THESIS

DEVELOPING INTEGRATED PEST MANAGEMENT (IPM) STRATEGIES FOR HEMP

RUSSET MITE (*ACULOPS CANNABICOLA* FARKAS) ON HEMP (*CANNABIS SATIVA* L.)

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ABSTRACT

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RUSSET MITE (*Aculops cannabicola* Farkas) ON HEMP (*Cannabis sativa* L.)

Cannabis sativa L. is a plant that is rapidly becoming a crop of global agricultural importance. However, because of the historical peculiar regulatory status of this crop little has been developed on the pests and pest management needs of the crop. Among the more serious pests that have become established with this plant is hemp russet mite (HRM) *Aculops cannabicola* Farkas (Acari: Eriophyidae).

In order to assess the efficacy of various IPM approaches to mitigating HRM infestations, a series of field and lab experiments were conducted including: 1) evaluation of the effects of sprays of sulfur on control of hemp russet mite, yield, and cannabinoid produduction; 2) evaluation of the efficacy of field release of the phytoseiid mites *Amblyseius andersoni*, *A. swirskii, Neoseiulus fallacis*, and *N. californicus* on HRM-infested hemp plants; and 3) evaluation of hot water immersion as a potential disinfestation method for HRM-infested cuttings used in propagation. The results of the sulfur sprays in field trials showed excellent ability to suppress HRM by up to 98%. Yields of treated plants improved by up to 33% and there was a further increase in the percentage of phytocannabinoids by up to 45% relative to untreated plants. Greatest effects were seen in all trials with plants receiving two applications, one during the vegetative period in July and the second at the initiation of flower production in August. Mass releases of *N. fallacis* and *A. swirskii*, but not *N. californicus* and *A. amblysieus*, did produce a significant reduction in HRM

populations, but no treatments significantly affected yield or percentage of phytocannabinoids, relative to untreated plants. No reproduction was observed of any of the released mites on HRM-infested plants. Immersion treatments to disinfest cuttings included use of a water bath at temperatures of 106°F or 109°F for 10 or 15 minutes, and dips in room temperature surfactant solution of Dr. Bonner's Pure-Castile lavender soap at 1.0% and 0.1% concentrations. All treatments were able to cause significant reduction of HRM on infested hemp cuttings, although none caused complete elimination. No phytotoxicity, as evidenced by effects on subsequent rooting, were observed with any treatment. This study provides novel effective approaches to mitigating HRM at multiple stages in hemp production operations. Outcomes of this research may provide hemp producers and other stakeholders with key pest management strategies needed to produce hemp plants that are free of HRM.

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DEDICATION

For Dr. Thomas Patrick O'Shaughnessy.

"*The more you know, the more you know the less you know*.

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CHAPTER 1- INTRODUCTION AND LITERATURE REVIEW

Introduction

Arthropod pest management in crops is a primary concern for agricultural producers across the industry. *Cannabis sativa,* or hemp, crops are no exception to this. Unlike other major crops, however, hemp has experienced a unique legal history in the United States that has restricted its access. As a result of its prohibition dating roughly from the passage of the Marihuana Tax Act in 1937 to the introduction of state pilot programs under the Agricultural Act of 2014, and ultimately federal legalization of industrial hemp under the Agricultural Improvement Act of 2018 (also known as the "2018 Farm Bill"), the agroecology of hemp in the US has gone under-studied until recent years (Cranshaw et al., 2019; Fike et al., 2020; Malone and Gomez, 2018; Mark et al., 2020; Rawson, 2005).

Under the protection of the Agricultural Act of 2014, universities in the United States were able to begin conducting research into domestic hemp production, and recent studies provide valuable insights into this system (Britt, 2021; Cranshaw et al., 2019). Prior to this period, much of the research conducted on hemp was limited to countries which were more permissive to its production, such as Canada, Israel, and the Netherlands (Mark et al., 2020). Much of the work researching hemp in the US is still ongoing, however, and vital research questions remain. The current study seeks to provide valuable insights into the management of one of the most prolific pests threatening hemp production in the US, *Aculops cannabicola*, the Hemp Russet Mite.

Background

Hemp is a crop with a history that long predates its prohibition in the twentieth century, with its versatile applications as a crop being documented throughout the centuries (Small and Marcus, 2002). Records of hemp being cultivated for fiber and medicine date back to ancient Egypt, and evidence of hemp fiber usage in China goes back to 10,000 BC. Taxonomic descriptions of *C. sativa* or "cultivated hemp" date back to the sixteenth century (Russo, 2007). In US history, accounts of founding fathers such as George Washington and Thomas Jefferson document the importance of hemp cultivation in the economic growth of the colonies, and during World War II the US government encouraged farmers to grow hemp for fiber and oil as part of the war effort (Deitch, 2003; Rawson, 2005).

In the mid-to-late twentieth century, however, public opinion surrounding hemp declined due to its potential use as a narcotic. This culminated in 1970 with the passage of the Controlled Substances Act, which categorized "marijuana" as a schedule 1 narcotic along with dangerous drugs like heroin, making it criminally illegal to cultivate or possess. Even today, "industrial hemp" is differentiated from "marijuana," which is still categorized as schedule 1 in spite of the two products being derived from the same plant species, *C. sativa*. This differentiation is based upon the content of the psychotropic chemical tetrahydrocannabinol, or THC, with "hemp" being defined as *Cannabis*-based products which may not exceed a concentration of 0.3% THC. This legal distinction was first proposed in 2005 and has gone on to allow for the passage of legislation in the twenty-first century that has permitted hemp to once again be cultivated nationwide in the US for its industrial applications (Malone and Gomez, 2018; Rawson, 2005).

As public opinion surrounding this crop has shifted from the late twentieth century into the twenty-first century (resulting from cultural factors such as the legalization movement, and successful lobbying from groups like the American Farm Bureau Federation), more and more states have introduced measures to legalize *Cannabis* for both industrial use, and medicinal or recreational use (Malone and Gomez, 2018).

While industrial hemp is primarily cultivated for fiber, its applications are incredibly versatile (Small and Marcus, 2002). Hemp may also be grown as a source of food, seed, oil, bedding or feed for livestock, paper, and more recently molded bioplastics, construction materials, and supercapacitors for the next generation of battery technology (EnergyTech Staff, 2022; Small and Marcus, 2002). Pre-prohibition estimates for the industrial applications of hemp describe over 25,000 hemp-based products (Malone and Gomez, 2018). In addition to its industrial applications, hemp cultivation has also been identified as having potential environmental benefits in regenerative agriculture, both as a source of phytoremediation of heavy metals and carbon sequestration (Adesina et al., 2020). Hemp has also been recognized as a possible economic alternative to crops like wheat and tobacco, and interest in the crop has grown as legislation has become more permissive (Mark et al., 2020).

Furthermore, hemp's usefulness in medicine has also been well documented (Russo, 2007). In addition to THC, hemp produces at least 60 other phytocannabinoids, some of which have been identified as being beneficial to human health (Clarke, 1981). Chief among these is cannabidiol, or CBD. The market for CBD-based products has grown in recent years, with applications in routine body care and nutrition, as well as treatment for diseases. Biopharmaceutical applications

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for hemp derived products represents an area of significant need for future research, with phytocannabinoids such as THC, CBD, cannabigerol (CBG), cannabinol (CBN), and cannabichromene (CBC) being identified as possible treatments for diseases like chronic pain, multiple sclerosis spasticity, insomnia, post-traumatic stress disorder, fibromyalgia, and Tourette syndrome (Anonymous, 2017; Williamson and Evans, 2000).

As legal markets opened in the US on a state-by-state basis following the introduction of the state pilot program in 2014, acreage of hemp production in the US rapidly expanded from 0 acres in 2013 to over 146,000 acres in 2019 (Mark et al., 2020). The USDA's National Agricultural Statistics Survey (NASS) estimates that the current acreage of hemp planted in the US has declined to approximately 54,000 acres in 2021 (Nseir, 2022). This has been attributed to regulatory hurdles faced by growers, in addition to an outpacing of supply relative to the demand for the crop, specifically in the market for CBD oil, which has seen significant imports from Canada and China (Mark et al., 2020). In the same survey, however, NASS estimated that hemp production in the US in 2021 valued a total of $824 million, highlighting the continued economic value of hemp as a domestic crop (Nseir, 2022).

The Hemp Russet Mite and its Impact on Hemp Production

In addition to the regulatory hurdles faced by growers, one of the greatest challenges cited by hemp producers is a lack of basic knowledge surrounding the crop (Mark et al., 2020). This inhibits crucial decision-making processing surrounding cultivation. One key area where basic decision-making is hindered is in the field of IPM, or Integrated Pest Management. This approach to pest management relies on the management of pests (including phytophagous arthropods, invasive weeds, and plant pathogens) through limited use of agricultural pesticides

and greater dependence on multi-faceted, integrated approaches (Apple and Smith, 1976). It is predicated on an understanding of the biological and ecological systems that define these pests (Apple and Smith, 1976; Kogan 1998). IPM relies on alternative controls to pest populations, such as cultural, and biological controls. In the case of hemp, further research will be instrumental to the development of best practices in IPM (Britt et al., 2022; Cranshaw et al., 2019).

The management of HRM on hemp is at the epicenter of this need for further research. HRM has been documented in Europe, Asia, and North America, yet HRM represents one of the most under-studied systems in hemp agronomy (Cranshaw et al., 2019; Edde, 2022). This is especially problematic for hemp producers due to the potential for damage caused by this pest (Britt, 2021; Cranshaw et al., 2019). HRM affects hemp crops in the field, in greenhouses, and in indoor cultivation. While HRM populations have been observed to rise in field plants over the course of a growing season and to cause significant injury, the risk to hemp production is greatest in indoor production systems, where unchecked mite populations may reach incredibly destructive levels (Schreiner and Cranshaw, 2020).

Hemp is an annual crop that is typically cultivated in the field during the summer months before being harvested in the fall, and indoors year-round. When present in field plants, HRM typically will have lower populations at the beginning of the growing season that gradually increase over the summer before declining prior to harvest (Schreiner, 2019). It is believed that naturally occurring enemies may play some role in suppressing HRM populations in the field, however studies into the efficacy of these enemies in controlling HRM are limited, and no commercially

available biological control agents have been shown to economically manage HRM (Britt et al., 2022). It is unknown how HRM bridge growing seasons in the field, as no description exists for the overwintering habits of this pest. It is unclear whether HRM undergo a period of diapause (a dormant, non-phytophagous period), or an alternate host period (Schreiner and Cranshaw, 2018). Individual mites are unable to survive in the absence of a host plant, so this remains a mystery. Attempts to rear mites on alternative host plants like fellow *Cannabaceae* family member *Humulus lupulus* L. (hops) have been unsuccessful; and attempts made in the course of this study to rear mites on alternative materials like wood, metal, glass, plastic, as well as possible overwintering hosts like bindweed (*Convolvulus arvensis* L.), and Canada thistle (*Cirsium arvense* L.) were all unsuccessful as well (Schreiner and Cranshaw, 2018). HRM populations have been observed on volunteer hemp plants in the field, and volunteer plants may play some role in promoting the re-colonizing of HRM in the field across growing seasons (Schreiner and Cranshaw, 2019).

Indoor hemp cultivation, however, is at risk to HRM year-round (Britt et al., 2022; Groves et al., 2020). In enclosed growing environments, non-pollinated female hemp plants are typically grown for CBD production (Adesina et al., 2020). This is achieved using high-powered grow lights, and periods of darkness that mimic shortened day lengths in the field. Hemp is highly photoperiod sensitive and will flower under conditions of darkness exceeding 12 hours of dark to 12 hours of light regardless of plant maturity (Backer et al., 2019). This allows hemp flowers to be cultivated indoors, with plants at various stages of maturity in different growing environments within a facility, thus allowing for continuous production. This poses a unique opportunity for

HRM to persist in indoor growing operations in a way it does not in the field (Pulkoski and Burrack, 2020).

Once mites have established in an indoor grow, they are able to proliferate continuously, and can be extremely difficult to eradicate. In these environments, HRM disperse on air currents, greatly assisted indoors by fans that are commonly used in growth facilities. HRM may also be transferred by humans who work on HRM-infested plants then move to other plants. (Schreiner and Cranshaw, 2020; Britt et al., 2022, Britt, 2021). In situations where HRM establish indoors and go undetected, populations may reach severe enough levels to cause plant death, or even crop failure, highlighting the importance of screening, and early detection for management in indoor production (Britt, 2021; Cranshaw et al., 2019). Due to the high risk of this pest in indoor cultivation, economic thresholds for action against HRM should be lower than in outdoor cultivation, and repeated screening should be conducted after taking action to ensure eradication of this pest before it is able to reach less manageable levels (Pedigo, 1999). Mites that disperse outdoors may not re-infest field plants, and instead be swept away by the wind. In indoor cultivation, mites are confined within growing spaces, and are far more likely to resettle on plants (Cranshaw, personal correspondence 2022).

When HRM has been established in an indoor cultivation facility, one measure for breaking the proliferation of this pest across growth cycles is to allow a host-free period, where plants are not present in growing spaces (Schreiner and Cranshaw, 2020). Since HRM is unable to survive in the absence of a host, allowing a period of up to a month without plants may lead to eradication of HRM. These efforts may be enhanced by maintaining high temperatures in previously infested

spaces, "heat treating" rooms with temperatures >100° F, requiring less time for pest populations to die off. This may be problematic for facilities, however, with groups of plants at varying levels of maturity. In these cases, preventing the spread of HRM to younger groups of plants, which may act as new hosts, is essential. When designing growth facilities, airflow patterns should be considered, and flow from areas housing older plants to areas housing younger plants should be avoided (Schreiner and Cranshaw, 2020).

Hemp Russet Mite Biology

Hemp russet mite is a vermiform mite in the arachnid order Trombidiformes and the family Eriophyidae (Farkas, 1960) (**Fig. 1.1**). They feed using chelicerate mouthparts that pierce plant cells, but due to their minute size feeding is limited to the epidermis of their host plant. (Schreiner and Cranshaw, 2018; Petanovic et al., 2007). The body of the HRM displays a number of setae which are useful in latching onto the surface of plants, fellow mites, or other arthropod species, and are also useful in dispersal via wind (Petanovic et al., 2007). HRM undergo arrhenotokous reproduction, whereby unfertilized females lay eggs which produce exclusively male offspring. Males then fertilize females, and fertilized females lay eggs which produce female offspring (Edde, 2022).

The HRM measures approximately 110-210 micrometers depending upon maturity. Its life history is similar to that of other eriophyid mites, with larvae measuring 110-112 micrometers hatching from eggs, before moving on to a nymphal instar which measures 170-172 micrometers, and ultimately an adult form which is 195-210 micrometers (Petanovic et al., 2007) (**Fig. 1.2**). Males are typically at the smaller end of this range, and females are typically at the larger end (Edde, 2022; Petanovic et al., 2007; Pulkoski and Burrack, 2020).

