Meeting the Challenges of Greenhouse Production and Propagation of *Cannabis sativa*

by

Kyle Thomas Owsley

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Approved by

Jeremy M. Pickens, Chair, Assistant Extension Professor Department of Horticulture
Katelyn A. Kesheimer, Entomology Team Lead, Bayer Crop Science
Daniel E. Wells, Associate Professor Department of Horticulture

Abstract

With the passing of the 2018 Farm Bill, it was federally legal to grow hemp, *Cannabis sativa* L. but still regulated on a state-by-state basis. In 2019, Alabama legalized the production of hemp. Since hemp is such a new crop, there is little published information about the production of *C. sativa*. Two separate research studies were carried out at the Auburn University Ornamental Horticulture Research Center in Mobile, AL to address concerns of Alabama hemp growers. The first study evaluated a plant growth regulator (PGR), uniconazole as a pre transplant liner soak on *C. sativa* L. 'Berry Blossom' hemp liners. In December of 2021 four treatment rates (0, 1, 3, and 5 mg/L uniconazole) and four different root-ball soak times (0, 30, 75, and 120 seconds) were evaluated in regards to plant growth. Results showed that there were no differences between rate and soak time. The trial was repeated in June of 2022 with soak time removed as a variable. Results showed plants receiving 3 ppm uniconazole were 1.3 times greater in calculated yield per area when compared to the untreated control.

The second study examined *Tetranychus urticae*, the two-spotted spider mite (TSSM), one of the most common pest found in greenhouse *Cannabis* crops. Three biological pesticides, one non-biological, and an untreated control were evaluated for efficacy in two trials.The results from both trials showed that there is potential to reduce TSSM populations when using Grandevo® and Venerate®.

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Table of Contents

List of Tables

List of Figures

Chapter I

Literature Review

Kyle Owsley

History of Hemp

Hemp, *Cannabis sativa* L. has been utilized for thousands of years across different cultures and continents; its uses have ranged from religious ceremonies and food to numerous medicinal purposes such as arthritis, pain, and depression. The wide distribution of *C. sativa* can be attributed to its ability to be grown in both temperate and tropical climates (Clarke, 1981). The *C. sativa* seed was one of the "major grains" harvested by the ancient Chinese and can be traced back to 8,500 years ago. Cultivation later spread throughout temperate Eurasia and, by Roman times, had grown to the majority of Europe. *C. sativa* was also historically grown in Africa for medicinal and agricultural utilizations (Clarke, 1981).

The introduction of hemp into North America can be traced back to the early 1600s for use in rope production for ship rigging (Cherney and Small, 2016; Schultes, 1970). Hemp played a vital role in colonial America to produce goods such as canvas and was viewed as an important fiber crop (Byrd, 2019; Small and Marcus, 2002). English colonists living in North America, especially in Connecticut, Massachusetts, and Virginia during the late 1630s, were required by law to grow hemp to help ensure supplies for the Royal Navy. Each family was required to plant a teaspoon worth of hemp seeds on their property (Deitch, 2003). The production of hemp fiber continued in North America for several hundred years, with a large amount of production located in the states of Kentucky, Illinois, and Missouri from the 1840s to the 1860s (Cherney and Small, 2016).

Hemp production increased during World War I across 12 states. The largest production occurred during World War II, when hemp production was needed for military supplies, with

59,000 hectares of hemp produced in 1943 (Cherney and Small, 2016). Currently, hemp of <0.3% tetrahydrocannabinol, (THC), content is produced in Asia, Europe, and the Americas for fiber, grain, and extraction of cannabinoids (Gauthier et al., 2019).

The U.S. government began to reduce the production of hemp in the 1930's due to concerns of hemp's relationship to its high-THC counterpart, marijuana. Hemp and marijuana are the same species, *C. sativa* L., however marijuana has a significantly higher THC content than hemp. The reduction in U.S. hemp production was achieved through the Marihuana Tax Act in 1937, placing hemp under the Department of Treasury's regulatory controls (Byrd, 2019; USDA, 2000). The Marihuana Tax Act did not make the cultivation of *C. sativa* illegal in the United States but instead regulated the shipping, cultivating, and possession of the plant. The politics surrounding the Tax Act of 1937 made the production of hemp on a commercial scale less profitable and production decreased until the 1940s (Cherney and Small, 2016; U.S. Customs and Border Protection, 2019). As a result of a Japanese blockade in the Philippines in World War II, hemp supplies became limited for the U.S. military, resulting in the U.S. government allowing hemp production to resume. Restrictions were reinstated after the war had ended (Byrd, 2019). The resurgence of hemp production in the United States during World War II produced 68,000 metric tons of hemp fiber in 1943 (Cherney and Small, 2016). In 1970, the United States government ruled that all forms of *C. sativa* were to be designated as a Schedule I drug under the Controlled Substance Act and ending the production of hemp as an agricultural crop (Byrd, 2019; USDA, 2000).

The Agricultural Act of 2014 allowed select research institutions and state departments to research pilot programs to evaluate the production of industrial hemp (Gauthier et al., 2019; Johnson, 2018). The Farm Bill of 2018 made hemp distinguishable from marijuana, which is the

psychotropic strain of *C. sativa*. Hemp is classified as *C. sativa* with a concentration of < 0.3% delta-9 tetrahydrocannabinol (THC), effectively removing hemp from the Controlled Substance Act (Johnson, 2018; Miller, 2018). The 2018 Farm Bill defines hemp as any portion of the plant, *C. sativa* L. plant that possesses a percentage less than 0.3% THC based on dry weight concentration. Tetrahydrocannabinol is the cannabidiol that produces a psychoactive effect. This definition effectively separated hemp from marijuana (Johnson, 2018; Miller, 2018). By the end of 2018, 23 states were producing over 78,000 acres of hemp grown. In 2019, hemp production was estimated to be over 500,000 acres across 45 documented states (Gauthier et. al., 2019).

In 2019, Alabama legalized the production of agricultural hemp and approved 10,000 acres for production with 150 licensed growers and 50 licensed processors (Kesheimer, 2019). Hemp can be produced for seed production, fiber production, cannabidiol (CBD) oil, or as a dual-purpose crop (Johnson, 2018). Cannabidiol oil extracted from hemp was a new and emerging market at this time, and represented 90% of the hemp grown in Alabama (Kesheimer, 2019, Weedmaps, 2021). The popularity of CBD hemp production resulted in an overabundance of CBD oil in the market followed by a significant decline in production. By 2023, hemp production in Alabama had reduced to 486 acres in production (Gail Ellis, personal communication, August 2023).

Any hemp crops that tests over 0.3 % THC is illegal to produce or possess. In the state of Alabama, a grower must notify the Alabama Department of Agriculture and Industries (ADAI) at least 14 calendar days prior to the grower's intended harvest date. An inspector will collect samples from every grow site and variety ready for harvest. The samples collected by the inspector are sent to the Food and Drug Lab in Montgomery, AL within 24 hours of collection and analyzed for THC concentrations. If the harvest tests above the allowable THC threshold,

then a second collection of samples will be collected at a cost to the grower. The crop must be destroyed if it tests over the allowable limit for a second time. Destruction of crops or hemp materials must be carried out under ADAI supervision. (Alabama Department of Agriculture and Industries, 2023). However, this phenomenon is rare; the majority of hemp crops produced in Alabama have tested below the legal limit for THC.

Production of Hemp in Alabama

The majority of hemp in Alabama is field-produced; however, greenhouse production is also used. Hemp produced from CBD focuses on floral production, as the flowers are the primary source of cannabinoids. Hemp produced for CBD extraction is also referred to as floral hemp. To maximize the number of flowers produced per plant, floral hemp is grown at a much lower density than fiber hemp (1-2 plants per m^2 vs 320-370 plants per m^2) (Danziger and Berstein, 2022). Floral hemp can be direct-seeded or transplanted from seedlings or clonal propagules derived from stem cuttings. For field production, the planting time is heavily dependent on the photoperiod of the species. Hemp is usually planted in the spring when daylight hours are longer to allow for a longer vegetative period and harvested in the fall.

Hemp is a photoperiod-specific crop, where vegetative growth and flowering growth stages are influenced by the period of time that is absent in light (Clarke, 1981). Vegetative growth in hemp requires short nights, but this is often referred to as long days or long periods of undisturbed artificial light that lasts 15 hours or more. To induce the flowering stage in *C. sativa*, the photoperiod that a hemp plant is exposed to needs to be naturally or artificially reduced to 14 hours or less.

For field production of hemp, research from North Carolina State University has shown that an area with a target pH of 6.2 requires a season total of 36-54 kg/4046 m^2 of nitrogen (N) and 68 kg/4046 m^2 of phosphorous (P), and potassium (K) (McGinnis, 2020). Soil tests should be performed for each location for field-grown hemp, as recommendations can change depending on the soil test results. The production of hemp requires two different fertility rates based on the plant's growing stage. The vegetative growth cycle varies in the requirements of N, P, and K compared to what is required at the flowering stage of the production cycle. Adjusting fertility concentrations is easier accomplished in greenhouse production where fertigation is utilized. While a hemp plant is in vegetative growth, the amount of N recommended is 200-250 ppm, and the recommended amount of P and K is 200-250 ppm, but when the plant reaches the flowering growth phase, the amount of N is reduced to 125 ppm and the amount of P and K is increased or kept at 250 ppm (Griffith, 2019). When fertilizing *C. sativa*, it is important to pay attention to fertility levels. If plants become under-fertilized, yields can be reduced, and the plant health suffers. If a plant is over-fertilized, salts can accumulate in the root zone, damaging the plant (Bar-Yosef, 1999).

Harvesting hemp is largely based on plant maturity and time of harvest. When growing outdoors, plants usually start to flower between late August and early October in Alabama because the nights become longer, meaning there is more uninterrupted darkness for the plant. Using natural day length results in harvesting taking place from October to November (Frank and Rosenthal, 1978). A decision on the harvest is primarily made based on when trichomes on the bud begin to change from white to milky white (Place, 2019).

Pest Management in Hemp

Prior to the Marihuana Tax Act of 1937, hemp was produced on a large scale in the U.S. before being made illegal. At that time of large-scale US hemp production, the only pest recorded was the European corn borer, *Ostrinia nubialis* (Cranshaw, 2017). Since World War II, hundreds of insects that have been previously recorded on other agricultural crops and have now been observed and documented as a pest of hemp. These insect pests include cutworms, grubs, flea beetles, aphids, fire ants, mites and thrips (Roth et. al., 2018; Cagle, 2019; Conner and Kesheimer, 2019). These insect pests can cause damage to a hemp crop in both field and greenhouse operations, and negatively impact the crop yield.

Some arthropod pests that have been observed in Alabama hemp operations are chewing pests such as fire ants, termites, corn earworm, yellow striped armyworm and grasshoppers. Other arthropod pests that cause damage are piercing and sucking insects such as the two-spotted spider mite, hemp russet mite, and cannabis aphid (Conner and Kesheimer, 2019). Insect pests are not the only threat to a grower's operation; pathogens can also damage or ruin a hemp crop. While there are known pathogens to hemp such as the hops latent viroid, Fusarium, powdery mildew, southern blight, leaf spot diseases, and damping off, there is little information available to growers in the state of Alabama on how to treat or prevent these issues (Conner, 2020, Warren et. al. 2019). Additional pathogens have been confirmed in Alabama such as Fusarium bud rot, damping off, southern blight, and leaf spot diseases (Conner, 2020). Growers can reduce the impact of these pests and minimize crop damage through the effective planning and execution of an integrated pest management (IPM) protocol.

Limitations of Pesticide Availability

Growers face limitations on what pesticides can be used on hemp. States differ on what pesticides are approved for hemp. This lack of availability, along with hemp being a new crop, has led to confusion among growers as to what they can apply to their hemp crops (Bessin, 2019).

