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# Science of Hemp: Production and Pest Management Meeting Proceedings (2022)

University of Kentucky
College of Agriculture, Food and Environment

# Science of Hemp: Production and Pest Management

Cannabis (*Cannabis sativa*) continues to establish across the US. Today, hemp (THC <0.3%) is legal, while marijuana (THV >0.3%) is still considered a Schedule 1 narcotic by the US Drug Enforcement Agency (DEA). Markets are becoming more stable, despite having experienced volatility in recent years. Hemp acreage in the US totaled just over 54,000 acres in 2021, down from over 0.5 million acres in 2019. Markets are recovering as grain and fiber are projected to reach record sales in the coming years. Marijuana production, now legal in 39 states, is expanding, although acreage is not well documented. As a result of 80 years of prohibition, cannabis research is in the early stages. Today's cannabis is vastly different from the fiber hemp of the past. Furthermore, production and pest management are both different from the time when hemp was legal.

In recent years, university and industry research has continued to expand. Now, eight years after reintroduction of hemp and over twenty years after the first states legalized marijuana, we are gaining better understandings of the crop and its challenges. Some of that research was shared at the third meeting of the Science of Hemp: Production and Pest Management held on November 3-4, 2022 at the University of Kentucky in Lexington, KY. Ninety-two agricultural scientists attended a series of scientific presentations. Topics included discipline-specific presentations in agronomy, entomology, horticulture, economics, and plant pathology, as well as general sessions. Attendees enjoyed a networking social at Pepper Campus, a revitalization project at the historic James E Pepper Bourbon Distillery. The evening included poster sessions and a hemp-themed dinner.

Organizers thank the University of Kentucky College of Agriculture, Food, and Environment for making this meeting possible. We also thank the countless volunteers from both the University of Kentucky and Auburn University for their tireless work and contributions that made this meeting a success.

Thank you, from the planning committee:

Nicole Gauthier
Katelyn Kesheimer
Kimberly Leonberger
Misbakhul Munir
Natalia Martinez
Magdalena Ricciardi
Paul Cockson
Raul Villanueva

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# Meeting Overview

| **Wednesday, Nov. 2, 2022** | |
|---|---|
| 6:00 pm - 9:00 pm | Welcome Social, Food & Drinks - Rosemont Station |
| | CannaBingo - Students play for prizes |

| **Thursday, Nov. 3, 2022** | |
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| 7:30 am - 8:15 am | Registration, Poster Set Up, Coffee + Pastries |
| | Student Coffee and Convos |
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| 8:15 am - 8:30 am | Welcome (Nicole Gauthier and Katelyn Kesheimer) |
| | |
| 8:30 am - 9:00 am | Fusarium Head Blight: An Emerging and Potentially Devastating Disease on Hemp - Misbakhul Munir and Henry Smith, University of Kentucky |
| | |
| 9:00 am - 11:30 am | Student Oral Presentations (Organizer: Misbakhul Munir) |
| 9:00 - 9:20 am | Evaluating Fertility and Biological Control on CBD Yield in Outdoor Hemp - Ivy Thweatt, Auburn University |
| 9:20 - 9:40 am | Phenology of Corn Earworm and Tobacco Budworm, and Their Parasitoids, on Hemp in Tennessee - Julian Cosner, University of Tennessee |
| 9:40 - 10:00 am | Can a Sweet Corn Trap Crop Contribute to Floral Hemp Yield? - Alejandra Velez Chavez, Auburn University |
| 10:00 - 10:20 am | CBD Resistant *Salmonaella* Strains are Susceptible to Epsilon 34 Phage Tailspike Protein - Ibrahim Iddrisu, Alabama State University |
| 10:20 - 10:30 am | Break |
| 10:30 - 10:50 am | Densification of Hemp Floral Biomass Pre- and Post-Extraction - Gary Lopez, University of Kentucky |
| 10:50 - 11:10 am | Initial Explorations in Hemp Root Phenotypes During the Early Establishment Phase - Paul Cockson, University of Kentucky |
| 11:10 - 11:30 am | Detection of *Fusarium* spp. across different planting dates and plant developmental stage - Erin Thomas, University of Kentucky |

| | |
|---|---|
| 11:30 am - 12:00 pm | Student Poster Session (Organizer: Misbakhul Munir) |
| 11:30 - 11:35 am | Strategies for managing *Botrytis* in outdoor hemp, Cora Yates, Auburn University |
| 11:35 - 11:40 am | Fungicide and Hemp: How Fungicide Application Affects Microbial Communities in Leaves - Allison Paolucci, University of Louisville |
| 11:40 - 11:45 am | Detection of *Fusarium* spp. in Non-Symptomatic Plant Tissues of Hemp - Oluwatoni Adedokun, University of Kentucky |
| 11:45 - 11:50 am | Trichome Density of CBD Hemp - Spencer Schuchman, Southern Illinois University Carbondale |
| 11:50 - 11:55 am | Understanding the Mechanisms of CBD Resistance in *Salmonella typhimurium* - Ayomide Adebanjo, Alabama State University |
| 11:55 - 12:00 pm | Methodological Approaches to Combat Pests in Hemp: A Greenhouse Study - Chelsea Lawrence, Auburn University |
| | |
| 12:00 pm - 1:00 pm | Lunch - Comete Alguito, Colombian Food Truck |
| | Welcome - Dr. Nancy Cox, University of Kentucky Vice President for Land-grant Engagement and Dean of the College of Agriculture, Food, and Environment |
| | |
| 1:00 pm - 5:00 pm | Bourbon Distillery Field Trip by Bus: Pepper Distillery and Pepper Campus |
| | |
| 5:30 pm - 6:00 pm | Student Trivia with Swag! (Moderator: Katelyn Kesheimer) |
| 6:00 pm - 9:00 pm | Banquet - Hemp Themed Dinner - Bayou Bluegrass Catering + Cocktails |
| 6:30 - 7:15 pm | State of the Hemp Industry Keynote: Opportunities Galore for Industrial Hemp: It's More Than Rope and Dope - Nick Walters, National Hemp Coop |
| 7:15 - 7:45pm | Student Competition Awards (Presenter: Misbakhul Munir) |
| 7:45 - 9:00 pm | Social & Poster Viewing, Drinks and Social |

| **Friday, Nov. 4, 2022** | |
|---|---|
| 8:00 am - 8:30 am | Coffee + Pastries, & Poster Viewing |
| | Student Coffee and Convos |
| | |
| 8:30 am - 9:00 am | General Session |
| 8:30 - 8:45 am | I Like Big Buds and I Cannot Lie: Corn Earworm Has a Taste for Hemp - Julian Cosner, University of Tennessee |
| 8:45- 9:00 am | Lessons from the Biological and Chemical Control of Corn Earworm on Hemp - Armando Falcon-Brindis, University of Kentucky (virtual) |
| | |
| 9:00 am - 12:00 pm | Breakout Sessions |
| **AGRONOMY** | |
| 9:00 - 9:20 am | Integrated Weed Management in Hemp - Karla Gage, Southern Illinois University Carbondale |
| 9:20 - 9:40 am | Fiber Hemp in Tennessee: Preliminary Results for Variety Performance, Planting Dates, and Retting Time - Mitchell Richmond, University of Tennessee |
| 9:40 - 10:00 am | Effect of Outdoor and Controlled Environment Drying on Quality of Hemp Flower grown for Cannabidiol - Noelle Joy, University of Georgia |
| 10:00 - 10:20 am | Impact of Planting Date and Seed Depth on Establishment, Growth, and Grain Yield - Tara Valentine, University of Kentucky |
| 10:20 - 10:40 am | Break |
| 10:40 - 11:00 am | Agronomic Evaluation of Diploid and Triploid Hemp Cultivars - Bob Pearce, University of Kentucky |
| 11:00 - 11:20 am | Hemp, Elvis, and Luke the Drifter- Eric Walker, Genamera |
| 11:20 - 11:40 am | Hemp Crop Compliance Sampling - Doris Hamilton, Kentucky Department of Agriculture |
| 11:40 - 12:00 pm | Open discussion |

| ENTOMOLOGY | |
|---|---|
| 9:00 - 9:20 am | Effect of Diet on Parasitism Rates of Corn earworm - Armando Falcon-Brindis, University of Kentucky |
| 9:20 - 9:40 am | Wild Wild West: Ecology and Management of Emerging Viruses of Hemp - Punya Nachappa, Colorado State University (virtual) |
| 9:40 - 10:00 am | Managing two-spotted spider mites, *Tetranychus urticae*, in greenhouse hemp in Alabama - Katelyn Kesheimer, Auburn University |
| 10:00 - 10:20 am | Managing hemp russet mite, *Aculops cannabicola* (Acarina: Eriophyidae) in industrial hemp in greenhouse and field - Ada Szczepaniec, Colorado State University (virtual) |
| 10:20 - 10:40 am | Break |
| 10:40 - 11:00 am | Hemp Russet Mite Control Using Organic and Conventional Acaricides - Raul Villanueva, University of Kentucky |
| 11:00 - 11:20 am | Potential Impacts of Climate Change on Industrial Hemp Pests - Olufemi Ajayi, Alabama State University |
| 11:20 - 11:40 am | Minute Pirate Bugs and Phytoseiids, What are they doing in Hemp? - Raul Villanueva, University of Kentucky |
| 11:40 am - 12:00 pm | Trends across the US: A Nationwide Survey of Insect Pests on Cannabis - Katelyn Kesheimer, Auburn University |

| PLANT PATHOLOGY | |
|---|---|
| 9:00 - 9:20 am | Downy Mildew Observations in Hemp Variety Trial - Marguerite Bolt, Purdue University |
| 9:20 - 9:40 am | A Statewide Hemp Survey: Preliminary Data and Observations from Kentucky - Henry Smith, University of Kentucky |
| 9:40 - 10:00 am | *Fusarium* species Threaten Postharvest Cannabis - Desiree Szarka, USDA (presented by Nicole Gauthier, University of Kentucky) |
| 10:00 - 10:20 am | A Survey of Hemp Diseases in Oregon and Washington between 2021 and 2022 - Hannah Rivedal, United Stated Department of Agriculture - Agricultural Research Service |
| 10:20 - 10:40 am | Detection of latent infection of *Fusarium graminearum* Across Different Planting Dates and Plant Development Stages - Misbakhul Munir, University of Kentucky |
| 10:40 - 11:00 am | Break |
| 11:00 - 11:20 am | Potential *Fusarium* species Infecting Hemp Seeds in Kentucky - Magdalena Ricciardi, University of Kentucky |
| 11:20 - 11:40 am | Wild Wild West: Ecology and Management of Emerging Viruses of Hemp - Punya Nachappa, Colorado State University (virtual) |
| 11:40 am - 12:00 pm | Trends across the US: A Nationwide Survey of Diseases of Cannabis - Nicole Gauthier and Misbakhul Munir, University of Kentucky |

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|---|---|
| 12:00 pm - 1:00 pm | Lunch - Stella's Deli & Poster Viewing |
| 1:00 pm - 2:00 pm | General Session |
| 1:00 - 1:15 pm | In-Field Diagnosing of Nutrient Disorders in *Cannabis sativa* L. - Paul Cockson, University of Kentucky |
| 1:15 - 1:30 pm | Hempseed as a Food Material: Carbohydrate Content in Edible Grain - Rachel Schendel, University of Kentucky |
| 1:30 - 1:45 pm | A Comparison of Industrial Hemp Yields in the US - Adjit Williams, University of Florida |
| 1:45 - 2:00 pm | Biocontrol Projects on Hemp Grown in Kentucky: Untangling Complexity - Magdalena Ricciardi, University of Kentucky |
| 2:00 pm - 2: 30 pm | Closing Comments, Discussion, Potential Collaborations (Katelyn Kesheimer and Nicole Gauthier) |
| 2:30 pm - 3:00 pm | Poster Teardown |

| **Posters** |
|---|
| Impact of Plant Growth Products on Cannabinoid and Terpenes - Kimberly Gwinn, University of Tennessee |
| Industrial Hemp By-Products as Potential Animal Feedstuffs - Alexander Altman, University of Kentucky |
| Hemp Powdery Mildew Fungicide Trials in Tennessee - Zachariah Hansen, University of Tennessee |
| Trichome Density of CBD Hemp - Spencer Schuchman, Southern Illinois University |
| Parasitoids of Corn Earworm in Western Kentucky - Armando Falcon-Brindis, University of Kentucky |
| Understanding the Mechanisms of CBD Resistance in *Salmonella typhimurium* - Ayomide Adabanjo, Alabama State University |
| Influence of fungicide on hemp rhizosphere microbial composition and enzymes - Junhan Xu, Alabama State University |
| Auburn Plant Diagnostic Lab Highlights - Cora Yates, Kassie Conner, and Katelyn Kesheimer, Auburn State University |

## Student Competition Winners

Congratulations to our student winners:

