The epidemiology and integrated management of pathogens and microbes on *Cannabis sativa* L. (cannabis) under greenhouse conditions

by

Liam R. E. Buirs

B.Sc. Horticulture, Kwantlen Polytechnic University, 2021.

Diploma Horticulture Technology, Kwantlen Polytechnic University, 2019.

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

in the
Department of Biological Sciences
Faculty of Science

SIMON FRASER UNIVERSITY

Fall 2024

Declaration of Committee

Name:	**Liam R. E. Buirs**
Degree:	**Master of Science**
Title:	**The Epidemiology and Integrated Management of Pathogens and Microbes on Cannabis sativa L. (Cannabis) under Greenhouse Conditions**
Committee:	**Chair:** Harald Hutter Professor, Biological Sciences
	Zamir K. Punja Supervisor Professor, Biological Sciences
	Sherryl Bisgrove Committee Member Associate Professor, Biological Sciences
	David Joly External Examiner Associate Professor, Biology Moncton University

Abstract

The cultivation of *Cannabis sativa L.* (cannabis) in greenhouses in Canada has increased the prevalence of several diseases, including bud rots, root rots, powdery mildew, and hop latent viroid. An integrated disease management (IDM) framework was developed by combining epidemiological insights and multifaceted suppression strategies. These include growing disease tolerant genotypes, maintaining pathogen-free stock plants, and employing cultural, environmental, and biological approaches. One major pathogen, *Botrytis cinerea*, which causes bud rot resulting in significant yield reductions, was studied in more detail. Artificial inoculation studies revealed that peak infection occurred during a consistent period in the flowering phase (33-41 days). Temperatures and high humidity microclimatic conditions that equated to seasonal weather conditions during June to November increased disease incidence. Effective management approaches included increased air circulation and multiple applications of fungal biocontrol agents such as *Trichoderma harzianum*. An integrated approach to management of cannabis diseases is required for effective suppression of the primary pathogens, ensuring high-quality cannabis production. Avenues for future research on cannabis IDM are discussed.

Keywords: *Cannabis sativa*; integrated disease management; greenhouse cultivation; plant pathogens; *Botrytis cinerea*; biological control

Dedication

This thesis is dedicated to the network of good people who worked to re-schedule and legalize this impactful plant through research and lobbying, enabling people to once again benefit from its medicinal and recreational properties, while providing opportunities for researchers like me to work on optimizing its production.

Acknowledgements

I would like to sincerely thank the many individuals who supported me throughout this research journey, which has pushed me to operate at my maximum potential. It revealed my limits and forced me to grow exponentially as a young professional. I would like to express my deepest gratitude to my parents especially, with whom I lived when I began the MSc program, and who supported me in countless ways, helping me reach a point in life where I was ready to take on a project of this magnitude.

I am equally grateful to my partner, Anja, for her patience and for sacrificing a significant amount of her time and energy to support me over the many evenings and weekends I spent working on this thesis. I would also like to thank my brother, Ryan, for providing the lighthearted comedic relief I often needed when I was stuck in my head, grappling with thesis or work-related problems. My family and friends also deserve thanks for understanding why I couldn't always attend gatherings or be at my best.

I also want to acknowledge and thank the cultivation team and colleagues at Pure Sunfarms for their support in accommodating and assisting in executing this research. I want to specifically mention Rob Baldwin for his overall support of this research initiative, as well as Mitch Fortier and Mike Gee for their implementation of the climate and cropping strategies that were used to cultivate the plants involved in this research and Ben Rogers for his assistance in developing the IPM program. I would also like to acknowledge the significant contributions of our SFU Plant Biotechnology Lab, which allowed for some important lab-based components of my research to be completed and specifically Samantha Lung for her help with much of the *Botrytis cinerea* work and overall research assistance.

Last, but certainly not least, I wish to thank Dr. Zamir Punja for his faith in my abilities, his regular guidance, and his unfiltered passion, which became a driving force behind my motivation to take on this endeavor and persist through the many challenges. I have learned a tremendous amount about myself and this discipline of horticulture through completing this project, and I hope it serves as an inspiration for others in the horticultural field to share their research questions and pursue them so that we can improve this extraordinary industry collectively.

This research was funded by an Alliance Grant (ALLRP 571270-21) from the Natural Sciences and Engineering Research Council of Canada (NSERC) with matching funding from Pure Sunfarms Corp.

Table of Contents

List of Tables

List of Figures

Figure 1. A mature non-diseased cannabis inflorescence of the cultivar ‘Ginger Dawg’ ready to be harvested.

Chapter 1. Introduction to *Cannabis sativa*

1.1. Ethnobotany and Legal History

Cannabis sativa L., referred to as cannabis hereafter, has been utilized by humans for millennia. It is believed to have originated in western or central Asia (Long et al., 2017; Small, 2017). The earliest evidence of cannabis cultivation comes from archaeological sites in China, dating back approximately 6,000 years (Li, 1973; Fleming & Clarke, 1998). Since then, humans have widely spread cannabis plants to various regions of Asia, the Middle East, Africa, and Europe through trade routes, leading to further domestication (Small, 2017). Over this time, cannabis has been used for multiple purposes: its fibers were used to make hemp, which was employed in producing textiles, ropes, and paper; its seeds were a source of nutrition; and the psychoactive properties of cannabis, particularly the resin from the inflorescences, were utilized for medicinal and spiritual purposes (Li, 1973; Abel, 1980; Russo, 2007; Clarke & Merlin, 2013).

The cultivation and practices associated with drug-type cannabis became illegal in Canada in 1923 with the passage of the Opium and Narcotics Act. During the countercultural movements of the 1950s and 1960s, cannabis prohibition became a topic of heated debate, leading to reduced penalties associated with its consumption. Since then, public opinion has continued to shift in favor of cannabis consumption, leading to its legalization for medical use in Canada in 2001 and for recreational use in 2018 under the Cannabis Act (Hathaway, 2022).

1.2. Taxonomy

The taxonomy of the cannabis plant has been a source of some disagreement, and debates on its classification are still ongoing. In an extensive review, Small (2017) argues that *Cannabis sativa* is the only species that should be officially recognized as a stand-alone species, with its variation driven by human domestication and hybridization, leading to confusion in classification. In Canada, cannabis used for medical or recreational purposes is typically categorized as products from "sativa," "indica," or "hybrid" plants (McPartland, 2017; Hazekamp, 2016). However, these terms do not align with some of the original cannabis taxonomic classifications, and due to hybridization, true landraces are uncommon. Additionally, there has been no documentation of illicit

cannabis breeding (McPartland, 2017).

To address this confusion, researchers are moving towards more precise classification systems based on genetic sequencing or chemovar profiles, which consider the plant's cannabinoid and terpenoid composition (Hazekamp & Fischedick, 2011; Hazekamp, 2016; McPartland, 2017; Zheng, 2022a). Human selection for fiber (hemp) and THC (drug) production has resulted in two distinct plant types that are recognized by governments. Health Canada (2018) defines hemp as cannabis containing less than 0.3% THC and drug-type cannabis as any cannabis plant exceeding 0.3% THC. A similar criterion is used in the USA. The drug-type *Cannabis sativa* is the focus of this thesis.

1.3. Botany

Cannabis plants are dioecious, with distinct male and female organs, although hermaphroditism can occur naturally or be induced by environmental stressors (Clarke & Merlin, 2013). Male plants produce flowers containing pollen, while female plants develop compound raceme inflorescences that contain desirable cannabinoids, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), as well as terpenes that contribute to the plant's aroma and therapeutic properties (Russo, 2007; Farag & Kayser, 2017). Female flowers consist of an ovary, style, and exposed stigmas (Raman et al., 2017). Collectively, these solitary flowers form a branched compound raceme inflorescence (Spitzer-Rimon et al., 2019). The inflorescences of female plants are covered with glandular resinous trichomes, which produce and contain many cannabinoids, predominantly THC-A, and terpenoids (Farag & Kayser, 2017; Radwan et al., 2017; Grassi & McPartland, 2017).

Male plants are used in breeding programs to introduce genetic diversity and develop new cultivars with desirable traits. However, for production operations, cultivators identify and remove male plants during the early flowering stage if grown from seed or take cuttings from female “mother” plants to ensure predominantly female crops, as the flowers are the desirable end product (Small, 2016; Barcaccia et al., 2020; Jones & Monthony, 2022). Seed development in female flowers reduces their quality and THC content.

1.4. Economic Importance

Since legalization, the regulated cannabis industry has become a significant economic driver, contributing an annualized 10.8 billion dollars to Canada's GDP as of July 2023, generating millions in tax revenue. The changes in regulation have led to safer products being sold, reducing purchases from the black market, with an estimated 70% of total cannabis consumption coming from a legal source in 2023, compared to 22% in 2018. Law enforcement costs have been reduced, as only 12% of cannabis-related drug offenses were due to possession in 2023, compared to 81% in 2016. No increase in the rates of cannabis use among 15- to 17-year-olds has been reported, despite access for Canadians increasing from 182 stores in 2018 to 3,332 stores in 2023 (Statistics Canada, 2023). The federally licensed area for cannabis production in Canada was 1,389,175 m² indoor and 6,240,000 m² outdoor as of March 2024 (Health Canada, 2024a), and the number of licensed producers exceeded 900 (Health Canada, 2024b).

1.5. Phytochemistry

Female inflorescences contain a rich cocktail of hundreds of cannabinoids, terpenes, polyphenols, and many other compounds (ElSohly et al., 2017; Bassolino et al., 2024; Pereira Francisco et al., 2024). The cannabinoids, particularly THC (tetrahydrocannabinol) and CBD (cannabidiol), are the most sought after currently (Small, 2017). THC offers an intoxicating recreational or medicinal psychoactive experience for consumers, while CBD provides a myriad of non-psychoactive benefits for general wellness and the healing of various ailments. When combined with THC, CBD has been shown to counteract some of the unwanted side effects of THC (Russo & Guy, 2006; Russo, 2011; Small, 2017). Other cannabinoids, often present in lesser degrees in female cannabis flowers, are currently being studied for their role in the cannabinoid entourage effect and as isolated medicinal compounds. Additionally, there is evidence that the terpenes in cannabis also impact the effects of the plant on individuals, contributing to the overall therapeutic profile (Russo, 2011; Pereira Francisco et al., 2024). Much of this research is still in its early stages due to the nascent nature of the legal industry and the significant hurdles researchers must overcome to study this plant (Small, 2017).

1.6. Greenhouse Production

1.6.1. Propagation and Vegetative Stage

Greenhouse cannabis cultivation involves multiple key stages to ensure the production of high-quality flower buds. The process begins with mother plants, which are kept in a vegetative state to provide a consistent source of cuttings or clones. These clones are then propagated under controlled conditions, treated with root stimulation products, and maintained in high humidity until they form strong roots. Once rooted, the clones undergo a hardening phase to acclimate them to greenhouse conditions before being transplanted into the growing medium of choice for vegetative growth. During the vegetative stage, plants receive extended periods of light (typically 18+ hours per day) to maximize biomass production (Chandra et al., 2017a; Jones & Monthony, 2022; Stasiak & Dixon, 2022; Fleming, 2023).

1.6.2. Flowering Stage

Following the vegetative phase, plants transition to the flowering stage, which is induced by reducing light exposure to an ~12-hour cycle. During this stage, plants are grown in carefully regulated environments that control parameters such as temperature, humidity, air circulation, carbon dioxide levels, light intensity, and irrigation. Strategically maintaining these factors are crucial to ensuring optimal photosynthetic activity and preventing diseases. The flowering phase typically lasts around ~7-10 weeks, with distinct early and late phases during which fertilizer regimens and pest management programs are customized to attain optimal flower quality (Chandra et al., 2017a; Stasiak & Dixon, 2022; Fleming, 2023).

1.6.3. Greenhouse Production Advantages

Greenhouses provide several advantages for year-round cannabis cultivation, including precision climate control, supplemental CO_2 availability, and protection from winter or extreme weather. However, they do come with challenges such as inconsistent natural lighting, higher potential for pest or pathogen outbreaks, and significant energy costs for heating. Advanced cannabis greenhouse systems often rely on technologies such as energy and blackout curtains, supplemental lighting, de-humidifiers, aggressive

air circulation fans, and automated control systems to manage these variables. This controlled environment approach helps achieve consistent yield and secondary metabolite production that is essential for medicinal and recreational cannabis products where uniform cannabinoid and terpene profiles are desired (Chandra et al., 2017a; Stasiak & Dixon, 2022; Fleming, 2023).

1.7. Greenhouse Pathogens

1.7.1. Propagation and Vegetative Stage

Cannabis cultivation is susceptible to various diseases at each developmental stage, requiring targeted management approaches to mitigate losses. In general, effective management involves maintaining pathogen-free stock plants with operation-wide sanitation practices, water quality management, strategic environmental set points, routine scouting, preventative reduced-risk product applications, and controlled access zones. During the mother plant and propagation stages, common diseases include root rots caused by *Fusarium* and *Pythium* spp., powdery mildew (*Golovinomyces ambrosiae*), and infections by hop latent viroid (HLVd), which can significantly impact plant vigor. Management of these pathogens involves breeding or buying pathogen-tolerant genotypes that are quarantined, tested for pathogens, or tissue cultured and refreshed routinely. During the vegetative growth stage, these same pathogens continue to pose risks, and similar management strategies are used with the addition of approaches like ultraviolet light treatment and pathogen-focused crop steering.

1.7.2. Flowering Stage

In the flowering stage, bud rot (*Botrytis cinerea*) becomes a significant concern, especially under high-humidity, fall-season conditions. Some key prevention strategies are the use of seasonal plantings for susceptible genotypes, the application of biological products, the execution of humidity-reducing cultural controls such as lowering planting density or reducing flowering time, and the timely removal of infected plant material. During the post-harvest processing stages, it is important to consider both the drying method and the trimming procedures; for example, hang-drying practices paired with dry trimming can minimize plant wounding and flower contamination. Scheduled quality control sampling can provide an accurate assessment of pathogen presence and steer

downstream processing. In cases where flower microbes are elevated, irradiation can be utilised to reduce microbial populations in processed inflorescences. Ultimately, an integrated approach that combines genetic, cultural, biological, physical, and environmental strategies is most likely to result in optimal plant health and high-quality cannabis production (Buirs & Punja, 2024; Scott & Punja, 2022).

1.8. Thesis Research Objectives

Canadian licensed producers of cannabis are laying the foundation for how high-quality cannabis can be grown commercially and are providing a case study for the positive impact legal cannabis production can have on citizens. However, the industry is still in its infancy, and therefore, there is much to be learned and applied. On the pathogen front, producers have few registered suppression products at their disposal and are lacking research to drive efficient pathogen management programs. The goal of this research was to improve the understanding of cannabis pathogen epidemiology and identify more efficient and effective strategies for their management to provide producers with protocols tested at scale in cannabis greenhouses.

The four primary objectives of this research, separated into chapters of this thesis, are as follows:

- To develop an integrated disease management protocol for greenhouse-cultivated cannabis, combining cultural, environmental, genetic, and biological control strategies to effectively mitigate key pathogens throughout the different plant developmental stages.

- To understand the fundamentals of *Botrytis cinerea* as a global pathogen and the best practices for bio-fungicide application.

- To explore the epidemiology of *Botrytis cinerea*-induced bud rot on greenhouse-cultivated cannabis and develop protocols for its management.

Chapter 2. Integrated Management of Pathogens and Microbes on *Cannabis sativa* L. (Cannabis) under Greenhouse Conditions

2.1. Abstract

The increased cultivation of high THC-containing *Cannabis sativa* L. (cannabis), particularly in greenhouses, has resulted in a greater incidence of diseases and molds that can negatively affect the growth and quality of the crop. Among them, the most important diseases are root rots (*Fusarium* and *Pythium* spp.), bud rot (*Botrytis cinerea*), powdery mildew (*Golovinomyces ambrosiae*), cannabis stunt disease (caused by hop latent viroid), and a range of microbes that reduce post-harvest quality. An integrated management approach to reduce the impact of these diseases/microbes requires combining different approaches that target the reproduction, spread, and survival of the associated pathogens, many of which can occur on the same plant simultaneously. These approaches will be discussed in the context of developing an integrated plan to manage the important pathogens of greenhouse-grown cannabis at different stages of plant development. These stages include the maintenance of stock plants, propagation through cuttings, vegetative growth of plants, and flowering. The cultivation of cannabis genotypes with tolerance or resistance to various pathogens is a very important approach, as well as the maintenance of pathogen-free stock plants. When combined with cultural approaches (sanitation, management of irrigation, and monitoring for diseases) and environmental approaches (greenhouse climate modification), a significant reduction in pathogen development and spread can be achieved. The use of preventive applications of microbial biological control agents and reduced-risk biorational products can also reduce disease development at all stages of production in jurisdictions where they are registered for use. The combined use of promising strategies for integrated disease management in cannabis plants during greenhouse production will be reviewed. Future areas for research are identified.

2.2. Introduction

Integrated disease management (IDM) incorporates the coordinated use of multiple approaches to reduce the impact of disease-causing agents (pathogens) on agricultural crops (Dik & Albajes, 2000). When applied in parallel or consecutively, these tactics can achieve control of multiple pathogens using different and sometimes synergistic suppression tactics. IDM builds upon the concept of integrated pest management (IPM), which has been widely utilized for decades to target and manage insect pests on agricultural crops and requires different strategies to be employed in a coordinated manner, often with resounding success (Razdan & Sabitha, 2009; Nicot et al., 2020). When IDM approaches are considered for cannabis (*Cannabis sativa* L., high THC-containing genotypes) grown under greenhouse conditions, several aspects several aspects of traditional IDM programs need to be modified.

First and foremost is the fact that there are no synthetic fungicides available for use on cannabis crops, thus eliminating a widely-used disease management strategy. Instead, only reduced-risk “biological” and “biorational” products are permitted. These products are mostly protective in action, i.e., non-fungicidal, so they are best suited for preventative applications, although some products can also be deployed as sanitizers. While claims of product efficacy and applications for disease reduction in cannabis may be made, not all are necessarily supported by data from replicated research trials or third-party evaluations. This adds to the difficulty in identifying the specific IDM approaches that are best suited for each pathogen. The recent expansion of hemp cultivation (*C. sativa*, low THC-containing cultivars) in the USA following federal government approval should provide useful information on disease and pest management approaches that could be extended to cannabis (Wang, 2021). The lack of synthetic fungicides for cannabis production has prompted the registration of several biological control products that can be used at different stages of production (Scott et al., 2021; Punja & Scott, 2022). However, efficacy data for these products are not always available, and the modes of action of the biocontrol agents are not often fully understood in the context of cannabis IDM, highlighting the need for further research in this area (Punja & Scott, 2022; Punja, 2021a). Fortunately, efficacy data may exist for many of these products on other crops, e.g., for organic production, and therefore, IDM approaches utilized in these crops can likely be extrapolated to cannabis crops (Awasthi, 2021).

A second challenge for IDM development in cannabis is that highly bred cultivars containing specific resistance genes against important pathogens are lacking. Instead, genetic selections (genotypes) that target higher yields of inflorescences and THC content and that display unique morphological traits have been made a priority (Grof, 2018). In most instances, these efforts have excluded the specific incorporation of disease resistance traits. Consequently, some high-yielding genotypes frequently show high susceptibility to various pathogens, as will be illustrated in this review. Fortunately, the broad genetic variation that currently exists among cannabis genotypes has led to the identification of resistance in various genotypes to specific pathogens, such as powdery mildew (Punja & Scott, 2022; Stack et al., 2021; Mihalyov & Garfinkel, 2021; Stack et al., 2024). The mechanisms underlying this resistance are currently under investigation (Sirangelo et al., 2023).

A third IDM challenge is that when cannabis is compared to other widely-grown greenhouse crops, such as tomatoes, cucumbers, and peppers, the optimal cultural and environmental conditions for cultivation have not yet been fully established. Since different cannabis greenhouse operations can experience variable growing conditions, standardized research trials are needed to establish these parameters. Recent research has identified integral aspects of controlled environment cultivation practices that can be used as a baseline reference (Zheng, 2022a; Fleming et al., 2023). The prevalent pathogens affecting cannabis crops in greenhouses have been recently characterized and described (Punja, 2021a), providing diagnostic information that is required for IDM implementation. Accurate diagnosis of the pathogen(s) involved in a disease syndrome is an important component of IDM, and several diagnostic methods have been described (Wang, 2021; Punja, 2021a; Punja, 2018; Punja et al., 2019; Jerushalmi et al., 2020a; Punja et al., 2023). In this section, we describe the most important pathogens of cannabis crops cultivated under greenhouse conditions and highlight the various growth stages at which IDM approaches can be implemented during the crop production cycle, which generally occurs over 12–15 weeks (Figure 2).

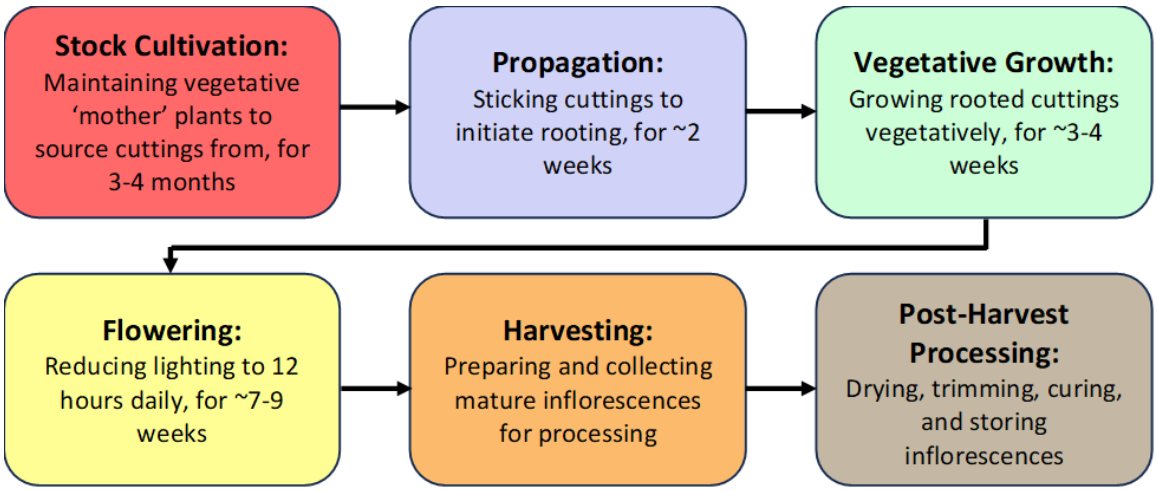


Figure 2. The different stages of cannabis production under greenhouse conditions. Each crop cultivation cycle from propagation to harvest spans ~12-15 weeks. This is followed by a final stage of post-harvest processing that includes drying, trimming, curing and storage.

The first stage of production of a cannabis crop is stock (mother) plant cultivation (Figure 3A), which provides a source of vegetative cuttings (Figure 3B). Once rooted, these are transferred to greenhouse growing conditions for 2-3 weeks (Figure 3C). The developing vegetative plants are then transferred to flowering rooms for 8 weeks (Figure 3D,E), after which time the inflorescences are harvested (Figure 3F).

Figure 3. The stages of cannabis crop development. (A) Stock plants. (B) Rooting of cuttings. (C) Vegetative plants. (D, E) Flowering plants. (F) Harvested inflorescences.

During each crop production year, up to 3-4 cropping cycles may take place per greenhouse compartment. The IDM approaches that can be developed include the selection of disease-tolerant genotypes, implementation of cultural practices, modification of environmental climate settings, and application of reduced risk products (Figure 4).