Fig. 1.1. Photograph showing a severe Hemp Russet Mite outbreak. This close-up image was taken from the petiole of a *Cannabis* plant at an Indiana University greenhouse. HRM tend to proliferate on petioles, stems, and the base of leaflets, on the underside of leaves. Image credit Karl Hillig.
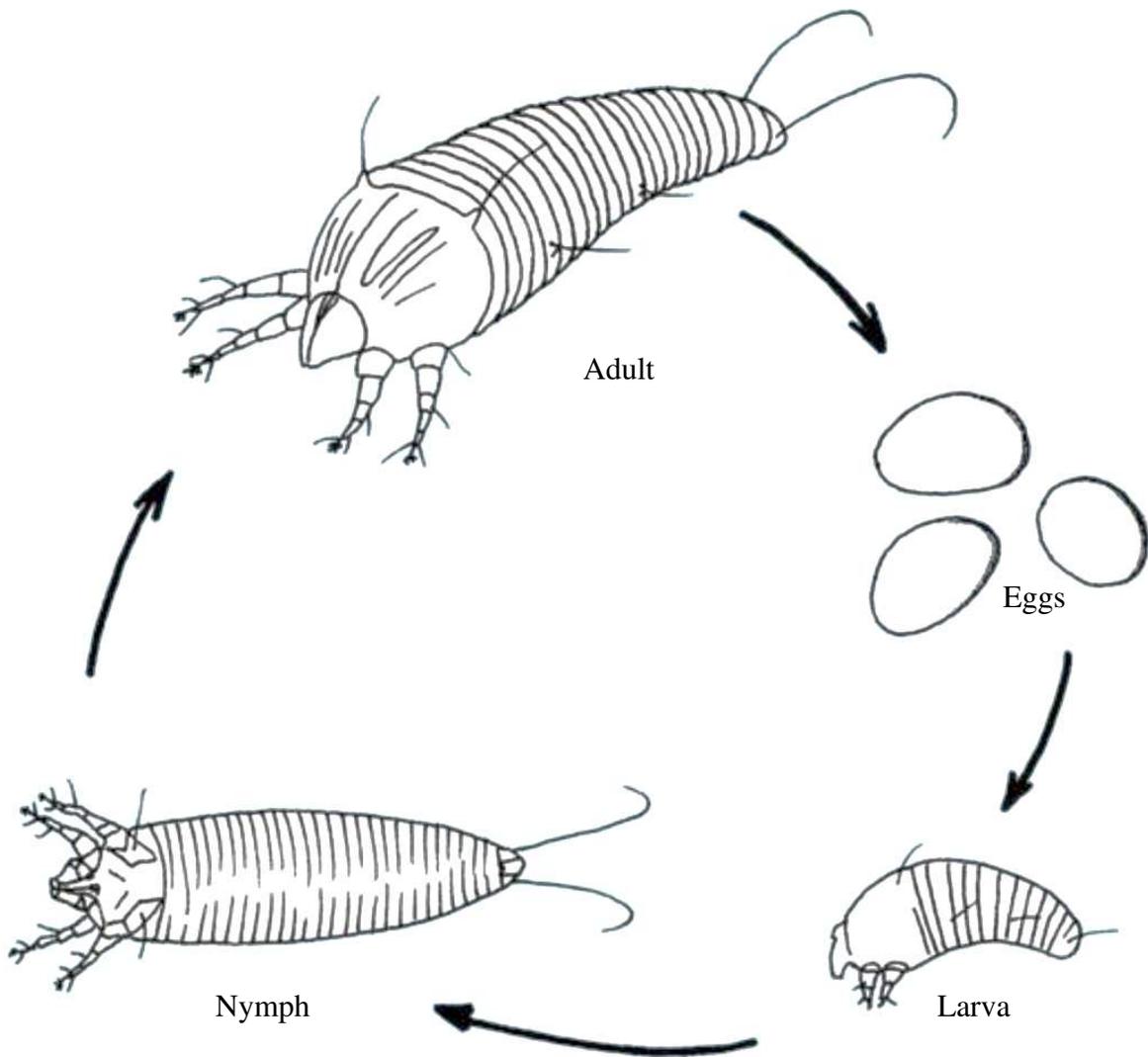
Fig. 1.2. Illustration depicting the life cycle of eriophyid mites. The HRM exhibits the life cycle common to most eriophyid mites, whereby larvae hatch from eggs, moult to produce nymphs, which moult once more to produce adults. Female adults lay eggs. Illustration modified from J.R. Baker, NC State University Extension.

Their size makes HRM invisible to the naked eye, and a microscope is required for observing this pest (Groves et al., 2020). When viewed under a microscope, earlier instars of this pest appear to be translucent and pill-shaped. As they reach adulthood, their bodies become more opaque and may take on a reddish-brown color. Furthermore, as they reach adulthood their bodies appear to narrow more dramatically towards their hind-end. Earlier instars seem to be less mobile than adults, which navigate the surface of leaves using their anterior legs. Mites also display a body-flicking behavior, whereby they latch onto the outer edge of leaves using their posterior setae and swing their bodies back and forth before releasing onto neighboring leaves or plants.

Mites will typically reside on the petioles and stems of plants and may congregate in whorls and crevasses of the leaf surface, such as below the veins of leaves. They typically appear at the highest density at the base of the leaflets, where the leaf meets the petiole, and on the underside of the leaf surface (Schreiner and Cranshaw, 2019). This area may be targeted when scoping for HRM during screening. Additionally, mites seem to favor newer plant growth, relocating from older leaves to fresher leaf tissue.

The types of injury associated with this pest are diverse and may vary based on the cultivar of host plants (**Fig. 1.3**). The symptom most commonly associated with HRM is an upward curling of the leaf surface (Britt et al., 2022; Edde, 2022). This upward leaf curl may not always be indicative of HRM infestation, however, as some cultivars display this phenotype under normal conditions. Other symptoms of HRM infestation include a discoloration of the leaves, causing

Fig. 1.3. Photograph showing hemp plant symptomatic of HRM infestation. This plant shows typical signs of HRM infestation, including stunting, rust-coloration, brittle leaves, and an upward curl along the leaf edge. Other symptoms of HRM infestation include malformation of flowers, and even plant death. Image credit Alabama A&M and Auburn University Extension.

them to turn gray, brown, or rust-colored (Schreiner and Cranshaw, 2018; Schreiner and Cranshaw, 2019; Schreiner and Cranshaw, 2020). Infested leaves may become brittle, crumbling easily when handled. HRM may also lead to abnormal development of hemp flowers, causing them to be severely stunted or malformed (Edde, 2022; Pulkoski and Burrack, 2020). During vegetative growth, stunting of whole plants may also be observed (Edde, 2022). In the most severe infestations, pockets of mites may become so abundant that they become apparent to the naked eye, appearing as a powdery tan substance (Schreiner and Cranshaw, 2020; Britt et al., 2022; Cranshaw et al., 2019). At low enough population levels, no visible signs of injury may occur, complicating detection of this pest (Schreiner and Cranshaw, 2020; Britt et al., 2022). Once more noticeable symptoms set in, populations have likely already reached high levels, and may have begun dispersal to new plants. Thus, early detection through routine screening for this pest is recommended as part of a hemp IPM strategy (Britt et al., 2022; Edde, 2022; Pulkoski and Burrack, 2020).

The life history of HRM is little described in the literature, with some authors suggesting life cycles ranging from 7 to 40 days (Cloyd, 2020; Edde, 2022; Pulkoski and Burrack, 2020). Life history assays conducted in the Nachappa lab have shown that mites produce anywhere from 3-19 eggs in a seven-day period, reaching adulthood after 5-9 days, and having a longevity of 7-9 days. Furthermore, results showed that the variety of host plant played a considerable role in determining the life history traits of HRM. Mites showed greater survival and fecundity on a fiber hemp variety (cultivar "Elite") relative to a CBD variety (cultivar "Unicorn"). This may be due to natural insecticidal qualities of volatile chemicals produced by varieties cultivated for

flower production. Differences in arthropod diversity between hemp cultivar types have been described in previous studies (Benelli et al., 2018; Pulkoski and Burrack, 2020; Schreiner, 2019).

Current IPM Practices for Hemp Russet Mite

In addition to the cultural control methods described above for indoor cultivation, a number of other cultural, as well as chemical, and biological control approaches are applied in managing HRM. As mentioned above, biological controls are not considered to be highly effective in the management of HRM (Schreiner and Cranshaw, 2020; Britt, 2021; Cranshaw et al., 2019). Some species of predators such as *Amblyseius swirskii, A. andersoni,* and minute pirate bugs (*Orius* spp.) are thought to feed on HRM (Edde, 2022; Pulkoski and Burrack, 2020). Additionally, the spider mite predator midge *Feltiella acarisuga* can sustain itself on HRM (Cranshaw, personal communication 2022). The value of these biological control measures in an IPM strategy for this pest, however, are not supported in the data. The effectiveness of biological control agents in mitigating HRM are investigated further in the course of the current study.

Limited research has been conducted into effective insecticidal controls for HRM. Among these, the mineral oil product Suff-Oil X, and the bioinsecticidal product Venerate (Marrone Bio-Innovations) were found to be effective in reducing HRM populations (Britt and Kuhar, 2019; Schreiner, 2019). Sulfur is believed to be another effective chemical control for this pest due to its miticidal properties and may be a practical solution for Colorado growers due to its approved use as an insecticide in hemp production (Schreiner and Cranshaw, 2020).

In terms of best practices for cultural control, a number of steps should be taken to mitigate HRM in hemp production. By preventing the introduction of HRM in indoor growth facilities,

growers may avoid serious outbreaks of this pest (Britt et al., 2022). This may be achieved through careful screening of introduced hemp plants, or clones, as well as implementing quarantine protocols to prevent infestations from occurring via newly introduced plants. When possible, plants identified as being infested with HRM should be quarantined, or destroyed, in order to prevent spread to healthy plants (Britt et al., 2022; Edde, 2022; Pulkoski and Burrack, 2020).

Eriophyid mites have been known to disperse via phoresis, being carried to new plant hosts by other, larger pest species (Brown et al., 2021). Therefore, by reducing overall arthropod pest pressure, the risk of HRM dispersal may also be reduced. Furthermore, HRM may be vectored by human traffic (Schreiner and Cranshaw, 2020; Pulkoski and Burrack, 2020). Growers should always avoid re-entry to uninfested grow spaces after being in an area infested with HRM, and should structure workflow around contamination levels, moving from areas of lower pest pressure to higher pressure. For best results, growth facilities may wish to implement "clean room" protocols, with growers showering and donning freshly laundered clothes prior to entering growing spaces and handling plants (Anglin, 2021).

In order to prevent the proliferation of mites across growth cycles, IPM strategies may be targeted at the propagation stage. HRM are unable to survive on seeds, and propagating plants from seed when possible may provide an option for obtaining HRM free plants (Schreiner and Cranshaw, 2020; Edde, 2022; Pulkoski and Burrack, 2020). When seed propagation is not possible, ensuring that mite populations do not persist from mature plants to juvenile plants is essential in eradicating this pest from facilities where an infestation has occurred. This may be

achieved via treatment of cuttings before planting, or by ensuring that cuttings are taken from mother plants that are free of HRM (Schreiner and Cranshaw, 2020; Edde, 2022; Pulkoski and Burrack, 2020). The current study investigates the efficacy of hot water immersion and surfactant dip treatments in managing HRM during asexual propagation, while assessing risk to clonal propagules.

In the field, similar tactics may be applied in terms of screening, quarantining, and treating juvenile plants prior to planting. This may reduce the risk of HRM colonizing field plants and becoming an issue later in the growing season. To further reduce the risk of re-colonizing plants across growing seasons, volunteer plants should be removed at the start of the season (Schreiner and Cranshaw, 2019). Equipment should also be cleaned between fields, and movements should be based on contamination levels, again moving from areas of lower pest pressure to higher pressure in order to reduce human mediated transport of this pest. Relative to indoor production, however, a lower risk to field plants has been described, with field plants tolerating some level of HRM with lowered risk to crop success. Further differences in hemp tolerance to this pest may derive from the type of hemp being cultivated (i.e., fiber vs. CBD) (Britt et al., 2022, Pulkoski and Burrack, 2020). These factors should be considered when determining economic thresholds for action in an outdoor IPM program (Pedigo, 1999).

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CHAPTER 2- DEVELOPING INTEGRATED PEST MANAGEMENT (IPM) STRATEGIES
FOR HEMP RUSSET MITE (*Aculops cannabicola* Farkas) ON HEMP (*Cannabis sativa* L.)

Introduction

Developing and implementing an IPM strategy is essential to crop success. Typically, this

includes preventative measures such as sanitation and waste removal, as well as direct measures

such as the mechanical removal of pests and infested plants, or pesticidal applications.

Depending on the ecology of pests, this may include the promotion of naturally occurring

predators, release of biological control agents, or the use of chemical controls such as herbicides,

insecticides, and repellents (Apple and Smith, 1976).

In the case of hemp russet mite (HRM), *Aculops cannabicola* Farkas, affecting hemp (*Cannabis*

sativa L.) pest management approaches have been little developed resulting in significant needs

of crop producers to help create effective IPM strategies in their hemp program (Cranshaw et al.,

2019). Hemp russet mite also poses special problems with detection, due to its minute size, and

infestations often are not identified until plants become symptomatic, infestation levels have

become severe (Britt et al., 2022), and the mites have often spread widely in the production

areas.

Earlier investigations into the management of this pest have pointed towards the efficacy of

mineral oils, bioinsecticides, and sulfur-based products in reducing HRM infestations (Schreiner

and Cranshaw, 2020; Britt and Kuhar, 2019; Schreiner, 2019, Cranshaw, personal

communication 2019). Fewer investigations exist into the effectiveness of biological control

agents in managing this pest (Britt et al., 2022). While some predatory species have been observed feeding on HRM, the effect of commercially available biocontrols in reducing this pest in an economic fashion has not yet been proven (Schreiner and Cranshaw, 2020; Edde, 2022; Pulkoski and Burrack, 2020).

In hemp production, the clonal propagation stage marks a pivotal step in this system. Cuttings are taken from mother plants, and then rooted in growing media in order to produce genetically identical stock. This practice is typical to CBD-variety cultivation systems, where clones are preferred to seed stock due to their genetic stability, and shorter development time relative to germinating seeds. Furthermore, hemp is dioecious, and unpollinated females are required for flower production. Asexual propagation of female plants via cloning is therefore a reliable way to exclude unwanted male plants from production (Clarke, 1981).

IPM tactics are often applied during this stage, as clones are highly susceptible to pest pressure (Pulkoski and Burrack, 2020). Additionally, disinfesting cuttings of pests provides a means for growers to disrupt proliferation of pests across growing cycles. These tactics are often weighed against risk of phytotoxicity, as clones are extremely sensitive during this process (Schreiner, 2019).

Immersion of plant tissue in hot water has been shown to be effective in mitigating arthropod pests in other crops (Lay-Yee et al., 1997; Melvin Couey, 1989). Warm water may dislodge pests from the surface of plant tissues, and warm enough water may damage the wax layer of the epicuticle, killing arthropod pests.

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In order to assess the efficacy of various IPM approaches to mitigating HRM infestations, a series of experiments were conducted including: 1) field evaluations during two seasons of a commercial sulfur-based product (Microthiol Disperss) both on control of HRM and hemp yield; 2) field evaluation of the efficacy of four phytoseiid mites used in biological control of spider mites and thrips during one season; and 3) laboratory evaluations of hot water immersion to disinfest hemp cuttings of HRM. I hypothesize that sulfur sprays will reduce HRM populations on treated plants relative to untreated plants, and that in turn the reduction in phytophagy will improve plant health, raising yields and phytocannabinoid content. I hypothesize that releases of biological control agents will not be as effective in reducing HRM populations, given the lack of evidence for the success of biological controls mitigating HRM in the literature. Furthermore, I hypothesize that hot water immersion treatments, and surfactant dips will reduce HRM populations on treated cuttings relative to untreated cuttings, and that any risk to clonal development due to phytotoxicity will be within an acceptable level, justifying the treatment of clones in order to mitigate this pest.

Materials and Methods

Field Evaluations of Sulfur

During 2020 and 2021 the hemp cultivar "Unicorn" was cultivated in the field at the Colorado State University Agricultural Research, Development and Education Center South facility (ARDEC South). Prior to planting, the transplants were grown under conditions to infest all plants with hemp russet mite. The plants were then planted into the field in 30 inch rows, with plants spaced 60 inches within the row. Between each row of hemp was a row of low growing brassicaceous vegetables, which allowed the individual hemp plants to be sufficiently separated so that they did not touch until near harvest. Plots consisted of individual plants, and the

experimental design was a randomized complete block. In 2020 there were six replications, and in 2021 there were five replications. The field was furrow irrigated weekly during the course of the growing season.

All sulfur treatments included the use of the commercial formulation Microthiol Disperss applied as a spray to individual plants at a rate of 5 lbs/A in a liquid volume of 20 gal/A. Treatments involved in both years were either a single application applied early in the growing season (15 July in 2020, 5 July in 2021), late in the growing season approximately at the beginning of flowering (17 August in 2020; 16 August in 2021), a combination of both applications, and an untreated check.