Oral Competition

1st Place – Paul Cockson, University of Kentucky

2nd Place – Julian Cosner, University of Tennessee

3rd Place – Gary Lopez, University of Kentucky

Poster Competition

1st Place – Spencer Schuchman, Southern Illinois University Carbondale

2nd Place – Chelsea Lawrence, Auburn University

3rd Place – Allison Paolucci, University of Louisville

# Meeting Proceedings

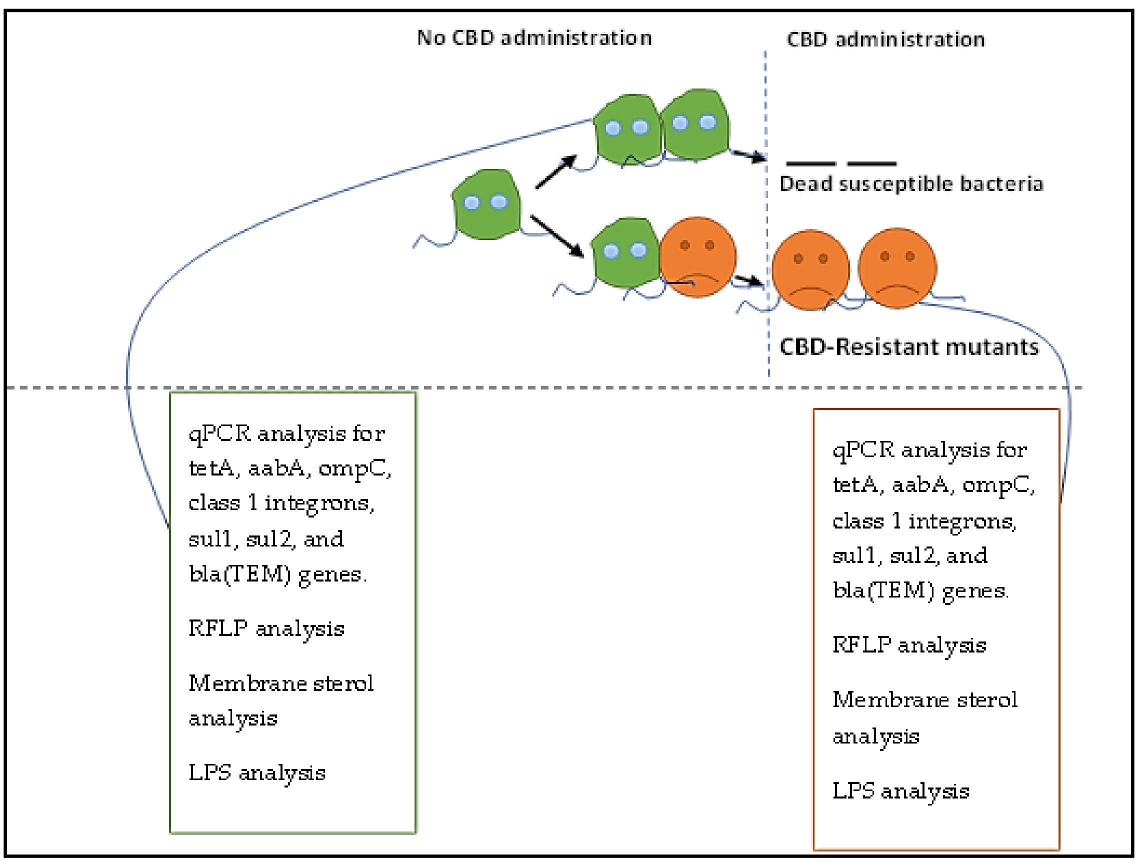
Following are forty-four meeting abstracts, arranged in alphabetical order by author. Oral and poster presentation abstracts are included herein.


Images: Katelyn Kesheimer, Auburn University

# Understanding the Mechanisms of CBD Resistance in *Salmonella typhimurium*

Ayomide Adebanjo*[1], Iddrisu Ibrahim[1], Joseph Ayariga[1], Junhuan Xu[1] and Olufemi Ajayi[1]
[1]Alabama State University, Montgomery, Alabama, United States of America

Isolates of the multidrug-resistant *Salmonella typhimurium* are a global public health issue. One of the goals of our Industrial Hemp Program is progressive research on the pathogenic microbes and cannabidiol (CBD) interface. Recently, we demonstrated that CBD is an effective antimicrobial against *S. typhimurium*, but our research indicated that the bacteria developed resistance after prolonged treatment with CBD. Thus, prompting this investigation into the possible involvement of genetic, enzymatic, metabolic, and structural alterations in *S. typhimurium* that led to the acquisition of CBD resistance. This research is especially critical if CBD should be further developed into a drug formulation. We hypothesized that CBD resistance is similar to antibiotic resistance, a continuous process that shapes and ensures the bacterial evolution and adaptation to the antibiotic. We explored the possible mechanisms by which CBD induces *S. typhimurium* resistance by examining experimentally specific known resistance factors in the bacteria using DNA fragment length polymorphism analysis, qPCR analysis, LPS modification analysis, and bacteria cell membrane sterols analysis. Thus, in brief, CBD-resistant *S. typhimurium* clones were made *in vitro* via culturing *S. typhimurium* in progressively higher concentrations of CBD for 7 days. The DNA, RNA, LPS and Sterol contents of the resistant isolates were extracted and examined against the susceptible wild type *S. typhimurium* isolates. For DNA analysis, restriction fragment length polymorphisms of resistant *S. typhimurium* strains were compared with that of the susceptible strains. The presence of any gene fragment indicating resistance to CBD were marked, cut and gel-purified for further analysis. The qPCR analysis for *tetA*, *aabA*, *ompC*, class 1 integrons, *sul1*, *sul2*, and *bla(TEM)* genes were investigated. The LPS and Sterol analysis were carried out via spectrophotometric method. The significance of the results is discussed.



# Detection and Isolation of *Fusarium* spp. from Asymptomatic Plant Tissues of Hemp

Toni Adedokun*[1], Erin Thomas[1], Rebecca Schroer[1], Jacqueline Reynolds[1], Justin Wong[1], Georgia Avery[1], Desiree Szarka[1], Henry Smith[1], Nicole Gauthier[1], and Misbakhul Munir[1]
[1]University of Kentucky, Lexington, KY, USA

Fusarium head blight (FHB) is an emerging disease of hemp in the US. Four *Fusarium* species including *F. avenaceum*, *F. graminearum*, *F. incarnatum-equiseti* species complex, and *F. sporotrichioides* have been reported to be associated with FHB in hemp in Kentucky. This study was conducted to determine *Fusarium* spp. potential in causing FHB from asymptomatic hemp tissues. In 2022, a total of 145 asymptomatic hemp samples (plant tips, seed head, or flowers) were collected from a hemp field and from across multiple different planting dates (PD, n=3), plant ages (PA, n=3), and cultivars (CV, n=3) with 4 minimum samples collected from each combination of PD*PA*CV. Samples were subjected to two detection methods: 1) plating onto potato dextrose agar (PDA); and 2) four species specific-qPCR assays detecting the four aforementioned FHB causal species. Detection between the two methods were compared and correlated using Chi-square test and correlation. Multiple *Fusarium* spp. were detected and recovered from asymptomatic hemp tissues. Percent of positive detection was 33% higher with qPCR method than plating method ($P<0.0001$). This can be due to qPCR being more sensitive than the plating method in detecting *Fusarium* spp. in plants. However, correlation of detection results between the two methods showed significant and moderately strong relationship ($r$) 0.47 ($P<0.0001$) indicating that positive detection on plating method was confirmed with the qPCR. Capability in detecting *Fusarium* species before symptom development (in asymptomatic tissues) can help in management of the disease (e.g., timing fungicide application and/or other control actions).

# Potential Impacts of Climate Change on Industrial Hemp Pests

Olufemi Ajayi[1]
[1]Alabama State University, Montgomery, Alabama, USA

There has been a resurgence in the cultivation of industrial hemp, *Cannabis sativa* L., in the United States since its recent legalization. This may facilitate increased populations of arthropods associated with the plant. Hemp pests target highly marketable parts of the plant, such as flowers, stalks, and leaves, which ultimately results in a decline in the quality. Industrial hemp can be used for several purposes including production of fiber, grain, and cannabidiol. Thus, proper management of pests is essential to achieve a substantial yield of hemp in the face of climate change. Here, we provide updates on various arthropods associated with industrial hemp in the United States and examine the potential impact of climate change on corn earworm (CEW) *Helicoverpa zea* Boddie, a major hemp pest. For example, temperature and photoperiod affect the development and diapause process in CEW. Additionally, drought can lead to a reduction in hemp growth. Host plant diversity of CEW may prevent populations of the pest from reaching outbreak levels. It is suggested that hemp varieties resistant to drought, high soil salinity, cold, heat, humidity, and common pests and diseases should be selected. Ongoing research on effective management of CEW in hemp is critical.

# Efficacy of Fungicides Against Hemp Powdery Mildew in the Greenhouse

Rufus Akinrinlola[1], Alyssa Counce*[2], and Zachariah Hansen[2]
[1]Microbial Discovery Group, Oak Creek, WI, USA
[2]University of Tennessee, Knoxville, TN, USA

Powdery mildew, caused by *Golovinomyces ambrosiae*, is a common disease of greenhouse-grown hemp. The list of fungicides registered for use on hemp is regularly changing and varies by state. The purpose of this study was to evaluate fungicides currently registered for use on hemp in the greenhouse in Tennessee for efficacy against hemp powdery mildew. Several products that are not currently registered for use on hemp in Tennessee were also evaluated. Four greenhouse studies were conducted between 2020 and 2022 at the University of Tennessee in Knoxville, TN. Nine fungicides were tested in repeated experiments, and four additional fungicides were tested in a single non-repeated experiment. 'BaOx2' or 'Sweetened' hemp cultivars, which are known to be susceptible to powdery mildew based on previous studies, were inoculated with a conidial suspension one day before or one day after (post-inoculation Regalia treatment only) the first fungicide application. Two additional fungicide applications were made at 7-day intervals using hand-held sprayers. Control plants were sprayed with water. Disease incidence and severity were assessed weekly three times. Disease index (DI) and the area under the disease progress curve (AUDPC) were calculated.

All fungicides evaluated in repeated experiments significantly reduced powdery mildew compared to non-treated controls. Bonide sulfur, Luna Experience, and MilStop reduced disease by 96% to 100%. Cinnerate, Exile, Regalia, and Sil-Matrix reduced disease by 86% to 95%. Defguard and Stargus reduced disease by 76% to 85%. Preliminary results from a non-repeated experiment suggest that Zerotol 2.0, PerCarb, OxiPhos, and Zonix also have good efficacy for managing powdery mildew, and reduced disease by 96%, 94%, 100%, and 89%, respectively. Phytotoxicity was not observed in any of the treatments.

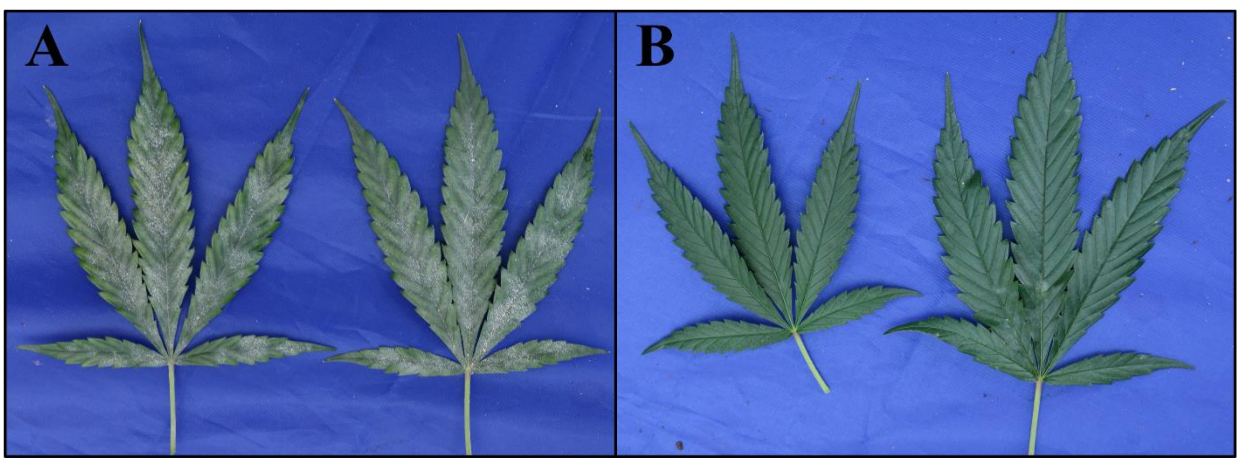



**Figure.** Hemp leaves removed from experimental plants 21 days after inoculation with *Golovinomyces ambrosiae* and following three fungicide applications. (A) Non-treated control. (B) Plants treated with Regalia.

# Industrial Hemp By-Products as Potential Animal Feedstuffs

Alexander Altman[1*], Coral Kent-Dennis[2], Kyle McLeod[1], Eric Vanzant[1], and David Harmon[1]
[1]Animal and Food Sciences Dept., University of Kentucky, Lexington, KY, USA
[2]USDA-ARS Forage-Animal Production Research Unit, Lexington, KY, USA

Since 2018, the number of unique and innovative products associated with industrial hemp (*Cannabis sativa*) production for cannabidiol (harvested from inflorescence), seed, and fiber has steadily increased. While innovation is good for a growing industry, one inevitable consequence of such advancement can be the creation of by-products that are discarded as waste. Such is the current fate of many by-products resulting from processing of industrial hemp inflorescence and seeds. Hemp fiber has been largely ignored as a potential feedstuff due to a nominal amount of available waste products and its popularity as construction and bedding materials. Current research suggests that hemp seed and inflorescence by-products may be suitable for inclusion in livestock diets providing a source of feed in a world demanding increased sustainability.

Of these two product types, seeds are the more prevalent and have received greater research interest. Hempseed is garnering attention as an attractive feedstuff due to its inability to produce cannabinoids. However, these plant secondary metabolites can be transferred during physical contact with trichomes located primarily in the inflorescence, and to a lesser extent also on leaves and stems. Several studies have reported hempseed as a potentially good source of protein and fat, with one noting this concentrate as an excellent source of rumen undegraded crude protein. Whole hempseed and its associated by-products have been used in both *in vivo* studies as a dietary ingredient for poultry, sheep, cattle, and swine, as well as *in vitro* studies examining formation of digestive products such as volatile fatty acids and methane gas.

By comparison, hemp inflorescence in livestock rations is a novel research topic, with most published studies relying on *in vitro* methods. Unlike seed, inflorescence from the hemp plant is the primary source of cannabinoids, with the psychoactive tetrahydrocannabinol compounds presenting the most concern. Hemp inflorescence is also known to produce other, non-psychoactive compounds such as terpenes and isoflavones. Unlike cannabinoids, terpenes and isoflavones are present in other forages and have been investigated for their antimicrobial properties in the animal and flavor-enhancing properties in the end-products (i.e., milk, cheese, meat, etc.) There are several studies quantifying the concentrations and prevalence of these bioactive chemicals in industrial hemp, but work remains as to their bioactivity in animals following consumption.

There are still many unanswered questions regarding the safety of feeding industrial hemp products to livestock destined for the human food supply. The primary concern remains the rate and extent to which cannabinoids are deposited in muscle and fat. Primary investigations have indicated that some of these compounds appear the bloodstream after entering the rumen and are later deposited in muscle, liver, and kidney tissues. However, it remains unknown what the rate of deposition and the half-life for withdrawal within the affected tissues is for cannabinoids.

