

**Insects Associated with Industrial Hemp, *Cannabis* sp. L., in Tennessee and
Development of *Helicoverpa zea* (Boddie) on a Hemp-Based Diet**

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Master of Science
Degree
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DEDICATION

To my parents Curtis and Debbie Seals, thank you for always believing in me. Life can be hard, they say it's even harder when you're stupid, but it's most likely the hardest when you have no support. Both of you have always been supportive and I never could have made it this far in life without the two of you. Thank you for everything.

I also dedicate this to my lovely fiancé Brandy Golden. Along with my parents, your support over these last few years has been invaluable to my ability to accomplish my goals and succeed both in graduate school and out.

Finally, I would like to dedicate this to all my friends and family who have barely seen me since my move to Knoxville to pursue this degree

I love you all and truly appreciate everything you've done for me.

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ABSTRACT

As production of industrial cannabis, or hemp, increases throughout the United States, knowledge on the insect interactions and insect guild communities associated with hemp will be essential to understanding beneficial and pest insects that affect this crop. Licensed farmers and hectareage in Tennessee are at its highest since the 2014 legalization. For the 2019 season approximately 2,800 farmers received a license, an increase of 1,100%+ from 2018. Placing insects into guild categories, such as predator and herbivore, will allow for distinctions to be made between the various insects identified during the two-year study. An insect study was performed in Tennessee during 2017 utilizing visual surveys, beat-sheet sampling techniques and pitfall traps. A similar study was performed again in 2018, but with the omission of pitfall traps.

The goals of this research were to 1) determine guild composition and seasonality of insect species on hemp in Tennessee, 2) assess the influence of hemp variety on insect incidence, and 3) determine development and survival of *Helicoverpa zea* (corn earworm) on hemp plant material. In 2017, three varieties were planted representing a grain variety, fiber variety, and dual-purpose. For 2018, three varieties bred for cannabinoid production were planted in Crossville, TN, and sampled weekly to assess insects and their pressure. Both crops were assessed to determine the most common insects associated both among and between crops. The most abundant guild found to interact with hemp was herbivores. While insect incidence between varieties did not produce significant results, more research into different varieties may prove to show varietal resistance to certain pests. Lepidopteran larvae were observed as the most damaging pest, corn earworm larvae, a commonly observed insect, were chosen for feeding trials involving hemp. Development of corn earworm on hemp was stunted when compared to the

artificial diet, with larvae showing lower weights and higher mortality on solely hemp-based diet.

Interactions determined from this study can be used to better interpret how pest insects may affect this crop. Understanding the insect pests that need to be mitigated will lead to more robust management plans increasing the yield and quality of hemp crops.

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CHAPTER I

INTRODUCTION

Hemp: Overview of Uses Past and Present

Food, fuel, and fiber generated by agricultural production systems empower this world, providing the materials needed for modern life. Food provides nourishment, fuel powers our machines and fiber is used to produce our textiles. Hemp, or industrial cannabis (*Cannabis* spp. L.), which belongs to the family Cannabaceae, can be utilized in each of these three areas of agriculture (Small and Marcus 2002).

Hemp is a dicotyledonous annual plant originally from Southeast Asia that has now been cultivated and spread by humans for several millennia. The cannabis plant is considered a cosmopolitan species with cultivation occurring in more than 30 countries (Clarke and Merlin 2013). The most recent assessment of total hemp hectarage throughout the world is approximately 81,000 ha (Johnson 2018).

Hemp is distinguished from other varieties of *Cannabis* spp. by the low concentrations of THC, tetrahydrocannabinol. Medical/drug type varieties of *Cannabis* spp. can contain greater than 25% of THC on a dry mass weight basis. THC concentrations found in hemp are much lower, averaging between 0.02 and 0.15% THC by dry mass volume (Mechtler et al. 2014). The low levels of THC found in hemp aided in its distinction from “marijuana” cannabis varieties and helped aid in its revival in 2014 due to the government recognizing this varietal difference. Federally, hemp is defined as containing 0.3% or less THC by dry mass volume (Agriculture Act. H.R.2642 2014).

Four main types of hemp varieties are grown for industrial applications: fiber, grain, dual-purpose, and flower. Fiber varieties are generally taller, narrower plants that are ideally

harvested before hemp seeds reach maturity. Grain varieties, grown for their seed, are generally smaller, bushier plants that are harvested once the seed has begun to mature, but before the seed begins to shatter too readily. Dual-purpose varieties enable growers to capitalize on both the fiber and grain properties of hemp and are reported to grow more similar to grain varieties than fiber varieties. Flower varieties have varied growth types, typically smaller than grain but larger than fiber, and are usually grown in female only fields to prevent pollination. Varieties grown for floral material may provide a source of legal cannabinoids, and higher revenue than other hemp types. Currently in Tennessee, the majority of hemp that is being produced is grown for cannabinoid production (Walker 2018).

The stalk of a hemp plant is a source of strong, durable natural fiber similar to kenaf (Chen et al. 2007). These strong fibers make it ideal for textiles, such as ropes and clothing, biodegradable plastics, animal bedding, and non-woven composites, where it is already featured in car brands such as BMW. Another important use for hemp fibers is in building materials like hempcrete and for use in creating bio-fuels (Keller 2013). Hempcrete is a bio-aggregate building material, similar to concrete, formed from the combination of hemp hurd (the pith), lime binder, and water. The hempcrete material is low in heat conductivity, serves as an effective moisture buffer, and has a low carbon footprint (Dhakal et al. 2017). Hempcrete can be applied in several ways with molding in premade castes being the most popular method currently available to producers. These possible advantages over some building materials have led to its increased popularity. For example, the first “hemp house” was constructed in 2010 and led to widespread media coverage (Woods 2018). Availability and costs are limiting factors for hempcrete though, with cannabinoid crops, not fiber, being highest in hectareage, thus limiting the hurd necessary to create hempcrete.

As a grain crop, hemp seed is typically consumed as either a whole grain, cold pressed into an oil, or hulled stripping the outer fibrous shell leaving the consumer with only the “meat” of the seed, typically referred to as the hemp heart. Hemp grain is high in oil content with 30% of the seed being constituted of oil, and over 80% of that oil is represented by polyunsaturated fatty acids (Russo and Reggiani 2015). Polyunsaturated fatty acids are important due to their human health attributes. Polyunsaturated fatty acids are considered healthier than saturated or trans fats, and may lead to lower LDL cholesterol reducing risk for heart disease (Eckel et al. 2014). The grain is a possible feed alternative for livestock, as well as a source of human food supplements. As a livestock feed supplement, hemp grain has shown to increase fatty acids in animal meat, which may have both negative and positive outcomes (Fike 2016). For human consumption, hemp is important due to it being the only known natural source of gamma-linolenic acid, an important supplement with several reported health benefits related to the skin (Fike 2016). The grain is also a high source of protein, with 30-40% of milled seed dry matter comprised of protein. Because of this high protein content found in a plant-based source, hemp is gaining in popularity as a protein-shake supplement alternative to whey protein (Russo and Reggiani 2015). Several amino acids are required for proper human nutrition. Asparagine, threonine, serine, glutamate/glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, and tryptophan are considered essential amino acids and all are contained within hemp seed (House et al. 2010).

History of Hemp Production

Humans began to cultivate hemp several thousand years ago, possibly as early as 8,000 BCE, making it one of the first crops to be intentionally cultivated (Small and Marcus 2002). Native to Central Asia, hemp was originally grown primarily for its fiber to be used in textiles and for seed as a grain crop. This ability to be used as an early grain crop most likely aided in the initial spread of hemp from its place of origin. As early farmers of this crop harvested the plants, they accidentally disseminated the seed as they transported the plant from production fields to early processing areas. Once production of hemp began to spread around the world, it was used for several millennia as one of the main fibers used in production of ropes, clothing and paper (Clarke and Merlin 2013).

Hemp is believed to have first been introduced to North America with the English separatists via the Mayflower on their voyage to begin Colonial America. Hemp was transported as seed and fiber to the New World. The seed was stowed for planting, and the fiber was used throughout the ship for sails and rope (Will 2004). The crop was important to early travelers, with an average English ship requiring several tons of hemp for rope, with even more of the hemp fiber needed for sails. This high need for hemp led the British to distribute the seed along their travel routes, especially in the New World. By law, many of the farmers in Colonial America had to devote at least a portion of their land to hemp production, which led to a deeply entrenched respect for the crop in Colonial America. In 1632, the Virginia Assembly ordered farmers to grow hemp on at least a portion of their land or face fines levied against them (Will 2004). Drafts of the Constitution were made on hemp, and taxes could even be paid in hemp. Hemp cultivation began in New England where the puritans from Europe began their colonies but later spread to the south where it grew well enough to support an industry. Kentucky alone

had over 160 factories where thousands of individuals were employed to work with fiber varieties of hemp creating ropes and bagging (Fortenberry and Bennett 2004)

Hemp was a staple for American farmers for centuries, with Popular Mechanics publishing a paper in 1938 describing hemp as the “Billion Dollar Crop.” This economic nomenclature did not come to fruition, as the federal Marihuana Tax Act of 1937, drafted by Harry Anslinger, was passed (Ferraiolo 2007). This Act was intended to control the production and movement of “marijuana”, the narcotic variety of *Cannabis* spp. Both are varieties of *Cannabis*, but hemp does not produce heavy concentrations of the compound THC, known for its medical and narcotic attributes.

Though it was made illegal in 1937, the United States government encouraged hemp production during World War II because of the need for a strong, durable fiber to produce ropes for the military. This need led to production of a war documentary/propaganda film titled “Hemp for Victory” by the United States government in 1942. The purpose of this film was to inform growers about hemp, its uses, and tactics for production and processing. During this time, hemp hectareage grew to 59,000 ha in 1943 (Cherney and Small 2016).

Many countries followed suit with the United States in making cannabis production illegal during the early 20th century. Since being made illegal, hemp has experienced an uphill battle to once again be cultivated in the world, with China as an exception. China never banned the production of hemp and is currently the largest producer of hemp with over 20 provinces cultivating the crop. China in 2004 cultivated more than 65,000 ha of hemp and production is reported to have grown since then. The European Union (EU) allows hemp production, which has steadily grown since 2004 when total hectareage was 14,932 ha, with France producing the most (ca. 8,500 ha) (Boulloc et al. 2013).

Resurgence of Hemp Production

Hemp production in the United States has resurged following the enactment of the 2014 Farm Bill, officially titled the Agriculture Act of 2014, which contained Section 7606 or the Legitimacy of Industrial Hemp Research (Agriculture Act. H.R.2642 2014). The USDA Farm Bill allowed any farmer, upon approval by his/her state government, to produce hemp for research under a hemp pilot program. This law rejuvenated hemp on the federal level and allowed individual states to decide if they wanted to allow farmers to grow hemp. After almost a century of prohibition in the United States, hemp was once again allowed to be grown. Thus, states were allowed to develop pilot programs that allowed farmers to grow hemp as part of research trials. This law also defined hemp and set legal guidelines that distinguish hemp from “marijuana”, as well as hemp products. This research is intended to examine the viability of hemp use and marketability as a new specialty crop for American agriculture. In 2015, hemp seed was sown and cultivated legally in Tennessee for the first time since World War II.

In Tennessee, production of hemp as fiber and grain was the focus of hemp plantings in 2015 and 2016, but a shift towards CBD production occurred in 2017 and 2018 (Johnson 2018). Across the United States, many businesses have now formed with the intention of either growing, processing, and/or selling CBD-rich hemp or hemp extract that has been processed to concentrate the CBD oil and reduce the overall plant matter. This new industry has led to a drastic increase in hemp hectareage applied for within the state of Tennessee from approximately 283 ha in 2017, to over 1,618 ha in 2018 (TDA 2017, 2018).

Hemp Production, Pests and Agronomic Practices

As hemp production is investigated in the United States, and hectareage continues to grow around the world, so will the need for knowledge on cultivation of this plant. A primary concern is the need to understand the pest species that may affect its growth, survival, and yield, as well as understanding the complex of natural enemies and pollinators that inhabit the plant. Farmers, as well as processors, need more research to better prepare for production. Many agricultural crops are billion-dollar industries, with years of agronomic research supporting them. For hemp to compete monetarily with yield values of other crops, more research into better management and good agriculture practices will be needed.

Activists for hemp will regularly say that hemp is free of any pests, though this statement is false, at least for production within the state of Tennessee. Agronomic reports for hemp production provided and collected by the Tennessee Department of Agriculture show that hemp is affected by many factors such as disease, weeds, insects, and weather. In the 2017 agronomic report, insect pests were listed more than any other type of pest when it came to plant damage (TDA 2017). Though insects were listed as the number one pest, growers lack the necessary resources to manage them properly. In addition, knowledge on how insect pests affect this crop is lacking. Regarding insect pests, the insecticides commonly available in other cropping systems are not approved for use on hemp. Due to cannabis having a DEA Schedule 1 status as of November 2018, the EPA has not been able to approve pesticides labeled for hemp. With the passing of the 2018 Farm Bill, hemp has now been removed from the Controlled Substance Act (CSA). Hemp's removal from the CSA should help alleviate issues such as EPA approving pesticides for use on hemp.

Current knowledge on insect pests affecting hemp plants is limited within the southern United States. Older publications researching hemp prior to its legalization state that hemp is rarely affected by insects or diseases, and that broom rape is the most damaging pest of field-grown hemp (Dewey 1913). Other publications have stated that insect pests are insignificant, and not considered widespread issues (Kaiser et al. 2015). This information is contrary to farmer testimonials recorded within the agronomic reports collected by the Tennessee Department of Agriculture (2017, 2018) which list many insect pests affecting the crop. Literature written specifically on the pests and diseases associated with hemp lists 155 insect or mite pests found to be associated with hemp cultivation (McPartland et al. 2000). Not all of the insects listed are considered pests, though many are injurious insects that affect the health and overall yield of a crop. Aphids, whiteflies, and lepidopteran larvae are listed as some of the primary insect pests, with whiteflies typically being listed as an indoor production pest. Of the Lepidoptera pests named, *Helicoverpa armigera* (Hubner) [Old World bollworm (OWB)], and *Helicoverpa zea* (Boddie) (corn earworm) are listed as the most damaging pests to hemp crops. OWB is not listed as an insect found in Tennessee with the last observation in the United States being confirmed within Florida in 2016 based on data entry in NAPIS (National Agricultural Pest Information System). With OWB currently not listed as present within Tennessee or the southeastern United States, *H. zea* may be the primary pest to contend with when producing a hemp crop.

Research Objectives

With hemp now grown again in Tennessee, it is imperative that research focuses on this crop to provide a solid research foundation to enable growers to produce a viable, profitable crop. This research project will investigate industrial hemp and the insects that are associated

with it in Tennessee. Beneficial insects, as well as damaging pests, will be studied to develop a more complete listing of insects associated with hemp grown in Tennessee. Thus, the specific objectives of this research are to:

- 1) Determine guild composition and seasonality of insect species on hemp in Tennessee,
- 2) Assess the influence of hemp variety on insect incidence, and
- 3) Determine development and survival of *Helicoverpa zea* (corn earworm) on hemp plant material.