Sampling of HRM was done using collections of five leaves per plant. Leaves were selected by taking the first fully formed leaf from the top growth of randomly selected branches to ensure conformity across plants. Leaves were continuously sampled throughout the season in order to monitor HRM population development. Numbers of mites present were determined using an alcohol collection technique (Appendix 1) during the first year of the study (2020), which involved field collections immediately placed in alcohol. In the second year of the study similar collections were made, but samples were enumerated by microscopic examination, which involved use of leaves collected into plastic bags or petri dishes and subsequently kept refrigerated until the whole leaf could be examined under the microscope.

Upon reaching maturity in early October, plants were harvested using a large pair of garden shears, and placed into 30 gallon paper bags to dry. Plants were given four weeks to dry before

removing stems and branches by shucking the whole plants. Leaf and flower material was then transferred to large plastic tubs to be weighed for final yield results.

After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by liquid chromatography-mass spectrometry (LC/MS) analysis to screen samples from the field studies for the concentration of 20 phytocannabinoids: Cannabichromene (CBC), Cannabichromenic Acid (CBCA), Cannabichromeorcin (CBCO), Cannabichromevarin (CBCV), Cannabidiol (CBD), Cannabidiolic Acid (CBDA), Cannabidivarin (CBDV), Cannabidivaric Acid (CBDVA), Cannabigerol (CBG), Cannabigerolic Acid (CBGA), Cannabicyclol (CBL), Cannabicyclolic Acid (CBLA), Cannabinol (CBN), Cannabinolic Acid (CBNA), Cannabitriol (CBT), delta-8-Tetrahydrocannabinol (D8THC), delta-9-Tetrahydrocannabinol (D9THC), delta-9-Tetrahydrocannabinolic Acid (D9THCA), Tetrahydrocannabivarin (THCV), and Tetrahydrocannabivarinic Acid (THCVA) (Happyana et al., 2013).

Samples from each plant were prepped for analysis by homogenizing using a mortar and pestle (2020) or bead beater machine (2021). 20 ± 0.5 mg of each sample was weighed out into a 2mL glass autosampler vial. 1mL of LC/MS grade MeOH was added to each vial, and samples were vortexed at 4°C for 1 hour. Samples were then centrifuged at 3500 rpm for 10 minutes at 4°C. The supernatant was transferred to a new set of 2mL glass autosampler vials and stored at -80°C until ready to continue processing. Supernatant samples were retrieved from the -80°C freezer and centrifuged at 4°C. 245μL of 80% LC/MS grade MeOH in LC/MS grade water spiked with 25.51 ng/mL CBD-d3 and D9THC-d3 was pipetted into a new set of 2mL glass autosampler

vials. 5µL of each supernatant sample was transferred to this set of vials, with the exception of a blank, and stored at 4°C until ready for final LC/MS analysis. A QC pool was made by transferring 55µL of each diluted supernatant sample, except the blank, to a glass media bottle. 175µL of the pool was transferred into a set of 2mL glass autosampler vials, and 3 QC's were placed at the beginning of the run, followed by randomized samples, with 1 QC placed after every 6 samples, and 1 QC at the end of the run.

Predatory Mite Releases

In 2020 a separate section of the field described above was planted similarly, but using a second cultivar, "SK", infested with HRM prior to transplanting, for use in trials to evaluate the potential of mass releases with the four species of phytoseiid mites. Specific treatments included mass releases of the species *Amblysieus andersoni* (Chant), *Neoseiulus fallacis* (Garman), *N. californicus* (MacGregor), and *A. swirskii* Athias Henriott – or an untreated check. Plots consisted of individual plants, separated from adjacent plants so they did not contact each other. Experimental design was a randomized complete block with four replications.

There were two sources of the phytoseiid mites. *Neoseiulus fallacis* and *A. swirskii* were provided by Beneficial Insectary (Redding, California) and were placed on plants on August 8. Each plant received approximately 5000 mites, which were introduced into Universal Release Boxes that were hung on the plants, with 4 boxes/plant. *Amblysieus andersoni* and *N. californicus* were later received from Biobest USA Inc. (Romulus, Michigan) and released August 26, at a similar rate also using the Universal Release Box for application.

Similar to the 2020 sulfur trial, numbers of HRM were determined based on collections of five leaves per plant throughout the growth season, placed immediately in alcohol, and subsequently enumerated. Upon reaching maturity, plants were harvested, and allowed to dry before being shucked, weighed, and sampled for LC/MS analysis in the same manner as the sulfur trial plants.

Water Immersion Trials

Cuttings (n= 30) were taken from clean mother plants (cultivar "Unicorn") reared in the CSU Plant Growth Facility and processed by removing leaves and nodes up to the top inch of growth, snipping away the tips of fan leaves to reduce crowding effects. Cuttings were placed in 50mL falcon tubes with 1cm^2 of leaf tissue uniformly infested with HRM, taken from the HRM colony in the Nachappa Lab. Cuttings with HRM infested tissue were then stored at 4° C for 48 hours to allow the mites to infest the cuttings before treatment. After this period, infested cuttings were placed in a room temperature water bath to prevent desiccation. An untreated control group was then directly removed from the bath and prepared for rooting.

Groups were then individually removed from the bath to receive treatments before being prepared for rooting. Four treatments included immersion of the cuttings into warmed water using an Anova Precision[R] Sous-vide Cooker: 10 minutes at 106° F, 15 minutes at 106° F, 10 minutes at 109° F, 15 minutes at 109° F. Two other treatments involved immersion of the samples in a dip of a surfactant solution (Dr. Bronner's Pure-Castile[R] Lavender Soap at either a 0.1% or a 1.0% concentration) in room temperature water for 10 seconds.

Upon treatment cuttings were prepared for rooting by snipping at the base of the stem (below the knuckle, at a 45-degree angle) and placing in a solution of Dip-n-Gro rooting hormone for 60 seconds to promote root development before being stuck stem-side-down in rockwool media soaked in a 1:1:1 250-300 ppm solution of Jack's Classic All-Purpose Fertilizer. The cuttings in the media were then stored in a plastic tray under a vented cloning dome with vents closed to maintain conditions of high humidity. They were placed under LED lights on a 20:4 hour light:dark schedule. After 72 hours, the domes were briefly removed to allow gasses to exchange and replaced on the tray. Once a day, the domes were briefly removed and replaced, and the rockwool was watered using the 1:1:1 250-300 ppm fertilizer solution as needed, gradually opening the vents.

After four weeks, the clones were scored based on their root development using a three-point scale. The clones were graded a 1 if no roots appeared on the outside of the rockwool cubes, a 2 if any roots appeared on the outside of the cubes, and a 3 if more than 12 roots appeared on the outside of the cubes (**Fig. 2.1**). Clones were then snipped away from the rockwool cubes at the base of the stem, and stored in 1:1 ethanol:DI water solution before counting mite populations using the alcohol collection technique (Appendix 1).

Fig. 2.1. Root scoring system for water immersion experiment. These images provide examples for rooted clone scores used in the hot water immersion experiment to determine the developmental success, and phytotoxicity risk of treated cuttings. The image on the left shows a clone that received a score of 1, no root tips extend beyond the growing medium. The image in the center shows a clone that received a score of 2, some root tips extend beyond the growing medium. The image on the right shows a clone that received a score of 3, more than 12 root tips extend beyond the growing medium.

Statistical Analysis

All statistical analysis was conducted using RStudio (2021.9.01). Single year yield and

phytocannabinoid data for all field trials (2020 and 2021 sulfur treatments, and 2020 biocontrol

releases) were assessed for normality based on histogram and qq-plot results. If data were found

to be normally distributed, one-way ANOVA tests were used to determine whether the effect of

treatments was statistically significant. When histogram and qq-plot results were ambiguous,

normality was confirmed using a Shapiro-Wilke's test. If data were found to be abnormally

distributed, normality was achieved via log10, exponential, or square root transformation and

analyzed using one-way ANOVA tests. If normality could not be achieved via transformation,

Kruskal-Wallis H tests were used as a non-parametric alternative to one-way ANOVA tests.

Two-year yield and phytocannabinoid data for sulfur trials were analyzed using two-way

ANOVA tests to determine whether the effect of treatments over a two-year period when year-

to-year effects were not significant. Additionally, to determine the effect of treatments on HRM populations, all mite count data was analyzed using repeated-measure ANOVA tests to account for data being repeatedly sampled over time from the same individuals. When significant effects were detected by ANOVA and Kruskal-Wallis H tests, post-hoc tests were conducted using Tukey's honestly significant difference tests, and Dunn's tests for multiple comparisons, respectively, in order to determine the relative effect of specific treatments.

Hot water immersion treatment data was assessed for normality using histogram and qq-plot data and was found to be abnormally distributed. To determine the effect of hot water immersion on mite counts, Kruskal-Wallis H tests were performed as a non-parametric alternative to ANOVA tests due to abnormal distribution. Since these were ordinal data, root scores were analyzed using a Chi-squared test. To analyze the relative efficacy of each treatment, a post-hoc Dunn's test for multiple comparisons was conducted on mite count data. Significance was accepted for $p < 0.05$.

Results and Discussion

Sulfur treatments
Weekly assessments of HRM populations (**Fig. 2.2a-b**) showed a gradual increase in HRM populations on foliage, peaking in late summer (2 September in 2020, 23 August in 2021) followed by a decline through to harvest [repeated measures two-way ANOVA: time ($F=9.23$, df=10, $p< 0.0001$) treatment ($F= 49.86$, df= 3, $p< 0.0001$) time:treatment ($F= 11.71$, df= 30, $p< 0.0001$)]. This pattern of population growth and decline has been described in a past survey of HRM on outdoor grown hemp (Schreiner and Cranshaw, 2019). Overall numbers of HRM recorded on plants varied greatly between seasons, being much lower in 2021 than in 2020, which may be an artifact of the different techniques used to sample plants in the two years.

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A
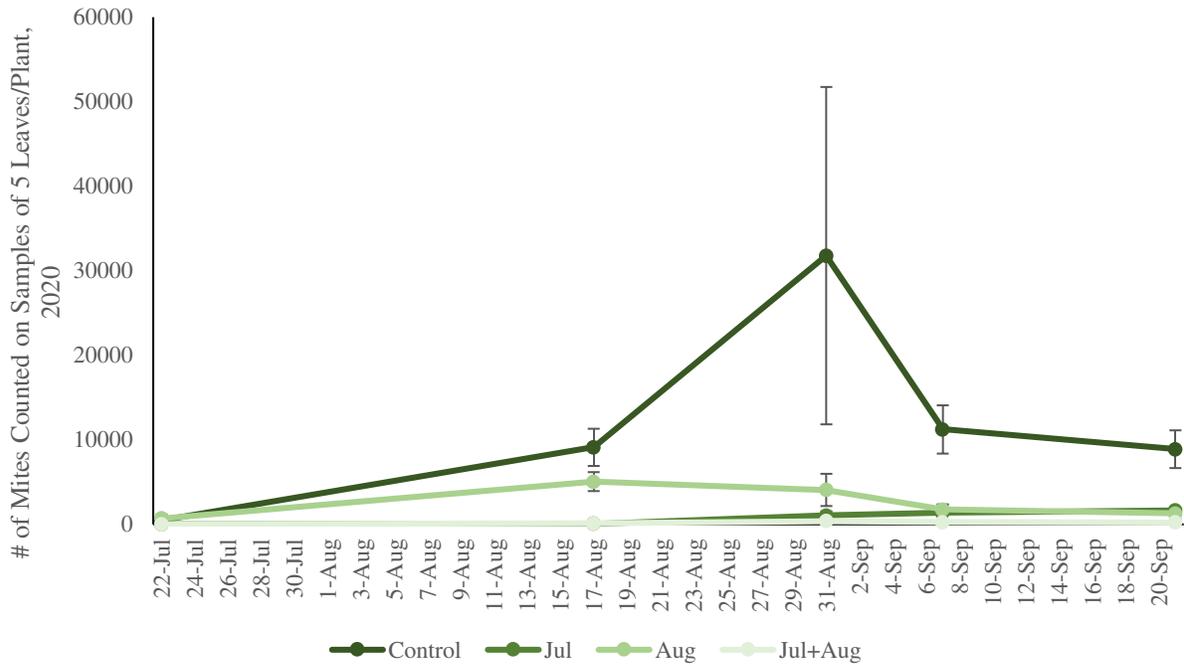
B
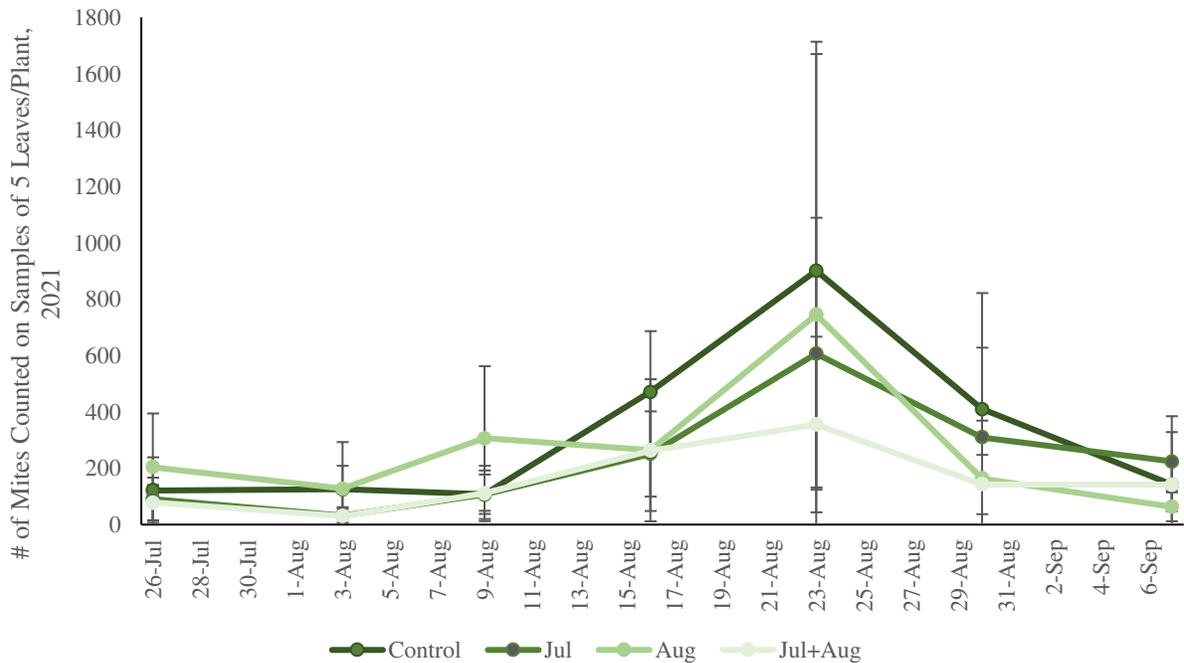
Fig. 2.2a-b. 2-year sulfur trial mite population dynamics over time on hemp plants infested with hemp russet mite. This figure shows average mite counts ±SE for each treatment – an untreated control, an early season sulfur spray (Jul), a late season sulfur spray (Aug), or an early and late season sulfur sprays (Jul+Aug) – sampled over time during the 2020(a) & 2021(b) Sulfur trials. Sampling of HRM was done using collections of five leaves per plant. Leaves were selected by taking the first fully formed leaf from the top growth of randomly selected branches

to ensure conformity across plants. Leaves were continuously sampled throughout the season in order to monitor HRM population development. Numbers of mites present were determined using a microscope counting technique (Appendix 1). Our results show that time had a significant effect on mite population levels, with numbers gradually rising over the course of the season before peaking in late August and dropping off before harvest [repeated measures two-way ANOVA: time (F=9.23, df=10, p< 0.0001) treatment (F= 49.86, df= 3, p< 0.0001) time:treatment (F= 11.71, df= 30, p< 0.0001)].

In both years effects of sulfur applications resulted in dramatic reductions in HRM numbers on plants with greatest reductions from the two application treatments (**Fig. 2.3a-c**) [repeated measures two-way ANOVA: year (F= 0.056, df= 1, p= 0.814) treatment (F= 22.97, df= 3, p< 0.0001) year:treatment (F= 19.99, df= 3, p<0.0001)]. There were differences in the magnitude of control between the two seasons, with reductions of 98% were recorded in 2020 and 43% in 2021, following two sulfur applications.