In short, although there are several studies available examining this unique plant as a potential source of feedstuffs for livestock, many insights regarding its influence on animal performance and the possibility for contaminating the human food supply remains unknown. This is particularly true with feeding inflorescence products due to their relationship with cannabinoid production. Future, long-term studies, with emphasis on answering these questions must be conducted before a decision regarding the suitability of industrial hemp by-products as viable feedstuff alternatives in commercial livestock operations can be reached.

Keywords: Hemp, livestock, feedstuff

# Downy Mildew Observations in Hemp Variety Trial

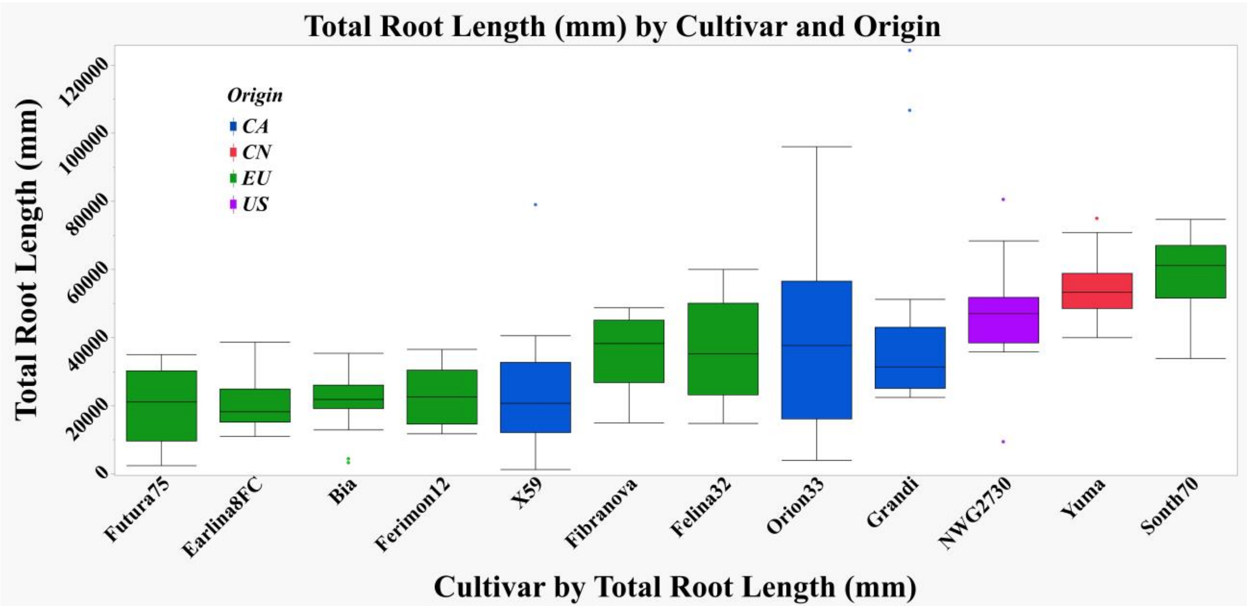
Marguerite Bolt*[1], Petrus Langenhoven[1], and Janna Beckerman[1]
[1]Purdue University, West Lafayette, IN, USA

Hemp (*Cannabis sativa* L.) has drawn significant interest since prohibition ended, with a surge in production. There are many production challenges associated with growing a new crop. One of these challenges is the identification of suitable varieties for different locations. Growers select varieties based on the amount of THC to remain compliant and CBD to maximize yield, while other plant traits may be overlooked. To evaluate traits across different hemp varieties, a trial was established in West Central Indiana. We evaluated 17 photoperiod-dependent cannabinoid hemp varieties for cannabinoid content, height, biomass yield, and disease pressure. Specifically, we measured the incidence and severity of hemp downy mildew (*Pseudoperonospora cannabina*) during the 2021 growing season. Leaf observations began two weeks after planting and occurred every two weeks until harvest began at the end of August. The 2021 season was wet and humid, contributing to moderate to high disease pressure from *P.cannabina* and other pathogens. Cover crops planted between rows created a humid environment, possibly exacerbating infection. Yield was low, largely due to storms that caused flooding and lodging of plants. The earliest maturing variety had the highest disease severity at the end of the trial. Disease severity was relatively low for all other varieties. Further observations of downy mildew need to be made to better understand why some varieties are more susceptible than others and what management practices can be used to reduce disease.

# Explorations into Hemp Root Phenotypes During Early Establishment

Paul Cockson*[1], Lindsey Moffit[1], David McNear Jr.[1], and Robert Pearce[1]
[1]University of Kentucky, Lexington, KY, US

This study aimed to quantify key root characteristics and phenotypes in a panel of industrial hemp (*Cannabis sativa* L.) cultivars. Key production areas (United States, Cannada, Central Europe, and China), industrial hemp (IH) types (grain, fiber, and dual purpose), and reproductive types (monoecious and dioecious) were utilized to select the 12 cultivars investigated. Plants were grown from seed in sand substrate in a greenhouse to control environmental factors. Twenty three days after sowing (DAS) plant roots were washed and subsequently photographed using a novel imaging system. Photos were then analyzed imaged using RhizoVision Explorer to extract 24 key root characteristics. The resulting analysis showed statistical differences among all four major categories with the greatest differences being seen in the following order: cultivar > production area > type > reproductive strategy. The results presented here will be a preliminary exploration of these data.



# In-Field Diagnosing of Nutrient Disorders in *Cannabis sativa* L.

Paul Cockson*[1], Robert Pearce[1], Patrick Veazie[2], and Brian Whipker[2]
[1]University of Kentucky, Lexington, KY, US
[2]North Carolina State University, Raleigh, NC, US

When seeking to diagnose nutrient disorders in the field, two main metrics prove useful. Firstly, the location of the deficiency symptom will indicate if the element is mobile or immobile. Mobile elements [Nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg), and zinc (Zn)] initially display symptoms on the lower portions or older leaves of the plant. Immobile elements [Calcium (Ca), boron (B), manganese (Mn), iron (Fe), molybdenum (Mo), and copper (Cu)] display deficiency symptoms on the upper portions or newer leaves. Only one element, sulfur (S), is semi-mobile and will display an overall yellowing of the plant. Secondly, the visual deficiency symptoms can be categorized into four main types: distorted growth and necrosis (B and Ca), overall yellowing (N and S), marginal yellowing and necrosis (P, K, Fe, Cu, and Mn), and overall yellowing and necrosis (Mg). By cross-referencing the nutrient deficiency location and the category one can narrow down possible deficiencies. The deficiency symptom location and category are useful in diagnosing potential nutrient disorders in a field setting when more precise equipment may not be present. Before corrective measures are enacted, soil and leaf tissue samples should be taken to confirm the diagnosis.

# I Like Big Buds and I Cannot Lie: Corn Earworm and Tobacco Budworm Have a Taste for Hemp

Julian Cosner[1], Jerome Grant[1], Mitchell Richmond[1]
[1]University of Tennessee Knoxville, Knoxville, Tennessee, USA

Industrial hemp, *Cannabis sativa* L., has been re-legalized in the U.S. and serves as a host plant for two major insect pests, the corn earworm, *Helicoverpa zea* Boddie, and tobacco budworm, *Chloridea virescens* F. This pest complex has been observed in several agricultural systems and these species are nearly identical in appearance during the larval stage. Unlike tobacco budworm, corn earworm larvae lack spinules, or microspines, at the base of the setae on the first, second, and eighth chalazae, also referred to as abdominal tubercles, and a retinaculum, or tooth, on the mandible. On hemp, these larvae devour foliage and developing floral clusters, known as buds in *Cannabis* culture, causing damage to the plant via herbivory leading to entry wounds for pathogens. The corn earworm has received notably more attention in cited literature than the tobacco budworm, but distinguishing between these two species is important for future research because their susceptibility to pesticides and preference from natural enemies differ. This presentation will outline distinctions between these insect pests and successful management strategies to reduce damage to hemp grown outdoors.

# Phenology of Corn Earworm and Tobacco Budworm, and Their Parasitoids, on Hemp in Tennessee

Julian Cosner[1], Jerome Grant[1], Mitchell Richmond[1]
[1]University of Tennessee Knoxville, Knoxville, Tennessee, USA

Production of industrial hemp, *Cannabis sativa* L., production has tremendously increased in the United States since its legalization through the Agricultural Improvement Act of 2018 (a.k.a. 2018 Farm Bill). Though fiber and grain production have great potential for the hemp industry, most growers concentrate on floral production for secondary metabolites, such as cannabinoids, because of the expected higher cash value potential per hectare. This emerging industry has been negatively impacted by larvae of corn earworm, *Helicoverpa zea* (Boddie), which feed on the developing inflorescences where cannabinoid concentrations are highest. However, in eastern Tennessee a Heliothinae complex, including corn earworm and the tobacco budworm, *Chloridea virescens* (F.), occurs on hemp. Few reports have made distinctions between these two insect pest species on hemp, but their known natural enemies and development patterns differ on other agricultural crops; thus, they are expected to differ on hemp. Differentiation of these two species is difficult until the adult wing patterns can be observed. To investigate the seasonal incidence of these pests on hemp, larvae will be collected from hemp grown in different regions of Tennessee during the 2022 growing season and reared to adulthood or parasitoid emergence. Adult moths and parasitoids will be identified. Results will be presented and discussed. Understanding the incidence of similar pest species and crop phenology will inform pest management decisions to increase the productivity of hemp in Tennessee and the southeastern United States.

# Effect of Diet on Parasitism Rates of Corn Earworm

Armando Falcon-Brindis[1], Raul T. Villanueva[1], and Zenaida J. Viloria[1]
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The corn earworm (CEW) *Helicoverpa zea* (Boddie) is considered a key pest in hemp crops across the United States, especially on cultivars aimed to produce cannabidiol (CBD). However, management options to control this pest are not well understood due to the recent legislation for hemp as a crop in North America. Since studying the performance of moth pests under different diets allow to better understand aspects related to their survival and dispersal, we aimed to study the effects diet on the mortality of parasitized CEW attacking hemp in western Kentucky.

During September and October in 2021 and 2022, we collected 1,040 CEW caterpillars between the 3rd and 6th instar. Both parasitized (with visible tachinid fly eggs attached – Figure 1A) and unparasitized (no visible eggs, i.e., the control group) larvae were removed from six different hemp fields of western Kentucky. In the laboratory, we recorded the weight and number of fly eggs attached to the body from each caterpillar. Then CEW were placed individually in plastic containers with either artificial diet (pinto bean mix) or pesticide-free flower buds of Felina 32 hemp. Caterpillars fed on each diet were distributed in size and parasitism intensity (number of eggs/host larva) to avoid biases. Larvae were monitored every two days to clean containers, provide food, and record mortality events.

There were two tachinid fly species associated with the mortality of corn earworm larvae: *Winthemia rufopicta* and *Lespesia aletiae* (Figure 1B-C). Overall, 54.2% of CEW-bearing eggs died, while mortality of the unparasitized larva reached 30%. The percentage of mortality differed between larvae fed with artificial diet (44%) and, hemp buds (58.1%), whereas parasitized CEW fed with artificial diet survived for longer periods. The mortality of CEW strongly depended on the nutrition, larval instar, and intensity of parasitism. Switching to a nutritious diet enhanced the survivorship of parasitized CEW. Apparently, hemp compounds may affect the performance of CEW larvae and their parasitoids.

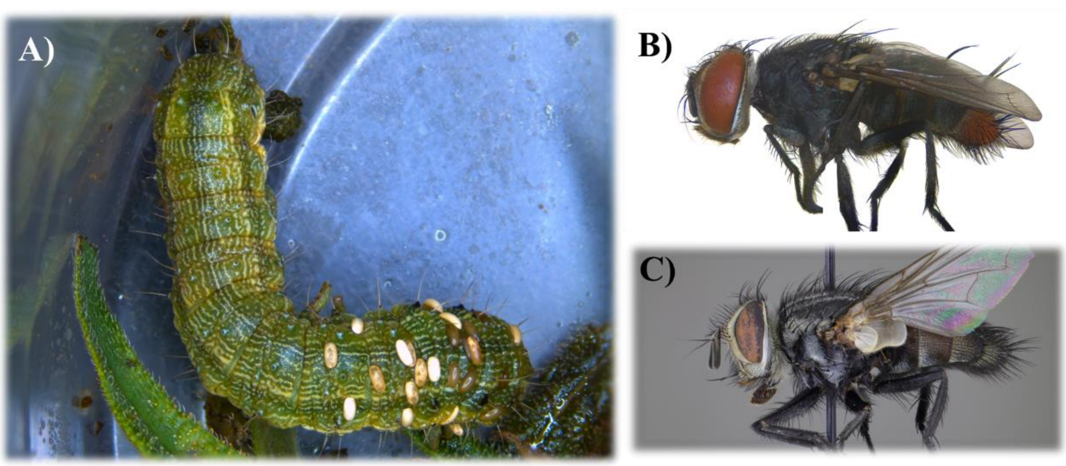


**Figure 1.** Corn earworm with eggs of tachinid flies attached to the body **(A)**. Adult flies parasitizing *H. zea* in hemp crops. *Winthemia rufopicta* **(B)** and *Lespesia aletiae* **(C)**.

# Lessons from the Biological and Chemical Control of Corn Earworm on Hemp

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Many decades of prohibition have restricted research on hemp regarding the chemical and biological control of pests, especially the corn earworm (CEW) *Helicoverpa zea* (Boddie) (Figure 1). In this work we provide an overview of both chemical and biological control methods tested against CEW attacking hemp outdoors and indoors during 2021 and 2022. The effect of insecticides to control CEW is not well understood, and regulations in the United States do not allow the use of conventional chemical insecticides in hemp. However, we have conducted tests in hemp using conventional insecticides registered in Kentucky for other commodities for experimental purposes. We also test the efficacy of organic insecticides (biologicals or plant-based) against CEW attacking hemp for CBD production. In addition, we described how parasitoids can help to control CEW in hemp fields. In outdoor conditions, we did not find treatment effect during 2021 trials, but in 2022, the conventional insecticides Baythroid, Besiege and Coragen kept the number of CEW per plot significantly lower than the biopesticides Agree, Gemstar, Heligen, Neemix and Dipel. The latter (*Bacillus thuringensis*) showed the best performance against CEW. Under laboratory conditions, chemical insecticides caused mortality faster than biological pesticides, where NPVs were highly efficient controlling early stages of CEW (2-4 larval stage), whereas neem oil and Bt were more efficient on later instars. Both timing of application and spray replication played a role determining the efficacy of pesticides. A second spray significantly reduced the populations of CEW. The first evaluation of parasitism of CEW by tachinid flies provided evidence that tachinid flies hold promise as biological control agents for populations of CEW attacking hemp (Figure 1). Mortalities of CEW bearing tachinid fly eggs reached 45.9%. Parasitized caterpillars survived 5 to 19 days depending on the number of eggs per individual. After rearing 1,742 CEW larvae collected during 2021 and 2022 in hemp fields of west and central KY, only two caterpillars were parasitized by the wasp *Campoletis sonorensis* (Ichneumonidae). The reason why parasitism of CEW by wasps are too low (0.1%) is still unclear, especially since other studies from Tennessee and Alabama reported the presence of different parasitic wasps attacking CEW in hemp.

