numbers of corn earworms were present from late June to early September months when suitable weather conditions were present for their life cycle, reproduction, diapause, and oviposition (Jackson et al. 2008).

Female corn earworm moths lay between 500 and 3,000 eggs on host plants (Hardwick 1965, Capinera 2000). These eggs hatch within three to four days, and the newly emerged larvae start feeding and molting on their host plants (Reay-Jones 2019). The larvae undergo six instars and are characterized by their orange to light brown head and their brown, green, yellow, or black body color (Hardwick 1965).

After reaching the final larval stage, larvae drop to the ground to pupate. The pupal stage is approximately 13 days for males and 12 days for females, and the pupa has a mahogany brown color (Hardwick 1965, Reay-Jones 2019). In the summer, adults emerge after two weeks, leading to a new generation. However, in the fall, the pupal stage remains dormant until the following growing season (Shrestha 2022).

Crop losses

Corn earworm larvae are a destructive insect pest in a wide range of plant hosts. They are commonly known to affect Fabaceae, Malvacea, Poaceae, and Solanaceae families (Capinera 2000). In addition, corn earworms are known to affect wild hosts such as crown vetch, red clover, and velvetleaf (Capinera 2000).

Sweet corn is the preferred host of corn earworm; adult moths lay eggs on fresh silks, with emerging larvae burrowing into the ear and feeding on the kernels (Rice 2022). As they feed, the larvae deposit frass-contaminated material, which can expose the ear to various pathogens and fungal diseases (Rice 2022). Annual yield losses in sweet corn and field corn are estimated to be as high as 50% and 2.5% respectively (Cook and Weinzierl 2004, Pioneer Agronomy Sciences 2021).

Corn earworm is an economic insect pest in cotton production across United States (Graham and Smith 2021). When left untreated, corn earworm can cause yield losses of 67% (Renneberg et al. 2017, Towles et al. 2021). The pest feeds on different structures of the plants such as the flowers, bolls, terminals, and fruiting squares of cotton plants, making it highly destructive (Towles et al. 2021).

In grain sorghum, corn earworm feeds on the seed heads, and the presence of even one larva can reduce yield by 5%. This damage increases if two larvae are feeding on the seeds, the yield loss can be as high as 9-10% (Rice 2022)

In soybean, corn earworm concentrates its feeding in the foliage, flowers, or pods (Reisig 2020). The most serious yield losses occur when large corn earworm larvae feed on soybean seeds that have achieved almost full size (Reisig 2020). Severe defoliation caused by large caterpillars may also impact yield in soybean (Reisig 2020).

Corn earworm in hemp

Similar to traditional row crops, hemp is grown throughout the summer months. Hemp flowering takes place in August and September in the southeast, as this crop is photoperiod dependent. Adult moths become attracted to hemp after corn has matured and is no longer suitable for oviposition (Cranshaw et al. 2019).

Female moths oviposit on hemp's reproductive structure, showing a tendency of egg lay in the upper part of the plant (Britt et al. 2021). This presents a unique challenge for pest management, as the structure of the hemp plant makes scouting for eggs significantly more difficult than other crops.

Newly emerged larvae concentrate their feeding on the flower buds, which is the marketable portion of the plant (Britt et al. 2021). The feeding damage caused by this insect pest creates an open wound, making it susceptible to pathogen infection and bud necrosis (Cranshaw et al. 2019). This further highlights the importance of effective pest management strategies in hemp cultivation, as failure to do so can result in significant economic losses for growers.

Integrated pest management (IPM)

The incidence and damage corn earworm has on a variety of crops have led to the development of different integrated pest management (IPM) strategies. IPM targets pests in an environmental approach relying on different strategies and using the pest's life cycle and the interaction with its environment (US EPA 2015).

The first approach when establishing integrated pest management is monitoring and identification of pests (US EPA 2015). Growers across United States have adopted monitoring techniques using pheromone-baited traps to monitor adult moths and target the eggs laid by them (Laurent 2007). The presence of five to ten moths per night indicates the need of control strategies such as foliar applications (Gauthier et al. 1920).

The use of foliar applications with different insecticides has been commonly used to control this pest in a variety of crops (Olmstead et al. 2016). The most common insecticides used to control corn earworm are carbamates, diamides, indoxacarbs, pyrethroids, and spinosads (Frank 2018). Chemical control has been adopted since the end of World War II (Olmstead 2015). The widespread adoption of these insecticides as a control strategy has increased the resistance of corn earworm leading to the development of new strategies.

Currently, growers rely on different integrated pest management strategies to aid in the control of this pest. One of the most common strategies used is the adoption of genetically modified plants with *Bacillus thuringiensis* toxins (Bilbo et al. 2018). This is commonly used in sweet corn, field corn and cotton production, providing control for corn earworm since its introduction in 1996 (Wold et al. 2001).

Varietal selection is used to control corn earworm in sweet corn. Sweet corn varieties that possess long, tight husks provide better control for corn earworm limiting their entrance to the husk (Bessin 2019). Varieties that have shown these characteristics are Country Gentlemen, Staygold, Golden Security, and Silvergent (Alston et al. 2011).

Another option to manage corn earworm is using a trap crop to divert the pest from the cash crop (Boucher 2021). This strategy has shown effective control for this pest while reducing insecticide use and preserving natural enemies (Holden et al. 2012).

Biological control agents such as *Coleomegilla maculata* (De Geer), *Harmonia axyridis* (Pallas), *Orius insidiosis* (Say), and *Geocoris punctipata* (Say) are generalist predators that have helped contribute to corn earworm population suppression (Olmstead 2015). Several parasitoids target corn earworm eggs, also aiding in the suppression of corn earworm populations (Alston et al. 2011). Some examples of commonly known parasitoids for this pest are *Trichogramma* *minutum* (Riley), *Campoletis sonorensis* (Cameron), *Hyposoter* spp., *Meloborus fuscifemora* (Graf), *Chelonus texanus* (Cress), and *Apanteles militaris* (Walsh) (Olmstead 2015).

Monitoring techniques

Corn earworm eggs are as small as a pencil point and measure approximately 1.27 – 1.52 cm diameter and 0.5 mm in height. The small size of these eggs makes scouting extremely challenging (Capinera 2000). Therefore, techniques to monitor other life stages are more routinely used.

Pheromone-baited traps are commonly used to monitor adult moths in various crops (Coop et al. 1992). This monitoring technique has a long history of success, with the traps identifying reproductively mature adult moths that will oviposit on the crop (Gauthier et al. 1920, Cranshaw et al. 2019). In addition, pheromone-baited traps can show patterns of moth flights throughout the growing season and in various crops. In Alabama, corn is the first host of corn earworm, followed by cotton, peanuts, or soybean (Graham and Smith 2021). With the relative newness of hemp, the timeline and preference for corn earworm moving into hemp is unclear.

In sweet corn, the use of pheromone-baited traps has been used to identify moth presence and reproductive activity (Chowdhury et al. 1987). Trap catches conducted in sweet corn show patterns of adult moths from July to September, with numbers steadily increasing during the silking period (Capinera 2000). Determining presence of corn earworm helps indicate to growers when insecticide applications need to take place to control this pest (Guerrero et al. 2014). Spraying insecticide during the egg-laying period can prevent newly hatched larvae from moving down into the husk, which can effectively control feeding damage caused by corn earworm (Barber 1941).

Monitoring in hemp

While pheromone traps have been effective in other crops, they have not proved effective in hemp. A study conducted by Britt et al. (2021) showed no correlation between larval presence and trap catches of adult moths in hemp. Given the ineffectiveness of pheromone-baited traps in this crop, visual inspection for corn earworm eggs and larvae is the most reliable method to determine insecticide applications in hemp (Britt et al. 2021).

Host plant resistance

History of Bacillus thuringiensis

The use of *Bacillus thuringiensis* (Bt) Cry protein-expressing sweet corn, field corn, and cotton varieties has been widely adopted since their introduction in 1996 for the control of several noctuid species (Wold et al. 2001). These crops showed effective control of corn earworm (*Helicoverpa zea*), European corn borer (*Ostrinia nubilalis*), western corn rootworm (*Diabrotica virgifera virgifera)*, fall armyworm *(Spodoptera frugiperda),* and western bean cutworm *(Striacosta albicosta*) (Milner 1994).

The strategy of using Bt Cry proteins to control pests was developed to provide a sustainable alternative to insecticide use (Lynch et al. 1999). Bt Cry proteins disrupt insect guts by activating gut proteases (Kurtz 2010). The proteins then bind to receptors on the brush border membrane of the midgut, which causes the formation of pores and ultimately leads to cell lysis (Kurtz 2010).

The introduction of Bt hybrids in sweet corn was originally made with a single Bt toxin (i.e., Cry1Ab or Cry1F) (Bilbo et al. 2018). Varieties expressing a single Bt toxin target a specific pest species (e.g., European corn borer, corn rootworm, corn earworm). However, the widespread use of these varieties with single toxins lead to the evolution of resistant alleles among different noctuid species (Bilbo et al. 2018).

The most recent Bt hybrids are a combination of multiple toxins, called pyramids (combinations of Cry1F, Cry1Ab, Cry1A.105, Cry2Ab2, and/or Vip3A20) (Bilbo et al. 2018). These varieties provide better control by including multiple toxins against the same pest, in an attempt to decrease the individuals with resistant alleles (Bilbo et al. 2018). Similar to corn, transgenic cotton (Bollgard) was first commercialized in 1996 expressing Cry1Ac (Luttrell and Jackson 2012). After its introduction, transgenic corn with two Bt toxins (Bollgard II) were commercialized, Cry1Ac + Cry2Ab2, and Cry1Ac + Cry1F (Tabashnik et al. 2009). Later on, Bollgard 3 expressing Cry1AC+Cry2Ab+ Vip3A20, and Widestrike 3 expressing Cry1AC+ Cry1F+VipA320 were released (Luttrell and Jackson 2012).

Bacillus thuringiensis resistance

Resistance to *Bacillus thuringiensis* toxins (Cry1Ab, Cry2Ab2, Cry1A.105, and Cry1Ac) has been documented in some corn earworm populations (Dively et al. 2016). Different factors could attribute this evolution, such as behavior and life history characteristics, Bt toxicity, trends in Bt acreage, and production practices (Dively et al. 2016). Whalon and Wingerd (2003) described three mechanisms of resistance. The most predominant one describes altered binding between Cry toxins and receptors localized in the insect's midgut. The second mechanism of resistance characterizes disruptions of Cry proteins proteolytic process. The third and final one suggests that septicemia is prevented through the regeneration of the insect's midgut (Whalon and Wingerd 2003).

Dively et al. (2016) characterized the field's evolved resistance to Bt toxins in a 10-year trial (1996-2016), showing reduction of field performance of Cry1A.105+Cry2Ab2 sweet corn. Neither Bt toxin provided control of larval infestations reaching 4th -6th instar and increasing the damaged kernel area of sweet corn (Dively et al. 2016, Venugopal and Dively 2017). Corn earworm resistance ratios have increased from 13.5 to >4,000 for Cry1A.105 toxin and from 0.26

to 33.7 for Cry2Ab2 toxin (Reay-Jones et al. 2020). Corn collected in Louisiana reported resistance ratios of 8 to >1,623 for Cry1A.105 toxin and 5 to 88 for Cry2Ab2 toxin (Kaur et al. 2019).

Overall, Vip3A20 is the Bt toxin that provides the best control of this pest (Burkness et al. 2010, Shelton et al. 2013). Vip3A20 is a novel class insecticidal protein that creates agricultural control against economically important pests such as black cutworm *(Agrotis ipsilon*), fall armyworm (*Spodoptera frugiperda*), tobacco budworm (*Heliothis virescens*), and corn earworm (*Helicoverpa zea*) (Kurtz 2010).

The production of resistant hemp varieties is not established. This is likely due, in part, to the end products produced by this crop. Without established resistance varieties, control of corn earworm in hemp relies mainly relies on insecticide applications, including with *Bacillus thuringiensis* (Britt et al. 2021).

The use of *Bacillus thuringiensis* to control corn earworm in several crops has led to the development of resistant alleles in corn earworm populations. Given that corn earworm is a multivoltine, mobile pest, the increase of resistant alleles runs through multiple generations. Relying on insecticides with *Bacillus thuringiensis* is not a reliable source for pest management in hemp.

Chemical control

Corn earworms are often managed using foliar applications of chemical insecticides. In crops such as cotton, sweet, field corn, and soybean corn earworm populations are controlled using insecticides with different modes of actions, predominantly pyrethroids, diamides, and spynosyns (Farias et al. 2013).

Pyrethroids have been a major group of insecticides used since 1970 for controlling economically important pests (Vijverberg and vanden Bercken 1990). Pyrethroids act as nerve poisons that interfere with sodium channels, leading to convulsion and rapid paralysis (Khambay and Jewess 2005). Although pyrethroids have been effective in controlling corn earworm in sweet corn and vegetables, they have provided less control since 2000 (Hutchison et al. 2007). A study conducted by Hutchison et al (2007) tested the decline of four common pyrethroids used to control corn earworm. The results showed that only 19.3% to 37.3% provided control against this pest, likely due to resistant alleles after years of insecticide exposure. The improper use of pyrethroids without rotating of modes of action resulted in the development of resistant individual capably to detoxify and pass resistant genes to their offspring (Khambay and Jewess 2005).

Spinosyns are commonly used to control lepidopteran species; this insecticide activates the nicotinic acetylcholine receptor, causing insect death by hyperexcitation of nervous system (Sporleder and Lacey 2013). This synthetic insecticide is commonly used to control corn earworm in sweet corn (Reay-Jones 2019).

Diamides belong to a new class of insecticides that target the ryanodine receptor; targeted insects undergo lethargy, muscle paralysis and death (Allen et al. 2022). A study conducted by Kuhar et al. (2010) showed how the use of diamides reduced corn earworm populations in tomatoes, as well as reducing crop injury. Viteri and Linares-Ramirez (2022) applied four different insecticides to control corn earworm in sweet corn, and they found that diamides were the most effective to control this pest. Overall, field testing has shown that diamides provide the highest mortality rates in corn earworm and therefore the best control (Olmstead et al. 2016).

Chemical control in hemp

Corn earworm is a major pest in hemp production and controlling it chemically has been a challenge. This is because the Controlled Substance Act categorizes hemp as a Schedule 1 drug, which had previously led to a lack of registration for pesticides on this crop (Cranshaw et al. 2019). Consequently, developing accurate pest management strategies for hemp has been slow (Britt et al. 2021).

The regulation of pesticide registration for hemp production varies from state to state. In some states, growers use pesticidal products exempt from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) registration requirements to manage pests. Other states have regulatory agencies that allow registration of specific insecticides for use on hemp (Cranshaw et al. 2019). These states follow registered pesticides from the Environmental Protection Agency (EPA), with 98 biopesticides and one conventional insecticide currently approved for hemp production as of 2023 (US EPA 2019).

Washington developed regulations in 2013 that have been adopted by many states, including Alabama, registering insecticides for use in hemp production (Cranshaw et al. 2019). These regulations include exempting active ingredients from tolerance requirements on all food crops and having broadly written label use directions (Cranshaw et al. 2019).

Insecticides used to control this pest are mainly botanically or biologically based, due to hemp’s unique uses (e.g., ingestion, dermal application) (Britt et al. 2021). Some registered insecticides allow this crop to be sold in an organic market, as well as being compatible with pollinators, predators, and parasitoids (Britt et al. 2021).

The most common insecticides used against corn earworm in hemp are nucleopolyhedrovirus (NPV) and *Bacillus thuringiensis* insecticides (Britt et al. 2021). NPV is a narrow-spectrum insecticide that is specific to certain species. When consumed by the insect, the occlusion bodies of NPV dissolve in the midgut's pH. This leads to the replication of occlusion bodies and ultimately results in the death of infected larvae within 5 to 7 days of exposure (Inceoglu et al. 2001). Field trials conducted in hemp measuring efficacy of biopesticides and conventional insecticides showed how the use of NPV insecticides and Bt insecticides did not provide an efficient control of corn earworm in hemp (Pulkoski and Burack 2023).

Laboratory bioassays with conventional and biopesticides were conducted with high mortality rates (95%-100) when exposed to conventional insecticides, specifically spinosyns. In addition, results from biopesticides with active ingredients such as nucleopolyhedrovirus virus (NPV) and *Bacillus thuringiensis* did not differ significantly from the control (Britt and Kuhar 2020). The lack of insecticides that provide efficient control against corn earworm in hemp requires us to explore novel integrated pest management strategies (Cranshaw et al. 2019).

Trap crops

The use of a trap crop may aid in decreasing corn earworm damage in hemp. Trap cropping is an environmentally friendly strategy that aims to reduce the overuse of insecticides (Poveda et al. 2008). This strategy diverts pests from the main crop and concentrates their feeding in the trap crop (Hokkanen 1991). Pests are lured to the trap crop, where they can be monitored and managed by using insecticides or creating a suitable environment for natural enemies to aid in pest suppression (Holden et al. 2012).

To be an effective pest management tool, a trap crop must be in an attractive phenological stage for adult oviposition (Badenes-perez et al. 2005). Achieving synchronization between attractive stages of trap crop and main crop increases the probability of the trap crop to be successful (Rhino et al. 2014). However, simply being attractive for oviposition is not enough for a trap crop to be successful, the trap crop must retain the targeted pest and prevent its development (Rhino et al. 2014).

A study conducted by Rhino et al. (2014) used sweet corn as a trap crop to control corn earworm in tomatoes. Results showed that female moths oviposited a greater number of eggs in the sweet corn resulting in less damage on fruiting tomato. A study conducted in Venezuela used sweet corn as a trap crop to control corn earworm in cotton, resulting in only 0.1% of damaged cotton (Javaid and Joshi 1995).

Research conducted by Javaid et al. (2005) showed the potential of sweet corn as a trap crop to suppress corn earworm populations in soybeans. Results show significant difference in corn earworm larvae presence in all treatments containing sweet corn. The reduction of corn earworm in soybeans resulted in an increase of soybean yield, indicating the effectiveness of sweet corn as a trap crop in soybean (Javaid et al. 2005).

Sweet corn is highly preferred by corn earworms for oviposition. Therefore, utilizing sweet corn varieties containing the Vip3A20 protein may prove to be an effective pest management strategy. This is because this protein has insecticidal properties against corn earworm.

Corn earworm in hemp is an ongoing challenge across United States. The newness of this crop and the difficulty in pesticide registration requires the development of different strategies that could potentially decrease damage of this insect pest in hemp. The objectives of this study are to: 1. Evaluate chemical control against corn earworm in hemp in field and laboratory settings, 2. Evaluate sweet corn as a potential trap crop to reduce corn earworm damage in hemp, and 3. Explore local hemp varieties as a cultural control strategy.

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Chapter 2

Evaluating chemical control against *Helicoverpa zea.*

Abstract

Corn earworm, *Helicoverpa zea* (Lepidotera: Noctuidae), has emerged as an injurious insect pest to hemp, *Cannabis sativa*. One of the biggest challenges in managing corn earworm in hemp is the limited number of effective pest management tools. Chemical control in this crop is challenging due to its complicated federal status and the different approaches taken by each state. Given the damage caused by this pest, we sought to explore the efficacy of insecticides in both field trials (2021-2022) and laboratory bioassays (2022). Hemp's unique architecture and environmental conditions in the southeast may both play a role in the efficacy of insecticide application in field trials. Therefore, laboratory bioassays were used to evaluate insecticide efficacy in the absence of these variables. Treatments in both trials consisted of a combination of biological insecticides currently registered in hemp and conventional standards used against corn earworm in other crops. In the field, insecticide applications commenced at the first sighting of corn earworm eggs or larvae. Three days after chemical applications, caterpillar numbers were assessed, and flower damage rated. In 2022, hemp plants were harvested, and fresh and dry weights taken. Laboratory bioassays were conducted using hemp leaves and flowers treated with low and high rates of insecticides to measure efficacy against multiple corn earworm larval instars. Results from field trials in both years showed no significant difference in damage ratings, caterpillar counts, or harvest data between insecticide treatments. In laboratory bioassays, results showed that conventional insecticides not currently registered for use in hemp were more effective than biological insecticides.

Introduction

In the United States, hemp was grown as a staple crop dating back to the colonial era (Cherney and Small 2016). Cultivation throughout history has taken place for multiple purposes, including fiber, seed, and cannabidiol (CBD) production (Fike 2019). Regardless of its multiple purposes, in 1937 the Marihuana Tax Act illegalized hemp due to its similarities with drug-type marijuana (Cherney and Small 2016). It wasn't until the 2014 Farm Bill (U.S. H.R. 2462 – 113th Congress [113-333]) that hemp was authorized for legal production. This was followed by the 2018 Farm Bill, which established Domestic Hemp Production along with removing hemp from the Controlled Substances Act (U.S. H.R. 2 – 115th Congress [115-334]). As a result, floral hemp production across the United States grew and by 2022, the estimated value was $179 million (USDA NASS 2022).

However, the resurgence of hemp production brought new challenges. Questions regarding the correct use of pest management strategies to control several species of insects and mites have not been answered. Corn earworm, *Helicoverpa zea,* is the most damaging pest in floral hemp production (Cranshaw et al. 2019, Britt et al. 2021). They concentrate their feeding on the flower bud creating an entry for pathogen infection (Britt et al. 2021). Historically, crops such as sweet corn, field corn, soybean, and cotton rely on genetically modified varieties or insecticide applications with different modes of action to successfully control this pest. Without these available control methods in hemp, control of *H. zea* in outdoor floral hemp is an ongoing challenge.

In floral hemp production, the use of conventional insecticides is restricted due to a lack of research on the potential contamination of CBD products, which are used by humans for their therapeutic and medicinal properties (Cranshaw et al. 2019). The Environmental Protection

Agency (EPA) regulates the use of pesticides and sets strict guidelines for their application. Insecticides that are labeled to control corn earworm are botanically or biologically based (Cranshaw et al. 2019). Registered insecticides include Nucleopolyhedrovirus (NPV) and *Bacillus thuringiensis* subsp. as active ingredients (Britt et al. 2021).

Biological insecticides used for this experiment were 1. Spear-Lep (GS-omega/kappa-Hxtx-Hv1a) (Vestaron, Stamford, CT, USA) + Leprotec (Bt subsp. *Kurstaki*) (Vestaron, Stamford, CT, USA), 2. Gemstar LC (NPV Insecticidal virus) (Certis Biologicals, Columbia, MD, USA) + BoteGHA ES (*Beauveria bassiana* strain GHA) (Certis Biologicals, Columbia, MD, USA), 3. Basin Flex (Insecticidal peptide U1-AGTXTa1b-QA) (Vestaron, Stamford, CT, USA) + Leprotec (Bt subsp. *Kurstaki*) (Vesatron, Stamford, CT, USA), 4. Heligen (*H. zea* NPV ABA-NPV-U) (AgBiTech, Fort Worth, TX, USA), 5. Heligen (*H. zea* NPV ABA-NPV-U) (AgBiTech, Fort Worth, TX, USA) + Dipel DF (Bt subsp. *Kurstaki*) (Valent BioSciences, Libertyville, IL, USA), 6. Heligen (*H. zea* NPV ABA-NPV-U) (AgBiTech, Fort Worth, TX,USA) + Xentari (Bt subsp. *Aizawai*) (Valent BioSciences, Libertyville, IL, USA).

Spear-Lep and Basin Flex have a mode of action in which peptides modulate the nicotinic acetylcholine receptor (nAChR) (IRAC 2023). These peptides only work when combined with *Bacillus thuringiensis kurstaki* such as Leprotec. The combination of both insecticides' modes of action lead to the rupture of the insects' gut cells, which allow the peptides to bind with the acetylcholine neurotransmitter (Guedot 2023). After binding occurs, insects' neurons depolarize and paralysis or death is achieved (Guedot 2023).

Gemstar LC and Heligen have nucleopolyhedrovirus (NPV) as the active ingredient that disrupts the insects' midgut. When consumed, the occlusion bodies of NPV dissolve in the midgut, leading to replication of occlusion bodies, and resulting in death.

Dipel DF and Xentari contain *Bacillus thuringiensis*. These insecticides target the insects' gut after ingestion by activation of gut proteases (Kurtz 2010). The midgut binds to receptors located on the brush border membrane, creating pore formation that leads to cell lysis (Kurtz 2010).

With these particular modes of actions, death of infected larvae is within four to seven days after exposure, but may be even shorter (Inceoglu et al. 2001). This statement is supported by Pingel and Lewis 1999 in which they measured the efficacy of *B. thuringiensis* products, with results showing larval death in one to three days after exposure. NPV products showed death after two to five days after ingestion (Pingel and Lewis 1999). In hemp, the timeframe between application and ingestion of insecticide and larval death gives the larvae several days to continue feeding, which creates open wounds susceptible for pathogen infection.

Conventional insecticides used in other are explored in this study. The conventional insecticides used were: Entrust SC (Spinosad) (Corteva, Indianapolis, IN, USA), Warrior II (Lamba-cyhalothrin) (Syngenta, Greensboro, NC, USA), Besiege (Lamba-cyhalothrin, 3 + Chlorantraniliprole) (Corteva, Indianapolis, IN, USA), Asana XL (Esfenvalerate) (Corteva, Indianapolis, IN, USA). As mentioned in Chapter 1, these insecticides have successfully controlled corn earworm damage in other crops.

The objective of this study is to measure the efficacy of biological and conventional insecticides to suppress corn earworm populations in the field. Specific objectives include: 1. Quantifying the efficacy of conventional insecticides compared to biological insecticides in outdoor floral hemp; 2. Evaluating the effect of conventional insecticides compared to biological insecticides in outdoor floral hemp yield; 3. Evaluating high and low rates of insecticide for control of corn earworm in hemp.

Materials and Methods

Insecticide trials

Establishment of hemp plants

Field experiments were conducted during the 2021 and 2022 growing season at E.V. Smith Research Center in Shorter, Alabama, USA (32.44562, -85.89010). On April 13, 2021, one thousand two hundred feminized BaOx variety seeds (Triangle Hemp, Raleigh, North Carolina, USA [34.53322° N, 83.04279° W]) were sown in the greenhouse at Plant Science Research Center in Auburn, Alabama, USA (32.58829, -85.48852). On April 15, 2022, one hundred feminized BaOx variety seeds were sown as seeds at Plant Science Research Center. In both years hemp seeds were placed in 36 cell count trays containing PRO-MIX 'BX' (Rivière-du-Loup City, Québec, Canada) potting soil and watered under a misting system for six weeks. The water regime shifted from thirty seconds of water every thirty minutes daily for the first four weeks to twice a day using a misting hose for the remaining two weeks.

Transplanting

On June 4, 2021, all plants were hand-transplanted into plastic beds covered with white mulch at E.V. Smith Research Center in Shorter, Alabama, USA (32.44562, -85.89010). Weed control was performed prior to laying plastic with the application of S-metolachlor (Dual Magnum, Syngenta, Greensboro, NC, USA). At cultivation, Ultrasol Multipurpose Plus 20-20-20 fertilizer (SQM North America) and 15.5-0-0 calcium nitrate were applied at a rate of 183.8 kg/ha. Throughout the growing season, weeds were manually removed weekly. Drip irrigation was used for water management and was applied as needed in all plots. Plots consisted of ten plants per plot, with 0.61 meters in row spacing and 1.83 meters between row spacing. Treatments used for the experiment included six biological insecticides registered for hemp and four conventional insecticides against corn earworm in other crops (Table 2.1). The ten treatments were replicated four times in a randomized complete block design.

In 2022, one hundred hemp plants were hand-transplanted into plastic beds with white mulch on June 9, 2022, at E.V. Smith Research Center. Agronomic practices were similar to those in 2021, except plots contained four plants each.

Data collection

In 2021 and 2022, scouting for corn earworm eggs and larvae began at flowering. As soon as corn earworm larvae were detected in the field, insecticide applications were made. In both years, a Lee Avenger high-clearance sprayer with AITT110002 spray nozzles was used with 16.84 milliliters per square meter of pressure.

In 2021, applications took place on August 5, August 10, and September 7, 2021. A five-day application interval was performed for the first two dates, whereas the third and last spray was delayed due to excessive rain. Three days after each application, corn earworm larval counts were conducted on one bud each from ten plants per plot. Damage ratings were assessed on the same buds where larvae were spotted. Damage ratings were performed using a 0-3 damage scale after Britt et al. (2021) (Figure 2.1). Criteria for this scale include 0 is no damage; 1 is visible damage, but still marketable; 2 is damage of 50% or less, making the bud unmarketable; 3 is damage to more of 50% making the bud unmarketable.

Data collection began on August 2 before the first spray application. Subsequently, three days after each foliar application on August 8, August 13, and September 10, 2021, data were collected. Excessive rain delayed the third application; therefore, an extra larval count and damage rating was done on September 3, 2021, prior to the final application.

In 2022, the first insecticide application was made on August 1, 2022. Insecticide applications followed the same methodology as 2021. Applications were made on August 5, August 10, August 15, and August 26, 2022. We conducted four sprays, which one was one more than in 2021 due to the consistent pest pressure. Data were collected three days after application on August 4, August 8, August 13, August 18, and August 30, 2022. Additionally, an extra larval count was conducted prior to harvest on September 10, 2022.

Harvest

To evaluate the effectiveness of each insecticide treatment, yield data were taken in 2022. Harvest data were not possible in 2021 due to severe damage to hemp plants from disease and weather.

On September 16, two plants per plot were harvested by cutting at the base of the plant using bond steel bypass lopper (Bond Manufacturing, Antioch, California, USA). Fresh weight was recorded from each plant with a hanging scale (Uline, Pleasant Prairie, Wisconsin, USA). After weighing, harvested plants were grouped together in bundles of two or three plants using a wool string and then hung upside down on Grip-Rite black annealed steel 16 Ga. tie wire (0.0508 centimeters D x 102.108 meters L) (Prime Source, Irving, Texas, USA) for two weeks to dry. On September 30, plants were weighed with a hanging scale (Uline, Pleasant Prairie, Wisconsin, USA) and the dry weight of each plant recorded.

Three randomly selected buds were collected from the two plants immediately prior to harvest. Three-centimeter-long buds were trimmed from the upper, middle, and bottom part of the plant and placed individually in Ziploc bags (Ziploc, San Diego, USA). Flower buds were evaluated for marketability using the same 0-3 damage scale. Fresh weight of each flower bud was recorded using a digital scale (VWR, International LLC, Radnor, Pennsylvania, USA). After weighing, flower buds were stored in Plant Science Research Center for drying. On October 16, 2022, the dry weight was measured using a digital scale (VWR, International LLC, Radnor, Pennsylvania, USA).