These data provide the first evaluations of sulfur for hemp russet mite in a field setting and show that sulfur-based products like Microthiol Disperss may be a useful tool in a hemp IPM program. Britt and Kuhar (2020) found a 78% reduction in HRM on indoor grown hemp at 22 DAT and sulfur is used to control other russet mites, notably tomato russet mite (Anonymous 2016).
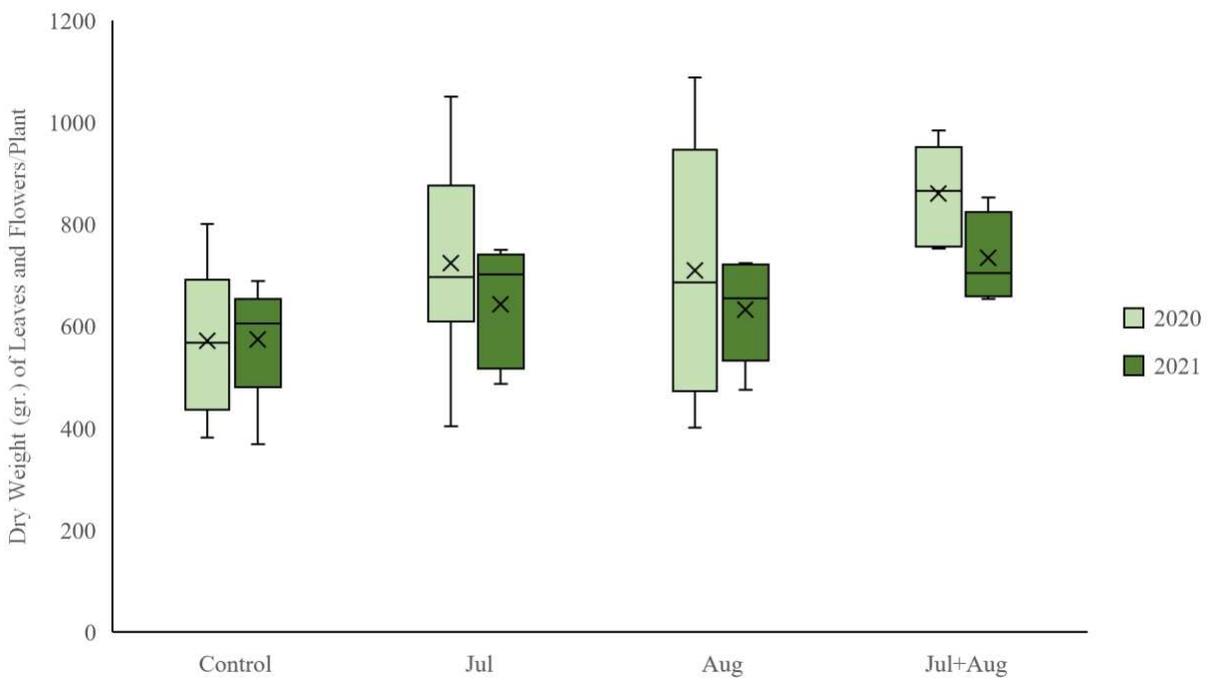
A

B

C
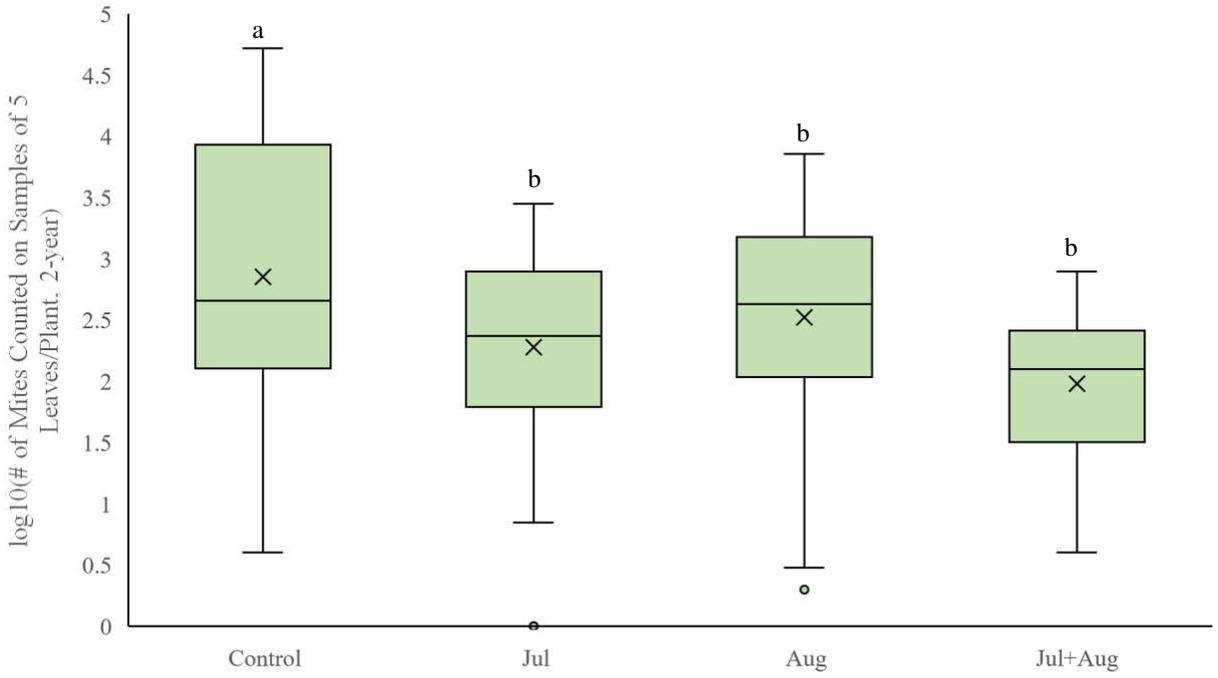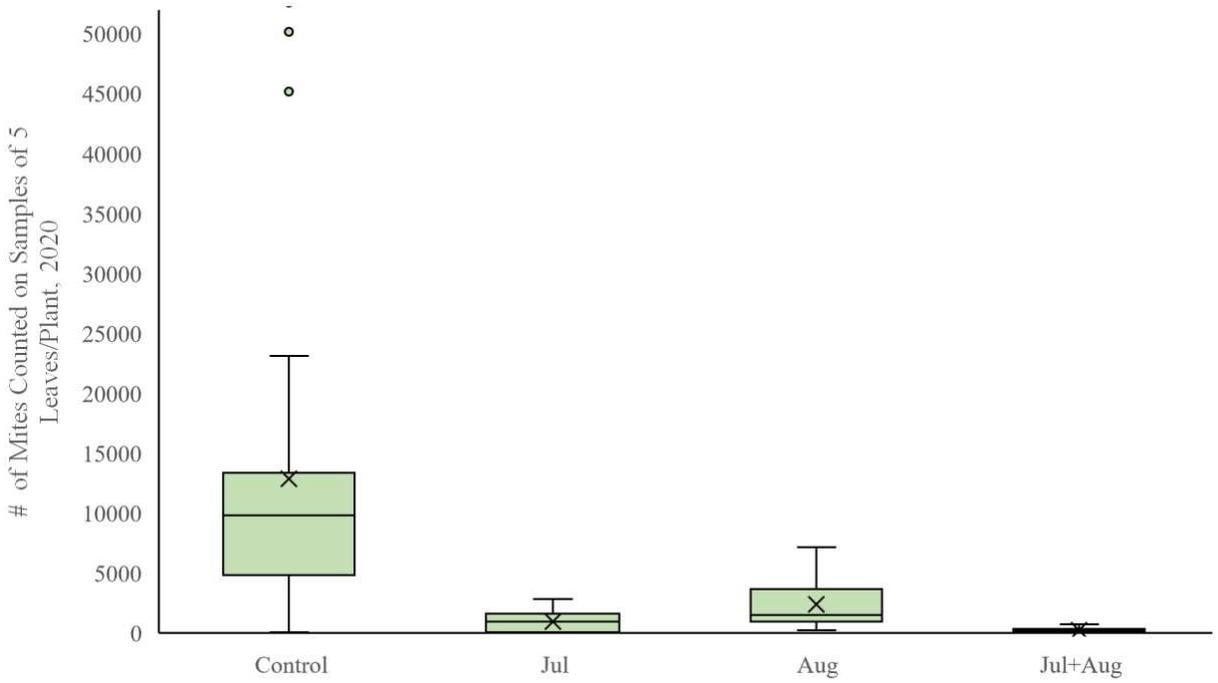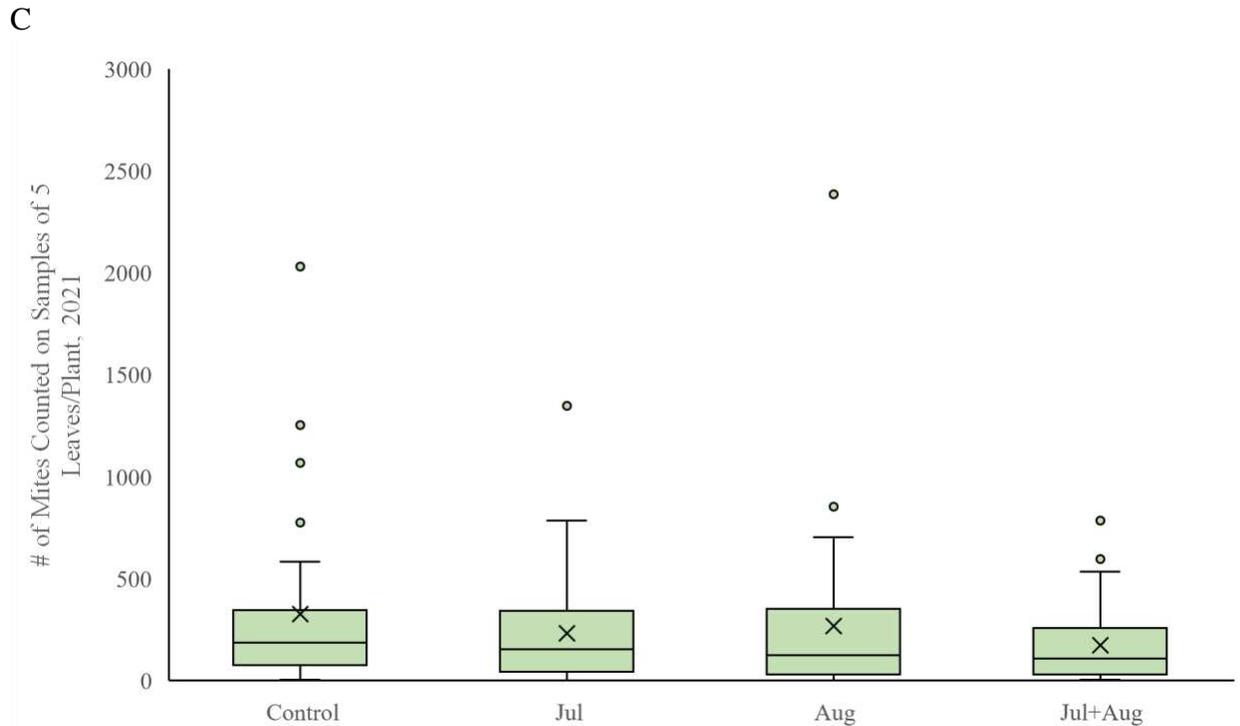
Fig. 2.3a-c. 2-year impact of sulfur treatments on mite populations of hemp plants infested with hemp russet mite. This figure shows the effect of 3 sulfur treatments, an early season spray (Jul), a late season spray (Aug), or early and late season sprays (Jul+Aug) on HRM populations on treated hemp plants relative to an untreated control group over the course of a 2-year study in the field (a). Sampling of HRM was done using collections of five leaves per plant. Leaves were selected by taking the first fully formed leaf from the top growth of randomly selected branches to ensure conformity across plants. Numbers of mites present were determined using an alcohol collection technique (Appendix 1) during the first year of the study (2020) (b) and using a microscope counting technique during the second year (2021) (c). Boxes display the first and third quartile, median (line), mean (x), outliers (points), and whiskers indicate variability outside upper and lower quartiles. Our results show that sulfur treatments significantly reduced HRM populations on treated plants relative to the control group [repeated measures two-way ANOVA: year (F= 0.056, df= 1, p= 0.814) treatment (F= 22.97, df= 3, p< 0.0001) year:treatment (F= 19.99, df= 3, p<0.0001)]. Values marked with the same letter are not significantly different.

Effects on dry weight yield from applications of sulfur also resulted in significantly improved

yield in both years [two-way ANOVA: year (F= 0.55, df= 1, p= 0.463) treatment (F= 3.27, df=

3, p= 0.032) year:treatment (F= 2.7, df= 3, p= 0.06)] (**Fig. 2.4**). Post-hoc analysis of individual

treatments revealed that the combination of early (July) and late (August) season applications

treatment significantly increased yield relative to the control group (p= 0.008, 95% C.I.= 49.56,

411.71) but individual treatments in July and August did not significantly affect yield (July: p=

0.34, 95% C.I.=-66.38, 295.76; August: p= 0.44, 95% C.I.= -79.01, 283.14). Dry weight

increases from the two sulfur applications averaged 33% in 2020 and 22% in 2021.

Fig. 2.4. Impact of sulfur treatments on yield of hemp plants infested with hemp russet mite. This figure shows the effect of 3 sulfur treatments, an early season spray (Jul), a late season spray (Aug), or early and late season sprays (Jul+Aug) on dry weight yields of treated hemp plants relative to an untreated control group. Upon drying after harvest, whole plants were shucked to remove stems and branches, and dry weight yields were taken using plastic tubs. Boxes display the first and third quartile, median (line), mean (x), and whiskers indicate variability outside upper and lower quartiles. Our results show that sulfur treatments significantly

improved dry weight yields compared to untreated plants [two-way ANOVA: year (F= 0.55, df= 1, p= 0.463) treatment (F= 3.27, df= 3, p= 0.032) year:treatment (F= 2.7, df= 3, p= 0.06)].