In Alabama, the Department of Agriculture and Industries (ADAI) must approve a pesticide product before its permitted use within the state (Kesheimer, 2023). The current criteria for a products use on hemp set forth by ADAI include the following: 1) the pesticide label must contain directions for the use of the pesticide on unspecified food crops, 2) the pesticide must either be tolerance exempt and be registered by the EPA, 3) registered by ADAI, 4) label must be broad enough to include hemp but not prohibit use on hemp (Conner and Kesheimer, 2019). The strict and limiting criteria set forth by ADAI are due to the prior legality of the crop, resulting in a lack of established pesticide residual tolerance levels for *Cannabis spp.* For this reason, many of the products allowed for hemp production in Alabama are tolerance-exempt. A pesticide is considered tolerance exempt when the active or inert ingredients are considered safe enough to use as described in the tolerance exemption, that a maximum permissible level doesn't need to be established (National Archives and Records Administration, 2024). A tolerance level is set by the EPA for a pesticide, which states the maximum amount of residuals that can remain in or on foods; an exemption can be set when the product is found to be safe.

Currently, hemp growers in the state of Alabama have very little chemistries available to them for use on pests, and many of the products that are approved are broad spectrum biological pesticides with low efficacy and quick degradation that require prophylactic and frequent applications. This further restricts the amount of chemicals available to effectively treat *C.*

sativa pests. Many growers in the state have become interested in using biological controls in their IPM strategies. While there is little available information about the efficacy of biological controls in hemp production, more research into these controls could potentially lead to additional tools for a grower's IPM plan.

Two-Spotted Spider Mites in Greenhouse Hemp and Transplants

The two-spotted spider mite, *Tetranychus urticae* is a major pest in the production of greenhouse-produced hemp and hemp transplants. All stages of the two-spotted mites are primarily found on the underside of leaves and are typically first observed on older leaves (Chong, 2015). As populations increase, mites can be found throughout the canopy and leaf surfaces. Without routine scouting, spider mites often go unnoticed until visible plant damage occurs. The first signs of damage appear as stippling. Stippling can be described as small blemishes of tissue where feeding has occurred. Severe damage can be described as a discolored, bleached, or washed look. Occasionally, webbing occurs on terminal branches in high populations.

Because spider mites are less than 2mm (about 0.08 in), a hand lens is often needed to identify the specific species and its life stage. Pesticide efficacy may also be impacted by life stage, including eggs, larva, nymph, and adult stages. Temperature significantly impacts the development of spider mites, with increasing temperature resulting in significantly shorter life cycles (Chong, 2022). Dry weather is also favorable to mite outbreaks.

Scouting is essential in preventing outbreaks, as populations can increase rapidly. Scouting is often done by looking at the underside of leaves or tapping canopies over white

paper. Treatments are recommended as soon as a population is detected, with further scouting utilized to determine discontinuation (Chong, 2015).

Miticides are insecticides that provide control over spider mite species. Some miticide products can be used on insect species, and some are specific to only mites. Miticide resistance is a concern due to spider mites' short life cycles and high reproduction levels (Chong, 2015). The Insecticide Resistance Action Committee (IRAC) has generated a coding system (IRAC numbers) to classify products by their modes of action. Modes of action should be rotated to prevent resistance from evolving. When applying pesticides, control can be reduced by irrigation, precipitation, growth flushes, and ultraviolet light. It is important to understand how a product will affect each life stage. Some products are effective in only two stages, while many provide control across eggs, nymphs, and adults. Currently, there are no miticides registered for use on hemp in Alabama (Kesheimer, 2023).

With no miticides approved to treat mites on hemp in Alabama, identifying broad spectrum, approved products is important. Additionally, coverage and pesticide rotation is important to maintain low populations. All life stages of spider mites can be found in a plant crop but are typically located on the underside of a leaf. Damage is usually found in older growth, and treatments are recommended as soon as a population is detected (Chong, 2015). Biological controls can be used in combination or alternative chemical treatment and include predatory mites, thrips, midges, and beetles, as well as biological control agents that consist of either fungal or bacterial pathogens of mite species (Chong, 2015). Biological controls are often also best used as a preventative control tactic. A drawback to using some biological control is the application frequency. Many biological control agents have a short residual time once applied and may need to be re-applied every few days.

Several biological pesticides are allowed by ADAI for use on hemp crops. Even though these biologicals are available, there has been little work on evaluating their efficacy. Approved pesticides in Alabama include Grandevo® (*Chromobacterium* subtsuge strain PRAA4-11 and spent fermentation media) (Pro Farm Group, Davis, CA, USA), Venerate®XC (*Burkholderai* spp. Strain A396) (Pro Farm Group, Davis, CA, USA), BoteGHA® ES (*Beauveria bassiana* strain GHA) (Certis USA LLC, Columbia, MD, USA), Sil-Matrix® (Potassium silicate) (Certis USA LLC, Columbia, MD, USA).

Chromobacterium subtsuge strain PRAA4-11 and spent fermentation media is a microbial-based insecticide that according to the manufacturer, repels pests, stops pest feeding, reduces reproduction, and induces mortality. In a 2020 study evaluating the toxicity of several biopesticides on two-spotted spider mites on green beans (*Phaseolus vulgaris*), Grandevo® decreased the fecundity rate as product rates were increased (Golec et. al., 2020), Additionally, only the Grandevo® treatment was able to prevent exponential population growth and had significantly higher mortality rates over the control. Conversely, in three other studies, Grandevo® provided no control of spider mites or russet mites (*Aculops cannibicola*) in hemp (Britt and Kuhar, 2020) and two-spotted spider mites in tomatoes (Walgenbach and Schoof, 2014; and Bilbo et al., 2020).

Venerate®XC is a biological insecticide for use on a wide range of ornamental and agricultural crops. Venerate® controls chewing and feeding insect pests by enzymatic degradation of exoskeletal structures and interference with the molting process, leading to mortality. It is used on a variety of foliar feeding pests such as caterpillars and soft-bodied pests like whiteflies, aphids, and mites. Venerate® has exhibited a high mortality rate against two-spotted spider mites in controlled studies (Cordova-Kreylos et. al., 2013; Ruiu, 2015). When

evaluated by Britt and Kuhar in 2019, Venerate® reduced hemp russet mites by 54% when compared to the untreated control at 22 DAT. Golec et al. (2020) reported that when Venerate® was compared to four other biopesticides and one non-biopesticide in all life stages of two-spotted spider mites, it displayed the overall weakest response. It was noted that Venerate® "exhibited no significant effects on reproductive traits." However, the study only lasted four days so results may be different if the trial ran for a longer duration. Venerate® is slow-acting and can potentially take up to 7 days to notice effect (Golec, 2020).

BoteGHA® is a contact pesticide containing live spores of a naturally-occurring fungus found in corn borers and other insects. After infection, the fungus penetrates through the insect cuticle, causing mortality after the rapid growth of the fungus inside the host. According to the product label, BoteGHA® is most effective when used early and before high insect populations develop. Qureshi and Kostyk (2019) evaluated 8 miticide treatments to reduce broad mite populations on greenhouse peppers. All treatments reduced the number of broad mite adults and eggs when compared to the untreated control except for BoteGHA®. BoteGHA® is currently federally labeled for use on hemp but is not labeled for use on mites in California.

The active ingredient in Sil-Matrix® is potassium silicate that provides the plant with a protective barrier to act as a fungicide, miticide and insecticide. The plant cuticle absorbs the potassium silicate to create a physical barrier of silicon crystals on the leaf surface. This barrier provides disease control by preventing pathogens from penetrating the plant surface. The silicon crystal deposits are reported to make fruit and foliage unpalatable to pests, resulting in reduced feeding. The potassium silicate also serves as a contact pesticide for soft-bodied insects. Direct contact of Sil-Matrix® with the pest results in desiccation and death. In (Morse,2008) study on Persea mites in avocados, Sil-Matrix® was not effective in reducing mite numbers. There was

only one application, and counts were performed on the mites in wide intervals (20-30 days). Multiple applications with shorter intervals may have yield different results. Sil-Matrix® is federally labeled for use on hemp and application is recommended as a preventative for optimum disease and insect control.

There has been a limited number of trials evaluating different biologicals on hemp in greenhouse production but previous research shows they are effective as part of IPM strategy for other crops. There is conflicting evidence on the efficacy of these products, but they may be more effective when used in combination. More research into the use of these products is needed.

Growth Regulators on Hemp

Transplants are typically moved to the field once the plant has established healthy roots in the container. Old transplants may have reached the physiological age where reproductive growth begins and initiates premature flowering, but can also result in lower yields (Schrader, 2000). Once transplants have hit a certain age, the subsequent growth and yield potential is negatively affected once they have moved into the field (Vavrina, 1998). Transplants left in the propagation trays too long can become root-bound and begin to girdle. This can cause the mature plant to choke itself out later in production, and will make it more difficult for the transplant to root once in its final container. Overly mature liners have a large shoot-to-root ratio, which leads to plant stress and the inability to take up enough water to establish.

Plant growth regulators (PGR) are used to create more consistency in liner production and reduce transplant shock and stress. Plant growth regulators occur naturally in plants to encourage or regulate growth in specific areas of the plant structure. They are either naturally

occurring or synthetic and do not have a nutritive value. PGRs, mostly in low doses affect the development or metabolic processes in plants (Rademacher, 2015). Since their discovery in the early 1900's, PGR's have been used in ornamental plant production, agriculture, viticulture to manipulate bud initiation, fruit set, plant height, branching, and quantitative and qualitative increases in yields. They also create lowered susceptibility from abiotic and biotic stress (Rademacher, 2015). PGR's can lower labor demands, such as pinching and pruning, in some ornamental crops. Some PGRs work as chemical pinchers by retarding vegetative growth through inhibition of gibberellin production, resulting in a shorter plant with less shoot production. Additionally, some PGRs can provide better control of budding time and allow for a more uniform flower set. Plant safety is a major concern when using PGR's as there is no one-size-fits-all product. Each product label must be evaluated for plant safety and efficacy before applying to a crop.

PGR's can be applied through foliar application, soil drench, or bulb/transplant soak. PGRs affect the balance of the hormones in plants that have been treated through the application of naturally occurring hormones or their synthetic counterparts. This inhibits and disrupts the biosynthesis of the plant's endogenous hormones or blocks the movement of hormones from the production to action site of the plant (Rademacher, 2015). The application type and concentration can influence the effectiveness. Some products may require additional or subsequent applications depending on plant age or active ingredient concentration. For example, applications of uniconazole in low concentrations with increased frequency may provide better control than a single heavy concentration in some species.

The type of media ornamental plants are grown in has been shown to change the effectiveness of paclobutrazole. Pine bark can significantly reduce the effects of paclobutrazol

drenches due to the decreased ability of absorption through the media, and it is recommended to follow through with subsequent drench applications or consider a foliar treatment instead (Latimer and Whipker, 2012).

PGR effectiveness can vary widely depending on the plant species and crop growth stage. Repeat applications of PGRs have been shown to increase the desired effects such as darker foliage and shorter stems in plants with rapid vegetative growth characteristics. Repeat applications may be required if plants are being grown in vigorous growing conditions including high fertility and temperature environments (Latimer and Whipker, 2012).

Uniconazole ((E)(+)(S)-1 (4-chlorophenyl)4,4-dimethyl-2(1,2,4-triazol-1 -yl)pent-1 -ene-3-ol)(Valent, 2008), is used extensively on a wide range of ornamental plants in greenhouse production to reduce plant height and create a more compact plant. Uniconazole can reduce internode elongation by disrupting the gibberellin biosynthesis that happens naturally in the plant, create darker foliage, increase stem strength, leaf thickness, chlorophyll content, and flower number and size (Valent, 2008).