Bioassays

Bioassays were conducted to measure the mortality of corn earworm larvae when exposed to different insecticides rates and modes of actions. The experiments consisted of leaf bioassays (#1 and #2) and flower buds bioassays; each had its own methodology but followed the same basic protocol. The experiment consisted of nine treatments, each replicated six times (Table 2.2). The rates used for this experiment were the minimum and maximum label rate and applied in 100 milliliters of water. All experiments were done in the laboratory with temperatures consistently between 22.8°C - 23.8° C.

Bioassay #1

Corn earworm (*Helicoverp*a *zea*) larvae were collected on July 28, 2022, from non-Bt sweet corn plots located at Tennessee Valley Regional Research and Extension Center, Belle Mina, Alabama, USA (34.69012, -86.88192). One hundred eight (108) larvae were extracted from corn silks and individually placed in trays containing 35 mL diet cups filled with Frontier Agricultural Sciences (Newark, DE, USA) all-purpose Lepidopteran diet. Trays containing 4th, 5th, and 6th instar larvae were transported in a cooler back to the laboratory.

On July 30, 2022, larvae were removed from diet cups relocated to new cups to starve for 48 hours. These cups consisted of only a moistened cotton ball. On July 31, 2022, the head capsule of each individual larva was measured using digital calipers (Uline, Pleasant Prairie, Wisconsin, USA). Head capsule measurement was used to determine larval instar after Capinera (2000). Mean head capsule measurements for one to sixth instar larvae are 0.29, 0.47, 0.77, 1.30, 2.12, and 3.10 mm (Capinera 2000).

Hemp leaves from variety BaOx were collected from E.V. Smith Research Station on August 1, 2022. Two hundred (200) leaves were collected in Ziploc bags and transported in a cooler to the laboratory. Leaves were placed individually in 108 Petri dishes (100 mm x 15 mm) (Fisher Scientific, Waltham, Massachusetts, USA). Each leaf was sprayed with insecticide until runoff. One corn earworm larva was placed in each Petri dish. Petri dishes were maintained at laboratory ambient light (8 hours light:16 hours dark) with temperatures of 25-27 °C. Mortality was monitored daily from August 2, 2022, to August 9, 2022. Mortality was assessed using soft forceps to see if the caterpillar responded to touch with movement.

Bioassay #2

Corn earworm eggs from Frontier Agricultural Sciences were used for the early instar bioassays. Emerged larvae (n=108) were placed individually in diet cups on February 3, 2023. Each diet cup contained Frontier Agricultural Sciences all-purpose Lepidopteran diet. On February 5, 2023, larvae were relocated to diet cups containing only a moistened cotton ball and starved for 48 hours. Head capsule measurements were made using the widest part of each corn earworm larvae ranging from 0.1-0.2 mm.

On February 5, 2023, 150 hemp leaves from variety BaOx were collected from a research greenhouse at Alabama State University, Montgomery, Alabama, USA (32.36373, -86.29428). The collected leaves were placed in flower tubes with water and transported in a cooler to the laboratory. On February 7, 2023, each hemp leaf was placed in Petri dish (100mm x 15mm) and treated following the methods from Bioassay #1. Mortality was recorded daily for seven days from February 8, 2023, to February 15, 2023.

Flower bud bioassays

On September 22, 2022, a collection of corn earworm larvae was collected from non-Bt sweet corn at Brewton Research Station, Brewton, Alabama, USA (31.14132, -87.04788). One hundred eight (108) larvae were placed in diet cups filled with All-Purpose Lepidopteran diet and transported to the laboratory. Larvae were removed and placed in new diet cups containing a moistened cotton ball on September 24, 2022 to starve for 48 hours. To determine the developmental stage of each individual larvae, head capsule measurements were conducted following the previously mentioned protocol. Instars ranged from 4th to 6th, with head capsule sized ranging from 1.4-3.57 mm.

Hemp flower buds (n=108) from the variety BaOx were collected on September 26, 2022, from E.V. Smith, Research Station. Three-centimeter-long flower buds were placed in Ziploc bags and transported in a cooler to the laboratory. Each flower bud was completely submerged in a 100 mL solution of each insecticide. Petri dishes were set up following the same guidelines from previous bioassays. Mortality was recorded daily from September 26 - October 1, 2022.

Low concentration rate flower bud bioassay

Cherry Blossom propagation process

Hemp clones, variety Cherry Blossom, were used for this experiment and propagated at the Ornamental Horticulture Research Center, Mobile, Alabama, USA (30.70205, -88.14550). Hemp clones were propagated by cutting plant material from the lower part of the plant. The cuts ranged from 2.5-5mm in diameter and 8 cm long. All sugar leaves from the cuttings were removed and two or three true leaves remained. Cuttings were dipped into Dip N Grow (Dip N Grow, Clackamas, OR) rooting hormone, with a concentration of 1:5 (Dip n Grow: Water) for five seconds. Hemp cuttings were placed into 50-cell count trays containing a propagation mix of perlite: vermiculite: and peat moss (1:1:0.5). Each cutting had one or two nodes under the media surface to aid in rooting. Hemp cuttings were placed under mist irrigation using Netafim CoolNet ProTem. For the first four weeks, water was applied for one minute every hour. The remaining two weeks a low-frequency mist was applied for one minute every eight hours. MWF 20-10-20 (Peters Professional 20-10-20 water-soluble fertilizer) was applied three times a week with 200 ppm used for six weeks.

Cherry Blossom clones were transported on September 29, 2022, in six-inch pots from H-C Companies (Twinsburg, OH, USA) from the Ornamental Horticulture Research Center to the laboratory at Auburn University. Pots were filled with PRO-MIX 'BX' (Rivière-du-Loup City in Québec, Canada). Cherry Blossom clones were watered twice a day using an industrial hose.

Bioassay set up

Corn earworm eggs from Frontier Agricultural Sciences were used for this bioassay. Newly emerged larvae (n=54) were individually placed in diet cups filled with All-Purpose Lepidopteran diet. On October 11, 2022, individual larvae were transferred to new diet cups with a moistened cotton ball and starved for 48 hours. Head capsule measurements were performed in each second instar larvae with measurements ranging from 0.32-0.45 mm at the widest part of the head.

Three-centimeter-long buds were cut from the Cherry Blossom clones on October 13, 2022. Flower buds were treated following the same guidelines from the first flower bud bioassay. and Petri dishes were used similarly. This experiment was initiated on October 14, 2022 and completed on October 21, 2022.

Statistical Analysis

All statistical analyses were conducted in SAS® 9.4 (Cary, NC, USA). Data from 2021 and 2022 were analyzed separately due to differences in treatments, application numbers, and plot size. The average caterpillar numbers and damage ratings in both 2021 and 2022 field trials were analyzed using a generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). To compare the treatments, least square means were separated using Tukey- Kramer. The fixed effect treatment was used to model the average caterpillar numbers and damage ratings. The identity link function and the Gaussian distribution were used. Degrees of freedom were calculated using the Residual Method. Means and standard errors were determined using PROC MEANS.

Average caterpillar numbers and damage ratings in both 2021 and 2022 were analyzed by each individual date using generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). To compare treatments by date, least square means were separated using Tukey- Kramer. Data were analyzed with Gaussian distribution and identity link function. The Residual Method was used to calculate the degrees of freedom. Means and standard errors were determined using PROC MEANS.

Harvest data were analyzed including the average fresh and dry weight of the plants, fresh and dry weight of the buds, and flower bud marketability. Variables were analyzed using a generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). Least square means of treatments were performed using Tukey- Kramer. The average fresh weight and dry weight of the two harvested plants, average bud fresh weight and dry weight, and the proportion of marketable and unmarketable buds were modeled as a function to the fixed effect treatment. Data were analyzed using Gaussian distribution along with the identity link function. Degrees of freedom were calculated using the Residual Method. Finally, means and standard errors were determined using PROC MEANS.

Data for leaf bioassays were analyzed using generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$) and least square means of treatments were performed using Tukey- Kramer.

Results

2021 Insecticide trial

Overall, in 2021 and 2022 there was no significant difference between treatments on average caterpillar numbers (2021: F= 0.590, P= 0.804; 2022: F= 1.280, P= 0.249) (Table 2.3, 2.5). In addition, damage ratings results for 2021 and 2022 showed no significant differences between treatments (2021: F= 0.360, P= 0.951; 2022: F= 0.190, P= 0.995) (Table 2.4, 2.6). However, there were significant differences on each sampling date (Table 2.8).

On each of the five sample dates in 2021, there were no significant differences observed in caterpillar numbers (Table 2.7). However, treatments provided a decline in caterpillars from the first date (F= 1.230, P= 0.239) (Figure 2.2) to the second date (F= 1.710, P= 0.130) (Figure 2.2a). On the third (F= 0.560, P= 0.819) (Figure 2.2b), fourth (F= 1.490, P= 0.1971) (Figure 2.2c) and fifth (F= 0.950, P= 0.497) (Figure 2.2d) sample dates, there were no significant differences between treatments. However, all products provided a numerical reduction in caterpillars compared to the untreated control.

There were significant differences in damage ratings between treatments across all dates (Table 2.8). In the first date (F= 10.1900, P= <.0001), Spear-Lep+ Leprotec, Heligen+ Dipel DF and Basinflex + Leprotec had significantly less damage than the untreated control, Heligen + Xentari, and Warrior II (Figure 2.3). Plots treated with Gemstar LC + BoteGHA ES, Heligen, Asana XL, and Besiege had significantly less damage than all other treatments.

On the second date (F= 7.660, P = <.0001), Basinflex + Leprotec, Asana XL, Gemstar LC + BoteGHA ES, Besiege, and Heligen all had significantly less damage than the untreated control. All other treatments did not differ significantly from the untreated control (Table 2.8, Figure 2.3a).

Results for the third date (F= 22.780, P= <0.001) showed that Besiege, Heligen, Heligen + Dipel DF, Asana XL, Basinflex+ Leprotec, and Gemstar LC + BoteGHA ES were not significantly different from each other (Figure 2.3b), but had significantly lower damage than Warrior II, Heligen + Xentari, Spear-Lep + Leprotec and the untreated control. The same pattern occurred on the fourth (F= 17.750, P= <.0001) and fifth date (F= 19.190, P= <.0001).

2022 Insecticide trial

On each of the eight sample dates in 2022, there were no significant differences in caterpillar counts or damage ratings between treatments (Tables 2.9, 2.10). Caterpillars or damage were not found on the first two dates of assessment (Table 2.9).

On the third date results for both caterpillar numbers (F= 0.6100, P= 0.7778) and damage rating (F= 0.610, P= 0.778) showed that Besiege had fewer caterpillars compared to the untreated control (Table 2.9, 2.10, Figure 2.4, 2.5). Plots treated with Asana XL, Entrust SC, Gemstar LC + BoteGHA ES, Heligen + Dipel DF, and Heligen + Xentari had a more caterpillars and higher damage compared to all treatments.

On the fourth date (F= 1.860, P= 0.099), plots treated with Besiege and Asana XL lower caterpillar numbers compared to the untreated control (Table 2.9, Figure 2.4a). However, there were no significant differences between treatments in damage ratings (F= 0.830, P= 0.595) (Table 2.10, Figure 2.5a).

On the fifth date, there were no significant differences between treatments for caterpillar numbers (F= 0.670, P= 0.725) (Table 2.9, Figure 2.4b) or damage ratings (F= 1.190, P= 0.339) (Table 2.11, Figure 2.5b).

There was an increase in both caterpillar numbers (F= 0.500, P = 0.866) and damage ratings (F= 0.360, P= 0.946) on the sixth date compared to the past dates (Table 2.9, Table 2.10, Figure 2.4c, 2.5c), although these were not significant increases. While there we no significant differences Besiege, Entrust SC, Heligen + Xentari, and Spear-Lep + Leprotec provided better control in caterpillar numbers (Table 2.9, Figure 2.4c). The treatments resulting in lower damage were Warrior II, Heligen + Xentari, and Entrust SC (Table 2.10, Figure 2.5c).

All treatments had lower caterpillar numbers compared to the control on the seventh date (F= 1.090, P =0.399), although this was not significant (Table 2.9, Figure 2.4d). Damage ratings (F= 0.340, P =0.953) on this date had similar results, with all treatments had a reduction in damage compared to untreated control, but these results were not significant (Table 2.10, Figure 2.4d).

On the last date of assessment, there were no significant differences between caterpillar numbers (F= 0.650, P =0.745) (Table 2.9, Figure 2.4e). Damage ratings (F= 0.580, P = 0.803) were not significantly different, but all treatments provided less damage compared to the untreated control (Table 2.10, Figure 2.5e).

Harvest

For the harvest data, fresh weight (F= 1.080, P= 0.406) and dry weight (F= 1.360, P= 0.249) were not significantly different between treatments (Table 2.11, 2.12). However, all treatments had higher fresh and dry weight compared to the untreated control. Similarly, analysis of fresh weight (F= 1.04, P= 0.4314) and dry weight (F= 0.660, P= 0.734) from the buds did not show any significant differences between treatments (Table 2.13, 2.14).

There were biological differences in the proportion of unmarketable buds (F=1.940, P=0.052) between treatments (Table 2.15, Figure 2.6), although these results were not significant. Entrust SC, Besiege, Asana XL and Heligel + Dipel DF had the lowest numbers of unmarketable buds compared to all treatments.

Flower bud bioassays

High concentration rates of nine insecticide treatments (Table 2.16) were used to assess mortality of six larvae per treatment in seven days. While results in corn earworm mortality varied among treatments, the three conventional insecticides Asana XL, Entrust SC, and Karate showed fastest knockdown one day after treatment (Table 2.16).

Data taken four days after biopesticide treatment are the most useful given the mode of action of these treatments and the time it takes to inflict larval death (Table 2.16). Mortality four days after treatment showed that most biopesticides worked against corn earworm larvae (Table 2.16). Surprisingly, Xentari was the only biopesticide that led to larval mortality in all replications seven days after treatment (Table 2.16).

Results on low concentration rates showed that the three conventional insecticides Asana XL, Entrust SC, and Karate provided the fastest knockdown one day after treatment (Table 2.17). Gemstar, Leprotec, and Heligen + Dipel DF were the biopesticides that provided the best results with knockdown at four days after treatment. Heligen and Xentari provided the slowest knockdown (Table 2.17).

Low concentration flower bud bioassay

These bioassays were conducted on second-instar larvae. One day after treatment, the majority of larvae were dead across all treatments (Table 2.18). As expected, the three conventional insecticides Asana XL, Karate, and Entrust SC worked faster than the other insecticides. Nevertheless, two days after treatments, all products had successfully killed all remaining larvae.

Leaf Bioassays

One day after treatment (F= 41.600, P= <.0001) Asana XL, Entrust, and Karate had significantly higher mortality than all treatments (Table 2.19). Xentari was the only biopesticide significantly different from the untreated control (Table 2.19).

At two (F= 29.700, P= <.0001), three (F= 7.010, P= 0.0042), four (F= 17.380, P= 0.0001), five (F= 19.400, P<.0001), six (F= 19.400, P<.0001) and seven (F= Infty, P = <.0001) days after treatment, all products resulted in significantly higher mortality than the untreated control (Table 2.19). Larvae exposed to Xentari and Heligen had the slowest knockdown with seven days after treatment (Table 2.19).

Results in low concentration bioassays showed that at one day after treatment (F= 198.000, P<.0001) Asana XL, Entrust SC, and Karate were significantly different among treatments, providing the fastest knockdown (Table 2.20). Among biopesticides, Leprotec led to significantly higher mortality than Heligen + Dipel DF, Gemstar, and Xentari.

At two days after treatment (F= 8.210, P= 0.002) Heligen, Heligen + Dipel DF, Gemstar, and Xentari provided better results in larval death compared to the untreated control (Table 2.20). Leprotec and the three conventional insecticides Asana XL, Entrust SC, and Karate had overall better results from all treatments.

Similar results were seen three days after treatment (F= 22.120, P<.0001), with all treatments being significantly different compared to the untreated control (Table 2.20). Xentari had the lowest mortality rate and was significantly different from biopesticides and conventional insecticides.

All treatments were significantly different from the untreated control four days after treatment (F= 151.00, P<.0001) (Table 2.20). Overall, all biopesticides exhibited slower larval death rates. Among the biopesticides, Gemstar and Heligen performed the best and were significantly different from Xentari.

At five (F= 36.000, P<.0001) and six (F= 25.750, P<.0001) days after treatment all treatments were significantly different from the control (Table 2.20). Heligen and Xentari were significantly different from the untreated control but required a few days to achieve complete larval death. Overall Xentari took the longest on achieving complete larval death, but by seven days after treatment (F= Infty, P<.0001) all larvae were dead.

Discussion

Field trials

Corn earworm, *Helicoverpa zea*, remains an economically important pest across multiple agricultural systems, posing economic challenges for growers. In hemp, chemical control for this pest is limited. This study evaluated conventional insecticides that are not labeled in hemp and biopesticides that are currently registered in hemp.

No significant differences were observed for control of caterpillar in field trials in 2021 or 2022, however there were significant reductions in damage ratings among treatments. In general, conventional insecticides tended to provide better control than biopesticides or the untreated check, however results varied between years.

Results from 2021 showed a consistent but non-significant trend in a reduction of caterpillar numbers provided by Besiege. This dual action insecticide exhibited the highest reduction among all treatments on the second, fourth, and fifth dates. Damage assessments revealed that Besiege, Heligen, Heligen + Dipel DF, Asana XL, Basinflex + Leprotec, and Gemstar LC + BoteGHA ES consistently resulted in less damage across all dates compared to the untreated control.

Overall, the field study showed variations in both caterpillar numbers and damage ratings. Despite the small presence of caterpillars, all plants were damaged at the end of the trial. This is due to the open wounds created by corn earworm while feeding. These open wounds are susceptible to pathogen infection, causing bud rot.

In 2022, while there were no significant differences observed between treatments. Besiege, Asana XL, Entrsut SC, Heligen, and Spear-Lep+ Leprotec were more successful in reducing caterpillar number and damage. Of all the treatments used in this study, only Heligen and Spear Lep + Leprotec can be legally used on this crop. Growers could include these biopesticides as a potential strategy against corn earworm. In both 2021 and 2022, despite the small numbers of caterpillars, all plants were damaged at the end of the trial.

Beyond the challenges hemp poses in pest management, there are few insecticides registered for hemp. In hemp, insecticides that are labeled to control corn earworm are botanically or biologically based (Cranshaw et al. 2019) and these insecticides take four to seven days to kill larvae after exposure. The time frame between exposure and larval death gives the larvae time to continue feeding, resulting in open wounds susceptible for pathogen infection.

The effectiveness of Dipel and Gemstar was evaluated by Little et. al (2016) in non- Bt cotton. Field trials were conducted with different concentrations of both insecticides. In the field study, results showed that larval numbers in non-Bt cotton were not significantly different compared to the control. This is similar to our results from the 2021 and 2022 hemp trials. NPV insecticides are susceptible to degradation in higher temperatures due to solar irradiation (Little et al. 2016), and this may have been a factor in our trials in Alabama. Average temperatures during the sprays in August and September, 2021 were 30°C and 23.3°C. Temperatures in 2022 were higher at 33.3°C and 29.3°C respectively.

In contrast to the findings of this experiment, biological insecticides have provided effective control in other crops. For instance, in cotton, field tests were conducted on 1974-1975 to control *Heliothis* spp. (Bell and Kanavel 1977). Field tests consisted of measuring the efficacy of NPV occlusion bodies against *Heliothis* spp. Results from field tests showed that 1.48xl0^{12} PIB/ha (pink bollworm/ha) had 59% less boll damage compared to the untreated control. In addition, *Heliothis* spp. populations did not reach economic thresholds compared to the untreated control (Bell and Kanavel 1977).

NPV was found to be successful against corn earworms in field experiments on lima beans conducted by Woodall and Ditman (1967). Results showed that control is increased when application is done weekly instead of biweekly (Woodall and Ditman 1967). Although insecticidal applications in our hemp trials were done on a five-day interval, results did not show any significant difference. The lack of significant differences in larval counts and damage ratings in the hemp trials could be attributed to the larger canopy area and complex architecture of hemp plants, which may result in inadequate coverage when applying foliar insecticides.

Given the poor efficacy provided by biopesticides in hemp, we sought to explore the efficacy of conventional insecticides. Pyrethroids such as Warrior II and Asana XL are a major group of insecticides that have been widely adopted in other agricultural systems (Vijverberg and vanden Bercken 1990). These insecticides cause mortality by disrupting the sodium channels of the targeted pests. Although they has proven to be successful in other crops, pyrethroids did not provide adequate control in our hemp trials.

The reasons for the results are unclear, but corn earworm populations have been reported to have resistant alleles against this class of insecticide in the southern United States (Hopkins and Pietrantonio 2010a). Pyrethroids have greater efficacy when targeting early instar larvae, and if full coverage was not achieved in the hemp plants, this may had played a role in corn earworm populations (Hopkins and Pietrantonio 2010b).

Besiege (diamides) and Entrust SC (spinosad) are novel class insecticides that have been successful in other crops. Steckel and Stewart (2016) showed plots treated with Besiege had a significant reduction in both corn earworm populations and yield loss (Steckel and Stewart 2016). In soybean, diamides are commonly used to provide successful control against corn earworm (Swenson et al. 2013).

In sweet corn, a study conducted by Farrar et al. (2009) measured the efficacy of spinosad to control corn earworm. Results showed a reduction of both larvae and damage when exposed to this insecticide, and provided the best results compared to other treatments (Farrar et al. 2009). In our experiment, Besiege and Entrust SC did not provide significant a reduction in caterpillars, applications resulted in the highest larval reduction and lowest damage in both years compared to all treatments. Replicated research on the efficacy of these insecticides is needed for hemp.

In hemp, there are no economic thresholds for corn earworm or other pests of economic importance. There is still a need for replicated research on economic losses in hemp to properly develop the economic thresholds and injury levels and advise growers. For this study, foliar applications were conducted at first sight of corn earworm larvae or eggs. Given the unique architecture of hemp plants, finding corn earworm eggs or early instar larvae is extremely difficult. Spray initiation timing and difficulty in scouting may play a role in our results as well as effective control. At this time, growers should conduct weekly scouting for this insect pest and initiate control measures when larvae are detected. This study highlights the importance of replicated research trials on efficacy of different insecticides across regions.

Harvest

Harvest was conducted in 2022 on two plants; harvest was not conducted in 2021 due to significant plant degradation from weather and disease pressure. Overall, results showed no significant differences in fresh or dry weight, or the proportion of unmarketable buds between treatments. However, compared to the untreated control, all treatments had higher fresh and dry weight compared to the untreated control. Besiege and Entrust SC had the highest weight among all treatments, although neither product is registered for use in hemp in the United States.

Furthermore, it is important to note that hemp dry weight is linked to plant's height, fan leaf production, internodal branching and abundance of flowers (Prats et al. 2022). At the end of the trial, all harvested hemp plants had some level of damage, which may explain why results were not significantly different. The number of plants harvested likely influenced the statistical power of fresh and dry weight; harvesting a larger number of plants in future trials may result in statistical significance.

There were variations in fresh and dry weight of harvested buds, although they were not significant. Among treatments, only Heligen, Heligen + Dipel DF, and Entrust had higher fresh weight compared to the untreated control. Results showed that Gemstar LC + BoteGHA ES had higher dry weight compared to the untreated control. Flower buds were collected from the upper, middle, and bottom part of the hemp plants. However, some hemp plants displayed a lack of middle and bottom buds due to damage. There is a possibility that there were differences in the marketability of buds based on their position on the plant, although we were unable to accurately test this given the damage.

The proportion of unmarketable buds differed between treatments (P= 0.0522), although this difference was not significant. Even though there was no statistical difference, all treatments had less unmarketable buds compared to the untreated control. Overall, there were less damaged buds in plots treated with Entrust SC, Besiege, Asana XL, and Heligen + Dipel DF. These results follow the trends in which Entrust SC, Besiege, and Asana XL had the best results in controlling damage and reducing unmarketable buds among treatments.

In contrast to these findings, (Hamm and Young 1971) found that application of NPV alone and in combination with Bt products successfully improved yield in early season sweet corn by 18.3 and 28.4%, respectively. Furthermore, field experiments using two strains of *Bacillus thuringiensis* (*B. thuringiensis* var. *kurstaki* and *B. thuringiensis* var. *aizawai*) were evaluated against corn earworm in grain yield of corn. Results from this study showed that both strains had an increase in grain yield compared to the untreated control (Torres-Cab et al. 2022). It is worth noting that in both studies, these insecticides did provide a significant difference in larval damage compared to the untreated control.

Although economic thresholds aren’t established in hemp, research is needed regarding yield loss from corn earworm damage. Field studies that focus on yield-loss relationships are crucial in determining the thresholds in pest and host phenology, damage and developmental rates, and using practical management strategies (Hunt 2015).

Bioassays

Laboratory bioassays were conducted to assess the efficacy of insecticides in low and high concentration rates under a controlled environment (Table 2.2). Larvae were susceptible to Karate, Entrust SC, and Asana XL one day after treatment in both low and high concentration rates. Given the modes of action from the biopesticides used, results four days after treatment were the most meaningful for this experiment. Similar to Pingel and Lewis (1999), pesticides containing NPV and Bt take a few days longer for effective knockdown compared to conventional insecticides. Overall, high concentration rates were more successful than low concentration rates four days after treatment in all treatments except for Xentari.

Low concentration rates were used in second instar larvae. Data show effective control from all treatments two days after treatment. A study conducted on European corn borer (Lepidoptera: Crambidae, *Ostrinia nubilalis*) showed that first instar larvae were more susceptible after exposure to Dipel than fourth and fifth instar larvae (Huang et al. 1999). Susceptibility on early instar larvae may be attributed to their smaller size which makes them less able to tolerate insecticides (Rock and Monroe 1983). In addition, the sublethal effect of pyrethroids was significantly different from corn earworm late instar larvae compared to first and second instar larvae (McClanahan 2012).In hemp, this is worth exploring to provide more insight on the behavior of corn earworm after exposure to biopesticides.

Leaf bioassays were conducted using the same treatments (Table 2.2). In low and high concentration rates, Asana XL, Karate, and Entrust SC provided control one day after treatment. Effective control among biopesticides varied among treatments at two and three days after treatment. However, low concentration rates at four days after treatment showed that most biopesticides had successfully killed all larvae; only Gemstar, Xentari and Heligen took additional days. High concentration rates at four days after treatment showed an improvement in Gemstar, but not in Heligen and Xentari.

The potential effect on increased susceptibility when two modes of actions are combined was measured with Heligen + Dipel DF. Results showed that the combination of both biopesticides did provide a better control than Gemstar, Xentari, and Heligen, but not Leprotec. Similar results were found by Bell and Romine (1986), in which the combination of NPV and Bt increased mortality of *Heliothis* spp. This statement was found also true, in a study conducted by

Qayyum et al. (2015) on *Helicoverpa armigera.* Second and fourth instar larvae were used to determine whether the combination of Bt and NPV. The interaction of both insecticides did provide better results than individual insecticides (Qayyum et al. 2015).

Similar findings were reported by Britt and Kuhar (2020), in which the conventional insecticide Entrust SC provided the fastest control among treatments. In this experiment, we found that Gemstar took more than four days to successfully kill all larvae. This may be attributed to the fact that Gemstar takes more than 96 hours to kill large corn earworm larvae (Opender Koul 2012, Britt and Kuhar 2020).

Laboratory bioassays were conducted by Little et al. (2016) in non-Bt cotton to evaluate the effectiveness of Dipel and Gemstar against corn earworm larvae. Results showed that both insecticides resulted in larval death at five days after exposure. This also shows how insecticides based on NPV and BT may work more slowly than other conventional products.

Overall, low, and high concentration rates of conventional insecticides (Asana XL, Entrust SC, and Karate) provided the fastest mortality rates among all treatments. These findings demonstrate that low concentration rates can be used, saving product and money for growers. While the biopesticides took longer to achieve complete larval death, Heligen + Dipel DF and Leprotec provided better control than the other biopesticides at four days after treatment in both low and high concentration rates. Heligen and Xentari were the slowest to achieve complete larval death, and better results were found at high concentration rates.

Conclusions

Chemical control in hemp is challenging due to the newness of this crop and the lack of pest management strategies. Results from this study showed that even though there were no significant differences among treatments, Besiege, Entrust SC and Asana XL worked best in controlling corn earworm damage. Alabama has high temperatures and a humid environment, resulting in the possible degradation of biopesticides. Regardless of the insecticide used, if larvae feed on the buds, damage will most likely occur.

While our laboratory bioassays showed high larval mortality using conventional insecticides, further research is needed on the efficacy of these insecticides in outdoor hemp. To provide better recommendations to growers, it is crucial to investigate application timing and coverage for optimal control.