CHAPTER II

Incidence, Seasonality, and Guild Composition of Insect Species on Hemp in Tennessee

Introduction

Industrial hemp, *Cannabis* sp. L. (Cannabaceae), is an annual plant native to Eastern Asia that is valued for its fiber, grain, and flower. During the 1990s and early 2000s, a rejuvenated interest in hemp cultivation spread throughout the United States. This growing interest led to specific wording related to hemp being included in the 2014 Farm Bill (Agriculture Act, H.R.2642 2014). Since the 2014 Farm Bill was passed, overall production of hemp has increased each year (Johnson 2018). Most hemp grown in the United States is grown with the intent of harvesting it for its floral material. When grown as a female plant only, the hemp flowers do not become pollinated which increases the production of sticky, glandular trichomes that contain valuable cannabinoids and terpenes.

Cannabinoids, such as cannabidiol or CBD, are currently in the spotlight and constitute the largest sector of hemp production in Tennessee. Hemp production focused primarily on floral material is partially attributed to the problematic hurdles associated with production of hemp grain and fiber. For example, grain production is difficult without the use of modified harvesting equipment, which may be unavailable or expensive. The main problem with grain production is the tough, long fibers of hemp that can bind within the machine during combining and eventually rub against each other causing heat from friction (Dietz 1999). This heat buildup can cause combine fires and complete destruction of farming equipment and harvested product.

Companies are now fabricating headers that are specifically designed for hemp to mitigate the fiber harvesting equipment issues. For fiber production, the main problem is the lack of post-harvest processing. Without proper machinery, such as a decorticator, tough hemp fiber cannot be converted into a useable textile ready to be made into clothing. Most decorticators are found overseas in either Europe or Asia and are in limited supply within the United States.

As hemp cultivation increases, so will the need for entomologists to assess the insects associated with this plant. Little research has been conducted within the southern United States on hemp-insect associations. The most relevant study examining insect interactions with cannabis was performed on marijuana strains of cannabis at the University of Mississippi and dates back to the 1980s. Within this research, they found that few lepidopteran pests affected outdoor grown cannabis (Lago and Stanford 1989), and these results vastly differ from recent preliminary research conducted in Tennessee. Determining the insect guild complex of hemp and categorizing the insects that may affect hemp will allow researchers to determine where to focus their management and control efforts. To enable farmers in Tennessee to compete economically at both the state and national levels, researchers will need to identify problematic pest species and develop appropriate mitigation tactics. Not only must pests be determined, and beneficial insects identified, but their seasonality must also be determined to better formulate pest management programs. Thus, the specific objectives of this research were to determine incidence, seasonality, and guild composition of insect species on hemp in Tennessee.

Materials and Methods

Plot Design and Planting

2017

Three varieties of hemp were planted on 21 June 2017 in Pall Mall, TN, with a walk behind seeder, Earthway Precision Garden Seeder Model #10001B (Fig. 2.1). The seeder comes with many “seeding plates” (Fig. 2.2), which are round plastic wheels with grooves and holes that are used in conjunction with the seeder to plant seeds of various sizes with distinct in-row spacing. Due to the recent revival of hemp as an agricultural crop, conventional farming equipment is not typically outfitted for hemp farming and may require major modifications to be used in hemp production. The Earthway Seeder followed the usual trend and did not include a hemp-specific seeding plate nor was one available to be used with the machine. To determine if one of the plates that accompanied the seeder could be used with hemp, the seed trough on the seeder was filled with hemp seed. Each of the seed plates that could have been used with hemp was evaluated. Seed plates, such as the lettuce and carrot plates, that were clearly too small were omitted. The okra seeding plate was chosen for planting the hemp seed because it was the most consistent at lifting and then depositing the hemp seed into the ground. The hole diameter for the okra plate was ~7.0 mm and had the most ideal in-row plant spacing at ~8.9 cm.

The three varieties planted were a fiber variety (Futura 75), grain variety (Fedora 17), and dual-purpose variety (Joey). The fiber variety was planted in ~10.2 cm rows, while the grain variety was planted in ~20.3 cm rows. The dual-purpose variety was planted similar to the specifications for grain production parameters at ~20.3 cm rows. The variety Joey did not have good germination in this study; this germination was typical of this variety as other farmers

experienced poor stands across the entire state of Tennessee in 2017. Futura 75 and Fedora 17 had good germination and developed sufficient stands at the Pall Mall location.

Varieties were arranged in a completely randomized design, with three replications of each variety. Each plot was 3.0 by 9.1 m, with 1.8-m alleys between plots (Fig. 2.3). Samples were collected and/or monitored using pitfall traps, visual observations, and beat sheets. Plots were sampled from 5 July to 28 September 2017. Three randomly distributed pitfall traps were installed in each of the eight plots (24 total traps) during the second week of observations on 13 July. Pitfall traps were constructed using three cups placed within each other, forming a funnel and liquid trap to catch and preserve ground insects. A plastic cup (532.3 ml) was placed in the ground and served as a protective cup around the inner cup, and as an overflow drain. The inner cup was a white foam cup (237 ml) which had the top 3.8 cm removed to allow for a funnel to be placed within the outer cup, funneling insects into the white foam cup. The funnel was constructed of a clear plastic cup (266 ml) that had the bottom section of the cup removed from overhead creating a hole to be used as a funnel. Propylene glycol-based antifreeze was poured into the white foam cup, filling it to within 1cm of the lip, to act as a preserving agent for insects that fell into the pitfall trap. Collections of pitfall trap samples began the week of 21 July 2017. Pitfall samples were collected bi-weekly and taken to the laboratory for processing. Specimens were tentatively identified to five Orders/Groups: Coleoptera, Diptera, Hemiptera, Hymenoptera, and Other Orders.

Sampling, using visual observations and beat-sheet sampling techniques, began on 5 July 2017 and was conducted weekly. Visual observations focused on 10 randomly selected plants per plot, and each plant was examined for two minutes, for a total of 20 minutes per plot. Attempts were made to collect at least one of each unique insect observed interacting with hemp

via hand collections or by using an aspirator. Insects collected were to be used as a voucher specimen; all insects were preserved in vials containing ~70% ethanol. Once visual observations were completed for each plant, beat-sheet sampling was used to monitor for pests, such as thrips and aphids. If thrips and/or aphids were observed, plants were labeled as having Thripidae and/or Aphididae populations. Thripidae and Aphididae were both collected and stored in vials containing ~70% ethanol. Thrips populations collected from hemp in 2017 were later identified as tobacco thrips, *Frankliniella fusca* (Hinds). None of the aphids collected were the recently introduced cannabis aphid, *Phorodon cannabis* (Halbert 2016, Cranshaw et al. 2018). First confirmed in Colorado during 2016 on industrial hemp, the cannabis aphid has now been confirmed in hemp fields within Virginia (Cranshaw et al. 2018). Each beat-sheet sample was examined on a sheet (21.6 cm long by 27.9 cm wide) of white paper.

2018

Varieties used for legal cannabinoid production were grown in 2018 at the University of Tennessee AgResearch Plateau Research and Education Center near Crossville, TN. Hemp varieties grown for cannabinoids typically have high levels of CBD, usually ranging between 10-15% CBD by dry mass volume. Three hemp varieties (Franklin, Stout, and Sweet) were planted on 22 June 2018. None of the three varieties had been produced previously in Tennessee and were selected because many farmers in Tennessee planned to grow these varieties in 2018.

The plot design in 2018 consisted of a randomized block design with 12 rows planted beside each other in four, three variety blocks (Fig. 2.4). Thus, each variety was replicated four times. Within each plot, 10 plants were spaced 1.2 m apart with plots separated by a 1.8 m buffer zone to reduce varietal overlap. White, pink, and orange flags were used to separate the three

varieties. Hemp was planted on 22 June 2018, and sampling was conducted from 3 July to 29 September 2018.

On each sampling date, five of the 10 plants in each plot were randomly selected using a random number generator to determine which five plants to sample. Once five plants were selected, observations were made for five minutes per plant for a total of 25 minutes per plot. Observations focused on insects, where they occurred on the plant (i.e., top, middle, or bottom third of the plant), type of damage they caused, etc. Most insects were recorded as the number observed on the plant, while some insects were simply classified as present or not present (i.e., Aphididae, Dolichopodidae, and Thripidae). Insects such as Dolichopodidae were classified as present or not due to their habits of dispersing when disturbed and then returning to plants multiple times, making it difficult to distinguish if each incidence was a new or returning organism. Thripidae and Aphididae were counted as present or not due to high population numbers.

Beat-sheet sampling was implemented to monitor for smaller insects, such as thrips or aphids. Once each visual observation was completed, beat-sheet sampling was then conducted at three different branches on each plant to determine if thrips or aphids were infesting plant material. If the first branch had both thrips and aphids, a second and third branch were not sampled. Similar to 2017, samples in 2018 were collected for 13 weeks for two varieties (Franklin and Stout) and for 12 weeks for the Sweet variety which matured faster than the other two varieties.

Results and Discussion

2017

Visual Observations

In 2017, 1,815 insects were observed on industrial hemp in Pall Mall, TN. Of those 1,815 insects, the eight most commonly observed insect orders, families, or species associated with hemp during the 13-week period were: Aphididae, Chrysomelidae, Cicadellidae, Coccinellidae, Lepidoptera, Miridae, Pentatomidae, and Thripidae (from most abundant to least abundant) (Fig. 2.5, Fig. 2.6) (Table 2.1). Tarnished plant bug (TPB) (n=394), *Lygus lineolaris* (Palisot de Beauvois), was the most commonly observed Miridae, as well as the most abundant insect. TPB was observed in highest abundance (n=111) during week 5 (2 Aug. 2017) of the growing season. Larvae and adults of various Coccinellidae species were observed to feed on insects affecting hemp late in the season but were not found to associate with hemp before week 10 (8 Sept. 2017). Their rapid population increases later in the season is most likely associated with the increase of populations of their prey (i.e., Aphididae), which peaked during week 11 (15 Sept 2017). At that time, aphid populations were found on 69 of the 80 plants sampled (~86% of all hemp plants sampled).

Lepidopteran pests found on hemp peaked (n=71) during week 7 (15 Aug. 2017), but were found throughout the entire season. Populations of Thripidae peaked during week 6 (8 Aug. 2017) with 38 of 80 plants (ca. 48%) infested with thrips. Densities of Cicadellidae similarly peaked (n=23) during week 7 (15 Aug. 2017), then declined until the end of the sampling period. Members of Pentatomidae were most abundant during week 11 (15 Sept. 2017), similar to aphids. Periods with the highest abundance of insects were weeks 13 (29 Sept. 2017) with the

highest, next highest for week 12 (22 Sept. 2017), and third highest during week 8 (24 Aug. 2017) .

Pitfall Samples

Pitfall traps collected ~6,000 insects across a 13-week sampling period with six trap collections. Of five main groups: Coleoptera, Diptera, Hemiptera, Hymenoptera, and Other Orders, the ‘Other’ group had the highest number of organisms collected (n=1,477) across the five groups. Diptera was the second most abundant (n=1,363) group, followed by Hymenoptera (n=1,283), Hemiptera (n=968), and Coleoptera (n=909) (Table 2.2). Coleoptera was the most abundant insect order collected within the first sampling period, which correlates with visual sampling during the same timeframe. Diptera was the most prevalent insect order collected during the third week, Hemiptera during the fifth week, and the ‘Other’ group for weeks seven and nine. The ‘Other’ group was mostly comprised of Orthoptera, Collembola, and Arachnida, with Gryllidae being the most prevalent orthopteran found. Due to few Orthoptera and no Collembola observed feeding on hemp, pitfall-trap samples were used to assess these guilds associated with hemp.

The majority of Diptera collected in pitfall traps were members of the families Chloropidae and Agromyzidae. Chloropidae reportedly have a varied diet, with the majority being phytophagous, typically focusing on cereals and other grasses (Kubík and Barták 2017). Agromyzidae are referred to as leafminers based on their feeding habits and can be polyphagous consuming more than one type of plant. In 2017, hemp was planted within a large pasture field (~49 ha) that consisted of a wide variety of plants, mostly grasses. Chloropidae were not observed to interact with hemp during visual surveys, and feeding damage caused by Agromyzidae was not observed on hemp at this location in 2017. This information, coupled with

an abundant habitat for both, suggest that these species of Diptera were ‘incidental species’ in pitfall traps and not insects that are associated with hemp. Due to the high number of probable incidental insects, Diptera was not a focus of using pitfall samples to determine dipteran associations with industrial hemp. Thus, Coleoptera, Hemiptera, and Hymenoptera were the focus of guild groupings (Table 2.3).

Carabidae (n=397) was the most abundant insect family collected within Coleoptera using pitfall traps. Carabidae are generalist predators of many other insect species found within agricultural systems although two species, the seedcorn beetle (*Stenolophus lecontei* (Chaudoir)) and slender seedcorn beetle (*Clivina impressifrons* (LeConte)), are also considered pests of seed, such as corn. It was not determined if either of these pest species were found in the samples. High numbers of predators known to occur in many cropping systems may also lead to protection of hemp from some insect pests.

Chrysomelidae (n=316) was the second most abundant insect family collected in Coleoptera using pitfall traps. Of those collected, 285 (~90%) were represented by flea beetles. Flea beetles were also a major pest group found during visual sampling. Though occurring often, flea beetle damage was not considered severe.

Cicadellidae (n=336) was the most commonly collected family of Hemiptera in pitfall-trap samples, although it was not among the top five most abundant insect families found during visual observations. One possible explanation is the ability of Cicadellidae to blend into and hide amongst plant material. Many of the planthoppers and sharpshooters were pale in color or a shade of green similar to that of hemp. It is possible that Cicadellidae were overlooked during later stages of hemp growth due to their size and ability to blend with hemp plant material. They were not observed fleeing from plants prior to visual inspection. Miridae (n=245) was the second

most abundant insect family found within pitfall traps; the genus *Spanagonicus* Berg (n=212) represented ~87% of all Miridae found. Their high abundance in pitfall traps differed greatly from visual sampling, which found few *Spanagonicus* spp. feeding upon hemp material. Hemp may have been an indirect food source for these insects, whose main food source was probably another plant species within the landscape. Their capture within the pitfall traps may have occurred from them traversing between hosts that were closer to the canopy floor.

Collections of Hymenoptera in pitfall traps were similar to those of Diptera in that the most abundant family found within the order was not found to be associated with hemp. The most common family of Hymenoptera was Formicidae (n=568). Though not found to be associated with hemp during visual observations, it is possible that the ants interacted with hemp at a terrestrial level in some way. In addition, ants are known to “farm” aphids and may have aided in the increase of aphid populations during the later stages of hemp growth. Some ant species have been observed to farm subterranean root aphids (Ivens et al. 2012). With root aphids being a known pest of hemp, it is possible in this case that root aphids went unnoticed along with the ants due to their below soil habitat. The second most observed Hymenoptera group was the superfamily of parasitoids Platygastroidea (n=112). Many lepidopteran larvae found during the final weeks of this study showed signs of parasitism in the field. High collections of parasitoids were to be expected following the parasitism rates observed during visual sampling.

