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Factors Influencing the Efficacy of Biological Control Agents Used to Manage Insect Pests in Indoor Cannabis (*Cannabis sativa*) Cultivation

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Current biological control strategies in cannabis (*Cannabis sativa*) cultivation have resulted in poor efficacy for managing certain insect pests. The cannabis industry has grown at a rapid pace, surpassing our ability to develop knowledge on the production systems for this crop. Currently, the research focus is on optimizing agronomic and environmental factors to maximize the yield and quality of cannabis. However, cannabis growers are increasingly challenged by severe insect pest pressure, with few effective options. Decades of research have optimized biological control strategies in other crops. The implementation of effective biological control strategies in cannabis is hindered by a variety of morphological, biochemical, and agronomic factors unique to this crop. Here, we review the rather limited literature relevant to insect pest management in indoor cannabis production. Further, we have identified three factors that we believe are primarily responsible for the ineffectiveness of biological control in cannabis: Plant morphology including trichome density and floral resources, effects of plant biochemistry on prey suitability, and finally the effects of supplemental lighting including photoperiod, intensity, and spectrum. We highlight the importance of prioritizing the evaluation of these factors to improve our understanding of the tritrophic interactions governing the success of biological control in cannabis cultivation. As intensive research efforts are underway to optimize agronomic practices for cannabis, it is also important to consider their relevance to biological control.

Keywords: *Cannabis sativa*, biological control, secondary metabolites, trichomes, lighting, tritrophic interactions

INTRODUCTION

In 2018, Canada became the second country to legalize the recreational use of cannabis. Thus, creating a new and rapidly expanding agricultural industry. As of September 2021, licensed cannabis cultivation in Canada exceeded 1.7 million m^2 indoors and 8.9 million m^2 outdoors (Government of Canada, 2022). The cannabis industry is experiencing a global expansion as additional countries continue to legalize medicinal and recreational uses. Until recently, the cultivation of *Cannabis sativa* received very little attention from the scientific community resulting in a paucity of reliable information on how to efficiently cultivate cannabis. In fact, within the available cannabis literature, plant science is the least represented scientific discipline (Aliferis and Bernard-Perron, 2020). Hemp[1] was prohibited in Canada until 1998 and the USA until 2014 and suffers from a similar lack of reliable scientific knowledge. Hemp is cultivated for industrial fiber, seed for oil and food, and more recently for specialty phytochemicals such as cannabidiol (CBD). It is important to make the distinction between drug-type cannabis (hereafter referred to as cannabis) and industrial hemp due to differences in cultivation methods, secondary metabolites, and end use. However, hemp grown for CBD or other specialty phytochemicals can be similar to cannabis.

A growing body of research is focused on optimizing agronomic practices to improve yield, quality, and the efficiency of cannabis cultivation (Backer et al., 2019). Due to the high value of cannabis and the competitiveness of the industry, cannabis growers have been early adopters of agricultural innovations to improve production efficiency (Owens, 2019). From indoor cultivation in warehouse-style facilities to hybrid greenhouses, growers are implementing new technologies to optimize the environmental parameters desired for cannabis cultivation (e.g., temperature, relative humidity, CO_2 concentration, light spectrum and intensity). For example, new and affordable LED (light emitting diodes) technologies have significantly changed the way crops are cultivated indoors (Cohen, 2021). However, the integration of agronomic practices and pest management has been largely ignored. With the absence of insecticides available as rescue treatments, integrated pest management (IPM) strategies must be optimized to prevent pest outbreaks and significant economic losses. The implementation of effective biological control strategies in cannabis is hindered by a variety of morphological, biochemical, and agronomic factors unique to this crop. In this paper, we review key knowledge gaps that may relate to the reduced performance of biological control agents (BCAs) used in IPM programs for insect pests (including mites) in indoor cannabis cultivation. We will first describe the state of insect pest management in cannabis and then focus on how cannabis morphology and its secondary metabolites may present unique challenges for BCAs in cannabis. Finally, as the science and technology of lighting and our understanding of its effects in cannabis cultivation is rapidly changing, we will discuss the impact lighting systems may have on BCAs. This will help shape our understanding of the tritrophic and environmental interactions involved in biological control of insect pests in cannabis that must be understood to ensure growers have reliable pest management solutions.