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Tables

Table 2.1

Trade Name, Manufacturer	Active ingredient(s), Mode of Action[x]	Rate/ha (liter or kg)	Years evaluated
UTC	NA	n/a	2021, 2022
Spear-Lep+Leptrotec, Vestaron	GS-omega/kappa-Hxtx-Hv1a, 32 + Bt subsp. Kurstaki, 11	2.34 + 1.16	2021, 2022
Gemstar LC+BoetGHA ES, Certis Biologicals	Insecticidal virus, 31 + *Beauveria bassiana* strain GHA, UFN	0.37 + 1.16	2021, 2022
Basin Flex+Leprotec, Vestaron	Insecticidal peptide U1-AGTXTa1b-QA, 32 + Bt subsp. kurstaki, 11	0.58 + 1.16	2021
Heligen, AgBiTech	*H. zea* NPV ABA-NPV-U, 31	0.09	2021, 2022
Heligen+Dipel DF, Valent BioSciences	*H. zea* NPV ABA-NPV-U, 31 + Bt subsp. Kurstaki, 11	0.09 + 1.12	2021, 2022
Heligen+Xentari, Valent BioSciences	*H. zea* NPV ABA-NPV-U, 31 + Bt subsp. Aizawai, 11	0.09 + 1.12	2021, 2022
Warrior II[y], Syngenta	Lamba-cyhalothrin, 3	0.14	2021, 2022
Besiege[y], Corteva	Lamba-cyhalothrin, 3 + Chlorantraniliprole, 28	0.73	2021, 2022
Asana XL[y], Corteva	Esfenvalerate, 3	0.7	2021, 2022
Entrust SC[y], Corteva	Spinosad, 5	0.37	2022

UTC= Untreated Control

[x] IRAC (IRAC 2022)

[y] Insecticides currently not registered for use in hemp

Table 2.2

Trade Name, Manufacturer	Active ingredient(s), Mode of Actionx	Low-rate (mL or gr/ 100mL)	High-rate (mL or gr/ 100mL)
UTC	NA	n/a	n/a
Gemstar LC, Certis Biologicals	Insecticidal virus, 31 + Beauveria bassiana strain GHA, UFN	0.78mL	1.95mL
Xentari, Valent BioSciences	Bt subsp. Aizawai, 11	0.05gr	0.37gr
Heligen, AgBiTech	H. zea NPV ABA-NPV-U, 31	0.23 mL	0.47 mL
Leprotec, Vestaron	Bt subsp. kurstaki, 11	0.20 mL	0.68 mL
Heligen + Dipel DF AgBiTech, Vesatron	H. zea NPV ABA-NPV-U, 31 + Bt susbsp. kurstaki,11	0.20 mL+ 0.23 mL	0.68 mL + 0.47 mL
Asana XL[y], Corteva	Esfenvalerate, 3	1.13 mL	1.88 mL
Entrust SC[y], Corteva	Spinosad, 5	0.29mL	1.13 mL
Karate[y], Syngenta	Lamba-cyhalothrin, 3	0.50 mL	0.75 mL

UTC= Untreated Control

[x] IRAC (IRAC 2022)

[y] Insecticides currently not registered for use in hemp

Table 2.3

Treatment	Mean number of Caterpillars per 10 plants ± S.E.M
UTC	0.137 ± 0.037
Asana	0.116 ± 0.038
Basin Flex + Leprotec	0.090 ± 0.039
Besiege	0.185 ± 0.039
Gemstar LC + BoteGHA ES	0.201 ± 0.050
Heligen	0.172 ± 0.053
Heligen + Dipel DF	0.116 ± 0.041
Heligen + Xentari	0.155 ± 0.072
Spear-Lep + Leprotec	0.143 ± 0.031
Warrior II	0.119 ± 0.041
P-value	0.8039

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.4

Treatment	Mean damage per 10 plants ± S.E.M [z]
UTC	0.345 ± 0.098
Asana	0.561 ± 0.109
Basin Flex + Leprotec	0.330 ± 0.087
Besiege	0.420 ± 0.120
Gemstar LC + BoteGHA ES	0.460 ± 0.126
Heligen	0.532 ± 0.130
Heligen + Dipel DF	0.431 ± 0.111
Heligen + Xentari	0.453 ± 0.150
Spear-Lep + Leprotec	0.463 ± 0.128
Warrior II	0.491 ± 0.138
P-value	0.9519

S.E.M= Standard Error of Mean

UTC= Untreated Control

[z] Scale for damage rating in hemp; 0: no damage, 1: visible damage, bud still marketable, 2: damage to 50% or less of material making bud unmarketable, 3: damage to more than 50% of material making bud unmarketable (Britt et al. 2021).

Table 2.5

Treatment	Mean number of corn earworms per 4 plants ± S.E.M
UTC	0.092 ± 0.031
Asana	0.039 ± 0.016
Besiege	0.043 ± 0.021
Entrust	0.044 ± 0.021
Gemstar LC + BoteGHA ES	0.096 ± 0.031
Heligen	0.053 ± 0.024
Heligen + Dipel DF	0.14 ± 0.052
Heligen + Xentari	0.054 ± 0.021
Spear-Lep + Leprotec	0.076 ± 0.025
Warrior II	0.107 ± 0.035
P-value	0.2491

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.6

Treatment	Mean damage per 4 plants ± S.E.M
UTC	0.412 ± 0.130
Asana	0.359 ± 0.131
Besiege	0.481 ± 0.122
Entrust	0.354 ± 0.115
Gemstar LC + BoteGHA ES	0.388 ± 0.148
Heligen	0.287 ± 0.095
Heligen + Dipel DF	0.429 ± 0.144
Heligen + Xentari	0.346 ± 0.117
Spear-Lep + Leprotec	0.334 ± 0.117
Warrior II	0.393 ± 0.120
P-value	0.995

S.E.M= Standard Error of Mean

UTC= Untreated Control

[z] Scale for damage rating in hemp; 0: no damage, 1: visible damage, bud still marketable, 2: damage to 50% or less of material making bud unmarketable, 3: damage to more than 50% of material making bud unmarketable (Britt et al. 2021).

Table 2.7

Treatment	Date of Assessment				
	2-Aug	8-Aug	13-Aug	3-Sep	10-Sep
UTC	0.025 ± 0.025	0.000 ± 0.000	0.175 ± 0.143	0.489 ± 0.059	0.550 ± 0.165
Asana	0.075 ± 0.478	0.025 ± 0.025	0.075 ± 0.047	0.225 ± 0.075	0.325 ± 0.170
Basin Flex + Leprotec	0.000 ± 0.000	0.050± 0.028	0.025± 0.025	0.090± 0.059	0.200± 0.070
Besiege	0.125 ± 0.075	0.025 ± 0.025	0.175 ± 0.025	0.105 ± 0.454	0.175 ± 0.143
Gemstar LC + BoteGHA ES	0.025 ± 0.025	0.000 ± 0.000	0.100 ± 0.057	0.100 ± 0.070	0.300 ± 0.122
Heligen	0.000 ± 0.000	0.100 ± 0.057	0.175 ± 0.103	0.286 ± 0.100	0.475 ± 0.149
Heligen + Dipel DF	0.025 ± 0.025	0.025 ± 0.025	0.050 ± 0.028	0.313 ± 0.183	0.225 ± 0.0478
Heligen + Xentari	0.050 ± 0.028	0.000 ± 0.000	0.025 ± 0.025	0.213 ± 0.113	0.325 ± 0.110
Spear-Lep + Leprotec	0.000 ± 0.000	0.000 ± 0.000	0.075 ± 0.047	0.075 ± 0.047	0.325 ± 0.062
Warrior II	0.025 ± 0.025	0.000 ± 0.000	0.225 ± 0.225	0.150 ± 0.086	0.375 ± 0.086
P-value	0.2395	0.1296	0.8194	0.1971	0.4976

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.8

Treatment	Date of Assessment [z]				
	2-Aug	8-Aug	13-Aug	3-Sep	10-Sep
UTC	0.165 ± 0.184^{a}	0.950 ± 0.086^{ab}	1.000 ± 0.912^{a}	1.000 ± 1.957^{a}	0.755 ± 0.110^{a}
Asana	0.100 ± 0.100^{c}	0.025 ± 0.025^{c}	0.025 ± 0.025^{b}	0.025 ± 0.025^{b}	0.025 ± 0.047^{b}
Basin Flex + Leprotec	0.150 ± 0.119^{c}	0.075± 0.047^{c}	0.025 ± 0.025^{b}	0.075± 0.047^{b}	0.025± 0.025^{b}
Besiege	0.000 ± 0.000^{c}	0.075 ± 0.047^{c}	0.000 ± 0.000^{b}	0.000 ± 0.000^{b}	0.125 ± 0.457^{b}
Gemstar LC + BoteGHA ES	0.125 ± 0.094^{c}	0.025 ± 0.025c	0.000 ± 0.000^{b}	0.025 ± 0.025^{b}	0.000 ± 0.000^{b}
Heligen	0.100 ± 0.040^{c}	0.000 ± 0.000^{c}	0.200 ± 0.135^{b}	0.050 ± 0.050^{b}	0.250 ± 0.095^{b}
Heligen + Dipel DF	0.600 ± 0.286^{bc}	0.175 ± 0.143^{bc}	0.050 ± 0.028^{b}	0.150 ± 0.064^{b}	0.025 ± 0.025^{b}
Heligen + Xentari	1.175 ± 0.286^{ab}	1.050 ± 0.210^{ab}	1.055 ± 0.104^{a}	0.811 ± 0.205^{a}	0.918 ± 0.144^{a}
Spear-Lep + Leprotec	0.700 ± 0.158^{bc}	1.193 ± 0.210^{a}	0.716 ± 0.172^{a}	1.611 ± 0.105^{a}	0.838 ± 0.127^{a}
Warrior II	0.775 ± 0.201^{bc}	1.225 ± 0.515^{a}	1.000 ± 0.187^{a}	0.875 ± 0.165^{a}	0.925 ± 0.154^{a}
P-value	<.0001	<.0001	<.0001	<.0001	<.0001

S.E.M= Standard Error of Mean

UTC= Untreated Control

[z] Scale for damage rating in hemp; 0: no damage, 1: visible damage, bud still marketable, 2: damage to 50% or less of material making bud unmarketable, 3: damage to more than 50% of material making bud unmarketable (Britt et al. 2021).

Means and S.E.M followed by the same letter are not significantly different.

Table 2.9

Treatment	Date of Assessment							
	18-Jul	25-Jul	4-Aug	8-Aug	13-Aug	18-Aug	30-Aug	7-Sep
UTC	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050	0.062 ± 0.062	0.000 ± 0.000	0.125 ± 0.125	0.312 ± 0.119	0.187 ± 0.119
Asana	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.125 ± 0.072	0.062 ± 0.062	0.125 ± 0.072
Besiege	0.000 ± 0.000	0.000 ± 0.000	0.100± 0.100	0.000 ± 0.000	0.000 ± 0.000	0.062 ± 0.062	0.062 ± 0.062	0.125 ± 0.125
Entrust	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.208 ± 0.125	0.083 ± 0.083	0.000 ± 0.000	0.000 ± 0.000	0.062 ± 0.062
Gemstar LC + BoteGHA ES	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.145 ± 0.085	0.000 ± 0.000	0.187 ± 0.187	0.187 ± 0.062	0.250 ± 0.102
Heligen	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050	0.062 ± 0.062	0.000 ± 0.000	0.125 ± 0.125	0.125 ± 0.125	0.062 ± 0.062
Heligen + Dipel DF	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.375 ± 0.072	0.062 ± 0.062	0.375 ± 0.375	0.187 ± 0.119	0.125 ± 0.072
Heligen + Xentari	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.062 ± 0.062	0.062 ± 0.062	0.000 ± 0.000	0.062 ± 0.062	0.250 ± 0.102
Spear-Lep + Leprotec	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050	0.125 ± 0.125	0.062 ± 0.062	0.062 ± 0.062	0.187 ± 0.119	0.125 ± 0.072
Warrior II	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050	0.187 ± 0.119	0.000 ± 0.000	0.125 ± 0.125	0.187 ± 0.119	0.312 ± 0.187
P-value	n/a	n/a	0.7778	0.0987	0.7253	0.8659	0.3995	0.7451

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.10

Treatment	Date of Assessment							
	18-Jul	25-Jul	4-Aug	8-Aug	13-Aug	18-Aug	30-Aug	7-Sep
UTC	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050	0.125 ± 0.125	0.000 ± 0.000	0.250 ± 0.250	1.687 ± 0.471	2.550 ± 1.388
Asana	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.312 ± 0.187	1.500 ± 0.684	1.062 ± 0.328
Besiege	0.000 ± 0.000	0.000 ± 0.000	0.100± 0.100	0.250 ± 0.176	0.437 ± 0.213	0.375 ± 0.375	1.500 ± 0.306	1.187 ± 0.425
Entrust	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.001	0.333 ± 0.333	0.000 ± 0.000	1.187 ± 0.471	1.312 ± 0.119
Gemstar LC + BoteGHA ES	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.145 ± 0.085	0.083 ± 0.083	0.250 ± 0.250	0.937 ± 0.543	1.687 ± 0.759
Heligen	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050	0.125 ± 0.072	0.000 ± 0.000	0.375 ± 0.375	0.812 ± 0.400	0.937 ± 0.312
Heligen + Dipel DF	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.062 ± 0.062	0.375 ± 0.214	1.437 ± 0.413	1.562 ± 0.664
Heligen + Xentari	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.270 ± 0.178	0.000 ± 0.000	1.000 ± 0.489	1.500 ± 0.270
Spear-Lep + Leprotec	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050	0.187 ± 0.187	0.125 ± 0.125	0.250 ± 0.176	1.312 ± 0.571	0.750 ± 0.478
Warrior II	0.000 ± 0.000	0.000 ± 0.000	0.050 ± 0.050	0.062 ± 0.062	0.000 ± 0.000	0.187 ± 0.187	1.375 ± 0.388	1.583 ± 0.220
P-value	n/a	n/a	0.7778	0.5949	0.3369	0.9466	0.9538	0.803

S.E.M= Standard Error of Mean

UTC= Untreated Control

^{z} Scale for damage rating in hemp; 0: no damage, 1: visible damage, bud still marketable, 2: damage to 50% or less of material making bud unmarketable, 3: damage to more than 50% of material making bud unmarketable (Britt et al. 2021).

Table 2.11

Treatment	Mean number of Wet weight per 2 plants ± S.E.M
UTC	1024.12 ± 538.653
Beseige	2381.36 ± 478.420
Entrust	2193.54 ± 331.469
Gemstar LC + BoteGHA ES	1559.22 ± 331.469
Heligen	1768.30 ± 226.454
Heligen + Dipel DF	1516.70 ± 312.542
Heligen + Xentari	1715.15 ± 470.052
Spear Lep + Leprotec	1970.29 ± 469.732
Warrior II	1523.79 ± 154.032
P-value	0.406

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.12

Treatment	Mean dry weight per 2 plants ± S.E.M
UTC	258.689 ± 145.522
Asana	276.408 ± 105.520
Besiege	712.282 ± 205.605
Entrust	620.146 ± 52.522
Gemstar LC + BoteGHA ES	377.202 ± 94.091
Heligen	577.622 ± 134.271
Heligen + Dipel DF	588.253 ± 187.783
Heligen + Xentari	382.651 ± 104.696
Spear-Lep + Leprotec	563.447 ± 138.146
Warrior II	510.291 ± 84.058
P-value	0.2499

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.13

Treatment	Mean bud fresh weight per 2 plants ± S.E.M
UTC	7.675 ± 1.582
Asana	5.530 ± 1.920
Besiege	6.125 ± 1.161
Entrust	8.166 ± 1.555
Gemstar LC + BoteGHA ES	6.639 ± 1.041
Heligen	8.400 ± 2.279
Heligen + Dipel DF	8.020 ± 2.840
Heligen + Xentari	4.318 ± 0.306
Spear-Lep + Leprotec	3.220 ±0.726
Warrior II	6.081 ± 1.541
P-value	0.4314

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.14

Treatment	Mean bud dry weight per 2 plants ± S.E.M
UTC	2.753 ± 0.571
Asana	1.720 ± 0.589
Besiege	1.835 ± 0.480
Entrust	2.391 ± 0.380
Gemstar LC + BoteGHA ES	2.764 ± 0.561
Heligen	2.248 ± 0.630
Heligen + Dipel DF	2.483 ± 0.908
Heligen + Xentari	1.897 ± 0.519
Spear-Lep + Leprotec	1.468 ± 0.383
Warrior II	1.654 ± 0.458
P-value	0.7338

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.15

Treatment	Mean proportion of unmarketable buds per two plants ± S.E.M
UTC	0.250 ± 0.115
Asana	0.083 ± 0.056
Besiege	0.083 ± 0.083
Entrust	0.041 ± 0.042
Gemstar LC + BoteGHA ES	0.333 ± 0.112
Heligen	0.291 ± 0.074
Heligen + Dipel DF	0.083 ± 0.056
Heligen + Xentari	0.166 ± 0.071
Spear-Lep + Leprotec	0.375 ± 0.108
Warrior II	0.166 ± 0.094
P-value	0.0522

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 2.16

Treatment	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
UTC	0	0	0	0	0.16667	0.16667	1
Asana	0.83333	1	1	1	1	1	1
Entrust	1	1	1	1	1	1	1
Gemstar LC	0.33333	0.33333	0.5	1	1	1	1
Heligen	0.16667	0.5	0.66667	1	1	1	1
Heligen + Dipel DF	0.33333	0.5	0.83333	1	1	1	1
Karate	1	1	1	1	1	1	1
Leprotec	0.16667	0.66667	0.83333	1	1	1	1
Xentari	0.5	0.66667	0.66667	0.66667	0.83333	0.83333	1

Table 2.17

Treatment	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
UTC	0	0	0	0	0.16667	0.16667	0.16667
Asana	0.83333	1	1	1	1	1	1
Entrust	1	1	1	1	1	1	1
Gemstar LC	0.33333	0.33333	0.5	1	1	1	1
Heligen	0.16667	0.5	0.66667	0.66667	0.66667	0.66667	1
Heligen + Dipel DF	0.33333	0.5	0.83333	1	1	1	1
Karate	1	1	1	1	1	1	1
Leprotec	0.16667	0.66667	0.83333	1	1	1	1
Xentari	0.33333	0.5	0.5	0.5	0.66667	0.66667	1

Table 2.18

Treatment	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT
UTC	0	0	0	0	0.16667	0.16667	0.16667
Asana	0.83333	1	1	1	1	1	1
Entrust	0.83333	1	1	1	1	1	1
Gemstar LC	0.66667	1	1	1	1	1	1
Heligen	0.83333	1	1	1	1	1	1
Heligen + Dipel DF	0.83333	1	1	1	1	1	1
Karate	1	1	1	1	1	1	1
Leprotec	1	1	1	1	1	1	1
Xentari	1	1	1	1	1	1	1

Table 2.19

Treatment	Date of Assessment						
	1DAT	2DAT	3DAT	4DAT	5DAT	6DAT	7DAT
UTC	0.000 ± 0.000^{c}	0.000 ± 0.000^{c}	0.000 ± 0.000^{b}	0.000 ± 0.000^{b}	0.000 ± 0.000^{b}	0.166 ± 0.000^{a}	0.166 ± 0.000^{b}
Asana	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Entrust	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Gemstar	0.333 ± 0.000^{cb}	0.333 ± 0.000^{bc}	0.750± 0.250 ^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Heligen	0.166 ± 0.000^{c}	0.583 ± 0.000^{b}	0.833 ± 0.166^{a}	0.833 ± 0.166^{a}	0.833 ± 0.166^{a}	0.833 ± 0.166^{a}	1.000 ± 0.000^{a}
Heligen + Dipel DF	0.333 ± 1.666^{cb}	0.583 ± 0.083^{b}	0.916± 0.083^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Karate	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Leprotec	0.166 ± 0.000^{c}	0.667 ± 0.000^{ab}	0.833 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Xentari	0.583 ± 0.083^{b}	0.667 ± 1.666^{ab}	0.833 ± 0.166^{a}	0.833 ± 0.166^{a}	0.916 ± 0.083^{a}	0.916 ± 0.083^{a}	1.000 ± 0.000^{a}
P-value	<.0001	<.0001	0.0042	<.0001	<.0001	<.0001	<.0001

S.E.M= Standard Error of Mean

UTC= Untreated Control

Means and S.E.M followed by the same letter are not significantly different.

Table 2.20

Treatment	Date of Assessment						
	1DAT	2DAT	3DAT	4DAT	5DAT	6DAT	7DAT
UTC	0.000 ± 0.000^{d}	0.000 ± 0.000^{b}	0.000 ± 0.000^{c}	0.000 ± 0.000^{d}	0.000 ± 0.000^{c}	0.166 ± 0.000^{c}	0.166 ± 0.000^{b}
Asana	0.916 ± 0.083^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Entrust	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Gemstar	0.333 ± 0.000^{b}	0.500 ± 0.166^{ab}	0.666 ± 0.166^{ab}	0.833 ± 0.000^{b}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Heligen	0.166 ± 0.000^{c}	0.583 ± 0.250^{ab}	0.750 ± 0.083^{ab}	0.750 ± 0.083^{b}	0.833 ± 0.166^{ab}	0.833 ± 0.166^{ab}	1.000 ± 0.000^{a}
Heligen + Dipel DF	$0.333 \pm 1.665^{E\text{-}7b}$	0.500 ± 0.000^{ab}	$0.833 \pm 1.666^{E\text{-}7ab}$	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Karate	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Leprotec	0.166 ± 0.000^{c}	0.667 ± 01.666^{a}	0.750 ± 0.083^{ab}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}	1.000 ± 0.000^{a}
Xentari	0.333 ± 0.000^{b}	0.500 ± 0.000^{ab}	0.500 ± 0.000^{b}	0.500 ± 0.000^{c}	$0.666 \pm 1.665^{E\text{-}7b}$	0.666 ± 0.000^{b}	1.000 ± 0.000^{a}
P-value	<.0001	0.0024	<.0001	<.0001	<.0001	<.0001	<.0001

UTC= Untreated Control

S.E.M= Standard Error of Mean

Means and S.E.M followed by the same letter are not significantly different.

Figures

Figure 2.1 Damage scale after Britt et al. (2021)

Figure 2.2

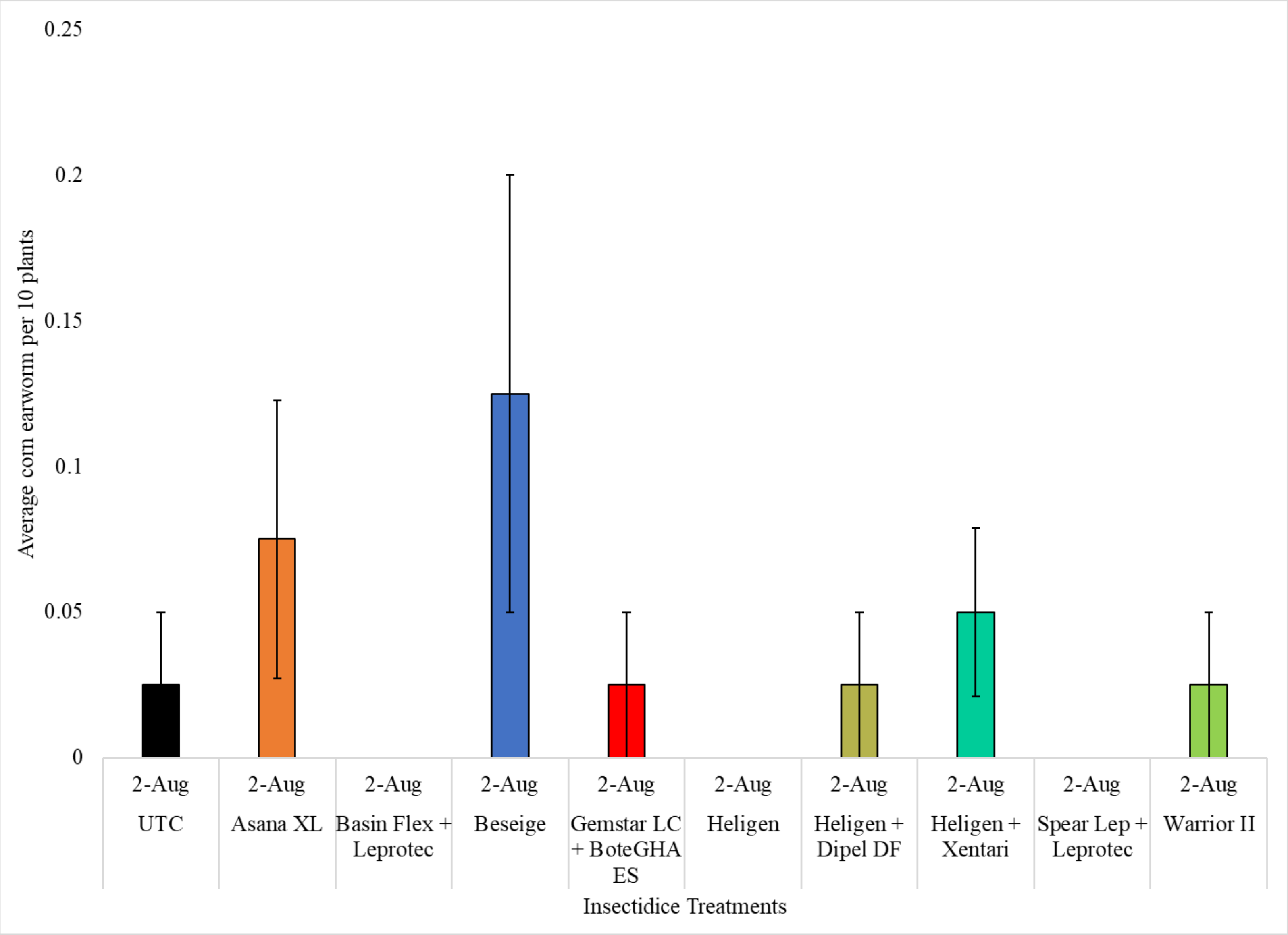

0.25
0.2
0.15
0.1
0.05
0
Average corn earworm per 10 plants
2-Aug
UTC
2-Aug
Asana XL
2-Aug
Basin Flex + Leprotec
2-Aug
Beseige
2-Aug
Gemstar LC + BoteGHA ES
2-Aug
Heligen
2-Aug
Heligen + Dipel DF
2-Aug
Heligen + Xentari
2-Aug
Spear Lep + Leprotec
2-Aug
Warrior II
Insectidice Treatments

Figure 2.2a

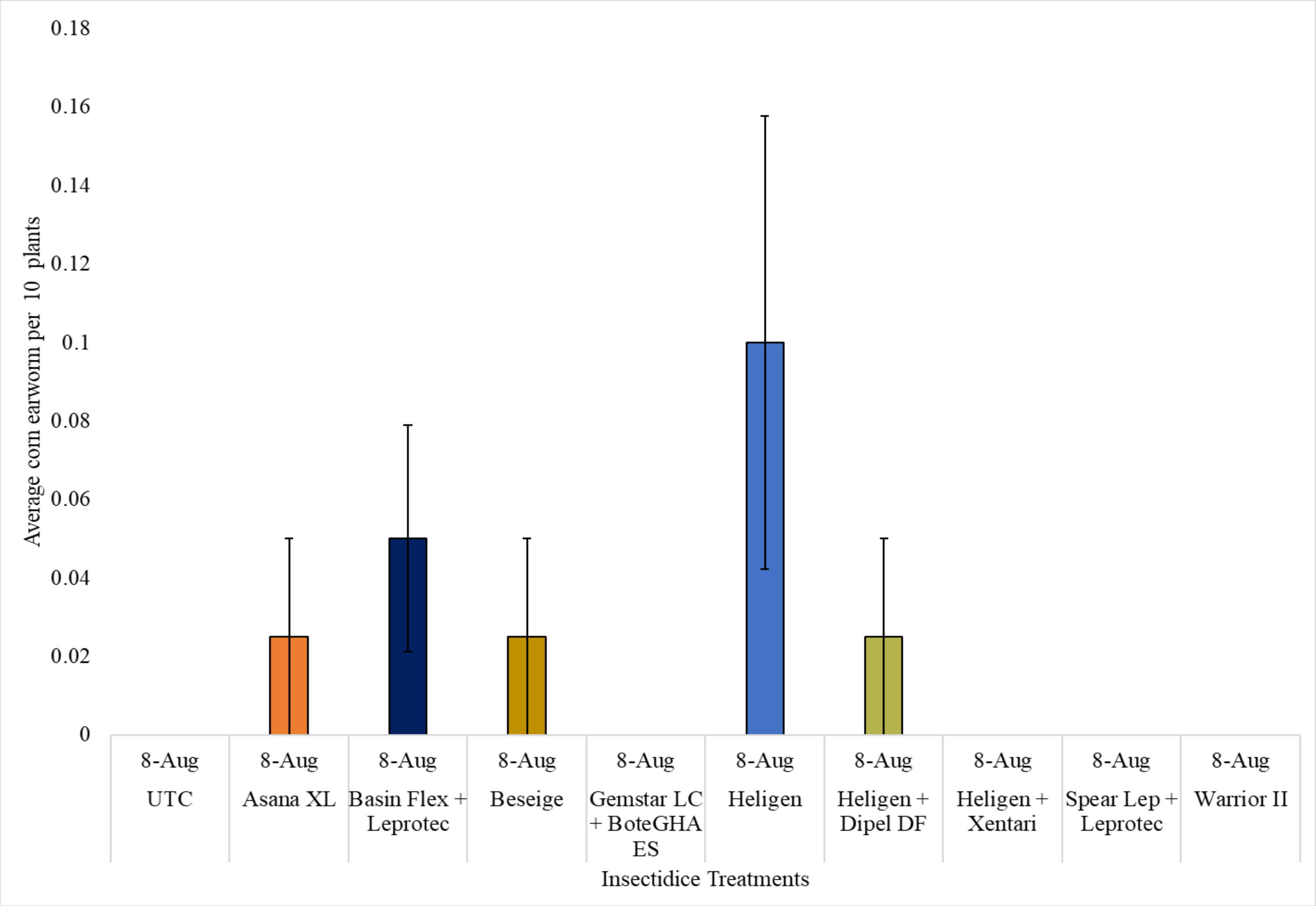

0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Average corn earworm per 10 plants
8-Aug
UTC
8-Aug
Asana XL
8-Aug
Basin Flex + Leprotec
8-Aug
Beseige
8-Aug
Gemstar LC + BoteGHA ES
8-Aug
Heligen
8-Aug
Heligen + Dipel DF
8-Aug
Heligen + Xentari
8-Aug
Spear Lep + Leprotec
8-Aug
Warrior II
Insectidice Treatments