There are four different application methods for uniconazole: foliar spray, drench, bulb or cutting dip, and pre-plant application to the soil surface (Valent, 2008). Since uniconazole and other members of the triazole-type chemicals do not readily move throughout the plant due to movement through the xylem and not the phloem, foliar applications may not move the chemical throughout the plant (Whipker, 2013). It is recommended that applications uniformly cover the stems for proper uptake unless a drench or bulb/liner soak is used. Uniconazole is quickly absorbed by plant stems, petioles, and roots with excess from spray treatments acting as a drench application to the substrate. Since uniconazole is one of the more potent and active triazole-type

chemicals, it also requires application rates 50% lower than what is recommended for paclobutrazol (Latimer and Whipker, 2012, and Whipker, 2013).

Uniconazole applications on mature transplants can have negative effects on plants, including flowering delay on bedding plants or uneven growth. Extreme stunting can also occur from foliar applications when runoff from the plant is too excessive onto the media since uniconazole is absorbed readily by the roots. Uneven growth can also occur from inconsistent coverage of the stems; to avoid differences in uniformity, drench applications are recommended when it is economically feasible for the grower (Latimer and Whipker, 2012). If the plants are large or dense and application to the stems may be difficult, growers may need to apply uniconazole at rates higher than the recommended volumes. Drench applications have several advantages over traditional spray methods in perennial production but due to higher application costs with drenches, most growers use foliar applications (Latimer and Whipker, 2012).

Presently, there is one uniconazole product with a supplemental label allowing for use on solanaceous crops such as tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annum*), and eggplant (*Solanum melongena* L.) which is registered as Sumagic® (Valent Biosciences, Libertyville, IL) (Agehara and Leskovar, 2015). When treating solanaceous crops, this product is used mainly to control the height of plants. It must be applied no later than 14 days after the two to four-leaf stage and total applications cannot exceed 10 $mg \cdot L^{-1}$ per crop (Valent U.S.A Corp, 2008). Studies have shown that the application of uniconazole on tomato transplants reduced the overall plant height but did not affect fruit yield (Villavicencio et. al. 2015; Zandstra et. al. 2006; Wang and Gregg, 1990). Research has shown that variety can impact the effects of uniconazole (Schnelle, 2009).

A 2000 study (Jung et. al., 2000) on potted *Chrysanthemum indicum* L. showed that uniconazole at a rate of 0.05 mg a.i./ pot suppressed plant height and stem diameter but also had the highest number of branches in the study. The 0.05 mg a.i./ container uniconazole drench used in the study created uniform and compact plants. The study also noted that when the highest concentrations of uniconazole were applied, resulting plants were too short to be marketable. In a study that evaluated three growth retardants (uniconazole, paclobutrazol, and propiconazole) in tomato seedlings, growth could be retarded and plants held as transplants for two months without affecting the quality of the plants (Miguel-Zarate et. al.,2021). Miguel-Zarate et. al., (2021) reported the lowest plant height at 31 days after sowing were those treated with uniconazole. Even at 49 and 66 days after sowing when paclobutrazol had the lowest plant height, uniconazole was able to maintain a much lower plant height when compared to the control. For that study, the ideal plant height was determined to be 20-25 cm. A single application of propiconazole and uniconazole was able to keep plants in the desirable size range, allowing for a more uniform growth of seedlings in the seedbed (Miguel-Zarate et. al.,2021).

Ornamental and vegetable plants (Miguel-Zarate et. al. [2021], Jung et. al. [2000], and Villavicencio [2015]), had more compact and uniform plants after uniconazole treatment. These studies lead us to hypothesize that hemp plants treated with uniconazole could produce more compact, sturdy, and uniform transplants. The use of uniconazole may reduce problems associated with overgrown transplants with poor root to shoot ratios, resulting in greater transplant survival and more uniform stands. Research has shown that this can be achieved with one application (Agehara and Leskovar, 2015: Miguel-Zarate et. al. 2021).

Propagation of hemp

Hemp transplants can be produced through seeds or stem cuttings. Stem cuttings is a form of asexual propagation resulting in identical genetic material as the source stock (clones). Advantages of using clones over seedlings are predictability, uniformity, and the ability to produce all female plants. (; Potter, 2009; Langston, 2003; Coffman and Gentner, 1979).

As *C. sativa* is a dioecious plant, both male and female plants can result from seedlings. For CBD or high THC floral crops, it is undesirable to have male plants within a crop due to the risk of pollination and seed production. Seed production in a pollinated crop will halt flowering and reduce flower biomass (Clarke, 1981). For seed produced transplants, seeds can be feminized through chemical applications, but the risk of growing a staminate plant by accident still exists (Mohan et. al., 1982). Plants grown from seed also run the risk of spontaneously developing pollen clusters or becoming a hermaphrodite, which is detrimental to the production of flowers (Clarke, 1981).

Some cultivars of hemp can only be grown from seed such as *Cannabis ruderalis*. *C. ruderalis* is a cultivar of hemp that photoperiod does not induce the flowering cycle, instead it starts to flower after only a few weeks of growth. These cultivars of hemp can also be known as "auto flowering" cultivars (Cervantes, 2006; Clarke, 1981).

Little published information exists on standards for cutting size and leaf number. Caplan et al., (2018), reported that Canadian marijuana growers utilized 2-3 leaf terminal cuttings. This also represents our observations among Alabama growers with 2-3 leaf cuttings with approximately 7.5 cm of stem. In some cases, the leaf tips are removed to reduce transpiration loss of water from the cutting; however, Caplan et al. (2018) indicated that leaf tip removal

reduced rooting success from 53 to 71%. In the same study, rooting percentage was similar between two and three leaf cuttings, suggesting that two leaves provide sufficient support when rooting cuttings. However, three leaf cuttings resulted in a 15% greater root quality.

Temperature and cutting size may influence rooting success. A large problem faced by Alabama hemp growers is the hot weather which can lead to increased transpiration rates, resulting in poor rooting. One study showed that using larger diameter cuttings (2.9 mm-3.2 mm) along with root-zone heating of 27.8ºC resulted in higher quality rooted cuttings (Cockson et. al., 2019). Cockson's (2019) study on 'BaOx' hemp speculated that larger caliper cuttings could contain more carbohydrate reserves located in roots and shoots that could result in increased rooting success. These findings back up (Chaturvedi, 2001) where larger caliper stems rooted in a vertical position resulted in increased rooting percentages. There is a relationship between temperature and the development of adventitious roots. The formation of adventitious roots is dependent on temperature but also the number of leaves left on the vegetative cutting and the rooting environment (Owen, 2018).

In cutting propagation, intermittent mist is used to increase humidity levels (75-90%), thereby reducing transpiration water loss from leaves by lowering the vapor pressure deficit (Casillas, 2016). By lowering the vapor pressure deficit, stomatal water loss is reduced, allowing the cutting to stay turgid and prevent desiccation (Owen, 2018). Moisture management in hemp transplant production must be closely monitored. Transplants left in heavy mist may lead to leaching of nutrients, overly saturated media, and increased disease pressure (Owen, 2018).

The ability of *C. sativa* to successfully root can also be influenced by the maturity of the stock plant. Cuttings taken from juvenile plants have better rooting success than mature plants (Caplan et. al., 2018). This difference in rooting could be caused by juvenile plants having

greater concentrations of endogenous auxins or by other rooting components when compared with a mature plant (Husen and Pal, 2006). The use of synthetic hormones has also been shown to improve rooting percentages in many plant species. The legality of rooting hormones in hemp propagation is dependent on labeling, regulations, or restrictions associated with organic production (Caplan et al., 2018; Kurtz et al., 2022).

Quality and Transplant Success

A quality hemp transplant should be pest-free, compact with a thick stem, well rooted, and of good color, indicating an adequate nutritional status. Transplant quality can directly impact total yields and overall crop success. If a transplant has become under-fertilized, neglected, or overly hardened, it can lead to a decrease in yields (McAvoy and Ozores-Hampton, 2015). Container size greatly affects transplant quality. Transplants will undergo physical and morphological changes in response to the reduction in rooting mass. This reduction in root volume directly correlates to root growth, shoot growth, plant biomass accumulation, nutrient uptake, respiration, flowering, and yield (NeSmith and Duval, 1998). The effects of a disproportioned root-to-shoot ratio caused by a small root volume can have both immediate and long-term consequences for plant health, such as restricted roots unable to actively take up water and nutrients, the inability to adapt to field planting, or appear soil moisture stressed even when there is adequate moisture in the ground (Krizek et al, 1985; Aloni et al, 1991).

Transplant Shock/ Hardening/Acclimation

When plants have not been hardened off or gradually eased from propagation settings to the final production environment, the plants can go into transplant shock, which greatly reduces

plant health and quality. When plants have been gradually eased out of the intermittent mist to manual watering, the possibility of transplant shock is reduced (Hartmann et al., 2002).

Substrate

Hemp production is achievable in all soil types, but is best adapted for well-drained loams with a pH range of 6 to 7 (Byrd, 2019). The basic components of transplant media are peat moss, perlite, vermiculite, and the addition of a wetting agent. Perlite is a volcanic material that has been processed, and it benefits the media by providing good drainage and helping the media hold air in the root zone. Since most of the water stays on the surface from perlite, it is easier for the plant to take up. Vermiculite is used as a mica-type mineral with the ability to retain large amounts of water, air, and nutrients (Kelley and Boyhan, 2003). Besides containing the basic requirements for a growing media, transplant substrates should be sterile, levigated, light in weight, and possess adequate water-holding capacity (Bailey et. al., 1998; Pierce, 1987).

The use of field soils in transplant production is not recommended. Field soils generally drain poorly and can be heavy clays or compacted soils, and this can result in poor yields and decreased emergence and development. Field soils are also discouraged because of potential contamination by diseases and weed seeds (Kelly and Boyhan, 2003; Roth et. al., 2018). Peat-based media, once dry, can be difficult to wet and could require pre-moistening or the addition of a surfactant. Heavy clays and soils are unsuitable for transplant production due to their poor drainage and is one of the reasons that peat based media is used in transplant production (Granberry and Boyhan, 2003).

Cell Size

Quality and location are not the only factors affecting transplant success. The size of the containers, both for rooting cuttings and finishing transplants, is critical for plant success. Since different species of plants require different spacing, nutrient, and water needs, this makes some cell sizes preferable to plant producers. The larger a cell is the greater the amount of media it can hold. With the increase in media, the chance of nutrient and water retention increases. Since the larger cells require less frequent watering, the chance of moisture or nutrient stress is decreased. Because of this, containers with larger cells tend to produce stockier and earlier plants due to a larger root volume. These larger cell containers can help reduce plug transplant shock because of the larger root volume, while smaller cells tend to have a higher chance of root damage and transplant shock (Kelley and Boyhan, 2003). Previous research into compact-growth tomato transplants showed that plants produced in larger cells flowered sooner after transplanting and produced higher early season yields than transplants that were produced using a smaller cell size (Kemble et. al., 1994; Weston and Zandstra, 1986). Cell size, transplant quality, and media are all major components in the production of hemp transplants. The use of small cell sizes in heavy clays may result in poor yields and quality, and transplant shock. Larger cell sizes, sterile peat-based media, and proper nutrient management can produce earlier yields and less transplant shock in the field (Roth et al., 2018; Bailey et al., 1998; Peirce, 1987). Larger transplants can reduce water demands and there is a reduction in the chance of the plant becoming pot bound.

Objectives

Overgrown liners are observed to have poor stands with reduced yields due to root girdling and severe transplant shock. Uniconazole has been shown to improve root to shoot ratios and allow growers to hold transplants longer before planting. Uniconazole is currently not

labeled for use in *C*. sativa but Sumagic® (Valent, 2008) has a supplemental label for use in *Solanaceous* vegetable transplants. Our study will explore the use of uniconazole as a pre transplant liner soak, by evaluating yield in hemp liners. The goal of the study is to produce a higher density hemp planting while also improving yield.