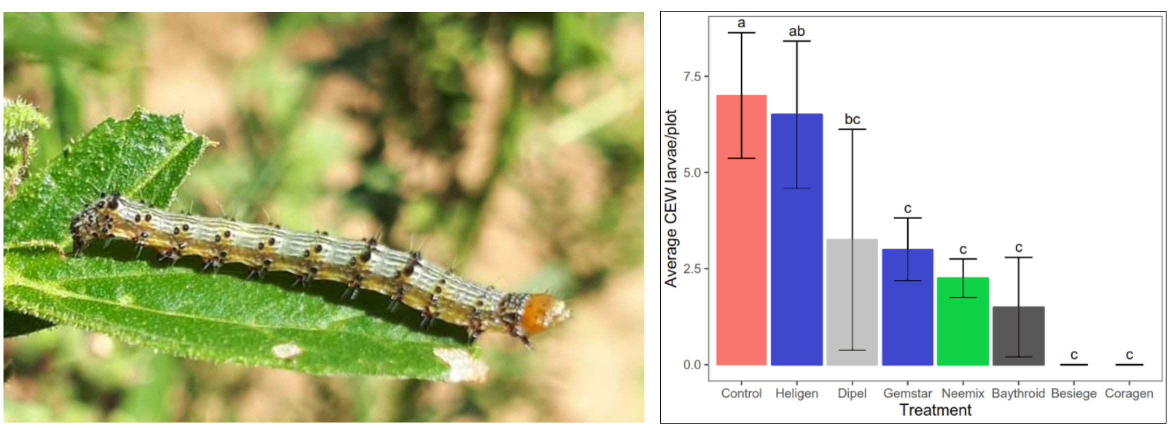


Figure 1. Corn earworm larva (left). Efficacy of chemical and biological insecticides to control CEW outdoors (right).

# Developing Best Management Practices for Integrated Weed Management in Hemp

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With decades of prohibition on hemp research, little is known about the impact of weeds on hemp growth. Weed competition typically reduces crop growth and yield through direct effects of competition for resources such as space, light, and nutrients. However, there are no published studies quantifying the effects of yield loss in hemp due to weeds. There are currently no registered herbicides at the federal level in the US. Limited research suggests that hemp may be an excellent competitor, and if established, may be minimally affected by weed competition. Planting of highly competitive fiber or dual-purpose hemp cultivars could provide a benefit in simplified agricultural systems which primarily rely on a corn-soybean rotation for diversified weed management. If hemp is a competitive crop, then diversifying corn and soybean rotations with a highly competitive crop such as hemp may suppress weed populations and promote seedbank decline. Any impact of weeds on hemp growth and yield may depend upon the Critical Weed Free Period (CWFP), which is defined as the period of crop growth during which the crop must be kept weed free to prevent yield loss due to weed interference. Two studies were established in Carbondale, Illinois, as part of a multistate project. Study 1: hemp management, sought to evaluate the efficacy of cultural and chemical weed management practices in a dual-purpose hemp crop and their impacts on quantity and quality of crop yields, and study 2: CWFP was designed to define the critical timing of weed management operations in relation to impacts on common and troublesome weeds. Study 1 (hemp management) was a 3 X 2 X 3 factorial, randomized complete block design with four replicates. Factors were herbicide [nontreated; ethalfluralin (1050 g ai $ha^{-1}$) followed by (fb) quizalofop (77 g ai $ha^{-1}$); and s-metolachlor (1423 g ai $ha^{-1}$) fb clethodim (76 g ai $ha^{-1}$)], row spacing (19 or 38 cm), and seeding rate (100, 200, and 300 plants $m^{-1}$). Data were collected on visible hemp injury, individual species weed control at 7, 14, and 21 days after preemergence and postemergence herbicide treatments (DAT) and at end of season (EOS), and EOS weed species above ground weed counts and biomass by weed species, crop height and stem diameter, and hemp fiber yield proxy (above ground biomass). Study 2 (CWFP) used three cultivars, planted at 34 kg pure live seed $ha^{-1}$ on 38 cm rows. There were 18 treatments (3 varieties x 6 weed-free periods) in a randomized complete block design with 4 replications. Plots were kept weed free via manual hoeing and the use of clethodim (76 g ai $ha^{-1}$) for grass control until 0, 7, 14, 28, and 42 days after emergence. Then plots were allowed to become weedy, and these were compared to a season-long (87 day) weed-free control. Data collected at EOS were: average crop height and stem diameter, weed density by species (in 0.5 m2 quadrat), above ground weed biomass by weed species, and hemp fiber yield proxy (above ground biomass), among other measures. For this presentation, stem diameter, plant height, hemp and weed aboveground biomass, and weed counts were analyzed in R using 3-way or 2-way ANOVA for study 1 and 2, respectively. Preliminary analyses in study 1 (hemp management) suggest that the use of an herbicide program resulted in less weed competition and was associated with an increase in stem diameter, increased heigh in female plants, and increased above ground biomass. Narrow row spacing resulted in increased hemp stem diameter, and low seeding rate also resulted in increased stem diameter. Preliminary analyses of study 2 (CWFP) suggest that weed removal all season (87 days) was not significantly different than control treatments with no weed removal. In conclusion, hemp growth characteristics are influenced by management, and management programs may be optimized to achieve the characteristics that are desirable for various processors and market applications of hemp product. The CWFP study suggests that hemp is a highly competitive crop, which may suppress weed growth, if established ahead of or with emerging weeds.

# Trends Across the US: A Nationwide Survey of Diseases of Cannabis

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Hemp and marijuana, both *Cannabis sativa* L., are revitalized crops to U.S. agricultural and horticultural industries. Hemp ($\Delta^9$-Tetrahydrocannabinol content <0.3%) was reintroduced in 2014 under a pilot research program and legalized in 2018. Hemp can now be grown in all 50 states. Marijuana ($\Delta^9$-THC content > 0.3%), though classified as a Schedule I narcotic by the U.S. Drug Enforcement Administration (DEA), is legal in 37 states for medical and/or recreational use. Legalization for medical marijuana use in 1996 in California and recreational use in 2012 in Colorado and Washington initiated a series of state laws legalizing marijuana production. Thus, cannabis acreage increased during the past decade, with hemp peaking at over 200,000 ha in 2019. Likewise, marijuana yields reached 3M kg in 2021. Although *C. sativa* is often promoted as a pest-free crop, multiple diseases and arthropod pests have been identified and confirmed in recent years. There are limited options for control of diseases and pests affecting hemp. A survey of diagnosticians, researchers, and industry leaders conducted in 2021-22 sought to determine the distribution and occurrence of 76 common diseases and pests on *C. sativa* across the U.S. A total of 148 responses were collected and grouped by U.S. region: Western, Great Plains, North Central, Northeastern, and Southern. Survey results suggest that while some pathogens and pests are widely distributed across the U.S., others occur more frequently in specific regions, which may represent variations in economic importance. Among the widespread diseases of cannabis are Botrytis bud rot/stem canker, powdery mildew, and Pythium root rot. Diseases caused by *Cercospora*, *Alternaria*, and *Fusarium* were also reported in all regions. Viruses, viroids, and nematodes were more frequently reported in the Western and Great Plains regions compared to other regions of the U.S. While bound by the limitations of a respondent-driven survey and unequal regional representation, results from this survey nonetheless provide a foundation for regional and national prioritization of research and regulatory activities.

This survey was submitted to Plant Health Progress for publication. Authors include Misbakhul Munir, Kimberly Leonberger, Katelyn Kesheimer, Marguerite Bolt, Marion Zuefle, Emma Aronson, Magdalena Ricciardi, Craig Schluttenhofer, David Joly, Henry Smith, Jacqueline Coburn, Jose Franco Da Cunha Leme Filho, Silvia I. Rondon, Christine D. Smart, Alyssa Collins, Andrea Garfinkel, Nicole Gauthier.

# *Fusarium* species Threaten Postharvest Cannabis

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As hemp becomes established as a commodity in the US, continued cultivation results in pathogen build up and increased disease severity. This also demands a greater understanding of the pathogens that affect the consumable portions such as flowers and grain. Several Fusarium species that are known to produce mycotoxins have been confirmed pathogenic on hemp in Kentucky. Several of the resulting toxins are regulated in grains used for human and animal consumption (DON, NIV, T-2), therefore Fusarium management is critical. Determining which Fusarium species infect hemp is the first step to producing safe material. While several studies are ongoing regarding field disease, there have been no studies regarding stored hemp. Postharvest material can remain in barns or supersacks for months or years before processing. Harvested and stored floral material for production of cannabidiol (CBD) were collected from 17 field sites across Kentucky from 2019 and 2020 harvests. Material was screened using a Fusarium-selective medium and DNA sequencing. At least 13 different species were confirmed, including known mycotoxin-producers F. equiseti, F. graminearum, F. incarnatum, and F. sporotrichioides. Additional research is essential to determine pathogenicity of these species and whether they can produce toxins dangerous for humans and animals. Such information is crucial to determine how to store hemp, manage infected material, and promote successful production of hemp products.

# Hemp Crop Sampling and Compliance

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Sampling and compliance testing of hemp crops are necessary to distinguish legal hemp from illegal marijuana. The concentration of Tetrahydrocannabinol (THC) is the only difference between the two types of cannabis and THC levels are not detectable by sight, smell, or touch.

The Agricultural Act of 2018 (2018 Farm Bill), 7 U.S.C. 1639, legalized the commercial production of hemp on the federal level and defined hemp. *"The term 'hemp' means the plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol [THC] concentration of not more than 0.3 percent on a dry weight basis."* The 2018 Farm Bill also removed "hemp" from the controlled substances list and established the USDA Domestic Hemp Production Program.

Although hemp is a legal crop, it may only be grown in the United States in accordance with the USDA Hemp Final Rule (USDA rule), 7CFR 990. These federal regulations include the USDA sampling and testing procedures and the sampling and testing requirements that must be included in each state or tribal hemp plan. The USDA rule requires that all compliance samples be collected from the top five to eight inches of the main stem flower no more than 30 days prior to harvest. Additionally, the USDA rule standardized the requirement for total delta-9 THC to be reported by the labs on a dry weight basis and requires each lab to calculate a "measurement uncertainty" (MU) to be considered in determining the "acceptable hemp THC level" for each sample.

Along with standardizing some aspects of the sampling and testing, the USDA rule also allows for significant differences from state to state. The MU is different for each lab and potentially different for each sample. A state plan may incorporate a "performance based sampling" plan that would allow them to sample less than 100% of the crops based on seed certification, compliant varieties, research, past compliance of a producer, or other factors. The state plans are also allowed to be stricter than the federal program. Some states do not allow for remediation and retesting and the implementation of performance based sampling varies widely from state to state.

Kentucky has had a hemp program since 2014 and has the most extensive set of THC test data. Kentucky THC test results for the initial pre-harvest sample had a non-compliance rate of 5.2% in 2018, but with the introduction of more high-CBD strains and inexperienced producers, non-compliance rose to 28% in 2020, and is back down to 16% in 2022. Because every high-CBD strain of cannabis grown as "hemp" will produce more than 0.3% THC, if allowed to fully mature, producer experience (knowing when to harvest) and variety/strain selection are key to crop compliance.

With deliberations underway for the 2023 Farm Bill, there are expected federal changes ahead. There are political initiatives advocating for a 1% THC limit, a separate THC limit for the crop and hemp products, an exemption from testing for grain and fiber crops, and an exemption from testing of crops grown from certified seed. Although the USDA program only regulates the crop production, the definition of hemp is being applied to all finished "hemp" products as well. This definition designed for the crop has created a perceived loophole that is allowing for highly intoxicating and potentially dangerous THC products to be labeled as "hemp". This situation is gaining attention and is likely to lead to federal changes and possible federal regulation of hemp cannabinoid products.

In summary, the USDA rule has standardized some aspects of the sampling and testing of hemp, but still allows for countless differences from state to state such as performance based sampling. There is no national regulatory framework for finished products and it is becoming clear that applying the crop THC concentration of "hemp" to cannabinoid products is not acceptable for public safety. With the hemp industry still in its infancy, changes are expected for sampling and compliance.

# CBD Resistant *Salmonella* Strains are Susceptible to Epsilon 34 Phage Tailspike Protein

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The rise of antimicrobial resistance is a global public health crisis that threatens the effective control and prevention of infections. Due to the emergence of pandrug-resistant bacteria, most antibiotics have lost their efficacy. Meanwhile, the development of new antimicrobials has stagnated, which leads to the creation of new and unconventional treatments. Bacteriophages or their components are known to target bacterial cell walls, cell membranes, and lipopolysaccharides (LPS) and hydrolyze them. Bacteriophages being the natural predators of pathogenic bacteria are inevitably categorized as “human friends”, thus fulfilling the adage that “the enemy of my enemy is my friend”. Leveraging on their lethal capabilities against pathogenic bacteria, researchers are searching for more ways to overcome the current antibiotic resistance challenge. Bacteriophages are one of the most effective alternative therapies for multidrug resistant bacteria. We hypothesized that phage protein-CBD combination exhibits antibacterial activity against *S. typhimurium* and *S. newington*. In this study, we expressed and purified epsilon 34-phage tailspike protein (E34 TSP) from the E34 TSP gene, which was previously cloned into a pET30a-LIC vector, then assessed the ability of this bacteriophage protein in the killing of two CBD-resistant strains of *Salmonella* spp. We observed that the combined treatment of CBD resistant strains of *Salmonella* with CBD and E34 TSP showed poor killing ability whereas the monotreatment with E34 TSP showed considerably higher killing efficiency. The results suggest that phage-CBD does exhibit potent antibacterial activity against *S. typhimurium* and *S. newington*. This warrants further research and development of phage-CBD as a potential antibacterial agent.