Figure 2.2b

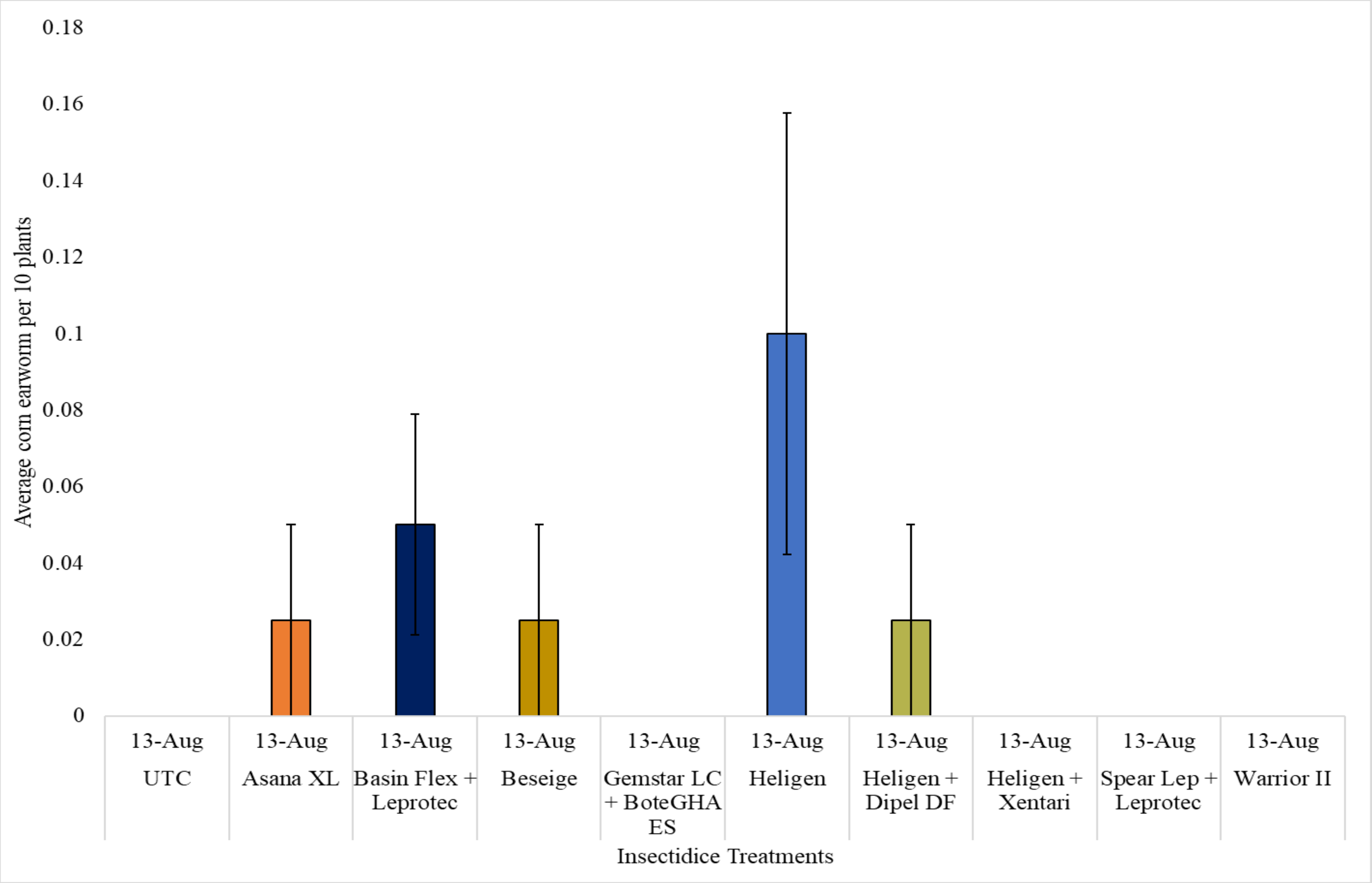
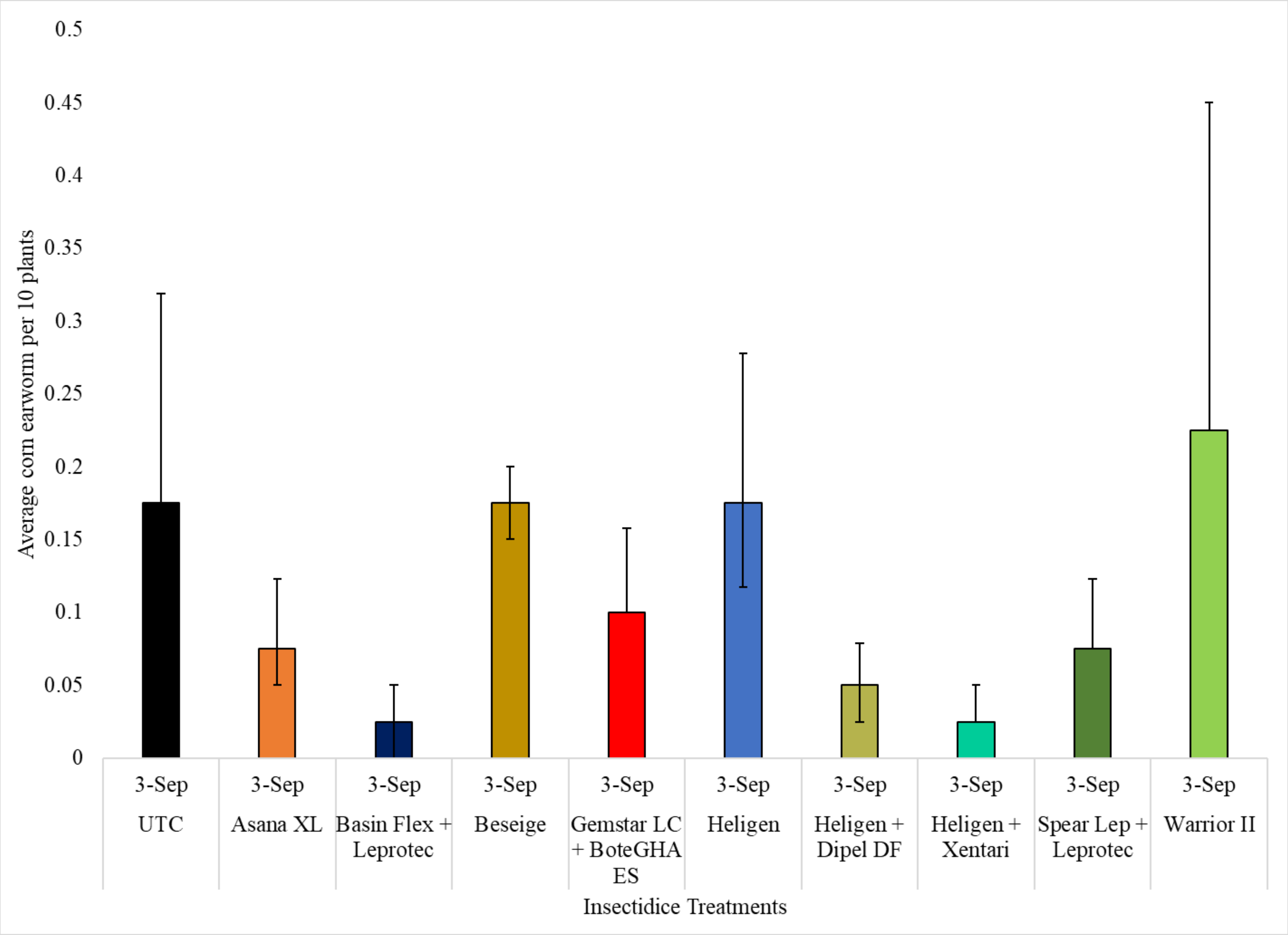

0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Average corn earworm per 10 plants
13-Aug
UTC
13-Aug
Asana XL
13-Aug
Basin Flex + Leprotec
13-Aug
Beseige
13-Aug
Gemstar LC + BoteGHA ES
13-Aug
Heligen
13-Aug
Heligen + Dipel DF
13-Aug
Heligen + Xentari
13-Aug
Spear Lep + Leprotec
13-Aug
Warrior II
Insectidice Treatments

Figure 2.2c



Average corn earworm per 10 plants
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
3-Sep
UTC
3-Sep
Asana XL
3-Sep
Basin Flex + Leprotec
3-Sep
Beseige
3-Sep
Gemstar LC + BoteGHA ES
3-Sep
Heligen
3-Sep
Heligen + Dipel DF
3-Sep
Heligen + Xentari
3-Sep
Spear Lep + Leprotec
3-Sep
Warrior II
Insectidice Treatments

Figure 2.2d

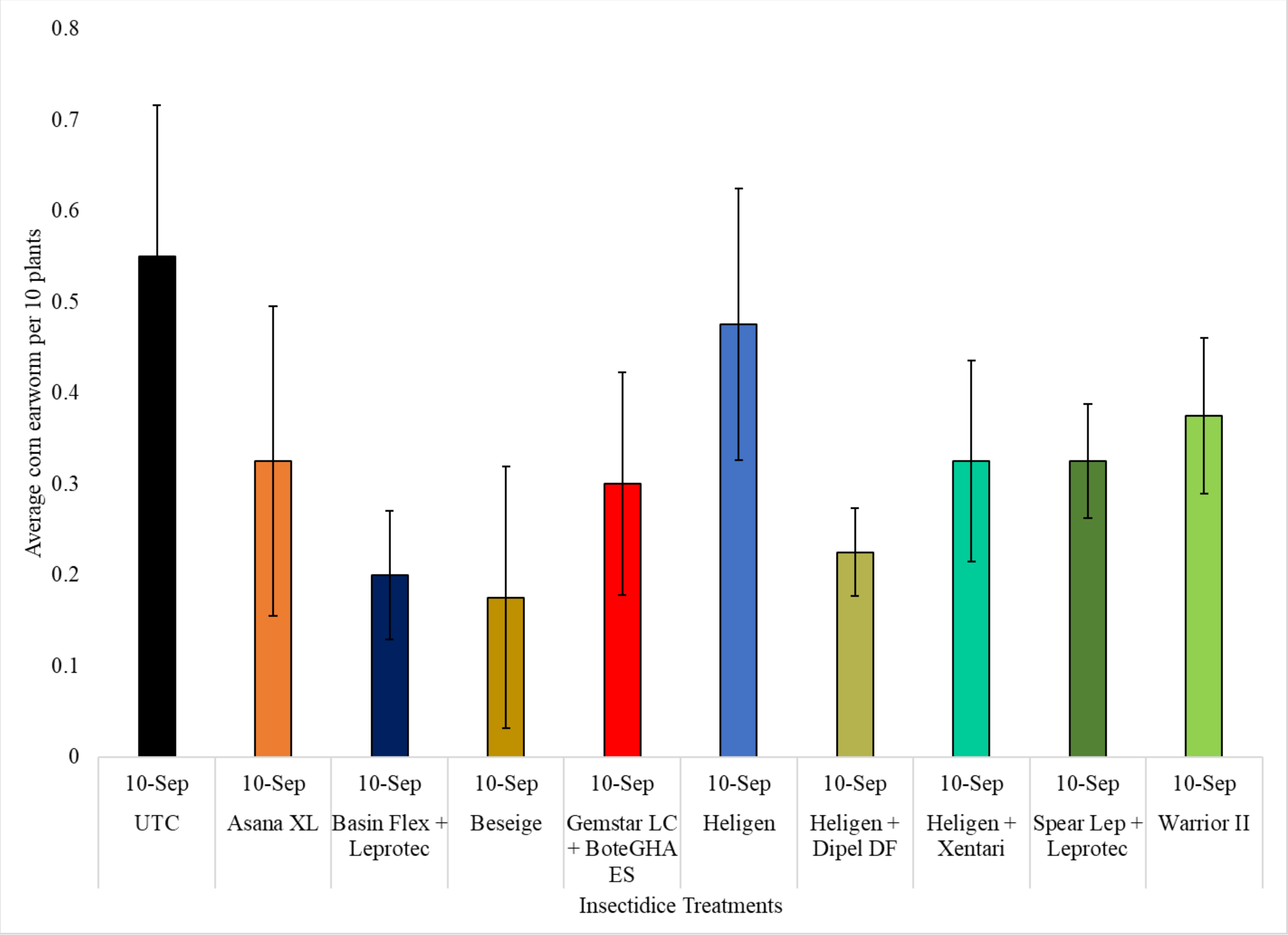

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Average corn earworm per 10 plants
10-Sep
UTC
10-Sep
Asana XL
10-Sep
Basin Flex + Leprotec
10-Sep
Beseige
10-Sep
Gemstar LC + BoteGHA ES
10-Sep
Heligen
10-Sep
Heligen + Dipel DF
10-Sep
Heligen + Xentari
10-Sep
Spear Lep + Leprotec
10-Sep
Warrior II
Insectidice Treatments

Figure 2.3

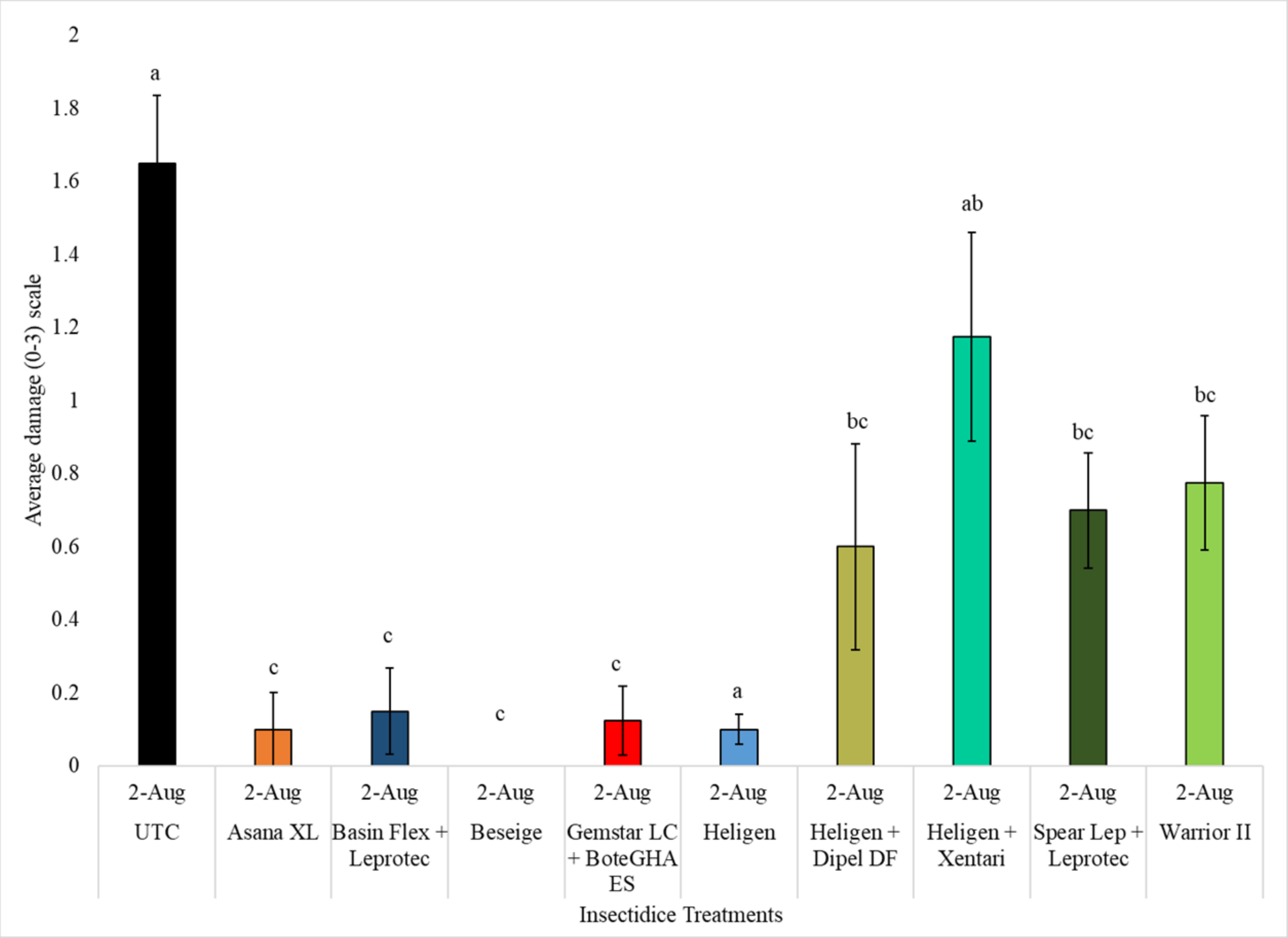

Average damage (0-3) scale
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
c
c
c
c
a
bc
ab
bc
bc
2-Aug
UTC
2-Aug
Asana XL
2-Aug
Basin Flex + Leprotec
2-Aug
Beseige
2-Aug
Gemstar LC + BoteGHA ES
2-Aug
Heligen
2-Aug
Heligen + Dipel DF
2-Aug
Heligen + Xentari
2-Aug
Spear Lep + Leprotec
2-Aug
Warrior II
Insectidice Treatments

Figure 2.3a

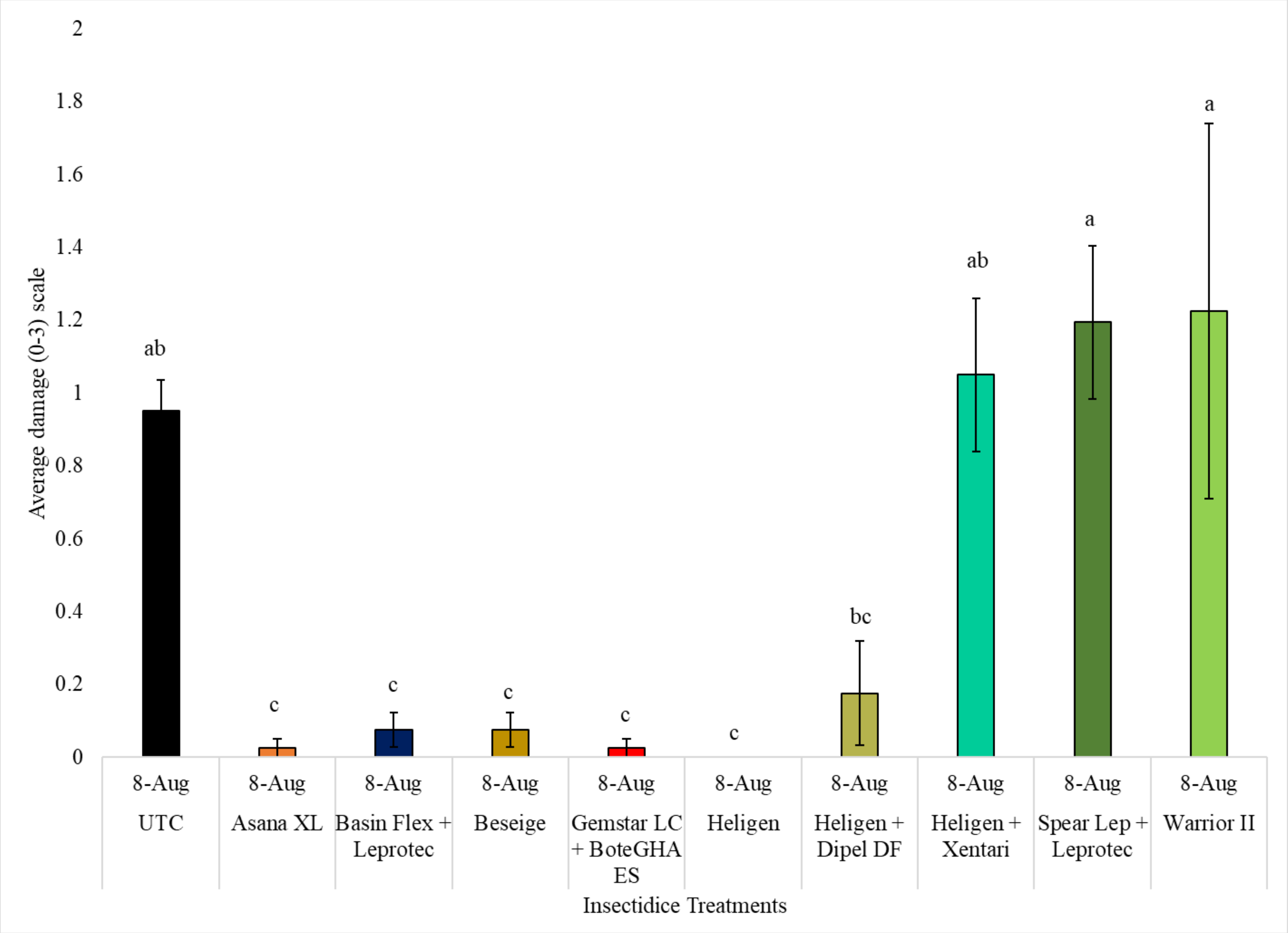

2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Average damage (0-3) scale
ab
c
c
c
c
c
bc
ab
a
a
8-Aug
UTC
8-Aug
Asana XL
8-Aug
Basin Flex + Leprotec
8-Aug
Beseige
8-Aug
Gemstar LC + BoteGHA ES
8-Aug
Heligen
8-Aug
Heligen + Dipel DF
8-Aug
Heligen + Xentari
8-Aug
Spear Lep + Leprotec
8-Aug
Warrior II
Insectidice Treatments

Figure 2.3b

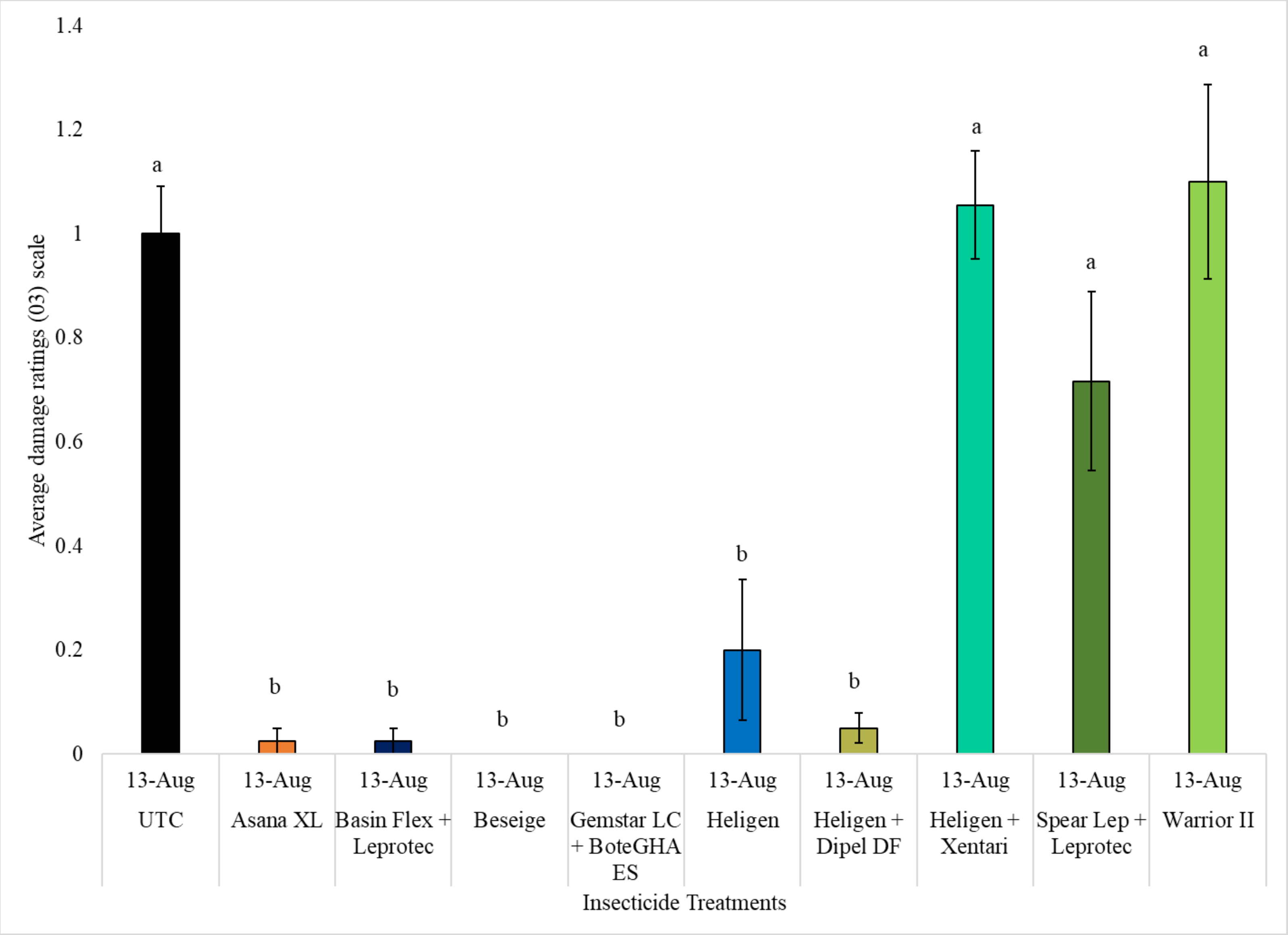

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Average damage ratings (03) scale
a
b
b
b
b
b
b
a
a
a
13-Aug
UTC
13-Aug
Asana XL
13-Aug
Basin Flex + Leprotec
13-Aug
Beseige
13-Aug
Gemstar LC + BoteGHA ES
13-Aug
Heligen
13-Aug
Heligen + Dipel DF
13-Aug
Heligen + Xentari
13-Aug
Spear Lep + Leprotec
13-Aug
Warrior II
Insecticide Treatments

Figure 2.3c

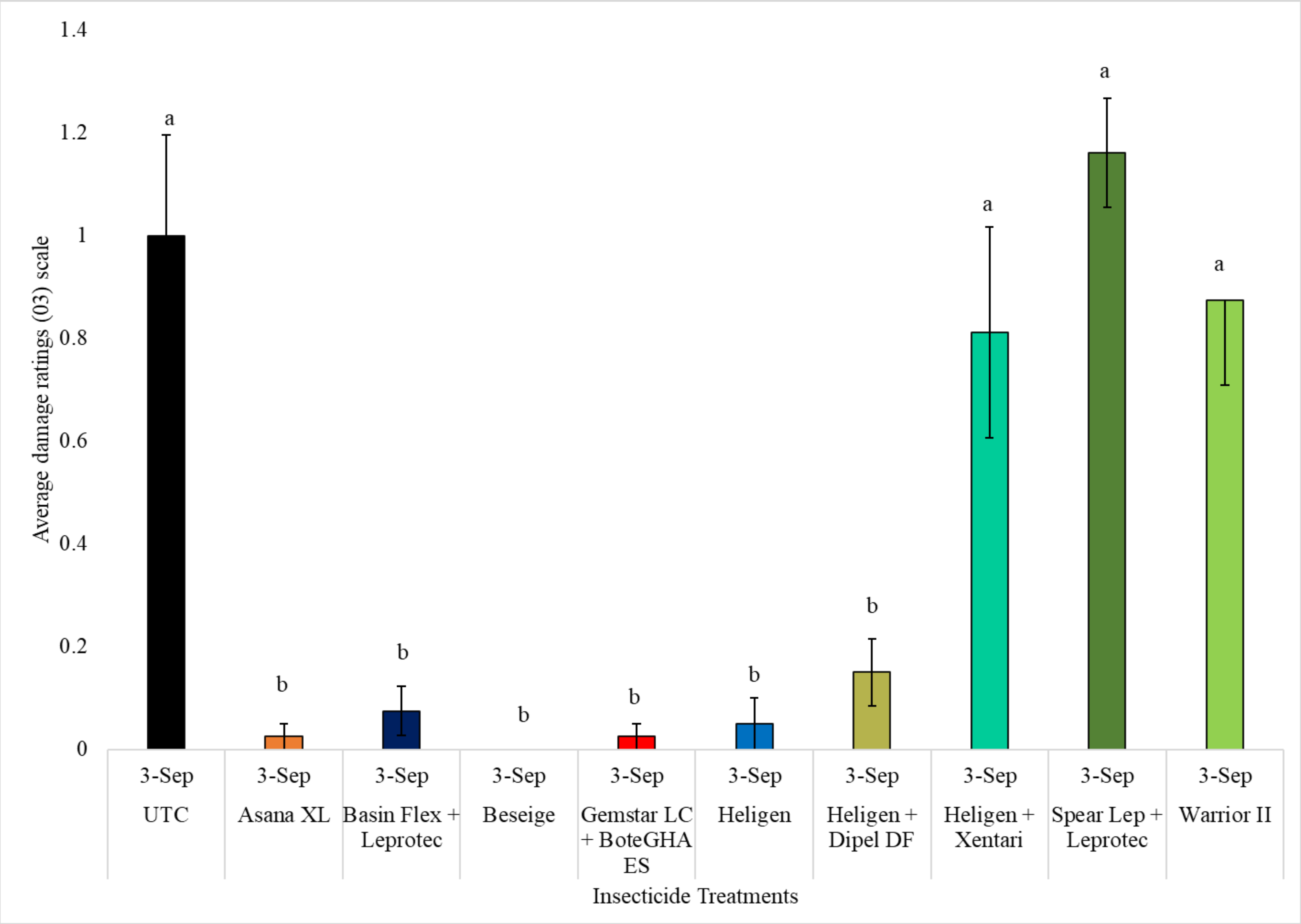

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Average damage ratings (03) scale
a
b
b
b
b
b
b
a
a
a
3-Sep
UTC
3-Sep
Asana XL
3-Sep
Basin Flex + Leprotec
3-Sep
Beseige
3-Sep
Gemstar LC + BoteGHA ES
3-Sep
Heligen
3-Sep
Heligen + Dipel DF
3-Sep
Heligen + Xentari
3-Sep
Spear Lep + Leprotec
3-Sep
Warrior II
Insecticide Treatments