Our second study examine the limited chemistries available for the control of TSSM in Alabama hemp by evaluating four miticides, three of which are biologicals and one non-biological which included: Grandevo® (*Chromobacterium* subtsuge strain PRAA4-11 and spent fermentation media), Venerate® XC *(Burkholderai* spp. Strain A396), BoteGHA® ES (*Beauveria bassiana* strain GHA), and Sil-Matrix® (Potassium silicate).

These products are tolerance exempt and are allowed for use on hemp by the ADAI. The goal of this study is to provide growers with efficacy data, enabling them to make more informative decisions on control TSSM.

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Chapter II

Uniconazole: A Potential Tool for High-Density Indoor Cannabis Systems.

Abstract

There have been inconsistencies in *C. sativa* transplants grown in the state of Alabama. Root-to-shoot ratios in liner production have varied greatly and transplant shock and crop damage have resulted from this issue of inconsistent liners. The use of uniconazole in ornamental nursery production is common and it is used to create more uniform and consistent plants. Our study is looking to use uniconazole to eliminate the variable heights in *C. sativa* transplants and study the effects of uniconazole on treated *C. sativa.*

On 6 December 2021, *C. sativa* L. 'Berry Blossom' transplants (50-cell tray) were treated with uniconazole to evaluate its effects on growth and yield. The trial evaluated four treatment rates (0, 1, 3, and 5 mg/L) and four different root ball soak times (0, 30, 75, and 120 seconds). The trial was repeated in June 2022 with 12 replications and with a 30 second soak time. In trial 1, the calculated yield per area was 2 to 2.5 times the yield when the lowest treatments (0 and 1 ppm respectively) were compared to the two highest-yielding treatments (3 and 5 ppm). In trial 2, plants receiving 3 ppm uniconazole were 1.3 times greater in calculated yield per area when compared to the untreated control. While yield per plant decreased with increasing rates of uniconazole, the calculated yield per area increased. Results suggest a potential benefit of increased yield per area using smaller uniconazole-treated plants and greater planting densities. Currently, uniconazole is not labeled for use on *C. sativa*. This study demonstrates the potential opportunities for the use of PGR's in *C. sativa* production.

Introduction

Because of the historically illegal nature of high THC cannabis, researched-backed production strategies are limited. Optimizing production through intensification is of significant

interest to cannabis industries. The intensity of cropping systems has increased over time and often involves increased planting density (Postma, 2021). Several specialty crops such as peaches (Oran et al., 2024), strawberries (Fiola 1998), pecan (Wood and Stahman 2004), and citrus (Chhetri and Kandel 2019) have moved to more intensive production systems. Optimizing plant density to maximize yields is also commonplace in greenhouse vegetable crops (Rodrigues et al. 2007, Amundson 2012). High-density crop systems are standard for most greenhouse crops due to the expense of growing in a climate-modified structure or facility. Equipment depreciation, loan interest, and energy costs also significantly impact the profitability of indoor *C. sativa* crops. Most cannabis species are genetically determined to make for a large plant (2 to 6 meters in height) (Clarke, 1981). Floral cannabis crops may have densities as low as one plant per square meter (Danziger and Berstein, 2022).

Due to the expense of transplants in outdoor floral cannabis, production often utilizes large spacing to maximize vegetative biomass per plant. Densities in these scenarios can be 3,500 to 5,172 plants per hectare and are typically planted in March and harvested in September/October in the U.S (Clarke, 1981). Little published information exists on common plant densities and corresponding yields for greenhouse-grown *C. sativa*. Through personal communication with the Dutch medicinal cannabis company Bedrocan®, Vanhove et al. (2011) reported plant densities of 2.33 plants per square meter yielded 251 g/m^2. Danziger and Bernstein (2022) investigated how yield was impacted when plant density increased from 1 to 2 plants /m^2 and reported increased yield per area by 28 to44%. Toonen et al. (2006) reported that the median plant density for Dutch illicit grow rooms was 15 plants per m^2 with 505 g/m^2. This high density is likely similar to the “Sea of Green” production, where floor space is maximized by high-density plants grown under shorter crop cycles (Cervantes 2006).

With high-density plantings, it is necessary to keep plants compact to reduce light competition; this is most often handled through pruning or pinching. Alden and Faust (2024) evaluated two methods to improve yields. These methods include withholding nutrients during the reproductive stages and increasing shoot number through manual pinching of terminal stems. Both factors significantly impacted yield but with differing tradeoffs. Alden and Faust suggested that shorter plants may reduce the need for supportive netting and allow growers to place grow lights closer to crop canopies. The latter is important for energy efficiency improvement as more photons reach the canopy, potentially allowing for increased space utilization as multi-tier or stacked vertical systems could be employed.

Plant growth regulators (PGR's) have been used high density fruit and nut production to reduce the high labor demand associated with pruning and training (Bisht et al., 2018; Chhetri and Kandel, 2019; Zhu and Stafne, 2019). Growth regulators are used across many crops to increase canopy compactness, allowing for higher densities. Nonetheless, there are currently no PGR products labeled for use on *C. sativa*.

The production of *C. sativa* has recently become legal with the Farm Bill of 2018. Since it is a relatively new crop, this has prevented the labeling of PGR products for use on *C. sativa* crops, and subsequently, there is little to no research on the effects of PGRs on *C. sativa*. The purpose of this study was to evaluate the PGR and uniconazole impact on the growth and yield of *C. sativa*. Uniconazole was selected as currently the only PGR labeled for vegetable transplants (Runkle and Blanchard, 2012).

Uniconazole ((E)(+)(S)-1 (4-chlorophenyl)4,4-dimethyl-2(1,2,4-triazol-1 -yl)pent-1 -ene-3-ol)(Valent, 2008), has been used extensively on a wide range of ornamental plants in greenhouse production for its ability to reduce plant height and create a more compact plant.

Uniconazole can reduce internode elongation by disrupting the gibberellin biosynthesis. Additionally, it can create darker foliage and increase stem strength, leaf thickness, chlorophyll content, and flower number and size (Valent, 2008). There are four different application methods for uniconazole: foliar spray, drench, bulb or liner dip, and pre-plant application to the soil surface (Valent, 2008). Uniconazole and other members of the triazole-type chemicals work primarily through the xylem tissue. Foliar applications are less effective (Whipker, 2013). It is recommended to ensure applications uniformly cover the stems for proper uptake unless a drench or bulb/root ball soak is used. Studies have shown that the application of uniconazole on tomato transplants reduced the overall plant height but did not affect fruit yield (Villavicencio et. al. 2015; Zandstra et. al. 2006; Wang and Gregg, 1990).

Given the potential for uniconazole use in hemp production, we sought to explore how to reduce growth inconsistencies in *C. sativa* plants grown in greenhouse settings. Plants treated with uniconazole should have a reduced height which could potentially increase the planting density per a given area. Besides an increase in plant density this study also plans to evaluate the effect uniconazole has on flower development in *C. sativa.*

Materials and Methods

Two trials evaluated the effects of the plant growth regulator, uniconazole-p as a pre-transplant liner soak on *C. sativa* L. hemp. This research was conducted at the Auburn University Ornamental Horticulture Research Center in Mobile, AL, USA (30.702628, -88.145606). The greenhouse used was pitched-roof glass with an east-to-west orientation. Temperatures were maintained between 27ºC and 38ºC using fan ventilation, forced air heaters, and 50% spray-applied shade.

Trial 1

On 6 December 2021, two-week-old transplants were treated with various concentrations and soaking times with uniconazole based on the experimental design. The experiment was a factorial design using three rates (0, 1, 3, and 5ppm uniconazole) and three soak times (30, 75, and 120 sec.). The product Concise® (Fine Americas, Inc. Walnut Creek, CA, USA) was used as the source of uniconazole for all treatments. An untreated control was also included in the trial. Transplants originated from cuttings taken from mother plants of C. *sativa* 'Berry Blossom'.

Treated transplants were rooted and grown in a 2:2:1 perlite, vermiculite, and peat moss in plastic 50-cell trays (90 cm^3) (Dillen Products™, Middlefield, OH, USA. Two-week-old, rooted transplants were submerged to the soil line of the main stem in a 3.7 L solution of their respective treatment concentrations. Transplants were submerged by hand and held submerged continuously using a stopwatch to measure time. Immediately after each transplant was submerged, the plant was potted into the final growing container for the experiment.

After treatment, transplants were transplanted into a 13.2 L plastic nursery container (Lerio Corporation, Mobile, AL, USA) filled with a 100% aged pine bark substrate amended with 5.06 kg/m^3 18-6-8 360 Nutricote (PROFILE Products LLC., Buffalo Grove, IL, USA), 1.81 kg/m^3 of dolomitic lime, and 0.44 kg/m^3 MicroMax ® (ICL Specialty Fertilizers, Summerville, SC, USA). Plants were arranged in a randomized complete block design with three blocks per treatment and a single plant serving as an experimental unit. Blocks were arranged perpendicular to the airflow created by greenhouse exhaust fans. Plants were irrigated by hand as needed with 200 ppm N using 20-20-20. Plants received five-times-a-week liquid fertilizer of 20-10-20 (Ultrasol Greenhouse Plus™, SQM North America, Atlanta, GA, USA) at 200 ppm. The trial was terminated on 24 March 2022, approximately 99 days after treatment (DAT).

Size index (SI) ([height+width1+width2]/3) and stem diameter were taken at 1, 8, 14, 24, 29, 66, and 99 DAT. The stem diameter was taken at 2.5 cm above the soil line. The number of lateral branches, referred to as breaks, were recorded at 30 DAT. Fresh and dry shoot and flower biomass were recorded at termination, 99 DAT. Flower biomass was divided into apical inflorescence (AI) and remaining inflorescence (RI). Apical inflorescence was comprised of the most apical flower. This was the largest bud on each plant and was harvested by removing the upper 14 cm of the flower. The stem diameter of the AI was recorded. All other flowers were considered RI. Bags were placed into a ShelLab SMO28G-2 forced air oven (Sheldon Manufacturing Inc. Cornelius, OR. USA) at 28ºC for 10 days.

Trial 2

The study was repeated on 14 June 2022. Soak time or the interaction between soak time and rate was not a significant factor in yield in trial 1. Therefore, soak time was removed in the second trial as a variable. The soak time used for the trial was 30 seconds for all treatments. Treatments consisted of 0, 1, 3, and 5 ppm uniconazole and an untreated control. The experimental design was a randomized complete block design with ten blocks per treatment. The second trial utilized the same rates as trial 1, and plants were grown as described previously. Plant height, stem diameter, and the number of nodes were counted on 8, 13, 31, 60, 72, and 111 DAT. Plants were destructively harvested for fresh and dry biomass using the same procedures in trial 1.

For both trials, calculated yield per area was used to simulate an increased plant density based on the average surface area occupied by each treatment. This yield per area calculation was used to show how an increased number of small uniconazole-treated plants in a given surface area could have larger total yields in that area when compared to a smaller number of

large plants in the same area. To analyze interactions, the untreated control was removed so data could be analyzed by mixed model analysis of variance (ANOVA) in JMP® Pro software (ver. 14, SAS Institute, Cary, NC). The main effects and their interactions included soak time, rate of uniconazole, and time after treatment and their interactions. Significant interactions were analyzed with post hoc means comparisons using Tukey's honest significant test (HSD) ($\alpha \leq 0.05$) and a means comparison was conducted using Tukey's honest significant differences test (HSD) ($\alpha \leq 0.05$). When interactions were not significant, treatment main effects were separated using Student's t test ($\alpha \leq 0.05$).