INSECT PEST MANAGEMENT IN CANNABIS

While more than 270 species of insects and mites have been recorded on *C. sativa,* few are recorded as economic pests (McPartland, 1996). Nevertheless, indoor cannabis cultivation is challenged by many of the same generalist insect pests as other North American greenhouse crops, including multiple aphid species, western flower thrips (*Frankliniella occidentalis* Pergande), and two-spotted spider mite (*Tetranychus urticae* Koch). Additionally, in North America, three insects have emerged as significant pests of cannabis: cannabis aphid (*Phorodon cannabis* Passerini), hemp russet mite (*Aculops cannabicola* Farkas), and rice root aphid [*Rhopalosiphum rufiabdominale* (Sasaki)] (Cranshaw et al., 2018; Lagos-Kutz et al., 2018; Cranshaw and Wainwright-Evans, 2020). The first two species are cannabis specialists, while rice root aphid is a generalist species that is rarely recorded as a pest in other crops. Pest complexes and IPM strategies in hemp are slightly better understood and can be used as a starting point to develop IPM strategies for greenhouse and indoor cannabis cultivation. McPartland et al. (2000) provide a detailed overview of IPM strategies, including pest identification tools, and management recommendations for many insects and pathogens in hemp. With a renewed focus on hemp cultivation in the USA, particularly for the production of phytochemicals, Cranshaw et al. (2019) provide a more recent review of the economically important insects associated with hemp, particularly in Colorado, Virginia, and Tennessee.

Few insecticides have been registered for use on cannabis. In Canada, growers rely on microbial biopesticide (e.g., formulated products containing *Beauveria bassiana*) as well as insecticidal soaps and horticultural oils. In addition to microbial biopesticides, soaps and oils, many growers rely on biological control to manage insect pests. To date, little research has been conducted on IPM in cannabis, impeding the knowledge transfer required for the development and adoption of effective biological control programs (Murphy, 2014). This lack of knowledge extends to most stakeholders, including researchers, growers, extension agents, and consultants. Notably, there are currently only six peer-reviewed scientific articles that discuss insect pests of indoor cannabis cultivation (Cranshaw et al., 2018; Britt and Kuhar, 2020; Cranshaw and Wainwright-Evans, 2020; Grammenos et al., 2021; McCune et al., 2021; Kostanda and Khatib, 2022). Despite these knowledge gaps, biological control has been rapidly adopted throughout the industry and is often used concurrently with biopesticide programs. Generalist predators, such as *Orius insidiosus* Say, *Chrysoperla carnea* (Fitch), aphid parasitoids (e.g., *Aphidius ervi* Haliday *and Aphidius matricariae* Haliday), and Phytoseiid mites (e.g., *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius swirskii* Athias-Henriot) are commonly used in cannabis cultivation. However, when used in cannabis cultivation, the effectiveness of many BCAs is reduced compared to other crops. Here, we will provide some context as to why BCAs have been less effective in cannabis than in other crops.

SECONDARY METABOLITES

The abundance of secondary metabolites produced by cannabis has led to it being called "*the plant of one thousand and one molecules*" (Andre et al., 2016). Secondary metabolites of interest in cannabis are primarily phytocannabinoids and terpenes. The

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[1] In Canada, like the USA, hemp is legally defined as *C. sativa* with $\leq 0.3\%$ $\Delta 9$-tetrahydrocannabinol (THC) in its foliage and inflorescence [Industrial Hemp Regulations (SOR/2018-145)].