# Effect of Outdoor and Controlled Environment Drying on Quality of Hemp Flower Grown for Cannabidiol

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Mature industrial hemp (*Cannabis sativa*) flower grown for cannabidiol (CBD) production was subjected to four different drying treatments and evaluated for cannabinoid profile, terpene yield, moisture loss and change in trichome morphology. Drying treatments included gravity oven drying at 40 $^{o}$C and 50 $^{o}$C, drying in an open-air barn in Watkinsville, Georgia, and in a controlled environment chamber maintained at 15.5 $^{o}$C and 60% relative humidity. Cannabinoid concentrations were not affected by drying treatment, but terpene yield was affected. Oven dried hemp flower had lower total terpene quantities than the barn and controlled environment dried hemp. For drying rates, oven dried hemp took an average of 26 hours at 40 $^{o}$C and 18 hours at 50 $^{o}$C, to dry from 73% to 10%, while and barn and controlled environment drying took 5 to 6 days to reach the same moisture level. Data suggests that differences in phenotype among varieties, specifically the density of leaves, influenced rate of drying. Scanning Electron Microscopy images were taken of hemp flower before and after drying. There was a noticeable difference in the morphology of multicellular stalked glandular trichomes between fresh and dried hemp, with the dried hemp exhibiting a desiccated appearance with trichomes more densely packed due to water loss. However, there were no visual differences in trichome morphology among drying treatments.

# Managing Two-Spotted Spider Mites, *Tetranychus urticae*, in Greenhouse Hemp in Alabama

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Alabama grew its first commercial hemp, *Cannabis sativa* L., crop in 2019 is scheduled to award its first medical marijuana, also *C. sativa*, licenses in 2023. Both hemp and marijuana are subject to high pest pressure from two-spotted spider mites, *Tetranychus urticae*. Two-spotted spider mites are especially problematic in indoor grow environments, but also plague outdoor hemp. Spider mites use their piercing-sucking mouthparts to feed on plant sap and feeding damage appears as stippling on the leaf surface. Heavy infestations can cause the entire leaf to become discolored, or even fall off. As their name implies, spider mites produce webbing on the plants and can significantly reduce flower yield and quality. Spider mites are able to grow their population exponentially due to their rapid life cycles and ability to reproduce parthenogenetically. Without proper scouting and monitoring techniques, growers may not realize there is a problem until infestations are very heavy. Therefore, we sought to identify effective chemical control treatments that Alabama growers can utilize for greenhouse hemp. We conducted two greenhouse trials at the Ornamental and Horticulture Research Center in Mobile, AL evaluating products that are approved for use on hemp in Alabama: Grandevo® (*Chromobacterium subtsuge* strain PRAA4-11 and spent fermentation media), Venerate™ XC (*Burkholderai* spp. Strain A396), BoteGHA® ES (*Beauveria bassiana* strain GHA), Sil-Matrix® (Potassium silicate), and an untreated control. Products were sprayed at the label rate on 7-day (Grandevo, BoteGHA, and Sil-Matrix) or 3-day (Venerate) intervals. The experimental design was a randomized complete block design with 10 replicates. In Trial 1, adult and egg counts were conducted on 3 cm leaf segments randomly selected from a top, middle, and bottom leaf of each plant. Damage was also assessed on a 0-5 rating scale. Grandevo® and Venerate™ maintained lower adult spider mite populations when compared to the control at 7 and 14 days after initial treatment, and had lower damage ratings at the end of the experiment. Egg counts did not differ significantly between treatments. In the second trial, mite counts were not done and only damage was assessed on a 0-5 scale. Venerate was the only product that had lower overall damage relative to the untreated control. Results of both trials demonstrated that Grandevo® and Venerate™ have potential to reduce mite populations and plant damage from an infestation of two-spotted spider mites. Future work will explore alternative integrated pest management strategies for indoor hemp, specifically biological control with predatory mites and insects.

# Trends Across the US: A Nationwide Survey of Insect Pests of Cannabis

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Hemp and marijuana, both *Cannabis sativa* L., are revitalized crops to U.S. agricultural and horticultural industries. Hemp ($\Delta^9$-Tetrahydrocannabinol content <0.3%) was reintroduced in 2014 under a pilot research program and legalized in 2018. Hemp can now be grown in all 50 states. Marijuana ($\Delta^9$-THC content > 0.3%), though classified as a Schedule I narcotic by the U.S. Drug Enforcement Administration (DEA), is legal in 37 states for medical and/or recreational use. Legalization for medical marijuana use in 1996 in California and recreational use in 2012 in Colorado and Washington initiated a series of state laws legalizing marijuana production. Thus, cannabis acreage increased during the past decade, with hemp peaking at over 200,000 ha in 2019. Likewise, marijuana yields reached 3M kg in 2021. Although *C. sativa* is often promoted as a pest-free crop, multiple diseases and arthropod pests have been identified and confirmed in recent years. There are limited options for control of diseases and pests affecting hemp. A survey of diagnosticians, researchers, and industry leaders conducted in 2021-22 sought to determine the distribution and occurrence of 76 common diseases and pests on *C. sativa* across the U.S. A total of 148 responses were collected and grouped by U.S. region: Western, Great Plains, North Central, Northeastern, and Southern. Survey results suggest that while some pathogens and pests are widely distributed across the U.S., others occur more frequently in specific regions, which may represent variations in economic importance. Almost all pests included in the survey were reported in all regions of the U.S., including flea and Japanese beetles, borers, thrips, whiteflies, fungus gnats, grasshoppers, spider mites, and several species of aphids and Lepidopterans. Regional differences were apparent with some pests, for example fire ants were frequently reported in the Southern and Western states but were not reported in the Northeast or North Central U.S. Beet leafhopper was restricted to the Western and Great Plains regions. Corn earworm, Helicoverpa zea, was the most reported Lepidopteran pest, but reports were especially high in the South with over 90% of respondents reporting damage. While bound by the limitations of a respondent-driven survey and unequal regional representation, results from this survey nonetheless provide a foundation for regional and national prioritization of research and regulatory activities.

This survey was submitted to Plant Health Progress for publication. Authors include Misbakhul Munir, Kimberly Leonberger, Katelyn Kesheimer, Marguerite Bolt, Marion Zuefle, Emma Aronson, Magdalena Ricciardi, Craig Schluttenhofer, David Joly, Henry Smith, Jacqueline Coburn, Jose Franco Da Cunha Leme Filho, Silvia I. Rondon, Christine D. Smart, Alyssa Collins, Andrea Garfinkel, Nicole Gauthier.

# Methodological Approaches to Combat Pests in Hemp: A Greenhouse Study

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Greenhouses are home to many pests that not only reduce plant vigor but also infect hemp. These insects act as vectors for plant viruses through mouthparts and production of honeydew which facilitates infection. A few examples of the pests that were looked at in this study were: two spotted spider mites, aphids and whiteflies.

This study highlights the importance of multi-faceted IPM strategies. IPM reduces damage by pests while simultaneously minimizing the damage to the environment and people. This sustainable form of pest management does include pesticide use, but only as a last resort. The treatments used were Neem oil, Canncontrol, and Blue Magic, all at high and low rates. Neem oil is a commonly used insecticide that is favored for its natural properties compared to conventional pesticides. The mode of action for Blue Magic and Canncontrol is repellency which is supposed to prevent insects from interacting with the plant. All treatments were applied as foliar sprays on the plants prior to introduction into bug dorms. Leaves were removed 3, 5, and 7 days after treatment to assess arthropod populations. Whitefly and aphid populations both declined by day 7, but this was not significantly related to treatment. Two-spotted spider mites were significantly affected by treatment. Blue Magic 4 oz had the most TSSM reduction, and showed a significant rate response. Arthropod counts were analyzed using a repeated measures analysis of variance (RMANOVA) in SAS 9.4.


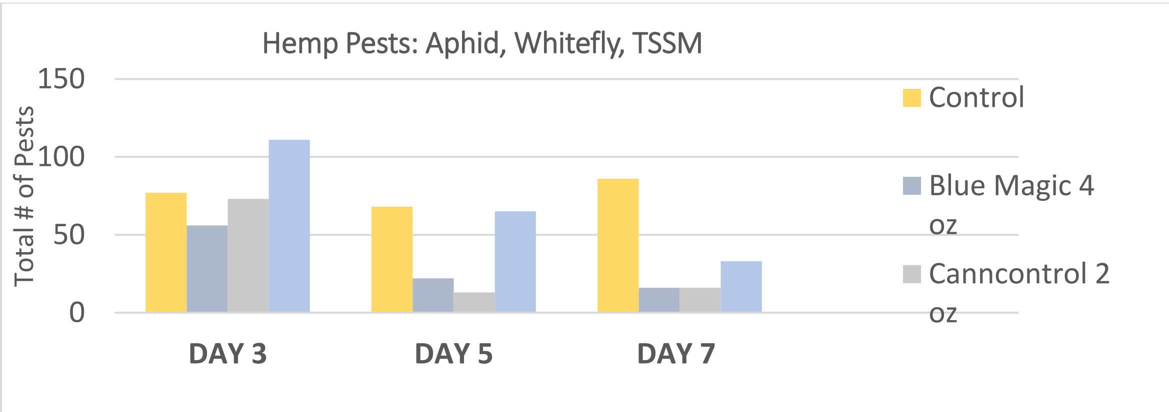


**Figure 4**. Most effective rates: Blue Magic 4 oz found to be most effective in reduction of pests.

# Densification of Hemp Floral Biomass Pre and Post-Extraction: Determination of Pellet Physical Characteristics

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The production of cannabinoids (CBD) derived products from hemp (Cannabis sativa L.) floral material is of great interest to the hemp industry. Hemp floral manifest a low bulk density which limits storage and handling options. Thus, hemp floral material both intact (pre-extraction) and supercritical CO2 extracted were pelleted to improve handling and storage characteristics. Intact floral material (BaOx) was passed through a hammer mill with a 5 mm screen; while, extracted floral material (Hawaiian Haze) was ball milled through a 60 mesh (250 microns) prior to extraction. Pelletization was conducted using a 3.7 kW pilot scale flat ring pellet mill. Prior to pelletization the floral material was condition to 10, 15, and 20% moisture content (MC). Across all initial MC, extracted floral pellets significantly increased pellet durability by 3%, elevated pellet yield by 4%, and increased bulk density by 14% when compared to intact floral pellets. However, extracted floral pellets required 3.6-fold more kWh/Mg of pellets produced and generated pellets at a 75% decreased rate when compared to intact floral material. For the extracted floral material, the 10% MC pellets were the most desirable; while, for intact pellets, the 15% MC pellets were the most desirable.

# Investigation of Critical Time for Managing Fusarium Head Blight (FHB) on Hemp Through Detection of Latent Infection of *Fusarium graminearum* Across Different Planting Dates and Plant Ages

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Fusarium Head Blight (FHB) caused by *Fusarium graminearum*, is an emerging disease on hemp in KY and across the US. Disease incidence and severity increase as the season progresses, but time of infection is unknown. This study was conducted to detect latent infection of *F. graminearum* on hemp planted at different planting dates and in various stages of development. In 2022, three hemp cultivars (CV) varying in flowering time (early, mid, and late-flowering) were planted at 3 different planting dates (PD) (April through July) with 4 replications in each planting date. Asymptomatic plant samples (plant tips, heads, or flowers) were collected 3 times in each planting date at plant age (PA) 3, 8, and 13 weeks after planting (WAP). Field environmental conditions throughout growing season were recorded. Samples were subjected to a *F. graminearum*-specific qPCR assay. Probability of positive detection across PD, PA, and CV were compared using binomial analyses. Positive detections were also plotted against field environmental conditions. Results concluded that PD, PA, and CV significantly affected the probability of positive detection (P<0.0001). Interaction of planting date and plant age (PD*PA) also significantly affected the probability of positive detection (P<0.0001). Detections were relevant to the environmental parameters affecting the pathogen life cycle. Detection of early infection of *F. graminearum* or at when symptoms have not been visually appeared in the field (latent infection) can help in determining critical time in managing FHB on hemp.

# Wild Wild West: Ecology and Management of Emerging Viruses of Hemp

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Hemp (*Cannabis sativa* L.) production has increased significantly in recent years; however, the crop has been understudied in the U.S. since its production declined in the late 1950s. Disease identification and management is an increasing challenge for hemp growers across the country. In 2019, beet curly top virus (BCTV) was first reported in hemp in Colorado. Hence, we were motivated to understand the diversity and prevalence of BCTV strains infecting hemp in Colorado. We detected BCTV at high incidence rate (81%) in leaf samples from 12 counties. We identified two different strains of BCTV, Worland (Wor) and Colorado (CO) present as a single or mixed infection in hemp leaf samples. Phylogenetic analysis revealed BCTV sequences from hemp formed a distinct group along with BCTV strains, CO and Wor. To determine other potential viral and viroid pathogens in hemp, we performed next generation sequencing (NGS). Virome analysis revealed the presence of both virus and viroid sequences that had high nucleotide sequence identity with GenBank accessions for cannabis cryptic virus, cannabis sativa mitovirus, citrus yellow vein associated virus, opuntia-like virus and hop latent viroid. In contrast, tobacco streak virus sequence was highly variable compared to sequences in GenBank suggesting a possible new genotype of this virus. The data presented here has important implications for the epidemiology and management of the various diseases of hemp and will lead to the development of integrated pest management strategies designed to interrupt transmission cycles and facilitate efficient crop production.