Results

Trial 1

Plant stem diameter and height were not influenced by a three-way interaction of rate, soak time, and time after treatment (Table 2.1). There was no interaction between rate and soak time. Stem diameter and height were influenced by the interaction of rate and soak time as well as rate and time after treatment. The final size index and the calculated area a treatment consumed were only influenced by rate (Table 2.1). At 14 DAT, the untreated control began to show differences in height compared to all uniconazole-treated plants (Figure 2.1). All uniconazole treatments began to differentiate from each other in height at 29 DAT (Figure 2.2). Plant canopies of uniconazole-treated plants receiving 3 and 5 ppm were four times smaller than the untreated control (Table 2.2). Plants receiving 1 ppm uniconazole were 25% smaller in canopy area than the untreated control. A similar trend was observed in the plant size index, with 1 ppm treated plants being 23% smaller than the untreated control. Plants receiving 3 and 5 ppm had approximately one-half the size index of the untreated control (Table 2.3).

There was no interaction between rate and soak time for total shoot biomass, AI, RI, total inflorescence, and calculated yield per area. Soak time had no effect on any of the previously mentioned parameters. Rate had an impact on biomass and yield across all parameters (Table 2.4). Plant shoot biomass was reduced with increasing rates of uniconazole, with 1, 3, and 5 ppm uniconazole being 30, 53, and 64% smaller than the untreated control, respectively. Apical inflorescence was positively affected by the higher rates of uniconazole (Table 2.3). Plants treated with 3 and 5 ppm uniconazole were 30 and 46%, respectively, greater in calculated yield per area than the untreated control but there was no difference found between 1 ppm and the untreated control. All remaining inflorescence demonstrated a reduction in yield per plant as rates of uniconazole increased. These reductions in yield of remaining inflorescence were incremental with 1, 3, and 5 ppm being 25, 53, and 71% less than the untreated control. Similar results were observed for total inflorescence. Yield per area was calculated based on the average diameter of each treatment and resulted in increasing yield per area as the rate of uniconazole increased. Plants treated with the highest rates of uniconazole (3 and 5 ppm) had the lowest yield per plant. However, the 3 and 5 ppm treatments had the highest calculated yield per area when compared to the untreated control and the 1 ppm treatment by a factor of two.

Trial 2

Similar to the first trial, stem diameter, and plant height were influenced by the interaction of uniconazole rate and time after treatment (Table 2.5). The untreated control plants showed a height difference from uniconazole one week earlier when compared to trial 1 (Figure 2.3). Plants treated with 1 ppm uniconazole began to differentiate from plants grown with 3 and 5 ppm between 31 and 62 DAT; however, the 3 and 5 ppm treated plants were similar throughout the study. At termination (111 DAT) height was reduced for uniconazole treatment by 64% to

77% across all treatments when compared the untreated control. No differences in plant height were observed among uniconazole treatments at 111 DAT (Figure 2.3). Plants treated with 5 ppm resulted in high mortality when plants were near harvest due to a pathogen that caused crop failure. This pathogen was only observed in the 5 ppm treatment plants.

Plant total shoot biomass followed a similar trend as observed in trial 1, as 1 and 3 ppm treatments were 41 and 61 % less than the untreated control. Total flower biomass per plant was also negatively influenced by uniconazole rates, with the 1 and 3 ppm rates resulting in 45 and 66% lower yield. When yield was adjusted for a calculated yield per area, the 3 ppm rate was greater than the untreated control by a factor of 1.3 (Table 2.6).

Discussion

Currently, uniconazole is not labeled for use on *C. sativa*, but this study has demonstrated some potential benefits for cannabis growers in terms of yield and more compact plants. In the present study, plants treated with 3 and 5 ppm of uniconazole applied as a root ball soak significantly reduced plant size and plant canopy area by a factor of four compared to the untreated control. Smaller plants at high densities may allow growers to harvest greater yields per area. Labor costs can also be reduced when using uniconazole due to its ability to reduce plant height and form a more compact plant (Rademacher, 2015).

More work is needed to determine the optimum rate of uniconazole. In the present study, the 3 ppm rate produced compact plants and high yields per area, whereas the over compactness of 5 ppm treated plants resulted in high mortality. The increased incidents of disease were likely due to the lack of airflow in the high level of compactness of flowers in the 5 ppm treated plants. In a study evaluating high-density blueberry plantings, similar issues with airflow through

compact plant canopies resulted in a limitation of outside air exchange and an increased risk of fungal foliar diseases (Fang et al., 2020).

Jung (2000) demonstrated that low rates of uniconazole (0.05 mg a.i./pot) on *Chrysanthemum indicum* L. reduced plant height and increased branching, while higher rates (0.10 and 0.15 mg a.i.) resulted in less desirable stunted plants. Schnelle (2009) found that pepper plants are highly responsive to uniconazole treatments. Plants treated with the lower rate of uniconazole (2.5 $mg \cdot L^{-1}$) successfully reduced the heights of bell peppers but when treated with the high rate of 10 $mg \cdot L^{-1}$ were severely stunted. These results were similar to what we found with the 1 ppm uniconazole treated *C. sativa*, the plant's height was reduced but not severely stunted and 5 ppm was severely stunted in our study. The highest treatment of uniconazole greatly reduced the height of the 5ppm treated plants.

Many studies have pointed out that the effects of PGR's can be highly influenced by cultivar (Villavicencio et al., 2015; Schnelle 2009, Giovinazzo and Souza-Machada 2001, and Biai 2011). Schnelle (2009) applied a single Sumagic® spray at 2.5 $mg \cdot L^{-1}$ to 'Hungarian Yellow Wax,' 'Big Bertha,' and 'Better Belle' pepper plants resulting in 41, 41, and 29%, respectively, shorter than the untreated control. In the same study, using 5 $mg \cdot L^{-1}$ of Sumagic® applied at 14 and 28 days after sowing resulted in significantly different heights at market ready stage (42 days after sowing) for 'Early Girl', 'Big Boy' and 'Champion II' tomatoes. The present study only evaluated the *C. sativa* cultivar 'Berry Blossom' which is noted to have rapid vegetative growth. Different cultivars of *C. sativa* could potentially show variable rates of growth when treated with uniconazole and there are cultivars of *C. sativa* that have short growth habits. Cultivars such as 'BaOx' have vigorous growth whereas 'Pipeline' is already a compact plant and treatments with uniconazole could potentially result in overly stunted plants. Cultivars of *C. sativa* could respond

the same to PGRs as Schnelle (2009) and result in variable levels of growth. In trial 1 plants treated with 3 and 5 ppm produced canopies that were smaller than the untreated control by a factor of four. Trial 2 resulted in similar reductions in growth. This is in contrast to other studies investigating the impact of plant density on high THC cannabis yield which found a reduction in yield per plant with increasing density. The opposite of the reduction in yield per plant was observed with the more economically important metric, yield per area (Benevenute et al., 2022; Danziger and Bernstein 2022). Similar effects were observed in the present study, but the influential factor was increasing rates of uniconazole which impacted compactness and increased densities. As uniconazole treatment concentrations increased, yield per plant decreased, but yield per area increased when calculated plant densities were considered. When smaller plants are cultivated in a dense planting area, the overall yields for that space are likely to surpass those achieved by a single larger plant occupying the same area. The more plants that can be placed into a smaller area should result in a higher total yield for the entire area.

It is essential to understand that calculated plant densities may not correlate to what might occur in actual production as the impact of light is not considered with this extrapolated method of determining yield per area. Light is the most limiting factor when increasing plant density (Postma et al., 2020). Since *C. sativa* is a photoperiod specific plant, the use of lights to trigger early or late flowering can make the growing cycle different than what would happen in production where plants were grown without supplemental light.

While flower yield may be the most critical *C. sativa* production, cannabinoid yield is a more critical factor for extraction-destined crops. Many factors can influence cannabinoid yield, such as light quantity, photoperiod, nutrition, and cultivars. Danziger and Bernstein (2022) demonstrated that plant architecture can also be a significant factor in cannabinoid yield and

reported a 90% lower cannabinoid yield when comparing axillary to apical inflorescence. While the present study did not evaluate cannabinoid concentrations, it is important to note that plants receiving 3 and 5 ppm of uniconazole had 30 and 46% greater yield in apical inflorescence when compared to the untreated control.

Currently, there are no PGR products labeled for use on cannabis crops. This work demonstrates the potential for uniconazole to be an impactful tool warranting more research. Future studies should incorporate cultivar response, plant density and impact of cannabinoid yield

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Tables and Figures

Table 2.1. Trial 1: Effect of rate, soak time, and their interactions on total plant dry biomass and yield on hemp transplants treated with uniconazole using the liner soak method.

	p values[Z]				
Main Effects	Plant Biomass[Y]	Apical Inflorescence[X]	Remaining Inflorescence[W]	Total Inflorescence[V]	Calculated Yield per Area ($g \cdot m^2$)[U]
Rate[T]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Soak Time[S]	0.1307	0.4204	0.1801	0.2373	0.3192
Rate x Soak Time	0.7983	0.9954	07212	0.6722	0.8259

[Z]Analysis of variance for main effects and their interactions ($\alpha = 0.05$). [Y]Plant biomass represents the total dry biomass of all shoot portions of the plant. [X]Apical inflorescence represents the most apical flower and largest bud and was harvested by removing the upper 14 cm of the flower. [W]Remaining inflorescence represents all remaining dry flower biomass when apical inflorescence was removed. [V]Total inflorescence represents the total dry flower biomass. [U]Calculated yield per area was calculated by adjusting yield per area to match an extrapolated plant density based on the average diameter of each treatments plant canopy [T]Rate = 0,1,3, and 5 ppm uniconazole. [S]Soak time = the time root balls were soaked for 0,30, 75 and 120 sec.

Table 2.2. Trial 1: Influence of uniconazole rate on hemp final size index and plant area when applied to transplants with the liner soak method.

Rate (mg·L)[Z]	Size Index[Y]	Plant Canopy Area (m^2)[X]
0	67.2 A[W]	0.4 A
1	51.1 B	0.3 B
3	32.8 C	0.1 C
5	27.6 C	0.1 C

[Z]Rate = 0,1,3, and 5 ppm uniconazole . [Y]Size index [(height + widest width + perpendicular width)/3]. [X]Plant Canopy Area was calculated by squaring the widest width. [W]Means were separated using the Tukey method for multiple comparison. Means followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 2.3. Trial 1: Influence of uniconazole rate on hemp size index and plant area when applied to transplants with the liner soak method.

Rate (mg/L)[Z]	Plant Biomass[Y]	Apical Inflorescence[X]	Remaining Inflorescence[W]	Total Inflorescence[T]	Calculated Yield per Area ($g \cdot m^2$)[U]
0	58.1 A[V]	3.2 C	31.4 A	34.6 A	87.6 B
1	40.1 B	2.9 C	23.5 B	26.3 B	96.4 B
3	27.0 C	4.6 B	14.6 C	19.2 C	192.6 A
5	20.9 D	6.0 A	9.0 D	15.0 D	218.1 A

[Z]Rate = 0,1,3, and 5 ppm uniconazole. [Y]Plant biomass represents the total dry biomass of all shoot portions of the plant. [X]Apical inflorescence represents the most apical flower and largest bud and was harvested by removing the upper 14 cm of the flower. [W]Remaining inflorescence represents all remaining dry flower biomass when apical inflorescence was removed. V Total inflorescence represents the total dry flower biomass. [U]Calculated yield per area was calculated by adjusting yield per area to match an extrapolated plant density based on the average diameter of each treatments plant canopy [S]Soak time = the time root balls were soaked for 0,30, 75 and 120 sec. [V]Means were separated using the Tukey method for multiple comparison. Means followed by the same letter are not significantly different ($\alpha = 0.05$).

Table 2.4.Trial 1: Effect of rate, soak time, and their interactions on plant height, size index, and stem diameter on hemp transplants treated with uniconazole using the liner soak method.