2018 Visual Observations

In 2018, 1,901 insects were observed on hemp throughout the growing season with the eight most common insect orders, families, or species representing 72% of observed insects

(n=1,370) (Table 2.4). Thrips represented the most abundant insect group observed (found on ca. 47% of all plants sampled; n=355 of 760 plants). However, thrips incidence was classified as occurrence data (i.e., present or not on the plant) and not as count data. Lepidoptera (n=258) was the second most observed insect group found on hemp, and Dolichopodidae (n=191) was the third most observed insect group. The next five most common insect groups were: flea beetles (Coleoptera: Chrysomelidae) represented by species within both the *Epitrix* and *Systema* genera (n=128), lady beetles (Coleoptera: Coccinellidae) represented by many species, such as *Coleomegilla maculata* and *Harmonia axyridis* (n=116), planthoppers and sharpshooters (Family Cicadellidae) (n=112), TPB (n=110), and Syrphidae (n=100).

More insects were observed in weeks 7 (15 Aug. 2018) (n=254), 8 (n=226) (22 Aug. 2018) and 11 (12 Sept. 2018) (n=228), accounting for ca. 37% of all insects observed (n=708), than in all other weeks. Of the eight most abundant insect or insect groups observed, populations of five of the eight groups peaked during weeks seven or eight. Insects that peaked during weeks seven or eight were: TPB (n=28), flea beetles (n=34), Cicadellidae (n=25), Thripidae (n=46), and Dolichopodidae (n=27). Populations of Lepidoptera (n=60) and Coccinellidae (n=20) peaked during week 12 (22 Sept. 2018) while populations of Syrphidae peaked in week 10 (n=18) (7 Sept. 2018) (Fig. 2.7, Fig 2.8).

Though aphids were not included in the most commonly observed insect groups, their high population numbers during the final weeks of hemp growth most likely contributed to the high lady beetle populations. Aphid populations were found throughout the season, but most (ca. 79%) of aphid incidence (n=64) occurred during the last four weeks. The pattern for both 2017 and 2018 illustrated a shift from heavy thrips pressure on plants to heavy aphid pressure during the final few weeks. Thrips were most abundant during weeks 7 – 10 (15 Aug. – 7 Sept. 2018).

Populations of thrips were present on ca. 23% of the plants during the last week, while aphids were present on ca. 58% of the plants sampled. This shift is possibly due to the influx of aphids following the harvest of earlier maturing crops, e.g., corn, soybeans, and tomatoes. The highest populations of corn earworms were also found towards the end of the season, possibly due to a mixture of harvesting mature crops and population increases following the movement of corn earworm into hemp fields during flowering. The hemp began to flower at approximately the fifth week of growth, which correlated with a stark increase in numbers of lepidopteran pests found on hemp.

While many pest insects seem to affect hemp and reduce yield, no other insect group thus far appears to cause the degree of damage that is associated with lepidopteran larval pests. Their feeding habits directly target the marketable portion (e.g., flowers and buds) of the cannabinoid hemp crop, and cause considerable damage to the plant even after they complete feeding. For example, the frass left behind by larvae appeared to maintain moisture within the flower/bud area after larvae completed development. This moisture facilitated rapid mold growth on the floral portions of the hemp. Hemp flowers are described as being raceme, or “a simple inflorescence (as in the lily of the valley) in which the flowers are borne on short stalks of about equal length at equal distances along an elongated axis and open in succession toward the apex” (Merriam-Webster 1785). The tight growth of the hemp flower seems to not only trap the insect frass and moisture within the buds, but also seems to act as protection for lepidopteran pests, such as the corn earworm. Even though no pesticides are currently labeled for use on hemp, the protection offered by the physiology and morphology of the hemp floral material may shield pests from pesticide spray applications if chemical options become available.

Guild Composition of Insects Associated with Hemp

The guild categories used for this study are herbivores, predators, pollinators, parasitoids, and incidentals (Table 2.5). Considering the insects most commonly encountered during visual surveys, the most abundant insect identified was tarnished plant bug (TPB). Though not a defoliator, TPB is most certainly a phytophagous insect and belongs inside the herbivore category. This herbivore guild, which had the highest abundance throughout the season, should be investigated further. The type and degree of damage due to feeding by TPB and other mirid plant bugs need to be investigated thoroughly in future studies.

The second insect family in highest abundance is primarily represented by predators. The Coccinellidae family, consisting of lady beetles, was the second most prevalent insect group. In 2017, coccinellids represented the only predator family to be found within the top 10 most abundant insects for visual surveys. Other herbivore pests include defoliators and Pentatomidae (sap feeders) and while some stink bugs are predators, none was observed on the hemp crop. Thus, Pentatomidae is only included in the herbivore guild.

Insects found within pitfall samples represented a larger array of guild classifications. For guild classifications the three orders chosen for placement were Coleoptera, Hemiptera, and Hymenoptera. For Coleoptera, the main family recovered within the pitfall traps was Carabidae, which contains members that are primarily ground-dwelling predators of many pests that may be at the terrestrial level of the crop. The second and third most prevalent beetle families were herbivores, Chrysomelidae and Staphylinidae. The Hemiptera were represented by all herbivores. Though Reduviidae were witnessed during visual observations, none was found in great enough numbers within pitfall samples to constitute placement within the top insects recovered. Formicidae is listed as an incidental due to the lack of plant insect interaction

observed with hemp. Though they were not witnessed on the plant in high numbers, they represented the majority of all insects found within pitfall traps (n=568). They are listed as incidentals due to their lack of obvious interaction with the plant. The second most prevalent insect group found were Phygadeuonidae, a superfamily of parasitoids. While caterpillar populations were high and destructive, if parasitoids could be managed and promoted, it may be possible to maintain lepidopteran larvae at tolerable levels. The third most prevalent Hymenoptera, Halictidae, is a family of pollinators but due to hemp being wind pollinated, they may serve little purpose to the plant. It is possible that hemp may benefit when halictids visit, but further research is needed to conclude if that is accurate.

Summary

Industrial hemp is a multi-use plant with the potential to be used as a fiber, grain, and cannabinoid crop. The fiber it produces is renowned for its strength, and the grain it produces contains many beneficial nutrients previously discussed in Chapter I. The speed of growth of this crop is currently limited by lack of hemp fiber and grain processors within the United States. As a cannabinoid crop it is grown for its legal cannabinoids, with the primary focus currently on cannabidiol or CBD. The cannabinoid CBD is used as a nutraceutical to treat a wide array of ailments, such as inflammation, seizures, anxiety, and insomnia (Costa et al. 2007) (Devinsky et al. 2014). In 2018 the first FDA-approved drug, Epidiolex created by GW Pharmaceuticals using the active ingredient of CBD, was approved for prescription use, spurring growth into the production of CBD hemp crops.

To aid farmers in maximizing yield potentials, all three types of hemp production were produced in Tennessee, and insects associated with each cropping system were observed,

collected, documented, and evaluated. In 2017, fiber and grain crops were produced, and visual and beat-sheet sampling methods were used to determine insects associated with the plant. In conjunction with these two sampling techniques, pitfall traps were used to determine insects that were moving through the systems below the canopy level. Insects from pitfall samples were taken to the laboratory for processing and divided into five main groups: Coleoptera, Diptera, Hemiptera, Hymenoptera, and Other Orders. For 2018, visual sampling and beat-sheet sampling methods were used to determine insects associated with hemp grown for legal cannabinoid production. Heavy populations of lepidopteran pests, as well as members of Thripidae, Aphididae, and Coccinellidae, were present in both years.

The misnomer that hemp is the “crop free of any pests!” is a regularly mentioned, but false statement. While damage did not seem overly severe in the fiber or grain crops, damage did occur, and quantifying it will be necessary to develop economic thresholds and injury levels. The cannabinoid crops did incur severe insect damage. The type of damage caused by lepidopteran larvae is problematic for large scale cannabinoid production hectarage. Without the availability of registered insecticides to reduce pest populations (although some biologicals have a vague enough label that makes their use debatable), production of large amount of hemp may result in severe losses. Though most likely still profitable, cannabinoid varieties are not inexpensive to plant, and these upfront expenses may be a massive, devastating loss if the crop is lost due to pest pressure. As of 2018, the expense of planting a cannabinoid field can cost as high as \$10,000, or more, per hectare based on the cost of plants alone, not including resources or labor. With high planting costs coupled with intensive labor needs throughout the season, and a lack of registered pesticides, caution is advised when planning large hectarage to avoid heavy fiscal losses related to crop losses.

CHAPTER III

Influence of Hemp Variety on Insect Incidence

Introduction

Industrial hemp has been bred to serve many uses, with many phenotypes produced to meet the needs of the end product. Fiber varieties are tall and slender, and grain varieties are shorter but typically still slender, both usually with a single apical branch comprising the main stalk. Varieties grown for their floral production are typically shorter than a fiber variety with several apical stems, as opposed to the single terminal branch, producing a bushier plant with many main branches referred to as “colas”. The breadth of differences observed among these hemp varieties in phenotypes and chemotypes have great potential to affect insects associated with this plant.

The value in fiber varieties reside in their ability to grow tall and straight, producing long bast fibers to be used in textiles. Boring and defoliating insects may pose the greatest threat to varieties grown for fiber. Insects that bore into the stalk will be direct pests, affecting the length of the fiber that can be used as well as possibly contributing to the death of the plant. Many insect borers have been found to affect hemp, with invasive pests, such as the Eurasian hemp moth, *Grapholita delineana* Walker (Lepidoptera: Tortricidae), already causing problems in areas where it has been detected (Miller 1982). Defoliators may also reduce the yield of fiber crops by consuming foliage, limiting the photosynthetic ability of the plant.

Similar to fiber varieties, grain varieties will also most likely be affected by defoliating and insect borers, but these insects may not be as troublesome as grain and sap-feeding insects. While insect borers that affect grain varieties should have similar issues to fiber varieties, the

grain types may not have stems that are as attractive to insect borers due to their short growth and lack of hurd or pith compared to fiber varieties. Defoliators, grain, and sap-feeding insects may pose the greatest risk to grain varieties. Defoliators limit photosynthesis needed to produce the high-energy-dependent seeds and may reduce grain yields indirectly, but grain feeders may affect the grain directly by consuming developing seed (Fig. 3.1). Research is needed to better understand insect associations with hemp, between cultivar types such as grain and fiber, but also among varieties within each production system. Thus, the second objective of this research is to assess the influence of hemp variety on insect incidence.

Materials and Methods

As discussed in Chapter II, three varieties were planted in Pall Mall, Tennessee on 21 June 2017. A grain variety (Fedora 17), a fibrous variety (Futura 75), and a dual-purpose variety (Joey). Two of the three varieties were evaluated (Fedora 17 and Futura 75); however, the Joey variety failed to germinate sufficiently to be used for research purposes. Plot layout and sampling methods were provided in Chapter II. Visual observations coupled with beat-sheet sampling were administered weekly and pitfall samples were collected and analyzed on a biweekly basis. Once an observation was completed for each plant, beat-sheet sampling was conducted to assess the presence and density of various insects. Collected insects were identified to family or lowest taxa possible and recorded. Thrips and aphids were recorded as occurrence data and not count data similar to Dolichopodidae mentioned earlier in Chapter II. Following in-field collections of pitfall-traps, the samples were taken to the laboratory for processing. An initial screening was administered to remove insects from the anti-freeze and mud slurry, separating them into five main groups. The categories selected were Coleoptera, Diptera, Hemiptera, Hymenoptera, and

Other Orders. Insects placed within the “Other” category included insects in orders such as Orthoptera, Lepidoptera, and Thripidae. Pitfall samples were separated into the five categories and then stored in screw-top Nalgene 250 ml bottles with ~70% ethanol. Insects for both visual and pitfall trap sampling methods were identified to order, with the most common insects identified to family, genera, or species (i.e., lowest taxa possible). In 2018, the methodology used was similar to that in 2017, except pitfall traps were not installed in 2018. In 2018, cannabinoid (CBD) production varieties were selected. Sweet, Franklin, and Stout had not been previously grown in the state of Tennessee but were some of the most widely planted varieties in 2018. Therefore, those three varieties were chosen for planting. The top 10 insect groups found to be associated with the three varieties were documented and assessed to determine different insect populations among the three varieties.

Results and Discussion

Data from 2017 and 2018 were analyzed separately due to separate years, separate locations, and different production type varieties grown (i.e. fiber, grain, dual-purpose, and cannabinoid). For comparison purposes, the top eight insect groups were determined and used for analyzing differences among varieties.

2017

A total of 1,815 insects were observed on hemp using visual sampling in 2017 at Pall Mall, TN with the eight most common insect orders, families, or species representing 98%) of observed insects (1,780). Of the insects observed, 67% (n=1,193) were associated with Futura 75 while 33% (n=587) were associated with Fedora 17 (Table 3.1). Tarnished plant bug (TPB) was found in greater abundance on Futura 75 crop, i.e., 58.6% of those detected (n=231) on

Futura 75, compared to 41.4% of them on Fedora 17 crop (n=163) (Table 3.1 and 3.2). The reason for the greater occurrence rate on Futura 75 could be due to the plant having larger surface area, or a higher abundance of pollen sacks appearing on the fiber variety. While grain varieties need adequate pollination for seed development, the larger size of the fiber variety may have greater numbers of pollen sacks. Pollen is nutrient rich and TPB may have been seeking the increased pollen load that could be associated with a larger plant. TPB are known to consume pollen in other crops and can be used as a marker for host plants (Jones and Allen 2012). TPB populations were not significantly different between the two varieties (Table 3.3).

Pentatomidae also had a greater incidental rate on Futura 75, with 69% (n=67) of all stink bugs being identified on this fiber variety. Similarities with pollen loads may have contributed to the higher population of stink bugs on Futura 75. Flea beetles also were found in highest number on the fiber variety, with 54% (n=67) occurring on Futura 75. As defoliators, flea beetles may have been more attracted to the variety with more foliage. Though fiber varieties are slender, during the population peak of the flea beetles in weeks 2 (13 July 2018) and 3 (21 July 2018) of the hemp's development, the fiber variety had more vegetative growth than the grain variety. This additional growth may have led to an increase in flea beetle populations.

Cicadellidae populations were similar to TPB and Pentatomidae in that they were found in greatest numbers on the fiber variety, Futura 75 (62%). Thripidae affected more fiber plants than grain (54%), and the reason for this difference may be the attractiveness of cannabinoids associated with grain varieties. Grain varieties have a larger, more resinous flower than fiber varieties. The trichomes which contribute to the resin production are quite sticky and, in some instances, may have acted similar to glue traps when the small thrips or other insects tried moving across the plants (Fig. 3.2). Aphididae may be similar due to their high incidences also

occurring on Futura 75, though the difference was not significant (Table 3.4). The aphid populations, similar to lepidopteran, increased towards the end of the season possibly due to other crops being harvested forcing insects to find refuge in hemp.

Populations of Coccinellidae, in this case predaceous lady beetles, were vastly higher on Futura 75 (98%). These high densities most likely correlated with the higher aphid populations and the cessation of Fedora 17. Coccinellidae populations peaked ($n=201$) during week 13 (Sept. 29, 2017) though they were only observed on Futura 75 during that final week due to Fedora 17 having senesced earlier. Week 12 (22 Sept. 2017) of observations saw the highest peak of aphid populations on Fedora 17, possibly contributing to the high Coccinellidae populations.