Effects of the sulfur applications also resulted in significant differences in the percentage of phytocannabinoids. Sulfur treatments significantly improved phytocannabinoid concentrations for 12 of the 20 cannabinoids screened in on 2020 samples (**Fig. 2.5a-l Tables 2.1, 2.2**). In 2021, sulfur treatments significantly improved the concentration of 13 of the 14 phytocannabinoids that were detected (**Fig. 2.6a-m Tables 2.3, 2.4**). Overall percentage of phytocannabinoids on plants receiving two sulfur applications increased an average of 45% in 2020 and 52% in 2021. In both years, the most abundant cannabinoid was CBDA, accounting for 83% of the total cannabinoids in year 1, and 79% in year 2. All sulfur treatments significantly improved the concentration of CBDA in year 1 (F= 12.291, df= 3, p= 0.0001; **Fig. 2.5d**), and the combination of early and late sulfur treatments improved the concentration of CBDA in year 2 (Z= -3.18, df= 3, p= 0.0088; **Fig. 2.6c**). Measured levels of phytocannabinoids also varied between seasons, being 93% higher in 2021. The reasons for these differences between seasons may, in part, be due to weather. Early in September 2020 record high temperatures were recorded and during this period there was also substantial changes in light and air quality due to nearby forest fires. Then, a heavy wet snow fell on 8 September. These events may have damaged the developing floral structures that produce phytocannabinoids.
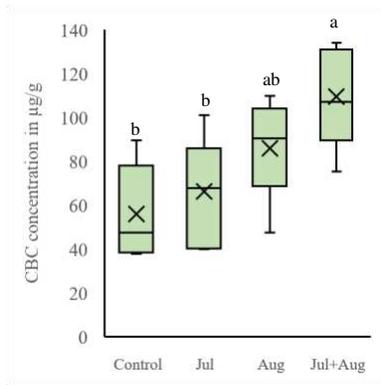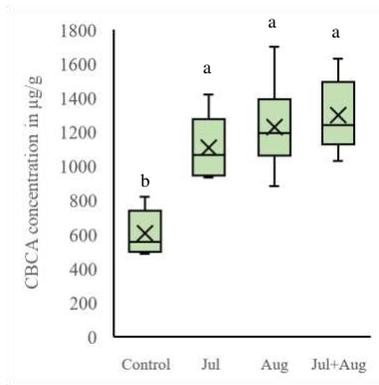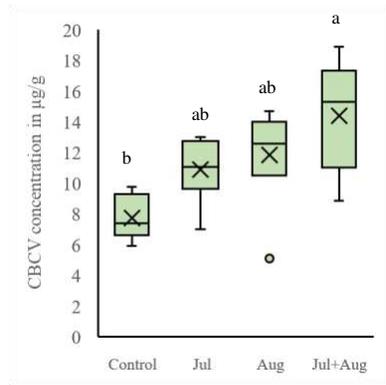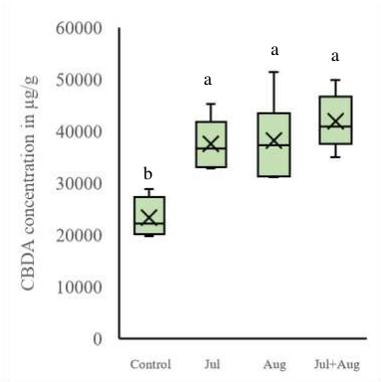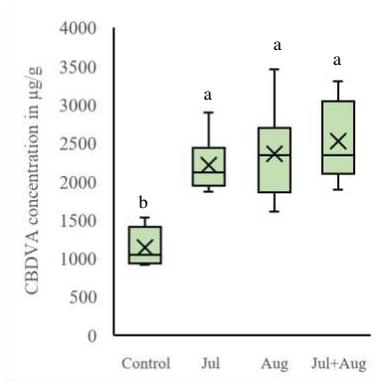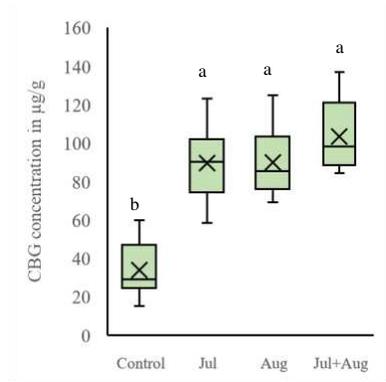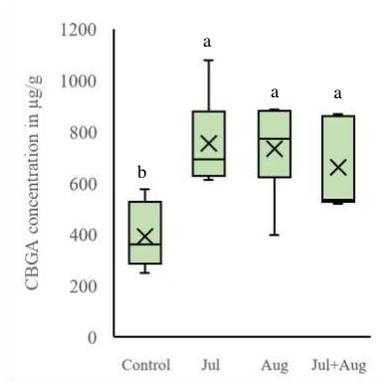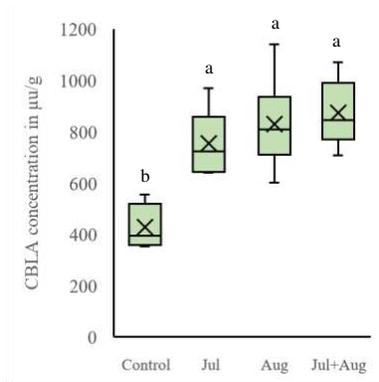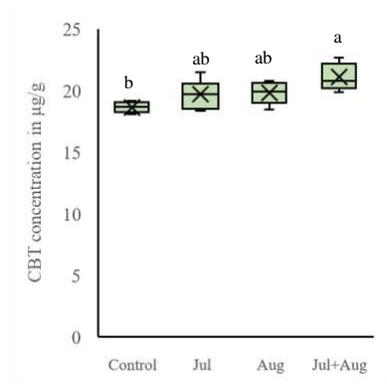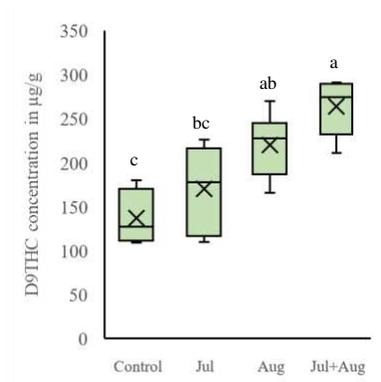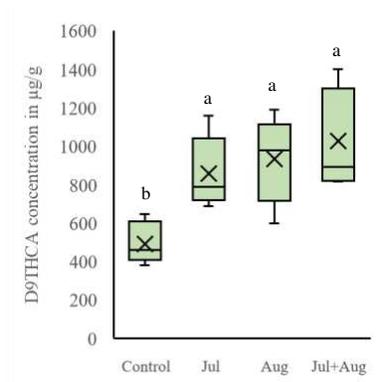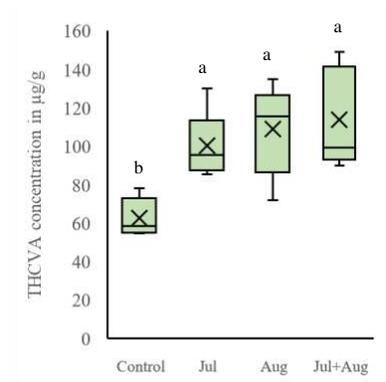
Fig. 2.5a-l. Impact of sulfur treatments on phytocannabinoid production of hemp infested with hemp russet mite in the 2020 field season. This figure shows the 3 sulfur treatments, an early season spray (Jul), a late season spray (Aug), or early and late season sprays (Jul+Aug), significantly improved the phytocannabinoid production of treated hemp plants relative to an untreated control group in 12 of the 20 sampled phytocannabinoids. After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by LC/MS analysis to screen samples from the field studies for the concentration of endogenous phytocannabinoids. Statistical outputs are provided in **Table 2.2**. Values marked with the same letter are not significantly different.

Table 2.1. Impact of sulfur treatments on average phytocannabinoid concentrations of hemp plants infested with hemp russet mite in the 2020 field season. This table shows the effect of 3 sulfur treatments, an early season spray (Jul), a late season spray (Aug), or early and late season sprays (Jul+Aug) on phytocannabinoid production of treated hemp plants relative to an untreated control group. After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by LC/MS analysis to screen samples from the field studies for the concentration of endogenous phytocannabinoids. Values shown are the average concentration of each phytocannabinoid in ng/g ± SE.

| Phytocannabinoid	Control	Jul	Aug	Jul+Aug
CBC	56000±21223	66283±23860	86033±22246	109660±23176
CBCA	605833±135570	1110833±189361	1230666±268034	1298000±220159
CBCO	45833±680	46116±661	45583±746	45960±939
CBCV	7750±1457	10881±2170	11851±3426	14414±3709
CBD	896500±207589	836833±213174	988500±174494	1174000±214312
CBDA	23333333± 3747354	37533333± 4808187	38250000± 7642970	41900000± 5432770
CBDV	71016±18458	71866±16436	82883±11678	94420±14286
CBDVA	1143666±248565	2210000±370135	2356666±625481	2524000±536031
CBG	33950±15369	89483±21143	89966±19759	103400±20242
CBGA	392666±131021	753833±176437	735166±180508	661800±182457
CBL	24466±366	24633±332	24366±382	24580±486
CBLA	427833±86165	753833±126532	829333±177523	873600±132020
CBN	31433±445	31766±332	31516±627	31820±712
CBNA	33583±696	35550±2956	34883±1871	35400±3128
CBT	18666±417	19716±1161	19833±891	21120±1096
D8THC	25166±366	25316±348	25033±403	25240±531
D9THC	137000±29953	170500±47660	220333±36037	264000±33105
D9THCA	493000±106015	859166±184561	932333±223200	1026600±261058
THCV	4756±1215	4665±1245	5716±1569	6072±1245
THCVA	62716±9784	100383±16689	108850±23303	113660±26172

Table 2.2. Impact of sulfur treatments on phytocannabinoid production of hemp plants infested with hemp russet mite in the 2020 field season. This table shows the effect of 3 sulfur treatments, an early season spray (Jul), a late season spray (Aug), or early and late season sprays (Jul+Aug) on phytocannabinoid production of treated hemp plants relative to an untreated control group. After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by LC/MS analysis to screen samples from the field studies for the concentration of endogenous phytocannabinoids. Statistical outputs are shown from one-way ANOVA tests when conditions were met or could be achieved via data transformation, or Kruskal-Wallis tests as a non-parametric alternative. Significant effects are indicated with an asterisk.

Phytocannabinoid	F- or H- statistic	df	p-value	Significance
CBC	$F= 5.945$	3	0.00489	*
CBCA	$F= 13.133$	3	7.07E-05	*
CBCO	$H= 1.519$	3	0.6779	
CBCV	$F= 5.392$	3	0.007425	*
CBD	$F= 2.834$	3	0.06573	
CBDA	$F= 12.291$	3	0.0001	*
CBDV	$F= 2.782$	3	0.069	
CBDVA	$F= 10.454$	3	0.0002787	*
CBG	$H= 13.53$	3	0.00362	*
CBGA	$F= 5.901$	3	0.005056	*
CBL	$H= 1.6028$	3	0.6587	
CBLA	$F= 13.018$	3	7.47E-05	*
CBN	$F= 0.6924$	3	0.5679	
CBNA	$H= 2.5512$	3	0.4661	
CBT	$F= 6.4291$	3	0.03446	*
D8THC	$H= 1.4768$	3	0.6876	
D9THC	$F= 12.193$	3	0.0001	*
D9THCA	$F= 7.9558$	3	0.00123	*
THCV	$F= 1.6047$	3	0.2215	
THCVA	$F= 8.0471$	3	0.00116	*
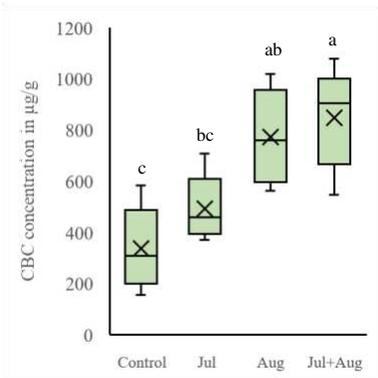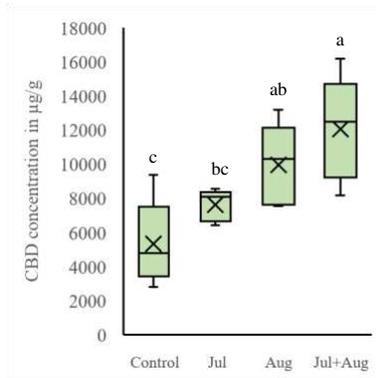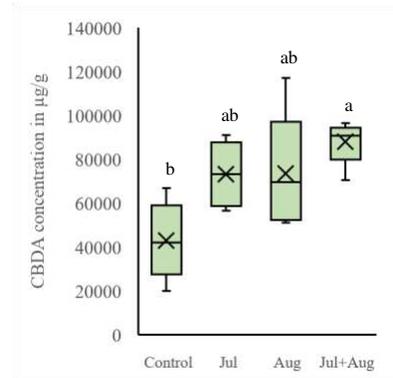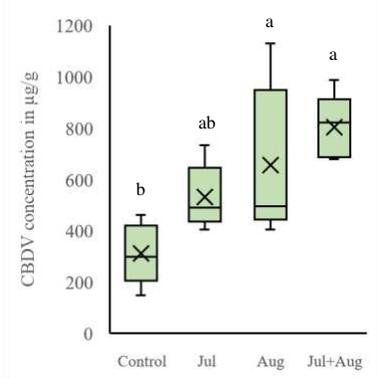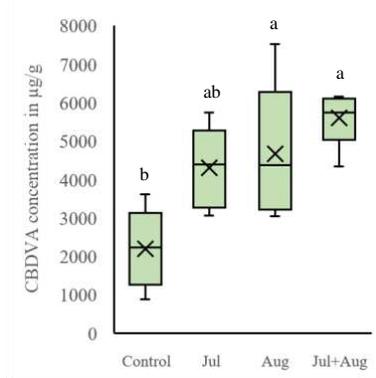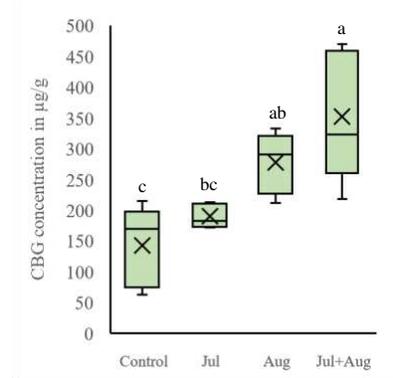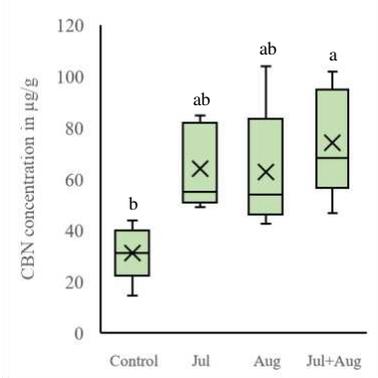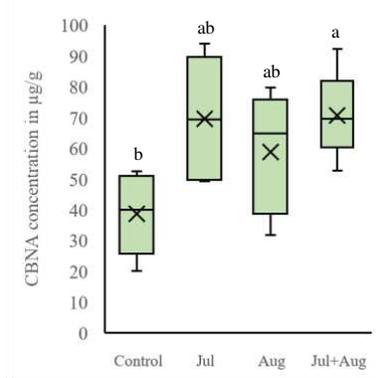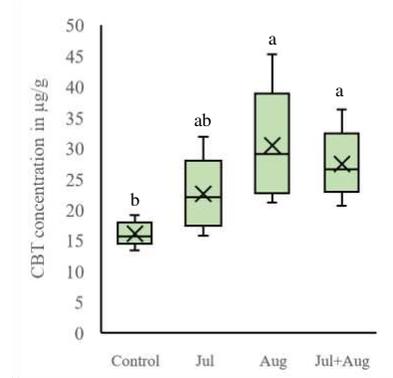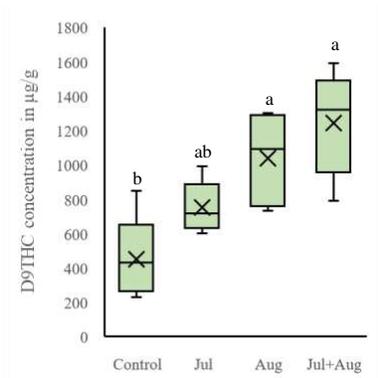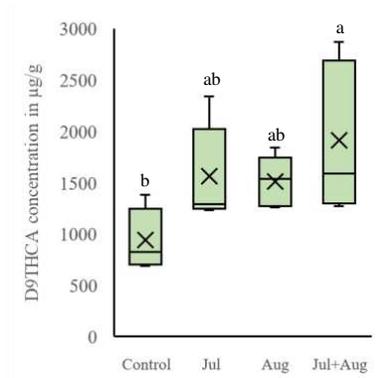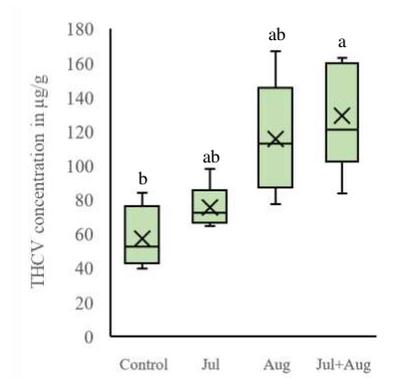
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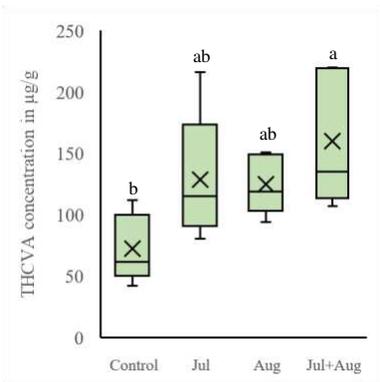
Fig. 2.6a-m. Impact of sulfur treatments on phytocannabinoid production of hemp infested with hemp russet mite in the 2021 field season. This figure shows the 3 sulfur treatments, an early season spray (Jul), a late season spray (Aug), or an early and late season sprays (Jul+Aug), significantly improved the phytocannabinoid production of treated hemp plants relative to an untreated control group in 13 of the 14 phytocannabinoids detected. After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by LC/MS analysis to screen samples from the field studies for the concentration of endogenous phytocannabinoids. Statistical outputs are provided in **Table 2.4**. Values marked with the same letter are not significantly different.