	ANOVA p values[Z]			
Main Effects	Stem Diameter	Plant Height	Final Size Index[Y]	(Plant Area)
DAT[X]	< 0.0001	< 0.0001	-	-
Rate[W]	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Soak Time[V]	0.9360	< 0.0001	0.2444	0.4662
Rate x Soak Time	0.0309	0.0435	0.9962	0.6585
Rate x DAT	< 0.0001	< 0.0001	-	-
Soak x DAT	0.8442	0.4636	-	-
Rate x Soak x DAT	0.0720	1.0000	-	-

[Z]Analysis of variance for main effects and their interactions ($\alpha = 0.05$). [Y]Size index [(height + widest width + perpendicular width)/3]. [X]DAT = days after planting. [W]Rate = 0,1,3, and 5 ppm uniconazole. [V]Soak time = the time root balls were soaked for 0,30, 75 and 120 sec.

Table 2.5. Trial 2: Effect of rate, soak time, and their interactions on plant height, size index, and stem diameter on hemp transplants treated with uniconazole using the liner soak method.

	p values[Z]	
Main Effects	Stem Diameter	Plant Height
DAT[Y]	< 0.0001	< 0.0001
Rate[W]	< 0.0001	< 0.0001
Rate x DAT	< 0.0001	< 0.0001

[Z]Analysis of variance for main effects and their interactions ($\alpha = 0.05$). [Y]DAT = days after planting. [W]Rate = 0,1,3, and 5 ppm uniconazole.

Table 2.6. Trial 2: Influence of uniconazole rate on dry biomass and flower yield on hemp plants when treated with the liner soak method.

Rate (mg/L)[Z]	Plant Biomass (g)[Y]	Total Flower Biomass (g)	Calculated Total Flower Biomass per Area ($g \cdot m^2$)[X]
0	357.8 A[W]	220.6 A	551.5 B
1	208.0 B	119.5 B	398.30 C
3	138.5 C	73.9 C	739.0 A
5	-	-	-

[Z]Rate = 0,1,3, and 5 ppm uniconazole. [Y]Plant biomass represents the total dry biomass of all shoot portions of the plant, [X]Calculated yield per area was calculated by adjusting yield per area to match an extrapolated plant density based on the average diameter of each treatments plant canopy. [W]Means were separated using the Tukey method for multiple comparison. Means followed by the same letter are not significantly different ($\alpha = 0.05$).

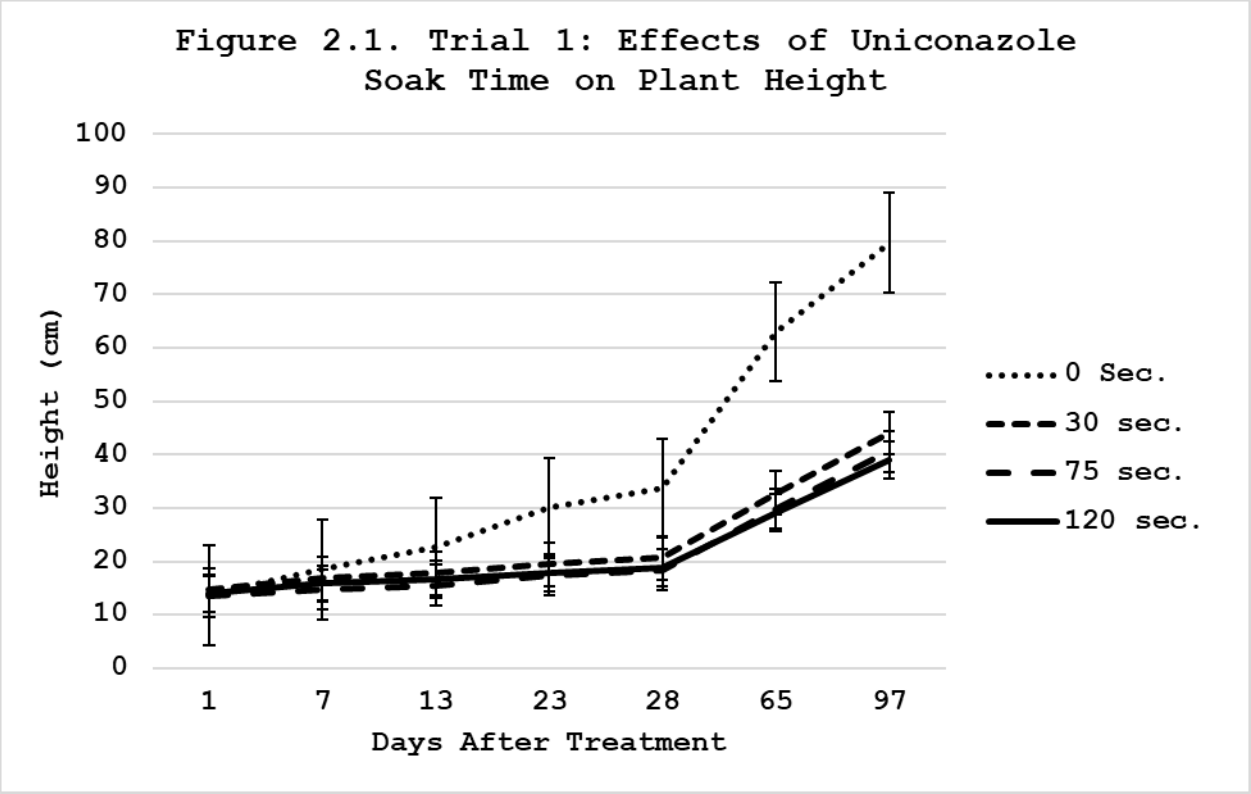


Figure 2.1. Trial 1: Effects of Uniconazole Soak Time on Plant Height
Height (cm)
100
90
80
70
60
50
40
30
20
10
0
1
7
13
23
28
65
97
Days After Treatment
0 Sec.
30 sec.
75 sec.
120 sec.

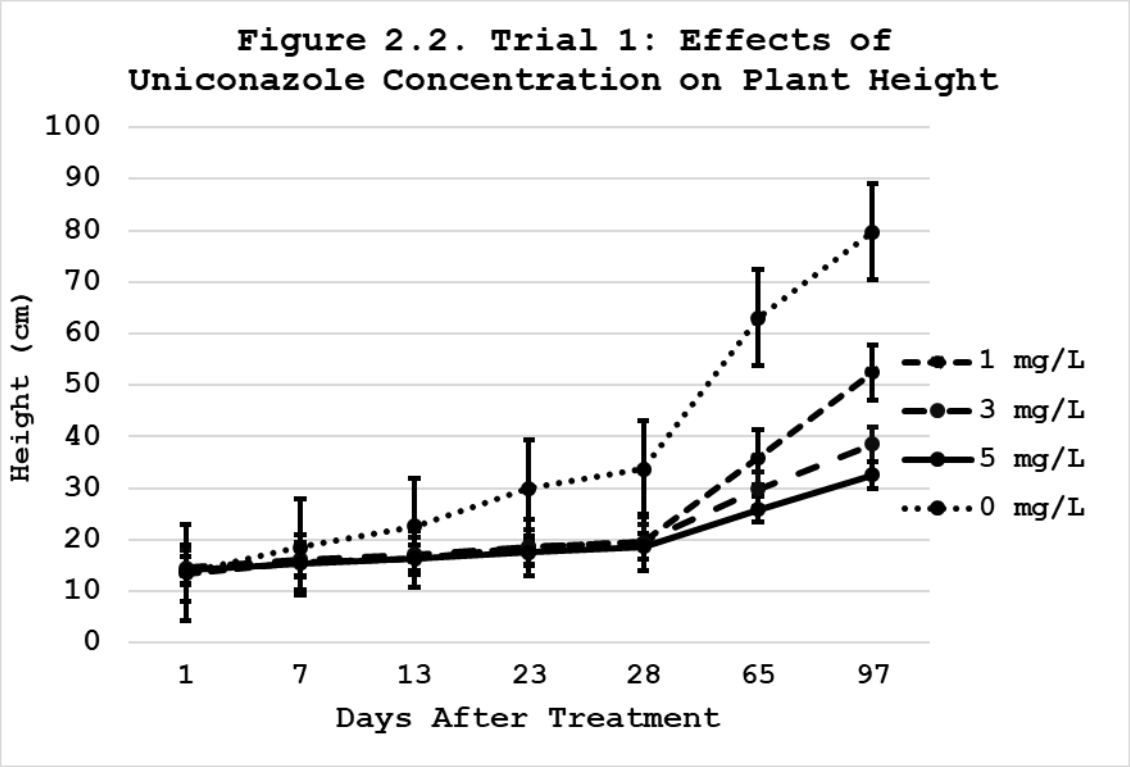
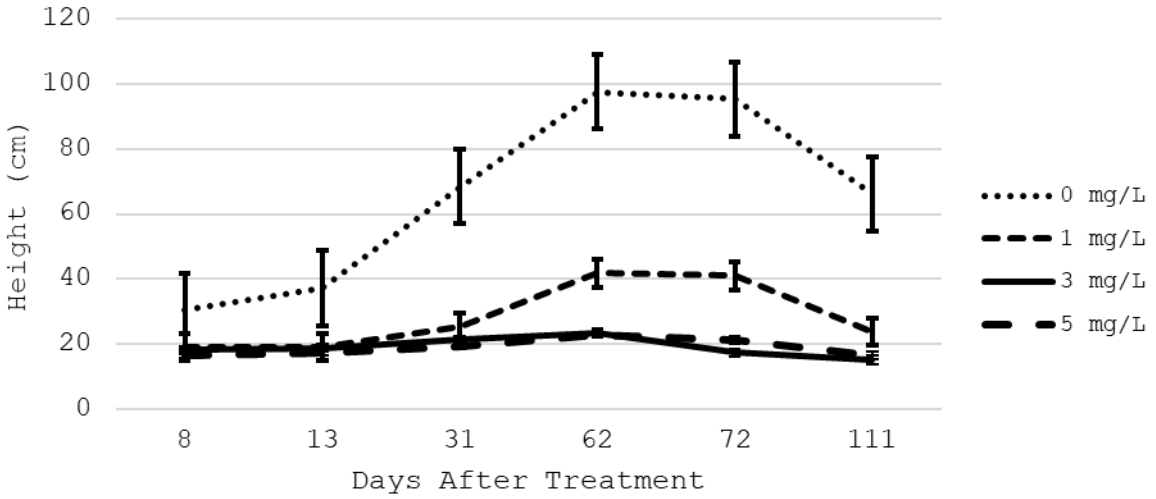


Figure 2.2. Trial 1: Effects of Uniconazole Concentration on Plant Height
Height (cm)
100
90
80
70
60
50
40
30
20
10
0
1
7
13
23
28
65
97
Days After Treatment
1 mg/L
3 mg/L
5 mg/L
0 mg/L

Figure 2.3. Trial 2: Effects of Uniconazole Rate on Plant Height of *Cannabis sativa* Treated Using the Rootball Soak Method.



Chapter III

Evaluation of the Efficacy of Biological Insecticides on Two-spotted Spider Mites on Greenhouse Grown Hemp

Abstract

Two-spotted spider mites (*Tetranchyus urticae*), (TSSM) are a major pest in nursery and greenhouse production and feed on over 3800 plant species. Rapid reproductive cycles and the ability for females to asexually reproduce, the population of TSSM can rapidly grow to an unmanageable size. Growers normally are unaware of TSSM until after the damage has occurred. Biological pesticides are of interest to Alabama cannabis growers but the efficacy of these products have not been evaluated. Our study looks to evaluate the ability to control populations of TSSM using biologicals available to Alabama growers.

Efficacy was evaluated for control of two-spotted spider mites TSSM in *C. sativa*: Grandevo, Venerate® XC, BoteGHA® ES and SilMatrix®. Each population was evaluated based on numbers of adults and eggs and damage ratings. A second trial was repeated but only damage ratings were collected. In trial 1, Venerate® and Grandevo® reduced the population of TSSM by 75 and 67% at 14 DAT, respectively. SilMatrix® and BoteGHA® were not significantly different from the untreated control. In trial two, only Venerate® at 14 DAT demonstrated efficacy on TSSM with a 39 % reduction in damage ratings compared to control.