Lepidoptera populations were greatest on Futura 75 at 59.1% ($n=169$). When comparing data from peak weeks of lepidopteran pests in weeks 5-9 (2 August 2017 - 31 August 2017), 126 larvae were found on Futura 75 and 102 larvae were found on Fedora 17. With grain production highest on Fedora 17, the expectation would have been to find lepidoptera larvae in highest populations affecting the grain crop. The size of Futura 75 may have given more surface area for larvae to exist, allowing for populations to exist in greater numbers than on the shorter, smaller grain variety. It is also possible that the grain variety has been bred for some degree of resistance based on possibly palatability, or the terpene profile. Though a higher Lepidoptera population was found on Futura 75, it is not significantly different (Table 3.5). Of the three major pests found on both Fedora 17 and Futura 75, all three were found in higher abundance on Futura 75 (Fig. 3.3).

2018

The eight most commonly encountered insect groups observed to be associated with the three cannabinoid varieties were: Thripidae, Lepidoptera (larvae), Dolichopodidae, Chrysomelidae (flea beetles), Coccinellidae, Cicadellidae, Mirdiae (TPB), and Syrphidae. Of the pest insect groups observed; Thripidae and Lepidoptera were the two most commonly occurring insects for all three varieties. Each variety had a unique third most commonly observed insect, with those three being TPB, Flea Beetles, and Cicadellidae (Fig. 3.4).

The insect most commonly observed on the Franklin variety was Thripidae (Table 3.6). Franklin had highest populations of Coccinellidae when compared across all three varieties. The Franklin variety was much larger than the other two varieties causing it to have greater foliage levels and ample food for heavy defoliators like Orthoptera. Franklin's size contributed to larger stems, stalks, and branches that were also more spread apart. While larger, it was also a less bushy plant with an airier, less compact phenotypic growth pattern compared to the Stout and Sweet. This type of growth could have been more advantageous to Cicadellidae pests but may have also allowed for easier observations of the insects, boosting visual sampling results.

Similar to Franklin, the most abundant insects found on Stout were Thripidae (Table 3.7). Chrysomelidae (flea beetles) (41%) and Cicadellidae (38%) were more abundant on Stout than the two other varieties. While the growth form of Stout was more similar to that of Sweet than Franklin, certain pests such as Chrysomelidae and Cicadellidae preferred Stout. While most likely not caused by phenotypical characteristics, the difference in pest pressure may reside in chemotypical differences.

Compared across all three varieties the Sweet variety had the highest populations of TPB (48%) (n=53), Thripidae (36%)(n=124), Dolichopodidae (41%)(n=71), Coccinellidae (39%)

(n=39), and Lepidoptera (39%)(n=76) with Thripidae being the most common insect group found (Table 3.8). Of the eight most commonly found insect groups, only one (Coccinellidae) was placed under a predator guild category. This beneficial group was highest on the Sweet variety. The high levels of beneficial insects may be in response to the high number of prey, such as thrips which may have also caused high populations of Dolichopodidae and Syrphidae. The most abundant insect groups were Lepidoptera and Thripidae, though neither group appeared in significantly greater numbers between the two varieties (Table 3.9, Table 3.10).

Terpenes play a major role in cannabis production. Many varieties are chosen more for their terpene and cannabinoid profiles than for pest resistance, vigor, or many other characteristics usually desired in crops. The terpene profile for each of the varieties are provided within Fig. 3.5. The various terpene profiles may influence which insects are attracted to or deterred by hosts, possibly more so than phenotypical differences (Paluch et al. 2010). The hemp plant is aromatic with certain terpenes like pinene being a major constituent of the terpene profile of some varieties. The importance of terpenes is that many insects are attracted to or are deterred by various terpene profiles and having a variety high in a particular terpene may cause greater pest pressure than if it wasn't in high abundance (Zhang et al. 2009, Loughrin et al. 1994). Breeding for a particular terpene profile may actually lead to development of a variety that deters or repels certain pest insects. More research into terpene profiles in relation to insect pressure, whether beneficial or pest, needs to be conducted to better understand the effects of terpenes on insect incidence.

Summary

Many uses for industrial hemp exist with new varieties being developed for each use. Fiber varieties grow tall and slender to produce more, longer fibers per hectare. Grain varieties grow shorter and focus on floral development aiding in creating larger seed heads capable of producing higher yields of grain. Cannabinoid varieties focus on floral development but are produced in female-only fields to prevent pollination, allowing for the flower to become engrossed with resinous bracts that form raceme-type flower groups. All of these different growth characteristics will most likely contribute to different pest pressures from indirect and direct feeding pests.

Defoliators were the most abundant type of insect found on hemp in Tennessee, with lepidopteran larvae considered to be the most threatening insect pest observed during both years. While lepidopteran larvae may not have been the most prevalent insect pest for each variety, their type of feeding caused the greatest damage observed in the field. Defoliation on leaves did not appear to be a major concern but feeding damage on floral material as a direct pest caused severe damage to many plants. Although this damage was observed in Tennessee, similar damage was reported by growers throughout the southern United States. The lepidopteran larvae feeding on the floral material caused not only direct damage by their consumption of the marketable portion of the plant, but frass left behind by larvae caused damage as well. Frass that was trapped within the developing bud material appeared to contain enough moisture to further damage the plant by causing rot and decay wherever larvae fed and frass was deposited. Development of methods to help in mitigation of pests, possibly through varietal trials and terpene analysis coupled with pest association, will be key to maximize hemp yields in Tennessee. The variety Franklin performed the best based on vigor and health, followed by

Sweet, then Stout. Sweet had produced colas both larger and earlier than Stout and Franklin which caused issues during the season due to mold growth, but abnormal rainfall and cloudy weather coupled with a lack of fungicides most likely compounded the mold issue. Stout had low vigor, severe leaf spotting, and was an overall less attractive plant. Several known Cicadellidae pests are carriers of major agricultural diseases (Bhowmick et al. 2016, Power 1992). With more Cicadellidae found on Stout than on the other two varieties, it is possible that Cicadellidae transferred a pathogen, which could have contributed to overall poor growth due to many Cicadellidae being known to transmit plant pathogens. Further research is needed into pathogen transmission between insects and hemp to better understand what insect-borne diseases may affect hemp.

CHAPTER IV

Development and Survival of Corn Earworm, *Helicoverpa zea* (Boddie), on Hemp

Introduction

Pests throughout the world contribute to major crop damage each year, with developing countries experiencing yield losses as high as 50%; yield losses in the United States may be as high as 25% (Oerke 2006). Insect pests are a large contributing factor to global crop losses annually. Hemp is not excluded from economic losses from pests, as it most likely suffers yield losses to multiple insect pests. It is important to evaluate the impact of major insect pests on hemp to better understand the threat they pose and how they may affect hemp growth and survival. One major pest that has been identified as a threat to hemp production in the United States is the corn earworm (CEW), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). CEW is a defoliator and pod feeder on hemp that appears to contribute heavy losses to hemp crops based on previous observations. While larvae can feed on plant material throughout plant development, major damage occurs during flowering.

During field studies in 2017 and 2018, CEW larvae were observed feeding on hemp in high abundance, with an increase in abundance as the season progressed. Varieties grown during the 2017 growing season were monoecious which allowed for the production of grain, both in the grain crop as well as fiber crop. Though not grown for grain, a fiber crop will produce grain due to its monoecious characteristics. Once plants began to develop seed, larvae consumed both leaves and calyxes that contained the grain. To better understand larval feeding habits and survivability of CEW on hemp, a feeding experiment was developed and conducted to examine

growth and survival of CEW larvae fed various hemp diets. This study will enhance our knowledge on how CEW develops on hemp, providing a better understanding of how CEW will affect this crop, and to which areas of the plant may be most affected.

Materials and Methods

To complete this feeding experiment, 1,000 CEW eggs that had been oviposited on a small white swatch of fabric was obtained from Benzon Research. Special thanks to Dr. Juan Jurat-Fuentes for assisting in obtaining CEW eggs for this study. Eggs were received in a 25 cm x 15 cm plastic bag, placed on a bench in a laboratory, and maintained at ~21C until hatching. Eggs began to hatch approximately 48 hours later. First-instar larvae were divided into two groups: 1) 40 larvae were placed on a hemp diet that consisted solely of cut hemp leaves, and 2) 60 larvae were placed on an artificial diet comprised mostly of soybean flower and wheat germ provided by Benzon Research.

Both larval groups were held on their respective diets for 3 days. After 3 days, 20 of the original 40 larvae remained on cut hemp leaves, while the remaining 20 larvae were moved to a combination of hemp leaves and hemp seed. This treatment was included to replicate the grain that larvae may consume in the field. The group consisting of 60 larvae was divided into three groups: 1) 20 larvae were left on artificial diet, 2) 20 larvae were moved from artificial diet to hemp leaves, and 3) 20 larvae were moved from artificial diet to a combination diet of hemp leaves and seed (Fig. 4.1, Fig. 4.2, Fig. 4.3). Groups were labeled as “Hemp”, “Hemp + Seed”, “Artificial Diet”, “Artificial Diet + Hemp”, and “Artificial Diet + Hemp + Seed”.

In all feeding combinations, larvae were placed individually into plastic cups (473.2 ml) covered with cheese cloth attached to the top with a rubber band to serve as a barrier to prevent

larval escape (Fig. 4.4). It was later determined that these cheese cloth-covered plastic cups were not an effective option to contain larvae. Thus, larvae were removed and transferred to clear, plastic cups (~60 ml) (Fig. 4.5). Foliage was checked daily and replaced if consumed or dry to the touch. Additional moisture was not added, because excess moisture may have increased the humidity too high within the closed cups. Larvae were held for 7 days; then weighed using a Mettler AC 100 balance; larval weights were recorded. Afterwards, larvae were weighed every 3 days until death or pupation.

Data Analysis

Larval weights, percent mortality, percent pupation, and development were all recorded every 3 days after day 7 on diet. The weights of each larvae were recorded for 22 days, then averaged across all larvae. Only live larvae were considered for averages. Data were analyzed using SAS/STAT[®] software (SAS 9.3). A Duncan's Multiple Range Test was used to determine statistical differences ($p \leq 0.05$) between larvae and among the five diets.

Results and Discussion

By day 7, larval mortality had occurred on two of the five diets tested. No larval mortality had occurred on artificial diet, artificial diet + hemp, and diet + hemp + seed by day 7. Larval mortality was highest (35%; 7 of 20 dead) on hemp + seed. Average larval weight was highest (mean = 0.0293 g) on artificial diet and lowest (0.0005 g) on the hemp (Table 4.1).

By day 10, no larvae had died on artificial diet. However, larval mortality had occurred on all other diets: hemp (38.9% mortality; 7 of 18 dead), hemp + seed (61.5% mortality; 8 of 13 dead), artificial diet + hemp (5.0% mortality; 1 of 20 dead), and artificial diet + hemp + seed (10% mortality; 2 of 20 dead). Again, average larval weight was highest (0.1379 g) on artificial diet and lowest (0.0007 g) on hemp + seed (Table 4.2).

By day 13, only 65% (65 of 100 larvae) of the larvae were still alive across all five diets. The first death of a larva on artificial diet was recorded (0.05% mortality; 1 of 20 larvae). No additional mortality of larvae on hemp was recorded while two more larvae died on hemp + seed (90% mortality; 18 of 20 dead), and three more larvae died on both the artificial diet + hemp (20% mortality; 4 of 20 dead) and on the artificial diet + hemp + seed (20% mortality; 4 of 20 dead). Average larval weight continued to be highest (0.3381 g) on artificial diet, and lowest (0.0014 g) on hemp + seed (Table 4.3).

By day 16, only 51 larvae remained alive across all five diets. Two more larvae died on hemp (55% mortality; 11 of 20 dead) and four more larvae died on the artificial diet + hemp (40% mortality; 8 of 20 dead) and the artificial diet + hemp + seed (40% mortality; 8 of 20 dead). No additional larvae died on hemp + seed or on artificial diet. Pupation was first observed on day 16; three of the larvae being fed artificial diet had pupated. Again, average larval weight

was highest (0.4254 g) on the artificial diet and lowest (0.00130 g) on the hemp + seed (Table 4.6).

By day 19, 44 larvae remained alive. No new larval deaths were found on artificial diet, artificial diet + hemp or on hemp + seed. Two larvae died on hemp (65% mortality; 13 of 20 dead) and on the artificial diet + hemp + seed (55% mortality; 11 of 20 dead). Three of the larvae fed artificial diet pupated. Average larval weight was highest (0.3570 g) on artificial diet; however, it is interesting to note that larval weight on artificial diet was highest on day 16 than on day 19. Average larval weight was lowest (0.0027 g) on the hemp, showing that grain may be an important source for nutritional needs (Table 4.4).

By day 22, only three of the original 20 larvae placed on artificial diet remained as larvae. Of the 20 larvae, one had died and 16 had either pupated or initiated pupation. No additional larval mortality was recorded on hemp or on artificial diet + hemp. One larva had died leaving only a single larva on hemp + seed (95% mortality; 19 of 20 dead) while three larvae died on the diet + hemp + seed (65% mortality, 13 of 20 dead). The final weights along with weights for previous weeks, and an average of all five diets are shown in Table 4.5.

SAS 9.3 was used to run a standard PROC ANOVA. Duncan's Multiple Range Test statistical analysis at an alpha of 0.05 was used to determine statistical differences in daily lepidopteran larvae weight among the diets (Fig. 4.6). Three distinct significant groups with an F value of 6.61 showed strong significant differences. The larva group fed an artificial diet represented one independent group separated from the other two groups. The next grouping was the 40 larvae that were fed an artificial diet for three days before being moved to the hemp leaf and hemp + seed. Larval weights and percent mortality of those that were raised on hemp for the entirety of their lives were significantly different from the other two groups that had received

artificial diet for at least a portion of their lives (Tables 4.7, 4.8). Whether the larval diets were combined with seed or not appeared to have no effect on statistical groupings. The key difference among the three groups was the presence of artificial diet incorporated into the food source at some point during larval development. Larvae fed artificial diet for the first three days of their life produced average larval weights that were more than nine times greater than their counterparts lacking artificial diet on day 22. Across the 22 days of this study, all three larval groups that were fed artificial diet for at least a portion of their life outperformed those larvae that were not fed an artificial diet as some component of their nutritional source. Average larval weight was highest for larvae that were fed an artificial diet whether as a sole food source or combined with plant materials. Mortality of larvae fed hemp + seed was highest across all five diets. Larval weights also were the lowest when fed hemp + seed through day 19, when the average weight surpassed those larvae fed only hemp.

Possible explanations for the lower weights and higher mortality observed with the seed incorporated diets could be due to a number of factors. The seed that was used would have been drier than fresh seed still found attached to the plant. This reduced moisture content within the seed may have led to it being undesirable to the lepidopteran larvae. It also may have contributed to a lower ambient moisture level within the cup, causing leaves to dry at a more rapid pace inside Petri dishes. If the seed had been treated with any pesticide that may have caused adverse effects, similar mortality results would have been expected in the diet + hemp + seed group. Another possible factor is that the seed crushed the larvae when cups were moved to assess larval weights. Due to their low weights, the seed jostling around may have damaged the larvae causing mortality. A pureed hemp seed diet fed to the larvae would remove the possibility of crushing them but would be inconsistent with what is found within the field.