Table 2.3. Impact of sulfur treatments on average phytocannabinoid concentrations of hemp infested with hemp russet mite in the 2021 field season. This table shows the effect of 3 sulfur treatments, an early season spray (Jul), a late season spray (Aug), or early and late season sprays (Jul+Aug) on phytocannabinoid production of treated hemp plants relative to an untreated control group. After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by liquid chromatography-mass spectrometry analysis to screen samples from the field studies for the concentration of endogenous phytocannabinoids. Values shown are the average concentration of each phytocannabinoid in ng/g ± SE.

| Phytocannabinoid	Control	Jul	Aug	Jul+Aug
CBC	336800±163046	494000±130608	773600±186959	847600±197972
CBCO	<LOD	<LOD	<LOD	<LOD
CBD	5338000± 2485180	7628000± 929607	9972000± 2386560	12074000± 3035684
CBDA	43020000± 17529318	73120000± 14808004	73680000± 26587158	88000000± 10178162
CBDV	310200±118478	530600±125611	657000±297961	804800±124294
CBDVA	2204800± 1025419	4298000± 1063870	4668000± 1779261	5608000± 732031
CBG	143000±64908	190000±19697	277600±49671	352600±105165
CBGA	725200± 383386	1148800± 460285	1220600± 349004	1478000± 543801
CBL	<LOD	<LOD	<LOD	<LOD
CBN	31220±10725	64120±16588	62680±24271	74240±21268
CBNA	38700±13294	69620±20256	58780±19677	70760±14121
CBT	16120±2088	22600±6093	30480±9375	27480±5742
D8THC	<LOD	<LOD	<LOD	<LOD
D9THC	450600± 240920	749400± 151417	1037400± 268171	1242200± 303058

Table 2.4. Impact of sulfur treatments on phytocannabinoid production of hemp infested with hemp russet mite in the 2021 field season. This table shows the effect of 3 sulfur treatments, an early season spray (Jul), a late season spray (Aug), or early and late season sprays (Jul+Aug) on phytocannabinoid production of treated hemp plants relative to an untreated control group. After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by LC/MS analysis to screen samples from the field studies for the concentration of endogenous phytocannabinoids. Statistical outputs are shown from one-way ANOVA tests when conditions were met, or could be achieved via data transformation, or Kruskal-Wallis tests as a non-parametric alternative. Significant effects are indicated with an asterisk.

Phytocannabinoid	F- or H- statistic	df	p-value	Significance
CBC	F= 9.6939	3	0.0006951	*
CBD	F= 7.727	3	0.002059	*
CBDA	F= 5.3552	3	0.009561	*
CBDV	F= 6.5461	3	0.004269	*
CBDVA	F= 7.0143	3	0.003173	*
CBG	F= 9.5611	3	0.0007447	*
CBGA	F= 2.5146	3	0.09525	
CBN	F= 4.8441	3	0.01386	*
CBNA	F= 3.7622	3	0.03223	*
CBT	F= 6.8431	3	0.003532	*
D9THC	F= 9.7249	3	0.0006841	*
D9THCA	H= 7.9117	3	0.04787	*
THCV	F= 11.34	3	0.01	*
THCVA	F= 3.7228	3	0.03329	*

Past studies into the effectiveness of pesticidal products for management of hemp russet mite were limited to efficacy measurements of mite count and do not show resulting effects on final yield or phytocannabinoid production (Britt and Kuhar, 2019; Schreiner and Cranshaw, 2019). This study was the first to quantify the effects of hemp russet mite on hemp production. It demonstrated that this mite is clearly able to greatly reduce not only dry weight yield, but also quality in the form of reduced percentage of phytocannabinoids. It is also of interest to note that the general appearance of plants in this study did not vary greatly with casual appearance and no obvious symptoms of HRM infestation were evident even on the untreated plants that supported high populations. This suggests that hemp russet mite damage is far less likely to be recognized by a producer than damage caused by most any other key arthropod pest of the crop, such as defoliators and caterpillars that tunnel into stems and flower buds (Cranshaw et al. 2019).

Predatory Mite Releases

Effects of mass releases of phytoseiid mites produced some modest reduction in populations of HRM measured on plants (**Fig. 2.7, 2.8**) [repeated measures two-way ANOVA: time (F= 7.22, df= 4, p< 0.0001) treatment (F= 8.05, df= 4, p< 0.0001) time:treatment (F= 1.7, df= 12, p= 0.09)]. Slight reductions were recorded on plants treated August 8 with *N. fallacis* and *A. swirskii*. No reductions were noted following the release of *N. californicus* and *A. andersoni*, released two weeks later. This discovery is still notable, however, given the lack of evidence in the literature for effective management of HRM using biological controls (Britt et al., 2022; Edde, 2022).
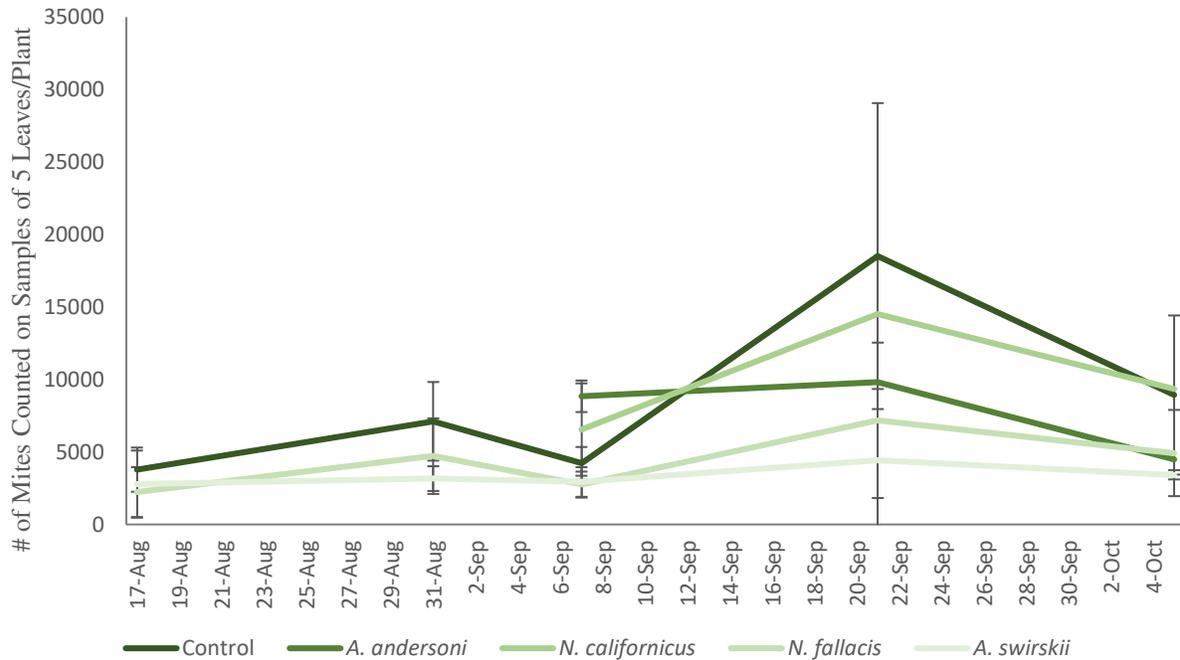
Fig. 2.7. Predatory mite release trial HRM population dynamics over time on hemp plants infested with hemp russet mite. This figure shows the effect of 4 biocontrol releases, *A. andersoni, N. californicus, N. fallacis*, and *A. swirskii*, on HRM populations on treated hemp plants relative to an untreated control group. All releases were at the rate of 5,000 mites/plant. Releases of *N. fallacis* and *A. swirskii* were made on 8 August; releases of *A. andersoni* and *N. californicus* on 25 August. Sampling of HRM was done using collections of five leaves per plant. Leaves were selected by taking the first fully formed leaf from the top growth of randomly selected branches to ensure conformity across plants. Numbers of mites present were determined using an alcohol collection technique (Appendix 1). Our results show that time had a significant effect on mite population levels, with numbers gradually rising over the course of the season before peaking in late August, and dropping off before harvest [repeated measures two-way ANOVA: time ($F= 7.22$, df= 4, p< 0.0001) treatment ($F= 8.05$, df= 4, p< 0.0001) time:treatment ($F= 1.7$, df= 12, p= 0.09)].
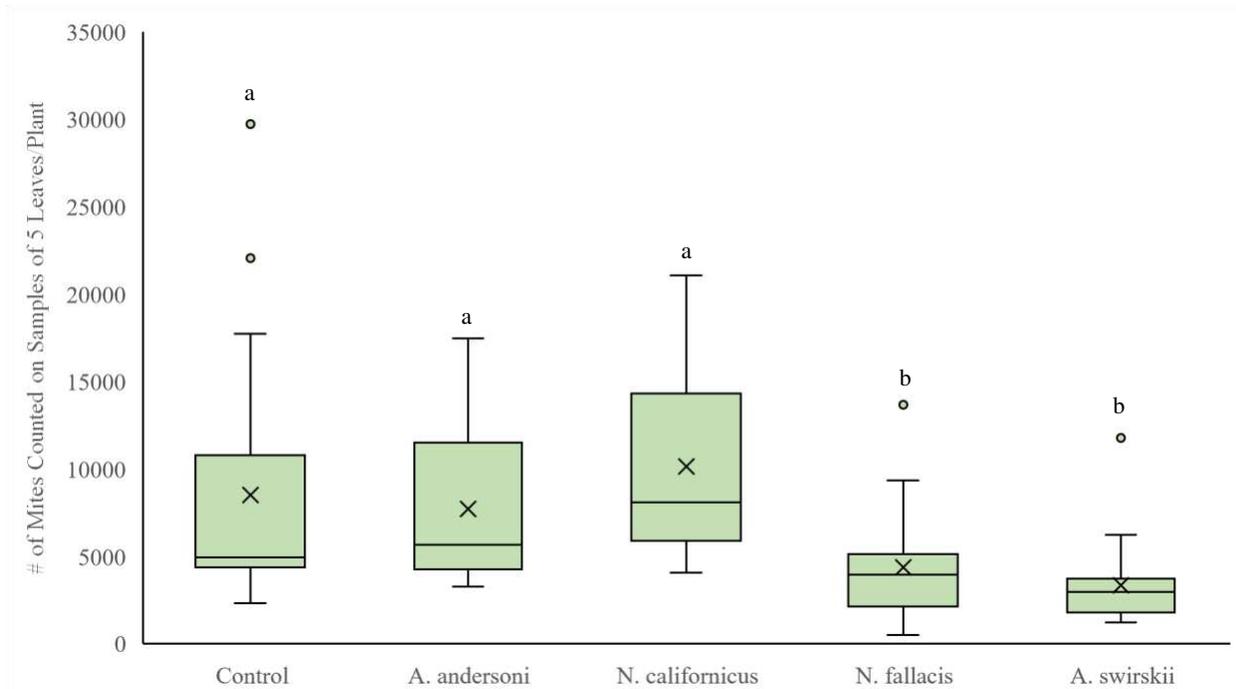
Fig. 2.8. Impact of predatory mite releases on mite populations of hemp infested with hemp russet mite. This figure shows the effect of 4 biocontrol releases, *A. andersoni, N. californicus, N. fallacis*, and *A. swirskii*, on HRM populations on treated hemp plants relative to an untreated control group. Sampling of HRM was done using collections of five leaves per plant. Leaves were selected by taking the first fully formed leaf from the top growth of randomly selected branches to ensure conformity across plants. Numbers of mites present were determined using an alcohol collection technique (Appendix 1). Boxes display the first and third quartile, median (line), mean (x), outliers (points), and whiskers indicate variability outside upper and lower quartiles. Our results show that biocontrol releases had a significant effect in reducing HRM populations on treated plants [repeated measures two-way ANOVA: time ($F= 7.22$, df= 4, p< 0.0001) treatment ($F= 8.05$, df= 4, p< 0.0001) time:treatment ($F= 1.7$, df= 12, p= 0.09)]. Values marked with the same letter are not significantly different.

Unlike the sulfur treatments, releases of pytoseiid mites did not significantly impact yield, as measured by dry weight of harvested leaves and flowers (F= 0.72, df= 4, p= 0.59) (**Fig. 2.9**). Effects of phytoseiid treatments were found to be significant in 5 out of 20 of the phytocannabinoids tested. However, in all the cases where there was significant difference in phytocannabinoids, concentrations were reduced compared to the untreated check (**Fig. 2.10a-e; Tables 2.5, 2.6**). CBDA was the most abundant phytocannabinoid in this trial as well, making up 84% of total cannabinoids. Results showed that releases of biological control agents significantly reduced the concentration of CBDA (F= 3.453, df= 4, p= 0.0366; **Fig. 2.10b**) with plants receiving releases of *A. andersoni* in late August seeing the greatest reduction in CBDA concentration (p= 0.02, 95% C.I.= -45706152, -3260515).

Furthermore, despite release rates used in this study that were far above that normally used with these phytoseiid mites on other crops, no reproduction by any of the species was ever observed, in the form of nymphs being recovered in samples. This indicates that any effect of release of phytoseiid mites for control of hemp russet mite will be due solely to the predation of the released individuals, and they lack ability to sustain on plants where hemp russet mite is the sole prey available. Given the extremely high cost of phytoseiid mites, particularly when contrasted with the cost of alternative highly effective treatments (e.g., sulfur, mineral oils), their use for management of hemp russet mite does not seem defensible for producers of hemp crops.
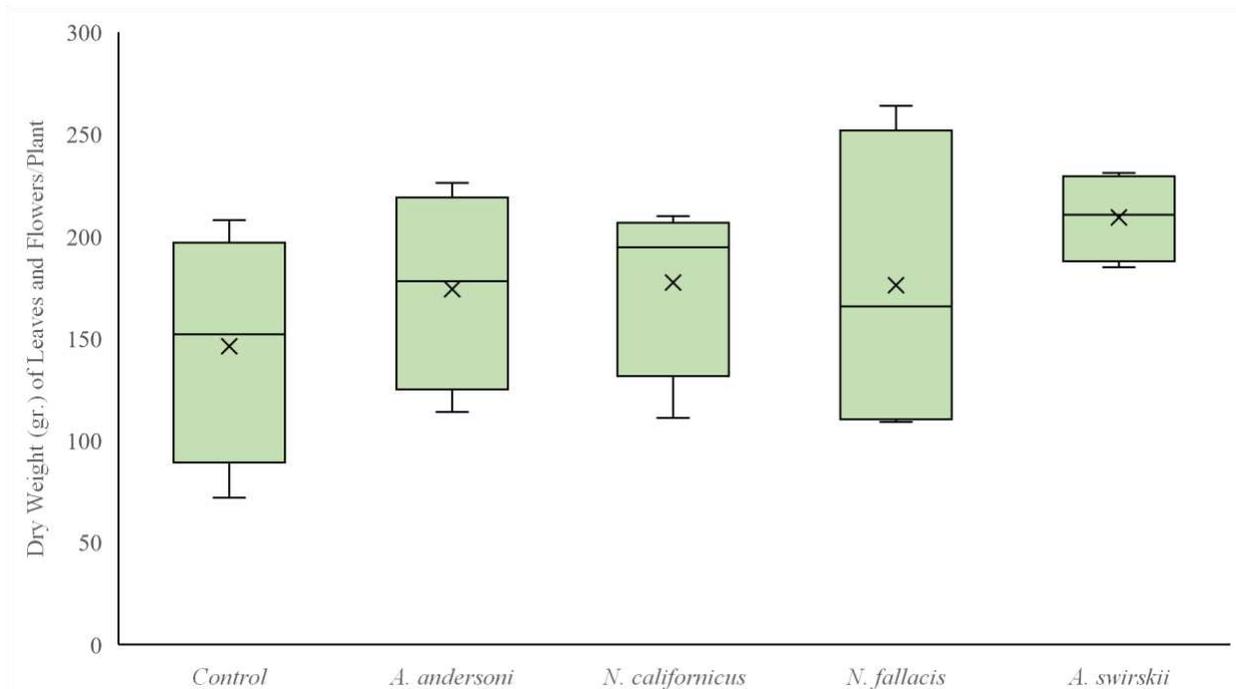
Fig. 2.9. Impact of predatory mite releases on yield of hemp infested with hemp russet mite. This figure shows the effect of 4 biocontrol releases, *A. andersoni, N. californicus, N. fallacis*, and *A. swirskii* on dry weight yields of treated hemp plants relative to an untreated control group. Upon drying after harvest, whole plants were shucked to remove stems and branches, and dry weight yields were taken using plastic tubs. Boxes display the first and third quartile, median (line), mean (x), and whiskers indicate variability outside upper and lower quartiles. Our results show that biological control agents had no significant effect on hemp yield relative to our control (F= 0.72, df= 4, p= 0.59).