# Fungicide Application and Pathogen-Inoculation Alter Leaf Microbial Communities in Hemp

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Plants are naturally colonized by microorganisms that can be beneficial, neutral, or antagonistic to plant health. These communities of microorganisms within plants can be altered by abiotic or biotic conditions including treatment with biological agents or introduction of novel microbial species. Here we investigated if inoculation with fungal pathogens or treatment with fungicide alters the microbial community that colonizes hemp (*Cannabis sativa*) leaves. We hypothesized that plants inoculated with fungal pathogens would have a higher abundance of pathogens and altered microbial community composition compared to fungicide-treated and control groups. We also hypothesized that fungicide-treated groups would have the lowest abundance of microbes compared to control and pathogen-inoculated treatments. To test our hypothesis, field-grown T1 hemp was separated into three treatment groups: control, pathogen inoculation, and fungicide treatment. The pathogen inoculation consisted of the common fungal pathogens *Cercospera* sp., *Septoria* sp., and *Bipolaris* sp., and the fungicide treatment was applied throughout the growing season following standard agricultural practices. Microbial community composition was not different between treatment groups ($F_{2, 74} = 4.72$, $P > 0.99$) but Shannon diversity differed between treatment groups ($F_{1,2} = 14.20$, $P < 0.001$). Our results indicate that abundance of microbial species is altered by abiotic and biotic manipulations in hemp. Future work should investigate how community composition of microbial species in hemp leaves affects plant health, including hemp chemistry and physiology.

# Biocontrol Projects on Hemp (*Cannabis sativa* L.) Grown in Kentucky: Untangling Complexity

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The 2021 field scouts of Hemp (*Cannabis sativa* L.) trials of the University of Kentucky at the Robinson Research Center (Jackson) and Spindletop Farm (Lexington) revealed two consistent problems – Fusarium head blight (*Fusarium* spp.) and corn earworm (*Helicoverpa zea*) inflorescences damage. Considering the impact of these problems on the hemp production yield and quality, two trials were established during 2022 to increase our understanding regarding their characteristics and management. Biological products as control alternatives were studied in both trials (1) Bio-fungicide screening to control *Fusarium* spp. infestation in hemp for grain, (2) Corn Earworm (*Helicoverpa zea*) management in hemp for cannabinoids. In the bio-fungicide (1) trial we had 11 treatments established in plots of 90 $f^2$ and 4 replicates (4 blocks), testing four biological fungicides, one positive control (only water) and one negative control (Miravis Ace® – Syngenta) in two different application plans. The biological options were: Double Nickel® LC (Certis), Cease® and Milstop® in-conjunction (BioWorks), PlantShield® HC (BioWorks) and Lalstop G46® WG and LalStim Osmo® in-conjunction (Lallemand). Rates for all the treatments were established considering the label directions. Application plan one consisted of one application every 7 days starting at floral initiation and continuing until most female inflorescences set seeds (70% of seeds and 30% of flowers in each inflorescence). This plan resulted in a total of 5 applications. Application plan two consisted of only two applications targeting anthesis of the entire trial (higher level of pollen release and receptive female flowers) – visually estimated by constant scouting. Variables measured were (i) incidence and (ii) severity of potential *Fusarium* spp. symptoms in inflorescences (10 plants/plot), evaluated each week since flower initiation and at harvest we registered (iii) grain yield (g/plot). Consequently, off-season laboratory evaluation of the *Fusarium* spp. seeds infection between treatments (100 seeds/plot), with representative pathogenic specie identification using molecular techniques (gene sequencing - translation elongation factor 1-alpha (EF-1α)). The Corn earworm management study (trial 2) included 5 different treatments: two biological alternatives - Gemstar® LC and BoteGHA® ES applied together (CERTIS) and Javelin® WG (CERTIS); a negative control using Coragen® (FMC Corporation) and positive control with only water. Each of these were applied every 14 days after the first larvae observed and a second treatment using Gemstar® LC and BoteGHA® ES every 7 days after the first larvae observed. Corn earworm presence was evaluated every week since floral initiation (25 inflorescences/plot considering 5 infl./plant, randomly selected). At harvest corn earworm damage was evaluated as percentage of each inflorescence damaged (25 inflorescences/plot considering 5 infl./plant, randomly selected). The results of these trials will expand our understanding of the integral control of these two main problems; however, we understand that it is necessary to continue these studies. We are looking forward to evaluating the inclusion of additional application plans, hemp cultivars and studying the effect of different environment conditions (year-effect-weather conditions).


Figures: (A) Bio-fungicide screening to control *Fusarium* spp. infestation in hemp for grain field trial during flowering, (B) Corn earworm management in hemp for cannabinoids trial.

# Potential *Fusarium* spp. Infection of Hemp (*Cannabis sativa* L.) Seeds in Kentucky: Expanding Our Understanding of the Infection Process

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During the 2021 season *Fusarium* spp. (*Fusarium graminearum*, among other species) infection was registered in inflorescences (bracts, leaves, and stem portions) of field grown hemp for grain in Fayette and Breathitt County, Kentucky. At least 80% of inflorescences inspected during field scouts pre-harvest presented a disease symptom (necrosis or shriveling of floral parts). In order to study the potential *Fusarium* spp. seed infection, representative samples were taken when approximately 75% of the seeds in each inflorescence were brown in color. Specific sterilization procedure was developed and applied to the seeds and cultured onto Nash-Snyder modified selective media, which resulted in confirmed infection by one or more *Fusarium* spp.  The objectives of this study were to: (i) confirm *Fusarium* spp. infection using multi-gene sequencing and fungal morphology (measuring colonies developed onto PDA media) and (ii) identify the seed structures involved in the infection process. We consider that the information generated in this study would contribute to our understanding of the disease characteristics and consequently, to the improvement of the Kentucky hemp industry sustainability.

# Fiber Hemp in Tennessee: Preliminary Results for Variety Performance, Planting Dates, and Retting Time

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Field trials were conducted at the University of Tennessee Highland Rim AgResearch and Education Center in 2022. A planting date trial evaluated four planting dates (28-Apr, 12-May, 31-May, 13-Jun) using fiber hemp varieties Futura83 and Santhica70 replicated four times in a randomized complete block design. Data from 2022 suggest earlier planting dates, prior to mid-May, should be targeted as a dry yields decreased with later planting dates. Percent bast fibers ranged from 30-40% with planting dates between 28-Apr and 31-May, however, the last planting date (13-Jun) was around 60% bast fiber. The June planting date also resulted in reduced yield, which is likely related to percent bast.

A variety trial was conducted using 11 fiber hemp varieties. Varieties were planted on 23-May in a randomized complete block design and replicated four times. Varieties included Fibror79, Futura83, Bama, HanFN-H, HanFN-Q, HanNE, Muka, Puma3, Puma4, Yuma, and Yuma2. Plant height at harvest ranged from 1.43 to 2.72 m, with Puma4 as significantly tallest and Muka as significantly shorter. Dry yield ranged from 0.34 to 4.78 tons/acre, with Yuma2 having significantly highest yield. Early maturing varieties with origins from Europe were over mature at time of harvest, whereas Chinese varieties were not.

A field trial evaluated retting time (0 – 28 d) in a randomized complete block design with four replications. The trial was established on 29-Apr using Futura83. Data were not collected for quality as metrics were not well established for performing quality evaluations at the time of this trial. Visually, bast fibers separated from hurd well in between the 7- and 14-day intervals. Fresh yield was 11.57 tons/acre at the time of harvest and reduced to 4.87 dry tons/acre after drying. After 7-days, yield ranged from 4.25 to 4.78 dry tons per acre. Bast fiber yields ranged from 1.43 to 1.76 dry tons per acre for the entire trial.

# A Survey of Hemp Diseases in Oregon and Washington Between 2021 and 2022

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Hemp (*Cannabis sativa* L.) grown in Oregon and Washington for flower, seed, and fiber is valued at $249 million. Growers face production challenges due to plant pathogens. An assessment of pathogen impact on hemp production has not been conducted for the Pacific Northwest (PNW). In 2021, a two-year survey was initiated to determine disease occurrence in PNW hemp crops. Fields were evaluated one to three times during the growing season. In 2021, 32 fields from Oregon and 11 fields from Washington were surveyed. In 2022, 25 fields in Oregon, and 8 fields in Washington were surveyed. One or two 100-plant transects were evaluated depending on field size. Each plant was visually examined for signs or symptoms of bacterial, fungal, oomycete, or virus-like diseases. Representative samples of suspect diseased tissue were collected for pathogen identification by morphological characteristics or genetic typing. Plant-parasitic nematode population densities were also considered from soil samples collected in autumn from 9 OR and 9 WA fields in 2021, with additional evaluations of all fields in 2022 in both the early season and late season sampling. In 2021, virus-like diseases were suspected in 75% of OR and 45% of WA fields. Worland-like strains of beet curly top virus have been confirmed via PCR from 38% and 91% of suspect OR and WA fields, respectively. Additional testing for other virus and virus-like organisms indicates that hop latent viroid and phytoplasmas are also detectable in hemp grown in the PNW. Late in the season, bud rot was found in 25% of OR and 72% of WA fields. Pathogen identifications are still underway for 2022 samples, but preliminary results from this survey indicate the presence of multiple pathogens that may impact hemp and other crops in the PNW landscape. This work will generate foundational data of yield-limiting diseases in PNW hemp.

# Hempseed as a Food Material: Carbohydrate Content in Edible Grain

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Hempseed has been used as a human food since ancient times. Recently, interest in incorporating hempseed as a food ingredient has grown, in part due to the nutrient composition of hempseed. Hempseed is rich in protein, fat, and dietary fiber. Hempseed protein has a good amino acid profile; however, it is only moderately digestible in the whole seed form, with dehulling having a beneficial effect on digestibility. Hempseed oil is very high in polyunsaturated fatty acids with a favorable omega-6: omega-3 fatty acid ratio. Compared to other seeds, hempseed is very low in carbohydrates digestible by human enzymes such as starch or free sugars. Instead, the majority of hempseed carbohydrates are classified as dietary fiber, e.g. indigestible by human enzymes.

Relatively little is known about the dietary fiber carbohydrates of hempseed. As dicots, they are expected to be rich in xyloglucans and xylans as their hemicellulosic carbohydrates and to have higher pectin levels than observed in monocotyledonous plants like cereal grains. Together, hemicellulosic carbohydrates and pectins help build a mesh network that tethers cellulose microfibrils and lignin polymers in the plant cell wall and creates structural strength. Differences in the carbohydrate composition of plant materials such as hempseed affects their fermentation behavior by both human and animal gut microbes and ruminant microorganisms, therefore, we have undertaken a detailed carbohydrate characterization of hempseed dietary fiber.

Hempseed fiber was isolated by defatting and destarching, and the monosaccharide composition was determined by sulfuric acid hydrolysis followed by separation and detection with a high-performance anion-exchange chromatography system incorporating pulsed amperometric detection (HPAEC-PAD). The branching pattern of the hempseed fiber was assessed by generating partially methylated alditol acetates (PMAAs) and examining their fragmentation pattern using GC-MS. Screening for unique arabinan, galactans, and xyloglucan structures was performed by enzymatic hydrolysis followed by HPAEC-PAD-based separation, detection, and quantification. Preliminary results show that hempseed cell walls are rich in cellulose and 1-4-linked xylans and contain small amounts of branched pectins. Future studies will screen carbohydrate structures of a hemp variety panel and explore the carbohydrates' fermentation properties.

# The Effect of Exogenously Applied Methyl Jasmonate on Glandular Trichomes of *Cannabis sativa* L.

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Most cannabinoids produced by Cannabis sativa L. are accumulated in glandular trichomes. Therefore, cultivation methods which increase glandular trichome density could increase yield quality. Glandular trichomes are also associated with ecological defense mechanisms in plants. As the plant growth regulator methyl jasmonate (MeJa) is involved in regulating plant defense responses, we hypothesized that exogenously applied MeJa would increase trichome density and thus potentially increase cannabinoid concentration in floral biomass. Our study investigated potential effects of various application rates of MeJa on trichome density in three cannabidiol (CBD) cultivars using a complete block randomized design. Three cultivars, ‘Cherry Citrus’, ‘Baox’, and ‘Super CBD’, were planted in a plasticulture production system with drip irrigation. Treatment applications of MeJa began at flowering stage with two additional applications biweekly. Treatments consisted of five concentrations of MeJa as well as one control, a carrier-only treatment of water + 0.1% (v/v) Tween-20. Concentrations were 0.125 mM MeJa + 0.1% Tween-20, 0.25 mM MeJa + 0.1% Tween-20, 0.5 mM MeJa + 0.1% Tween-20, 1 mM MeJa + 0.1% Tween-20, and 2 mM MeJa + 0.1% Tween-20. Two subtending leaflets from the top 6 cm of the terminal inflorescence from each plant were collected. Each leaflet was photographed using a camera connected to a dissecting microscope, and then the trichomes from each image were counted within a 1 mm2 area using the software ImageJ. The goals of the study were to 1) evaluate the effects of MeJa on trichome density, cannabinoid levels, and plant growth and yield parameters, and 2) identify the optimal application rate. There were no significant differences in trichome density between the treatments of MeJa. However, there was a difference in the trichome density between the three cultivars, where ‘Cherry Citrus’ had a higher trichome density as compared to ‘Baox’ and ‘Super CBD’. This may suggest higher cannabinoid content in floral biomass of ‘Cherry Citrus’ than the other cultivars, irrespective of the concentration of MeJa received. Sample processing for cannabinoid concentration is ongoing to address the additional study goals.

# A Survey of *Fusarium* spp. Associated with Fusarium Head Blight of Kentucky Hemp

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Hemp is a multipurpose crop that is cultivated for a variety of cannabinoids, fiber, and/or grain. An emerging threat to hemp production is Fusarium head blight (FHB). In response, a statewide survey was conducted during the 2022 growing season to determine incidence across the state and identify the distribution of causal species. A total of 25 fields were surveyed across 15 counties; 15 of the fields were sampled twice and 3 were sampled three times. Surveyed fields ranged from backyard ¼ acre plots to 200-acre plastic bed commercial operations. Thirty subsamples from floral tissues were taken from each field. A total of 441 *Fusarium* isolates were collected from 1380 collected samples, resulting in a 32% isolation rate. Each *Fusarium* isolate was categorized based upon morphological characteristics including pigment production and mycelial structure. Representative isolates from each group were identified by DNA sequencing. Isolates included species within the Fusarium *fujikuroi, incarnatum-equiseti, lateritium, oxysporum, sambucinum,* and *solani* species complexes. Many members of these species complexes are known mycotoxin producers, especially *F. graminearum* and *F. sporotrichioides* contained within the *sambucinum* complex. Future research is directed at understanding parameters that impact infection and subsequent disease development.