An interesting observation was the occasional feeding patterns of larvae when they were placed on hemp leaves. In some cases, larvae fed on leaf tissue closest to the leaf midrib vein, eating tissue around the vein and leaving the remainder of the leaf unscathed (Fig. 4.7). The leaf midrib may have contained more optimal nutrition or moisture levels, which made it more favorable to larvae. Another possibility is that the midrib may lack trichomes, which may act as a self-defense mechanism (War et al. 2012). An issue with this theory though is the abundance of CEW larvae feeding on trichome rich floral material in the field (Fig. 4.8). If larvae can consume floral tissue late into the flowering period, they should be able to consume trichomes found on the leaf surface.

Overall, larvae fed a diet consisting of hemp underperformed on mortality and weight gain when compared to larvae fed solely an artificial diet. The nutrient profile, or lack thereof, possibly mixed with volatiles from the hemp leaf, deter feeding by CEW larvae, preventing them from consuming the entire leaf, causing them to feed instead on the leaf midrib. Though they fared poorly on just hemp leaf material, larvae within hemp fields flourished and were cited by many farmers as their greatest insect pest. Lepidopteran pests are a major contributor to crop loss in Tennessee and will need to be properly managed to avoid major yield losses.

Summary

Lepidopteran species, specifically the CEW, are pests of hemp in Tennessee and have contributed to major yield losses. To determine survival rates and development of CEW raised on a hemp diet, 100 larvae were placed on various diets composed of artificial diet, hemp leaves, hemp seeds, or both leaves and seed. Of the 100, 60 were first placed on an artificial diet for 3 days before transferring 40 of the 60 onto hemp-based diets. Of these, 20 were placed on a hemp

leaf only diet, and 20 were placed on a diet consisting of hemp leaves and seed. Of the 60, 20 remained on artificial diet, while 40 larvae were raised on a hemp-based diet after hatching with 20 of the 40 having hemp grain included into their diet. Larval weight gain was higher and mortality lower on artificial diet compared to the other four groups. The artificial diet had a single death, while all larvae raised on hemp-based diets died. Larvae fed artificial diet for at least a portion of their lives had larger weight gains compared to those larvae that had never been exposed to an artificial diet during their larval lifetime.

Further research needs to be conducted on hemp flower-based diets, which may have the necessary nutrients required for CEW development. While this experiment yielded high mortality on hemp-based diets, lepidopteran larvae thus far have been major pests of field-grown hemp. Volatiles produced by the hemp leaf may have deterred feeding by the larvae, but more research is needed. If volatiles are not produced by hemp leaf material, nutritional requirements may be lacking from the leaf. Without proper nutrition, larval mortality or lack of proper pupation may become more prevalent on hemp-fed larvae than on those fed an artificial diet. Trials with mixed artificial diet and hemp leaf may help to determine if hemp leaves produce volatiles making them undesirable to the larvae.

While this study utilized leaves, future studies should focus on floral material. Both studies will allow researchers to better understand if lepidopteran larvae can survive solely on hemp, or if an alternate host is necessary for lepidopteran larvae to develop, survive, and reproduce.

CHAPTER V

Conclusions

Hemp is a diverse crop with many industrial uses. To fill the needs of these many uses, hemp has been bred to grow in many different forms, with almost each form produced in a different manner. Fiber crops are grown in extremely tight rows, with high populations per hectare. These large populations and dense canopies could be beneficial to pest insects and plant diseases. Grain varieties are grown similar to fiber varieties, but with heavy seed production a different pest load is to be expected. Fiber varieties are likely to be impacted by borers and defoliators. While grain varieties may also be impacted from similar pests, defoliators or grain feeders may cause greater impact due to them targeting the marketable portion of the crop (i.e., grain/seed). The grain is nutrient rich, and instances have occurred where a healthy plant is actually devoid of much of the grain that would have been harvested. Insects such as lepidopteran larvae may have been the culprit, but further research into feeding habits of insects associated with grain varieties is needed.

While fiber, grain and cannabinoid varieties will have many overlapping pest species, it is likely that each will have specific organisms that affect the crop based on its intended purpose. It is unclear what type of management style will be used in the future in relation to production of hemp. Will the crop be more similar to a tomato crop, with high cash value but more intensely managed or more similar to a corn crop with a lower cash value but less intensely managed? Because cannabinoid crops currently have a high cash value, pest organisms can greatly impact the profit potential of a hemp harvest. Therefore, determining methods for mitigating pest insects associated with hemp will most likely be a major interest of producers of hemp. Many hemp

farmers in Tennessee this year quoted insects as being their greatest problem and number one contributor to yield loss.

Once approved, commercial sprays will be used to help reduce pest incidence. However, one caveat to using pesticides is the final intended use of many cannabinoid crops. Most crops are currently grown for processing into full-spectrum oils. A full spectrum oil is a concentrated oil from the hemp plant that is terpene and cannabinoid rich. Concentrating cannabinoids will also lead to a concentration of any pesticide residue still found on or within the plant. The intended use of the hemp plant is something to be weary of when deciding what to spray, when to spray, and whether chemical control is necessary. Most people use the oils as a nutraceutical, similar to daily vitamins, for a wide array of reasons. One common theme though is wellness. When a consumer is wanting to consume a product with their health and wellness in mind, the idea of it being laden with chemical pesticides is unattractive to most, if not all current users of hemp extracts and oils. The organic market for hemp will most likely be a major component due to this pesticide and extract problem.

An examination of other countries that have been producing hemp for decades, if not longer, will be key for increasing hectares of hemp efficiently. Many nuances surround this crop that have yet to be fully understood such as drying and storing of the harvested hemp. The cannabinoid production model fits tobacco well, but few barns are in working condition, causing a bottle-neck during harvest. Once plants are harvested, pest insects as well as diseases still continue to diminish yields during storage of this crop. Lepidopteran larvae have been found to feed on hanging hemp, especially if the moisture content is still high. Mold issues are often associated with high moisture. In 2018, moisture from rainfall brought on by late-season storms caused heavy losses even post-harvest. To maximize yields, a better understanding of how to dry

and store hemp once it has been harvested will be necessary with cannabinoid crops. Grain and fiber have fewer storage issues but harvesting large-scale hectarage is still an issue for these model types.

Many facets of hemp production are still being investigated. Along with production, further research is needed to understand how cannabinoids and terpenes may affect insect pest pressure within the field or greenhouse. Terpene profiles may be associated with pest deterrence or attractiveness, based on the constituents of the profile. To deter pests without chemical pesticides is a massive advantage for any crop, and for a crop such as hemp that is looked at as something akin to medicine, that may prove to be even more important.

A wealth of research opportunities reside with hemp, it's cultivation, and as an agricultural product. In 2019, growers will produce hemp for the fifth season that hemp has been produced in Tennessee since it's inaugural season in 2014, and production methods ranging from management to harvest have already seen major changes. For this crop to continue its expansion into modern day agriculture, research will need to be performed to better understand this crop. Research into planting dates, varieties, fertility, and many other facets will help increase our knowledge into this crop and play a crucial role in developing pest management plans. As more farmers begin to farm larger hectares of this crop, mitigating pests to increase yields and overall profits will be necessary for hemp to compete with other crops commonly grown throughout the range of hemp farming. Determining insect pests will allow farmers to better understand what problems will need to be mitigated allowing them to better compete on both a national and international scale.

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APPENDIX

Chapter II



Figure 2.1 The Earthway Precision Garden Seeder Model walk behind seeder used to plant the 2017 hemp research plot.



Figure 2.2 Example of round seeding plate used in conjunction with walk behind seeder.

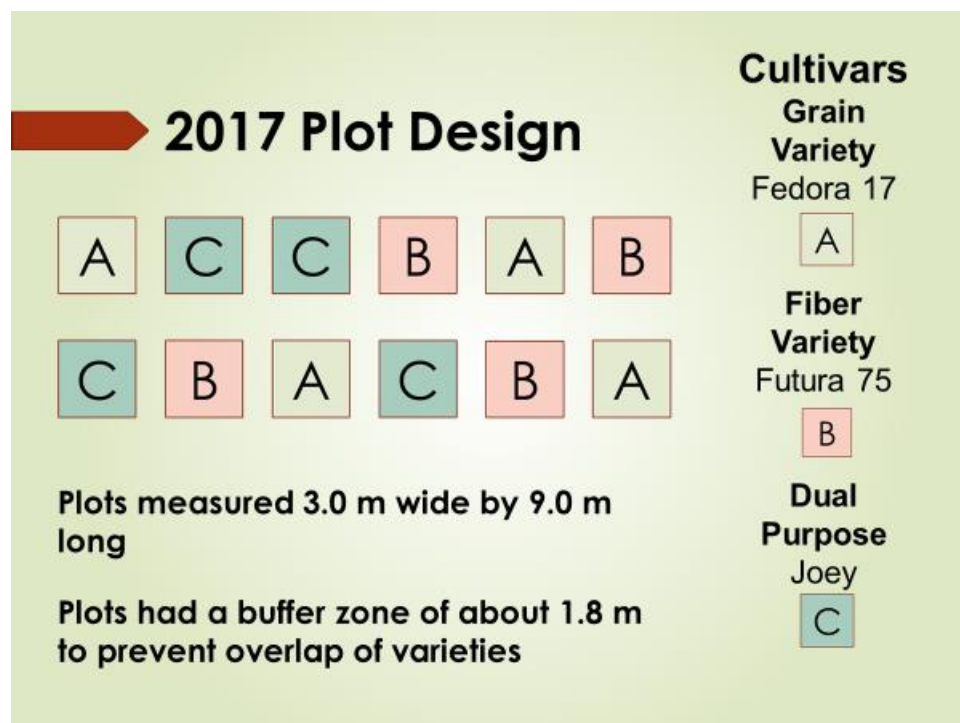


Figure 2.3 Design of 2017 research plot using three varieties: Fedora 17, Futura 75, and Joey.

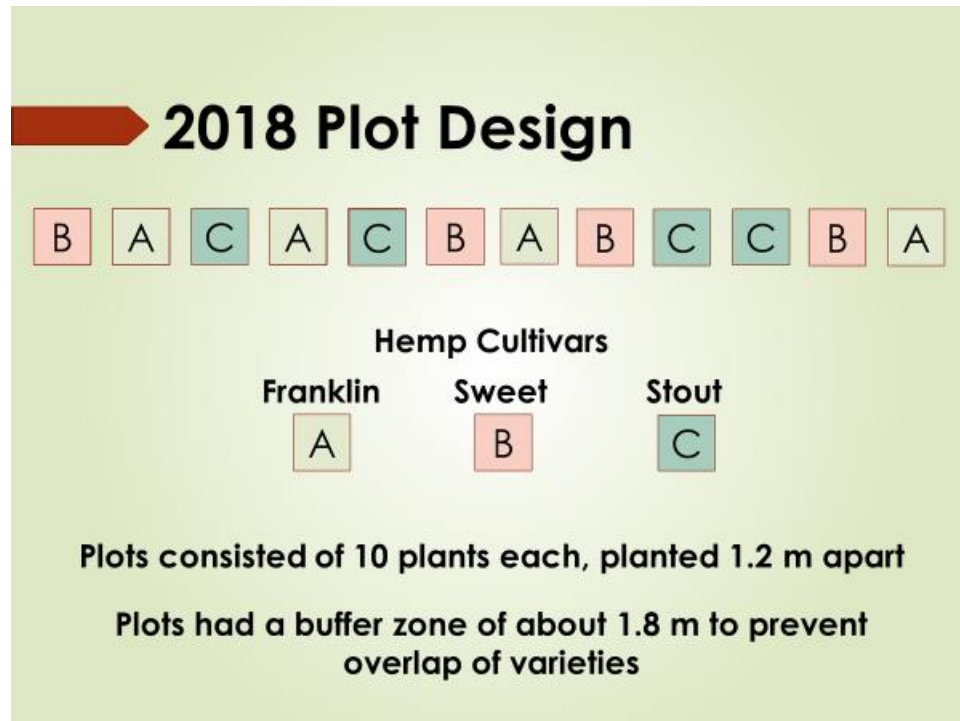


Figure 2.4 Design of 2018 research plot using three varieties: Franklin, Sweet, and Stout.

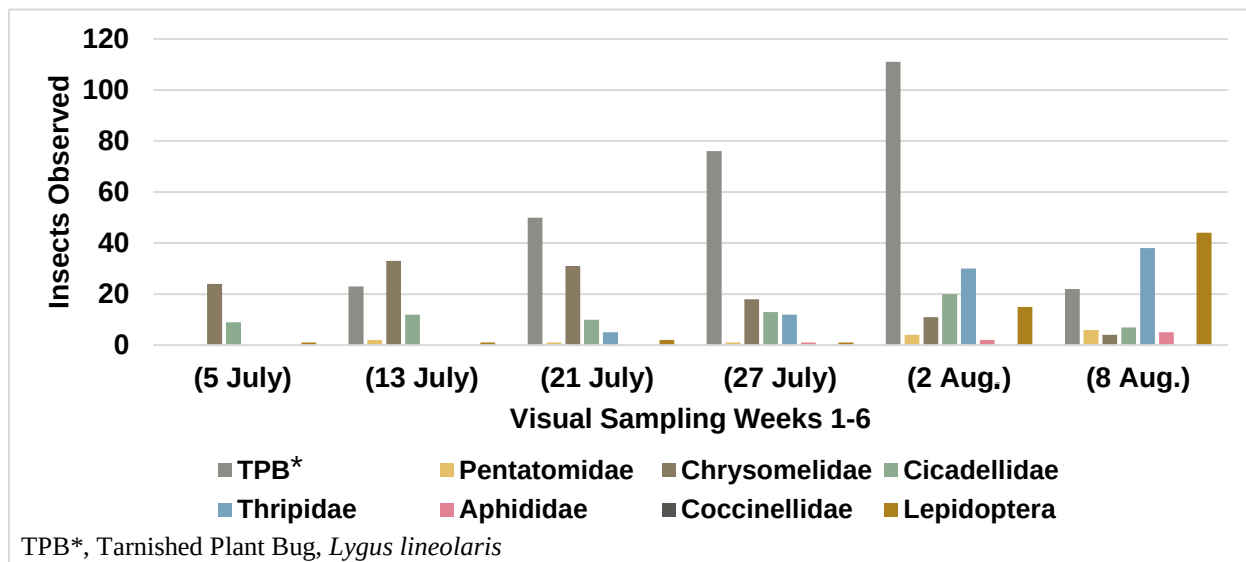


Figure 2.5 Insects observed across the varieties during visual sampling in 2017, weeks 1–6, Pall Mall, TN.