Fig. 2.10a-e. Impact from releases of predatory mites on phytocannabinoid production of hemp infested with hemp russet mite. This table shows the 4 biocontrol releases, *A. andersoni, N. californicus, N. fallacis*, and *A. swirskii*, reduced phytocannabinoid production of treated hemp plants relative to an untreated control group in 5 of the 20 sampled phytocannabinoids. After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by LC/MS analysis to screen samples

from the field studies for the concentration of endogenous phytocannabinoids. Statistical outputs are provided in **Table 2.6**. Values marked with the same letter are not significantly different.

Table 2.5. Impact from releases of four predatory mites on average phytocannabinoid concentration of hemp infested with hemp russet mite. This table shows the effect of 4 biocontrol releases, *A. andersoni, N. californicus, N. fallacis*, and *A. swirskii*, on phytocannabinoid production of treated hemp plants relative to an untreated control group. After final yields were taken, leaf and flower tissue samples were collected using 50 mL Falcon tubes for final cannabinoid profile analysis. This was done by LC/MS analysis to screen samples from the field studies for the concentration of endogenous phytocannabinoids. Values shown are the average concentration of each phytocannabinoid in ng/g ± SE.

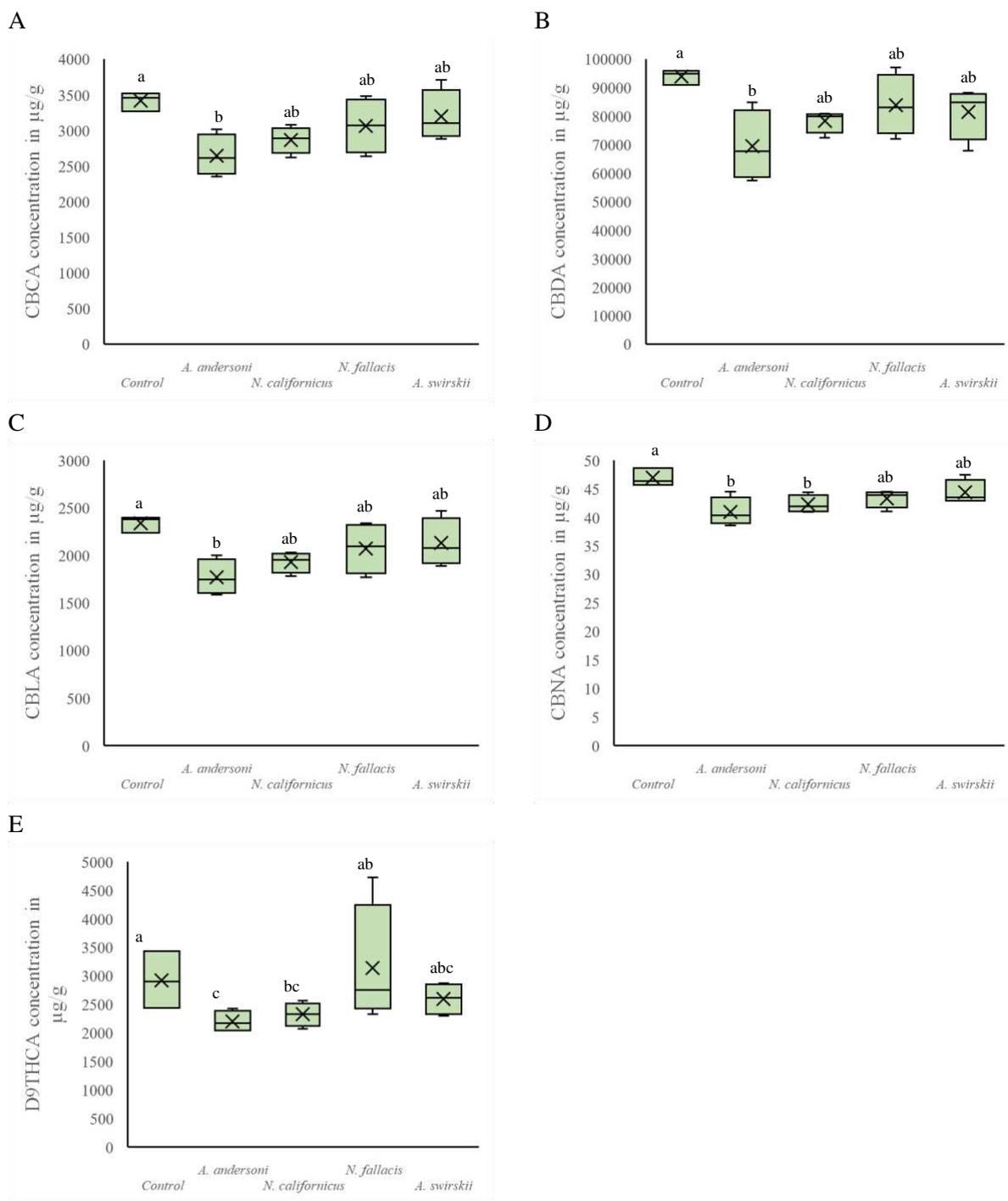| Phytocannabinoid	Control	Andersoni	Californicus	Fallacis	Swirskii
CBC	270666± 27300	205750± 30103	194000± 33931	218750± 52117	280250± 65622
CBCA	3416666± 130511	2647500± 288718	2870000± 189384	3065000± 391024	3200000± 358050
CBCO	46000±655	45225±377	45425±537	45375±330	45525±512
CBCV	4926±917	4312±1589	3935±1258	4075±1352	3935±358
CBD	2310000± 226053	1782500± 210930	1797500± 298482	1987500± 456973	2367500± 412744
CBDA	93933333± 2589079	69450000± 12347874	78300000± 3908964	83900000± 10607858	81550000± 9293904
CBDV	31600±1571	25950±1905	26425±2585	28500±4767	30575±2905
CBDVA	684666± 47437	470750± 66092	550000± 38790	608000± 136262	600250± 102053
CBG	312666± 24193	229000± 34292	241750± 62275	276250± 55661	299000± 44504
CBGA	3926666± 255799	2907500± 1202590	3452500± 806365	3710000± 476235	3552500± 894813
CBL	24566±305	24150±191	24300±316	24250±191	24350±251
CBLA	2340000± 87177	1770000± 183484	1930000± 108012	2075000± 269876	2130000± 252322
CBN	32733±351	31825±450	31925±543	32000±416	32625±826
CBNA	46933±1569	40950±2514	42300±1518	43375±1552	44375±2137
CBT	26300±1609	23050±806	23375±1497	23950±2433	25775±1774
D8THC	25233±351	24825±170	24925±287	24875±189	24975±262
D9THC	665000± 77929	520500± 91660	521750± 99221	604500± 170721	682750± 120192
D9THCA	2926666± 500533	2202500± 182825	2327500± 207585	3142500± 1077199	2600000± 282488
THCV	2300±317	2975±2065	1882±319	2032±265	2447±294
THCVA	40966±1650	36250±1619	38425±1357	40625±4723	39325±2634

Table 2.6. Impact from releases of four predatory mites on phytocannabinoid production of hemp infested with hemp russet mite. This table shows the effect of 4 biocontrol releases, *A. andersoni, N. californicus, N. fallacis*, and *A. swirskii*, on phytocannabinoid production of treated hemp plants relative to an untreated control group. Statistical outputs are shown from one-way ANOVA tests when conditions were met, or could be achieved via data transformation, or Kruskal-Wallis tests as a non-parametric alternative. Significant effects are indicated with an asterisk.

Phytocannabinoid	*F*- or *H*- statistic	df	p-value	Significance
CBC	*F*= 2.7576	4	0.06995	
CBCA	*F*= 3.5559	4	0.03346	*
CBCO	*F*= 1.2266	4	0.3439	
CBCV	*F*= 0.3976	4	0.8071	
CBD	*F*= 2.4722	4	0.09254	
CBDA	*F*= 3.453	4	0.03666	*
CBDV	*H*= 7.7138	4	0.1026	
CBDVA	*F*= 2.8513	4	0.06393	
CBG	*F*= 2.0947	4	0.1358	
CBGA	*H*= 2.6789	4	0.6129	
CBL	*F*= 1.2539	4	0.3338	
CBLA	*F*= 4.0358	4	0.02214	*
CBN	*F*= 2.112	4	0.1334	
CBNA	*F*= 4.7413	4	0.01253	*
CBT	*F*= 2.6155	4	0.08032	
D8THC	*F*= 1.2849	4	0.3226	
D9THC	*F*= 1.5852	4	0.2328	
D9THCA	*H*= 9.5518	4	0.04869	*
THCV	*H*= 6.112	4	0.1909	
THCVA	*F*= 1.778	4	0.1894	

Water Immersion Trials

The results of the hot water immersion trial showed that treatments had a significant effect in reducing HRM population counts (H= 84.483, df= 6, p<0.0001) (**Fig. 2.11**). While each treatment had a significant effect in reducing HRM, the 109° F immersion for 15 minutes was found to be the most effective in reducing HRM relative to the control group (Z= -7.878, p= 6.99e-14), followed by the 1.0% surfactant solution dip (Z= -6.747, p= 3.022e-10). While HRM populations were reduced, treatments were not found to significantly affect overall rooting success (X^2= 9.647, df=12, p= 0.6469) (**Fig. 2.12**).

The current study is the first to investigate the efficacy of hot water immersion treatments at a range of temperature and time intervals, as well as surfactant dips at high and low concentrations, in reducing HRM populations on infested hemp cuttings. This performance was evaluated relative to the phytotoxicity risk of treatments in order to determine whether this approach may be implemented in growing operations without posing a risk to clonal development based on rooting success (Caplan et al., 2018).

The results of the hot water immersion experiments showed that this approach may be an effective measure to significantly reduce HRM populations during clonal propagation. The most effective treatment, the 109° F immersion for 15 minutes, reduced average mite count by 75%. Furthermore, the least effective treatment, the 106° F immersion for 15 minutes, still reduced average mite count by 48%. While hot water immersion and surfactant dip treatments have not been studied on HRM in the past, a previous study has shown that temperature and surfactant treatments were effective in reducing eriophyid mites on garlic (Hoepting, 2019).
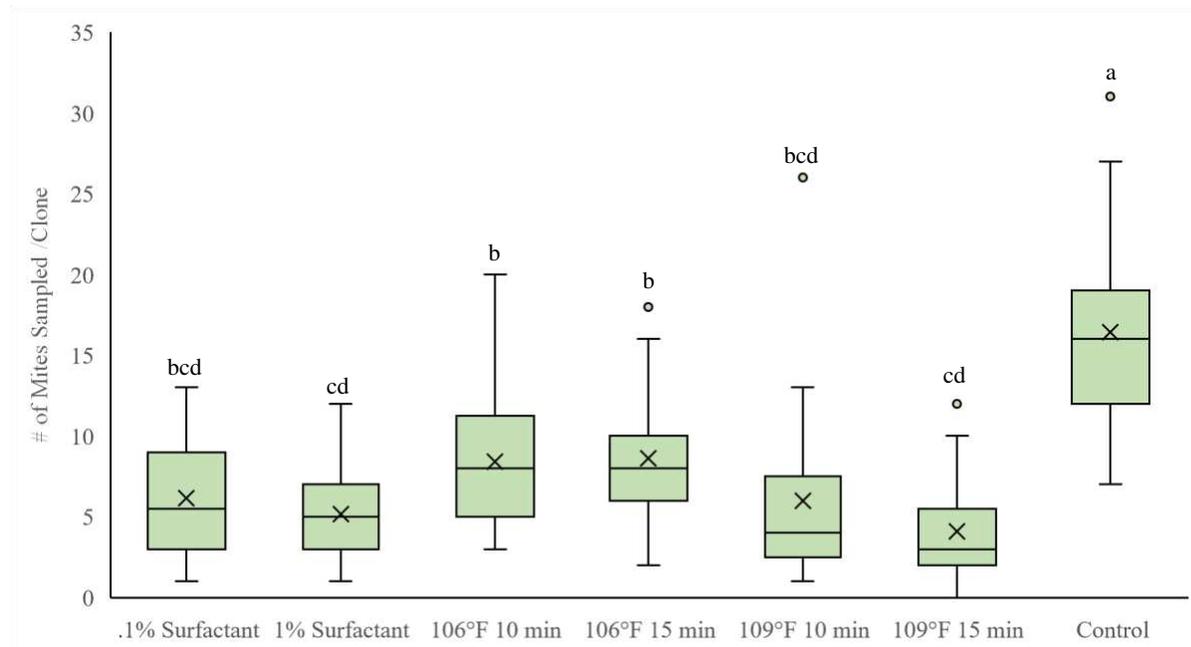
Fig. 2.11. Impact of water immersion treatments on mite populations of hemp clones infested hemp russet mite. This figure shows the effect of 4 hot water immersion treatments: immersion at 106°F for 10 minutes, at 106°F for 15 minutes, at 109°F for 10 minutes, at 109°F for 15 minutes; and 2 surfactant dips, a low concentration at 0.1% and a high concentration at 1.0%, relative to an untreated control group. Cuttings were infested prior to treatment and allows 4 weeks to develop before being collected. Mites were enumerated using the alcohol collection technique (Appendix 1). Boxes display the first and third quartile, median (line), mean (x), outliers (points), and whiskers indicate variability outside upper and lower quartiles. Our results show that treatments had a significant effect in reducing HRM counts compared to the untreated control group (H= 84.483, df= 6, p<0.0001). Values marked with the same letter are not significantly different.
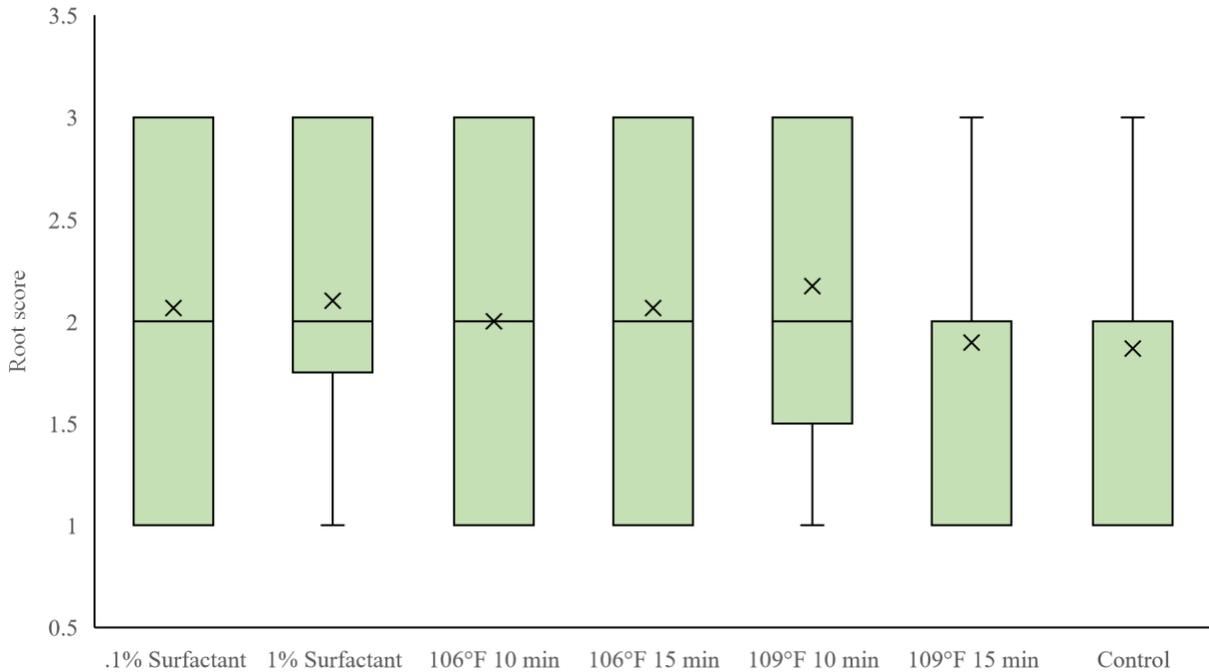
Fig. 2.12. Impact of water immersion treatments on rooting success of hemp russet mite infested clones. This figure shows the effect of 4 hot water immersion treatments: immersion at 106°F for 10 minutes, at 106°F for 15 minutes, at 109°F for 10 minutes, at 109°F for 15 minutes; and 2 surfactant dips, a low concentration at 0.1% and a high concentration at 1.0%, relative to an untreated control group. Boxes display the first and third quartile, median (line), mean (x), and whiskers indicate variability outside upper and lower quartiles. Our results show that treatments had no effect on the ability of clones to successfully develop roots (X^2= 9.647, df=12, p= 0.6469).