insecticidal, miticidal, and repellent potential of *C. sativa* is well-documented in the literature (see McPartland and Sheikh, 2018, for a review). However, secondary metabolites in plants can also affect the third trophic level. Jalali and Michaud (2012) suggest that nicotine, produced by tobacco plants used to rear green peach aphid [*M. persicae* (Sulzer)] and tobacco aphid (*Myzus persicae nicotianae* Blackman), decreases the survival and fecundity and increases the development time of the Coccinellid aphid predator *Adalia bipunctata* L. Multiple instances of Coccinellids rejecting aphids as a result of phytochemicals produced by the host plant have been recorded. For example, *A. bipunctata* rejected *Macrosiphum aconitum* vander Goot feeding on *Aconitum sp.* due to aconitin produced by the host plants (Hodek and Evans, 2012). Additionally, host plants can influence prey suitability regardless of the presence of toxic secondary metabolites. *Acyrthosiphum pisum* Harris reared on alfalfa (*Medicago sativa* L.) rather than on faba beans (*Vicia faba* L.) resulted in better prey for *Coccinella septempunctata* L., due to greater lipid storage (Giles et al., 2002).

To locate prey, predators and parasitoids use volatile terpenes produced by plants in response to herbivory known as herbivore-induced plant volatiles (HIPV) (Dicke et al., 1990). For example, (E)-β-farnesene (Zhu et al., 1999) and (E)-β-caryophyllene (Sasso et al., 2007) elicit oriented flight behavior and increased landing in parasitoids. These volatile terpenes are also produced by *C. sativa* in the absence of herbivory. It is possible that the presence of these terpenes interfere with the host finding behavior of parasitoids. It is therefore crucial to determine how volatile secondary metabolites produced by *C. sativa* influence foraging BCAs.

CANNABIS MORPHOLOGY

Microstructures such as trichomes on *C. sativa* potentially complicate biological control programs. Plant pubescence plays an important role in plant defense against insects (Levin, 1973). Increased densities of glandular trichomes have been found to reduce aphid feeding and infer greater resistance against aphids in various crops including potato (Cho et al., 2017), tomato (Simmons et al., 2003), and melon (Sarria et al., 2010).

Cannabis possesses both glandular and non-glandular trichomes (Ledbetter and Krikorian, 1975). Yet there have been few if any efforts to determine trichome densities across cannabis or hemp genotypes. Trichomes, especially glandular trichomes, are often detrimental to both beneficial and pestivorous insects. For many predatory insects, including some BCAs used in cannabis biological control programs, trichomes were found to do more harm than good (Riddick and Simmons, 2014). However, trichomes are generally beneficial for Phytoseiid mites as they provide refuge from predators, trap additional pollen to be used as a food source, and can facilitate the capture of prey (Schmidt, 2014). Yet, trichomes can also be detrimental to these mites. Notably, *Amblyseius andersoni* (Chant), prefers glaborous varieties of grapes over pubescent varieties (Camporese and Duso, 1996). Cédola et al. (2001) found that *Neoseiulus californicus* McGregor, consumed fewer prey on two tomato hybrids with glandular trichomes than reported by Laing and Osborn (1974) on strawberry without glandular trichomes. Finally, Krips et al. (1999) found that at prey densities of 1.3–2.5 eggs/cm^2 the predation rate of *P. persimilis* was negatively affected by trichomes. At prey densities of 8.0 eggs/cm^2 no differences between trichome density treatments were observed suggesting that *P. persimilis* could be less effective at low pest densities in trichome dense crops such as cannabis. Even non-glandular trichomes can reduce the effectiveness of many BCAs, as they struggle to move and find prey on foliage (Cortesero et al., 2000; Stavrinides and Skirvin, 2003; Madadi et al., 2007; Buitenhuis et al., 2014). The aphidophagous midge *Aphidoletes aphidimyza* was found to preferentially oviposit in areas with denser trichomes, possibly for protection against intra-guild predation (Lucas and Brodeur, 1999).