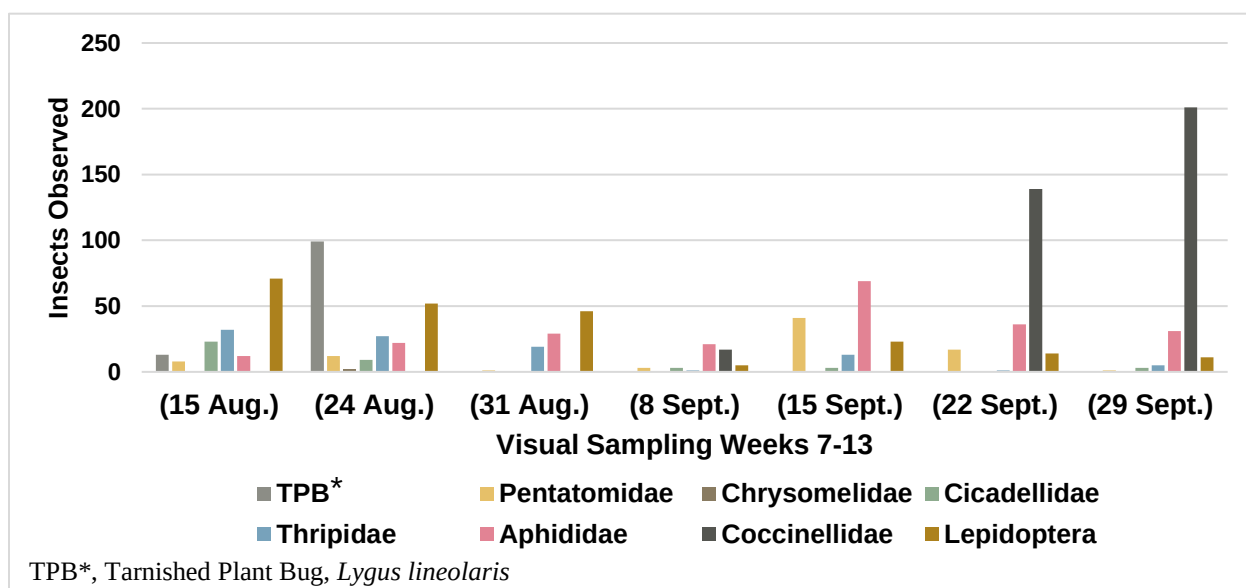


Figure 2.6 Insects observed across the varieties during visual sampling in 2017, weeks 7–13, Pall Mall, TN.

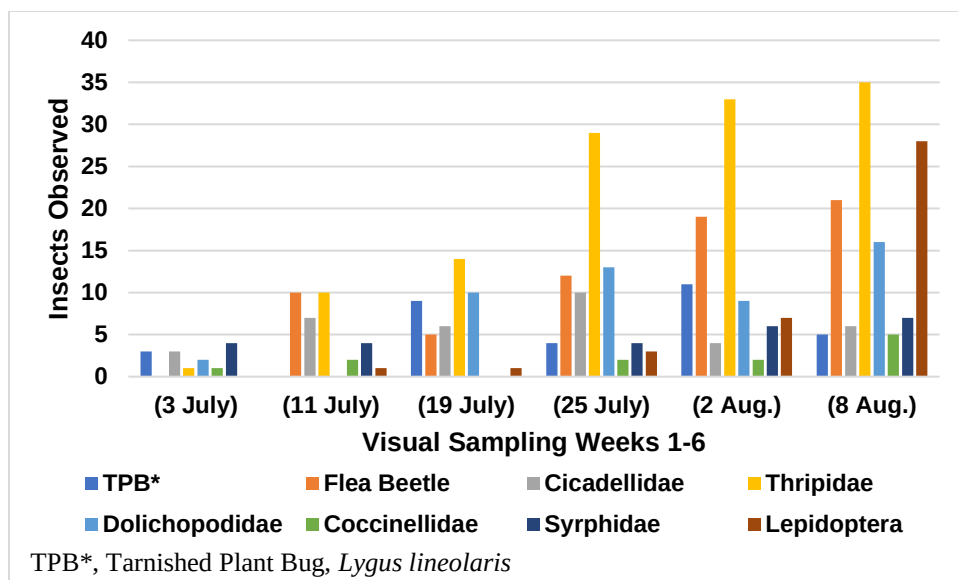


Figure 2.7 Insects observed across the varieties during visual sampling in 2018, weeks 1-6, Crossville, TN.

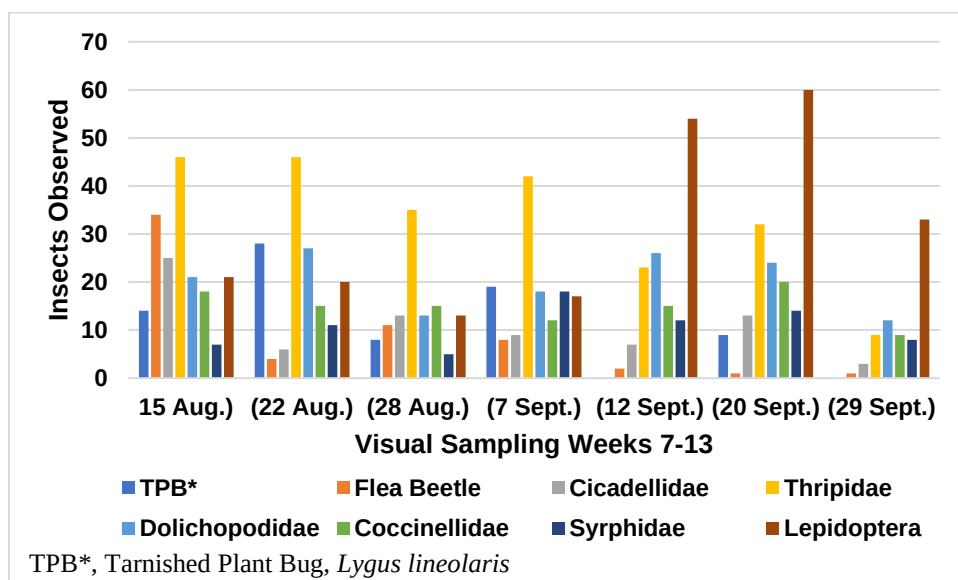


Figure 2.8 Insects observed across the varieties during visual sampling in 2018, weeks 7-13, Crossville, TN.

Table 2.1 Eight most commonly observed insect orders, families or species using visual sampling, Pall Mall, TN, 2017 (Total = 1,780).

2017 Visual Totals	TPB*	Pentatomidae	Flea Beetle	Cicadellidae	Thripidae	Aphidae	Coccinellidae	Lepidoptera
Week 1 (5 July 2017)	0	0	24	9	0	0	0	1
Week 2 (13 July 2017)	23	2	33	12	0	0	0	1
Week 3 (21 July 2017)	50	1	31	10	5	0	0	2
Week 4 (27 July 2017)	76	1	18	13	12	1	0	1
Week 5 (2 Aug. 2017)	111	4	11	20	30	2	0	15
Week 6 (8 Aug. 2017)	22	6	4	7	38	5	0	44
Week 7 (15 Aug. 2017)	13	8	0	23	32	12	0	71
Week 8 (24 Aug. 2017)	99	12	2	9	27	22	0	52
Week 9 (31 Aug. 2017)	0	1	0	0	19	29	0	46
Week 10 (8 Sept. 2017)	0	3	0	3	1	21	17	5
Week 11 (15 Sept. 2017)	0	41	0	3	13	69	0	23
Week 12 (22 Sept. 2017)	0	17	0	0	1	36	139	14
Week 13 (29 Sept. 2017)	0	1	0	3	5	31	201	11
Totals	394	97	123	112	183	228	357	286

*TPB, Tarnished Plant Bug, *Lygus lineolaris*

Table 2.2 Pitfall sampling totals collected from two hemp varieties in 2017, Pall Mall, TN.

Pitfall Order Totals						
		Coleoptera	Diptera	Hemiptera	Hymenoptera	Other*
Week 1		318	207	214	256	275
Futura 75		154	94	99	123	128
Fedora 17		164	113	115	133	147
Week 3		306	678	342	635	366
Futura 75		155	341	189	181	164
Fedora 17		151	337	153	454	202
Week 5		16	84	189	55	130
Futura 75		10	30	104	23	54
Fedora 17		6	54	85	32	76
Week 7		212	325	147	270	519
Futura 75		101	137	75	160	242
Fedora 17		111	188	72	110	277
Week 9		57	69	76	67	187
Futura 75		26	31	37	30	97
Fedora 17		18	29	35	43	73
	Totals	909	1363	968	1283	1477
*‘Other’ represents insects collected from orders not shown here.						

Table 2.3 Guild assessments for the four most commonly collected (in pitfall-traps) insect families or genera for Coleoptera, Hemiptera, and Hymenoptera.

Order	Families	Guild Classification				
		Herbivores	Predators	Pollinators	Parasitoids	Incidental
Coleoptera						
	Carabidae		397			
	Chrysomelidae	316				
	Chrysomelidae – Flea Beetle	285				
	Staphylinidae	20				
Hemiptera						
	Cicadellidae	336				
	Miridae	245				
	Miridae – <i>Spanagonicus</i> spp.	212				
	Alydidae	9				
Hymenoptera						
	Formicidae					568
	Platygastroidea				112	
	Halictidae			23		
	Ichneumonidae				20	

Table 2.4 Eight most commonly observed insect orders, families or species using visual sampling, Crossville, TN, 2018 (Total = 1,370).

	TPB*	Flea Beetle	Cicadellidae	Thripidae	Dolichopidae	Coccinelidae	Syrphidae	Lepidoptera
Week 1 (3 July 2018)	3	0	3	1	2	1	4	0
Week 2 (11 July 2018)	0	10	7	10	0	2	4	1
Week 3 (19 July 2018)	9	5	6	14	10	0	0	1
Week 4 (25 July 2018)	4	12	10	29	13	2	4	3
Week 5 (2 Aug. 2018)	11	19	4	33	9	2	6	7
Week 6 (8 Aug. 2018)	5	21	6	35	16	5	7	28
Week 7 (15 Aug. 2018)	14	34	25	46	21	18	7	21
Week 8 (22 Aug. 2018)	28	4	6	46	27	15	11	20
Week 9 (28 Aug. 2018)	8	11	13	35	13	15	5	13
Week 10 (7 Sept. 2018)	19	8	9	42	18	12	18	17
Week 11 (12 Sept. 2018)	0	2	7	23	26	15	12	54
Week 12 (20 Sept. 2018)	9	1	13	32	24	20	14	60
Week 13 (29 Sept. 2018)	0	1	3	9	12	9	8	33
Totals	110	128	112	355	191	116	100	258

*TPB, Tarnished Plant Bug, *Lygus lineolaris*

Table 2.5 Guild examples found during 2017 and 2018 visual surveys, Pall Mall and Crossville, TN, respectfully. (Shaded area represents guild presence)

Insect	Herbivores	Predators	Pollinators	Parasitoids	Incidentals
Tarnished Plant Bug					
Pentatomidae					
Mirid – Flea Beetle					
<i>Diabrotica undecimpunctata</i>					
Cercopidae					
Cicadellidae					
Lampyridae					
Orthoptera					
Thripidae					
Aphidae					
Dolichopodidae					
Coccinellidae					
Minute Pirate Bug					
Syrphid					
Membracidae					
Lepidoptera					

Chapter III



Figure 3.1 Grain eaten from seed pods formed on hemp.



Figure 3.2 Diptera caught within a hemp trichome.

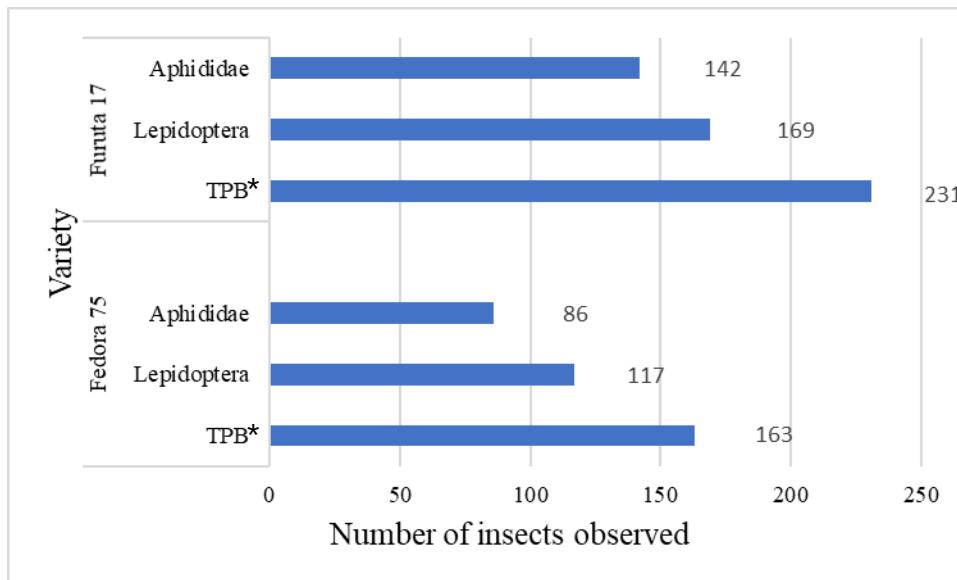


Figure 3.3 Three most commonly observed insect orders, families, or species using visual sampling surveys, Pall Mall, TN, 2017. *TPB, Tarnished Plant Bug, *Lygus lineolaris*

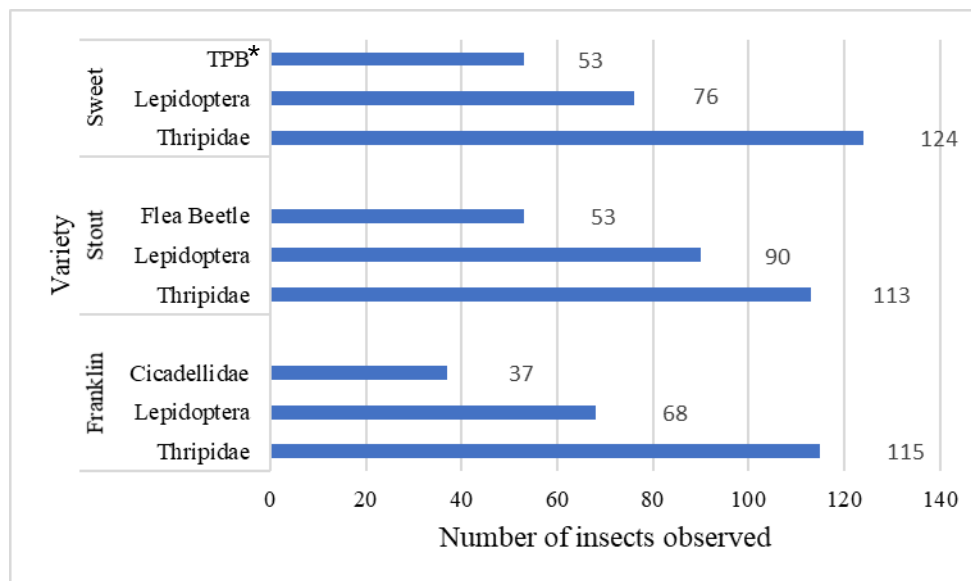
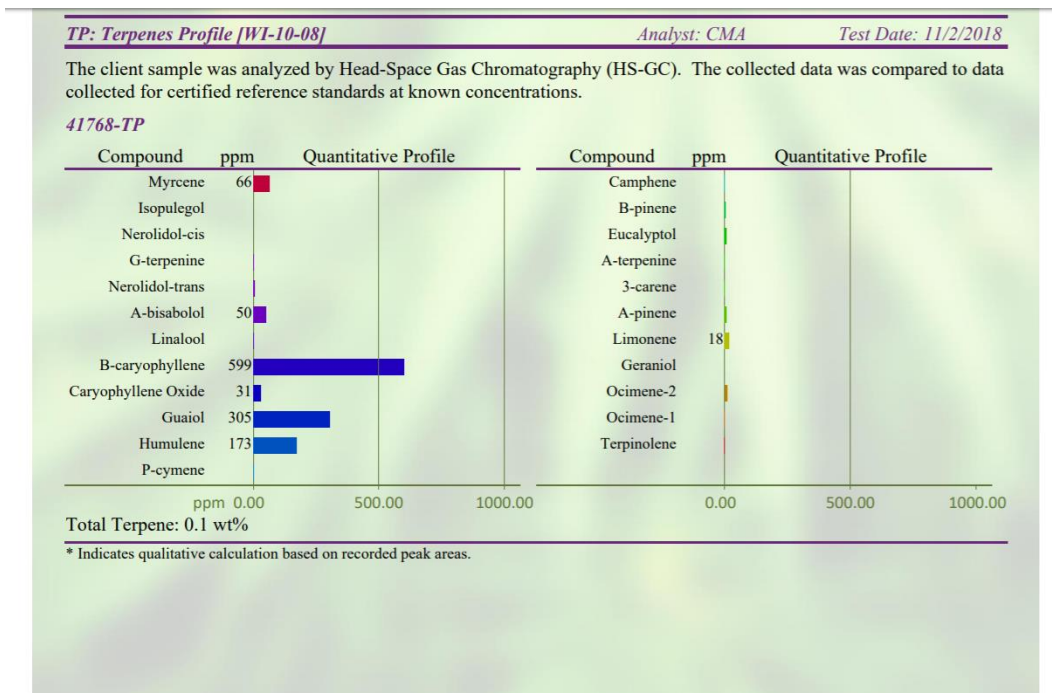


Figure 3.4 Three most commonly observed insect orders, families, or species using visual sampling surveys, Crossville, TN, 2018. *TPB, Tarnished Plant Bug, *Lygus lineolaris*.