Figure 2.3d

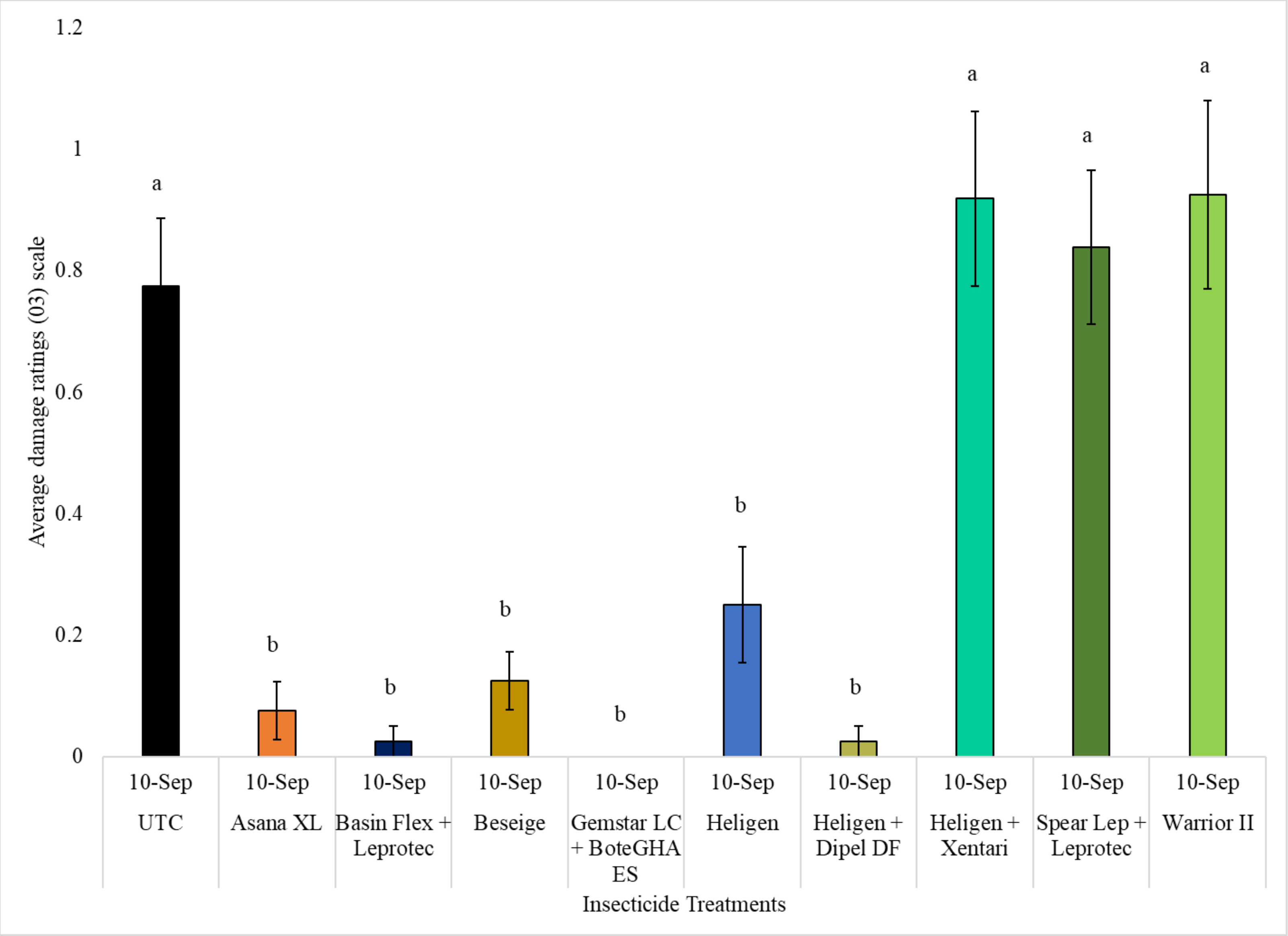
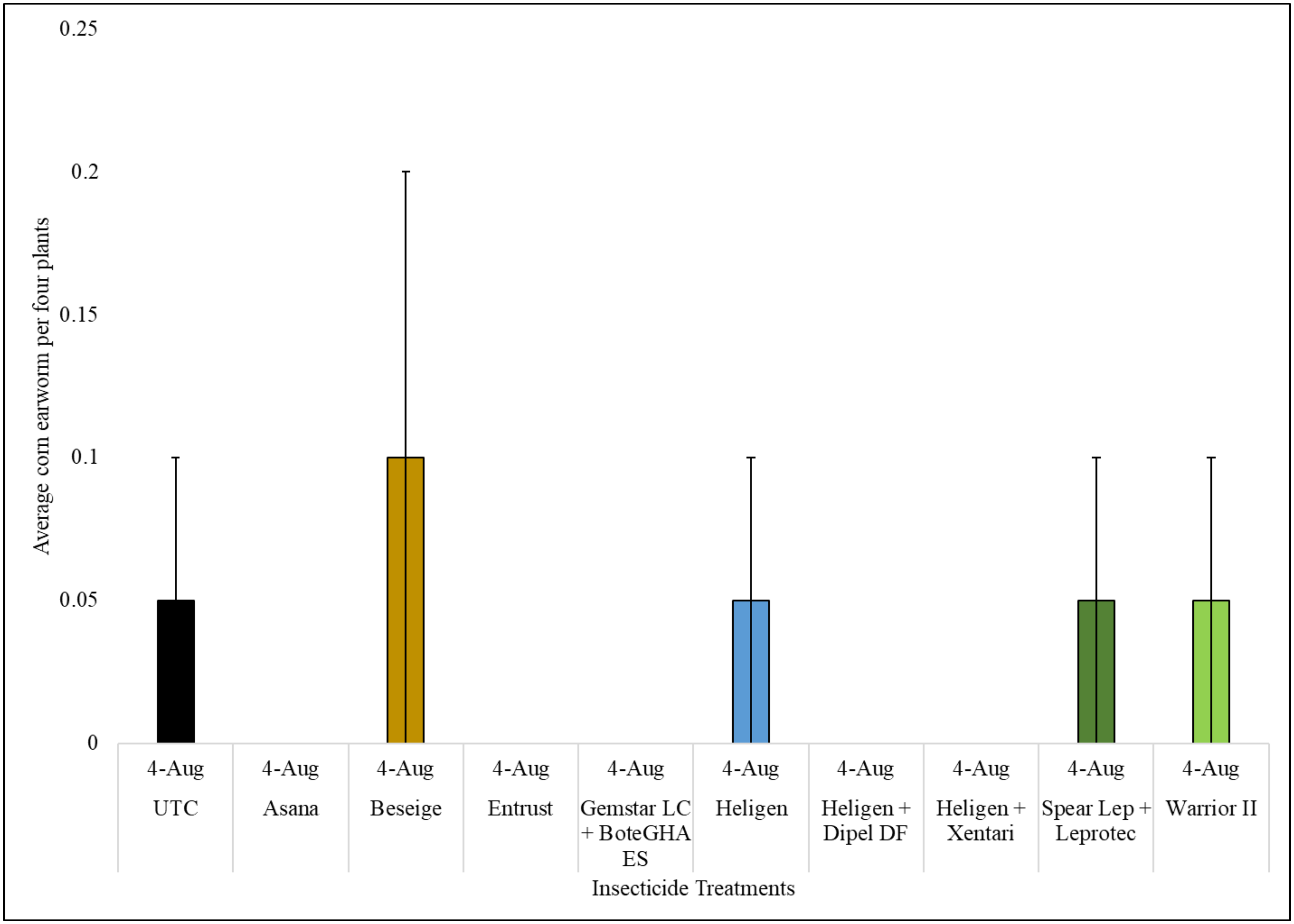
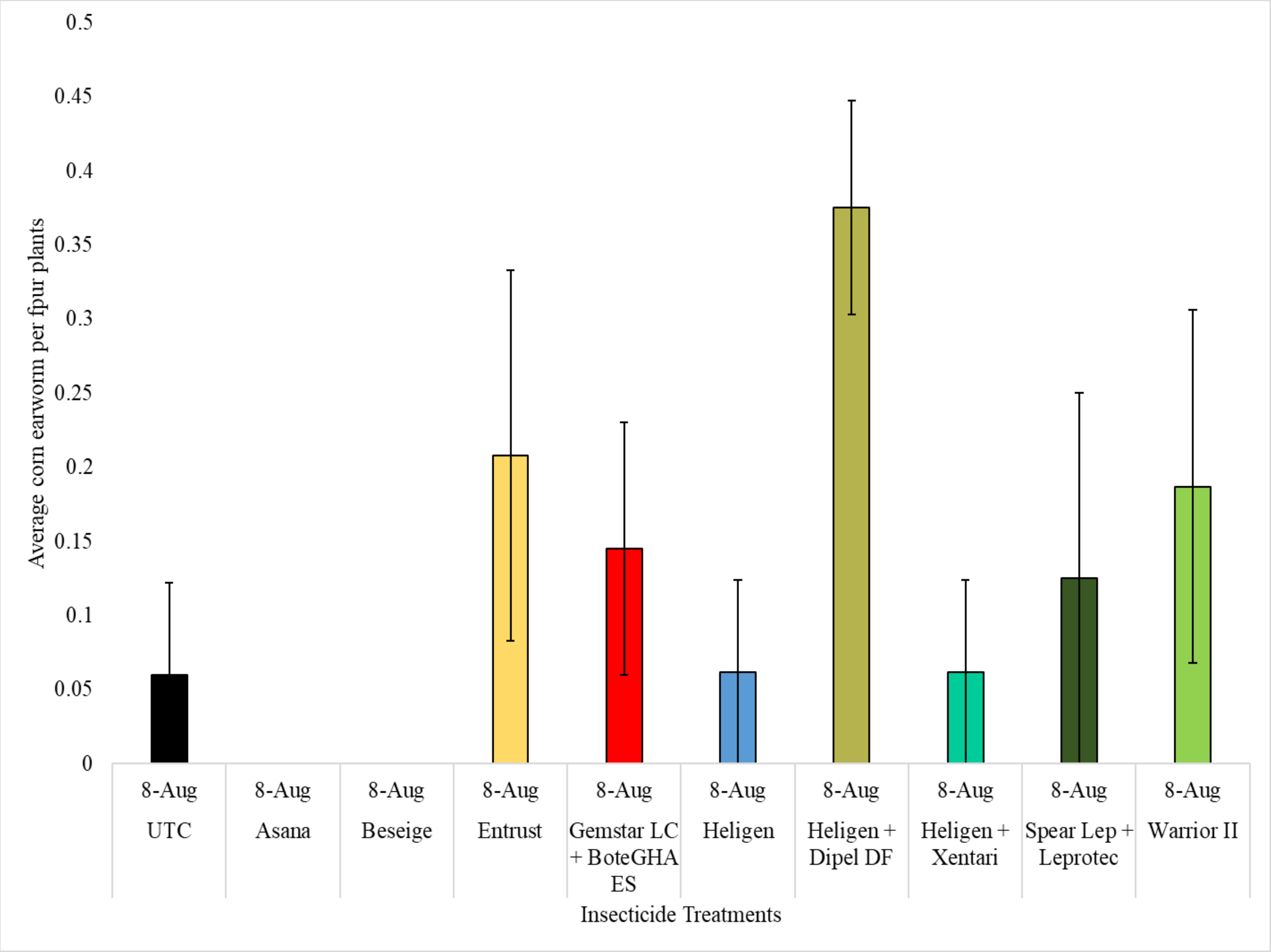

1.2
1
0.8
0.6
0.4
0.2
0
Average damage ratings (03) scale
a
b
b
b
b
b
b
a
a
a
10-Sep
UTC
10-Sep
Asana XL
10-Sep
Basin Flex + Leprotec
10-Sep
Beseige
10-Sep
Gemstar LC + BoteGHA ES
10-Sep
Heligen
10-Sep
Heligen + Dipel DF
10-Sep
Heligen + Xentari
10-Sep
Spear Lep + Leprotec
10-Sep
Warrior II
Insecticide Treatments

Figure 2.4



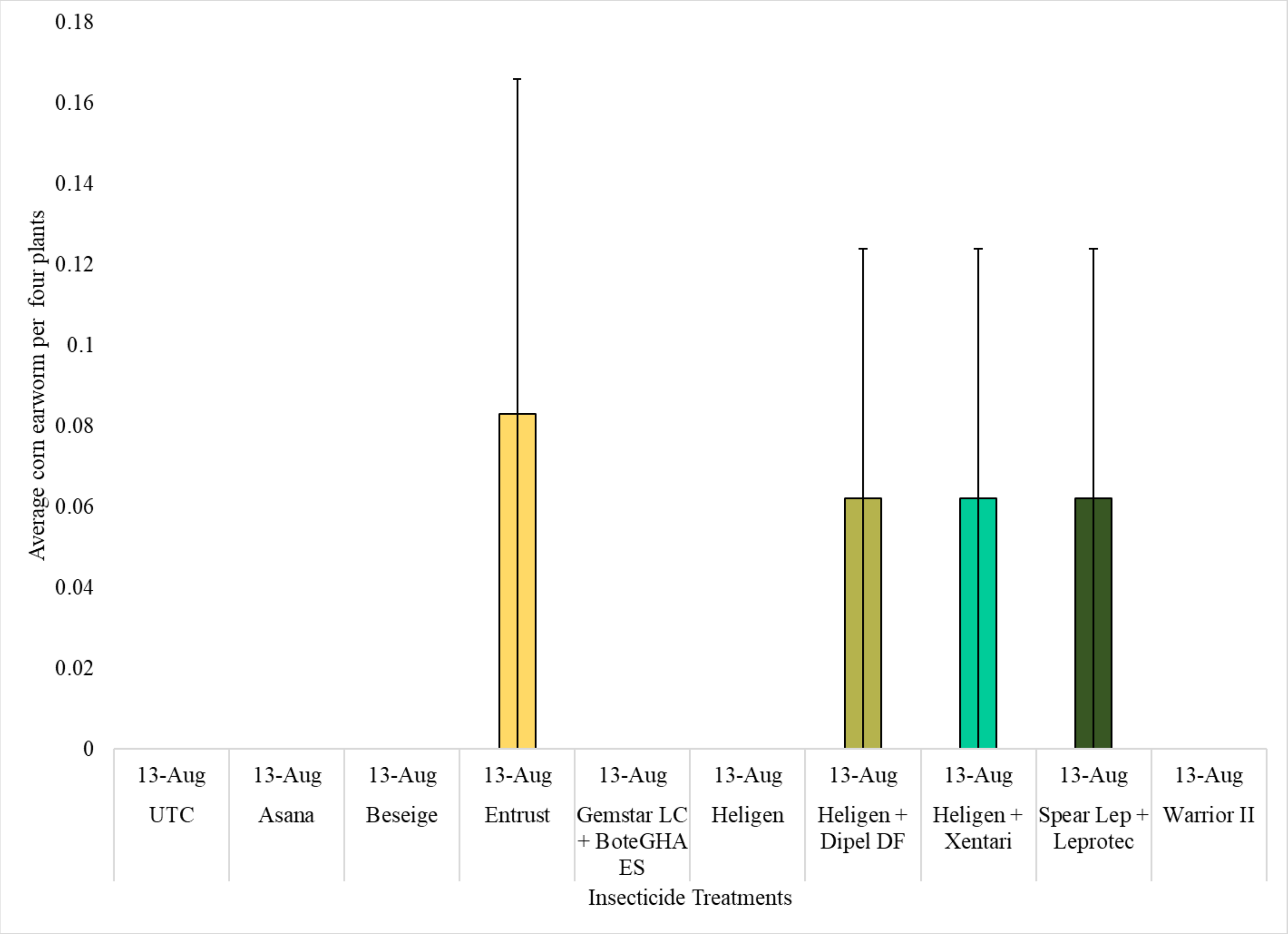
0.25
0.2
0.15
0.1
0.05
0
Average corn earworm per four plants
4-Aug
UTC
4-Aug
Asana
4-Aug
Beseige
4-Aug
Entrust
4-Aug
Gemstar LC + BoteGHA ES
4-Aug
Heligen
4-Aug
Heligen + Dipel DF
4-Aug
Heligen + Xentari
4-Aug
Spear Lep + Leprotec
4-Aug
Warrior II
Insecticide Treatments

Figure 2.4a



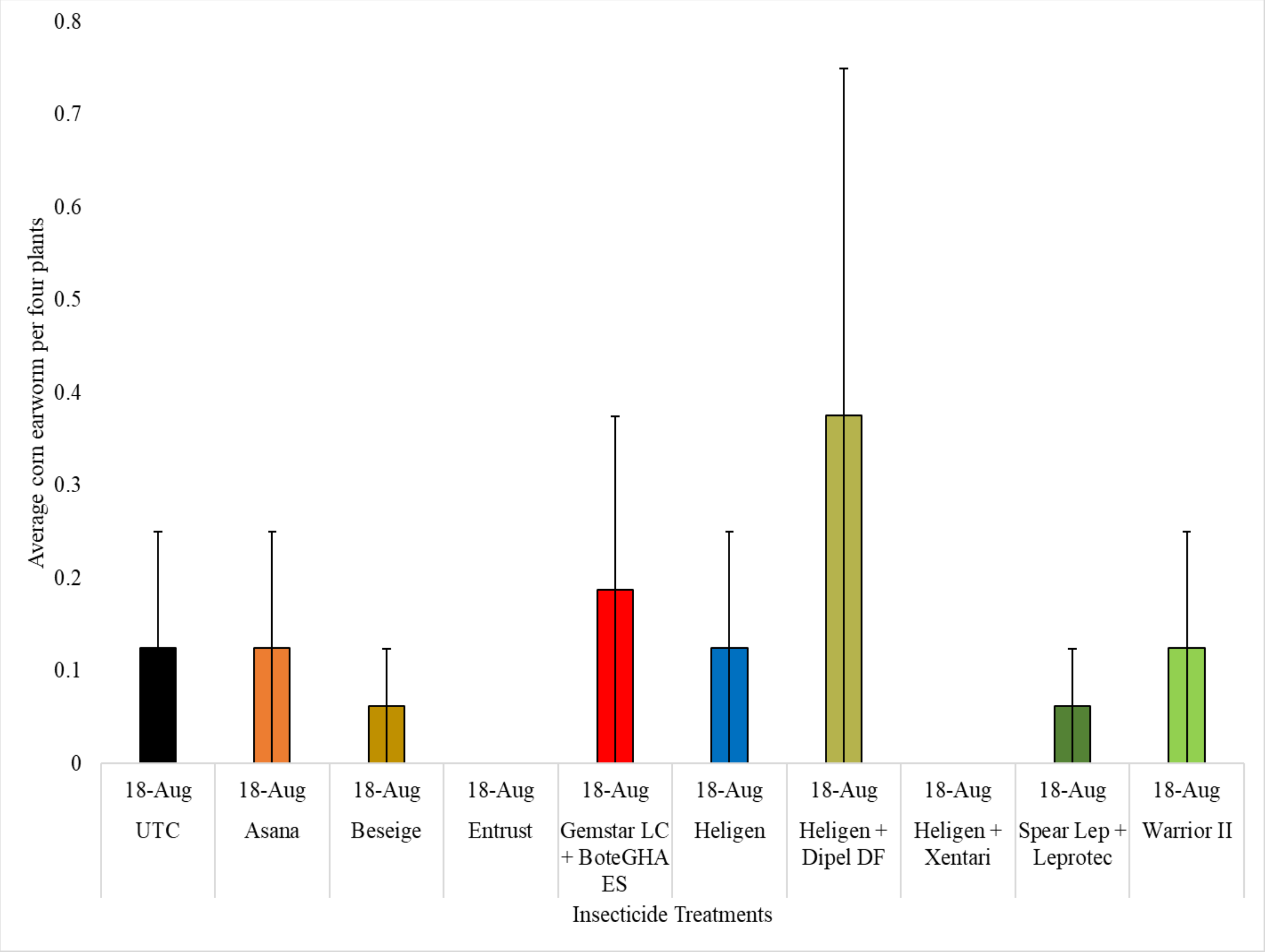
Average corn earworm per fpur plants
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
8-Aug
UTC
8-Aug
Asana
8-Aug
Beseige
8-Aug
Entrust
8-Aug
Gemstar LC + BoteGHA ES
8-Aug
Heligen
8-Aug
Heligen + Dipel DF
8-Aug
Heligen + Xentari
8-Aug
Spear Lep + Leprotec
8-Aug
Warrior II
Insecticide Treatments

Figure 2.4b



0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Average corn earworm per four plants
13-Aug
UTC
13-Aug
Asana
13-Aug
Beseige
13-Aug
Entrust
13-Aug
Gemstar LC + BoteGHA ES
13-Aug
Heligen
13-Aug
Heligen + Dipel DF
13-Aug
Heligen + Xentari
13-Aug
Spear Lep + Leprotec
13-Aug
Warrior II
Insecticide Treatments

Figure 2.4c



0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Average corn earworm per four plants
18-Aug
UTC
18-Aug
Asana
18-Aug
Beseige
18-Aug
Entrust
18-Aug
Gemstar LC + BoteGHA ES
18-Aug
Heligen
18-Aug
Heligen + Dipel DF
18-Aug
Heligen + Xentari
18-Aug
Spear Lep + Leprotec
18-Aug
Warrior II
Insecticide Treatments

Figure 2.4d

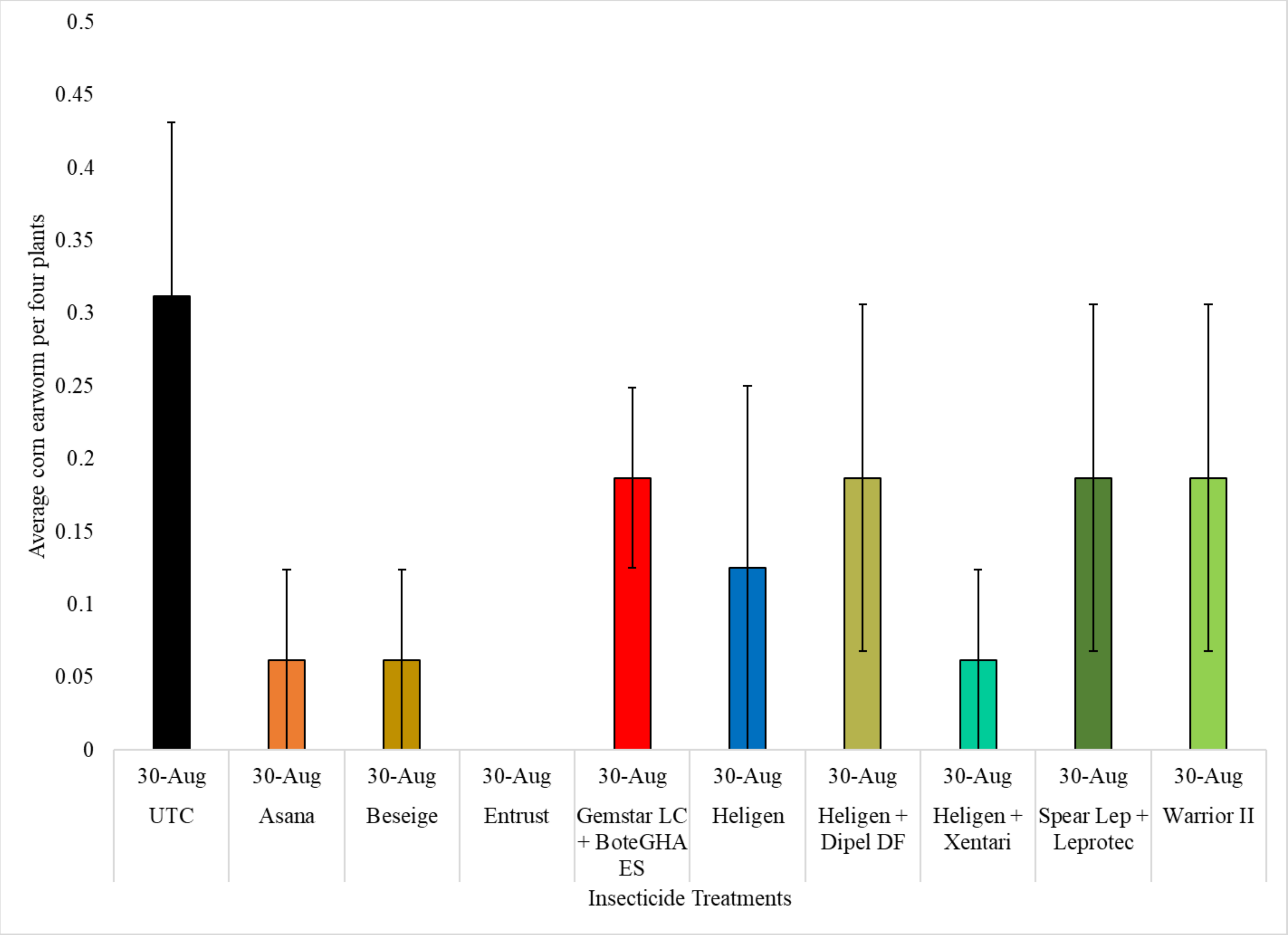

0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Average corn earworm per four plants
30-Aug
UTC
30-Aug
Asana
30-Aug
Beseige
30-Aug
Entrust
30-Aug
Gemstar LC + BoteGHA ES
30-Aug
Heligen
30-Aug
Heligen + Dipel DF
30-Aug
Heligen + Xentari
30-Aug
Spear Lep + Leprotec
30-Aug
Warrior II
Insecticide Treatments

Figure 2.4e

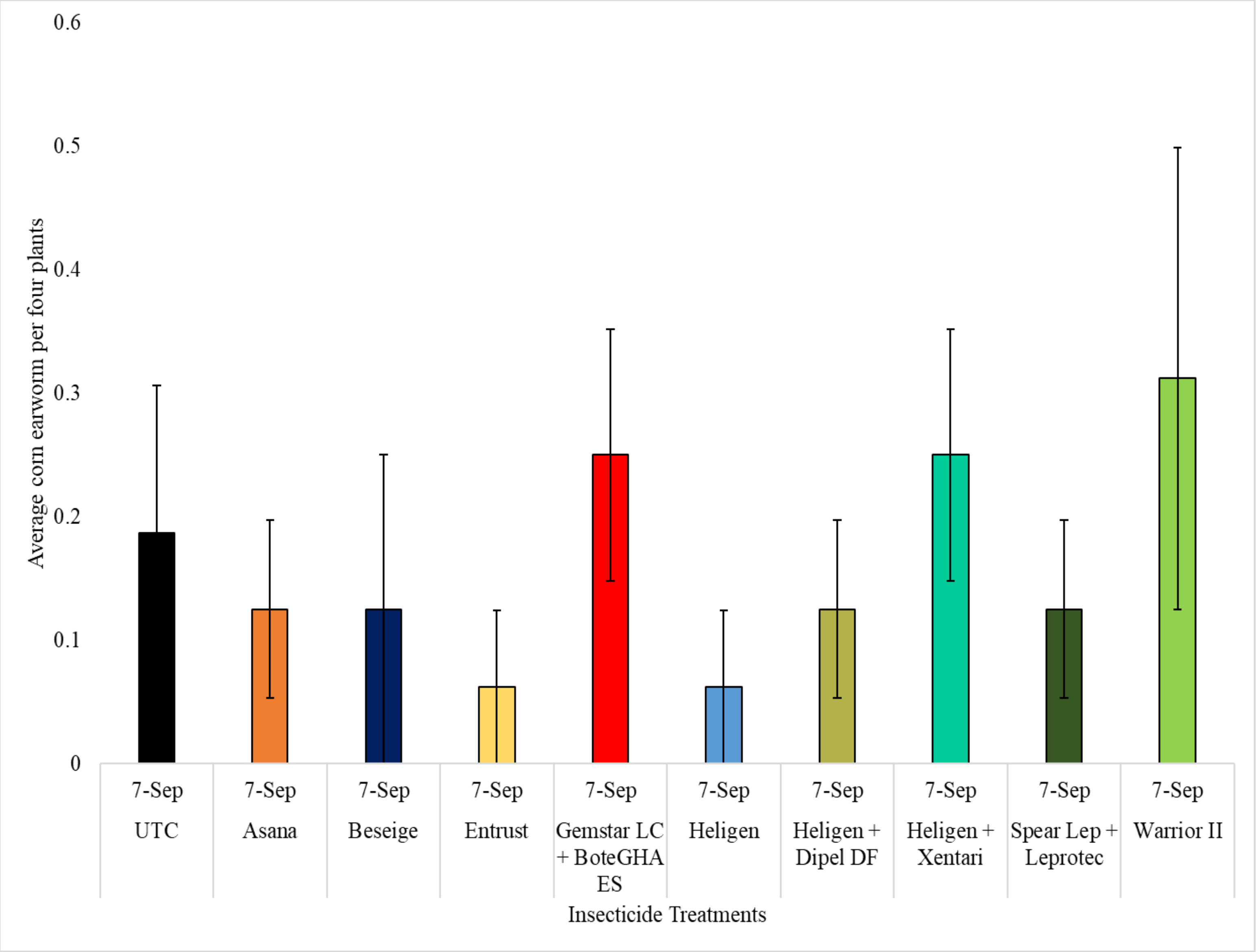

0.6
0.5
0.4
0.3
0.2
0.1
0
Average corn earworm per four plants
7-Sep
UTC
7-Sep
Asana
7-Sep
Beseige
7-Sep
Entrust
7-Sep
Gemstar LC + BoteGHA ES
7-Sep
Heligen
7-Sep
Heligen + Dipel DF
7-Sep
Heligen + Xentari
7-Sep
Spear Lep + Leprotec
7-Sep
Warrior II
Insecticide Treatments

Figure 2.5

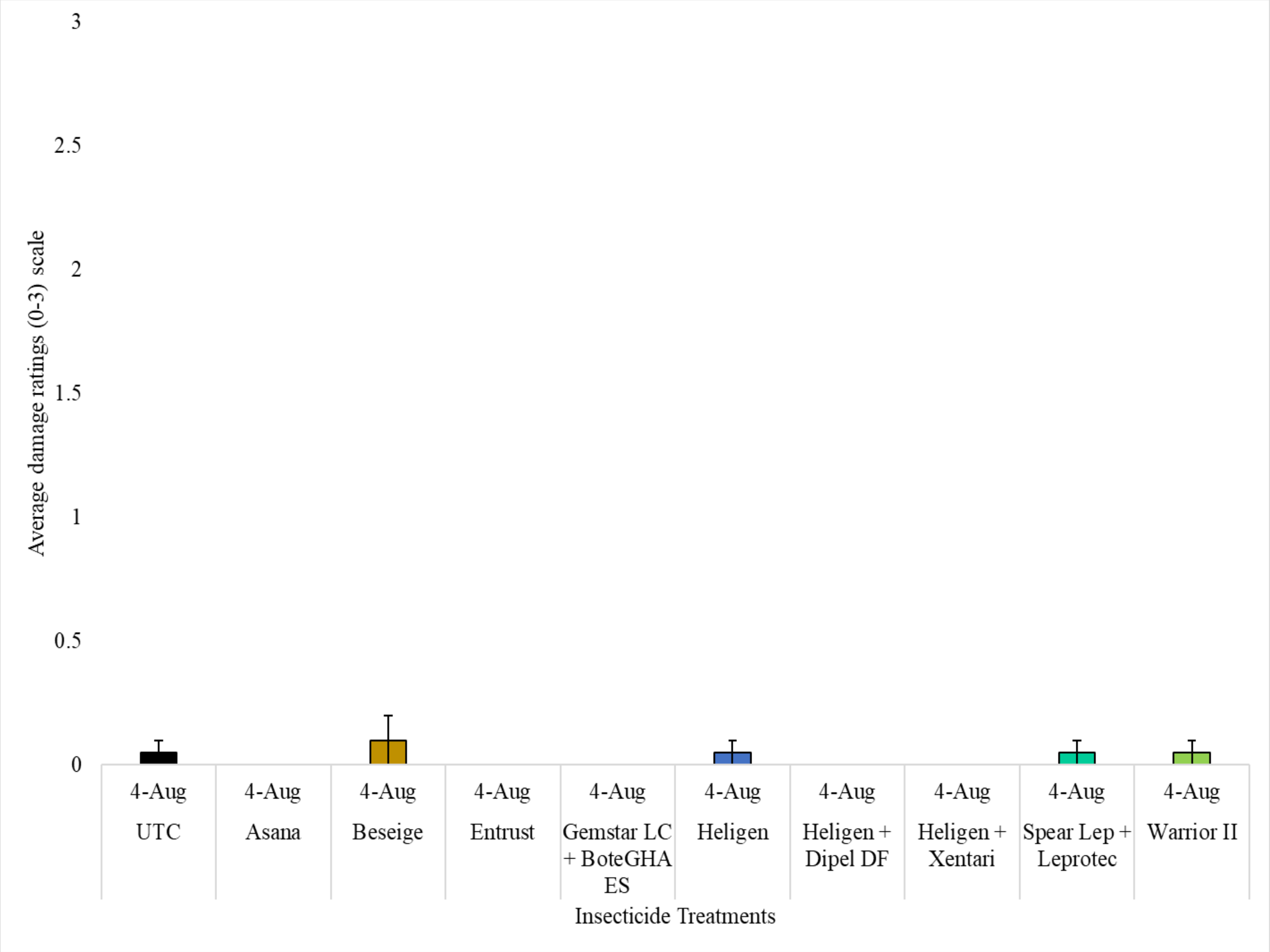

3
2.5
2
1.5
1
0.5
0
Average damage ratings (0-3) scale
4-Aug
UTC
4-Aug
Asana
4-Aug
Beseige
4-Aug
Entrust
4-Aug
Gemstar LC + BoteGHA ES
4-Aug
Heligen
4-Aug
Heligen + Dipel DF
4-Aug
Heligen + Xentari
4-Aug
Spear Lep + Leprotec
4-Aug
Warrior II
Insecticide Treatments