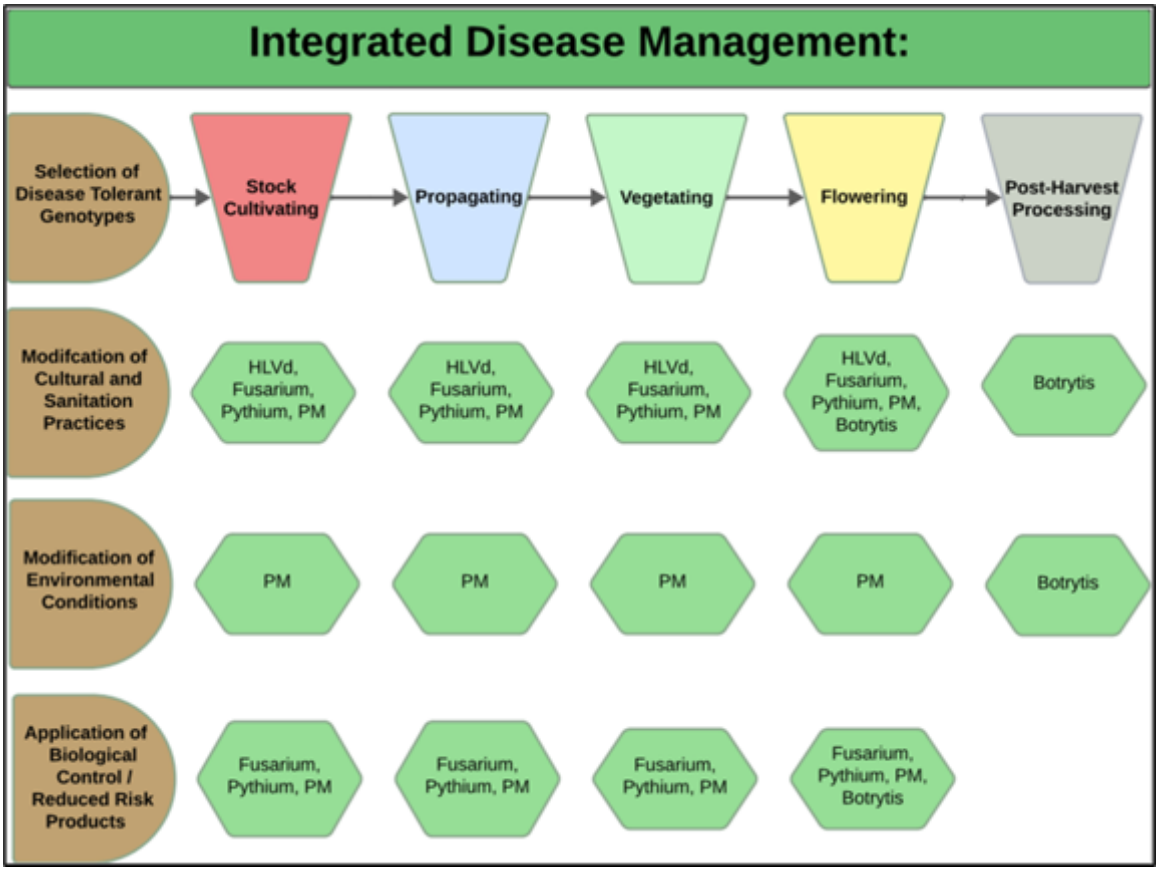


Figure 4. Integrated disease management strategies (left, in brown) are developed according to the crop development stage (top). The hexagons (in green) illustrate the specific diseases being targeted, which are discussed in more detail below. HLVd = Hop latent viroid, PM = powdery mildew, Botrytis = bud rot.

We also discuss aspects of the microbial colonization of cannabis inflorescences by yeasts and molds and propose IDM strategies to reduce the total microflora present. The monitoring of microbial colonization of inflorescences is an important quality step in controlling the quality of cannabis which is under strict regulatory control and thereby presents a unique and challenging component of crop management that is not found in most other crops (Punja et al., 2023; Gwinn et al., 2023). This review should aid in the design or refinement of further IDM programs in greenhouse-cultivated cannabis operations. Detailed descriptions of the symptoms caused by various pathogens at different stages of cannabis growth during commercial production and the approaches that can be taken to manage them are described below.

2.3. Cannabis Pathogens: Symptoms and Management Approaches at Different Stages of Growth

2.3.1. Stock Cultivation Stage

Stock (mother) plants provide a source of vegetative cuttings which are commonly used in commercial cannabis production. These plants generally constitute a range of genotypes that are chosen for their desired phenotypic characteristics and biochemical profiles. They are grown in designated areas within the greenhouse or in separate indoor rooms. The physical separation of stock plants from those planned for larger-scale commercial production is important to prevent the spread of pathogens. The ages of these stock plants can vary, and typically range from 3 to 12 months, depending on the facility. In the context of disease development, older plants often exhibit signs of declining growth, such as reduced shoot growth, leaf yellowing, and poor root development (Figure 5A). These symptoms may be indicative of sub-lethal infections by *Fusarium* and *Pythium* spp. or Hop latent viroid (Figures 5 and 6).

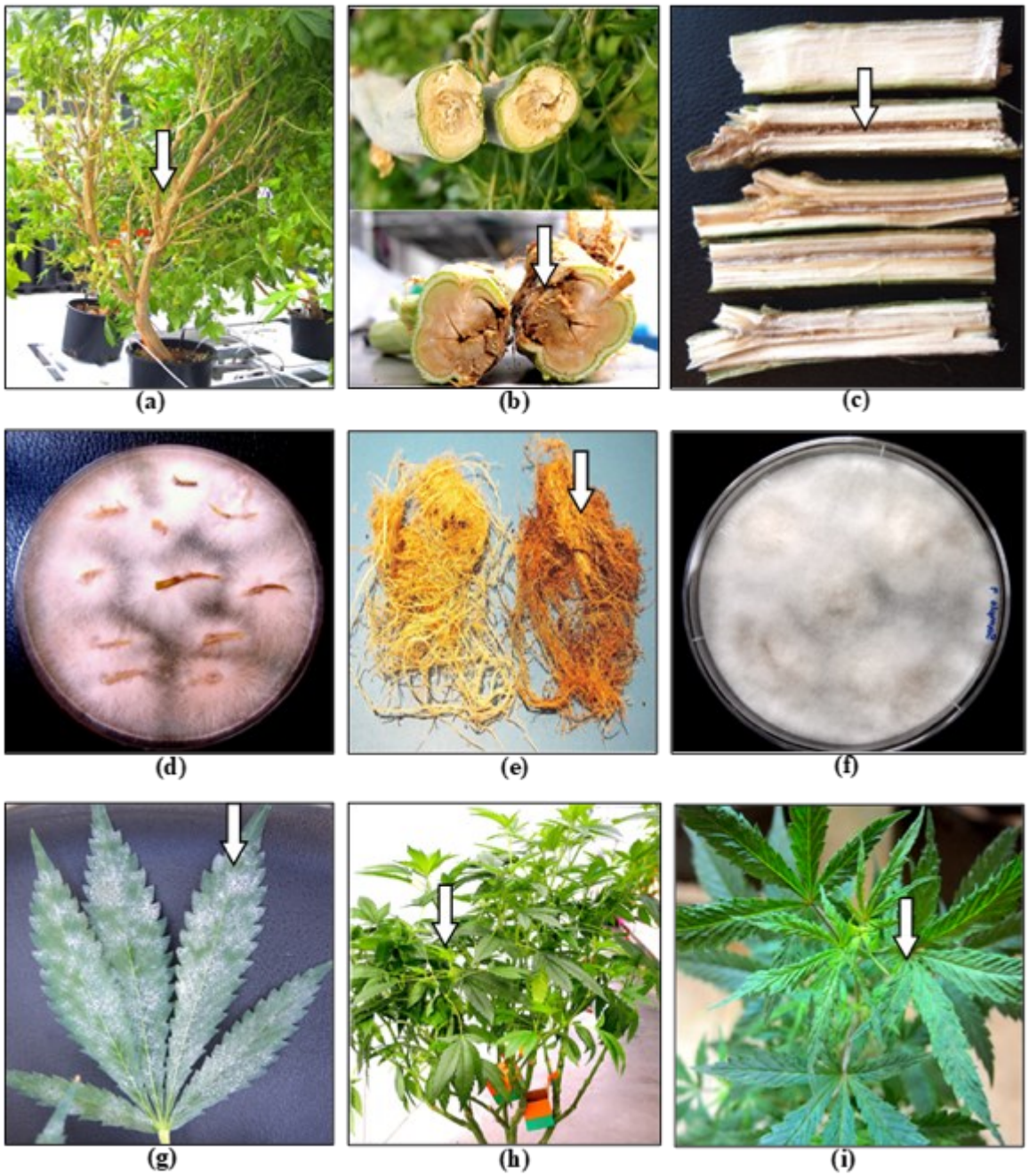


Figure 5. The symptoms of infection by a range of pathogens commonly observed on cannabis stock plants. (A) Declining growth with reduced vigour. (B, C) Internal stem discoloration due to F. oxysporum infection. (D) Isolation of colonies of F. oxysporum from diseased tissues. (E) Browning of roots due to Pythium infection. (F) Isolation of Pythium colonies from diseased roots. (G) Powdery mildew infection on leaves. (H, I) Infection by Hop latent viroid may cause reduced vigor and curling of young leaves.

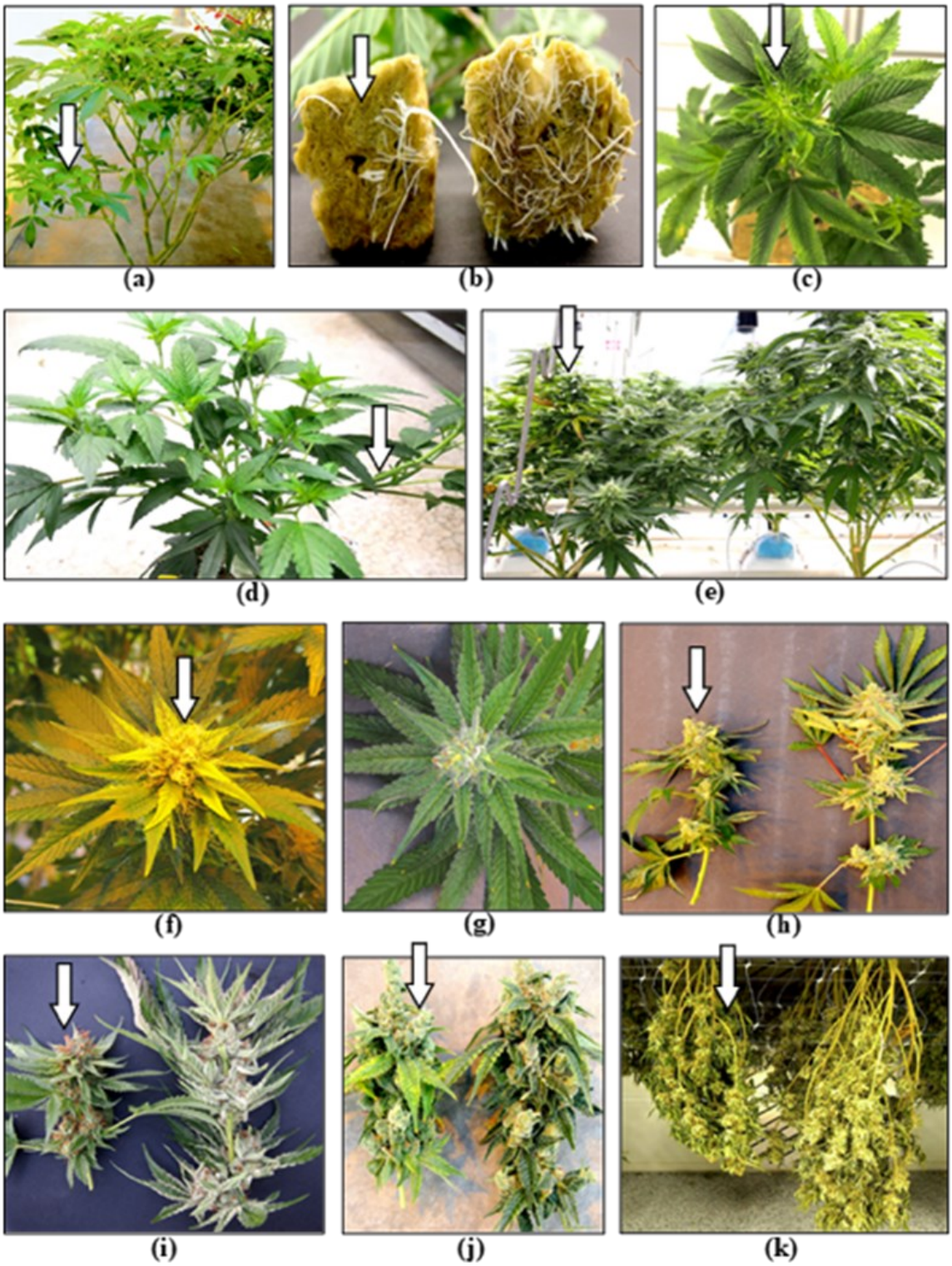


Figure 6. The symptoms of Hop latent viroid infection during the propagation, vegetative growth and flowering stages of the cannabis crop cycle. (A) Infected stock plants may show unthrifty growth and smaller leaves. (B) A comparison of root development on cuttings derived from a HLVd-infected stock plant (left) and a healthy plant (right).

(C) Vegetative plants may show curling and distortion of the youngest leaves. (D) Lateral branching may be seen on a HLVd-infected vegetative plants. (E) Stunted growth of a HLVd-infected flowering plant (left) compared to a healthy plant (right). (F, G) A HLVd-infected inflorescence with yellowing compared to a healthy one, respectively. (H, I, J) Reduced inflorescence development in three different genotypes of cannabis resulting from a HLVd infection. In all photos, the infected plant is shown on the left. (K) Dried inflorescences from a HLVd-infected plant (left) compared to a healthy plant (right).

A closer inspection of the stems of diseased plants will often reveal internal discolouration in the pith and xylem tissues (Figure 5B,C), a symptom of *Fusarium* infection and/or root browning that can be caused by *Fusarium* or *Pythium* species (Punja & Rodriguez, 2018; Punja et al., 2021; Punja, 2021b; Punja et al., 2022). Excessive water clogging may also cause root browning on cannabis plants. Accurate pathogen diagnosis at this stage is critical to determine the most effective IDM strategies to implement. Stock plants are also susceptible to powdery mildew, which is clearly visible as white colonies on the upper surfaces of leaves (Figure 5G). A significant challenge in maintaining healthy stock plants is the recent emergence of hop latent viroid (HLVd) (Adkar-Purushothama et al., 2023; Punja et al., 2024; Atallah et al., 2024), which is mostly asymptomatic on stock plants but which may cause occasional curling or mottling on the youngest leaves (Figure 5H,I). The impact of HLVd infection in stock plants is seen when the rooting frequency and the vigor of cuttings derived from them are examined (Figure 6). HLVd infection leads to poor root growth (Figure 6B) that continues to impact plant growth at the vegetative stage (Figure 6C,D) and that can also impact flowering (Figure 6E). HLVd-infected flowering plants derived from infected stock plants display reduced inflorescence growth as well as lower levels of cannabinoid production (Punja et al., 2024). This underscores the importance of maintaining pathogen-free stock plants during commercial production. Routine scouting for the presence of disease symptoms and testing stock plants for the presence of HLVd, *Fusarium*, and *Pythium* spp. are highly recommended.

2.4. IDM Approaches at the Stock Cultivation Stage

During the stock plant cultivation stage, various IDM strategies can be implemented to minimize the development of plant pathogens. The following are examples of some commonly used practices.

2.4.1. Biosecurity and Quarantine Inspection

Biosecurity practices, which include implementing foot baths, requiring protective clothing, and removing pruned leaves and diseased plants, are standard in most horticultural greenhouse operations (Kruidhof & Elmer, 2020); these practices should therefore be implemented for cannabis growing operations. In addition, it is important to establish a quarantine protocol in cases where plant materials, such as unrooted cuttings or whole plants, are brought in from an external source (Razdan & Sabitha, 2009). Such precautionary measures can prevent pathogen introduction and are standard biosecurity protocols in commercial crop production (Van Lenteren & Nicot, 2020). When applied to cannabis, these precautions necessitate an isolation period of 3–4 weeks, during which plants are monitored for disease symptoms and tested for the presence of potential viruses and other pathogens (Punja & Scott, 2022). Testing for cannabis pathogens can be achieved by polymerase chain reaction (PCR) methodologies, and testing for viruses or viroids can be achieved using reverse transcription polymerase chain reaction (RT-PCR); many laboratories currently offer this service for an array of cannabis pathogens (Wang, 2021; Punja & Scott, 2022; Punja, 2021a). After the plants are confirmed to be free of detectable pathogens, they can be used for commercial propagation and any infected plants should be destroyed.

2.4.2. Cultural and Environmental Management

Environmental management is a component of IDM across all stages of cannabis growth since climatic conditions can influence both plant growth and pathogen growth. Standard cannabis cultivation environmental setpoints, which are established for baseline pathogen management during low disease pressure periods, have been described (Fleming et al., 2023; Stasiak & Dixon, 2022). Conditions that are unfavorable for disease development while at the same time supporting optimal plant growth are required. This often necessitates lowering temperature and humidity levels below the optimal set points for plant development to reduce the vapour pressure deficit (VPD) in the crop environment during periods of high disease pressure. Optimal environmental conditions vary across the different developmental stages of cannabis. In the cloning or seedling stage, temperatures should be kept between 20 ◦C and 24 ◦C with relative humidity above 90%. For the stock plant and vegetative stages, temperatures should range from 25 ◦C to 28 ◦C to promote rapid growth, with relative humidity maintained

between 65% and 75% (equating to a VPD of 1.1 to 0.94). During the flowering stage, temperatures should be set between 23 ◦C and 28 ◦C to facilitate the transfer of photosynthates to the flowers, with relative humidity between 50% and 70% (resulting in a VPD of 1.4 to 1.13). These environmental parameters can be achieved by modifying venting, heating, and air circulation strategies (Stasiak & Dixon, 2022). Seasonal adjustments may also need to be made, as warmer temperatures with higher humidity in the summer months may increase the incidence of root-infecting pathogens, such as *Fusarium* and *Pythium* spp. Similarly, cooler and more humid conditions during winter seasons may contribute to the development of powdery mildew infections. The impact of environmental conditions on HLVd development is currently unknown.

2.4.3. Sanitary Practices

Thorough sanitation of the growing environment before planting a new cannabis crop is important to reduce residual pathogen inoculum, which can be spread by water or air, by tools, or potentially by clothing, gloves, or shoes. This is a common practice used on most greenhouse crops, especially where viruses are of concern (Punja & Scott, 2022; Kruidhof & Elmer, 2020). To reduce pathogen transmission, all surfaces and equipment, as well as gutters, tables, floors, drip emitters, and pots, should be cleaned with reduced-risk sanitary products. These products include hydrogen peroxide with peracetic acid (Sanidate® or Zerotol®), dodecyl dimethyl ammonium chloride (Chemprocide® or KleenGrow®), isopropyl alcohol, and bleach (Punja & Scott, 2022). The efficacy of these products in inhibiting pathogen growth can vary depending on the pathogen, product, and concentration used. A comparison of two products used at four concentrations against the growth of two pathogens is shown in Figure 7A,B. At increasing concentrations, both Zerotol® and hypochlorous acid (a product containing 1000 ppm that was diluted) reduced pathogen growth, but Pythium showed a greater sensitivity compared to Fusarium (Figure 7C). These products can also potentially negatively affect the growth of beneficial Trichoderma species applied as biocontrol agents (Figure 7D). Therefore, care must be taken to consider the potential impact of applying reduced-risk products in conjunction with biocontrol products. These types of evaluations are important to conduct for any reduced-risk product targeted for the cannabis market to demonstrate efficacy and determine possible non-target effects.

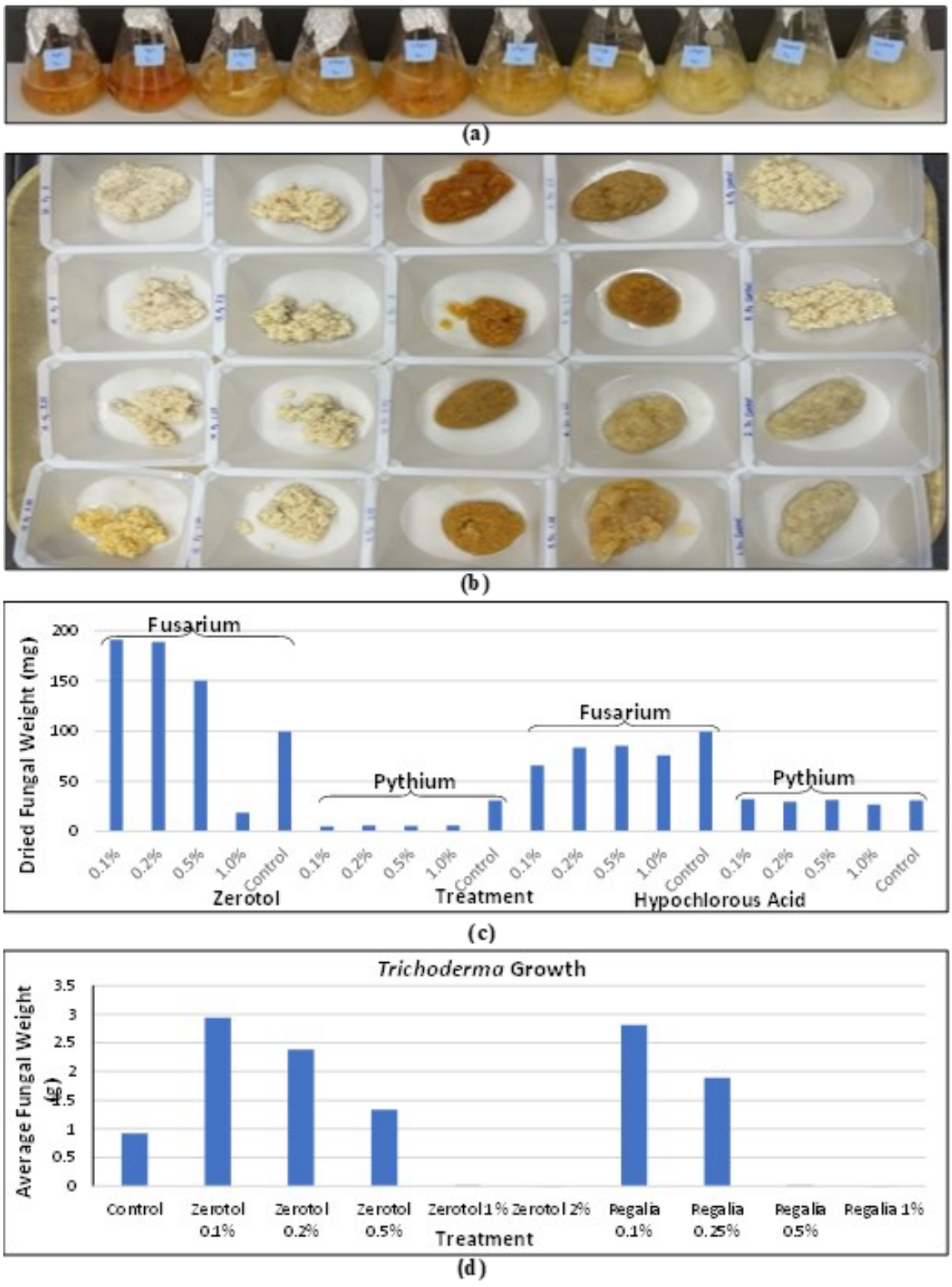


Figure 7. The effect of reduced-risk products on pathogen growth can be evaluated under laboratory conditions by testing a range of

concentrations in a liquid culture medium. (A) Growth in a potato dextrose broth containing a range of concentrations of individual products is measured by obtaining mycelium dry weights after a 7-day exposure. (B) The effect of Zerotol® and hypochlorous acid on the growth of two pathogens at increasing concentrations is shown. Both *Fusarium* and *Pythium* are reduced at higher concentrations, but the growth of *Pythium* shows greater sensitivity compared to *Fusarium*. (C) The growth of *Trichoderma* can also be reduced by the presence of specific compounds.

2.4.4. Testing for Pathogen Presence and Eradication

Early detection of disease symptoms in stock plants is important to prevent pathogens from spreading within the growing environment. There are several diagnostic approaches that have been developed to detect cannabis pathogens, and a number of commercial laboratories provide testing services for a range of pathogens (Wang, 2021; Punja, 2021a; Punja et al., 2023; Punja et al., 2024). The practice of culling and replenishing stock plants is a standard component of IDM programs when diseased plants are detected (Konstantinidou et al., 2022). Stock plants should be replaced after several (3-4) months of production with new, pathogen-free plants, which is key to maintaining a healthy and vigorous stock plant population. Plants infected with *Fusarium*, *Pythium*, or HLVd should be promptly removed from a facility. The eradication of diseased plants, particularly those infected with HLVd, is essential. When regular (weekly) pathogen testing is followed by the destruction of those plants infected by HLVd, a gradual decline in the occurrence of diseased stock plants can be achieved (Figure 8). After many rounds of testing performed over a 6-month period, this strategy was shown to reduce HLVd frequency in stock plants from 22% to 1% (Figure 8). Peaks of infection can still be seen, which are attributed to the re-introduction of diseased plant material that went undetected initially and was inadvertently used as a source of cuttings. Removing this material upon detection resulted in the continued downward trend of infection.