Franklin



Stout

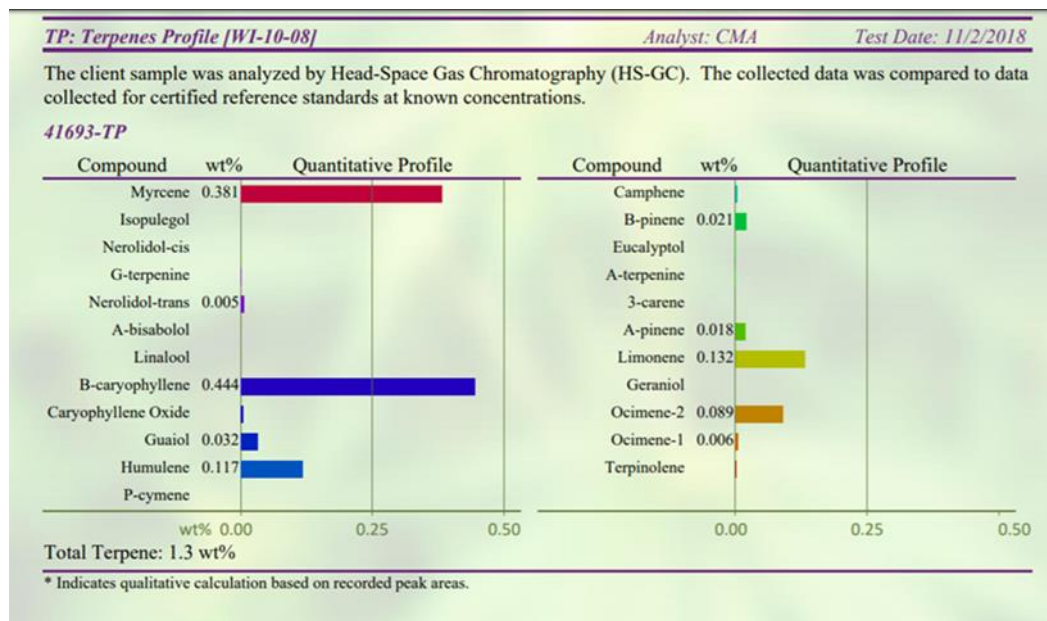


Figure 3.5 Terpene analysis performed on three varieties, A) Franklin, B) Stout, and C) Sweet, Crossville, TN, 2018.

Sweet

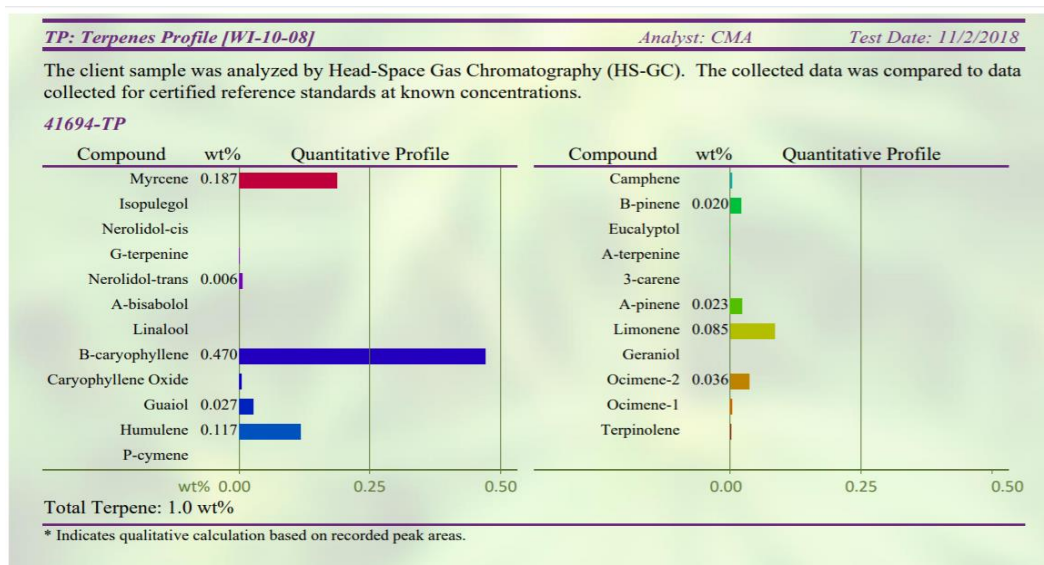


Figure 3.5 (continued)

Table 3.1 Eight most commonly observed insect orders, families or species using visual sampling on Futura 75, Pall Mall, TN, 2017 (Total = 1,193).

Futura 75 Variety	TPB*	Pentatomidae	Flea Beetle	Cicadellidae	Thripidae	Aphidae	Coccinellidae	Lepidoptera
Week 1 (5 July 2017)	0	0	12	4	0	0	0	1
Week 2 (13 July 2017)	15	1	18	8	0	0	0	1
Week 3 (21 July 2017)	31	0	17	4	3	0	0	0
Week 4 (27 July 2017)	41	0	7	9	7	0	0	1
Week 5 (2 Aug. 2017)	66	1	7	15	18	0	0	7
Week 6 (8 Aug. 2017)	11	3	4	4	18	2	0	19
Week 7 (15 Aug. 2017)	9	7	0	16	14	5	0	40
Week 8 (24 Aug. 2017)	58	9	2	5	15	14	0	31
Week 9 (31 Aug. 2017)	0	0	0	0	9	9	0	29
Week 10 (8 Sept. 2017)	0	0	0	0	0	10	10	1
Week 11 (15 Sept. 2017)	0	28	0	1	8	35	0	14
Week 12 (22 Sept. 2017)	0	17	0	0	1	36	139	14
Week 13 (29 Sept. 2017)	0	1	0	3	5	31	201	11
Totals for each week	231	67	67	69	98	142	350	169

TPB*, Tarnished Plant Bug, *Lygus lineolaris*.

Table 3.2 Eight most commonly observed insect orders, families or species using visual sampling on Fedora 17, Pall Mall, TN, 2017 (Total = 587).

Fedora 17 Variety	TPB*	Pentatomidae	Flea Beetle	Cicadellidae	Thripidae	Aphidae	Coccinellidae	Lepidoptera
Week 1 (5 July 2017)	0	0	12	5	0	0	0	0
Week 2 (13 July 2017)	8	1	15	4	0	0	0	0
Week 3 (21 July 2017)	19	1	14	6	2	0	0	2
Week 4 (27 July 2017)	35	1	11	4	5	1	0	0
Week 5 (2 Aug. 2017)	45	3	4	5	12	2	0	8
Week 6 (8 Aug. 2017)	11	3	0	3	20	3	0	25
Week 7 (15 Aug. 2017)	4	1	0	7	18	7	0	31
Week 8 (24 Aug. 2017)	41	3	0	4	12	8	0	21
Week 9 (31 Aug. 2017)	0	1	0	0	10	20	0	17
Week 10 (8 Sept. 2017)	0	3	0	3	1	11	7	4
Week 11 (15 Sept. 2017)	0	13	0	2	5	34	0	9
Week 12 (22 Sept. 2017)	0	0	0	0	0	0	0	0
Week 13 (29 Sept. 2017)	0	0	0	0	0	0	0	0
Totals	163	30	56	43	85	86	7	117

TPB*, Tarnished Plant Bug, *Lygus lineolaris*.

Table 3.3 ANOVA: Single Factor Excel output for tarnished plant bug observed during visual sampling, Pall Mall, TN, 2017.

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Fedora 17	11.00	163.00	14.82	307.76		
Futura 75	13.00	231.00	17.77	557.03		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	51.89	1.00	51.89	0.12	0.74	4.30
Within Groups	9761.94	22.00	443.72			
Total	9813.83	23.00				

Table 3.4 ANOVA: Single Factor Excel output for Aphididae observed during visual sampling, Pall Mall, TN, 2017.

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Fedora 17	11.00	86.00	7.82	113.16		
Futura 75	12.00	111.00	9.25	172.75		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	11.77	1.00	11.77	0.08	0.78	4.32
Within Groups	3031.886	21.00	144.38			
Total	3043.652	22.00				

Table 3.5 ANOVA: Single Factor Excel output for Lepidoptera observed during visual sampling, Pall Mall, TN, 2017.

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Fedora 17	11.00	117.00	10.64	123.65		
Futura 75	13.00	169.00	13	177.67		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	33.28788	1.00	33.29	0.217403	0.65	4.30
Within Groups	3368.545	22.00	153.12			

Table 3.6 Eight most commonly observed insect orders, families or species using visual sampling on Franklin, Crossville, TN, 2018 (Total = 403).

Franklin Variety	TPB*	Flea Beetle	Cicadellidae	Thripidae	Dolichopodidae	Coccinellidae	Syrphidae	Lepidoptera
Week 1 (3 July 2018)	0	0	1	0	0	0	1	0
Week 2 (11 July 2018)	0	6	2	5	0	0	1	1
Week 3 (19 July 2018)	1	1	2	5	1	0	0	0
Week 4 (25 July 2018)	2	2	2	9	7	0	1	0
Week 5 (2 Aug. 2018)	2	9	2	8	0	0	0	1
Week 6 (8 Aug. 2018)	2	4	2	9	5	1	2	5
Week 7 (15 Aug. 2018)	4	10	9	10	6	6	1	1
Week 8 (22 Aug. 2018)	2	4	4	16	7	4	3	4
Week 9 (28 Aug. 2018)	2	2	3	12	4	6	1	6
Week 10 (7 Sept. 2018)	1	4	3	13	8	4	3	5
Week 11 (12 Sept. 2018)	0	2	2	12	9	6	2	17
Week 12 (20 Sept. 2018)	6	0	5	11	8	8	6	13
Week 13 (29 Sept. 2018)	0	0	0	5	2	4	0	15
Totals	22	44	37	115	57	39	21	68

TPB*, Tarnished Plant Bug, *Lygus lineolaris*.

Table 3.7 Eight most commonly observed insect orders, families or species using visual sampling on Stout during 2018, Crossville, TN, 2018 (Total = 450).

Stout Variety	TPB*	Flea Beetle	Cicadellidae	Thripidae	Dolichopodidae	Coccinellidae	Syrphidae	Lepidoptera
Week 1 (3 July 2018)	1	0	1	0	0	0	0	0
Week 2 (11 July 2018)	0	1	4	3	0	1	0	0
Week 3 (19 July 2018)	4	3	2	5	6	0	0	0
Week 4 (25 July 2018)	0	6	3	7	1	0	0	0
Week 5 (2 Aug. 2018)	1	6	2	9	0	1	1	1
Week 6 (8 Aug. 2018)	0	15	3	11	4	1	2	7
Week 7 (15 Aug. 2018)	2	14	9	17	8	4	3	18
Week 8 (22 Aug. 2018)	12	0	0	13	7	7	3	10
Week 9 (28 Aug. 2018)	3	5	5	12	3	1	0	3
Week 10 (7 Sept. 2018)	11	2	2	14	2	3	5	5
Week 11 (12 Sept. 2018)	0	0	2	8	9	3	4	13
Week 12 (20 Sept. 2018)	1	0	7	10	5	6	3	15
Week 13 (29 Sept. 2018)	0	1	3	4	10	5	8	18
Totals	35	53	43	113	55	32	29	90

TPB*, Tarnished Plant Bug, *Lygus lineolaris*.

Table 3.8 Eight most commonly observed insect orders, families or species using visual sampling on Sweet during 2018, Crossville, TN, 2018 (Total = 467).

Sweet Variety	TPB*	Flea Beetle	Cicadellidae	Thripidae	Dolichopodidae	Coccinellidae	Syrphidae	Lepidoptera
Week 1 (3 July 2018)	2	0	1	1	2	1	3	0
Week 2 (11 July 2018)	0	3	1	2	0	1	3	0
Week 3 (19 July 2018)	4	1	2	4	3	0	0	1
Week 4 (25 July 2018)	2	4	5	13	5	2	3	3
Week 5 (2 Aug. 2018)	8	4	0	16	9	1	5	5
Week 6 (8 Aug. 2018)	3	2	1	15	7	3	3	16
Week 7 (15 Aug. 2018)	8	10	7	19	7	8	3	2
Week 8 (22 Aug. 2018)	14	0	2	17	13	4	5	6
Week 9 (28 Aug. 2018)	3	4	5	11	6	8	4	4
Week 10 (7 Sept. 2018)	7	2	4	15	8	5	10	7
Week 11 (12 Sept. 2018)	2	1	1	11	11	6	5	32
Week 12 (20 Sept. 2018)	0	0	0	0	0	0	0	0
Week 13 (29 Sept. 2018)	0	0	0	0	0	0	0	0
Totals	53	31	29	124	71	39	44	76

TPB*, Tarnished Plant Bug, *Lygus lineolaris*.

Table 3.9 ANOVA: Single Factor Excel output for Lepidoptera observed during visual sampling, Crossville, TN, 2018.

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Franklin	13.00	68.00	5.23	36.03		
Stout	13.00	90.00	6.92	50.24		
Sweet	11.00	76.00	6.91	89.49		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	23.97	2.00	11.98	0.21	0.81	3.28
Within Groups	1930.14	34.00	56.77			
Total	1954.11	36.00				

Table 3.10 ANOVA: Single Factor Excel output for Thripidae observed during visual sampling, Crossville, TN, 2018.