Not only did all treatments in this study significantly reduce numbers of HRM on plants, but no treatment posed a significant risk to rooting success, a useful metric for overall clonal development (Caplan et al., 2018). In fact, all treatments slightly improved average root scores relative to the untreated group. This result is notable given the importance of phytotoxicity as a consideration when treating hemp cuttings (Schreiner and Cranshaw, 2019). These results suggest that hot water immersion treatments, and dipping cuttings in surfactant solutions may offer hemp growers a low-cost, low-risk approach to managing HRM during clonal propagation compared to costly insecticides, which may pose a greater risk of phytotoxicity.

Conclusions and Future Research

Reliable pest management options are vital to hemp producers' success. Given the potential for damage from Hemp Russet Mites, further studies into the best practices for managing HRM will be necessary to provide stakeholders with the most effective means for mitigating damage caused by this pest. Information is essential to developing a successful IPM program, and a lack of information surrounding this crop is one of the greatest challenges faced by hemp producers (Mark et al., 2020).

The current study investigates novel approaches to managing an understudied pest in a system of rising importance. This research provides evidence for the efficacy of sulfur applications, releases of biological control agents, hot water immersion, and surfactant dips in reducing HRM populations. Furthermore, our findings demonstrate that reduced HRM infestations as a result of a combination of sulfur applications both early and late in the growing season may increase yields and phytocannabinoid production. Our results also show that hot water immersion treatments and surfactant dips are not only effective in reducing HRM during clonal propagation,

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but that these treatment approaches are safe to use, posing no risk to the rooting success of treated cuttings. Further research into IPM approaches for managing this pest is essential, as this system is still woefully understudied given its economic impact. Future studies may wish to investigate the effectiveness of other cultural controls, such as fumigation at high levels of CO_2, alternative pesticides that may be able to become registered on hemp, and other biological controls, notably entomopathogenic fungi, such as *Isaria fumosorosea* Wize and *Hirsutella thompsonii* Fisher.

Discussion provided in this study of the biology, life history, and current state of IPM of HRM may inform key decision-making processes surrounding this pest. While this summary provides valuable insights into the agroecology of HRM, it underscores the lack of available information. Future research into HRM biology may wish to investigate vital unanswered questions about this pest, such as its overwintering habits, and possible alternate hosts, as well as establishing economic thresholds, and improving scouting techniques.

The tools and information in this study will provide stakeholders and producers with effective IPM tactics for combatting one of the most important pests affecting hemp crops. As hemp production becomes more widespread in the US, further research into this crop, including its pest ecology will be imperative. While these efforts have been set back by hemp's unusual regulatory history, studies such as this will continue to build upon our understanding of the science of this crop.

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APPENDIX

APPENDIX 1- EVALUATION OF COUNTING METHODS FOR HEMP RUSSET MITE

Introduction

In order to study the success of pest management efforts in the HRM system, the ability to most accurately and efficiently assess HRM populations on plants was identified as a key need for many purposes, including determinations of efficacy of various management treatments. Due to the minute size of this pest, various techniques have been used to assess mite population counts under the microscope (Britt and Kuhar, 2019; Edde, 2022; Pulkoski and Burrack, 2020; Schreiner, 2019).

Hemp russet mite colonies were reared in the Nachappa lab at CSU Plant Sciences in order to source mites for scientific studies in a controlled fashion. Colony plants were regularly maintained and scoped using a Dyno-X handheld microscope to ensure uniform mite infestation and colony health. In order to assess the best practices for mite sampling in future studies, three sampling techniques were devised, and assessed for their accuracy and efficiency.

Materials and Methods

In this study, 25 uniformly infested leaves were sampled from colony plants and mite populations were counted using each technique. A stopwatch was used to time each process and further assess the best workflow for future experiments. The process was repeated once for each technique.

In the first technique, HRM infested leaf tissue was taken and stored on wet paper towels, or filter paper, to prevent leaf tissue from desiccating. The infested tissue and wetted paper towel

was moved on to a petri dish or glass slide, and placed directly under a dissecting microscope at approximately 150x magnification. Section by section, mites on the leaf surface were counted using a clicker counter.

In the second technique, HRM infested leaf tissue was run through a Bioquip Mite Brushing Machine. Mites on the surface of the leaves were brushed onto a glass slide with a grid, and this slide was placed under a dissecting microscope and counted using a clicker counter.

In the third technique, HRM infested leaf tissue was placed in 50mL Falcon tubes, and stored in an ethanol solution. Leaves in solution were agitated using a vortex machine and leaf tissue was discarded. Mites suspended in solution were then centrifuged and excess ethanol was discarded. The mite pellet was then resuspended and placed on a petri dish with a small grid. Mites in solution were then placed under a dissecting microscope and counted using a clicker counter.

Microscope Technique

Sampled leaf tissue was collected in Ziploc bags, or petri dishes along with a wet paper towel to prevent leaf tissue desiccation. If not already collected onto a petri dish, leaf tissue was transferred into a dish along with a moist paper towel. The sample was then placed directly under a dissecting microscope where mites were counted using a clicker counter, adding water to the paper towel as needed to avoid tissue desiccation while counting.

Bioquip Mite Brushing Machine Technique

Sampled leaf tissue was collected in Ziploc bags, or petri dishes, along with a wet paper towel to prevent leaf tissue desiccation. The Bioquip Mite Brushing Machine Glass Slide was prepared by affixing the grid sheet with clear tape, and uniformly spreading the surface with liquid detergent using a glass stirring rod to ensure that released mites are captured onto the slide. The prepared glass slide was then placed on the rotating dish of the brushing machine, and the machine was powered on. Leaf tissue was then passed through the rotating brushes 5-10 times, releasing any mites onto the detergent coated slide below. The machine was then powered off, and the slide was placed under a dissecting microscope where mites were counted using a clicker counter.

Alcohol Collection Technique

100% EtOH was diluted to 50% with DI water to make a 50% solution. Sampled leaf tissue was collected in 50mL falcon tubes and submerged in 50% EtOH solution. Falcon tubes with leaf tissue samples and EtOH solution were vortexed for 10 seconds to release mites from the leaf surface. Leaf tissue was then removed from the EtOH/mite solution using forceps or tongs and discarded. The EtOH solution with suspended mites was then centrifuged for 1 minute and 30 seconds at 5000 rpm to form a mite pellet. Excess EtOH solution was then collected using a motorized pipette machine, P5000 and P1000 pipettes, respectively, taking care not to disturb the pellet, leaving behind ~1mL of EtOH solution. The pellet was then resuspended in solution using a P1000 pipette and transferred into a 2mL microcentrifuge tube and centrifuged at 16000g for 2 minutes. Excess EtOH was then discarded using a P100 pipette, leaving the mite pellet and ~.1mL of EtOH solution. The pellet was then resuspended using a P100 pipette and transferred

onto a petri dish lined with grid markers for mite counting under a dissecting microscope using a clicker counter.

Results

Microscope Technique

In the first trial, 14816 mites were counted using the microscope technique. The process took a total of 7 hours and 40 minutes. In the second trial, 30570 mites were counted, and the process took 8 hours and 10 minutes. (**Fig. A.1** & A.**2**)

Bioquip Mite Brushing Technique

In the first trial, 6462 mites were collected using the mite brushing technique. The process took a total of 39 minutes. In the second trial, 12640 mites were collected, and the process took 33 minutes.

Alcohol Collection Technique

In the first trial, 6617 mites were collected using the ethanol collection technique. The process took a total of 1 hour 14 minutes. In the second trial, 13140 mites were collected, and the process took 1 hour and 3 minutes.

Discussion

Across the two trials, the results showed a similar pattern. The microscope counting technique yielded the highest counts, but also took the greatest amount of time to conduct. The alcohol collection and mite brushing techniques were less efficient relative to the microscope technique

in terms of the mites counted, but much more efficient in terms of the amount of time required to carry out the counts.
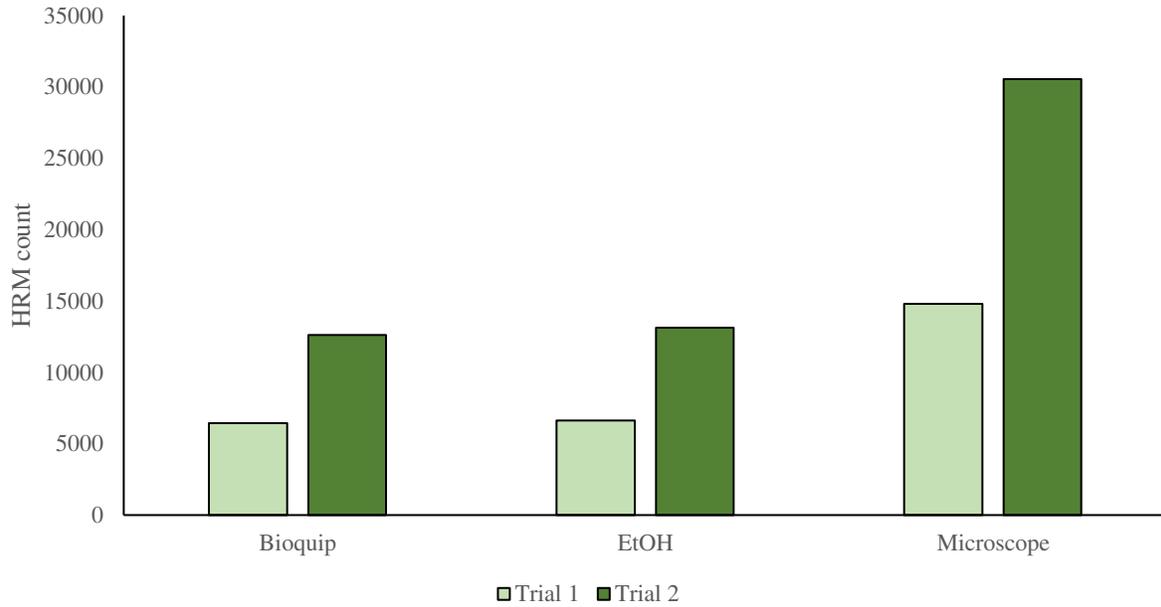

Fig. A.1. Effect of Different Techniques Used to Sample Hemp Russet Mite (HRM) on Leaves. This figure depicts average mite count versus counting technique for uniformly infested samples across 2 trials. Our results show that mite counts were lower in the Bioquip Mite Brushing Machine technique (Bioquip) as well as the alcohol collection technique (EtOH) relative to the microscope technique (Microscope). All counts increased proportionately between the first and second trial.
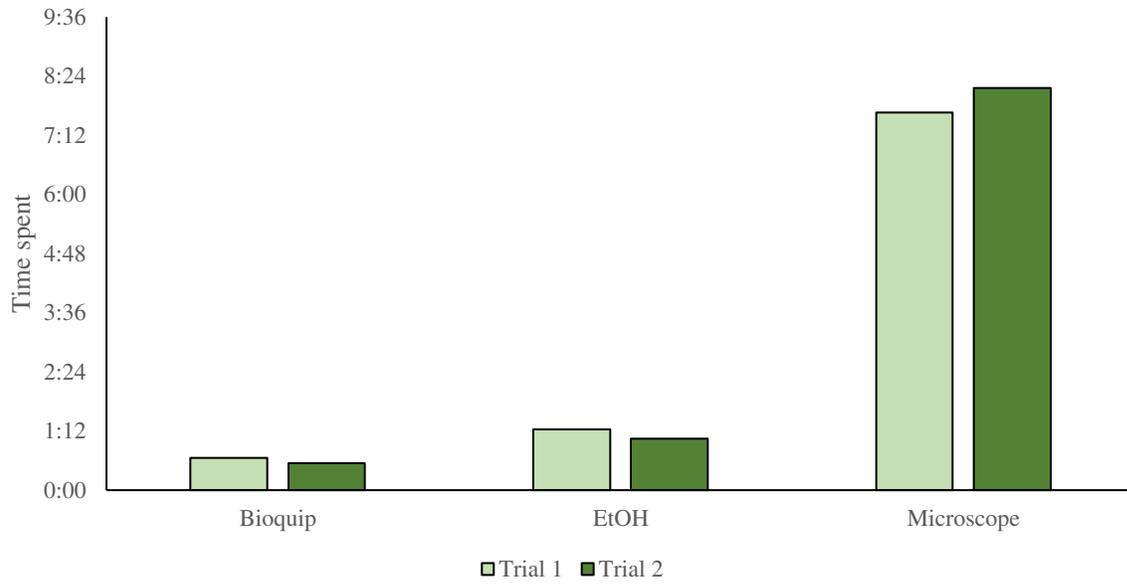
Fig. A.2. Requisite Time for Counting Techniques. This figure depicts the time spent versus counting technique across 2 trials. Our results show that while the microscope counting technique (Microscope) produced the highest mite counts, this technique is much less efficient than the Bioquip Mite Brushing Machine technique (Bioquip) as well as the alcohol collection technique (EtOH) in terms of the time required to carry out the counts.

The reduced mite counts of the alcohol and mite brushing techniques relative to the microscope technique are likely due to the biology of HRM. This pest resides below the glandular trichomes on the leaf surface, and in whorls and crevasses of leaves. This likely caused fewer mites to release from the leaf surface than were actually present. This effect, however, would be constant in a set of similarly cultivated plants, and these techniques would still be useful in determining the relative effect of a set of treatments in an experimental setting.

These results indicate that when time and labor are not of concern, directly counting mites on the leaf surface may be the best approach to garnering the actual mite population level. If time and labor are limited, however, or if the tissue surface is not visible under a microscope (such as in hemp flowers or meristems), either the alcohol collection, or mite brushing machine techniques should be used. In determining which approach would be best when designing an experiment, the maturity and type of leaf tissues being sampled should be considered. If sampling fan leaves, it may be most efficient to pass these leaves through the mite brushing machine to quickly release the mites. If sampling cuttings, inflorescences, or meristematic tissue, the alcohol collection technique may be the most effective at releasing mites from the crevasses of the plant tissue.

Note that sub-sampling may also be useful in minimizing the amount of time required to conduct mite population counts. Using grids in the case of the mite brushing machine and alcohol collection techniques allows a smaller number of cells to be selected at random, and the counts to be multiplied based upon the total number of cells. In the case of the microscope counting technique, the degree to which sub-sampling is possible is much smaller. Due to the bi-lateral symmetry of fan leaves and leaflets, the right or left surface of a whole leaf, or individual leaflets

could be counted, and the population doubled, or quadrupled to account for this. However due to the varying size of individual leaflets, this may be done only once, or twice respectively.

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