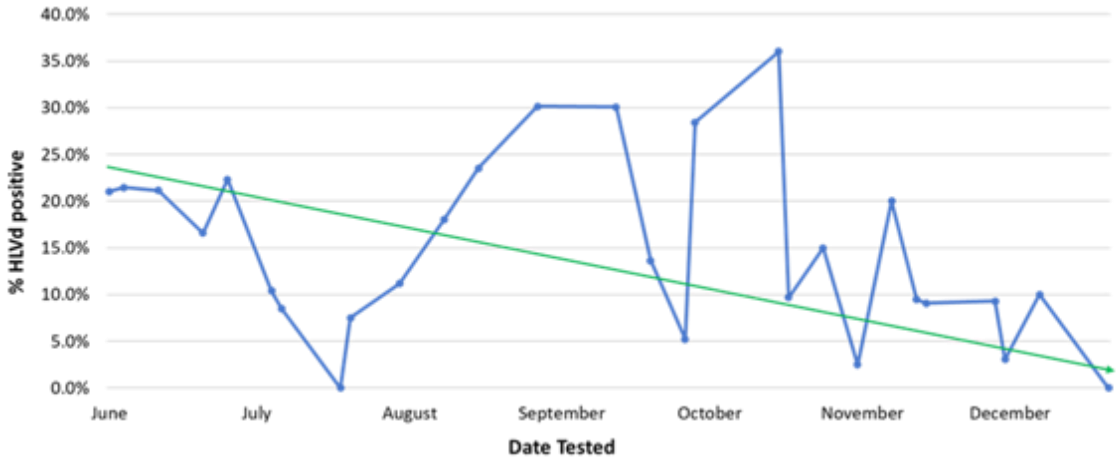


Figure 8. The impact of the eradication of HLVd-infected stock plants on the frequency of positively infected plants over a 6-month duration. The blue line shows the actual incidence of infected plants, which fluctuates over time. The solid green line indicates the general trend that shows a decline in the number of infected plants.

2.4.5. Utilizing Disease-Tolerant Genotypes

The utility of disease-tolerant genotypes that may have been developed through selective breeding and genotype screening is an important aspect of IDM for stock plants. Disease-tolerant genotypes of cannabis have been identified for a number of pathogens, including root rot (*Fusarium oxysporum*) (Punja, 2021b), powdery mildew (*Golovinomyces ambrosiae*) (Stack et al., 2021; Mihalyov & Garfinkel, 2021; Stack et al., 2024; Sirangelo, 2023), leaf blight (*Neofusicoccum parvum*) (Roberts & Punja, 2022), and bud rot (B. cinerea) (Punja & Li, 2021; Mahmoud et al., 2023) (Figure 9). Recent research suggests that specific defense genes may play a role in certain host–pathogen interactions, leading to a resistant phenotype](Mihalyov & Garfinkel, 2021; Stack et al., 2024; Sirangelo et al., 2023; Balthazar et al., 2020). The impact of cannabis genotype on disease development at the flowering stage will be discussed later in this review. The continued evaluation of cannabis genotypes for pathogen response is a critical component of an IDM program.

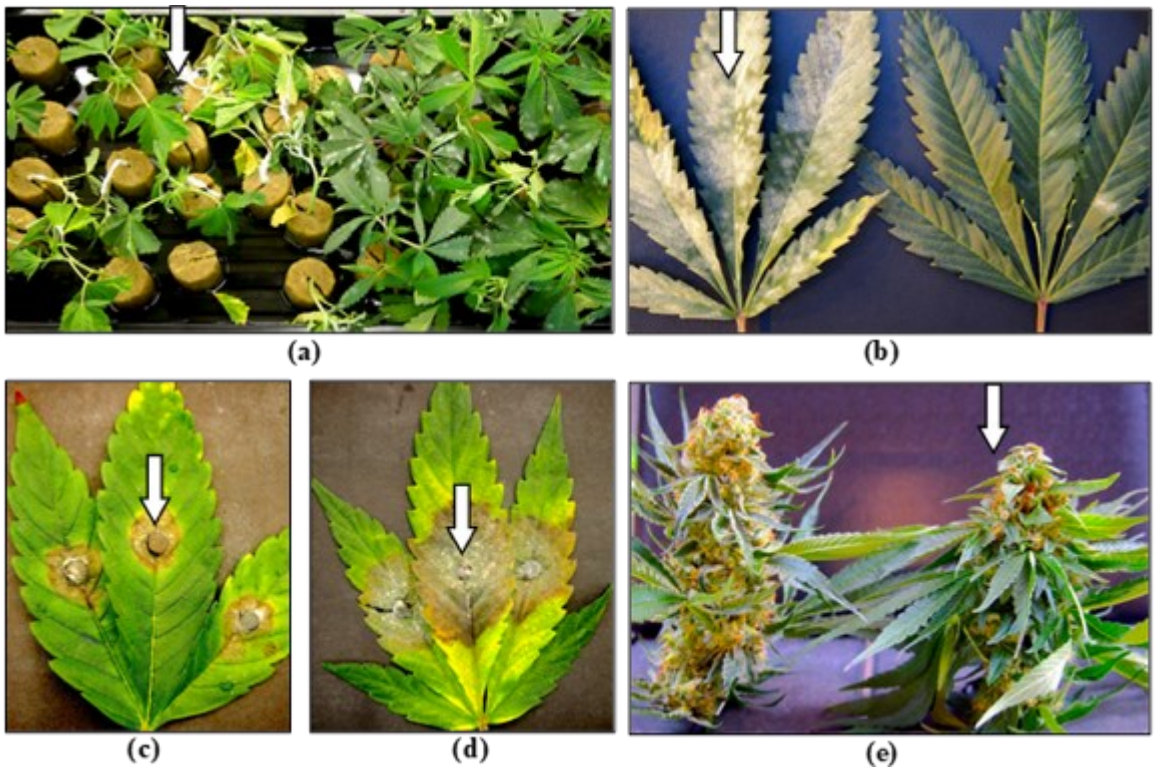


Figure 9. Examples of cannabis genotypes that exhibit a level of disease tolerance to different pathogens. (A) Fusarium damping-off, with susceptible genotype on the left and tolerant genotype on the right. (B) Powdery mildew, with susceptible genotype on the left and tolerant one on the right. (C, D) Alternaria leaf blight, with tolerant genotype on the left and susceptible one on the right. (E) *Botrytis* bud rot, with tolerant genotype on the left and susceptible one on the right.

2.5. Propagation Stage

Cannabis plants can be initiated either from seeds or from vegetative cuttings, that originate from stock plants, but the latter is more commonly used in commercial production. Routine testing for pathogens that may be present in seeds is not currently a standard practice in the cannabis industry, which can result in the spread of seed-borne pathogens. Cannabis and hemp seeds are known to harbour species of *Alternaria*, *Fusarium*, and several post-harvest molds (Roberts & Punja, 2022; Dumigan & Deyholos, 2022), as well as HLVd (Punja et al., 2024; Atallah et al., 2024). Implementing stringent sanitation protocols and testing for fungal or bacterial pathogens using PCR and for viruses or viroids using RT-PCR, as described previously, are important IDM

approaches during plant propagation in greenhouse crops, including cannabis (Wang, 2021; Punja, 2021a; Jones & Monthony, 2022; Munkvold & Gullino, 2020). These steps can minimize the subsequent spread of fungal, bacterial, and viral/viroid pathogens. Vegetative cuttings used for propagation are required to be rooted under high-humidity conditions over a two-week period. This environment is conducive to the spread of pathogens such as *Fusarium* spp. and *B. cinerea* (Figure 10), as well as to a number of bacteria that can be spread by water or in the air. Testing conducted in the rooting environment by swabbing surfaces, sampling water and air, or plating surface-sterilized plant material can be used to assess total microbes that may be present (Punja & Rodriguez, 2018; Punja et al., 2021; Punja, 2021b). Cuttings may harbour inoculum of *Fusarium* spp., and they are more likely to develop powdery mildew or HLVd if the original stock plants were infected (Punja & Scott, 2022; Punja, 2021a; Punja, 2021b; Punja et al., 2024). Cuttings taken from stock plants infected internally by *Fusarium* species can result in the spread of the pathogen, resulting in damping-off symptoms, particularly in susceptible genotypes (Figure 10). The infection causes the pith and xylem tissues to collapse, resulting in the death of the cuttings. Powdery mildew symptoms may also appear on cuttings from inoculum either carried over from the stock plants or introduced at the propagation stage. The most significant pathogen affecting root development and growth of cuttings is HLVd, which originates from infected stock plants (Punja et al., 2024). Additionally, under high humidity conditions, vegetative cuttings may be affected by gray mold (*B. cinerea*) and common saprophytic fungi, including *Penicillium* spp., which can potentially reduce the appearance and quality of the cuttings (Punja, 2021a; Punja, 2021b). Many of these fungal pathogens that affect cannabis cuttings, as well as other stages of plant development, produce large numbers of spores, which can be spread by water, air, and tools throughout the growing facility (Figure 11). These spores can serve as sources of initial inoculum and can be challenging to manage. The inclusion of HEPA filters and HVAC systems is advisable to reduce the total counts of air-borne fungal propagules.

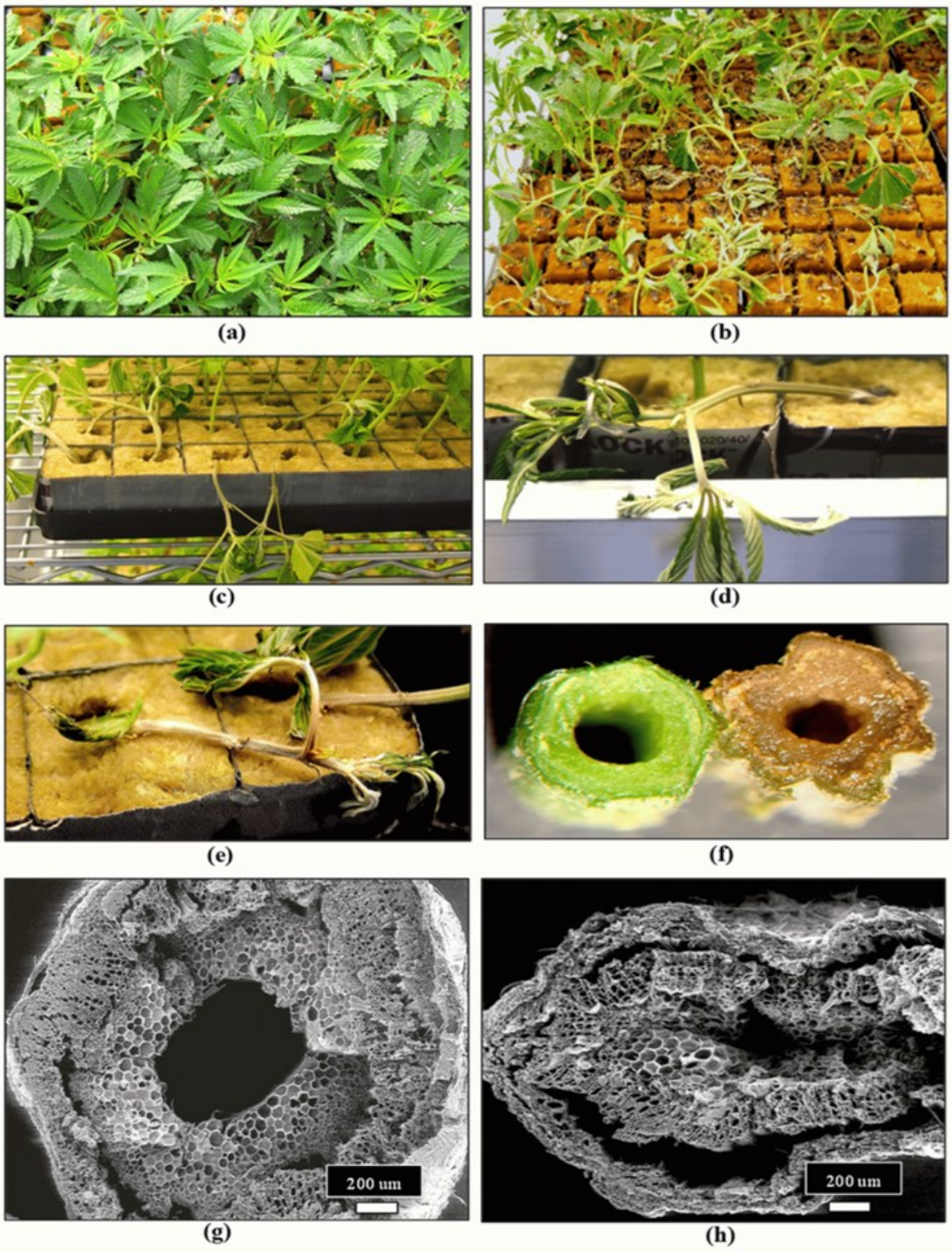


Figure 10. The propagation of cannabis from vegetative cuttings and the development of Fusarium damping-off. (A) A tray of healthy cuttings. (B) A tray of cuttings infected with *Fusarium oxysporum*. (C, D, E) Close-up views of damped-off cuttings. (F) A cross-sectional view of the stem a healthy cutting (left) compared to a diseased one (right) in which tissue browning can be seen. (G) A scanning electron microscopic view of a section through the stem of a healthy cutting. The central pith can be seen. (H) A collapsed stem of a diseased cutting viewed through the scanning electron microscope. The central pith has collapsed as well as the surrounding cells.

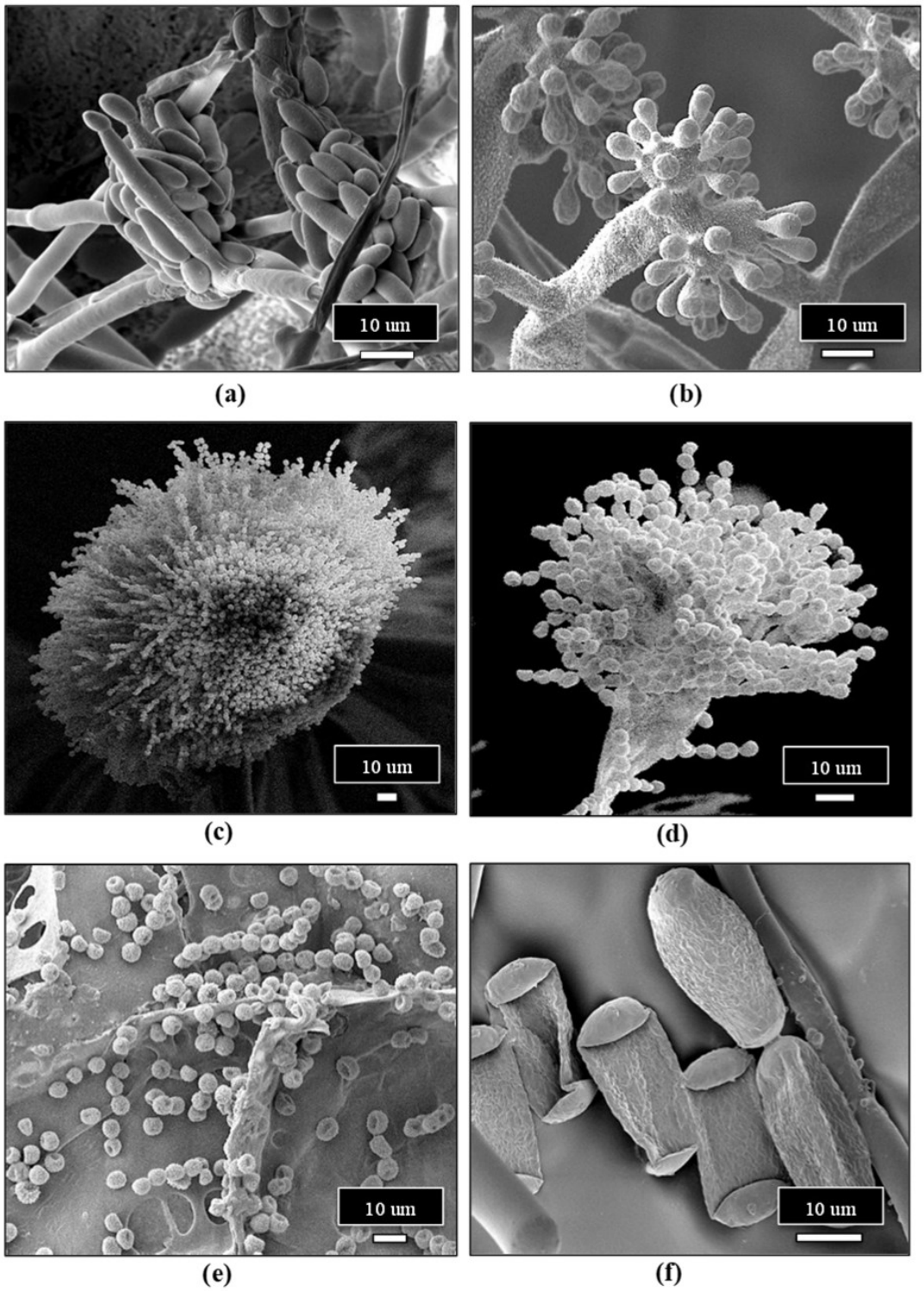


Figure 11. The spores of a range of pathogens that can affect cannabis plants at various stages of crop growth. (A) *Fusarium oxysporum* micro-conidia. (B) *Botrytis cinerea* spores. (C) A large cluster of spores of *Aspergillus* sp. (D, E) Chains of spores of *Penicillium* sp. (F) *Golovinomyces cichoracearum* spores.

2.6. Propagation Stage IDM Approaches

2.6.1. Cultural and Environmental Management

During the propagation stage, ensuring that cuttings are obtained from healthy stock plants reduces the probability of pathogens being transferred through these cuttings. In particular, the incidence of *F. oxysporum* is reported to be greater in cuttings taken from the base of the plant compared to those taken from higher locations of the plant (Punja, 2021a; Punja, 2021b). Therefore, cuttings from the uppermost part of stock plants may limit transmission of this pathogen and possibly of HLVd, although sufficient data is lacking at the present time for this latter pathogen. Ensuring that cuttings are acclimatized in a transitional environment prior to resuming vegetative growth reduces stress on the rooted plants (Jones & Monthony, 2022; Munkvold & Gullino, 2020).

2.6.2. Application of Biological Control Agents

Several biological control products containing *Trichoderma* spp. or *Gliocladium catenulatum* are registered for use on cannabis in Canada (Scott et al., 2021). These products are classified as “reduced risk” and provide an alternative in the absence of registered synthetic fungicides. They can be used at all stages of cannabis crop growth but are particularly useful for managing damping-off caused by *Fusarium* spp. on cuttings. When applied at the vegetative stage of plant growth, they can reduce mortality due to *Fusarium* and *Pythium* species (Scott & Punja, 2023). Several weeks after application, the biocontrol agents can be recovered from cannabis tissues, which indicates that they are able to survive for a period of time (Figure 12). Their effectiveness is depends on being applied prior to the pathogen infection, ideally as a drench or as a dip when cuttings are being rooted or as a drench at later stages of crop growth. The benefits of late applications should be evaluated, as most biocontrol products are costly to use at large scales. Biocontrol agents protect susceptible root tissues from infection by root pathogens and can colonize cuttings internally, possibly functioning as endophytes; they can potentially enhance root and shoot growth in addition to providing protection against pathogens (Scott & Punja, 2023). *Trichoderma* spp. also exhibits direct antagonism to *F. oxysporum* in dual culture (Figure 13). However, the optimal conditions for maximizing the efficacy of registered biocontrol agents in cannabis cultivation remain unexplored. Nevertheless, several biocontrol agents have been

demonstrated to be effective against root-infecting pathogens when applied preventatively and in accordance with their label on cannabis plants (Scott & Punja, 2023), indicating their adaptability to various environmental conditions.

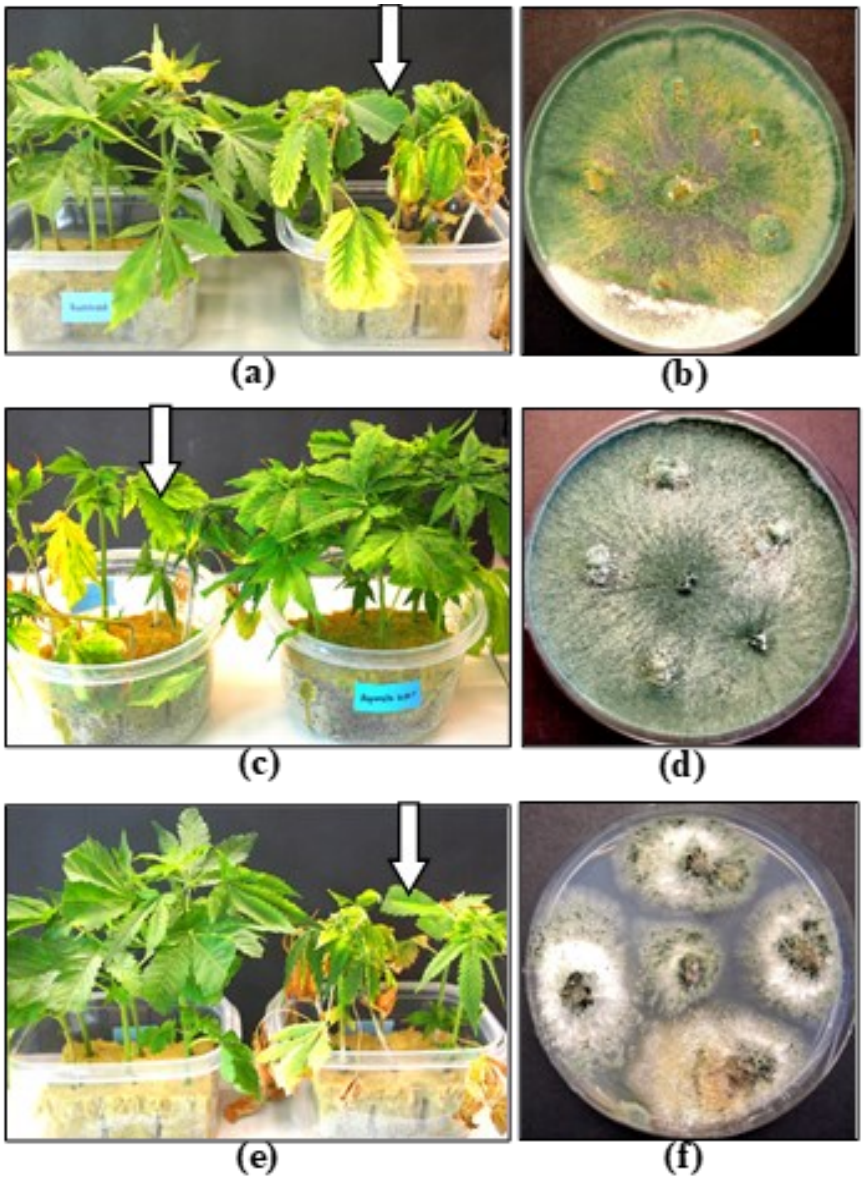


Figure 12. The application of biological control agents provides protection to cannabis cuttings against *Fusarium* damping-off. (A) Rootshield-treated cuttings (left) show greater survival compared to pathogen-only (right). (B) Growth of *Trichoderma harzianum* from Rootshield-treated cuttings. (C) Asperello-treated cuttings (right) show greater survival compared to pathogen-only (left). (D) Growth of *Trichoderma asperellum* from Asperello-treated cuttings. (E) Prestop-treated cuttings (left) show greater survival compared to pathogen-only. (F) Growth of *Gliocladium catenulatum* from Prestop-treated cuttings.

Figure 13. The growth of *T. asperellum* (top) is observed to stop the growth of *Fusarium oxysporum* (bottom) when both are placed on a Petri dish. After a few days, the biocontrol agent continues to grow over and inhibit further growth of the pathogen.

2.7. Vegetative Growth Stage

After rooted plants are established from cuttings, the plants are allowed to continue to grow vegetatively for an additional 2–3 weeks before being transferred to flowering rooms. During this growth stage, root-infecting pathogens, including *Fusarium* and *Pythium* species, as well as HLVd, may continue to develop and spread. The development of powdery mildew may also become more severe at this stage of production. Internal stem infections by *Fusarium* spp. in rooted cuttings can significantly reduce the growth and development of vegetative plants. Symptoms such as yellowing, stunted growth, browning of roots, and plant death often signal infection by *Fusarium* and *Pythium* species (Figure 14). The development of these pathogens can be exacerbated by root damage and excessive watering or flooding, which can also spread the pathogen inoculum and cause further development of disease. The testing of recirculated water for pathogen presence is an important aspect of IDM.