SUMMARY						
<i>Groups</i>	<i>Count</i>	<i>Sum</i>	<i>Average</i>	<i>Variance</i>		
Franklin	13.00	115.00	8.85	18.14		
Stout	13.00	113.00	8.69	23.40		
Sweet	11.00	124.00	11.27	39.02		
ANOVA						
<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	48.60	2.00	24.30	0.93	0.40	3.28
Within Groups	888.64	34.00	26.14			
Total	937.24	36.00				

Chapter IV



Figure 4.1 Diet consisting of hemp leaves and hemp seed fed to larvae (Hemp + Seed).



Figure 4.2 Diet consisting of only hemp leaves fed to larvae (Hemp).



Figure 4.3 Artificial diet fed to larvae (Artificial Diet).



Figure 4.4 Plastic cups covered with cheese cloth and secured with a rubber band.



Figure 4.5 Modified experiment using clear plastic cups (60 ml) that held larvae.

The SAS System

The ANOVA Procedure

Dependent Variable: caterpillar

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00203626	0.00067875	6.61	0.0028
Error	20	0.00205420	0.00010271		
Corrected Total	23	0.00409046			

R-Square	Coeff Var	Root MSE	caterpillar Mean
0.497806	83.51263	0.010135	0.012135

Source	DF	Anova SS	Mean Square	F Value	Pr > F
diet	3	0.00203626	0.00067875	6.61	0.0028

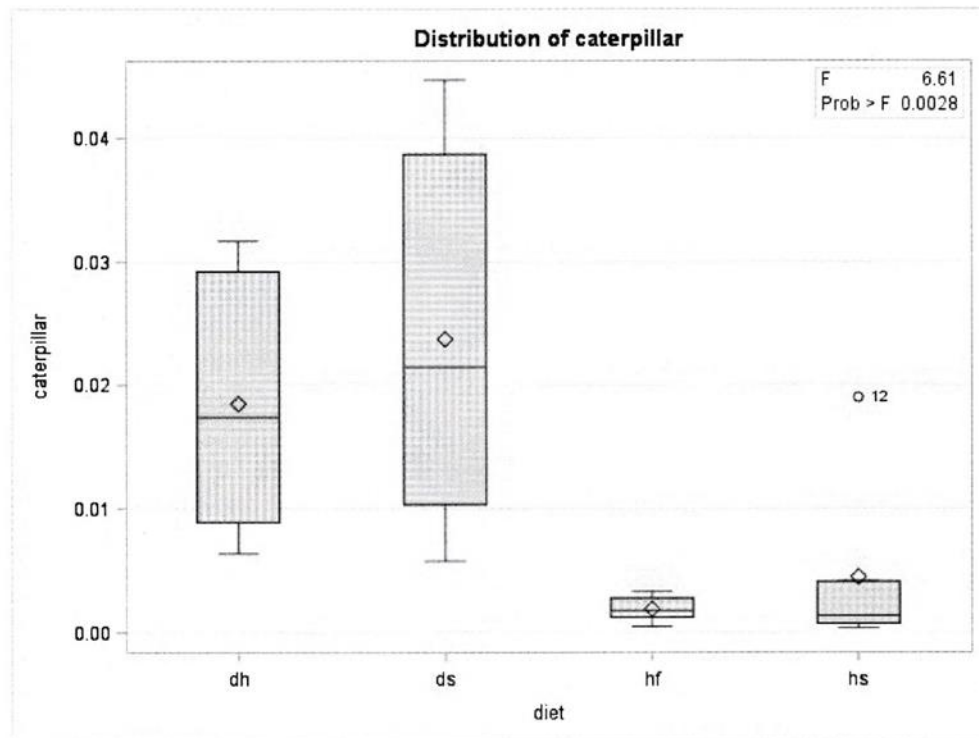


Figure 4.6 SAS output showing comparisons of larval weights of corn earworm on five diets.



Figure 4.7 Leaf tissue closest to the leaf midrib vein consumed, remainder of the leaf unscathed, by larvae.



Figure 4.8 Corn earworm larvae feeding on trichome rich floral material in the field.

Table 4.1 Individual corn earworm (CEW) larval weights taken on day 7 of diet experiments.

CEW Larvae ¹	Hemp Diet	Hemp Diet + Seed	Artificial Diet	Artificial Diet + Hemp	Diet + Hemp + Seed
1	0.0010	0.0007	0.0112	0.0040	0.0041
2	0.0005	0.0007	0.0251	0.0071	0.0066
3	0.0002	*	0.0098	0.0058	0.0037
4	0.0004	*	0.0100	0.0059	0.0073
5	0.0006	0.0002	0.0840	0.0050	0.0075
6	0.0009	*	0.0235	0.0122	0.0040
7	0.0006	0.0006	0.0710	0.0060	0.0047
8	*	*	0.0339	0.0040	0.0050
9	0.0003	0.0010	0.0454	0.0069	0.0092
10	0.0005	0.0006	0.0531	0.0051	0.0068
11	0.0006	*	0.0097	0.0132	0.0045
12	0.0005	*	0.0291	0.0032	0.0046
13	0.0003	0.0004	0.0319	0.0071	0.0073
14	0.0005	*	0.0140	0.0052	0.0029
15	0.0002	0.0003	0.0243	0.0104	0.0043
16	0.0005	*	0.0237	0.0064	0.0104
17	0.0002	0.0006	0.0263	0.0079	0.0051
18	0.0008	0.0005	0.0206	0.0040	0.0039
19	*	0.0009	0.0106	0.0043	0.0081
20	0.0006	0.0002	0.0287	0.0035	0.0046
AVG CEW Larval Weights	0.0005	0.0006	0.0293	0.0064	0.0057
¹ Individual CEW larval weights are represented in g. *Dead larvae					

Table 4.2 Individual corn earworm (CEW) larval weights taken on day10 of diet experiments.

CEW Larvae ¹	Hemp Diet	Hemp Diet + Seed	Artificial Diet	Artificial Diet + Hemp	Diet + Hemp + Seed
1	0.0013	*	0.1548	0.0073	0.0040
2	0.0007	0.0007	0.2278	0.0105	0.0087
3	0.0007	*	0.1285	0.0062	0.0120
4	*	*	0.1488	0.0132	0.0173
5	0.0005	*	0.0967	0.0071	0.0315
6	0.0012	*	0.0946	0.0108	0.0207
7	*	0.0007	0.2561	0.0130	0.0031
8	*	*	0.1485	0.0080	0.0075
9	*	*	0.1955	0.0093	0.0193
10	*	0.0008	0.1900	0.0059	0.0078
11	0.0010	*	0.0610	0.0135	0.0031
12	0.0023	*	0.1631	*	0.0081
13	0.0027	*	0.1699	0.0070	0.0078
14	*	*	0.0584	0.0067	*
15	0.0010	*	0.1452	0.0105	0.0052
16	*	*	0.0822	0.0073	0.0126
17	*	*	0.1501	0.0147	0.0036
18	0.0008	*	0.1302	0.0038	0.0035
19	*	*	0.0546	0.0092	0.0099
20	0.0010	0.0006	0.1018	0.0051	*
AVG CEW Larval Weights	0.0012	0.0007	0.1379	0.0089	0.0103
¹ Individual CEW larval weights are represented in g. *Dead larvae					

Table 4.3 Individual corn earworm (CEW) larval weights taken on day 13 of diet experiments.

CEW ¹ Larvae	Hemp Diet	Hemp Diet + Seed	Artificial Diet	Artificial Diet + Hemp	Diet + Hemp + Seed
1	0.0023	*	0.3279	*	0.0037
2	*	0.0008	0.5357	0.0114	0.007
3	*	*	0.3864	*	0.51
4	*	*	0.2887	0.013	0.0209
5	0.0011	*	0.4228	0.0132	0.0416
6	0.0012	*	0.5812	0.0063	0.0256
7	*	0.0019	0.5822	0.0211	0.003
8	*	*	0.1976	0.0045	0.008
9	*	*	0.5461	0.0112	0.0391
10	*	*	0.5005	0.0106	0.008
11	0.0015	*	0.1501	0.0361	*
12	0.0027	*	0.3137	*	0.01
13	0.0023	*	0.3435	0.0048	0.0053
14	*	*	*	0.0164	*
15	0.002	*	0.2981	0.0217	0.0097
16	*	*	0.1382	0.0041	0.0117
17	*	*	0.2902	0.0217	*
18	0.0011	*	0.1561	*	0.0053
19	*	*	0.1331	0.0077	0.0061
20	0.0011	*	0.2306	0.0043	*
AVG CEW Larval Weights	0.0017	0.0014	0.3380	0.0130	0.0447
¹ Individual CEW larval weights are represented in g. *Dead larvae					

Table 4.4 Individual corn earworm (CEW) larval weights taken on day 16 of diet experiments.

CEW ¹ Larvae	Hemp Diet	Hemp Diet + Seed	Artificial Diet	Artificial Diet + Hemp	Diet + Hemp + Seed
1	0.0020	*	0.5359	*	0.0038
2	*	0.0014	*	0.0179	*
3	*	*	0.5751	*	*
4	*	*	0.5419	0.0194	0.0230
5	0.0013	*	0.3722	0.0138	0.0451
6	0.0012	*	0.3671	*	0.0265
7	*	0.0012	*	0.0343	*
8	*	*	0.4555	0.0052	0.0081
9	*	*	*	0.0226	0.0483
10	*	*	0.5022	0.0118	0.0071
11	0.0010	*	0.3230	0.0507	*
12	0.0037	*	0.5439	*	0.0093
13	0.0034	*	0.5701	*	0.0060
14	*	*	*	0.0135	*
15	0.001	*	0.5261	0.0374	0.0092
16	*	*	0.2152	*	0.0090
17	*	*	0.4993	0.0246	*
18	0.0011	*	0.2327	*	0.0087
19	*	*	0.2728	0.0099	*
20	0.0011	*	0.2741	*	*
AVG CEW Larval Weights	0.0018	0.0013	0.4254	0.0218	0.0170
¹ Individual CEW larval weights are represented in g. *Dead larvae					

Table 4.5 Individual corn earworm (CEW) larval weights taken on day 19 of diet experiments.

CEW ¹ Larvae	Hemp Diet	Hemp Diet + Seed	Artificial Diet	Artificial Diet + Hemp	Diet + Hemp + Seed
1	0.0015	*	0.4046	*	*
2	*	0.0060	*	0.0275	*
3	*	*	0.3824	*	*
4	*	*	0.4268	0.0223	0.0334
5	0.0032	*	0.2811	0.0195	0.0414
6	0.0013	*	0.3258	*	0.0465
7	*	0.0022	*	0.0307	*
8	*	*	*	0.0048	0.0066
9	*	*	*	0.0382	0.0619
10	*	*	*	0.0135	0.0151
11	0.0010	*	0.3343	0.0778	*
12	0.0040	*	0.3940	*	0.0163
13	0.0059	*	0.4223	*	*
14	*	*	*	0.0153	*
15	0.0023	*	0.3837	0.0406	0.0079
16	*	*	0.2991	*	*
17	*	*	0.4037	0.0392	*
18	*	*	0.3389	*	0.0043
19	*	*	0.2447	0.0213	*
20	*	*	*	*	*
AVG CEW Larval Weights	0.0027	0.0041	0.3570	0.0292	0.0259
¹ Individual CEW larval weights are represented in g. *Dead larvae					

Table 4.6 Individual corn earworm (CEW) larval weights taken on day 22 of diet experiments.

¹ CEW Larvae	Hemp Diet	Hemp Diet + Seed	Artificial Diet	Artificial Diet + Hemp	Diet + Hemp + Seed
1	0.0019	*	Pupa	*	*
2	*	*	*	0.0351	*
3	*	*	Pupa	*	*
4	*	*	Pupa	0.0198	0.0453
5	0.0068	*	Pupa	0.0230	0.0459
6	0.0019	*	Pupa	*	0.0727
7	*	0.0190	Pupa	0.0245	*
8	*	*	Pupa	0.0046	0.0062
9	*	*	Pupa	0.0394	0.0671
10	*	*	Pupa	0.0133	0.0167
11	0.0012	*	0.3212	0.0733	*
12	0.0058	*	Pupa	*	0.0171
13	0.0038	*	Pupa	*	*
14	*	*	*	0.0235	*
15	0.0015	*	Pupa	0.0535	*
16	*	*	0.2473	*	*
17	*	*	Pupa	0.0530	*
18	*	*	Pupa	*	*
19	*	*	0.2365	0.0172	*
20	*	*	Pupa	*	*
AVG CEW Larval Weights	0.0033	0.0190	0.2683	0.0317	0.0387
¹ Individual CEW larval weights are represented in g. *Dead larvae					

Table 4.7 Average daily weights of corn earworm (CEW) larvae fed five diets.

	Day 7	Day 10	Day 13	Day 16	Day 19	Day 21
Hemp	4.6 n=18	12.0 n=11	17.0 n=11	17.6 n=9	27.4 n=7	32.7 n=7
Hemp + Seed	3.4 n=13	7.0 n=5	13.5 n=3	13.0 n=2	41.0 n=2	190.0 n=1
Artificial Diet	293 n=20	1378.9 n=20	3380.4 n=19	4254.4 n=16	3570.3 n=13	2683.3 n=3
Artificial Diet + Hemp	63.6 n=20	89.0 n=19	130.1 n=16	217.6 n=12	292.3 n=12	316.8 n=12
Diet + Hemp + Seed	57.3 n=20	103.2 n=18	446.9 n=16	170.1 n=12	259.3 n=10	387.1 n=7

*Average CEW larval weights are represented in mg.

Table 4.8 Percent mortality of corn earworm (CEW) larvae fed five diets.

Mortality rates of CEW	Day 7	Day 10	Day 13	Day 16	Day 19	Day 21
Hemp	10%	45%	45%	55%	65%	65%
Hemp + Seed	35%	75%	85%	90%	90%	95%
Artificial Diet	0%	0%	5%	10%	10%	10%
Artificial Diet + Hemp	0%	5%	20%	40%	40%	40%
Diet + Hemp + Seed	0%	10%	20%	40%	50%	65%

VITA

William Chester Cody Seals was born on June 15, 1993, in Nashville, TN, to his parents Curtis and Debbie Seals. As a child, he enjoyed being outdoors playing around the woods behind his house and splashing throughout the seasonal creek that ran through those woods. His parents regularly took him camping, hiking, and fishing helping to cultivate this love for the outdoors past even just the childhood years.

In June 2011, he graduated from Mt. Juliet High School in Mt. Juliet, TN, a city on the far west side of Wilson County bordering Davidson County, home of Nashville, TN. Once graduating high school, Cody attended Middle Tennessee State University (MTSU) majoring in Plant and Soil Science. While at MTSU Cody became involved with the Plant and Soil Science Club (PSSC), traveling to many conferences to compete in various undergraduate competitions. Cody eventually rose to President of PSSC and served for two semesters before graduating. During his time at MTSU he began to work with the University of Tennessee on CAPS (Cooperative Agricultural Pest Survey). The CAPS program helped him to discover his passion for pest insects and their plant interactions.

Around this time, hemp became legal once again for production in Tennessee and Cody began to become increasingly interested in the insect interaction that may affect this new crop. This interest led him to the University of Tennessee Department of Entomology and Plant Pathology where he pursued a Master's Degree researching the ecology of beneficial and pest insects that may affect hemp. He completed his degree in August of 2019.