Figure 2.5a

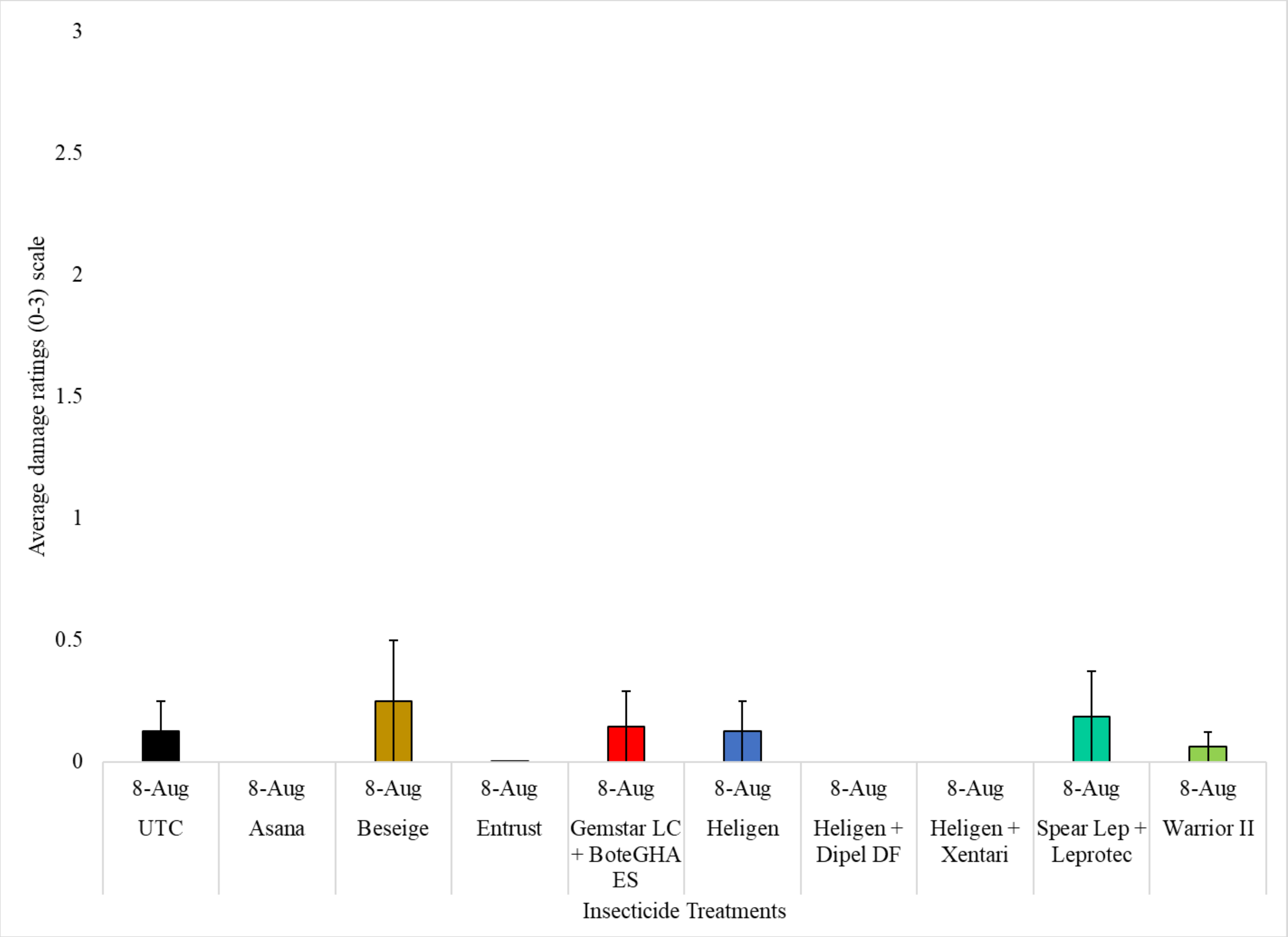
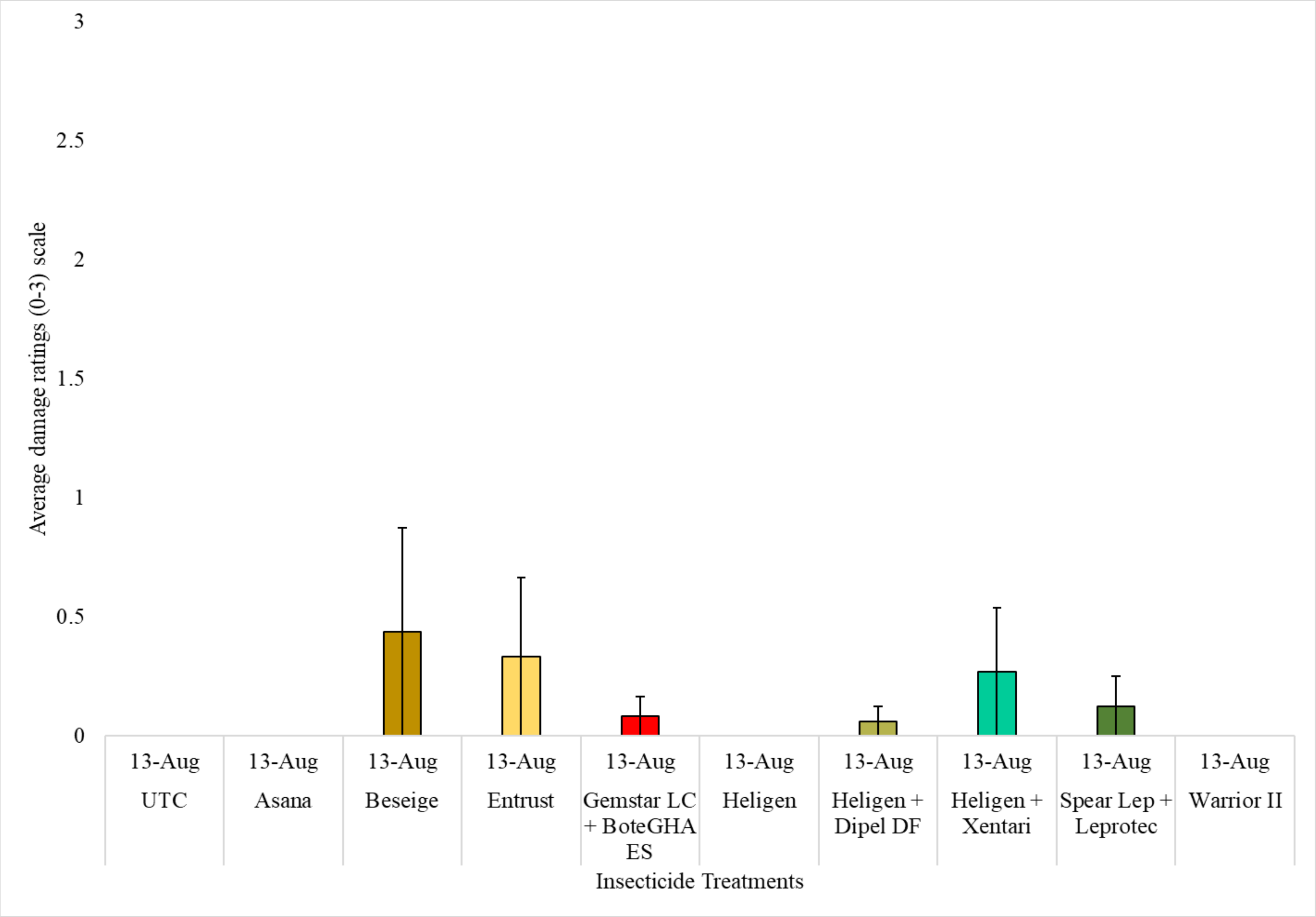
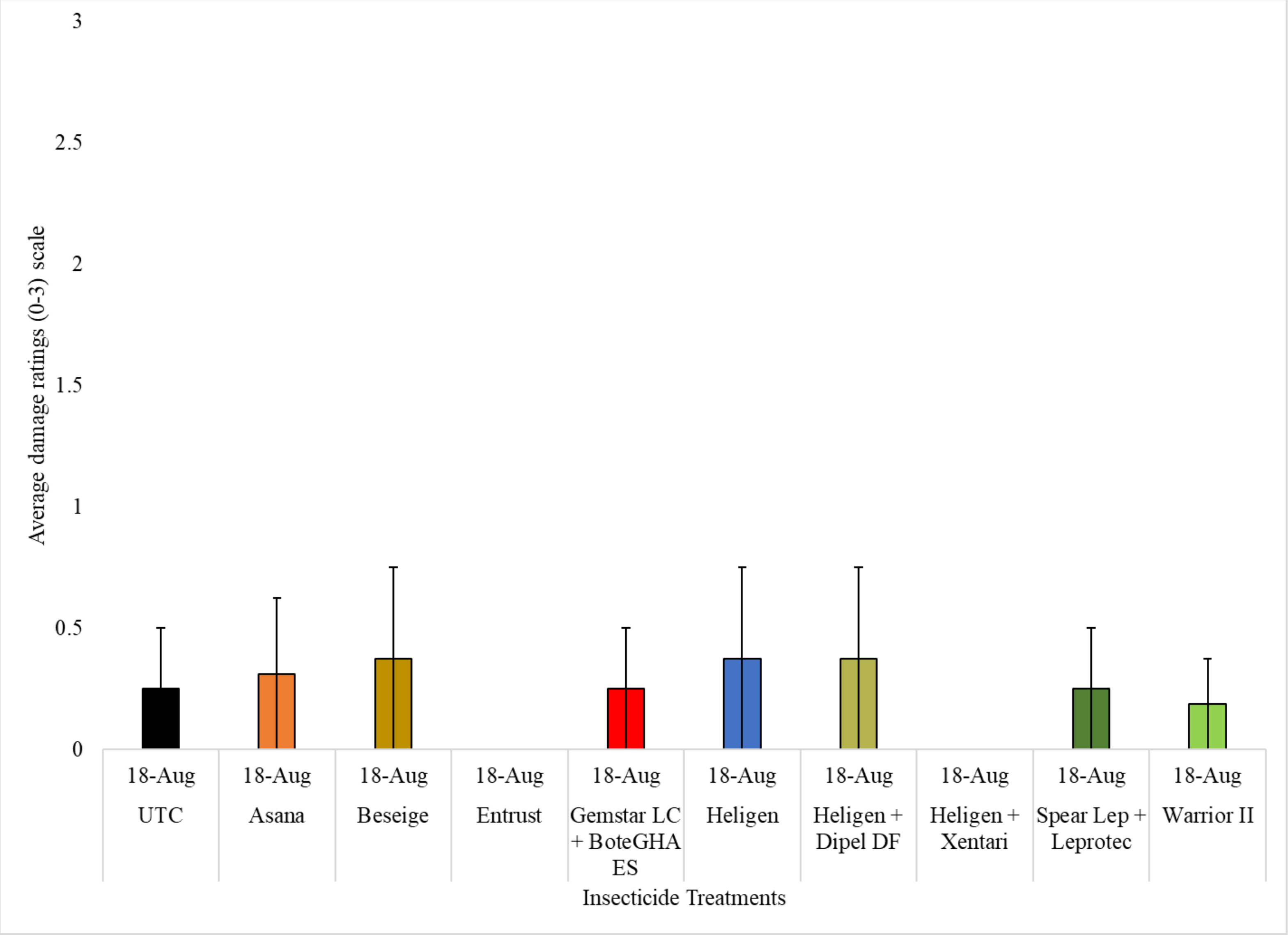

3
2.5
2
1.5
1
0.5
0
Average damage ratings (0-3) scale
8-Aug
UTC
8-Aug
Asana
8-Aug
Beseige
8-Aug
Entrust
8-Aug
Gemstar LC + BoteGHA ES
8-Aug
Heligen
8-Aug
Heligen + Dipel DF
8-Aug
Heligen + Xentari
8-Aug
Spear Lep + Leprotec
8-Aug
Warrior II
Insecticide Treatments

Figure 2.5b



3
2.5
2
1.5
1
0.5
0
Average damage ratings (0-3) scale
13-Aug
UTC
13-Aug
Asana
13-Aug
Beseige
13-Aug
Entrust
13-Aug
Gemstar LC + BoteGHA ES
13-Aug
Heligen
13-Aug
Heligen + Dipel DF
13-Aug
Heligen + Xentari
13-Aug
Spear Lep + Leprotec
13-Aug
Warrior II
Insecticide Treatments

Figure 2.5c



3
2.5
2
1.5
1
0.5
0
Average damage ratings (0-3) scale
18-Aug
UTC
18-Aug
Asana
18-Aug
Beseige
18-Aug
Entrust
18-Aug
Gemstar LC + BoteGHA ES
18-Aug
Heligen
18-Aug
Heligen + Dipel DF
18-Aug
Heligen + Xentari
18-Aug
Spear Lep + Leprotec
18-Aug
Warrior II
Insecticide Treatments

Figure 2.5d

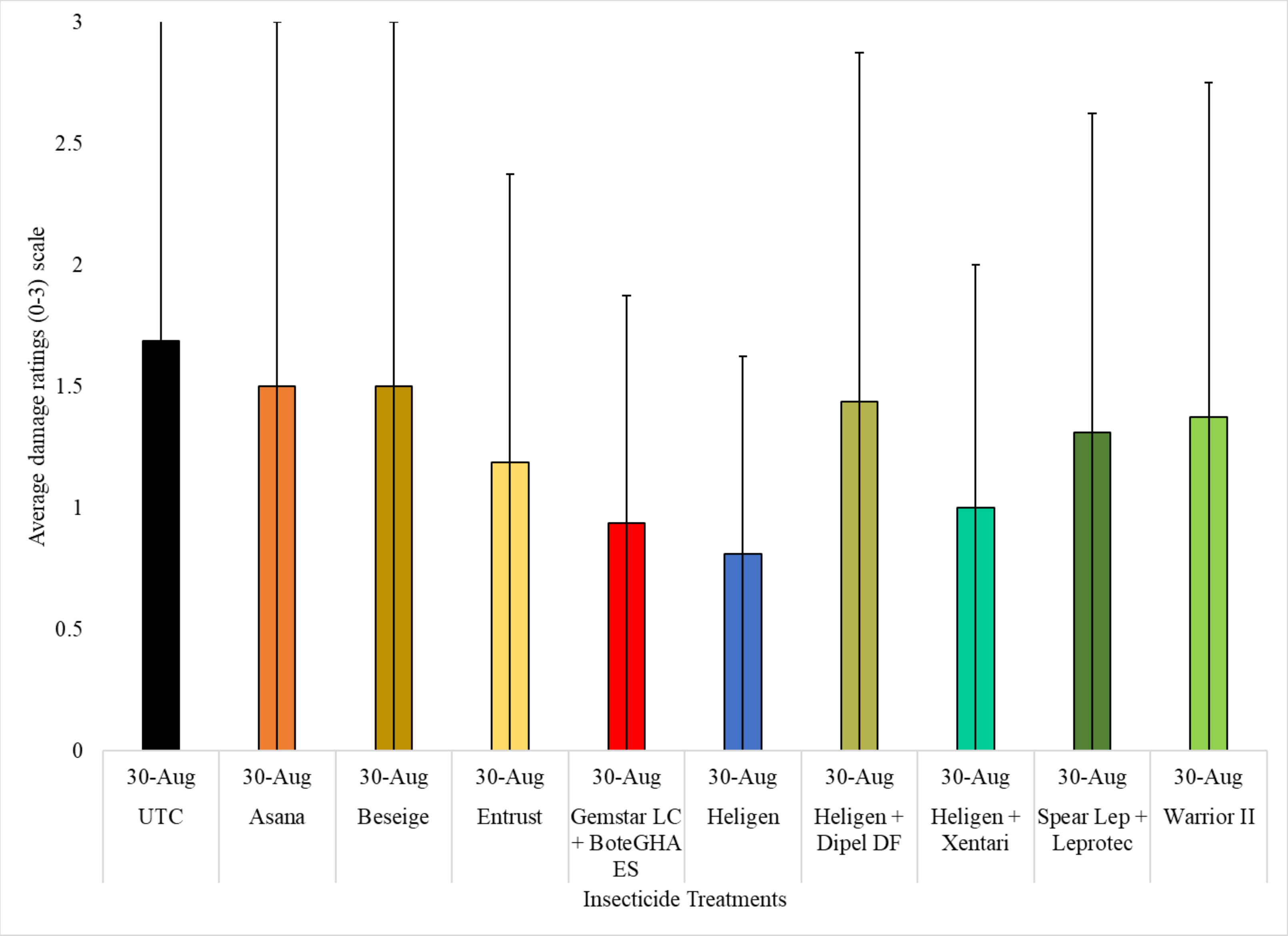

3
2.5
2
1.5
1
0.5
0
Average damage ratings (0-3) scale
30-Aug
UTC
30-Aug
Asana
30-Aug
Beseige
30-Aug
Entrust
30-Aug
Gemstar LC + BoteGHA ES
30-Aug
Heligen
30-Aug
Heligen + Dipel DF
30-Aug
Heligen + Xentari
30-Aug
Spear Lep + Leprotec
30-Aug
Warrior II
Insecticide Treatments

Figure 2.5e

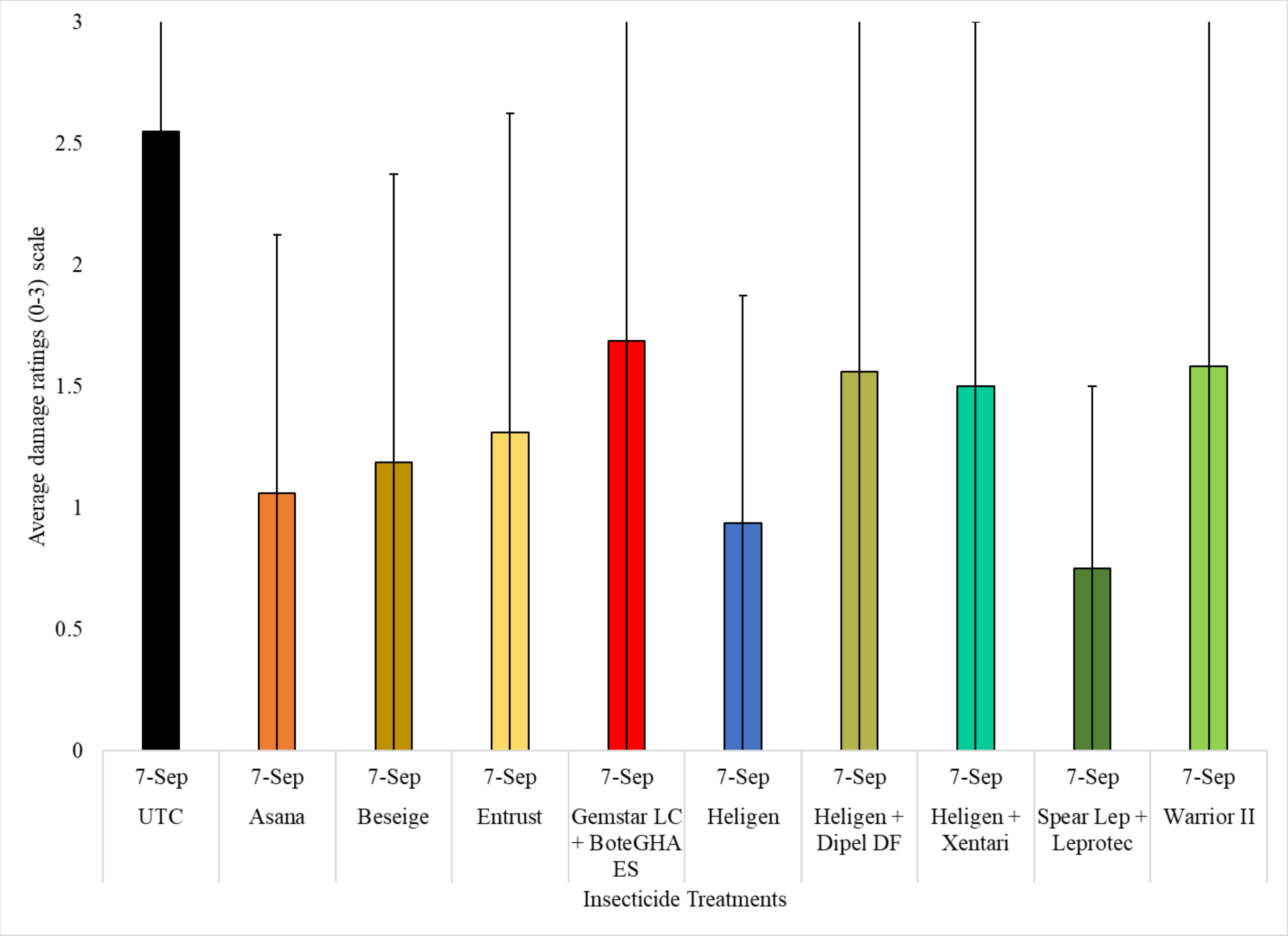
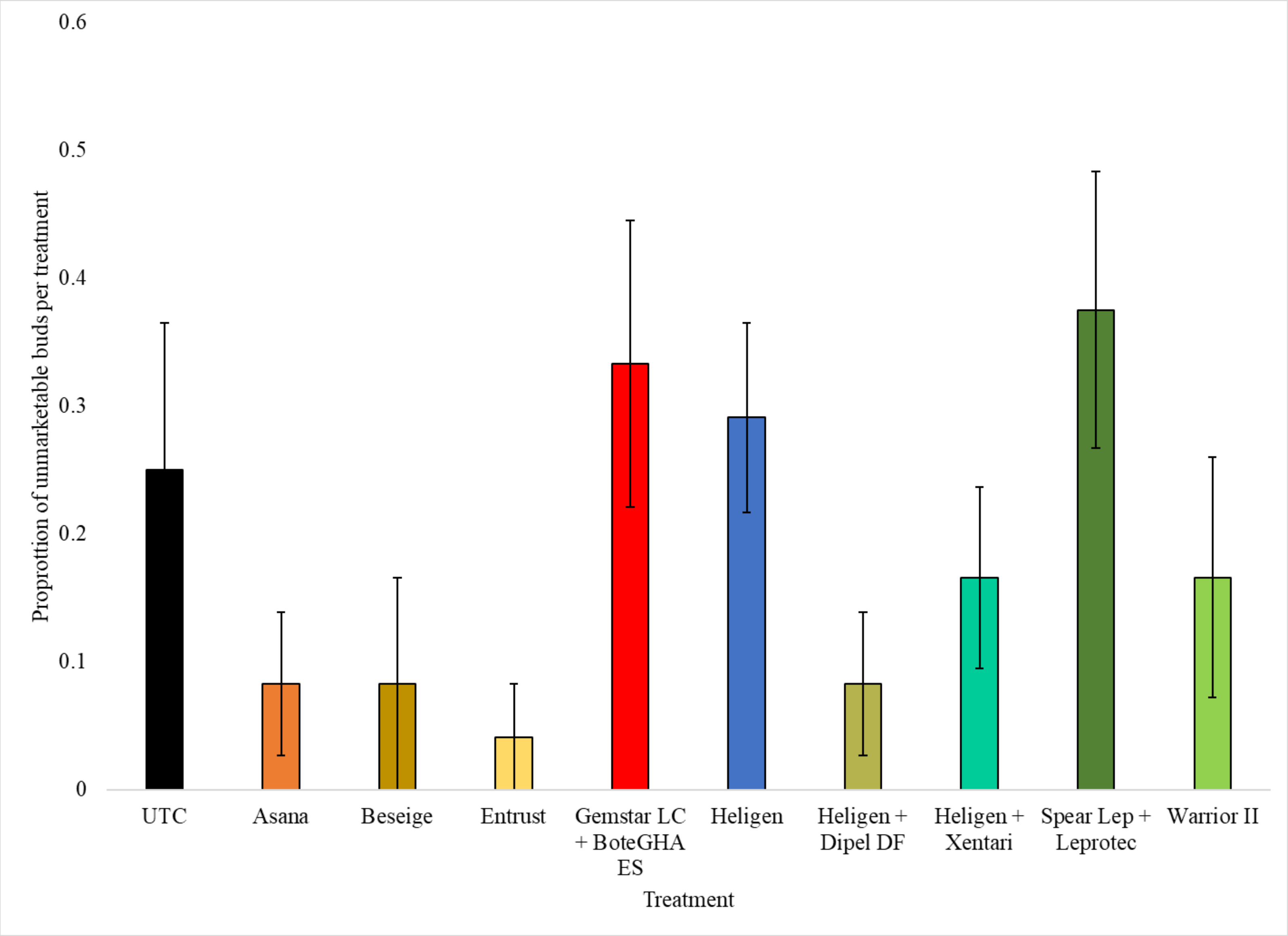

Average damage ratings (0-3) scale
3
2.5
2
1.5
1
0.5
0
7-Sep
UTC
7-Sep
Asana
7-Sep
Beseige
7-Sep
Entrust
7-Sep
Gemstar LC + BoteGHA ES
7-Sep
Heligen
7-Sep
Heligen + Dipel DF
7-Sep
Heligen + Xentari
7-Sep
Spear Lep + Leprotec
7-Sep
Warrior II
Insecticide Treatments

Figure 2.6


0.6
0.5
0.4
0.3
0.2
0.1
0
Proprottion of unmarketable buds per treatment
UTC
Asana
Beseige
Entrust
Gemstar LC + BoteGHA ES
Heligen
Heligen + Dipel DF
Heligen + Xentari
Spear Lep + Leprotec
Warrior II
Treatment

Chapter 3

Variety selection and trap cropping as potential cultural control strategies for corn earworm, *Helicoverpa zea,* in hemp.

Abstract

Corn earworm, *Helicoverpa zea*, is an insect pest that feeds on a variety of crops, including hemp and sweet corn. In hemp (*Cannabis sativa* L.), corn earworms feed on the flower buds which is the marketable portion of the plant. Due to the lack of pesticides allowed for use in hemp, exploring different management strategies is crucial to control corn earworms. Therefore, two field experiments on cultural control strategies were conducted in 2022 at the E.V. Smith Research Center in Shorter, AL, USA. The first study focused on the potential for a sweet corn trap crop to reduce corn earworm damage in hemp. Transgenic sweet corn expressing the Vip3A20 *Bacillus thuringiensis* toxin was used as a trap crop. Sweet corn was planted in staggered planting dates in border rows around hemp to attract adult moths for oviposition, hatching, and mortality due to the insecticidal properties of transgenic sweet corn. Five treatments were assessed including a control with no trap crop and four planting dates each seven days apart in a replicated complete block design. A second experiment was conducted with four hemp varieties (Cat Daddy, Southern Luck, Belle, and BaOx) to assess varietal preference by corn earworm. In the trap crop experiment, corn earworm egg and larval counts were conducted in sweet corn and hemp. In both trials, caterpillar numbers and damage were assessed on hemp. Results showed that the first sweet corn planting date had a greater number of eggs in hemp, resulting in greater damage and more unmarketable buds at harvest. In the variety trial, there were significant differences in plant measurements, cannabinoid concentrations, caterpillar numbers, and damage ratings. Variety BaOx performed generally better than the other varieties.

Introduction

Corn earworm (*Helicoverpa zea*) is a polyphagous insect species that is distributed across the Western Hemisphere (Hardwick 1965). Corn earworm has a variety of plant hosts including traditional row crops, vegetables, and fruits. Sweet corn has traditionally been the preferred host for female moth oviposition and larval development (Chowdhury et al. 1987). The use of different integrated pest management strategies has been developed to control corn earworm populations. The most common strategies are genetically modified varieties expressing *Bacillus thuringiensis* toxins and chemical control with different insecticides. Cultural control strategies such as varietal selection, planting dates, and trap crops have shown positive results in mitigating this pest.

In recent years, hemp (*Cannabis sativa* L.) has experienced a resurgence due to its diverse applications in various industries (Fike 2016). However, this crop faces several challenges, one of which is the lack of pest management strategies against corn earworm. Currently, many growers rely on the use of biopesticides to aid in the control of corn earworm damage. Chemical control in hemp, as mentioned in Chapter 2, has not proven to be effective in controlling this pest. Therefore, exploring different integrated pest management (IPM) strategies such as cultural control is crucial to reduce damage caused by this pest.

Varietal selection is a widely practiced cultural control strategy used in several agricultural systems (Flint and Bosch 2012). In hemp, the selection of an appropriate hemp variety may help reduce pest pressure while still thriving in local environmental conditions. This approach is crucial in hemp due to the genetic diversity this crop possesses (Clarke and Merlin 2016).

Growers across the United States prefer varieties containing high levels of cannabidiol (CBD) while still maintaining the legal limits of tetrahydrocannabinol (THC). In terms of pest management, varieties that show plant traits that are tolerant to herbivore damage should be used (Mitchell et al. 2016, Pappas et al. 2017). Exploring different varieties may address questions regarding the production of high CBD levels, legal THC levels, and plants with resilience against regional insect pests.

Another commonly used cultural control strategy is the use of trap crops. Trap cropping is a sustainable strategy that diverts insect pests from the cash crop to the trap crop, therefore reducing damage and increasing yield (Hokkanen 1991). For this strategy to be successful, the trap crop must retain the targeted pest and prevent its development and subsequent movement into the cash crop. As previously mentioned, sweet corn is highly preferred by corn earworms for oviposition. The synchronization of moth laying, hemp flowering, and sweet corn silking may help divert moth oviposition in sweet corn instead of hemp. Sweet corn varieties with insecticidal properties such as Vip3A20 can help prevent their development.

The objectives of this study are to: 1. To explore the potential of sweet corn as a trap crop to reduce corn earworm damage and thus yield loss in hemp, 2. Explore four hemp varieties as a cultural control strategy against corn earworm, and 3. Examine the interaction between levels of CBD/THC related to insect herbivory.