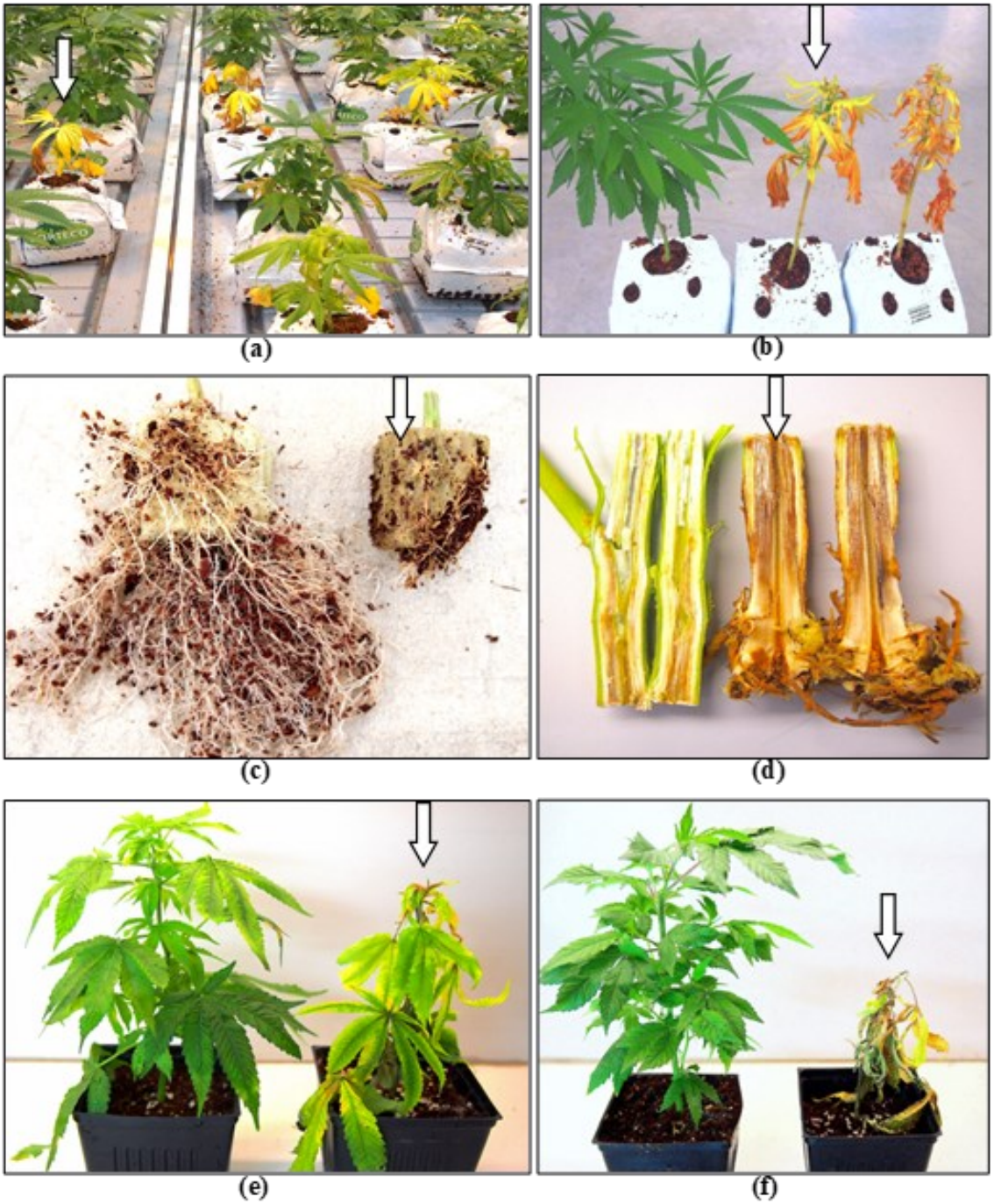


Figure 14. *Pythium* and *Fusarium* infection on vegetative plants of cannabis. (A) The symptoms of yellowing of the foliage are indicative of root infection by these pathogens. (B) The death of rooted cuttings due to *Fusarium* infection. (C) The root development on healthy plant (left) compared to one infected by *Fusarium* (right). (D) Internal stem discoloration is indicative of infection by *Fusarium*. (E, F) Infection by *Pythium* can cause significant stunting of plant growth and death (right) compared to healthy plants (left).

Infection of the rooted cuttings with HLVd can adversely affect root development and plant growth at the vegetative stage, leading to reduced plant size, particularly in susceptible genotypes (Figure 6). Molecular diagnostic methods should be used to ensure that vegetative plants are not infected by this viroid (Wang, 2021; Punja et al., 2024). In greenhouse environments where the recycling of nutrient solutions is practiced, monitoring for the presence of *Fusarium* and *Pythium* inoculum is necessary since both are known to be present in hydroponic nutrient solutions (Punja & Rodriguez, 2018). Regular testing of electrical conductivity (EC) and potential hydrogen (pH), coupled with testing of drip and drain nutrient ratios, will ensure that the nutrient profiles remain within the optimal range for crop development, preventing nutrient deficiencies that could lead to a predisposition to pathogen infection (Sonneveld & Voogt, 2009; Zheng, 2022b). In addition, monitoring water temperature and oxygen levels can reduce extremes that can enhance root infection by pathogens (Zheng, 2022b). The treatment of recirculated water with reduced-risk products, such as those indicated in Section 2.2.3, can reduce the incidence of root pathogens (Figure 7). The regular monitoring of plants for symptom development should be conducted.

2.8. Vegetative Growth Stage IDM Approaches

2.8.1. Cultural and Environmental Management

Root pathogen development in vegetative plants can be minimized by increasing the interval between watering events, leading to fewer and shorter irrigation events as long as adequate moisture is provided for optimal root development. This strategy has been used to reduce root pathogen development in various crops (Razdan & Sabitha, 2009). The exposure of plants to ultraviolet radiation, especially UV-C light (234 nm wavelength), can suppress powdery mildew mycelium development and spore germination *on the foliage*, when applied routinely at an appropriate dosage with good coverage of the upper leaf surfaces (Scott & Punja, 2020). Night-time exposure enhances pathogen susceptibility by limiting light-activated DNA repair mechanisms (Janisiewicz et al., 2016). Exposure to UV-C may also enhance plant defense responses, including the accumulation of reactive oxygen species (Urban et al., 2016), although the effect on cannabis plants has not been determined. To avoid phytotoxicity, plants should be exposed to UV-C gradually over several weeks, according to the

manufacturer's guidelines. Treated plants may show a reduction in plant height and increased lateral branching, as has been observed in some ornamental plant species (Darras et al., 2012; Bridgen, 2016). Additional research is required to demonstrate the potential benefits of exposing cannabis plants to UV-C.

2.8.2. Application of Biological Control Agents

Biological control agents can also be applied as drenches to vegetative plants to reduce the severity of root pathogens, similar to treatments made at the propagation stage (Scott & Punja, 2023). The extent to which these agents can survive following application at this stage has not been determined. The colonization of the rapidly growing roots by the biocontrol agent is required for adequate reduction in pathogen development.

2.9. Flowering Stage

After vegetative plants have been transferred to greenhouse compartments where the photoperiod is reduced from 18:6 h light:dark to 12:12 h or other iterations of light:dark (Zheng & Llewellyn, 2022; Ahrens et al., 2023), the onset of inflorescence development is triggered within 1–2 weeks. At this stage of crop development, symptoms of root infection by *Fusarium* or *Pythium* spp. originating from the propagation/vegetative stage may rapidly become apparent. These symptoms include leaf yellowing, plant wilting, crown and root rot, and stunted growth (Figure 15). There is no evidence that new infections from residual inoculum *occur* in flowering plants if all sanitary practices have been followed and recirculated water *is* not used. Symptoms attributed to HLVd infection, which may have been previously undetected on vegetative plants, will typically manifest within 1–3 weeks after transfer to the flowering room. These symptoms are distinct, appearing as reduced inflorescence size, yellowing of the bract leaves, and stunted plant growth (Punja et al., 2024) (Figure 6). The environmental conditions during inflorescence development, which include higher humidity due to increased plant biomass, may also promote the development of powdery mildew, particularly in more susceptible genotypes. Closer to the harvest period, when inflorescences begin to mature, bud rot caused by *B. cinerea* is likely to become visible, depending on environmental conditions and the genotype. This can lead to significant

reductions in inflorescence quality and yield (Figure 15). The development of pathogens in cannabis plants during the flowering phase is deemed to have the most significant impact on economic returns and can be the most difficult to manage.

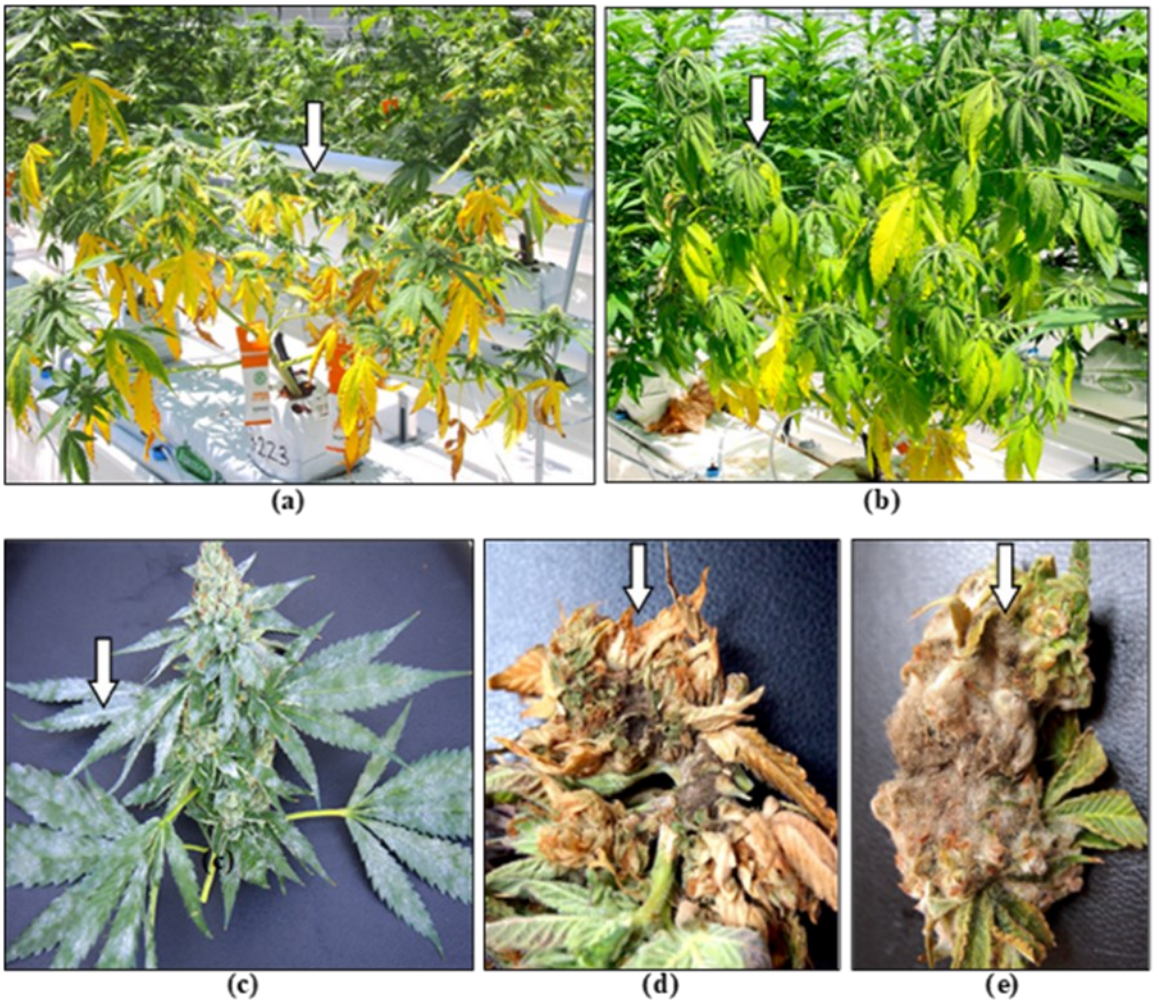


Figure 15. The symptoms due to pathogen infection on flowering cannabis plants. (A) The yellowing of the foliage and stunted growth due to infection by *Fusarium*. (B) The wilting of plants and the yellowing of foliage due to infection by *Pythium*. (C) Powdery mildew development on inflorescences and surrounding leaves. (D, E) Bud rot caused by *B. cinerea* destroys the inflorescence.

In addition to the pathogens that infect the crop during the flowering stage, the colonization of inflorescences by yeasts and molds prior to harvest is common and generally remains undetected until after harvest when quality tests are performed. On the inflorescence tissues, the most commonly encountered fungal genera include *Penicillium, Alternaria, Cladosporium,* and *Fusarium* (Figure 16). These microbes can be

detected by conducting bud swab tests as described in recent studies [19,20,50] (Punja et al., 2023; Gwinn et al., 2023; Punja, 2021c). This buildup of yeasts and molds can lead to the final dried product failing to meet quality standards by exceeding colony-forming unit thresholds or in some cases by increasing the level of mycotoxins (Gwinn et al., 2023; Punja, 2021c). Various factors influence the levels of yeast and mold contamination, which are discussed in the following sections. Testing for the presence of yeasts and molds on cannabis inflorescences prior to harvest is not routinely performed, although research studies have shown that this can provide useful information on the population levels and species that may be present (Punja, 2023). These populations are influenced by many factors, including the genotype of cannabis being grown, the environmental conditions prior to harvest, particularly temperature and relative humidity, the presence of excessive leaf litter, and the time of year (Punja, 2023).

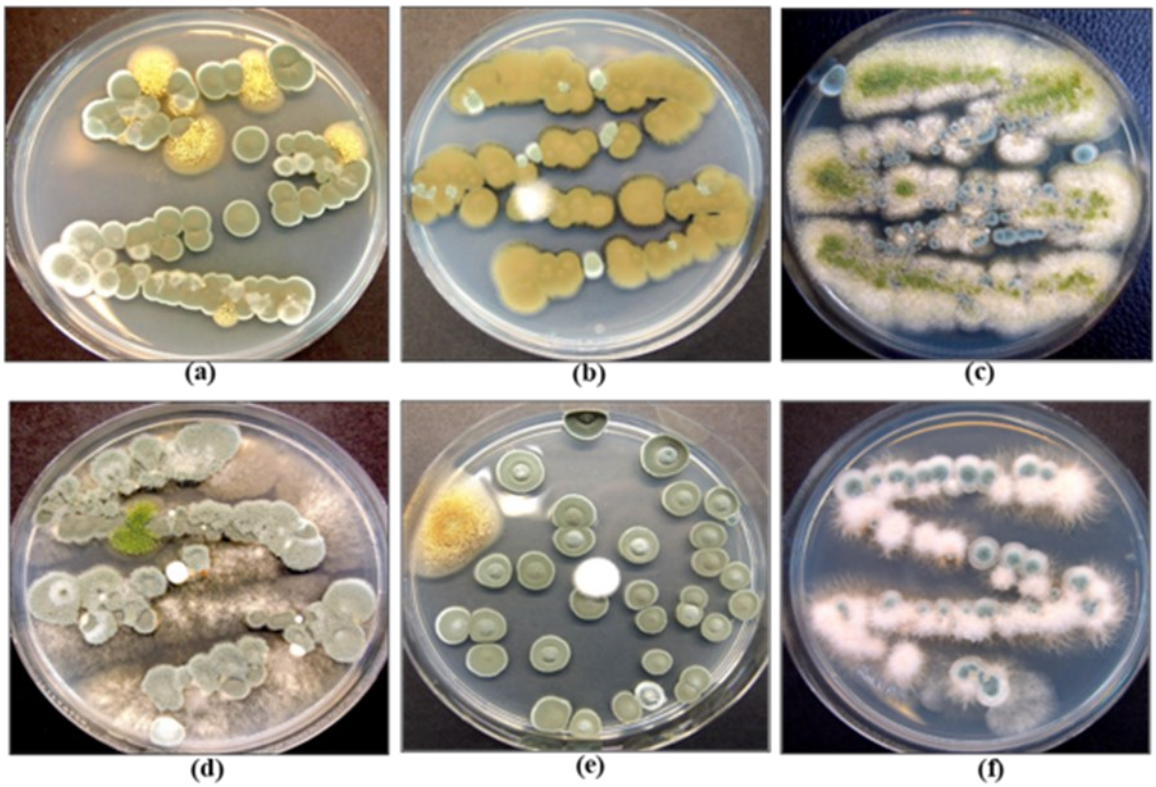


Figure 16. The most common fungi recovered from inflorescences of cannabis plants. The Petri dishes show the result from swabbing of samples and plating onto an agar medium that allows growth of yeasts and molds to occur. On top row – (left to right) *Penicillium, Cladosporium, and Aspergillus*. On bottom row – (left to right) *Botrytis, Penicillium, and Fusarium.* Photos were taken after 7 days.

2.10. Flowering Stage IDM Approaches

2.10.1. Cultural and Environmental Management

The increased plant biomass resulting from plant development during the flowering stage creates challenges for the maintenance of consistent environmental conditions, particularly with regard to ambient humidity. Reducing plant densities can significantly lower humidity levels in the greenhouse and also allow for better light penetration and ease of application of disease control products. However, lower plant densities can decrease overall yield per unit area of production (Mahmoud et al., 2023; Matzneller et al., 2022). A lower ambient relative humidity can also be achieved by increasing air circulation with circulating fans placed near the plants in the weeks leading up to harvest. Maintaining air movement at 0.5–1.0 m/s appears to be an optimal target for microbial suppression in cannabis (Matzneller et al., 2022). Under experimental conditions, enhanced air flow around maturing inflorescences was demonstrated to significantly reduce the populations of various microbes within the tissues of genotype 'PH' (Figure 17). This reduction in humidity, combined with appropriate climate control settings, can mitigate the severity of diseases such as bud rot (*B. cinerea*) and powdery mildew during high-risk periods. The cost and practicality of this approach during greenhouse production need to be evaluated, but it provides opportunities for disease management in indoor controlled environments.

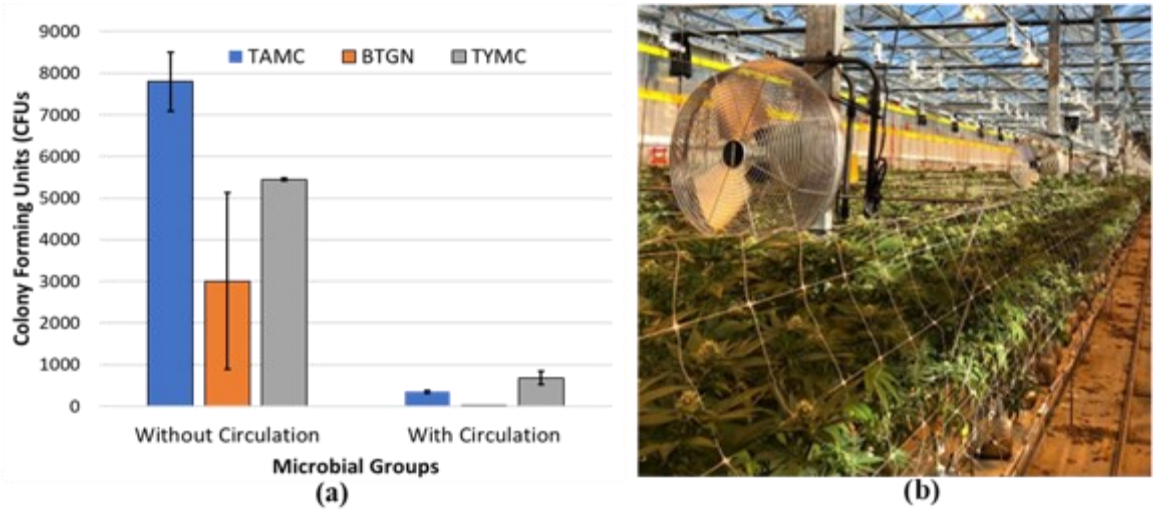


Figure 17. (A) The effect of enhanced air flow around cannabis plants using circulating fans on total colony-forming units of microbes in these tissues. The vertical bars show the total colony-forming units of total

aerobic count (TAMC), bile-tolerant Gram-negative count (BTGN), and total yeast and mold count (TYMC) with and without air circulation. (B) Fans were positioned 35 cm above the crop to circulate air continuously at ~7 m/s over ~40 plants, beginning in week 2 of the flowering period until harvest. The trial was replicated three times in different greenhouse compartments. The inflorescences were dried prior to microbial analysis.

In relation to seasonal effects on disease development in the greenhouse, *B. cinerea* bud rot development was shown to be influenced by external vapour pressure deficits that impacted moisture levels in the air and, hence, in the ambient humidity (Mahmoud et al., 2023). To avoid periods of high disease pressure brought on by external environmental conditions, one IDM strategy is to alter the time of seasonal plantings. By scheduling planting and harvest times to avoid periods of high disease pressure brought on by conducive environmental conditions, particularly on desirable but susceptible cannabis genotypes, producers can reduce the impact of seasonal pathogens such as B. cinerea (Mahmoud et al., 2023), as well as reduce the build-up of total inflorescence microbes that are also impacted by seasonal environmental fluctuations (Figure 18).

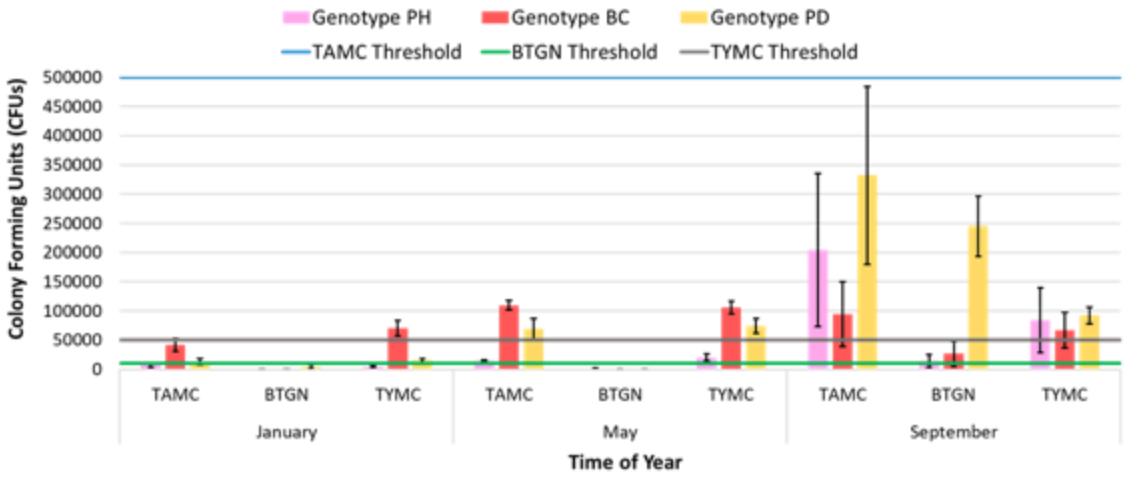


Figure 18. The influence of cannabis genotype and time of year (season) on total microbes present in dried cannabis inflorescences. The vertical bars denote the total aerobic microbial count (TAMC), bile-tolerant Gram-negative count (BTGN), and total yeast and mold count (TYMC). Samples were taken from three genotypes during three harvests in each season (fall, winter, summer season) of the same year. The highest microbial counts were observed in the September harvest period, corresponding to late-summer production. The failure thresholds for each microbial group are shown by the horizontal lines. The genotype 'PD' contained the highest microbial levels.

An alternative approach to reduce disease development in cannabis is to harvest inflorescences after a shorter crop development period to avoid prolonged exposure to environmental conditions that favour disease development at the maturation stage. For example, harvesting at 6 weeks of inflorescence development instead of 8 weeks can reduce B. cinerea bud rot incidence but could result in compromised yield and potency in certain genotypes unless they are close to maturity (Mahmoud et al., 2023; Caplan et al., 2022). Areas within a greenhouse that have localized disease or “hot spots” should be identified, followed by the eradication of the affected plants to minimize pathogen spread. The location of the diseased plants should be recorded, and if the causal pathogen is unclear, diagnostic testing should be performed, typically through the submission of samples to a diagnostic laboratory (Wang, 2021; Punja, 2021a). In addition to the visualization of these areas with the naked eye, the utility of infrared (IR) and artificial intelligence (AI)-powered scouting technologies could be of value as they have been used in a range of other crops (Sankaran et al., 2010; Mahmud et al., 2020; Anagnostis et al., 2021; Fountas et al., 2022; Shakeel et al., 2022), but further evaluation of how these technologies could be modified for application to cannabis is needed. A discussion of these technologies is presented in Section 2.10.5 and Section 2.10.6 of this article.

2.10.2. Utility of Disease-Tolerant Genotypes

The cannabis genotype being grown can have a profound impact on the development of certain pathogens, especially under disease-conducive conditions. The impact of genotypes on disease development at the stock cultivation and propagation stages was described previously. A similar significant effect of genotypes on pathogen infection can also be demonstrated at the flowering stage. A comparison of the response of six genotypes to four pathogens is shown in Figure 19. The genotype ‘LO’ showed high susceptibility to powdery mildew but low susceptibility to HLVd, *B. cinerea* bud rot and root pathogens. A second genotype, ‘LB’, showed high tolerance to all four pathogens, while the remaining genotypes varied in their response to these specific diseases. These data were collected from observation trials under natural infection rather than from replicated trials. They demonstrate, however, that cannabis producers have the option to select those genotypes that show tolerance to several important pathogens under the specific cultivation conditions of greenhouse production. While the

genetic basis for this level of tolerance has not been determined, it indicates there is a basis on which to establish breeding programs that can lead to the development of disease-tolerant cannabis cultivars.