# Fusarium Head Blight: An Emerging and Potentially Devastating Disease on Hemp

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Fusarium head blight (FHB) caused by *Fusarium graminearum*, is an emerging disease on hemp in Kentucky and across the US. *Fusarium* species, including *F. avenaceum*, *F. sporotrichioides*, *F. graminearum*, and *F. incarnatum-equiseti* species complex, have been reported to be causal agents of FHB of hemp in Kentucky and in other regions in the US. Yield losses can be caused from blight/necrosis and from potential mycotoxin contamination.

<u>Mycotoxin testing.</u> Using commercial immunoassay kits, we detected and quantified DON and T2 in floral and grain hemp collected from research trials and commercial fields in Kentucky in 2021 and 2022. In samples positive for *F. graminearum*, DON levels ranged from 0.57 ppm to 6.1 ppm in flowers from hemp grown for cannabinoids and 0.2 ppm to 1.5 ppm in seeds from hemp grown for grain. In samples positive for *F. sporotrichioides*, T2 toxin levels were as high as 263 ppb in flowers from floral hemp and 164 ppb in seeds from grain hemp. There was no mycotoxin detection in some grocery store hemp food products such as hemp hearts and chocolate candy. Future study is aimed to validate these detection and quantification results using a more sensitive and accurate method (e.g., HPLC-MS).

<u>Detection of latent infections.</u> Our study also focused on investigating time of infection of *Fusarium* species causing FHB on field hemp and the dynamic of pathogen populations in fields. Using qPCR, we detected multiple *Fusarium* species on asymptomatic hemp tissues in research fields. While investigating the effect of planting dates and plant developmental stages on latent infection of FHB causing species, we found that infection was consistent with environmental conditions (temperature, moisture) affecting pathogen life cycles. Final results of this study are expected to inform and improve FHB management strategies on hemp.

# Suppression of Hemp Russet Mite, *Aculops cannabicola* (Acarina: Eriophyidae) in Industrial Hemp in Greenhouse and Field

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Hemp russet mites, *Aculops cannibicola* Farkas (Acarina: Eriophyidae), is a primary pest of concern among hemp growers. Hemp russet mite feeds primarily on new growth and can reach high densities, frequently exceeding a thousand mites per leaf. The most serious damage occurs when the developing flower buds are infested, leading to a decrease in CBD yield. The objective of this experiment was to determine the efficacy of commercially available foliar insecticides in managing hemp russet mites in a greenhouse and field. Hemp (var. Unicorn) was exposed to leaves heavily infested with hemp russet mites, and once mite densities reached an average of 50 mites per leaf, the following insecticides were applied to the plants: abamectin, etoxazole, fenpyroximate, rosemary oil, two concentrations of a mineral oil, and a mixture of the concentrations of the mineral oil and potassium bicarbonate. An application of sulfur was also added to the field experiment. Treatments were replicated nine times in the greenhouse and six times in the field. Each of the pesticides significantly reduced hemp russet mite densities in the greenhouse, with all treatments resulting in significant decrease in mite populations 27 days after initial treatment. On the other hand, only fenpyroximate, sulfur, and rosemary oil provided strong and effective suppression of the mites in the field. These outcomes indicate that several pesticides available for organic crop production can provide effective control of hemp russet mites.

# Detection of *Fusarium* spp. Across Planting Dates and Plant Ages of Hemp

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Fusarium head blight (FHB) is an emerging disease on hemp in Kentucky and across the US. Four *Fusarium* species including *F. avenaceum*, *F. sporotrichioides*, *F. graminearum*, and *F. incarnatum-equiseti* species complex have been reported to be associated with FHB in hemp in Kentucky. This study was conducted to detect latent infection of the four aforementioned FHB causing species on hemp planted at different planting dates and in various stages of development. In 2022, three hemp cultivars (CV) varying in flowering time (early, mid, and late-flowering) were planted at 3 different planting dates (PD) (April through July) with 4 replications in each planting date. Asymptomatic plant samples (plant tips, heads, or flowers) were collected 3 times from each PD at plant age (PA) 3, 8, and 13 weeks after planting (WAP). Samples were subjected to four species specific-qPCR assays to detect the four aforementioned FHB causing species. Probability of positive detection across PD and PA were compared using binomial analyses. Results concluded that probability of positive detections among species were not significantly different at the earliest PD (PD1, Apr 22) ($P=0.0960$). However, probability of positive detections among species were significantly different at PD2 (May 31) ($P=0.0175$) and PD3 (Jul 16) ($P<0.0001$). At both PD2 and PD3, *F. graminearum* and *F. incarnatum-equiseti* species complex had consistently higher probability of detection compared to *F. avenaceum* and *F. sporotrichioides*. When detection probability among species were compared across PD and PA, *F. graminearum* and *F. incarnatum-equiseti* species complex showed higher probability compared to *F. avenaceum* and *F. sporothrichioides*. This one-year, single field study suggested that *F. graminearum* and *F. incarnatum-equiseti* are the predominant species in the experimental field across PD and PA. Further studies are needed to investigate factors influencing prevalence of species within a field throughout a growing season and to determine whether species prevalence varies among fields.

# Evaluating Fertility and Biological Control on CBD Yield in Outdoor Hemp

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Hemp, Cannabis sativa L, is one of the oldest cultivated plants. Following the Marihuana Tax Act and other restrictive laws, hemp has not been grown commercially for decades. The 2014 and 2018 Farm Bills legalized hemp production in the United States. Over the last 7 years we have seen a resurgence primarily because of its byproducts, including cannabidiol (CBD). CBD can use for human consumption and is often sold as a wellness product. As a result, there is pressure on growers to use fewer chemical pesticides. Without this information, growers may apply costly inputs without a return on their investment. Multiple insect pests feed on different parts of a hemp plant, but the most damaging outdoor pest is corn earworm, *Helicoverpa zea* Previous research has shown that management strategies such as fertility and biological control may influence insect damage and yield crops such as cotton and hops. The objective of this study is to identify the potential of cultural and biological control strategies on reducing pest-based losses in CBD hemp. We conducted two field experiments to evaluate these strategies in outdoor hemp at the E.V. Smith Research Farm in Shorter, AL. In one experiment, hemp variety BaOx was grown with 0, 51, 76, 109, or 164 lbs/acre of nitrogen applied at planting in a second experiment, four different species of wildflowers were used: purple coneflower (*Echinacea purpurea*), butterfly milkweed (*Asclepias tuberosa*), red shade yarrow (*Achillea millefolium*), and black-eyed- susan (*Rudbeckia hirta L.*) Treatment included individual species of wildflowers, a mix of all four species, and untreated control. In both experiments, caterpillar damage was conducted weekly. For harvest both experiments hemp plants were taken from each plot on the same day. The results for the experiments showed that nitrogen or wildflowers plot did influence insect and yield. Furthermore, the results will discuss the effects of nitrogen and biological control on insect damage and yield to identify potential management strategies for growers.

# Impact of Microbial Inoculants on Hemp Growth, Cannabinoid Concentration, and Terpene Concentration

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Manipulation of the *Cannabis sativa* soil and plant microbiota by the addition of microbial inoculants [plant growth-promoting rhizobacteria (PGPR), biocontrol fungi, and/or mycorrhizae] has the potential to increase plant yield and alter secondary metabolism. Even though many growers believe that the addition of these products results in superior yields, there have been few controlled studies. The objective of this study was to determine if addition of microbial inoculants impacted hemp growth and secondary metabolism. Seed for 'Alpen Gleaux', an autoflowering variety of *C. sativa* (high CBD) (High Alpine Genetics, Glenwood Springs, CO). Seed were pre-germinated in moistened paper towels, then planted in 5-gallon pots (1 seed/pot). Experiment was designed as a RCB with 4 treatments (Fig. 1) and 11 blocks. Three biostimulant products, selected based on grower recommendations, were applied according to label directions. Great White Premium Mycorrhizae is a complex mixture containing three species of ectomycorrhizal fungi, 12 species of beneficial bacteria, one species of *Saccharomyces*, and two species of *Trichoderma*. Azos contains a single species (*Azospirillum brasilense*). Mykos consists of a single species of *Rhizophagus intraradices*. Approximately 80 days after germination, bud was harvested, dried, trimmed, and weighed. There were no differences among treatments for plant weight (data not shown), cannabinoid concentration (Fig. 1), or terpene concentration (Fig. 2). In all treatments, concentration of CBD was approximately 8% of the concentration of CDBA, and levels of CBGA were minor components. Because there was also no evidence of mycorrhizal colonization on selected root systems, these data should be interpreted with caution; more research is needed before recommendations are considered. Funding for AV and CH was provided by the USDA –ELI (TEN2017-06430).

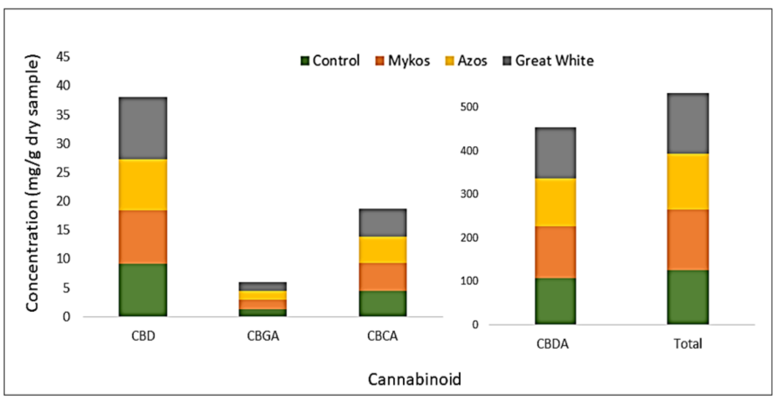


Figure 2. Concentration of cannbinoids in dry harvested flowers. Microbial treatment did not alter cannabinoid concentration as determined by LC-MS (P>0.05).

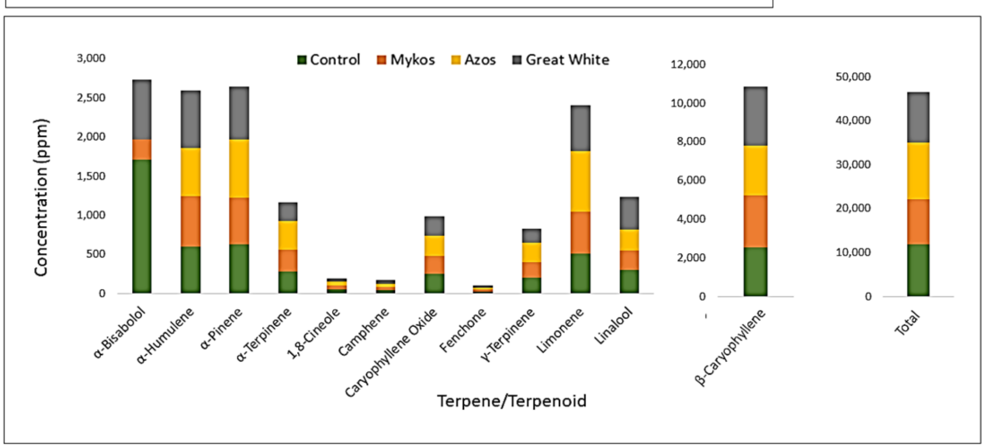


Figure 2. Concentration of terpenes in dry harvested flowers of 'Alpen Gleaux'. Microbial treatment did not alter terpene concentration as determine by GC-MS

# Impact of Planting Date and Seed Depth on Establishment, Growth, and Grain Yield of Hemp in Kentucky

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The urgency to investigate industrial hemp as a crop increased with the 2018 U.S. "farm bill" that removed hemp from the Schedule I listing, potentially opening-up the hemp market in Kentucky and throughout the country. Grain or dual-purpose industrial hemp has a moderate return potential with several possible end uses. In the first few years of hemp research, many have found inconsistent establishment and growth with direct-seeded industrial hemp. To improve the economic potential of hemp for growers, it is essential that we determine the optimum planting date and depth for hemp to produce consistent stand establishment and improve yields. This trial took place at the University of Kentucky's Agricultural Research Farm in Lexington, KY. Historically, this farm contains a silt-loam soil, a soil type identified as ideal for industrial hemp production due to its efficiency in drainage and potential for high organic matter content. This study was a randomized split block design where each main treatment block for six different planting dates ranging from early April to late June every two weeks were split in two randomized plots for cultivar (Bialobrzeskie- Polish origin, and NWG 2730 - U.S. origin). Each plot is split into two subplots for planting depth with a shallow (0.25-0.5") and deep (0.75-1") planting depth. Each block was randomly replicated four times with enough buffer space between blocks to get into plots at the different planting dates (n=96). Planting rate was targeted at 680,000 live seed per acre (adjusted using preliminary germination test) which computes to 20-25 lbs/acre for the chosen cultivars. Seeds were planted using a Great Plains 3P606NT Drill modified by Kincaid Manufacturing with a Zero-Max Cone Drive seeder at 7.5" row spacing. Soil moisture was determined at planting as many studies found that soil moisture played a role in the ideal planting depth. After emergence, plants were fertilized with 150 pounds of N per acre as Urea (46-0-0). Stand count data determined establishment, height data monitored growth, and yield was determined by hand harvest and seed separation using a Wintersteiger LD 350 rotary lab thresher. Additionally, data was collected on initial flowering date and male:female flowering ratios. It has been noted that bird predation may also be playing a role in field germination and has been observed at the time of planting and during seed set. Comparing 2021 and 2022 stand count data showed that the effect of seeding depth is greatly influenced by precipitation. Emergence was increased in the deeper planting depths in both 2021 and 2022 in the April and May planting dates, but the emergence of the June planting dates was influenced by rainfall in June. Grain yield results from 2021 showed that the high June precipitation increased yield of later planting dates, especially in the deep planting depths (shallow plots were washed out). The 2022 grain yield data reflects the very dry conditions where overall yield was reduced from 2021. Generally, the deep planting depths had higher yields than the shallow. Population (at emergence) did not correlate with overall grain yield. With still so many inconsistencies, planting date and depth of hemp needs to continue to be evaluated across the country to determine differences affected by changes in soil and climatic conditions.