Introduction

There is little published information on controlling two-spotted spider mites, (TSSM) in *C. sativa* crops. Additionally, hemp growers in Alabama have few pesticide options and are primarily limited to tolerance exempt materials. Biological pesticides may offer an alternative for hemp growers as these products are often tolerance exempt, have relatively short pre-harvest intervals, and are often labeled for organic production. Previous studies on crops such as avocados, hemp, peanuts and tomatoes have shown varying levels of control when treating for TSSM (Britt and Kuhar, 2020; Bilbo et. al., 2020; Morse, 2008; Majumdar and Price, 2019).

Cordova-Kreylos (2013) and Ruiu (2015), found that Venerate® produced high mortality against TSSM. When applied on strawberries, Grandevo® and Venerate® had fewer TSSM when compared to the untreated control, but results were not statistically signifiant (Dara, 2015). The same study also reported a significant TSSM egg reduction after the first application of Venerate. Throughout Dara's 2015 study, Grandevo® was not significantly different from the untreated control, and TSSM egg counts were higher than all other treatments except the untreated control after seven days after treatment. In a study examining TSSM control on green beans, Golec (2020), observed that Venerate® was not different from the untreated control. Across several other studies, Grandevo® performed the same as the untreated control (Britt and Kuhar, 2020; Walgenbach and Schoof, 2014; Bilbo et al., 2020). When evaluated by Ray and Hoy (2014), Grandevo® did not harm two predatory mite species study but failed to suppress populations of TSSM. Golec et. al. (2020) evaluated five biopesticides and noted that as the rates of Grandevo increased, fecundity of TSSM was reduced.

Morse, (2008) reported that SilMatrix® was not effective in reducing Persea mite in avocados, however, there was only one application and counts were performed on mites in wide intervals (20-30 days). When evaluating SilMatrix® efficacy on TSSM, Majumdar and Price (2019) observed no difference from untreated control.

Some biologicals may also reduce fecundity of TSSM. When using *B. bassiana* for TSSM control on cucumbers, Kheradmand et. al. (2021) observed "considerable pathogenicity" but also sub-lethal effects including reduced total lifespan and fecundity in females. Yuan et al. (2018) hypothesizes that due to female TSSM high energy requirements, decreased feeding caused by fungal infections could lead to decreases in egg laying. Application of *B. bassiana*

reduced of TSSM eggs on strawberries and also decreased hatching by 53.3% (Khoury et. al., 2019).

Biologicals and other soft chemistry insecticides have low residual control and often require more frequent or even prophylactic applications. Greater control might be achieved through increased spray frequency instead of single applications. Another factor that might have impacted these results was TSSM counts were performed every 20-30 days. When evaluating biological products, the frequency of data collection can also impact results.

Considering the short residual time and the need for a high degree of spray coverage, biological products may provide better control when used as a preventative control rather than a curative. Application timing and rate could also impact the efficacy of these products.

The objective of this study was to evaluate Grandevo®, Venerate® XC, BoteGHA® ES, and SilMatrix® efficacy on TSSM in greenhouse-grown *C. sativa* crops.

Materials and Methods

The research consisted of two trials that evaluated the efficacy of four products on TSSM of greenhouse grown hemp Products evaluated were three biologicals including Grandevo® (*Chromobacterium* substage strain PRAA4-11 and spent fermentation media), Venerate® XC (Burkholderia spp. Strain A396) and BoteGHA® ES (Beauveria bassiana strain GHA). SilMatrix®, a potassium silicate product, was also evaluated.

Trials were conducted at the Auburn University Ornamental Horticulture Research Center in Mobile, AL (30.702628, -88.145606). The facility used for the research study was a glass-pitched roof greenhouse. Application of a spray-on shade provided 50% solar radiation protection (ReduSol, Lumiforte, The Netherlands). To maintain the vegetative stage in hemp

plants, daylight-LED bulbs provided supplemental 24-hour lighting with string lights above the plants (SOUTHWIRE, Carrollton, GA, USA).

C. sativa L. 'Berry Blossom' (Kayagene LLC. Hollister, CA, USA) was grown in a 1-gallon (5.7 L) nursery container (Lerio Corporation, Mobile, AL, USA) using growing media consisting of 5.06 kg 18-6-8 360 Nutricote (PROFILE Products LLC., Sarasota, FL, USA) /m^3, 1.81 kg Lime (Austinville Limestone, Austinville, VA, USA) /m^3, 0.44 kg MicroMax (ICL Specialty Fertilizers, Summerville, SC USA) /m^3 aged pine bark (Longleaf Mulch, Semmes, AL, USA). Potted hemp plants were irrigated by hand as needed and received a five-times-a-week fertigation of 200 ppm N liquid using a 20-10-20 at (Ultrasol Greenhouse Plus™, SQM North America, Atlanta, GA, USA). Populations of TSSM were established by physical contact from heavily infested lemon verbena plants *Aloysia citrodora* placed into the blocks of 'Berry Blossom'.

The experimental design was a complete randomized block design with ten replicates. The application frequency and rates of the products were based on the label rates with seven day application intervals for Grandevo® 3.363g/m1800L, BoteGHA®1.78mL/1800mL, and SilMatrix®18mL/1800mL. Venerate® 9.37mL/1800mL was the only product to be applied at three-day intervals. Plants were arranged by treatment into a 0.92 m^2 square and treated with an overhead spray provided by a Model T4 CO_2 backpack sprayer (R&D Sprayers, Opelousas, LA) using a TeeJet® 8004VS nozzle (TeeJet Technologies Southeast, Tifton, GA) at 20 PSI for 15 seconds.

Trial 1

The study was initiated on 13 July 2021, when TSSM infested *C. sativa* plants had the aforementioned treatments applied. Adult and egg counts were performed prior to the first application, three days post-application, and seven days post-application. Mites and egg counts were performed by taking a 3 cm long leaf segment selected randomly from the top, middle, and bottom portions of the plant. The 3 cm long leaf segment was collected from the terminal end of the leaf and was treated with 70% isopropyl alcohol to kill any mites present on the leaf. Leaf segments were then placed under a Bausch and Lomb Stereo Zoom 7 microscope (Bausch + Lomb, Laval, Canada) and the number of mites and eggs was counted for each 3 cm leaf segment. The total number of mites and eggs was recorded for the leaflets collected from the top, middle, and bottom of the plant. Mite and egg numbers were recorded by keeping count with a laboratory counter (Fisher Scientific, Hampton, NH).

Plants were evaluated for plant damage seven days post-application based on a 0-5 rating scale. The rating scale was determined by: (0) no damage present on the plant, (1) a few small mite colonies and chlorotic spots along the midrib of lowest leaves, (2) mite colonies are spread out from midrib on lowest leaves with noticeable damage, (3) mites have killed at least 1 leaf; heavily infested and/or damaged leaves above lower leaves, (4) mites have killed several bottom leaves; mites and noticeable damage present on majority of leaves, and (5) little green left or plant is dead. This study ran for exactly 21 days.

Trial 2

Trial 2 was initiated on 27 July, 2021 and used the same protocol as trial 1 except only damage ratings and plant height were recorded. Damage ratings were taken at 7, 14, 22, and 28

DAT. The same damage rating scale was used for both trials. Trial 2 was terminated after 28 days.

Statistical analysis

To analyze interactions, the untreated control was removed so data could be analyzed by mixed model analysis of variance (ANOVA) in JMP® Pro software (ver. 14, SAS Institute, Cary, NC). For trial 1, the main effects and their interactions included treatment and location and their interactions. Significant interactions were further analyzed with post hoc means comparisons using Tukey's honest significant test (HSD) ($\alpha \leq 0.05$) and means comparison was conducted using Tukey's honest significant differences test (HSD) ($\alpha \leq 0.05$).

Results

Trial 1

There was no interaction between the location of the plant where the spider mite counts were taken (top, middle, or bottom) and the area of the plant where treatments were applied (Table 3.1). At 7 and 14 DAT Grandevo® (3.5 at 7 DAT and 5.0 at 14 DAT) and Venerate® (3.2 at 7 DAT and 3.9 at 14 DAT) treatments showed a lower adult spider mite population when compared to the untreated control (Figure 3.1). Grandevo® had a 28 and 20% reduction at 7 and 14 DAT, respectively, when compared to the untreated control (Table 3.2). Venerate® had a 28 and 17% reduction at 7 and 14 DAT, respectively, when compared to the untreated control. TSSM egg counts did not differ significantly between the treatments (Table 3.3). There was no significant difference between egg counts and location (Table 3.4). At 21 DAT, all treatments were statistically similar to the untreated control in regards to egg population (Figure 3.2). At both 7 DAT and 14 DAT, all four treatments resulted in lower mite counts than the untreated

control but at 21 DAT only Venerate® was able to provide a 58% reduction in adult TTSM population compared to the untreated control (Table 3.2).

At 7 DAT and 14 DAT, both Grandevo® and Venerate®, had significantly lower damage than all other treatments (Table 3.5). At 7 and 14 DAT, BoteGHA® and SilMatrix® had similar damage ratings as the untreated control. At 21 DAT, all biological products had similar damage ratings compared to the control (Figure 3.3).

Trial 2

Venerate® was the only treatment with damage ratings to be significantly different than the untreated control at 7 DAT (Table 3.5). Damage ratings for Grandevo® was similar to both the untreated control and Venerate® (Figure 3.4). At 14 DAT and 21 DAT only Venerate® was different from the untreated control (Table 3.5). There was no difference between all products and the untreated control at 28 DAT with the exception of Venerate® (Figure 3.4).

Discussion

This study sought to evaluate biological insecticides for greenhouse grown hemp. Two trials were conducted examining two spotted spider mite populations and subsequent plant damage following insecticide applications.

Across both trials, Venerate® showed the greatest potential for control of TSSM when compared to the other treatments. Venerate® no longer reduced mite populations 14 DAT but had the lowest damage ratings across both trials. Compared to the other treatments in the study, Grandevo® and Venerate® demonstrated the greatest potential to control mite populations. There is conflicting research to the efficacy of Venerate®, in Golec's (2020) study Venerate® exhibited the weakest overall response to one or more life stages of TSSM, Grandevo® in the

same study was shown to only be moderately toxic to all life stages of TSSM. Cordova-Kreylos (2013) found that when using *Burkholderia rinojensis,* the same active ingredient in Venerate®, was noted to have 93% mortality rate on TSSM adults, 3 days after exposure. Mertoglu (2019) observed a 47% adult TSSM mortality rate with Venerate® and a 44% adult TSSM mortality rate with Gradevo® during the same time interval as Cordova-Kreylos (2013). Our study did not have as high of a mortality rate in adult TSSM as reported by Cordova-Kreylos (2013).

In the present study, Venerate® was more efficient compared to the other treatments. Venerate® was the only treatment to be significantly different than the other treatments and the untreated control. Golec's (2020), observed that Grandevo® reduced adult fecundity and total eggs per cohort by 72.5% when compared to the control 7 DAT whereas in the present study, the TSSM egg counts was decreased by 84% and significantly different than the untreated control after 7 DAT. Venerate® was also able to decrease TSSM egg counts by 77% when compared to the untreated control at 7 DAT. Golec (2020) also noted that Grandevo® and Venerate® are slow acting and can take up to 7 days to show visible effects. Similar to findings reported by Golec (2020), Mertoglu (2019) also reported no significant difference in egg hatchability between Grandevo®, Venerate® and the untreated control on days four to ten. This may explain the higher TSSM egg counts for Grandevo® at 14 DAT in the present study.

SilMatrix® was not statistically different from the untreated control, which follows the same results reported by Morse (2008).