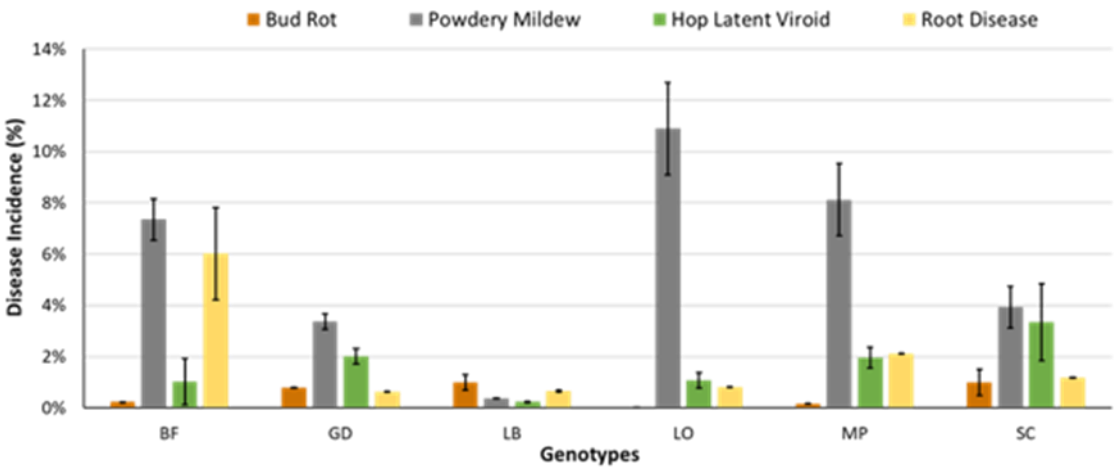


Figure 19. A comparison of disease incidence on six cannabis genotypes to four pathogens, demonstrating variation in the susceptibility to *Botrytis* bud rot, powdery mildew, hop latent viroid, and *Pythium* or *Fusarium* root diseases. Incidence data were obtained from scouting reports made during the cultivation of batches of genotypes in comparable greenhouse compartments over three production cycles in the summer season.

2.10.3. Application of Biological Control Agents.

As described for cuttings during the propagation and during vegetative growth of plants, biological control agents also show promise in reducing specific diseases at the flowering stage of cannabis plants. The diseases of importance that can be targeted are *B. cinerea* bud rot and powdery mildew. The application of several biological control products and reduced-risk chemicals at weekly intervals as a fine spray, at full label rates, onto developing inflorescences of the genotype ‘PH’ was observed to reduce the development of B. cinerea bud rot. This was true under both low pressure and the high disease pressure that resulted from natural infection during the fall growing season (Figure 20).

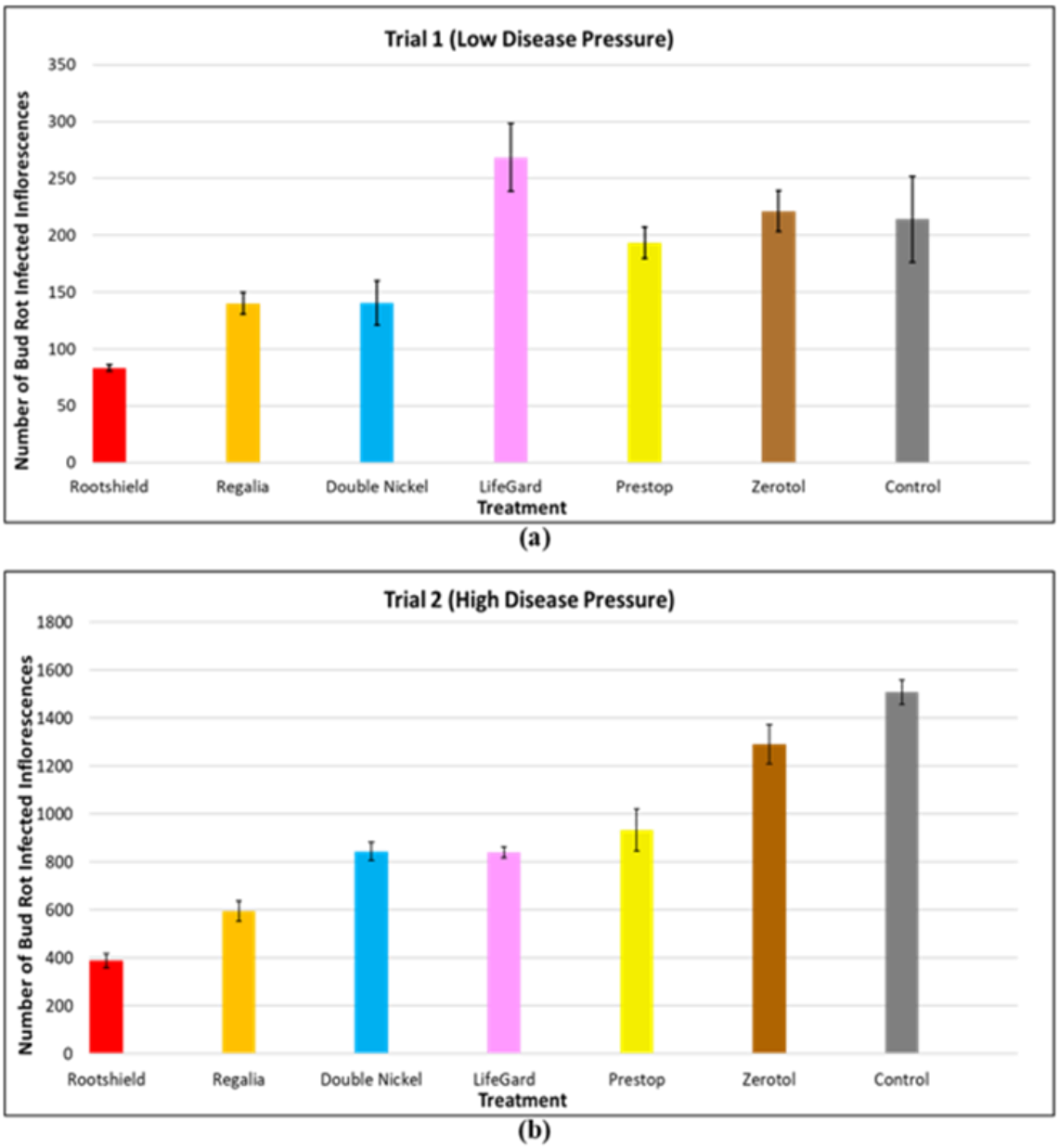
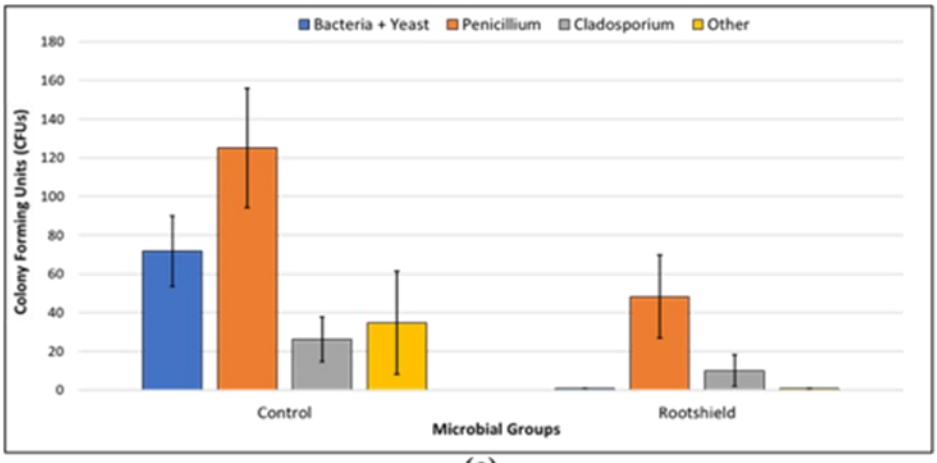
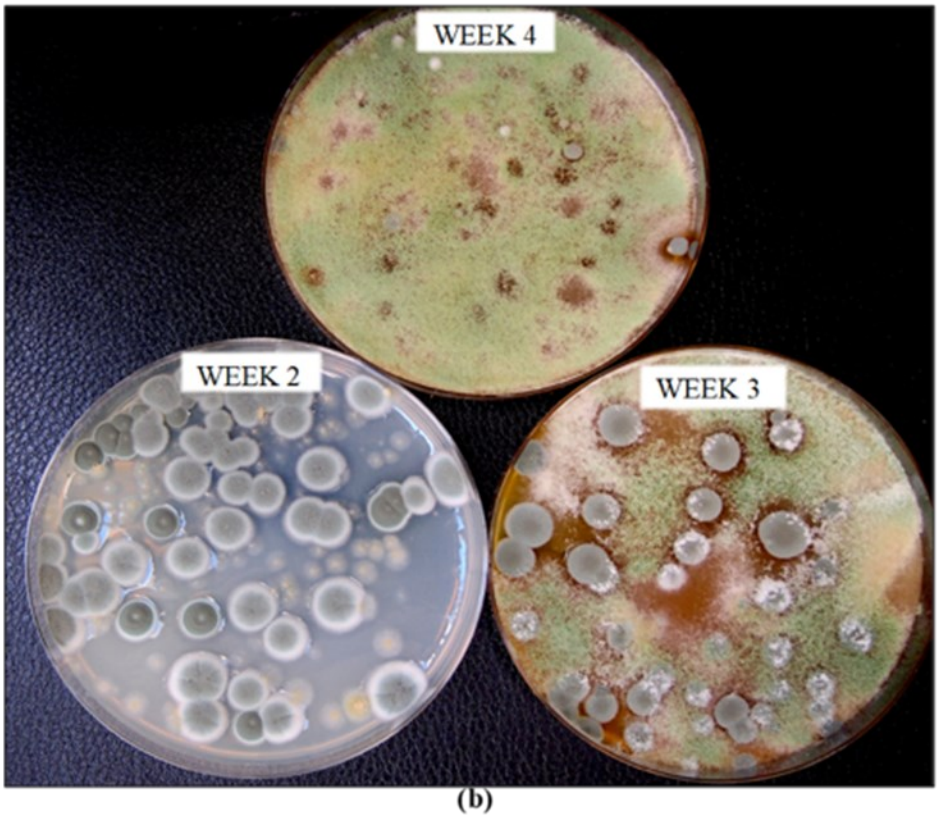


Figure 20. The comparative efficacy of six biological control products and reduced-risk chemicals on *Botrytis* bud rot development on flowering cannabis plants. Three applications were made at weeks 2, 3, and 4 of the flowering period at maximum label rates. The sprays were applied to ca. 216 plants using a robotic pipe rail sprayer that delivered ~60 mL of product to each plant. Disease assessments were made at harvest (week 8) in a greenhouse compartment with low and high *Botrytis* bud rot pressure from natural inoculum. (A) A low disease pressure flower room. (B) A high disease pressure flower room.

The most effective product for controlling *B. cinerea* was Rootshield HC® (containing *Trichoderma harzianum*), followed by Regalia® (*Reynoutria sachalinensis*), Double Nickel® (*Bacillus amyloliquefaciens*), Lifegard® (Bacillus mycoides), and Prestop® (Gliocladium catenulatum). Zerotol® (hydrogen peroxide) did not show an effect (Figure 20). The efficacy of the various biocontrol agents likely stems from their ability to pre-emptively colonize the inflorescence tissues and compete with the pathogen, a mode of action also reported on other crops (Elad, 1996; Vos et al., 2015). The application of *T. harzianum* was also found to suppress the development of other microbes naturally present within the inflorescences, including *Penicillium* spp., and this was reflected by a reduction in all three categories of microbial counts (Figure 21).



(a)



(b)

Figure 21. The effect of Rootshield HC® (*T. harzianum*) applications made at weeks 2, 3, and 4 of the flowering period on the final microbial levels in harvested cannabis inflorescences. (A) The total counts of all microbes in both untreated and sprayed plants are shown. The total microbes were reduced following Rootshield applications. (B) The growth of microbial colonies after the blending of the treated inflorescences in distilled water and subsequent plating onto agar medium. A comparison is shown of samples following applications of Rootshield made at weeks 2, 3, and 4 of the flowering period. Samples treated at week 4 show maximum suppression of *Penicillium* growth compared to week 2 where there is no suppression and no colonies of *Trichoderma* were recovered.

A second trial demonstrated that applying *T. harzianum* (Rootshield HC®) thrice to the foliage of flowering cannabis plants also reduced the development of powdery mildew compared to untreated plants, as shown in Figure 22. These results indicate that a single biological control agent may simultaneously target two important diseases affecting cannabis, namely *B. cinerea* bud rot and powdery mildew. *Trichoderma* applications have been shown to suppress powdery mildew in several crops (Elad, 2000; Ahmed, 2018; Esawy et al., 2021). The ease of application of the product and the potential to increase microbial counts in inflorescences of treated plants may determine the extent to which cannabis producers are willing to apply biocontrol agents to flowering plants. Other registered biocontrol products need to be assessed to determine whether they can provide similar efficacy in suppressing disease.

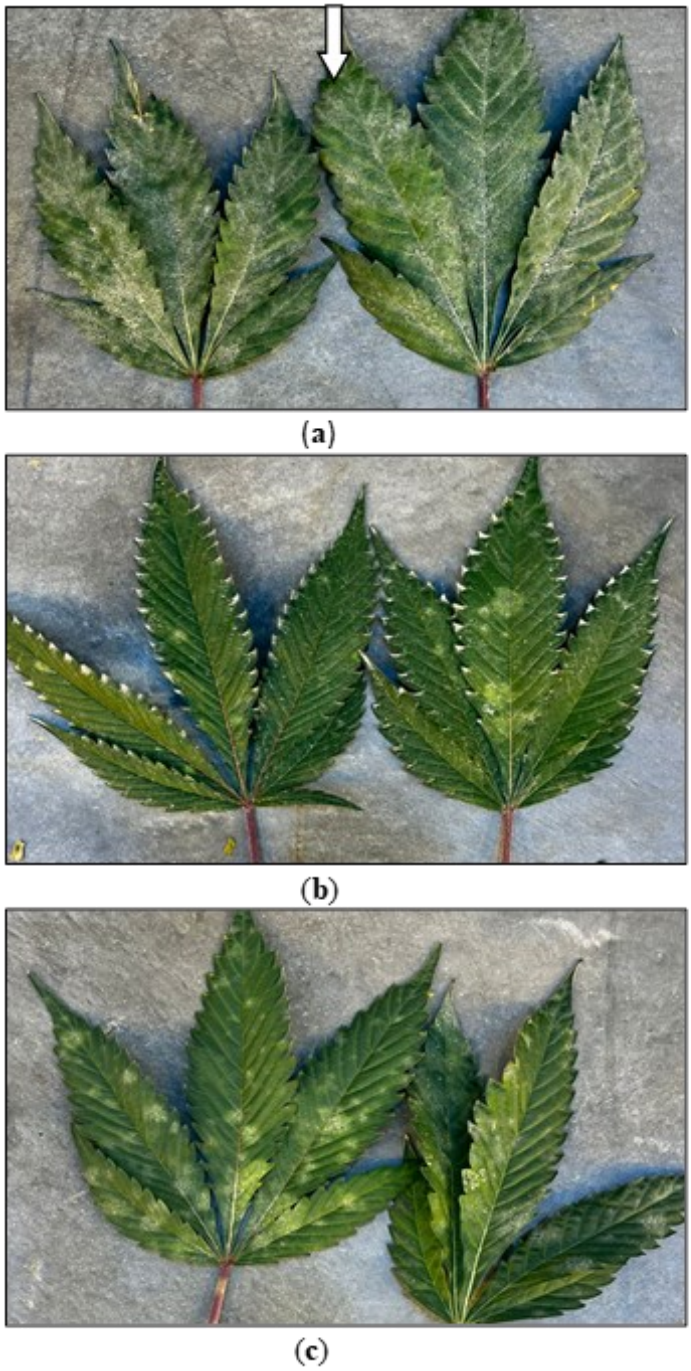


Figure 22. The effect of Rootshield HC® applications on the development of powdery mildew. Three weekly applications were made to the foliage of flowering plants as preventative treatments and compared to an untreated control and a water control. (A) Untreated control leaves. (B) Rootshield HC® treated leaves. (C) Water treated leaves.

2.10.4. Application of Reduced-Risk Products

A number of reduced-risk products are available for use on cannabis plants at the flowering stage. During this phase of crop development, care must be taken to avoid damage to inflorescence tissues and to avoid visual quality changes. Some of the products for reducing powdery mildew, include Agrotek vaporized sulfur®, Regalia Maxx®, Suffoil-X®, and Milstop® (Scott & Punja, 2020). Sulfur is applied via vaporizing pots, a method that ensures uniform dispersal and is commonly used on many other greenhouse crops (Konstantinidou et al., 2022), while the remainder of products are applied as sprays. In a comparative study to evaluate these and other products for powdery mildew control on flowering cannabis plants, nine products were applied thrice at days 0, 7, and 14 of the flowering period (~60 mL per plant) during the spring season on 'MP', a susceptible cannabis genotype, prior to disease appearance. Subsequently, disease severity was rated visually using a leaf infection coverage scale as follows: 0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100% (Figure 23A–D). Results showed that Suffoil-X® applied at a rate of 10 mL/L and Regalia Maxx® applied at a rate of 2.5 mL/L were the best preventative products (Figure 23E). In a subsequent trial with the same genotype, flowering plants visibly infected with powdery mildew (disease rating of 1) received one application of seven products at their maximum label rates made at day 42 of the flowering period to evaluate their curative potential. The findings showed that Milstop® applied at a rate of 3 g/L and Cyclone® applied at a rate of 12 mL/L were the best products for curative treatments (Figure 23F). The remaining products provided varying levels of disease reduction. No phytotoxicity was observed in any of the treatments. The active ingredients in Milstop® (potassium bicarbonate), Cyclone® (citric and lactic acid), and Suffoil-X® (mineral oil) are all considered to be 'physical' in their mode of action, altering either leaf surface pH and osmotic pressure or the desiccating/coating mycelium and spores (Punja & Scott, 2022). The active ingredient in Regalia Maxx® is an extract from the giant knotweed *Reynoutria sachalinensis* and was shown to be effective against pathogens such as *B. cinerea* and powdery mildew on cannabis as well as on various other crops (Konstantinidou-Doltsinis, 1998; Avila-Adame et al., 2008; Esquivel-Cervantes et al., 2022; Margaritopoulou et al., 2020). This product enhances plant defense responses through the salicylic acid (SA)-dependent pathway by inducing the accumulation of plant defense chemicals such as hydrogen peroxide and the formation of mechanical plant defenses such as callose papillae (Margaritopoulou et

al., 2020; Abdu-Allah & Abo-Elyousr, 2017). Additional research is needed to explore the extent to which Regalia Maxx® can control other pathogens and the duration of the protection offered following application.

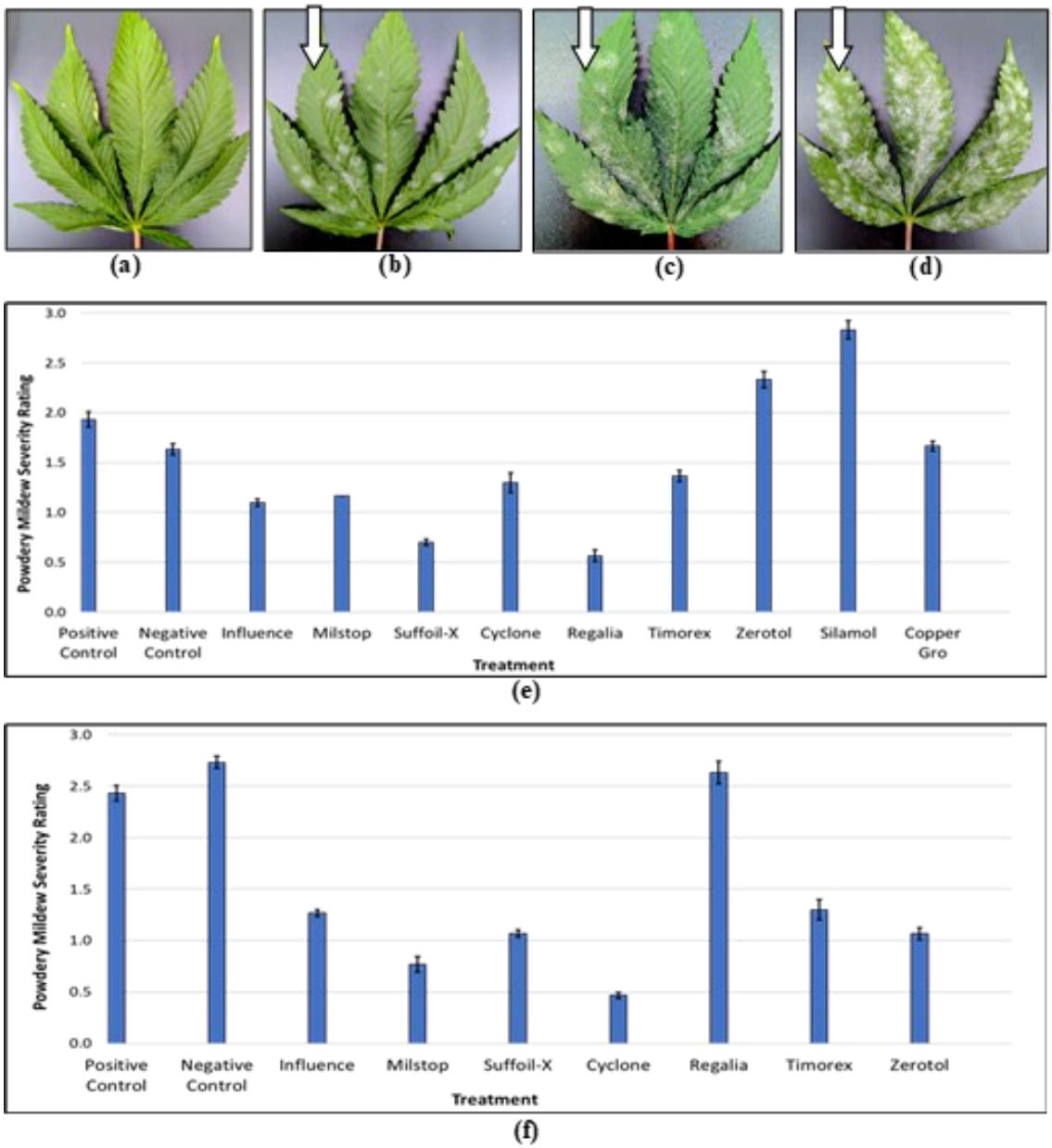


Figure 23. The comparative efficacy of reduced-risk products at managing powdery mildew development on cannabis genotype 'MP'(A-D). Disease was rated according to the scale shown, from 0 (A) to 3 (D). (E) Products were applied as preventative treatments at days 0, 7, and 14 of the flowering period. (F) Products were applied as a curative treatment, once at day 42 of the flowering period, after the onset of disease development. The trials were conducted during the spring growing season.

A summary of the IDM approaches that can be used against four important pathogens of cannabis is provided in Table 1.

Table 1. Summary of IDM strategies for four important pathogens affecting cannabis plants.

	HLVd Stunting Disease	**Fusarium/Pythium Root & Crown Rot**	**Botrytis Bud Rot**	**Powdery Mildew**
Prevention	Test propagative materials and stock plants; utilize pathogen-free planting materials.	Test propagative materials and stock plants; utilize pathogen-free planting materials.	Reduce canopy humidity by adjusting planting density and enhancing air circulation.	Maintain an even climate, above 21°C, and vaporize sulfur nightly.
Sanitation	Clean equipment and bench surfaces; destroy diseased plants.	Clean equipment and bench surfaces; actively remove dead or diseased tissues.	Fog growing environment with reduced risk products prior to planting.	Fog growing environment with reduced risk products prior to planting.
Protection	Isolate propagative materials and stock plants in controlled access areas.	Apply *Trichoderma harzianum* and *Gliocladium cantenulatum* as a drench to rooted cuttings and plants.	Apply Rootshield HC® on developing inflorescences, from day 14 to day 28 of flowering.	Preventatively spray reduced risk products such as Suffoil-X, Regalia Maxx, on susceptible genotypes.
Monitoring	Scout regularly for symptoms; routinely sample water and suspect plants.	Scout regularly for symptoms; routinely sample water and suspect plants.	Conduct daily scouting for bud rot from the sixth week of flowering onwards.	Conduct weekly scouting at all plant development stages.
Eradication	Immediately remove and safely dispose of diseased plants at all stages of growth.	Immediately remove and safely dispose of diseased plants at all stages of growth.	Remove and dispose of infected inflorescences; perform post-drying bud rot severity checks.	Remove and dispose of infected leaves; spot spray with reduced risk products.
Genotype Selection	Avoid highly susceptible genotypes; evaluate tolerant genotypes.	Avoid highly susceptible genotypes; evaluate tolerant genotypes.	Avoid planting highly susceptible genotypes during *Botrytis*-prone periods; evaluate tolerant genotypes.	Avoid highly susceptible genotypes; evaluate tolerant genotypes.