Few studies have evaluated the consequences of trichome densities on parasitoids. *Aphidius colemani* was found to have a greater rate of parasitism on a less pubescent variety of chrysanthemum (Soglia et al., 2006). The residency time and searching rate of the whitefly parasitoids *Encarsia formosa* Gahan and *Eretmocerus eremicus* (Rose and Zolnerowich), as well as the potato leafhopper parasitoid *Anagrus nigriventris* Girault have been found to decrease with increasing trichome densities, and this effect appears to be more pronounced in the presence of glandular trichomes (Sütterlin and van Lenteren, 1997; Lovinger et al., 2000; Gruenhagen and Perring, 2001).

In some instance, insects closely associated with a single plant can overcome specific plant defenses, as Eisner et al. (1998) reported for the aphid *Macrosiphum mentzeliae* Wilson on its highly pubescent host plant *Mentzelia pumila* Nutt. ex. Torr. & A. Gray. The authors found many insects stuck to the glandular trichomes on *M. pumila* but no *M. mentzeliae*. Due to its close association with *C. sativa*, cannabis aphid has an apparent ability to avoid being trapped by glandular trichomes similar to *M. mentzelia* on *M. pumila*. Given the importance of glandular trichomes in the production of secondary metabolites, there is interest for breeding programs to increase trichome density in cannabis (Andre et al., 2016). Identifying BCAs capable of avoiding trichomes could provide significant improvements for the management of cannabis aphid and other pests.

Finally, as cannabis cultivation uses exclusively female plants, there is normally no pollen in cultivation facilities. Furthermore, cannabis is wind pollinated and therefore does not produce nectar. Both pollen and nectar are important resources for maintaining BCA populations in the absence of pests (van Rijn et al., 1999). The absence of supplemental food creates a situation where it may be difficult to maintain inoculative populations of certain BCAs, forcing growers into expensive inundative biological control programs. In other crops, factitious foods such as cattail (*Typha latifolia* L.) pollen, *Ephestia kuehniella* Zeller eggs, or *Artemia* spp. cysts are used to supplement the diets of BCAs when pest populations are low, improving their establishment (Labbé et al., 2018). However, these products can be challenging to use in cannabis as they cannot be applied directly on flowering plants. Alternatively, banker plants can be used to provide resources for certain BCAs such as *O. insidiosus* and aphids parasitoids in the absence of pests (Brownbridge et al., 2013; Payton Miller and Rebek, 2018). However, banker plant programs require a long-term commitment and technical knowledge to be successfully implemented.

LIGHTING

Electric lighting is used for supplemental light in greenhouse cannabis cultivation, and as sole source lighting in warehouse-style cultivation (Zheng, 2021). Additionally, it can be used to control photoperiod (Potter, 2014), increase light intensity (Rodriguez-Morrison et al., 2021) and manipulate light spectrum (Hawley et al., 2018; Magagnini et al., 2018). The manipulation of light intensity and spectrum have become more common with the rapid advancement in LED technologies, especially with the increasing efficacy and affordability of LED fixtures (Cohen, 2021). However, photoperiod, intensity, and spectrum can impact insects (Vänninen et al., 2012). Cannabis cultivation uses a distinct photoperiod for each growth stage: 18–24 h during the propagation and vegetative stages; and 12 h to initiate and maintain the 6–12 weeks flowering stage (Carpentier et al., 2012; Potter, 2014; Yep et al., 2020). Short daylengths ($\leq$12 h) induce diapause in many predators, including *O. insidiosus* (Ruberson et al., 1991) and *A. aphidimyza* (Boulanger et al., 2019). Additionally, extended photoperiods have been found to increase the parasitism rate of *A. ervi* (Cochard et al., 2019). Thus, the short photoperiod used during the flowering period can be detrimental to certain BCAs.