Our trials were conducted under heavy TSSM pressure, a prophylactic approach using biological insecticides with a preventative goal might provide better long term control than using these products in a curative approach. Heavy mite pressure may explain the lack of control provided by SilMatrix® compared to the control reported by Majumdar and Price (2019) on

peanuts. Further studies should evaluate these products when used on plants with no TSSM population as a preventative treatment.

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Tables and Figures

Table 3.1. Trial 1: Efficacy of select biological insecticides on mite adult and egg counts and plant location.

	p values[Z]					
	Adults			*Eggs*		
Main Effects	7 Days[Y]	14 Days	21 Days	7 Days	14 Days	21 Days
Treatments[X]	<0.0001	0.0016	0.0018	0.3317	0.0002	0.0928
Location[W]	<0.0001	<0.0001	<0.0001	<0.0001	0.0772	0.3368
Treatment x Location	0.9428	0.3134	0.1089	<0.0001	0.1302	0.5665

[Z] Analysis of variance for main effects and their interactions ($\alpha = 0.05$), [Y]Days after treatment, [X]Treatments consist of; BoteGHA, SilMatrix, Venerate, and Grandevo,[W]Location represents top,middle, and bottom portions where leaf samples were taken.

Table 3.2. Trial 1: Efficacy of select biological insecticides on mite adult and egg counts and plant location. (Sum of all three locations)

	Adults[Z]			*Eggs*[Y]		
Treatment	7 Days[X]	14 Days	21 Days	7 Days	14 Days	21 Days
Untreated Control	54.7 A[W]	46.9 A	30.4 A	24.4 A[V]	3.8 B	4.3 A
BoteGHA	28.6 B	32.3 AB	22.1 AB	9.1 B	7.0 AB	1.5 A
SilMatrix	19.6 B	27.0 BC	29.7 A	19.5 B	1.8 B	3.4 A
Venerate	9.6 B	11.8 C	12.6 C	5.6 B	5.5 B	2.6 A
Grandevo	10.4 B	15 BC	22.5 AB	3.9 B	15.0 A	4.4 A

[Z]Adult two-spotted spider mites, [Y]Two-spotted spider mite eggs, [X]Days after initiation, [W]Adult two-spotted spider mite counts, [V]Two-spotted spider mite egg counts

Table 3.3. Trial 1: Efficacy of select biological insecticides on mite adult and egg counts, averaged across all locations.

	Adults[Z]			*Eggs*[Y]		
Treatment	7 Days[X]	14 Days	21 Days	7 Days	14 Days	21 Days
Untreated Control	18.2 A[W]	15.6 A	10.1 A	8.1 A[V]	1.3 B	1.4 A
BoteGHA	9.5 B	10.7 B	7.4 AB	3.0 B	2.3 AB	0.5 A
SilMatrix	6.5 BC	9.0 BC	9.9 A	3.8 B	0.6 B	1.1 A
Venerate	3.2 C	3.9 D	4.2 B	1.9 B	1.8 B	0.9 A
Grandevo	3.5 BC	5.0 CD	7.5 AB	1.3 B	5.0 A	1.0 A

[Z]Adult two-spotted spider mites, [Y]Two-spotted spider mite eggs, [X]Days after initiation, [W]Adult two-spotted spider mite counts, [V]Two-spotted spider mite egg counts

Table 3.4. Trial 1: Mite and egg population as relates to sample location

	Adult Mite Counts			Mite Egg Counts		
Sample Location[Z]	7 Days[Y]	14 Days	21 Days	7 Days	14 Days	21 Days
Top	5.6 B	11.2 A	11.4 A	3.3 A	2.9 A	1.0 A
Middle	5.2 B	6.3 B	5.8 B	4.4 A	2.5 A	1.3 A
Bottom	13.8 A	9.0 AB	6.2 B	3.1 A	1.2 A	0.9 A

[Z]Location represents top, middle, and bottom portions where leaf samples were taken, [Y]Days after treatment

Table 3.5. Comparisons of treatment effects on damage ratings.

	Trial 1[Z]			*Trial 2*[Y]			
Treatment	7 Days[X]	14 Days	21 Days	7 Days	14 Days	21 Days	28 Days
Untreated Control	3.8 A[W]	4.0 A	4.0 A	2.5 A	3.3 A	3.8 A	4.4 AB
BoteGHA	4.0 A	3.9 A	4.1 A	2.3 AB	2.9 A	3.7 A	4.5 A
SilMatrix	3.7 A	3.9 A	4.1 A	2.4 AB	2.8 A	3.7 A	4.2 AB
Venerate	2.7 B	3.3 B	3.8 A	1.3 C	2.0 B	2.8 B	3.7 C
Grandevo	2.7 B	3.2 B	4.0 A	1.8 BC	2.5 AB	3.5 A	4.1 B

[Z]Trial 1, initiated on 13 July, 2021, [Y]Trial 2 initiated on 21 July, 2021, [X]Days after initiation, [W]Damage ratings

Figure 3.1. Trial 1: Total Adult Mite Counts

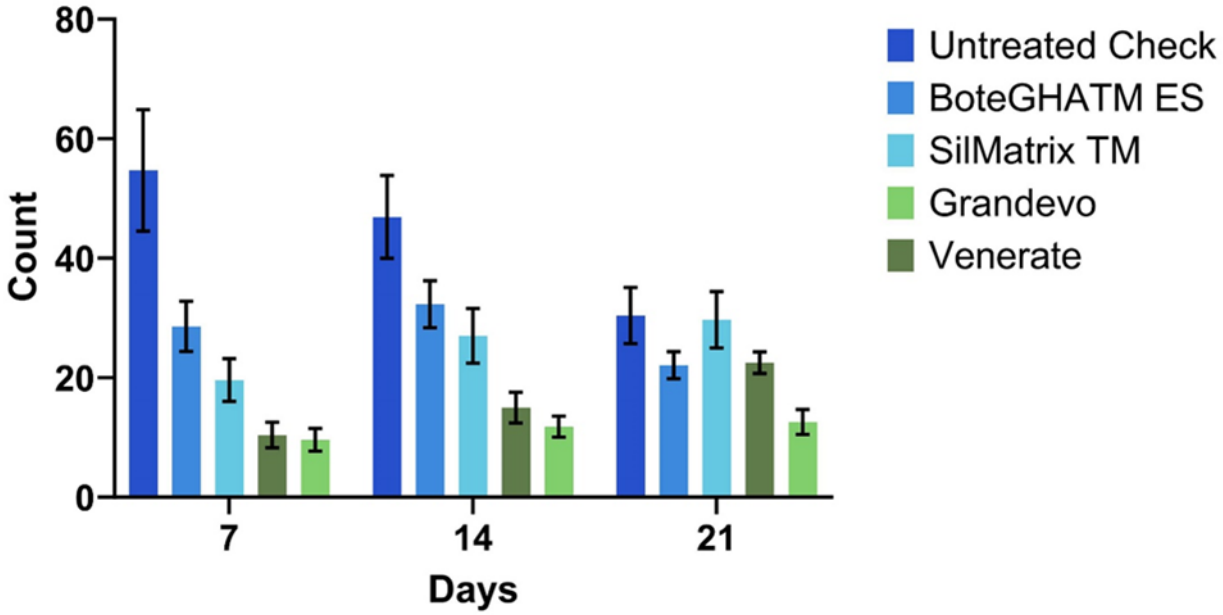

Figure 3.2. Trial 1: Total Mite Egg Counts

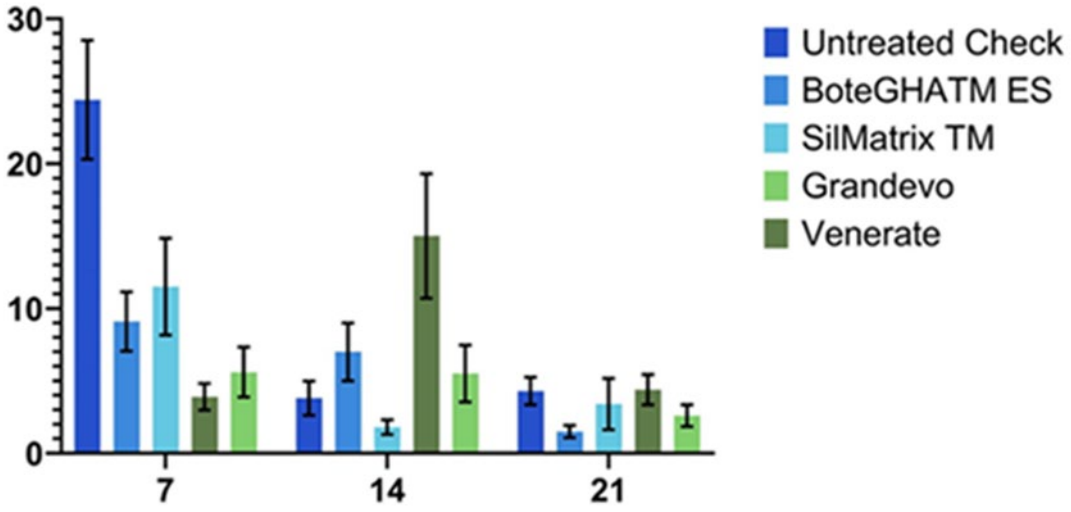

Figure 3.3. Trial 1: Damage Ratings

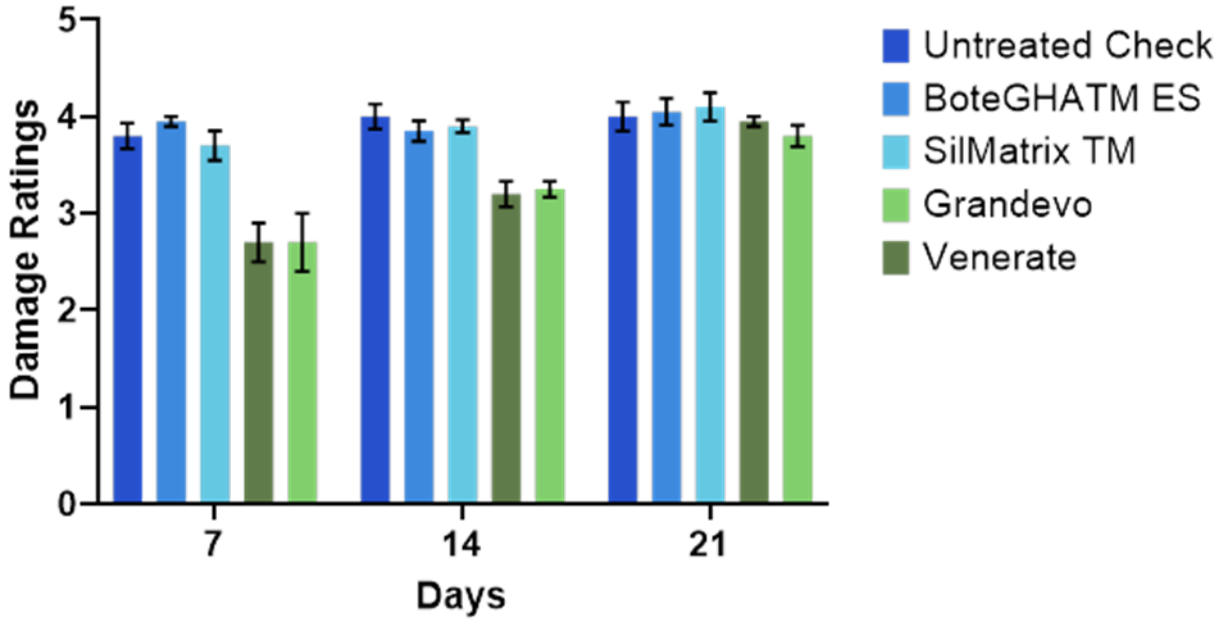

Figure 3.4. Trial 2: Mite Damage Ratings