2.11. Post-Harvest IDM Approaches

Following the harvest of cannabis inflorescences, they undergo a phase of drying to reduce the moisture content to levels designed to minimize the development of microbes (Punja et al., 2023; Gwinn et al., 2023; Caplan et al., 2022), following which they are trimmed and prepared for packaging and stored prior to shipment. During each of the post-harvest processing stages, there is the potential for microbial contamination to be increased, which is measured in total yeasts and molds (TYM), total aerobic microbial count (TAMC), and bile-tolerant Gram-negative count (BTGN). Some of these microbes likely originated from the original freshly harvested inflorescences while in the greenhouse or otherwise may have been picked up through contamination during harvesting and post-harvest processing stages. Detailed studies are lacking regarding at which specific stages the levels of microbes build up to cause the final product to potentially fail to meet regulatory standards. However, pre-harvest, it has been shown that cannabis genotype and growing conditions can significantly influence TYM build-up; in addition, post-harvest drying methods and handling practices can affect TYM levels (Punja et al., 2023; Gwinn et al., 2023; Caplan et al., 2022). A number of commonly encountered fungi have been identified on dried cannabis products pre- and post-harvest (Figure 16), and they contribute to TYM levels (Punja et al., 2023).

The implementation of integrated disease management (IDM) approaches to reduce total yeast and mold (TYM) is complicated by several pre-harvest variables. For example, TYM levels tend to be higher in the summer season than in winter, while certain cannabis genotypes tend to accumulate much higher TYM than others (Punja et al., 2023). Post-harvest handling practices also influence TYM levels (*given that* hang-dried inflorescences have lower TYM than those that are rack-dried) (Punja et al., 2023). Managing these factors to minimize microbial build-up depends on the appropriate use of IDM strategies that were previously outlined for stock plants *as well as those that apply to* propagation, vegetative growth, and flowering. Post-harvest processing practices, such as reducing moisture by hang-drying plants at a high vapour pressure deficit (VPD) and trimming only after inflorescences are dried, along with thorough cleaning of post-harvest processing equipment using sanitizing agents, can significantly reduce microbial load on inflorescences. Additionally, conducting detailed inspections at each stage of post-harvest processing to detect the presence of molds is critical. This may involve various standard practices, including predefined in-process acceptable

quality level (AQL) checks, to ensure that any quality issues are identified and addressed prior to shipment, as is commonly carried out in many food processing plants (International Commission on Microbiological Specifications for Foods, 2018).

The irradiation of cannabis products with gamma and electron beams has been shown to be an effective option for producers. This method can be used to sterilize commercial batches of inflorescences without major changes in quality, but they are costly (Hazekamp, 2016; Jerushalmi et al., 2020b; Majumdar et al., 2023). Irradiation is typically used in cases where microbial levels have exceeded regulatory limits or where a zero tolerance is recommended, e.g. for medical patients with immunocompromised immune systems that rely on cannabis (Gwinn et al., 2023). Other approaches have been described that require more in-depth studies to demonstrate their commercial utility (Dhillon et al., 2022; Frink et al., 2022). A summary of the various approaches that can be implemented as a part of an IDM program for greenhouse-cultivated cannabis is presented in Figure 24. These are organized according to the growth stages of the cannabis crop. These approaches can be readily implemented, and examples of their successful use have been included in this review. Additional potential IDM approaches for cannabis that require further research, but which have shown potential in other crops, are described below.

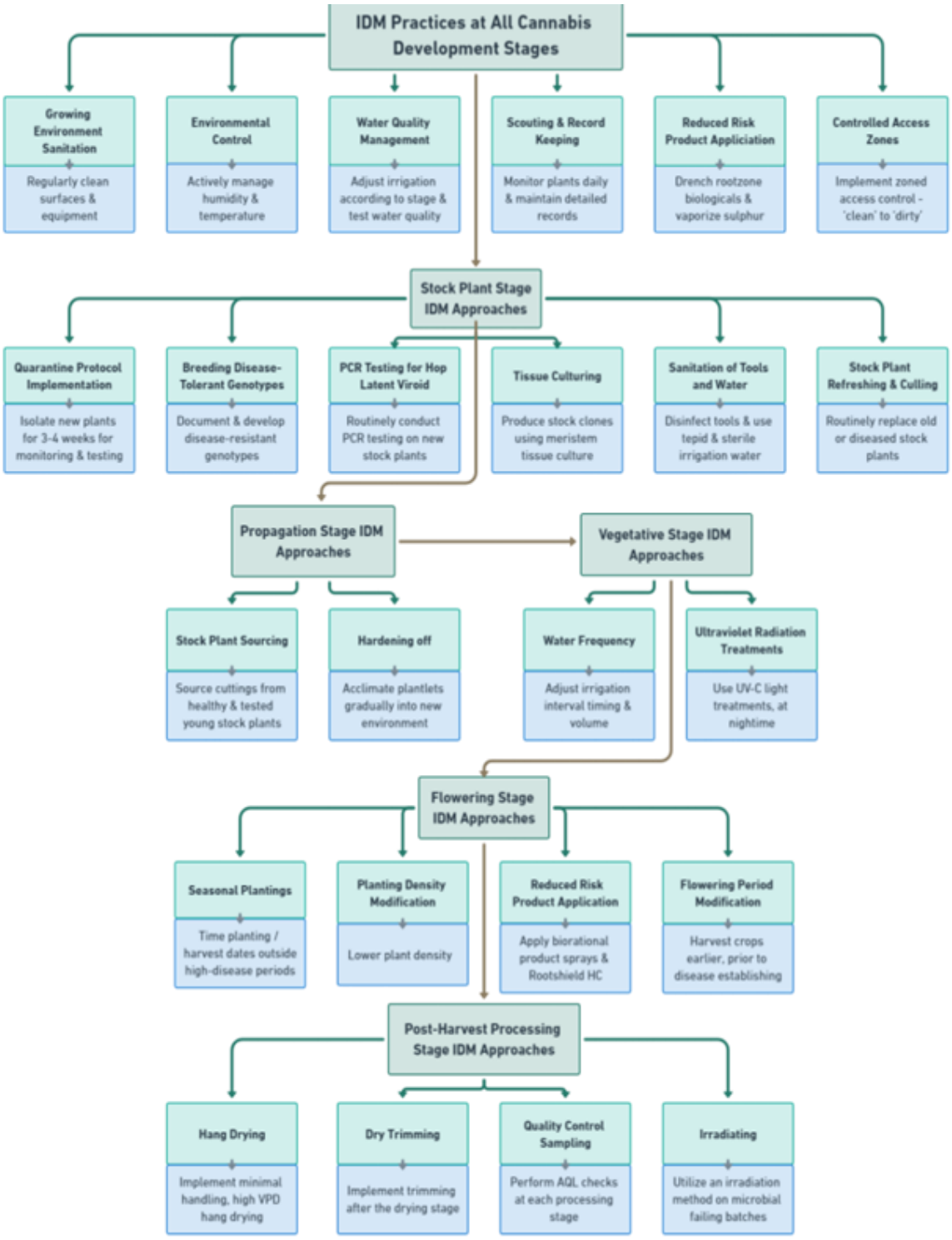


Figure 24. An operational flow chart for various IDM approaches that can be incorporated into an IDM program according to cannabis cultivation stage.

2.12. Future Potential Areas for IDM Development for Cannabis

2.12.1. Evaluation of Endophytes and Microbial Antagonists in Cannabis

Endophytes, consisting primarily of fungal and bacterial species, are present within various tissues and organs of cannabis and hemp plants and vary in species composition, depending on the tissue source, such as roots, stems, petioles, leaves, flowers, and seeds (Dumigan & Deyholos, 2022; Scott & Punja, 2023; Gautam et al., 2013; Kusari et al., 2013; Taghinasab & Jabaji, 2020). Various plant growth-promoting rhizobacteria, including species of *Bacillus* and *Pseudomonas*, have also been reported to be present in the roots of cannabis plants and can inhibit the growth of root pathogens (Scott & Punja, 2023; Balthazar et al., 2022). These endophytes can potentially improve plant growth and development (Lyu et al., 2019; Comeau et al., 2021), although research evaluating their growth benefits in cannabis and hemp plants is currently lacking. Dumigan and Deyholos (Dumigan & Deyholos, 2022) reported that seed-borne bacterial endophytes, including *Bacillus subtilis* and *Bacillus inaquosorum*, showed inhibitory activity in dual culture assays against fungal pathogens, including *Alternaria* and *Fusarium* species. These endophytes were also present in hemp seeds and included *Bacillus velezensis* and *Paenibacillus polymyxa*, which were also inhibitory to the growth of *Alternaria, Aspergillus, Fusarium, and Penicillium* species in vitro. *Pseudomonas* species have also shown growth inhibition of *Fusarium* species in vitro (Scott et al., 2018). In previous research, antagonism to *B. cinerea* in dual culture assays was demonstrated for several cannabis-derived endophytes (*Paecilomyces lilacinus* and *Penicillium* spp.) (Kusari et al., 2013) and for several hemp-derived endophytes (*Pseudomonas fulva* and *Pseudomonas orientalis*) (Scott et al., 2018). In a study conducted by Gabriele et al. [81] investigating the endophytes present in seeds and young plants of a cannabis cultivar, a unique resistance to the plant's own antimicrobial compounds was discovered, along with an enhancement of nutraceutical aspects such as polyphenol content and antioxidant activity in the plants. This finding suggests the potential for introducing these endophytes as natural biostimulants and biological control agents against pathogenic microbes, unhindered by the plant's inherent antimicrobial properties. Such symbiotic relationships underscore the potential of endophytes in cannabis cultivation, but further research is needed to establish their

potential applications. The antagonistic properties of endophytic bacteria have been attributed to antibiotic production, host defense response induction, growth promotion, competition, parasitism, and quorum signal interference (Kusari et al., 2014; Eljounaidi et al., 2016; Whipps, 2001; Fadiji & Babalola, 2020). Despite these promising studies, however, whole plant assays demonstrating the benefits of these bacteria and other fungal endophytes are presently lacking for cannabis. It should be noted that fungal endophytes can also be present in stem tissues of mother plants, including those shown in Figure 25, and they could negatively impact the health of these plants over time and complicate attempts to initiate tissue cultures using explants from these plants (Holmes et al., 2021). The inoculation of exposed cut surfaces on stems on cannabis plants with these endophytic fungi showed that species of *Fusarium, Penicillium* and *Trichoderma* rapidly colonized the tissues internally and were recovered at distances away from the point of inoculation (Punja et al., 2019). Fungal endophytes that are consistently present in stems of cannabis plants include species of *Penicillium* and *Chaetomium*, as well as others (Figure 25). Bacterial endophytes include species of *Bacillus* and *Pseudomonas* (Holmes et al., 2021). Although commonly recovered from different genotypes of cannabis grown under commercial conditions, the influence of genotype on the frequency of occurrence of these endophytes is unknown. Similarly, the impact of growing conditions, including the substrate used for plant growth, on these internalized microbes has not been determined. Under experimental conditions, the application of a systemic fungicide to growing cannabis plants was shown to reduce the frequency of occurrence of fungal endophytes (Holmes et al., 2021). This approach was used to reduce the occurrence of endophytic microbes that were encountered as contaminants in tissue culture experiments (Holmes et al., 2021).

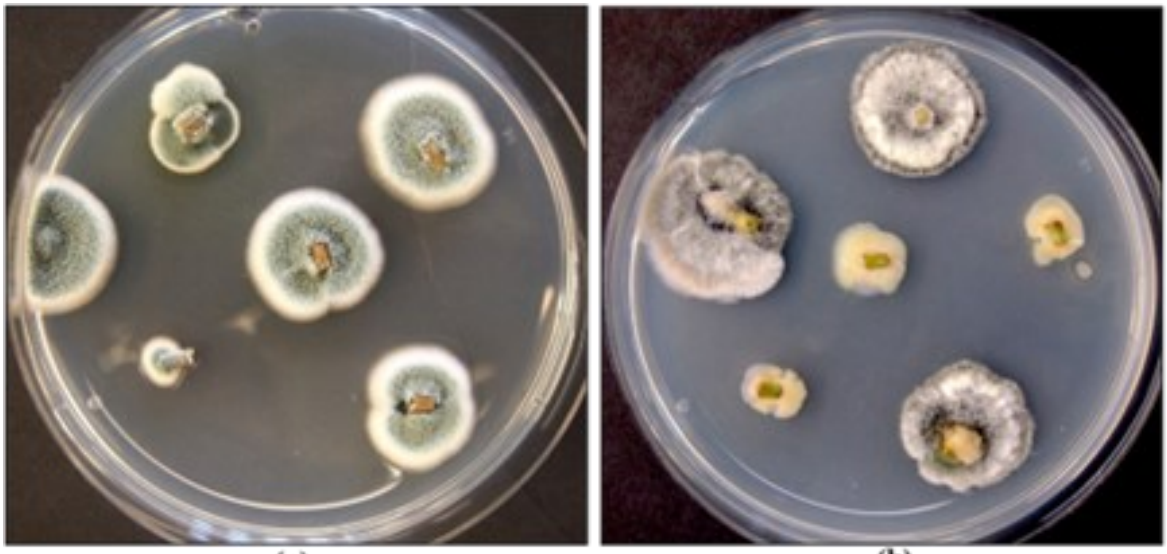


Figure 25. Examples of endophytic fungal and bacterial species recovered from cannabis stem segments following sterilization. In the left Petri dish are *Penicillium* species and in the right are *Chaetomium* and bacterial species.

Another aspect of potential microbial antagonism against fungal pathogens infecting cannabis that requires research is the presence of diverse microflora that develop in organic soils compared to conventional hydroponic cultivation. Punja and Scott (Punja & Scott, 2023) reported that a diverse range of microbes were recovered from cannabis inflorescences grown in organic soil compared to the cocofibre medium commonly used in hydroponic cannabis production. These communities were comprised of pathogenic, saprophytic, and beneficial microbes. Among the beneficial microbes detected, *Trichoderma harzianum* and *Metharzium anisopliae* are currently used as biological control agents for root disease suppression and insect suppression, respectively. *M. anisopliae* may hold some potential for cannabis pathogen suppression as well (Gupta et al., 2022). In the context of disease management, similar microbes that originate from organic soils and that exhibit general antagonistic properties, such as mycoparasitism, host defense response induction, competition, and antibiotic production, are worthy of evaluation (Fadiji & Babalola, 2020; Gupta et al., 2022; Busby et al., 2016; De Silva et al., 2019). Cannabis plants grown in ‘living soil’ or growing media amended with ‘compost teas’ may foster greater colonization of roots by these beneficial endophytes, although more research is needed to demonstrate their utility in an IDM program. It is likely that many of these microbes comprise a range of bacterial species.

Caution should be exercised to ensure these microbes do not colonize the inflorescences internally or externally, potentially leading to a failure of the product due to an excessive buildup of microbes.

2.12.2. Tissue Culture Applications for Cannabis

There has been increasing interest in the tissue culture of cannabis because producers are in search of a source of clean plant materials that are free of pathogenic microbes, as well as fungi, viruses, and viroids. Detailed methods have been described from several laboratories (Jones & Monthony, 2022; Holmes et al., 2021; Adhikary et al., 2021; Monthony et al., 2021). Cannabis producers are interested in utilizing tissue culture methods in order to obtain pathogen-free plants and minimize pathogen re-introduction into commercial production facilities. This is particularly relevant in the context of hop latent viroid, which is known to be spread through vegetative cuttings taken from infected mother plants (Punja et al., 2024). Meristem tip culture technology has been used for many decades to eliminate the potential for virus introduction in other vegetatively propagated crops, such as potatoes, bananas, and strawberries (Slack & Tufford, 1995; Rao, 2014; Sharma et al., 2019). Meristem and shoot-tip culture techniques have been utilized not only for virus elimination but also for rapid clonal multiplication and germplasm preservation of many vegetatively propagated crops (Nehra & Kartha, 1994; De Jesús Romo-Paz et al., 2021). In some cases, these methods are augmented with cryotherapy (cold treatment), thermotherapy (heat treatment), chemotherapy (anti-viral chemical treatment), electrotherapy (electrical current treatment), and shoot-tip grafting (micrografting technique) to enhance the chances of obtaining pathogen-free planting materials (Zapata et al., 1995; Bhojwani & Dantu, 2013; Wang et al. 2008; Singh et al., 2022; Kanwar et al., 2019). Research to evaluate the applicability of these methods in the search for pathogen-free planting materials, particularly for HLVd, is still in the early stages of evaluation and development. Tissue culture-derived plants can be obtained from meristems and nodal explants of cannabis, resulting in the shoot growth of a number of genotypes in vitro (Figure 26). However, confirmation of the eradication of pathogens of importance requires additional research. Hence, while tissue culture approaches hold promise for potential inclusion in an IDM program for cannabis, more effort to generate high frequencies of plants confirmed to be pathogen-free on an economically feasible scale is needed. The

confirmation of pathogen-free planting materials could be utilized for certification programs for cannabis, similar to many agriculturally important crops.

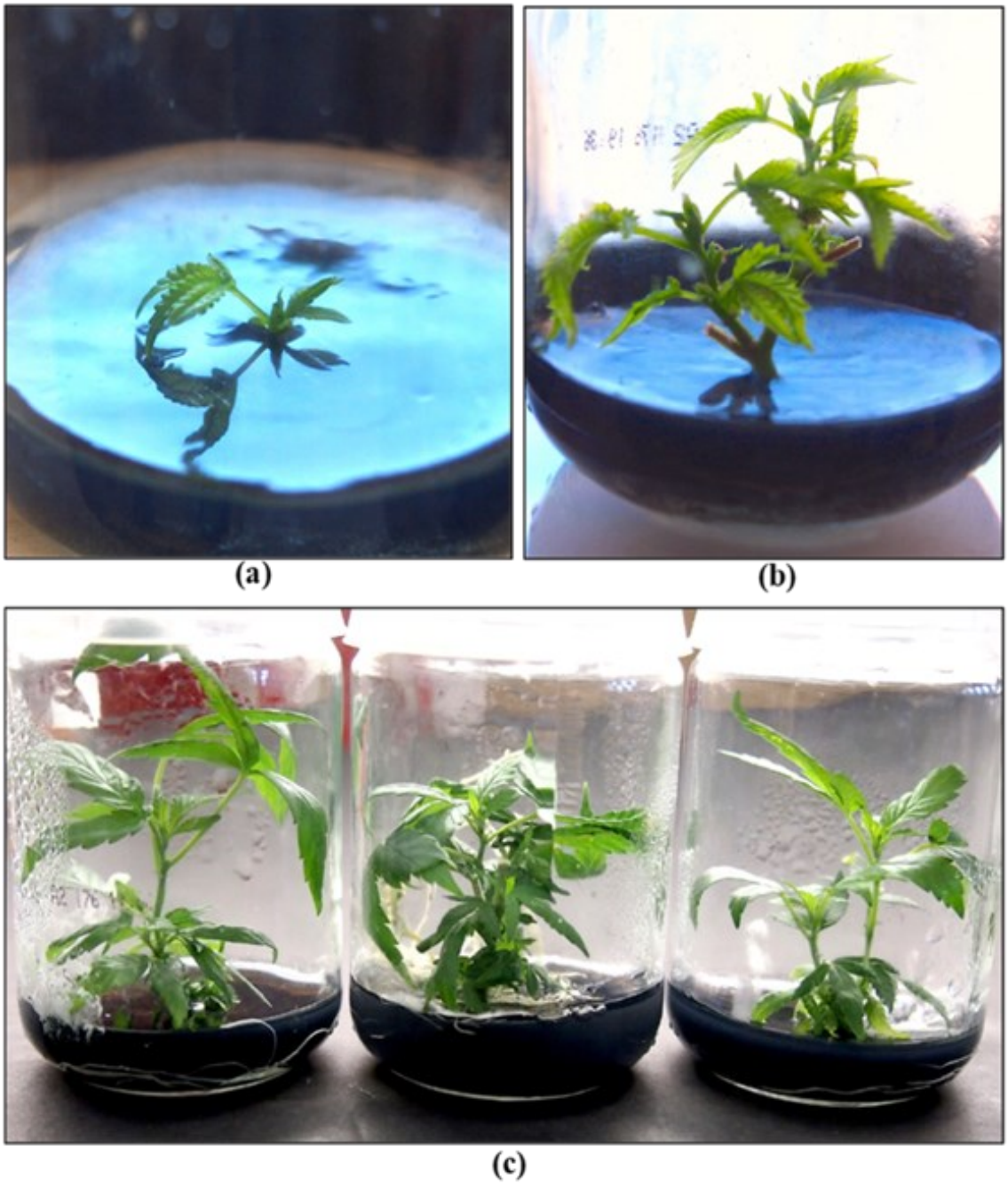


Figure 26. Tissue-culture derived plants of cannabis can be obtained from meristem tips. (A) and nodal explants. (B), resulting in the growth of a number of genotypes. (C) The feasibility of generating large-scale production of pathogen-free planting materials awaits further research and development.

2.12.3. Registration of Pathogen Control Products for Cannabis

To evaluate new products aimed at managing fungal pathogens in agricultural crops, screening for pathogen growth inhibition is an important first step. For example, effective concentration (EC50) values are determined to establish fungicide levels needed to inhibit 50% of the pathogen's growth in vitro. However, such studies are less commonly reported for products intended for use on cannabis. EC50 studies, which are relatively straightforward to conduct, as demonstrated in Figure 7, provide key information about the potential of new products to inhibit the growth of specific pathogens affecting cannabis, especially when followed by whole plant assays. These studies can also determine if there are any secondary effects on biocontrol fungi, such as *Trichoderma* spp. (Figure 7). Recent evaluations of products for powdery mildew control in organic hemp production (Akinrinlola & Hansen, 2023) serve to identify products that may be acceptable for registration in cannabis. Such products could be utilized for pathogen control at the stock plant and propagation stages, which is critical to ensure that the subsequent vegetative and flowering stages do not carry over the pathogen inoculum and to reduce concerns about product residues in the finished flower. Currently, the majority of products registered for use on cannabis can be applied up until harvest, as outlined by Scott et al. (Scott et al., 2021). Several commercial products have since been added to the registered list that are not included in (Scott et al., 2021). For soil fumigation, Pic Plus Fumigant® (Chloropicrin—85.1%), Chloropicrin 100 Liquid Soil Fumigant® (Chloropicrin—85.1%), and Mustgrow Crop Biofumigant® (Oriental Mustard Seed Meal—100%) can be used pre-plant. For powdery mildew suppression, Vegol Crop Oil® (Canola Oil—96%), Suffoil-X® (Mineral Oil—80%), Purespray FX (Mineral Oil—80%), and General Hydroponics Suffocoat (Canola Oil—96%) can be applied as foliar sprays. For suppression of *B. cinerea*, powdery mildew, and *Sclerotinia sclerotiorum*, Timorex Gold® (Tea Tree Oil—23.8%) can be used. For *Phytophthora* spp. and *Verticillium* dahlia suppression, Foretryx® (*Trichoderma asperellum* strain ICC 012 and *Trichoderma gamsii* strain ICC 080) can be used. While many of these products are different formulations of the same active ingredient, unique products have been added each year since the legalization of cannabis production in Canada. A current list of registered products can be found at Health Canada—Pesticide Label Search (https://pr-rp.hc-sc.gc.ca/ls-re/index-eng.php).

2.12.4. Nutrient Supplements for Cannabis Disease Suppression

Nutrient amendments have been shown to impact plant susceptibility to infection by a range of pathogens, often reducing disease development through various mechanisms. These involve a wide range of macronutrients and micronutrients. In hydroponic greenhouse cultivation, nutrient levels are carefully monitored to prevent deficiencies; thus, additional nutrient supplements must be approached cautiously to avoid phytotoxicity or imbalances. Formulations and rates are critical factors when considering using these nutrient amendments for disease management (Tripathi et al., 2022; Datnoff et al., 2023). The use of nutrient supplements containing copper, silicon, and calcium shows particular promise for cannabis and can be applied via the roots or foliage, as discussed below.

Copper has a long history of use as a bactericide and fungicide for various crops against numerous pathogens since the discovery of the 'Bordeaux mixture' in 1885 utilized to prevent grapevine mildew infections. Copper disrupts fungal cell membrane integrity and interferes with key enzyme activities, thereby inhibiting pathogen growth and survival (Lamichhane et al., 2018; Flemming & Trevors, 1989). Copper can be applied to cannabis plants as root zone drenches, foliar sprays, or seed treatments. For instance, Mayton et al. (Mayton et al., 2022) assessed different seed treatments to manage damping-off caused by *Pythium* and *Fusarium* spp. on industrial hemp. Seeds treated with a copper-containing product, Ultim® at 0.05 mg Cu/seed, showed efficacy that was comparable to fungicide treatments. Moreover, copper nanoparticles have been successfully applied as dips and foliar treatments on tomatoes and watermelons to reduce Fusarium infection (Borgatta et al., 2018; Shen et al., 2020). A copper formulation, Copper CropTM, reduced powdery mildew on melons (Freires et al., 2022). This suppression aligns with the conventional use of copper sulfate pentahydrate as a foliar fungicide on plant species such as roses and dogwood (Newman et al., 1999; Mmbaga & Sauve, 2004). On grapevines, copper citrate effectively reduced *B. cinerea* infections (Aleksic et al., 2019). The diverse range of pathogens suppressed by copper formulations suggests its potential for use in cannabis; however, copper is not currently registered for this purpose.