Cannabis has been found to benefit from extremely high light intensities (Chandra et al., 2008; Potter and Duncombe, 2012; Eaves et al., 2020; Rodriguez-Morrison et al., 2021). Inflorescence yield was found to increase linearly with light intensity up to photosynthetic photon flux densities of 1,800 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Rodriguez-Morrison et al., 2021). With a 12 h photoperiod, this represents a daily light integral (DLI) of 78 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, which is greater than the outdoor DLI anywhere in North America at any time of the year (Torres and Lopez, 2010). Most research evaluating the effects of light intensity on insects used intensities lower than what is used in cannabis cultivation ($<$500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Identifying the effects of high light intensities on pests and BCAs will be very important as the general trend is that increasing intensity results in increases to desirable traits for BCAs such as dispersal, host feeding, fecundity, and longevity (Zilahi-Balogh et al., 2006, 2007, 2008; Schirmer et al., 2008; Johansen et al., 2011; Wang et al., 2013; Tan et al., 2014).

LED technologies can provide many different spectra and spectral combinations. Manipulating light spectra is an emerging topic for indoor agriculture as different spectra have different effects on plant morphology, growth, yield, and quality. In cannabis, the potential to manipulate cannabinoids and terpenes through changes in light spectrum has been investigated (Hawley et al., 2018; Magagnini et al., 2018; Wellhoffer, 2020). The effects of different light spectra on BCAs are still not well-understood. Different light spectra are known to affect beneficial insects, including the longevity and fecundity (Labbé and McCreay, 2020), and movement speed (Wang et al., 2013) of *Orius*, as well as sex ratios (Cochard et al., 2019), locomotor activity (Cochard et al., 2017), and oviposition behavior (Battaglia et al., 2000) of *Aphidius* wasps. Importantly, Cochard et al. (2019) found that an increase in the proportion of red light resulted in a male biased sex ratio in *A. ervi*, reaching 80% males at 100% red light. This could have serious impacts on the effectiveness of *A. ervi* due to a reduction in oviposition. Light spectrum can also have indirect effects on insects through changes to plant secondary metabolite (Lazzarin et al., 2021). For example, light spectra can affect phytohormone mediated plant defenses (El-Esawi et al., 2017; Makowski et al., 2019; Mirzahosseini et al., 2020). Additionally, decreases to the ratio of red to far red light have been found to alter the emission of defensive volatile organic compounds, down regulate induced defenses, and reduce glandular trichome density in tomatoes (Cortés et al., 2016). Manipulating light spectra can also impact other pest management strategies such as sticky card monitoring and mass trapping due to changes in how pests perceive colored traps (Jandricic, 2021). As research is currently focused on optimizing light spectra for cannabis cultivation, it would be advantageous to consider its effects on BCAs, given their necessity as a pest management tool.

CONCLUSION

Cannabis has morphological and biochemical properties that, in other crops, have been found to reduce the efficacy of many BCAs. Unfortunately, some of these properties, such as dense glandular trichomes, are desirable in cannabis production. Further, as there is considerable effort underway to better understand the agronomic factors that affect the quality and yields of cannabis cultivation, we must consider their effects on the BCAs used to manage pests. Due to the importance and broad adoption of biological control in cannabis, there has never been a more important time to ensure the effectiveness of this management tool. Given the rapid increase and high costs associated with indoor cannabis cultivation, it is imperative that we focus interdisciplinary research efforts into a more systemic approach to crop production and crop protection. Otherwise, focusing solely on crop production could create a situation where decreases in yields are realized due to a lack of effective pest management options. With the seemingly endless number of genotypes cultivated by cannabis growers, breeding is unlikely to provide adequate solutions to these pest problems as market demands are a major driver in selecting cultivars. It is important to note that the factors discussed here are not the only agronomic factors relevant to biological control programs. For example, humidity and air flow are abiotic factors that are known to affect the performance of BCAs (see Prado et al., 2015, for a review of the factors that affect aphid parasitoids). A better understanding of all these tritrophic interactions will ensure that more optimized IPM programs can be deployed across the entire industry. This review has identified three major knowledge gaps associated with the effective use of biological control as part of IPM programs in cannabis. To successfully implement these management options, we must consider the effects of plant morphology, secondary metabolites, lighting, and likely other agronomic factors on the BCAs used to manage insect pests.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JL wrote the manuscript. YZ and CS-D provided critical feedback and contributed to the identification of priority knowledge gaps. All authors contributed to the article and approved the submitted version.

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