Materials and methods

Trap crop trial

Establishment of hemp plants

The field experiment was conducted during the 2022 growing season at E.V. Smith Research Center in Shorter, Alabama, USA (32.44562, -85.89010). On April 15, 2022, one hundred feminized BaOx variety seeds were sown as seeds at Plant Science Research Center in Auburn, Alabama (32.58829, -85.48852). Hemp seeds were placed in 36 cell count trays containing PRO-MIX 'BX' (Rivière-du-Loup City, Québec, Canada) potting soil and watered under a misting system for six weeks. The water regime shifted from thirty seconds of water every thirty minutes daily for the first four weeks to twice a day using a misting hose for the remaining two weeks.

Hemp transplanting

On June 9, 2022, one hundred hemp plants were hand-transplanted into plastic beds with white mulch at E.V. Smith Research Station, Alabama, USA (32.44562, -85.89010). S-metolachlor (Dual Magnum, Syngenta, Greensboro, NC) was applied on the field to control weeds prior to laying plastic. Ultrasol Multipurpose Plus 20-20-20 fertilizer (SQM North America) and 15.5-0-0 calcium nitrate were applied at a rate of 183.8 kg/ha. Throughout the growing season, weeds were manually removed weekly. Drip irrigation was used for water management and was applied as needed in all plots. Plots consisted of five plants per plot, with dimensions of 0.61 meters in row spacing and 6.096 meters between row spacing.

Corn seeding

For this experiment, seeds from the sweet corn variety Attribute II (Syngenta, Greensboro, NC, USA) were used. Sweet corn was planted in staggered plantings in border rows around hemp plots (Figure 3.1). All corn seeds were sown on the bare ground using a hand-pushed seed pusher (JP-1, Jang, South Korea). Corn seeds received the same fertilization and weed control regime as the hemp plants. Drip irrigation was established prior to seeding and water was applied as needed. Throughout the growing season, weeds were manually removed.

This experiment sought to explore different sweet corn planting dates. The treatments consisted of four sweet corn planting dates and an untreated control with no corn. These planting dates were established to achieve synchronization between hemp flowering and sweet corn silking. Treatments were replicated four times in a randomized complete block design. Planting dates followed a seven-day interval. The first planting date was on June 2, 2022, seven days before hemp transplant. The second planting date was on June 9, 2022, which is the same day as the hemp transplant. The third planting date was on June 16, 2022, and the fourth planting date on June 23, 2022.

Data collection

Sweet corn growth stages were conducted on each plot on July 26, August 1, and August 8, 2022. Growth stages were recorded following Licht 2017.

The number of corn earworm eggs on both crops was assessed starting on July 25, 2022 then continued on August 1, August 8, and August 15, 2022. In sweet corn, silks on five randomly selected plants were scouted for corn earworm eggs. In hemp, egg numbers were counted from three random buds from each plot.

Damage ratings were conducted on the same buds where larvae were spotted using the 0-3 damage scale after Britt et al. (2021). Criteria for this scale include 0 is no damage; 1 is visible damage, but still marketable; 2 is damage of 50% or less, making the bud unmarketable; 3 is damage to more of 50% making the bud unmarketable. Data were collected on July 18, July 25, August 1, August 8, August 15, August 26, August 29, September 8 and September 12, 2022.

Harvest

Three hemp plants from each plot were harvested on September 16, 2022 using bond steel bypass lopper (Bond Manufacturing, Antioch, California, USA) to cut at the base of the stem. Fresh weight was recorded using a hanging scale (Uline, Pleasant Prairie, Wisconsin, USA). After weighing, the harvested plants were grouped together using a wool string and hung upside down on a Grip-Rite black annealed steel 16 Ga. tie wire (0.0508 centimeters D x 102.108 meters L) (Prime Source, Irving, Texas, USA) for two weeks. Dry weight was measured on September 30, 2022, using a hanging scale (Uline, Pleasant Prairie, Wisconsin, USA).

Three randomly selected flower buds were collected from the harvested plants. Three-centimeter-long buds were collected from the upper, middle, and bottom parts of the plant and marketability assessed using the 0-3 damage scale. Fresh weight was recorded buds using a digital scale (VWR, International LLC, Radnor, Pennsylvania, USA) before being transferred in Ziploc bags (Ziploc, San Diego, USA) to Plant Science Research Center. On October 16, 2022, the weight of the dried buds was measured using a digital scale.

Sweet corn could not be harvested due to severe coyote damage (Figure 3.2).

Variety trial

Hemp transplant

On June 27, 2022, four hemp varieties were hand transplanted into plastic beds covered with white mulch following manual weed removal at E.V. Smith Research Station, Alabama (32.44562, -85.89010). Ultrasol Multipurpose Plus 20-20-20 fertilizer (SQM North America) and

15.5-0-0 calcium nitrate were applied at a rate of 183.8 kg/ha. Drip irrigation was used for water management and was applied as needed in all plots.

Seeds of variety BaOx were sown at Plant Science Research Center in Auburn, Alabama (32.58829, -85.48852). Hemp seeds were placed in 36 cell count trays containing PRO-MIX 'BX' (Rivière-du-Loup City, Québec, Canada) potting soil and watered under a misting system for six weeks. The water regime shifted from thirty seconds of water every thirty minutes daily for the first four weeks to twice a day using a misting hose for the remaining two weeks.

The three remaining varieties used for this experiment were Cat Daddy, Belle, and Southern Luck (Hemp Mine, Fair Play, South Carolina 34.53322° N, 83.04279° W). These three varieties were received as rooted clones and hand-transplanted into the field. Plots contained five plants each and were arranged in a randomized complete block with four replications. Each plot had 0.61 meters in row spacing and 1.829 meters between row spacing.

Data collection

Height and width measurements were conducted on all five plants per plot. Height measurements were taken from the base of the plant to the apical meristem. Width measurements of the stem were conducted using digital calipers by measuring at the base of the plant near the soil line. These measurements were taken on July 5, July 11, July 18, July 25, August 1, August 8, and August 26, 2022.

Caterpillar numbers and damage ratings were conducted from three randomly selected buds in each plot. Damage ratings were performed using a 0-3 damage scale after Britt et al. (2021). Criteria are the same as described above.

Cannabinoid testing

On September 7, 2022, one flower bud per plot was removed prior to harvest for potency analysis. Buds were taken by clipping the top twenty centimeters of the plant's primary stem. After clipping, plants were placed in paper bags and sent to ACS Laboratory, Sun City Center, Florida, USA (27.71360, -82.36988). Each flower bud was tested for eleven cannabinoids including Tetrahydrocannabinol (THC), Cannabichromene (CBC), Tetrahydrocannabinol acid (THCA), Tetrahydrocannabivarin (THCV), Cannabidiol (CBD), Cannabidiolic acid (CBDA), Cannabidivarin (CBDV), Cannabigerol (CBG), Cannabigerolic acid (CBGA), and Cannabinol (CBN). The samples were then analyzed via Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry using a UV detector to quantify cannabinoid concentrations of the hemp flower (ACS Laboratory, Sun City Center, FL, USA).

Harvest

On September 28, 2022 two plants per plot were harvested and dried following the same methods as above. Buds were collected, measured, and dried following the methods from the trap crop experiment.

Statistical Analysis

Trap crop

All statistical analyses were conducted in SAS® 9.4 (Cary, NC, USA). The average caterpillar numbers and damage ratings in hemp plants were analyzed using a generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). To compare the treatments, least square means were separated using Tukey-Kramer. The fixed effect treatment was used to model the average caterpillar numbers and damage ratings. The identity link function and the Gaussian distribution were used. Degrees of freedom were calculated using the Residual Method. Means and standard errors were determined using PROC MEANS.

Eggs numbers in both sweet corn and hemp were analyzed using a generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). Least square means of treatments were performed using Tukey-Kramer. The fixed effect treatment was used to model the egg numbers. The identity link function and the Gaussian distribution were used. Degrees of freedom were calculated using the Residual Method. Means and standard errors were determined using PROC MEANS. The preference/choice data from egg numbers in both crops were analyzed by using PROC TTEST procedure.

A linear regression using PROC REG was done in egg numbers of sweet corn. Egg numbers were the dependent variable, and the independent variables were treatments, growth stages and the interaction between both.

In addition, harvest data were analyzed including the average fresh weight and dry weight of the three harvested plants, as well as the average fresh weight and dry weight of the buds from these plants. The proportion of unmarketable buds from the three harvest plants was analyzed. The previously mentioned variables were analyzed using a generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). Least square means of treatments were performed using Tukey-Kramer. The average fresh weight and dry weight of the two harvested plants, average bud fresh weight and dry weight, and the proportion of marketable buds and unmarketable buds were modeled as a function to the fixed effect treatment. Data were analyzed using Gaussian distribution along with the identity link function. Degrees of freedom were calculated using the Residual Method. Finally, means and standard errors were determined using PROC MEANS.

Variety trial

Caterpillar numbers and damage ratings were analyzed by each individual date using generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). To compare the treatments by date, least square means were separated using Tukey-Kramer. Data were analyzed with Gaussian distribution and identity link function. The Residual Method was used to calculate the degrees of freedom. Means and standard errors were determined using PROC MEANS.

Height and width measurements and cannabinoid concentrations were analyzed using a generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). Data were analyzed using Gaussian distribution along with the identity link function. Degrees of freedom were calculated using the Residual Method. Least square means of treatments was performed using Tukey-Kramer. Means and standard errors were determined using PROC MEANS.

Furthermore, all the data from harvest were analyzed using a generalized linear mixed model, PROC GLIMMIX (significant $P \leq 0.05$). Least square means of treatments was performed using Tukey-Kramer. The average fresh weight and dry weight of the two harvested plants, average bud fresh weight and dry weight, and the proportion of marketable buds and unmarketable buds were modeled as a function to the fixed effect treatment. Data were analyzed using Gaussian distribution along with the identity link function. Degrees of freedom were calculated using the Residual Method. Means and standard errors were determined using PROC MEANS.

Results

Trap crop results

Egg numbers

There were no significant differences between planting dates and the untreated control in egg numbers found on hemp (F= 0.660, P= 0.630) (Table 3.1). While there were no significant differences, all planting dates showed less egg numbers compared to the untreated control. From all planting dates, the fourth date was the one that had the highest egg numbers (Table 3.1).

In sweet corn, there were no significant differences in egg numbers between planting dates and the untreated control (F= 1.570, P= 0.248) (Table 3.2). However, female corn earworm moths showed a preference in egg laying on the first planting date and eggs numbers declined on the remaining planting dates. Additionally, in hemp, the average egg numbers were the lowest in the first planting date (Table 3.1).

Results showed that on all planting dates, there were significantly more eggs found in sweet corn than hemp (P< 0.0001) (Table 3.3, Figure 3.3).

A linear regression was conducted to investigate the potential relationship between eggs numbers in sweet corn and the interaction between independent variables: planting dates, growth sages of sweet corn, and planting dates combined with their growth stages (planting dates x growth stages) (Table 3.4). Results for this linear regression showed an overall statistical significance (F = 2.940, P = 0.046). However, there is a low R^2 (0.192) as well as lack of significance for some individual coefficients (Table 3.4).

Caterpillar numbers and damage ratings

There were no significant differences between planting dates in the mean number of caterpillars found in hemp (F= 0.450, P= 0.773) (Table 3.5). While there were no significant differences shown, there was a reduction in caterpillar numbers from the first planting date to the fourth planting date (Table 3.5). In addition, compared to the untreated control, all planting dates had lower caterpillars in hemp.

The mean damage ratings showed similar results. While there were no significant differences between sweet corn plantings in hemp damage (F= 0.190, P= 0.940), plots with sweet corn border rows had less damage compared to the untreated control with no sweet corn (Table 3.6, Figure 3.4).

Harvest

There were no significant differences between planting dates in hemp fresh weight (F= 2.850, P= 0.061) (Table 3.7). Hemp plants with sweet corn border rows had overall higher fresh weight than plants in the untreated control. Hemp plots with the second sweet corn planting date had the highest fresh weight.

The dry weight of three hemp plants was also analyzed, with results showing similarities to fresh weight data (Table 3.7). There were no significant differences between sweet corn planting dates (F= 1.060, P= 0.411) and to the untreated control. Overall, compared to the untreated control, all planting dates provided higher dry weight (Table 3.8). The second planting date of sweet corn had a higher dry weight of hemp plants.

Hemp bud fresh (F= 1.460, P= 0.226) and dry (F= 1.270, P= 0.292) weights were not significantly different between sweet corn planting dates. In the second planting date, results showed a higher bud weight among all planting dates and compared to the untreated control (Table 3.9, 3.10).

The proportion of unmarketable hemp buds from the selected flower buds showed no significant differences between sweet corn treatments (F= 1.270, P= 0.293) (Table 3.10, Figure

3.5). From all planting dates, the third planting date had the lowest number of unmarketable buds (Table 3.10, Figure 3.5). All plots with sweet corn border rows had lower numbers of unmarketable hemp buds compared to the untreated control.

Variety trial

Plant height differed significantly differences between varieties (F= 11.200, P= <.0001) (Table 3.12). Of all varieties, Belle was the shortest and Southern Luck was the tallest (Table 3.12). Plant stem width differed significantly between varieties (F= 6.540, P= 0.0003) (Table 3.13). BaOx had the widest stems and Belle had the narrowest.

There were no significant differences between varieties in caterpillar numbers by treatments (F=1.010, P= 0.386) (Table 3.14), although Southern Luck and Cat Daddy had higher numbers of caterpillars. Damage did not significantly differ between varieties (F= 0.750, P= 0.525) (Table 3.15). However, higher damage was found in Belle and Southern Luck compared to BaOx and Cat Daddy (Table 3.15). However, time did have an effect on caterpillar numbers and damage.

On the first sample date, caterpillar numbers were significantly different between varieties (F= 2.80, P= 0.046) (Table 3.16, Figure 3.6). Southern Luck had the highest number of caterpillars, followed by Cat Daddy (Table 3.16). There were significant differences between varieties in damage ratings (F= 1.000, P= 0.298) (Table 3.17, Figure 3.7). On the second sampling date, there were no caterpillars or damage found in the hemp plants (Table 3.16, 3.17, Figure 3.6, 3.7).

On the third sampling date caterpillar numbers were significantly different between varieties (F= 4.580, P= 0.005) (Table 3.16, Figure 3.6). On this date, Cat Daddy had the highest number of caterpillars, while BaOx and Belle had the lowest (Table 3.16). Results for damage ratings on this date showed significant differences between varieties (F= 4.450, P= 0.006) (Table 3.17, Figure 3.7). Cat Daddy had the highest damage of all varieties and was significantly higher than BaOx and Belle.

On the fourth sampling date the number of caterpillars (F= 0.370, P= 0.773) or damage ratings (F= 1.650, P= 0.185) were not significantly different between varieties (Table 3.16, 3.17, Figure 3.6, 3.7). Belle had the highest numbers of caterpillars, followed by Southern Luck (Table 3.16), with Southern Luck having higher damage (Table 3.17).

On the fifth sampling date the number of caterpillars (F= 0.330, P= 0.801) or damage ratings (F= 1.040, P= 0.378) were not significantly different between varieties (F= 0.330, P= 0.801) (Table 3.16, 3.17, Figure 3.6, 3.7).

Cannabinoid testing

Flower buds from each variety were tested for ten cannabinoids including Tetrahydrocannabinol (THC), delta-8-Tetrahydrocannabinol (Delta 8-THC), Tetrahydrocannabinol acid (THCA), Tetrahydrocannabivarin (THCV), Cannabidiol (CBD), Cannabidiolic acid (CBDA), Cannabidivarin (CBDV), Cannabigerol (CBG), Cannabigerolic acid (CBGA), Cannabinol (CBN), and Cannabichromene (CBC). Cannabidivarin (CBDV), Cannabinol (CBN), and Tetrahydrocannabivarin (THCV), were not found in any variety.

There were no significant differences between varieties in CBD percentage on a dry weight basis (F= 2.200, P = 0.166), although Southern Luck had the highest concentration of CBD (Table 3.18). Results for THC percentage did not show any significant differences between varieties (F= 0.910, P=0.477), although Belle presented the highest concentration of THC among all varieties (Table 3.18).

There were significant differences in CBG (F= 4.410, P= 0.029) and CBGA (F = 4.420, P=0.029) (Table 3.18). BaOx had significantly lower concentrations of CBG and CBGA compared to other varieties.

There were no significant differences between varieties in CBC (F=1.360, P=0.306), CBDA (F= 1.51, P= 0.2667), THCA (F= 1.28, P=0.3306), or other cannabinoids (F=1.36, P= 0.3062) (Table 3.18).

Harvest

Results from the fresh (F= 3.010, P= 0.072) and dry (F= 0.360, P= 0.783) weights of the two harvested plants showed no significant differences between the four varieties (Tables 3.19, 3.20). While there were no significant differences, Cat Daddy had the highest fresh weight and dry weight among varieties and Belle had the lowest fresh and dry weight.

Flower bud fresh (F= 1.330, P= 0.310) and dry (F= 0.760, P= 0.587) weights showed no significant differences between varieties (Table 3.21, 3.22). From these results, BaOx had the highest fresh weight, and Southern Luck the highest dry weight. Cat Daddy showed the lowest number in both fresh and dry weight.

The proportion of unmarketable buds did not differ significantly between varieties (F= 1.690, P= 0.182) (Table 3.23, Figure 3.8). Overall, Cat Daddy had the highest numbers of unmarketable buds.

Discussion

Trap crop trial

Corn earworm, *Helicoverpa zea*, is a common pest sweet corn, showing high oviposition preference by female moths (Olmstead et al. 2016). Cultural control methods, such as the adoption of trap crops, are a pest management strategy that has been studied and shown positive results in agricultural systems (Hokkanen 1991). To establish a successful trap crop, several factors should be considered such as the attractiveness the trap crop to the insect pest, planting dates, weed control, and if necessary, the use of foliar applications to retain the targeted pest and prevent its development (Holden et al. 2012).

For this experiment, transgenic sweet corn variety Attribute II containing the VIP3A20 Bt toxin was used as a trap crop. Sweet corn was chosen as a host plant as it is a preferred oviposition site for female corn earworm adults. Further, the VIP3A20 toxin has insecticidal properties and will prevent larval corn earworm (Barber 1943, Burkness et al. 2010, Kurtz 2010). Four planting dates were used to synchronize hemp flowering time and sweet corn silking, the most attractive timing for oviposition. This followed a sequential trap cropping, in which the trap crop is planted before or after the main crop to enhance attractiveness of the desired pest (Shelton and Badenes-Perez 2006).

Overall results on this study showed no significant difference in average caterpillar numbers, average damage ratings, and yield. However, interesting findings were seen and will be summarized.

The data suggest an oviposition preference by female moths in sweet corn compared to hemp. This was expected due to similar findings in other crops such as tomatoes and soybeans

(Javaid et al. 2005, Rhino et al. 2014). Oviposition preference was seen by Rhino et al. (2014) with higher number of eggs in sweet corn than in tomato fields.

While our sweet corn was attractive to the target pest, the successful establishment of a trap crop may be difficult given several environmental conditions affecting establishment (Shelton and Badenes-Perez 2006). Environmental stressors may have impacted our results, such as extremely high temperatures at planting. Sweet corn was planted for this experiment in June 2022, during which temperatures ranged from 28.33°C - 37.77°C. To achieve optimal germination temperatures should range from 15.55°C - 29.44°C (Sideman 2016). In this study, the first planting date was sown on the coolest temperature which was 28.33°C, and it also had the highest germination success compared to other planting dates. This may explain why the first planting date had the highest egg numbers in sweet corn, and the remaining dates had lower egg numbers.

Furthermore, the effect of temperature was seen on the fourth planting date, which was sown on June 23, 2022, at 37.77° C. These temperatures compromised the germination and overall fewer corn plants were seen on these plots (personal observation). The outcome of these environmental stressors were overall higher egg numbers on the second, third, and fourth planting date in hemp plants and fewer on sweet corn plants.

Additionally, literature suggests that even if the targeted pest is attracted to the trap crop, low proportions of the trap crop will not be sufficient to reduce pest pressure (Sequeira 2001). For example, to control the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in cabbage, yellow rocket has been used as a trap crop (Badenes-perez et al. 2005). For this trap crop to show successful, 5 to 13% of the crop area has to be yellow rocket (Badenes-perez et al.

2005). In this study, the proportion of sweet corn in hemp plots was inconsistent and not factored into the analyses.

While results for this experiment were not as expected, similar findings were found by Parsons and Ullyett (1934) in the adoption of corn to control *Helicoverpa armigera* in cotton fields. Researchers reported that planting dates were interrupted due to rain, leading to a late planting date which did not provide effective control of the target pest (Parsons and Ullyett 1934).

Based on our results, the second planting date had the best performance. Hemp plants from this treatment were less damaged and had the highest fresh and dry weights. However, it is important to further investigate the use of sweet corn as a trap crop given the environmental stress on corn and inconsistent data from this trial. Additionally, other crops and planting dates are worth exploring.

Variety trial

Varietal selection plays a crucial role in pest management strategies (Kogan 1998). The correct choice of variety can impact the susceptibility of crops to pest attacks and the effectiveness of pest management strategies. In hemp, there is an extensive genetic diversity that plays a role in adopting a hemp cultivar depending on the environment. This trial was conducted to explore four hemp varieties bred and sold in the southeastern United States. We hypothesized that the tallest plant with low cannabinoid levels will result in the highest herbivory and subsequent damage by corn earworm larvae.

There were significant differences among the four hemp varieties in terms of plant measurements, caterpillar numbers, and damage ratings. While there were no significant differences in harvest parameters, physiological traits and plant chemistry did show a pattern with corn earworm feeding and overall yield.

In terms of plant measurements, the height and width of the hemp plants varied among the four varieties. The initial hypothesis for this experiment was that corn earworm will show feeding preference in the variety that had the biggest plants. In this trial, Southern Luck was the tallest, and Belle was the shortest. For plant width, BaOx exhibited the widest plants, and Belle the narrowest.

Caterpillar numbers and damage were variable on each sampling date. Southern Luck and Cat Daddy had the highest caterpillar numbers and Belle had higher damage. Among all varieties, BaOx had the lowest numbers in both damage ratings and caterpillar numbers. These findings suggest that plant architecture may influence insect herbivory in hemp plants, given the larger plant architecture of Southern Luck. Literature shows that architecture and vegetation structure influence the movement and provide shelter for herbivores (Obermaier et al. 2008, Chen et al. 2015). In addition, plants that have a larger architecture complex have shown results of more insect herbivory in galling insects (Araújo et al. 2006).

The percentages of cannabinoids found in the plants differed between varieties. While there were no significant differences in cannabidiol (CBD) and tetrahydrocannabinol (THC), Southern Luck had the highest concentration of cannabidiol (CBD), and Belle the highest concentration of tetrahydrocannabinol (THC). Little is known about the role of CBD and THC on insect herbivory. These results may suggest that caterpillars were less repelled to this variety and higher damage was the outcome. Hemp terpenoids may have played a role against insect herbivory by repelling or deterring their attack. Aljobory and Chen (2016) stated that hemp's terpenoids have an indirect effect on insect herbivory.

Terpenoids are volatile organic compounds that can act as a repellent for herbivores, and aid in attraction of natural enemies (Punja and Ni 2021). Hemp is considered a relatively pest tolerant plant possibly due to terpenoids (Benelli et al. 2018). A study conducted by Benelli et al. (2018) showed that cannabinoid extracts can be used as a possible organic insecticide against houseflies (*Musca domestica*).

While the interaction needs to be further studied, literature suggests a well-established relationship with terpenoids repelling herbivores. A study conducted by Agliassa and Maffei (2018) tested the effect of oregano (*Origanum vulgare)* terpenoids against Egyptian cotton leafworm (*Spodoptera littoralis)*. Results showed that higher levels of oregano terpenoids decreased larval survival and growth (Agliassa and Maffei 2018). Another study showed how cotton plants with inducible terpenoids showed more resistance to herbivory compared to varieties that didn't induce these volatile compounds (Hagenbucher et al. 2013). In hemp, this relationship needs to be further studied, given that terpenoids possess antimicrobial properties which contribute to plant defense against pathogens (Hammerbacher et al. 2019).

Yield differed between varieties but was inconsistent with fresh and dry biomass and bud marketability. The most significant findings were that Belle had the lowest fresh and dry weight, and Cat Daddy had the lowest flower bud fresh weight and dry weight. Cat Daddy and BaOx had the lowest proportion of unmarketable buds and Southern Luck had the highest.

In conclusion, this study shows the need for replicated research on varietal selection for hemp growers in Alabama. Varieties with higher cannabidiol (CBD) concentrations will produce a larger profit for growers, however the chosen variety must show resilience to pest attack. In this study Southern Luck had the highest cannabidiol (CBD) levels, but also the highest numbers of unmarketable buds. Overall, BaOx may be a preferred variety for growers in Alabama given that it showed the lowest numbers of caterpillars, damage ratings, and unmarketable buds among varieties.

Conclusion

Cultural control strategies such as trap crops and varietal selection play an important role in managing corn earworms in other crops. This study highlights the challenges of establishing a successful trap crop and synchronizing trap crop growth stages in a hemp ecosystem. While the results were not significant, this strategy needs further investigation with the adoption of different crops and researching different planting dates to enhance its effectiveness.

The variety trial showed the importance of selecting the right hemp cultivar to decrease pest pressure. Several parameters were taken into consideration such as plant measurements, in which results suggest corn earworm is more attracted to larger hemp plants. This attraction may be driven by hemp terpenoids, but this relationship is poorly understood and requires further investigation.

By the end of both trials, plants were highly damaged which led to differences in yield and plant quality. Trials were plagued by environmental stressors, such as high temperatures and wildlife damage. These studies highlight the need for future research on optimizing cultural control strategies, as well as exploring the relationship between terpenoids and insect feeding in hemp.

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Tables

Table 3.1

Treatment	Mean egg numbers per 5 hemp plants ± S.E.M
UTC	0.166 ± 0.021
First Planting Date	0.166 ± 0.083
Second Planting Date	0.333 ± 0.220
Third Planting Date	0.416 ± 0.166
Fourth Planting Date	0.500 ± 0.288
P-value	0.6306

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.2

Treatment	Mean egg numbers in sweet corn ± S.E.M
First Planting Date	7.000 ± 3.082
Second Planting Date	2.500 ± 1.258
Third Planting Date	2.000 ± 0.912
Fourth Planting Date	2.250 ± 1.600
P-value	0.2479

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.3

Treatment	Egg Numbers		*P-value*
	Corn	Hemp	
UTC	0.000 ± 0.000	0.083 ± 0.083	<.0001
First Planting Date	8.250 ± 2.023	0.166 ± 0.112	<.0001
Second Planting Date	9.083 ± 2.493	0.333 ± 0.887	<.0001
Third Planting Date	7.083 ± 2.441	0.416 ± 0.193	<.0001
Fourth Planting Date	4.333 ± 1.061	0.500 ± 0.358	0.0012

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.4

Variable	Parameter Estimate	SE	Pr(>|t|)
Intercept	16.93614	5.43139	0.0035
Growth Stage	-1.47403	1.61178	0.3664
Treatment	-2.06728	2.08763	0.3285
Growth Stage x Treatment	0.27633	0.47392	0.5634

R-squared: 0.1923

P-value : 0.0459

UTC= Untreated Control

SE= Standard Error

Table 3.5

Treatment	Mean number of corn earworm per 5 hemp plants ± S.E.M
UTC	0.294 ± 0.062
First Planting Date	0.248 ± 0.049
Second Planting Date	0.228 ± 0.056
Third Planting Date	0.209 ± 0.057
Fourth Planting Date	0.200 ± 0.054
P-value	0.7725

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.6

Treatment	Mean damage per 5 hemp plants ± S.E.M [z]
UTC	0.553 ± 0.253
First Planting Date	0.546 ± 0.287
Second Planting Date	0.311 ± 0.172
Third Planting Date	0.419 ± 0.239
Fourth Planting Date	0.425 ± 0.168
P-value	0.9396

S.E.M= Standard Error of Mean

UTC= Untreated Control

[z] Scale for damage rating in hemp; 0: no damage, 1: visible damage, bud still marketable, 2: damage to 50% or less of material making bud unmarketable, 3: damage to more than 50% of material making bud unmarketable (Britt et al. 2021).

Table 3.7

Treatment	Mean fresh weight per 3 plants ± S.E.M
UTC	1354.50 ± 482.077
First Planting Date	1450.25 ± 371.786
Second Planting Date	3045.25 ± 222.802
Third Planting Date	2142.75 ± 532.139
Fourth Planting Date	2194.75 ± 388.130
P-value	0.0612

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.8

Treatment	Mean dry weight per 3 plants ± S.E.M
UTC	262.250 ± 101.661
First Planting Date	326.250 ± 64.474
Second Planting Date	527.750 ± 158.120
Third Planting Date	459.500 ± 133.443
Fourth Planting Date	562.000 ± 146.530
P-value	0.4106

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.9

Treatment	Mean bud fresh weight per 3 plants ± S.E.M
UTC	9.521 ± 1.495
First Planting Date	7.1454 ± 1.512
Second Planting Date	10.128 ± 0.843
Third Planting Date	6.517 ± 1.602
Fourth Planting Date	7.257 ± 0.981
P-value	0.2256

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.10

Treatment	Mean bud dry weight per 3 plants ± S.E.M
UTC	2.794 ± 0.470
First Planting Date	2.883 ± 0.654
Second Planting Date	3.806± 0.487
Third Planting Date	2.715 ± 0.346
Fourth Planting Date	2.422± 0.4701
P-value	0.292

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.11

Treatment	Mean proportion of unmarketable buds per 3 plants ± S.E.M
UTC	0.319 ± 0.127
First Planting Date	0.348 ± 0.099
Second Planting Date	0.305 ± 0.110
Third Planting Date	0.069 ± 0.047
Fourth Planting Date	0.236 ± 0.095
P-value	0.2931

S.E.M= Standard Error of Mean

UTC= Untreated Control

Table 3.12

Treatment	Mean height (cm) per 5 hemp plants ± S.E.M
BaOx	50.547 ± 3.330^{a}
Belle	36.745 ± 2.570^{b}
Cat Daddy	51.992 ± 2.395^{a}
Southern Luck	56.166 ± 2.375^{a}
P-value	<.0001

S.E.M= Standard Error of Mean

Means and S.E.M followed by the same letter are not significantly different.

Table 3.13

Treatment	Mean width (mm) per 5 hemp plants ± S.E.M
BaOx	13.016 ± 1.050^{a}
Belle	8.275 ± 0.811^{b}
Cat Daddy	12.416 ± 0.756^{a}
Southern Luck	10.077 ± 0.749^{ab}
P-value	0.0003

S.E.M= Standard Error of Mean

Means and S.E.M followed by the same letter are not significantly different.