Silicon is effective against various bacterial, fungal, and viral pathogens, since it can strengthen cell walls via silicon deposits and also induce plant defense responses (Sakr, 2016; Islam et al., 2020). Scott and Punja (Scott & Punja 2020) reported that

multiple weekly sprays of potassium silicate, Silamol®, on vegetative cannabis plants significantly reduced powdery mildew development. In contrast, three preventative applications made during the flowering stage to a single genotype showed no effect in the present study (see Figure 23). Akinrinlola et al. (Akinrinlola & Hansen, 2023) reported that Sil-Matrix®, a fungicide with potassium silicate, significantly reduced hemp powdery mildew by 88%. Dixon et al. (Dixon et al., 2022) demonstrated that root-applied silicon at a rate of 600 kg/ha significantly reduced powdery mildew severity in hemp. Similar benefits of silicon supplementation have been observed in crops such as cucumbers, roses, and strawberries (Samuels et al., 1991; Liang et al., 2005; Shetty et al., 2021; Shetty et al., 2012; Kanto et al., 2004; Liu et al., 2020). Further assessments of silicon-containing products for use in cannabis are needed to establish rates and times of application and to demonstrate efficacy against pathogens affecting greenhouse crops.

Calcium application has been shown to reduce pathogen infection by strengthening plant cell walls, thereby providing greater structural integrity against fungal and bacterial infections (Reekie & Punja, 2023). However, its effectiveness in reducing the pathogens that affect cannabis has not been studied. There are no reports of a direct toxic effect of calcium-containing compounds on fungal pathogens affecting cannabis, suggesting that their action may stem from its reduction of host susceptibility or another unkown mechanisms. In some crops, root-zone supplementation of calcium nitrate was reported to reduce *B. cinerea* severity in beans and tomatoes, although higher doses increased disease in beans (Elad & Volpin, 1993). Supplementing roses with calcium nitrate and adding calcium chloride or calcium sulfate to solutions for harvested flowers reduced *B. cinerea* incidence under conducive disease conditions (Volpin & Elad, 1991). Similarly, increasing calcium and reducing nitrogen levels in the irrigation water for sweet basil plants reduced both sporulation and the infection severity of *B. cinerea* (Yermiyahu et al., 2006). Whether enhanced calcium supplementation can influence the development of B. cinerea in cannabis plants remains to be determined. In addition, its potential for reducing infection by root pathogens such as *Fusarium* and *Pythium* should be explored.

2.12.5. Artificial Intelligence (AI) Technologies for Cannabis Disease Detection

The use of recently developed robotic and imaging technologies to scouting for disease presence has garnered interest from cannabis producers. Various options are available with pros and cons, depending on the greenhouse scale, layout, and operations system. For small-scale greenhouses, fixed-crop monitoring cameras or AI-powered phone scouting apps are often utilized. Several cannabis-focused scouting apps include Koppert's Natutec Scout app (https://www.koppert.com/natutec-scout/) (accessed on 22 January 2024), BioBest's Crop-scannerTM app (https://www.crop-scanner.com/) (accessed on 22 January 2024), GrowDoc AI's app (https://growdoc.ai/) (accessed on 22 January 2024), and the IPM ScoutekTM app (https://ipmscoutek.com/) (accessed on 22 January 2024). In contrast, larger greenhouse operations, with a more consistent layout, have trialled autonomous robotic scouting carts or booms with cameras attached to crop carts, such as IUNU's LUNA AI scouting above-crop cameras or scouting carts (https://iunu.com/luna-ai) (accessed on 22 January 2024), Ecoation's OKO or ROYA scouting carts (https://www.ecoation.com/integrated-pest-management) (accessed on 22 January 2024), and Budscout AI's Budscout above-crop cameras (https://budscout.ai/budscout/) (accessed on 22 January 2024). The current challenge for AI and imaging solutions currently is that they may not reliably distinguish between symptoms and those caused by various pathogens and those caused from nutrient deficiencies and other environmental stressors. Supplemental and tailored training are likely needed to achieve accurate results. In the broader agricultural sector, significant progress is being made in the robot AI-assisted vision space (Fountas et al., 2022). An example of the training process and customizability of AI scouting technology is demonstrated in the following two studies. Anagnostis et al. (Anagnostis et al., 2021) aimed to build a fast and accurate object detection system to identify anthracnose-infected leaves (*Colletotrichum* spp.) in a commercial walnut orchard. The study involved segmenting high-resolution images into smaller sub-images and training an object detector to recognize disease-specific features. This deep learning approach achieved high accuracy under real-field conditions. Similarly, Mahmud et al. (Mahmud et al., 2020) focused on developing an innovative machine vision system to accurately detect powdery mildew in strawberry fields. This system utilized real-time image processing and artificial neural networks (ANNs) to distinguish between diseased leaves and

healthy ones. The study demonstrated the system's adaptability to field conditions and showed high accuracy in detecting powdery mildew. These examples point to the potential for applications in integrated disease management and early disease intervention in different agricultural settings.

2.12.6. Infrared (IR) Technologies for Cannabis Disease Detection

Infrared imaging (IR), a spectrum used in some remote sensing technologies, identifies variations in crop or leaf temperatures to reflect reduced transpirational activity or metabolic functions, signaling the potential presence of stressors, including disease (Sankaran et al., 2010; Shakeel et al., 2022; Traversari et al., 2021). In cannabis, leaf surface temperature changes due to root diseases or poor root development when tested at different developmental stages can be detected with handheld devices such as a FLIR E8 ProTM infrared camera (https://www.flir.ca/products/e8-pro/?vertical=condition+monitoring&segment=solutions) (accessed on 10 January 2024). This method showed definitively that poorly developed root systems on affected plants were correlated directly with a reduced rate of transpiration and, hence, a build-up of leaf surface temperature that was detectable with the IR camera (Supplementary Figure B.1.). However, when powdery mildew-infected plants or cannabis plants affected by hop latent viroid were similarly compared to healthy plants using an IR camera, these plants did not show a corresponding reduced transpirational activity pattern, suggesting that the IR camera was unable to detect physiological changes in these diseased plants. It is unknown whether infrared or other spectrums could be used to effectively detect hop latent viroid; limited research has been carried out on virus detection with infrared, but there may be potential applications (Berdugo et al., 2014; Xu et al., 2006). Vagelas et al. (Vagelas et al., 2021) utilized a low-cost infrared camera and a standard RGB web camera to analyze vine, chrysanthemum, and rose leaves that had been infected with various fungi. Results showed that infected leaves exhibited temperature deviations from uninfected ones, which occurred before visible symptoms developed. Specifically, infected vine and rose leaves showed a decrease in temperature, while chrysanthemum and another set of rose leaves demonstrated an increase, compared to healthy tissue. Lindenthal et al. (Lindenthal et al., 2005) used infrared thermography to detect downy mildew infection in cucumbers. In controlled environments, the study showed that the maximum temperature difference in a leaf could be used to distinguish between healthy

and infected tissues. Under natural environments, while leaf temperatures and transpiration rates were similar in both healthy and infected plants, diseased leaves showed more varied transpiration rates depending on the severity of the symptoms. Liaghat et al. (Liaghat et al., 2014) utilized Fourier transform infrared (FT-IR) spectroscopy to detect *Ganoderma* infections in oil palm trees. This method involved analyzing leaf samples from both healthy and infected trees, examining the infrared spectra of these samples, and using a statistical model for classification. The researchers successfully identified differences linked to the disease, accurately identifying infected trees at early, symptomless stages. Therefore, there is a growing body of evidence to demonstrate that IR approaches could be applied to cannabis for early detection of infection by foliar pathogens, but additional studies are required to validate this approach.

2.12.7. Electronic Nose Technologies for Cannabis Disease Detection

The use of “electronic nose systems” (e-nose systems), also known as electronic olfactory systems, for the early detection and diagnosis of plant diseases across various crops is receiving increased attention. This technology involves multiple sensors that are sensitive to a variety of volatile compounds that generate electrical signals upon exposure to these molecules, which are then digitized and analyzed using machine learning-based pattern recognition algorithms. By leveraging the unique volatile organic compound (VOC) signatures emitted by plants under disease stress, the technology compares detected patterns to known odour profiles to identify the presence and intensity of diseases in an air sample. This approach has demonstrated potential for the early detection and diagnosis of plant diseases across various crops, providing a rapid, non-invasive, and field-deployable solution (Sankaran et al., 2010; Wilson, 2018; Mohammad-Razdari et al., 2022). However, despite its advantages in non-destructive and bulk sampling, e-nose technology is considered less sensitive and specific than traditional diagnostics like PCR, suggesting it is currently best suited as a supplementary tool in an IDM diagnostics program rather than a stand-alone solution (Cellini et al., 2017). Numerous proof-of-concept studies have applied different methodologies to detect specific pathogen-induced VOC signatures. The Bloodhound® ST214′s efficacy in detecting disease presence by analyzing VOCs emitted by tomato plants infected with powdery mildew (*Oidium neolycopersici*) in greenhouse settings

compared to healthy controls was demonstrated (Laothawornkitkul et al., 2008). Similarly, a low-cost, portable e-nose combined with machine learning algorithms was used to accurately detect *Fusarium oxysporum* in tomato plants and soil samples (Feng et al., 2022), while Sun et al. (Sun et al., 2018) were successful in detecting *B. cinerea* infection in tomatoes. A PEN 3, Win Muster Air-sense Analytics E-nose was used to identify infections caused by three fungi—*Botrytis* spp., *Penicillium* spp., and *Rhizopus* spp.—in strawberries (Pan et al., 2014). A custom-built e-nose device was also used for early detection of fungal infections in garlic, distinguishing between garlic infected by three different fungi (*Fusarium oxysporum, Alternaria embellisia,* and *Botrytis allii*) (Makarichian et al., 2022). Hazarika et al. (Hazarika et al., 2020) utilized the Alpha MOSFOX 3000 e-nose system to identify Khasi Mandarin orange plants infected by citrus tristeza virus (CTV) with high accuracy by analyzing essential oils extracted from leaves. These studies collectively highlight the versatility of e-nose systems in agricultural applications, underscoring their potential utility in diagnosing cannabis diseases. However, to date, there have been no studies to demonstrate whether pathogen-induced volatile compounds can be separated from the natural constituents present in healthy cannabis plants and whether this technology can be used for early disease prediction.

2.12.8. Induction of Plant Defense Responses in Cannabis

The potential for inducing plant defense responses before pathogen infection has not yet been developed to a practical level for cannabis. However, as discussed earlier, Regalia Maxx (*Reynoutria sachalinensis*), when applied to cannabis plants, can reduce pathogens such as B. cinerea and powdery mildew, confirming reports in the literature of its efficacy through presumed induction of defense responses and pathogen reduction (Konstantinidou-Doltsinis & Schmit, 1998; Avila-Adame et al., 2008; Esquivel-Cervantes et al., 2022; Margaritopoulou et al., 2020; Abdu-Allah & Abo-Elyousr, 2017). Weekly applications are recommended to ensure the ongoing protection of cannabis plants. The role of endophytic microbes in the growing medium in promoting plant health and reducing pathogen infection in cannabis awaits more research. Previous reports demonstrated the defense-boosting properties of endophytic organisms on various plant species (Kusari et al., 2014; Elijounaidi et al., 2016; Whipps, 2001; Fadiji & Babalola, 2020; Morelli et al., 2020). The utility of a biological control product containing

Trichoderma spp. appears to be promising when applied preventatively to the root zone or to inflorescences; however, additional research is necessary to establish whether the induction of defense responses in cannabis can be confirmed as it has been in previous studies on numerous plants (Sharma & Sharma, 2020; Guzmán-Guzmán et al., 2023). The application of compounds such as salicylic acid and jasmonic acid to cannabis plants to potentially induce disease resistance is also worthy of study.

2.13. Conclusion

This comprehensive review of integrated disease management (IDM) approaches for greenhouse-cultivated cannabis underscores the significance of developing a multifaceted approach to control the various pathogens of economic concern. The review highlights the importance of pre-emptive measures, including a selection of disease-tolerant genotypes and the use of stringent sanitation practices, in minimizing pathogen incidence. The utilization of biological control agents and reduced-risk products along with the modification of cultural and environmental conditions, have shown promising results in suppressing *B. cinerea* bud rot, powdery mildew, *Pythium* and *Fusarium* root diseases, and hop latent viroid-causing stunt disease. Moreover, the exploration into alternative strategies, including the utility of endophytes, tissue culture, nutrient supplementation, and technology-aided scouting, offers potentially new avenues for enhancing plant health. This review underscores the need for studies on plant defense response induction and modes of action of biological control agents. It shows the dynamic nature of IDM in cannabis cultivation and emphasizes the continuing need for research and for the adoption of sustainable strategies to meet the evolving challenges in disease management within the greenhouse cannabis industry. Such strategies should receive support from governmental regulatory agencies to ensure they meet the criteria set forth by the appropriate jurisdictions. Flexibility in allowing additional disease management products to be registered for use by cannabis producers is essential to allow the industry to meet the continual challenges imposed by plant pathogens.

Chapter 3. Introduction to *Botrytis cinerea* and Bio-fungicide Application Best Practices

3.1. Brief History

Botrytis cinerea (grey mold), referred to as *B. cinerea* hereafter, has been affecting crops for hundreds of years. The earliest recorded instance of what was likely *B. cinerea*-induced rot dates back to 77 AD, when Pliny the Elder (Gaius Plinius Secundus) of the Roman Empire described the rot of cultivated grapes in his book *Natural History*. This fungal pathogen was formally named in 1729 by the Italian botanist Pier Antonio Micheli, who discovered fungal spores. Micheli combined the Greek word ‘bótrus,’ meaning 'a bunch of grape berries,' with ‘itis,’ a suffix indicating disease, to create the name *B. cinerea*. The species *Botrytis cinerea* was first described by the Swiss scientist Albrecht von Haller in his 1771 work, *Synopsis Methodica Fungorum* (Rosslenbroich & Stuebler, 2000).

3.2. Distribution Worldwide

B. cinerea is a globally distributed pathogen, thriving in a wide range of climates, from very cold to very hot environments. It can proliferate wherever host plants are cultivated, although the majority of infections occur in crops grown in temperate climate zones. *B. cinerea* infects a wide variety of plants, including 170 families containing 586 genera. Of these, 582 genera belong to the division *Magnoliophyta*, with the majority (419) being Eudicots, while the remaining genera consist of 114 Monocots and 4 unranked clades. Genera utilized for human and animal food or ornamental purposes exhibit the highest number of species affected by *B. cinerea*. The most commonly reported symptom in affected plant genera is rot, followed by blight, and in some cases, lesions (Elad et al., 2015). In cannabis, *B. cinerea* infections predominantly occur during the flowering stage, manifesting as bud rot. However, they can also be found during the propagative rooting stage of cuttings, though this is of far less economic significance (Mahmoud et al., 2023).

3.3. Economic Importance

There are 28 different *Botrytis* species of economic significance, but *B. cinerea* is the most impactful (Abbey et al., 2019). It is one of the most extensively researched plant pathogens globally, with estimates suggesting that it causes crop losses ranging from $10 to $100 billion annually (Watkinson et al, 2016). The significant economic damage caused by this opportunistic pathogen is largely due to its polyphagous nature, quiescent life stage, environmental adaptability, genetic plasticity, and lack of host resistance (Carisse, 2016). As a result, *B. cinerea* has been designated by many fungal pathologists as the second most economically important plant pathogen worldwide with suppression efforts costing the industry over $1 billion (Dean et al, 2012). This impact extends to cannabis, where *B. cinerea* infections in the flowers have caused substantial losses, partly due to the limited research on the *Cannabis-Botrytis* pathosystem and the lack of registered suppression products (Mahmoud et al., 2023).

3.4. Life Cycle

3.4.1. Classification and Reproductive Stages

B. cinerea has traditionally been classified as a necrotrophic pathogen, but evidence suggests it can also exist as an endophyte. *B. cinerea* represents the asexual reproductive stage (anamorph) of the genus, and it can remain in this anamorphic stage as long as environmental conditions are favorable. The less common sexual reproductive stage (teleomorph) is *Botryotinia fuckeliana* (Beever & Weeds, 2007). *B. cinerea* produces haploid macroconidia (conidia) through mitosis on specialized hyphal branches called conidiophores. These conidiophores grow from mycelium actively feeding on disintegrated plant tissues. When temperatures increase and humidity decreases, conditions typically observed during early morning, conidiophores dry out and release conidia into the air (Williamson et al., 2007). This spore release can also be facilitated by water movement (Blanco de santos et al., 2006), insects (Fermaud & Le Menn, 1992), or other physical disturbances.

3.4.2. Germination and Infection Process

Once conidia land on aerial plant tissues, they may remain quiescent until conditions become favorable for germination or colonization (Williamson et al., 2007). Germination is significantly influenced by the availability of nutrients, such as pollen, in the immediate environment (Chou & Preece, 1968). Conidia can remain latent until host tissues ripen or otherwise become favorable for infection (Sanzani et al., 2012). Alternatively, conidia may germinate and subsist saprophytically until simple food sources are depleted. When the microclimate becomes optimal and the host plant weakens, such as due to insect wounding, *B. cinerea* can transition into an opportunistic necrotroph (Fermaud & Le Menn, 1992). The survival of conidia prior to germination depends largely on environmental factors, including temperature, moisture, microbial activity, and light levels (Dewey & Grant-Downton, 2015).

Upon germination, a conidium produces a germ tube that attaches to plant tissue, forming an appressorium. This structure generates a penetration peg, which invades the plant cuticle. However, this process typically cannot occur through turgor pressure alone, as no septum separates the appressorium from the germ tube. The penetration peg secretes hydrogen peroxide at its tip and releases enzymes such as cutinases and lipases to degrade the cuticle. Microscopic cracks or wounds may also provide a more direct route for entry if a conidium lands near these access points. Once inside, the penetration peg invades epidermal cells, using pectinolytic enzymes to degrade the pectin-rich cell wall; plants with lower pectin content in their cell walls show greater resistance to *B. cinerea* infection.

To kill host cells, *B. cinerea* releases a variety of chemicals and metabolites, including botrydial, oxalic acid, and host-selective toxins (HSTs), as well as enzymes like cellulases and hemicellulases, which disintegrate the cell wall and allow nutrient absorption. This infection triggers the plant's oxidative burst response, releasing reactive oxygen species and initiating a hypersensitive response, in which surrounding cells die in an attempt to halt the spread of infection. However, this strategy is ineffective against *B. cinerea*, as the pathogen is necrotrophic and thrives on senescent tissues. The infection cycle repeats, leading to tissue colonization, lesions, and, eventually, rot (Watkinson et al., 2016).

3.4.3. Survival Structures and Host Pathogen Interactions

In regions with cold winters, *B. cinerea* can produce sclerotia—melanized overwintering structures capable of surviving lower temperatures and greater environmental exposure compared to mycelium (Holz et al., 2007). In the spring, warming temperatures trigger sclerotia to produce mycelium and conidia, allowing *B. cinerea* to spread again (Hsiang & Chastagner., 1992). During periods of drought, *B. cinerea* can form chlamydospores, which are produced at the tips of hyphae and can survive for up to three months in dry conditions. When moisture returns, the chlamydospores germinate, producing mycelium and microconidia (Urbasch, 1983).

While the full scope of *B. cinerea*'s infection process is still not completely understood, growing research points to significant crosstalk between the pathogen and the host plant's immune response during infection (Dewey & Grant-Downton, 2015). Plants utilize small interfering RNAs (siRNAs) to silence specific pathogen genes via RNA interference (RNAi) through the process of host-induced gene silencing, one of their defense mechanisms. However, *B. cinerea* also produces siRNAs that serve as effector molecules, exploiting the plant's RNAi response to silence its own defense genes, thus enhancing the pathogen's virulence (Weiberg et al., 2013).

3.5. Bio-fungicide Management

3.5.1. Definition

Biofungicides, in the context of disease management, refer to the use of non-toxic living organisms or naturally derived agents to suppress disease, with minimal impact on the environment or its inhabitants. As a result, these agents are often employed in organic production or applied close to harvest (Nicot et al., 2016). The use of biofungicides to suppress *B. cinerea* infection has been studied since the 1950s (Wood, 1951) and has proven to be an effective suppression strategy when applied under optimal conditions and as part of an integrated management approach (Dewey & Grant-Downton, 2015).

3.5.2. Market Growth of Biofungicides

Despite increasing research efforts, few biofungicidal products were commercialized or widely adopted (Nicot et al., 2011). However, in the last three decades, the biological control products market has experienced significant growth, with a compounded annual growth rate of approximately 15.6% between 2008 and 2014. In 2009, biological control products accounted for about 3.5% of the global pesticide market (Glare et al., 2012), and by 2022, they were projected to represent 7% ($6.6 billion). Over the past decade, the biological control market has grown at double the rate of the conventional pesticide market, and it is expected to reach parity by 2050 (Birch & Glare, 2020).

3.5.3. Factors Influencing Biofungicide Efficacy

The efficacy of a biofungicide is determined by several factors, all of which must be optimized to achieve maximum effectiveness. Given the wide variety of biofungicides available, many of which are microbial-based, proper usage depends on understanding the mode of action and adhering closely to label instructions (Nicot et al., 2011). When applying microbial-based biofungicides, it is essential to consider the environmental conditions, delivery systems, application timing, the host plant, variability in *B. cinerea* susceptibility, and the stability and dosage of the active ingredient. Environmental conditions, particularly temperature and humidity, are key determinants of efficacy for microbial biocontrol agents (MBAs), even when products are applied according to label directions. Small changes in these factors can significantly impact the success of MBAs (Hannusch & Boland, 1996). The delivery system and application timing also influence the product's inoculation coverage. The timing of application affects the stage of crop development at which MBAs are introduced, as well as the time available for them to colonize plant tissues before the pathogen becomes active (Raziq & Fox, 2004; Reeh & Cutler, 2013). Host-plant factors can also impact the success of MBAs in suppressing *B. cinerea*. Some MBAs provide effective control across a wide variety of crops (Köhl, 2004), while others show varying levels of suppression depending on the crop or even the specific strain of a given crop (Nicot et al., 2016; Tucci et al., 2011). Evidence suggests that combining biocontrol agents can enhance suppression efficacy against *B. cinerea* (Rotolo et al., 2018; Sylla et al., 2015) and that the presence of natural microbial communities can also contribute to this effect (Mercier & Wilson, 1994). Variability in the

susceptibility of *B. cinerea* to the modes of action of MBAs is an area of ongoing research. Ajouz et al. (2010) found significant variability in the sensitivity of 204 *B. cinerea* isolates to a common antibiotic produced by MBAs. Similar results were observed when 40 isolates were tested on tomato and lettuce leaves in the presence of *B. amyloliquefaciens.* The stability (quality) and dosage of the active ingredient are critical factors influencing efficacy during storage and application. Stability largely depends on the formulation of the product (Gotor-Vila, 2017; Wong et al., 2019), while dosage affects the concentration of the active ingredient and thus its potency and control capacity (Nicot et al., 2002; Raziq & Fox, 2004). While optimizing the efficacy of MBA strains already registered for use is an important focus for growers, further strain improvement and production research may yield additional benefits. For example, Masmoudi et al. (2017) enhanced the suppression abilities of *B. amyloliquefaciens* by subjecting the strain to several rounds of mutagenesis, selecting for more aggressive strains with higher metabolite production. This approach, combined with culturing on an optimized medium, resulted in a strain with a 12-fold increase in antifungal metabolite production, leading to significantly improved suppression of *B. cinerea* and other pathogens compared to the original strain.

3.6. Future Research Directions

New research directions could focus on identifying the microorganisms present in the phyllosphere and rhizosphere of crops and studying their interactions with *B. cinerea* (Lindow & Brandl, 2003; Compant et al., 2019; Karlsson et al., 2017). Equally important is to gain a better understanding of the plant host-*B. cinerea* interaction, as the full dynamics of how plants respond to this fungal pathogen remain unclear (Veloso & van Kan, 2018; van Kan, 2006). Unraveling these responses could help researchers determine whether crops can be modified through gene editing to enhance their defense against infection, aligning with strategies such as induced systemic resistance (Zhou & Zeng, 2021). Another promising but underexplored area is that of RNA mycoviruses, which inhabit and replicate in fungal cells, including those of *B. cinerea*. These viruses have been shown to reduce the growth and pathogenicity of *B. cinerea*, potentially opening new avenues for biocontrol (Zhang & Nuss, 2016; Yu et al., 2010). An emerging field with great potential is nanotechnology, which involves the use of nano-materials such as nanoparticles, nano-based kits, nano-capsules, and nano-biosensors to

recognize and deliver targeted agents to plants (Fraceto et al., 2016; Elmer & White, 2016). Nano-capsules are particularly relevant, as they can encapsulate fungicides and deliver them with higher absorption and application efficiency compared to conventional sprays. The analysis of the *B. cinerea* genome will undoubtedly lead to new insights and may assist in breeding resistant cultivars. Identifying the protective mechanisms and the genes responsible for them could prove valuable and may be incorporated into gene-editing efforts to develop more 'natural' resistance solutions (Tian et al., 2016; Poland et al., 2009).