# Can a Sweet Corn Trap Crop Contribute to a Floral Hemp Yield?

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Corn earworm, *Helicoverpa zea*, is an insect pest that feeds on a variety of crops. One of the main crops corn earworms are attracted to is sweet corn in its silking stage. Another host is hemp grown for CBD, *Cannabis sativa* L. In hemp, corn earworms feed on the flower buds which is the marketable portion of the plant. Damage in flower buds can create open wounds which have increased susceptibility to pathogen infections that cause yield and quality loss. Due to the lack of pesticides allowed for use in hemp, exploring different management strategies is crucial to control corn earworms. Therefore, this experiment sought to examine the potential for a sweet corn trap crop to decrease yield loss in hemp. VipA20, transgenic sweet corn with *Bacillus thuringiensis* was used because its toxins have an antibiosis effect on corn earworm larvae causing death after feeding. Sweet corn was planted in staggered plantings in border rows around hemp plots for oviposition and development of corn earworm in sweet corn. Five treatments were used, a control with no trap crop and four planting dates each 7 days apart in a replicated complete block design. Corn earworm eggs and larvae were assessed from July to September in EVS Research Station. Then at harvest, wet and dry weights of three hemp plants per plot were conducted. Random buds from three hemp plants were collected from the upper, middle, and lower parts of the plant, using a 0-3 damage scale these buds were identified as marketable or unmarketable. Results show a tendency in the data between planting dates showing how the first planting date had a greater number of eggs oviposited in hemp, resulting in greater damage and unmarketable buds in yield.

# Minute Pirate Bugs and Phytoseiids, What Are They Doing in Hemp?

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Summary. Preliminary observation had linked the populations of minute pirate bugs (*Orius insidiosus*, Hemiptera:Anthocoridae) to the presence of aphids (*Phorodon cannabis*) and hemp russet mites (*Aculops cannabicola*, Acari: Eriophyidae) in hemp grown in open fields and indoors in Kentucky. As well, an unknown species of a predatory phytoseiid mite was observed in hemp buds infested with hemp russet mites. The minute pirate bug is a generalist polyphagous predator that preys on all stages of aphids, eggs and early stages of lepidopterans, thrips, whiteflies, and mites. After hatching, minute pirate bug nymphs are whitish to yellow, and as they develop their color change to orange or red-brown, and the adult is black. All these instars were observed here. Phytoseiids have been associated preying upon whiteflies, thrips and spider mites rather than russet mites.

To tally minute pirate bugs, hemp aphids and hemp russet mite 15-cm hemp shoot with flower buds (*n*=10) were used (Fig. 1). Single buds were washed in 250 ml of 50% ethanol and transferred into a 12-cm diameter by 2-cm deep Petri dish (Figure 1). Positive regression trends were observed between minute pirate bugs (Figure 2a) vs. aphids, and minute pirate bugs vs. hemp russet mites (Figure 2b) although the $R^2$ were low in both cases. Phytoseiids also were observed on the ethanol washed hemp buds however, the numbers were low, we found less than 5 phytoseiids. The presence of phytoseiids were tallied searching in shoots in plants grown outdoors and indoors but the numbers were low (Figure 3). In both cases the only preys observed in these fields at the time the samples were taken, were hemp russet mites and aphids in indoor conditions, and hemp russet mites and corn earworm in outdoor conditions. Under the conditions studied in 2022, minute pirate bugs might played an important role to regulate aphids or can be an important natural enemy to control this pest in indoor grown hemp. Minute pirate bugs might be preying upon hemp russet mites but as it is shown in Figure 2b, hemp russet mites were high> than 1000 in all cases, hemp russet mites may be a complementary source of food for this predatory species. Phytoseiids were observed indoor and outdoor however, the species present in this system need to be identified and their role to reduce hemp russet mites need further studies.


Figure 1. Hemp flower shoot and Petri dish. Hemp russet mites were counted on 1/16 of dish (orange area) and Orius and aphids in entire disk/shoot

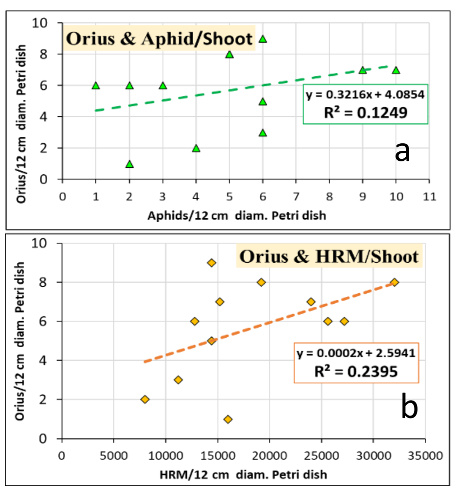


Figure 2. Regression of *O. insidiosus* vs. (**a**) hemp aphids or (**b**) hemp russet mites per shoot washed in 50% ethanol

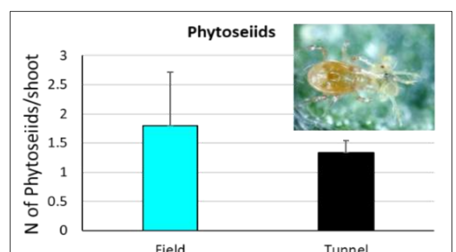


Figure 3. Numbers of phytoseiids /hemp shoots in untreated hemp plants grown in field or tunnel.

# Hemp Russet Mite Control Using Organic and Conventional Acaricides

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SUMMARY. Field tests were conducted to evaluate the efficacy of plant based, biological, mineral oils and conventional acaricides to control the hemp russet mite (HRM) in 2021 and 2022 (Table 1). Study was conducted at the University of Kentucky's Research and Education Center at Princeton, KY. Plots had 3 rows with 4 plants per row (12 plants per plot) and replicated 4X (Figure 1). The cultivar used was BaOX. The pesticides are shown on Table 1.

Two sprays, 7 d apart, were conducted with the respective pesticides on each plot, except the conventional acaricides that were applied only once. All solutions were calculated using 100 gal of water/A. Water was used as control. Tallies were conducted using digital images (Figure 2). The most effective compounds were the conventional acaricides ABBA Ultra, Portal Fujimite, and Kanemite. BoteGHA and BW280 1% were also effective reducing HRM populations in 2021 and 2022. The acaricide Stifle SC used in 2021 did not suppress HRM populations, similar results were obtained with Green Cleaner, TetraCURB MAX and Debug Optimo. For BoteGHA and BW280 1% a second application reduced HRM populations. Additional application every 7 to 10 day may improve the efficacies of plant- based, biologicals, and oils to reduce HRM populations.


Figure 1. Hemp cv. Baox: 3 rows, and 4 plants/row, 4X.

Table 1. Rates of pesticides used in 2021 and 2022. Plant-, biological-, mineral oils-based, and conventional acaricides.

| Type | Commercial name | A.I. | GPA H2O | Rate | 2021 | 2022 |
|---|---|---|---|---|---|---|
| | Control | - | - | - | ✓ | ✓ |
| Plant based | Green Cleaner® | Soybean oil +Sodium Lauryl Sulfate | 100 gal/A | 0.5 fl.oz | ✓ | - |
| | TetraCURB® MAX | Rosemary oil 50% | 100 gal/A | 128 fl oz | ✓ | |
| | RM-1964K® | Edible blended oils | 100 gal/A | 41.5 fl oz | - | ✓ |
| | Debug Optimo® | Fats and Glyceredic oils, Margosa (15.4%) + azadiracthin (0.7%) | 100 gal/A | 32 fl | ✓ | - |
| Biological | BoteGHA™ ES | *B. bassiana* strain GHA (11.3%) | 100 gal/A | 1 qt/acre applied | ✓ | ✓ |
| Mineral oil | BW280® 1% | Mineral Oil 80% (SuffOil-X) | 100 gal/A | 94.6 mL BW280 in 2.52 Gal $H_2O$ | ✓ | ✓ |
| Conventional acaricides | ABBA®ULTRA | Abamectin | 100 gal/A | 8 fl oz | ✓ | ✓ |
| | Fujimite® SC | Fenpyroximate | 100 gal/A | 3 pts | ✓ | - |
| | Stifle® SC | Etoxazole | 100 gal/A | 6 fl oz | ✓ | - |
| | Porta®l XLO | Fenpyroximate | 30 gal/A | 3 pints/100 gal | - | ✓ |
| | Kanemite®5SC | Acequinocyl | 30 gal/A | 31 fl oz/A | - | ✓ |

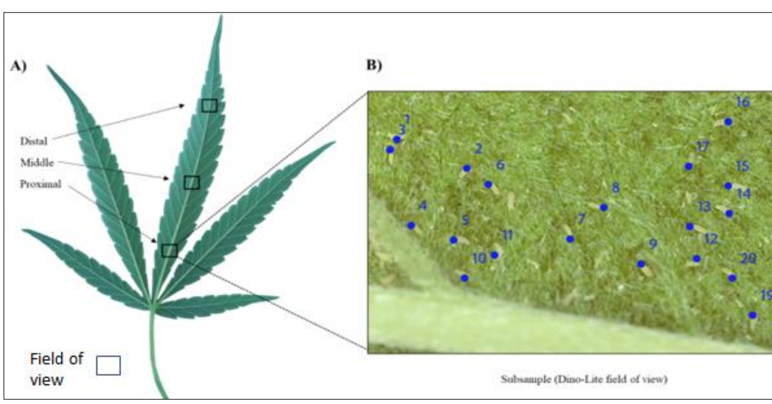


Figure 2. Image-based mite counting showing, A) selected central leaflet sampling unit with subsamples (squares) at proximal, middle, and distal sections, and B) example of marked and labeled mites using the Adobe Photoshop counting tool (numbers and dots).

# Hemp, Elvis, and Luke the Drifter

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Hemp (*Cannabis sativa* L.) has been a vital agricultural commodity, primarily as a fiber, at critical times in the United States, including its settlement and establishment, the mid-1800s, WWI, and WWII. Due to availability of more economical and suitable fibers for specific applications, combined with legislation that repressed and later banned hemp production, hemp was not grown commercially in the U.S. for nearly 60 years. When hemp was authorized by the Agricultural Act of 2014 to again be grown in the U.S., hemp was primarily regarded as a fiber and oilseed crop. Shortly thereafter, cannabis strains were identified that produced high levels of cannabinoids yet met the criteria for hemp as stated in federal and state laws, and legal cannabinoid production became the primary reason for hemp cultivation in the U.S. Because hemp evolved from not only a food, feed, and fiber crop, but also a source of cannabinoids, it now serves as a seldom-acknowledged vanguard for all cannabis in USDA-funded, university-led research, teaching, and outreach relating to production, market preparation, product safety and development, and effects of consumption on animals and humans. A positive impact of this will be an accelerated abundance and availability of sound scientific findings on cannabis that can be used to improve public health, wellness, safety, and policy as it relates to cannabis.

# Comparing the Agronomic Yield Potential of Industrial Hemp Varieties Grown in the United States.

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Industrial hemp is a multipurpose crop used by ancient civilizations as early as 10,000 BCE for fiber, grain, and flower . Although hemp genotypes are reported to have been cultivated in diverse environments and climatic conditions, regional evaluations on crop performance are crucial for its adoption as an agricultural commodity. The aim of this study is to compare harvest yields from varying hemp genotypes grown in the United States and assess their suitability for production across environments. Governed under the USDA-NIFA Hatch Program, the S-1084 Multistate Research Project involves academic collaboration amongst 30 institutions nation-wide. In 2019, varietal trials were established to test the suitability of thirteen certified hemp genotypes planted yearly during the summer growing season across participating locations. Initial yield performance for straw and grain averaged 1,600 kg/ha and 700 kg/ha while 2021 yields averaged 3,050 kg/ha and 815 kg/ha, respectively. The data suggests that hemp genotypes are better suited for north latitude conditions but historical breeding of industrial hemp varieties from Europe and Asia may explain hemps preference for those climate regions. Further investigation on genotype x location x year interactions can help scientist and plant breeders understand hemps physiological response to environmental factors and climatic conditions. Findings from the S-1084 project may provide stakeholders with the scientific evidence needed to evaluate hemp's suitability for production in the U.S.

# Influence of Fungicide on Hemp Rhizosphere Microbial Composition and Enzymes

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Microorganisms and enzymes play an important role in the soil and plant rhizosphere ecosystem's function. Soil microbes and enzymes transform organic matter, release nutrients, and degrade xenobiotics through biologically and biochemically mediated processes. However, the application of pesticides may damage microbes, destroy the soil ecosystems, and decrease microbial diversity and abundance in the plant rhizosphere due to the adverse effect of pesticides on microbial proliferation, biotransformation, nitrogen fixing, phosphorus solubilizing, and other functions in the soil. Currently, there are few studies on how different fungicides affect hemp plant rhizosphere microbial diversity and abundance, and enzymatic activities. We hypothesized that fungicides and different hemp cultivars impact the diversity and abundance of microbes, and enzymes in the hemp rhizosphere. In this study, we collected soil samples around hemp rhizosphere of four hemp cultivars (Otto2, BaOx, Cherry Citrus, and Wife) with three different fungicide treatments (natural infection, fungal inoculation, and fungicide-treated). DNA was extracted from the samples, 16S rDNA was sequenced, and data were analyzed for diversity and abundance at different treatments and hemp cultivars. The results show that fungicide alters the diversity and abundance of the hemp rhizosphere microbial community. The abundance of the phyla Archaea and Rokubacteria was highly increased. In contrast, those of the phyla Proteobacteria and Gemmatimonadetes were highly decreased in treatments with fungicides compared to those without fungicide in the four hemp cultivars. In addition, hemp cultivars affected the diversity and abundance of rhizosphere microbial community. Cherry Citrus was found to have less effect on the diversity and abundance of hemp rhizosphere microbial community compared to the other three hemp cultivars (Otto2, BaOx, and Wife). Meanwhile, fungicide treatment affected enzymatic activities in the hemp rhizosphere. More enzymes were significantly decreased in all four hemp cultivars treated with fungicides compared to non-fungicide treatment. Enzymes such as dehydrogenase, dioxygenase, hydrolase, transferase, oxidase, carboxylase, and peptidase significantly decreased in hemp rhizosphere treated with fungicides compared to those not treated with fungicides in the four hemp cultivars. These enzymes are involved in the function of metabolizing organic matter and degrading xenobiotics. Thus, fungicides impact ecosystem in the hemp rhizosphere. The ecological significance of the results is discussed.