Table 3.14

Treatment	Mean number corn earworm per 5 hemp plants ± S.E.M
BaOx	0.130 ± 0.033
Belle	0.160 ± 0.044
Cat Daddy	0.220 ± 0.048
Southern Luck	0.220 ± 0.051
P-value	0.3863

S.E.M= Standard Error of Mean

Table 3.15

Treatment	Mean damage per 5 hemp plants ± S.E.M
BaOx	0.350 ± 0.095
Belle	0.550 ± 0.112
Cat Daddy	0.380 ± 0.982
Southern Luck	0.440 ± 0.101
P-value	0.5248

S.E.M= Standard Error of Mean

Table 3.16

Treatment	Date of Assessment				
	11-Aug	18-Aug	26-Aug	8-Sep	15-Sep
BaOx	0.200 ± 0.091^{ab}	0.000 ± 0.000	0.000 ± 0.000^{b}	0.100 ± 0.068^{a}	0.000 ± 0.000^{a}
Belle	0.050 ± 0.050^{b}	0.000 ± 0.000	0.000 ± 0.000^{b}	0.200± 0.091^{a}	0.000 ± 0.000^{a}
Cat Daddy	0.300 ± 0.105^{ab}	0.000 ± 0.000	0.250 ± 0.099^{a}	0.100± 0.068^{a}	0.000 ± 0.000^{a}
Southern Luck	0.500 ± 0.170^{a}	0.000 ± 0.000	0.050 ± 0.050^{ab}	0.150 ± 0.081^{a}	0.083 ± 0.083^{a}
P-value	0.0457	n/a	0.0053	0.773	0.8013

S.E.M= Standard Error of Mean

Table 3.17

Treatments	Date of Assessment				
	11-Aug	18-Aug	26-Aug	8-Sep	15-Sep
BaOx	0.000 ± 0.000[a]	0.000 ± 0.000	0.000 ± 0.000[b]	0.550 ± 0.256[a]	1.200 ± 0.337[a]
Belle	0.150 ± 0.150[a]	0.000 ± 0.000	0.000 ± 0.000[b]	1.100 ± 0.289[a]	1.500 ± 0.344[a]
Cat Daddy	0.000 ± 0.000[a]	0.000 ± 0.000	0.750 ± 0.298[a]	0.400 ± 0.210[a]	0.750 ± 0.298[a]
Southern Luck	0.000 ± 0.000[a]	0.000 ± 0.000	0.200 ± 0.155[ab]	1.050 ± 0.328[a]	0.950 ± 0.285[a]
P-value	0.3976	n/a	0.0062	0.1853	0.3782

S.E.M= Standard Error of Mean

Table 3.18

Potency Analysis	Treatments				*P - Value*
	Mean ± S.E.M				
	BaOx	Belle	Cat Daddy	Southern Luck	
CBC	0.010 ± 0.007	0.050 ± 0.006	0.038 ± 0.017	0.069 ± 0.043	0.4450
CBDA	2.171 ± 0.262	5.601 ± 0.403	4.245 ± 0.944	4.917 ± 1.338	0.3594
CBGA	0.075 ± 0.013^{ab}	0.325 ± 0.401^{ab}	0.317 ± 0.053^{ab}	0.345 ± 0.088^{a}	0.0610
Other cannabinoids	0.010 ± 0.006	0.050 ± 0.600	0.038± 0.017	0.069 ± 0.031	0.3060
THCA	0.076 ± 0.008	0.179 ± 0.021	0.150 ± 0.034	0.151 ± 0.031	0.3935
Total CBD	2.164 ± 0.283	2.635 ± 0.261	4.179 ± 0.074	5.138 ± 01.646	0.3890
Total CBG	0.076 ± 0.014^{b}	0.343 ± 0.037^{ab}	0.330 ± 0.052^{ab}	0.373 ± 0.104^{a}	0.0650
Total THC	0.100 ± 0.012	0.242 ± 0.011	0.189 ± 0.048	0.223 ± 0.071	0.0441

S.E.M= Standard Error of Mean

CBC= Cannabichromene

CBDA= Cannabidiolic acid

CBGA= Cannabigerolic acid

THCA= Tetrahydrocannabinol acid

CBD= Cannabidiol

CBG= Cannabichromene

THC= Tetrahydrocannabinol

Means and S.E.M followed by the same letter are not significantly different

Table 3.19

Treatment	Mean fresh weight per 2 plants ± S.E.M
BaOx	2625.87 ± 381.815
Belle	946.17 ± 343.787
Cat Daddy	2647.14 ± 546.614
Southern Luck	1623.01 ± 590.865
P-value	0.0723

S.E.M= Standard Error of Mean

Table 3.20

Treatment	Mean dry weight per 2 plants ± S.E.M
BaOx	506.748 ± 102.277
Belle	326.020 ± 160.578
Cat Daddy	520.923± 144.018
Southern Luck	434.693 ± 217.962
P-value	0.7831

S.E.M= Standard Error of Mean

Table 3.21

Treatment	Mean bud fresh weight per 2 plants ± S.E.M
BaOx	9.264 ± 0.285
Belle	8.241 ± 2.756
Cat Daddy	5.188 ± 0.654
Southern Luck	8.217 ± 1.098
P-value	0.3096

S.E.M= Standard Error of Mean

Table 3.22

Treatment	Mean bud dry weight per 2 plants ± S.E.M
BaOx	2.847 ± 0.184
Belle	2.873 ± 1.007
Cat Daddy	1.999 ± 0.228
Southern Luck	3.097 ± 0.513
P-value	0.5868

S.E.M= Standard Error of Mean

Table 3.23

Treatment	Mean proportion of unmarketable buds per two plants ± S.E.M
BaOx	0.125 ± 0.226
Belle	0.208 ± 0.257
Cat Daddy	0.125 ± 0.226
Southern Luck	0.333 ± 0.235
P-value	0.1821

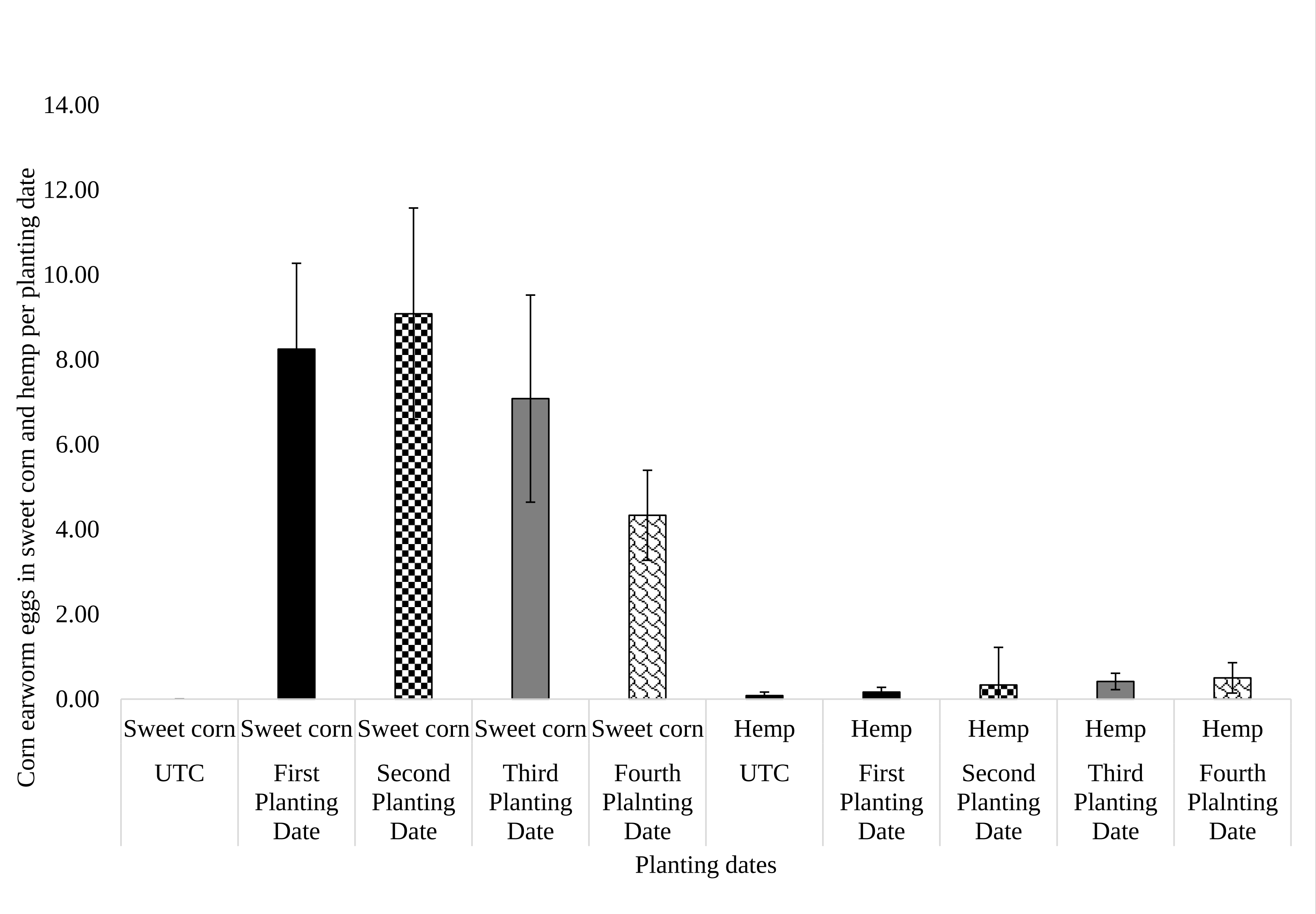
S.E.M= Standard Error of Mean

Figures

Figure 3.1

Figure 3.2

Figure 3.3



Corn earworm eggs in sweet corn and hemp per planting date
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Sweet corn UTC
Sweet corn First Planting Date
Sweet corn Second Planting Date
Sweet corn Third Planting Date
Sweet corn Fourth Plalnting Date
Hemp UTC
Hemp First Planting Date
Hemp Second Planting Date
Hemp Third Planting Date
Hemp Fourth Plalnting Date
Planting dates

Figure 3.4

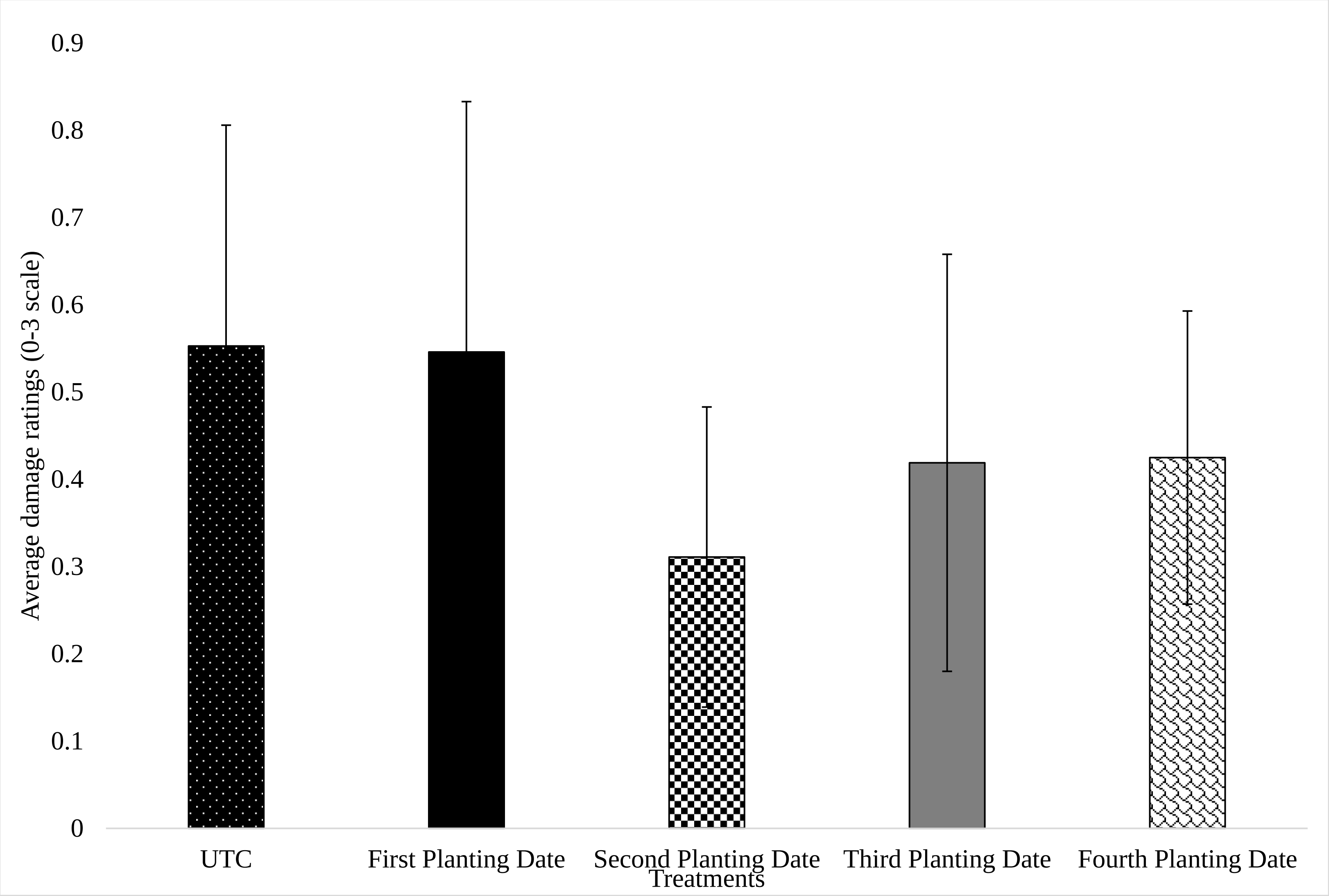

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Average damage ratings (0-3 scale)
UTC
First Planting Date
Second Planting Date
Third Planting Date
Fourth Planting Date
Treatments

Figure 3.5

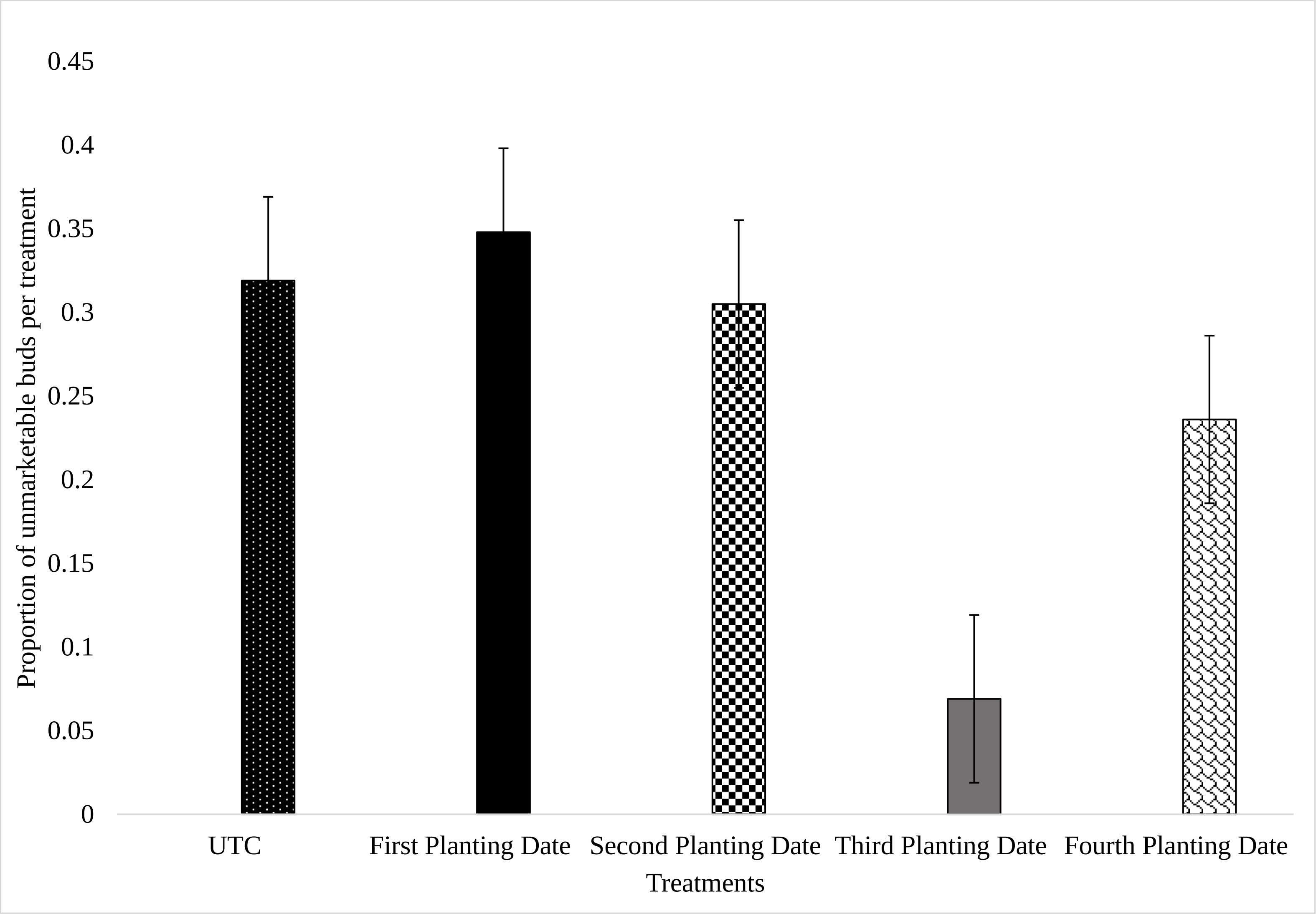
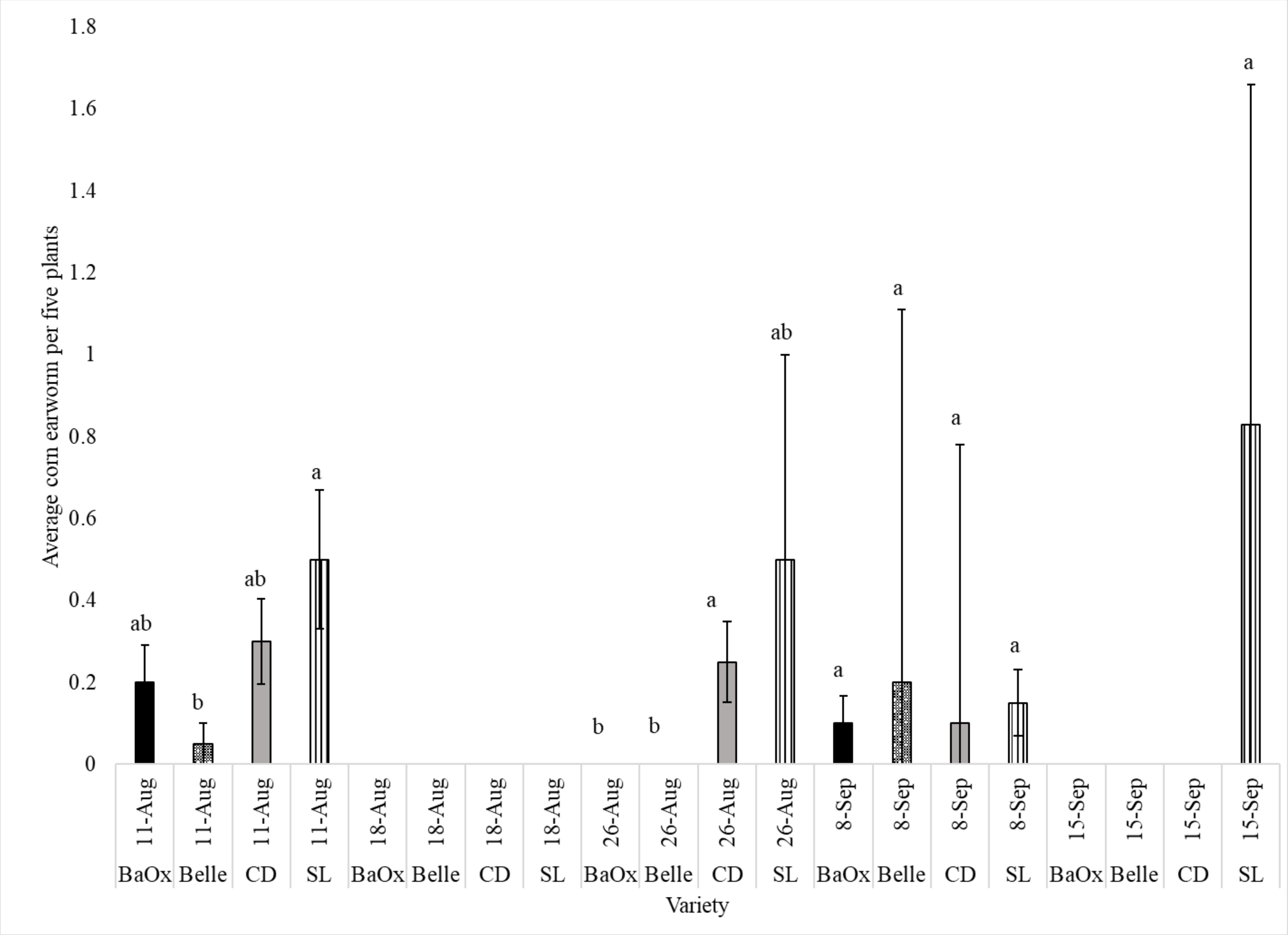

0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Proportion of unmarketable buds per treatment
UTC
First Planting Date
Second Planting Date
Third Planting Date
Fourth Planting Date
Treatments

Figure 3.6



1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Average corn earworm per five plants
ab
b
ab
a
b
b
a
ab
a
a
a
a
a
11-Aug
11-Aug
11-Aug
11-Aug
18-Aug
18-Aug
18-Aug
18-Aug
26-Aug
26-Aug
26-Aug
26-Aug
8-Sep
8-Sep
8-Sep
8-Sep
15-Sep
15-Sep
15-Sep
15-Sep
BaOx
Belle
CD
SL
BaOx
Belle
CD
SL
BaOx
Belle
CD
SL
BaOx
Belle
CD
SL
BaOx
Belle
CD
SL
Variety

Figure 3.7

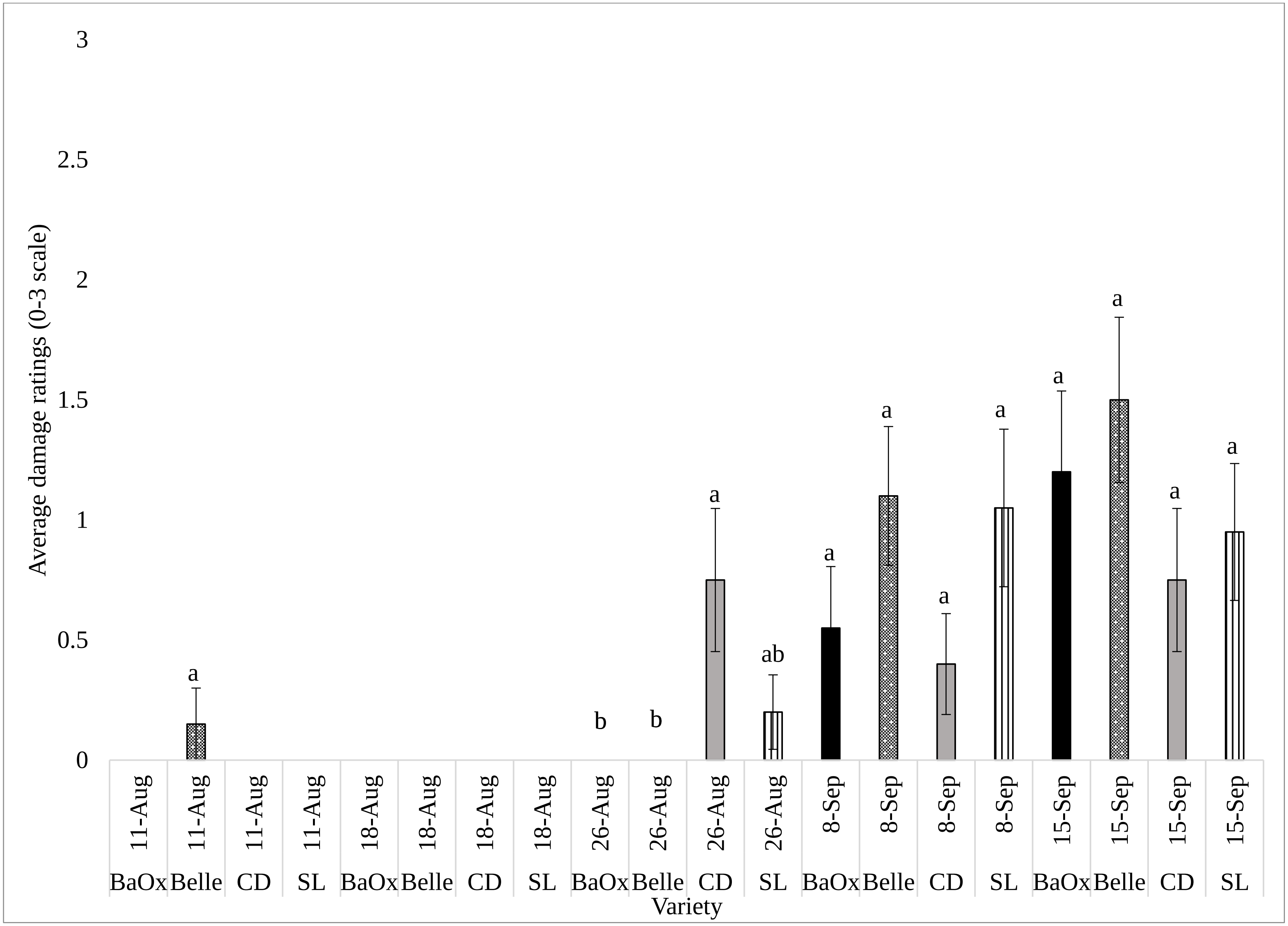
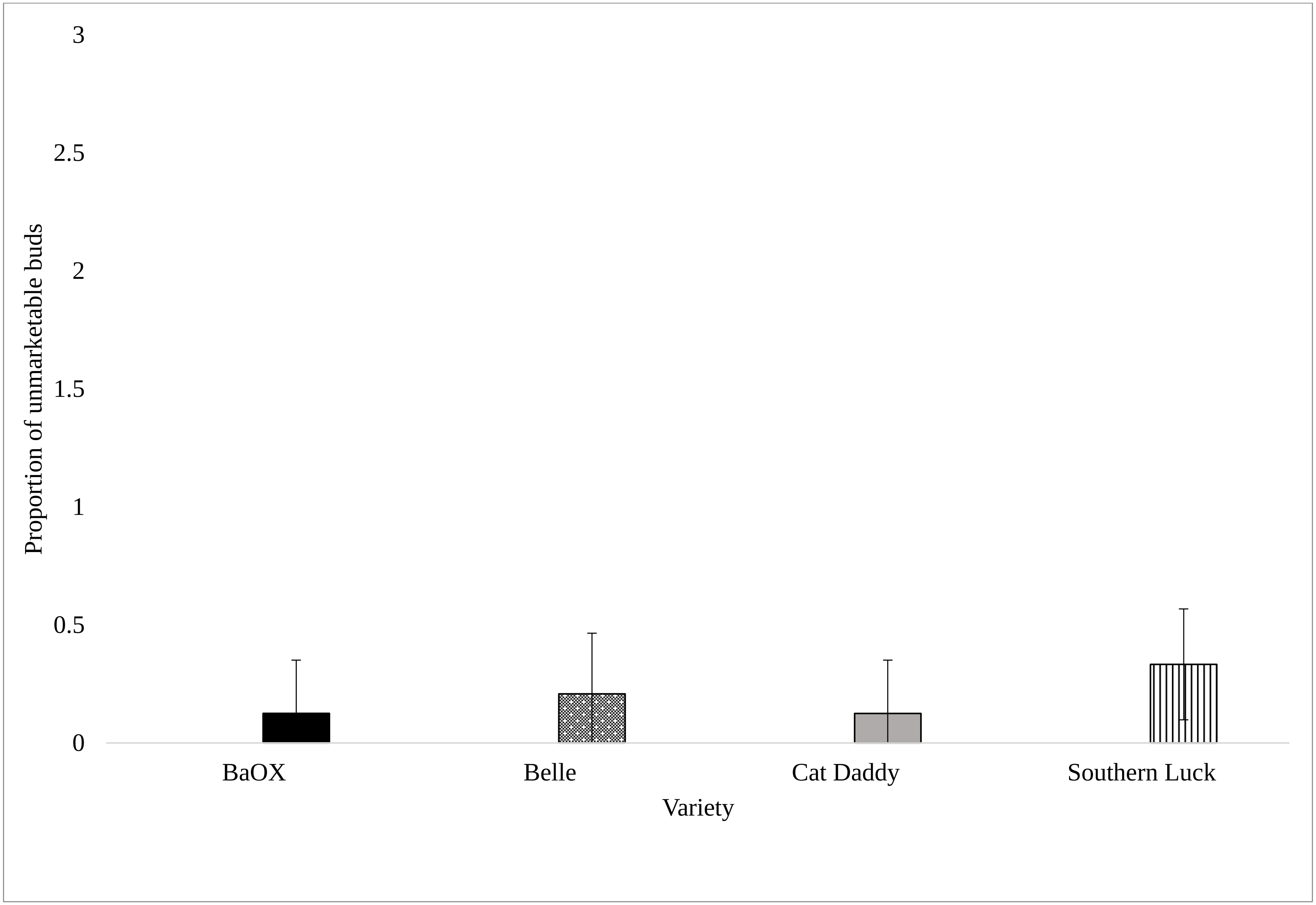

Average damage ratings (0-3 scale)
3
2.5
2
1.5
1
0.5
0
a
b
b
a
ab
a
a
a
a
a
a
a
a
11-Aug
11-Aug
11-Aug
11-Aug
18-Aug
18-Aug
18-Aug
18-Aug
26-Aug
26-Aug
26-Aug
26-Aug
8-Sep
8-Sep
8-Sep
8-Sep
15-Sep
15-Sep
15-Sep
15-Sep
BaOx
Belle
CD
SL
BaOx
Belle
CD
SL
BaOx
Belle
CD
SL
BaOx
Belle
CD
SL
BaOx
Belle
CD
SL
Variety

Figure 3.8



3
2.5
2
1.5
1
0.5
0
Proportion of unmarketable buds
BaOX
Belle
Cat Daddy
Southern Luck
Variety