Chapter 4. Epidemiology and management of *Botrytis cinerea* causing bud rot on greenhouse cultivated cannabis (*Cannabis sativa* L.)

4.1. Abstract

Botrytis cinerea Pers. causes bud rot on *Cannabis sativa* L. (cannabis) inflorescences, significantly reducing yield and quality. We investigated the timing of *B. cinerea* spore infection during the 49-day flowering period (FP) and how environmental conditions and host genotype influence disease development. Artificial spore-inoculations made at 14, 21, or 28 days of the FP resulted in the highest disease development compared to inoculations made at 7 or 35 days. Visible mycelial growth within inflorescences was observed at 33-41 days, regardless of inoculation time. The disease severity under greenhouse conditions from natural inoculum was highest during summer and fall seasons (June to November), which resulted in bud rot incidence of 1-13%, depending on the genotype; lower disease incidence occurred at other times. The highest disease was observed during September and October, which corresponded to average daily outdoor absolute humidity of 14 g/m³ and temperatures of 20-22°C. Notably, humidity and temperatures within 49-day-old inflorescences were higher by 15.4% and 2.5°C, respectively, compared to ambient conditions. These findings suggest that bud rot development is strongly influenced by environmental conditions within and outside the greenhouse, which can impact spore germination and subsequent infection. Among management practices evaluated, enhanced air circulation around inflorescences reduced bud rot incidence by 66-92%. Additionally, applications of Rootshield HC (*Trichoderma harzianum*, 10 g/L) on days 14, 21, and 28 of the FP reduced disease by 47-91%. Treatments with Double Nickel (*Bacillus amyloliquefaciens*), LifeGard (*Bacillus mycoides*), Prestop (*Gliocladium catenulatum*), and Regalia Maxx (*Reynoutria sachalinensis*) provided varying levels of disease reduction.

4.2. Introduction

Cannabis (*Cannabis sativa* L.) is cultivated commercially under greenhouse and field conditions across various geographic regions of Canada following its legalization for recreational and medicinal purposes in 2018. It is primarily grown for its inflorescences (buds or flowers), which produce a variety of cannabinoids, phenolic, and terpene compounds whose levels have been significantly enhanced through selective breeding (ElSohly and Slade 2005; Chandra et al. 2017b; André et al. 2016; Hazekamp et al. 2016; Small 2016). Under greenhouse production, the plant is affected by a range of pathogens that infect the roots, foliage, and inflorescences (Punja 2018; Punja et al. 2019; Punja, 2021a). One of the most important diseases currently affecting cannabis crops is grey mold (bud rot), caused by *Botrytis cinerea* Pers. (Punja, 2021a; Mahmoud et al. 2023; Buirs and Punja 2024). This necrotrophic pathogen infects over 1,000 plant species globally (Dean et al. 2012; Elad et al. 2015; Mahmoud et al. 2023). On cannabis, it causes decay of the compact inflorescence tissues and leads to abundant mycelial growth and sporulation under high humidity conditions in greenhouses and in the field (Punja and Ni 2021; Mahmoud et al. 2023). Recent research has explored aspects of *B. cinerea* development on cannabis (Punja, 2021a; Punja and Ni 2021; Mahmoud et al. 2023; Buirs and Punja 2024) but information on disease management is currently lacking. It is well known that moisture and temperature are two primary environmental factors influencing *B. cinerea* spore germination and development that lead to grey mold development (Mahmoud et al. 2023; Buirs and Punja 2024). Other factors that influence *B. cinerea* disease development on susceptible hosts include air circulation, light levels, nutrient levels, and plant growth stage (Bulger et al. 1987; Thomas et al. 1988; Shtienberg 1998; Korner et al. 2014; Kozhar and Peever 2018; Gossen and Lan 2021). Commercially cultivated cannabis genotypes are reported to vary in their susceptibility to infection (Buirs and Punja 2024). Denser and more compact inflorescences may be more susceptible to infection (Mahmoud et al. 2023). In some plants, *B. cinerea* can also develop as an endophyte, where mycelial growth occurs internally without obvious external symptoms or induction of host defense responses (Dewey and Grant-Downton 2015). Endophytic (latent) development can progress to a disease-causing phase when the host plant matures. This has been observed in several crops, including strawberries (Bristow et al. 1986), raspberries (Williamson et al. 1987), and grapes (Coertze and Holz 1999). Endophytic growth of *B. cinerea* in the absence of disease symptoms has been

reported to occur in cannabis stem tissues (Punja and Scott 2023), though the significance of this latency for disease development on inflorescences has not been established. There have been no reports of endophytic growth of *B. cinerea* within cannabis inflorescences. Notably, only cannabis inflorescences show significant susceptibility to infection, although damping-off, stem cankers, and leaf blighting can occur under high humidity conditions (Punja and Ni 2021; Mahmoud et al. 2023).

The influence of environmental conditions, host growth stage, and genotype on bud rot development in cannabis plants has not been published prior. In particular, the effect of temperature and relative humidity on disease development remains unclear. Symptoms caused by *B. cinerea* on cannabis plants first appear during the later stages of inflorescence development (at 5 weeks of the 7-8 week flowering period), although the range of susceptibility of cannabis inflorescences during development is not well understood. There have been no previous studies to evaluate disease mitigation practices to reduce the impact of this pathogen on greenhouse-grown cannabis plants. This lack of information on the epidemiology and management of *B. cinerea* has led to significant economic losses for cannabis producers both pre- and post-harvest.

In the present study, epidemiological factors influencing *B. cinerea* development on cannabis and disease management approaches were investigated. Spore inoculations were conducted at various stages of inflorescence development, and disease incidence was monitored to assess the timeline of susceptibility of cannabis inflorescences to *B. cinerea*. Environmental conditions within and outside the greenhouse were monitored to determine their influence on disease progression. Lastly, several management approaches were investigated, including genotype susceptibility to infection, enhanced air circulation via fans, and applications of biological control products and reduced-risk chemicals (biorational products) currently registered for cannabis use (available at: https://pr-rp.hc-sc.gc.ca/ls-re/index-eng.php). The findings from this study identify effective strategies for managing *B. cinerea* on cannabis that can be implemented in greenhouses or controlled environment facilities.

4.3. Materials and methods

4.3.1 Stages of Inflorescence Development

The cannabis inflorescence is a complex structure made up of an aggregate of pistillate flowers that form a branched compound raceme (Spitzer-Rimon et al. 2019). Glandular trichomes develop prolifically on the bract tissues of these inflorescences, producing the cannabinoid and terpene compounds that contribute to the distinctive olfactory and chemical characteristics of cannabis plants (Punja et al. 2023). To monitor the stages of inflorescence development over time, genotype 'OG', which is susceptible to infection by *B. cinerea* and which is consistently grown year-round, was selected for the study. Plants were propagated from vegetative cuttings, and once rooted, were cultivated hydroponically under a 12:12 hr photoperiod to induce inflorescence development (Buirs and Punja 2024). These growth conditions were consistently replicated within the ranges specified for all greenhouse trials conducted in different compartments during this study (Supplementary Table A.1). At weekly intervals, starting when the plants were placed under the 12:12 hr photoperiod, photographs of the developing inflorescences were taken over the ensuing seven weeks until the plants were harvested (Figure 27).

4.3.2. Pathogen Inoculation

An isolate of *B. cinerea* recovered from infected cannabis inflorescences and identified using PCR as described by Punja and Ni (2021) was grown on potato dextrose agar containing 140 mg/L of streptomycin sulphate under ambient laboratory conditions (temperature range of 21-23^0 C and 12-14 hr of fluorescent lighting). After approximately 4 weeks of growth when the cultures were sporulating, sterile distilled water (25 mL) was added to the Petri dish, and a glass rod was used to dislodge the spores. A suspension was made by combining spores from 20 cultures in 500 mL of water and the concentration was determined using a hemocytometer. The final spore concentration (after adjustment if needed) was in the range of 0.5-1 x 10^5 spores/mL. Approximately 20 mL of inoculum was sprayed onto the terminal (leading) inflorescences of each of five replicate plants of genotype 'OG'. Different groups of plants were inoculated on days 7, 14, 21, 28, and 35 of the 49-day flowering period. The plants were grown as per recommended commercial practices until harvest. Untreated control plants served as a

negative control and were exposed to any natural airborne inoculum. Following application of spore suspensions, small tissue pieces were obtained from the inflorescences and subjected to scanning electron microscopy to visualize the deposition and subsequent germination of *B. cinerea* spores as described by Punja and Ni (2021). Three inoculation trials were conducted during August and September 2022 and March 2023 to provide different harvest times.

4.3.3. Disease Assessments

Following each inoculation treatment, disease assessments were made at 2-day intervals from sprayed and control inflorescences in each group of plants (n=5). Visual ratings were made according to the following scale: 0 = no visible infection, 1 = browning of inflorescence tissues, 2 = visible presence of mycelium, 3 = necrosis of interior inflorescence tissues, 4 = necrosis of exterior inflorescence tissues. To quantify disease progression, the mean day within the flowering period when the average disease rating reached 2 (visible mycelium) was calculated for each group of plants. This time point was then compared across the different inoculation treatment groups to determine which treatments resulted in the fastest development of disease. The data from the three trials are presented separately due to differences in disease pressures.

4.3.4. Effect of Genotype and Seasonal Harvest Date on Bud Rot Severity

Bud rot development due to natural infection (i.e., on uninoculated plants in the greenhouse) was monitored monthly from June to November 2022 on two cannabis genotypes, 'BC' and 'SC'. Plants representing each genotype were grown in staggered plantings to achieve successive monthly harvests after a 7-week flowering cycle. During the week preceding harvest in each trial, inflorescences that were visibly affected by bud rot (Figure 28) were manually removed and weighed. To estimate the percentage of loss due to disease, the total weight of bud rot-affected inflorescences was divided by the total yield (kg fresh weight of inflorescences) from all plants in the trial. The data were averaged to compare the susceptibility of the two genotypes to bud rot during each month. To evaluate the susceptibility of a larger number of genotypes, disease development was assessed as described above on multiple plantings of nine genotypes (‘A5’, ‘BC’, ‘CD’, ‘DB’, ‘GD’, ‘GP’, ‘M1’, ‘PS’, and ‘SC’), each harvested during July.

Throughout the year, outdoor absolute humidity and temperature were measured using PRIVA weather station sensors (Pro 21 model) (https://www.priva.com/horticulture/solutions/priva-weather-station), which were placed on the greenhouse roof. Measurements were recorded on PRIVA office software, graphed, and compared to the *B. cinerea* infection trend.

4.3.5. Measuring Temperature and Relative Humidity Within Inflorescences

The relative humidity and temperature within the inflorescence tissues of genotype 'OG' plants were measured from day 28 to day 49 of the flowering period. The probe on a handheld Reed psychrometer, model 8706 (Newmarket, Ontario), was gently inserted into the center of the inflorescence tissues and held for 5 seconds to obtain a reading. Ambient readings were similarly obtained from within the greenhouse at the same time. Measurements were made daily at 6:45 a.m., 10:45 a.m., 2:45 p.m., and 6:45 p.m. from three replicate plants. The experiment was then repeated in six greenhouse compartments to assess the range of values obtained. A FLIR infrared camera, model E8 (Thousand Oaks, California), was used to capture infrared images of inflorescences from three different genotypes ('LB,' 'PD,' and 'DO') to compare surface temperatures. All measurements were taken at 1:00 p.m. on a sunny day (25°C outdoors) in August. The "hot spot" software mode was used to identify the hottest point of each inflorescence in the images. Additionally, images of a group of 'DO' inflorescences were taken 30 min before the daily blackout period, which is used in the greenhouse to provide a 12:12 hour photoperiod for flowering. Another measurement was taken 30 min after this period using the "thermal" software mode to visually compare the inflorescences and foliage at both time points.

4.3.6. Effect of Enhanced Air Circulation on Bud Rot Development

Plants of genotype 'OG' were grown with enhanced air circulation achieved by placing fans above the plants starting on day 14 of the flowering period. The fans were positioned 25 cm above the plant canopy and ran continuously until harvest, providing an airflow of 7 m/sec. This group consisted of 40 plants. An adjacent group of plants was grown without the air circulation from fans. One week prior to harvest, inflorescences visibly affected by bud rot within each group were removed and weighed, and the percent loss due

to disease was determined as described previously. This experiment was repeated three times in different greenhouse compartments, and the data were averaged. Measurements of relative humidity in control inflorescences and those with enhanced air circulation were taken thrice daily (9:00 a.m., 1:00 p.m., 5:00 p.m.) in each group of plants over a 5-day period before harvest, using the same psychrometer method described previously.

4.3.7. Efficacy of Biological and Reduced Risk Products in Reducing Bud Rot Development

Spray applications of several biological and reduced-risk products were made at 14, 21, and 28 days of the flowering period to assess their impact on bud rot development. A handheld spray gun was used to deliver approximately 60 mL of each product per plant. The tested products included Double Nickel LC (*Bacillus amyloliquefaciens*) at 4 mL/L (Certis Biologicals, Columbia, Maryland), Regalia Maxx (*Reynoutria sachalinensis*) at 2.5 mL/L (Pro Farm Group, Davis, California), Rootshield HC (*Trichoderma harzianum*) at 10 g/L (BioWorks Inc., Victor, New York), and Timorex Gold (tea tree oil) at 5 mL/L (Belchim Crop Protection, Guelph, Ontario). All products are approved for use on cannabis for *Botrytis* management by Health Canada. Three replicate groups of 10 plants per treatment were arranged in each greenhouse compartment in a randomized block design. Treatments were applied between 7 a.m. and 10 a.m. under shade. In the week before harvest, inflorescences that were visibly affected by bud rot were removed and counted. The results were compared to control rows that did not receive any treatment to assess the efficacy of the products. These trials were repeated in three different greenhouse compartments on genotypes 'BC' and 'SC,' which were harvested in early September, mid-September, and late October.

Two large-scale commercial trials were conducted to confirm the efficacy of applying Rootshield HC (*Trichoderma harzianum* Rifai strain KRL-AG2, formulated at 1.0 × 10^7 colony-forming units /g dry weight) in reducing bud rot. These trials followed the methodology used in the spray trials conducted by Buirs and Punja (2024). The product was applied at 10 g/L to three rows of 216 plants and 16 rows of 216 plants of genotypes 'PD' and 'BC', respectively, in two separate greenhouse compartments. Applications were made on days 14, 21, and 28 of the flowering period. The rows were sprayed using a greenhouse pipe rail spray robot (Buitendijk-Slaman, C.C.T 500 mm model; https://www.aisgreenworks.com.au/buitendijk-slaman-spray-robot/) equipped with flat-fan TEEJET 8002VK nozzles that delivered approximately 60 mL of solution per plant.

Treatments were applied between 7 a.m. and 10 a.m. under shade. The infected inflorescences were collected, counted, and compared to the untreated control plants. Harvesting occurred in late September for the 'PD' genotype and late October for the 'BC' genotype.

4.3.8. Statistical Analysis

Statistical analyses for all trials where statistical significance is presented were performed using one-way analysis of variance (ANOVA) to compare mean differences among the experimental groups. Tukey's honest significant difference (HSD) post-hoc test was used to control for Type I errors and to identify statistically significant pairwise group differences, with significance levels set at $p < 0.05$ for all tests. In cases of unequal sample sizes across groups, the Tukey-Kramer method was used to balance these discrepancies. All applicable data were expressed as mean ± standard error (SE) to provide a clear understanding of data variability around the mean. Statistical analyses were conducted separately for each trial since experiments were performed independently at different times during the season and were, thereby, influenced by plant developmental stages, genotypes being grown, and microclimatic differences in greenhouse compartments.

4.4. Results

4.4.1. Stages of Inflorescence Development

The stages of inflorescence development in cannabis genotype 'OG' over a 7-week flowering period are shown in Figure 27. At the time the plants were placed under a 12:12 hr photoperiod to induce flowering, no visible flower development was observed (Figure 27A). After one week, barely discernible bract leaf development appeared at both apical and axillary nodes (Figure 27B). After two weeks, the bracts were approximately 1 cm in length, and white stigmas had begun to develop (Figure 27C). From three weeks onward, visible clusters of flowers with numerous stigmas and inflorescence leaves produced the characteristic inflorescence structure (Figure 27D,E). By weeks 5-7 (Figure 27F-H), large fan and inflorescence leaves had formed, and the flower clusters has expanded to form a compact inflorescence. At harvest, the

inflorescence and fan leaves were trimmed to better illustrate the density of a mature inflorescence (Figure 27I).

Figure 27. The stages of development of the inflorescences of cannabis genotype 'OG' progressing from the initiation of flowering (week 1) to harvest (week 7). Morphological differences that can be seen include

the development of bract leaves, stigmas, carpels, fan leaves and inflorescence leaves surrounding the inflorescence at later stages of development. The following times represent when the images were taken during the flowering period. (A) Day 0 of flowering. (B) Day 7 of flowering. (C) Day 14 of flowering. (D) Day 21 of flowering. (E) Day 28 of flowering. (F) Day 35 of flowering. (G) Day 42 of flowering. (H) Day 49 of flowering. (I) Finished product - dried and trimmed.

Symptoms of *B. cinerea* infection on developing cannabis inflorescences from pre-existing inoculum sources in the greenhouse were generally seen from week 5 of the flowering period onward (Figure 28). This stage corresponded to an almost fully formed inflorescence structure where fan and inflorescence leaves were visible (Figure 27F). Extensive necrosis often developed within the center of the inflorescence (Figure 28A-C), depending on the origin of the initial infection. The fan and inflorescence leaves subsequently turned chlorotic and became necrotic. In severe infections under suitable environmental conditions, which included high relative humidity, the entire inflorescence was consumed by the pathogen and destroyed within a week (Figure 28D-F).

Figure 28. The late-stage bud rot symptoms of a natural *B. cinerea* infection on different genotypes. (A) Genotype ‘OG’. (B) Genotype ‘PD’. (C) Genotype ‘CD’. (D) Genotype ‘PE’. (E) Genotype ‘SC’. (F) Genotype ‘PC’. The severity of symptoms varies depending on the timing of the infection and the susceptibility of the genotype.

4.4.2. Disease Assessments Following Artificial Inoculation

The inoculation procedure using a hand-held spray bottle delivered a fine mist onto the inflorescence, and the inoculum was applied to run-off (Figure 29A). Scanning electron microscopic observations showed the cluster of stigmatic hairs on the bifurcate stigmas (Figure 29B), and spores of *B. cinerea* could be observed lodged in the crevices, some of which had begun to germinate (Figure 29C,D). Visible mycelium then developed and was observed growing around trichome gland heads (Figure 29E).

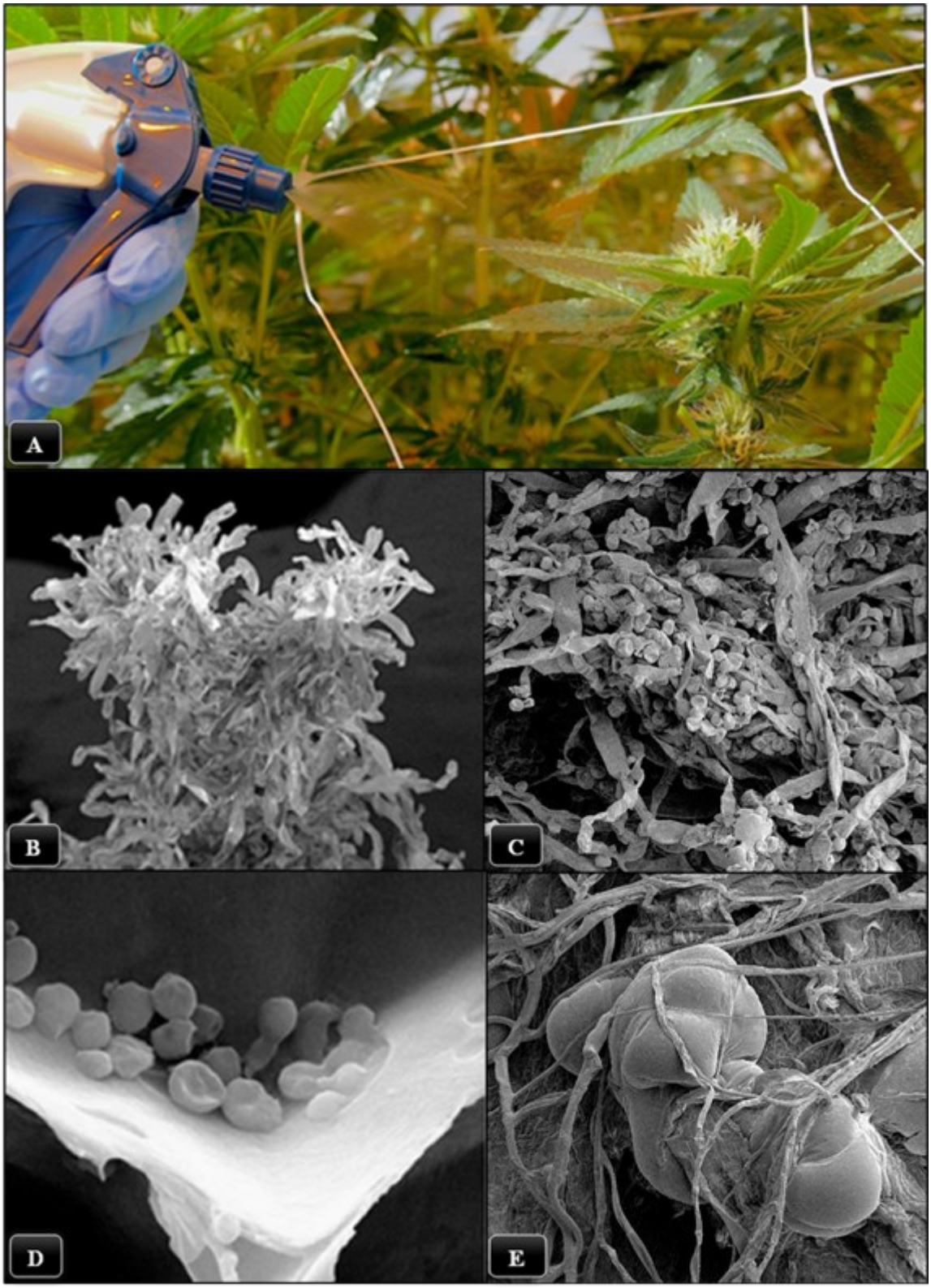


Figure 29. The artificial inoculation of cannabis inflorescences ‘OG’ with a spore suspension of *B. cinerea*. (A) The inflorescence being sprayed with a spore suspension using a hand-held sprayer. (B) The scanning electron

micrograph of inflorescence stigmas showing the bifurcate (two-sided branched) structure. The stigmatic hairs appear dried due to the preparation procedure used. (C) The scanning electron micrograph of *B. cinerea* spores deposited on inflorescence tissues. (D) The scanning electron micrograph of stigmatic tissue showing *B. cinerea* spores. (E) The scanning electron micrograph of trichome heads showing *B. cinerea* mycelium growing over them.

The first evidence of successful infection resulting from the spore suspensions applied to cannabis inflorescences was the development of mycelium which could be observed with the unaided eye. The mycelium generally developed during the time period of 33-41 days in the flowering cycle (5-6 weeks), with a median time of 34 days (5 weeks) (Figure 30A), regardless of the inoculation time. This correlated well with the time period of symptom development from natural inoculum, as described in Figure 28; symptoms were first observed in week 5 of the flowering period. With later spore inoculations on day 28, the onset of visible mycelium was expedited, and it took less time for the pathogen to cause infection and become visible. For example, inflorescences inoculated on days 7, 14, 21, 28, and 35 of the flowering period showed mycelial development, on average, after 34, 19, 12, 6, and 5 days, respectively (Figure 30B). This trend was consistent for inoculation trials conducted during August and September. In a trial conducted in March, the onset of infection was delayed for the early inoculations conducted on days 7, 14, and 21 but was not different for days 28 and 35 (Figure 30B). The bud rot severity ratings shown in Figure 30C confirmed that inoculations conducted on days 14, 21, and 28 of the flowering period resulted in the most severe disease, with less bud rot development when inoculations were made at days 7 and 35 of the flowering period. The most severe infections were recorded after week 5 of the flowering period (Figure 30C).

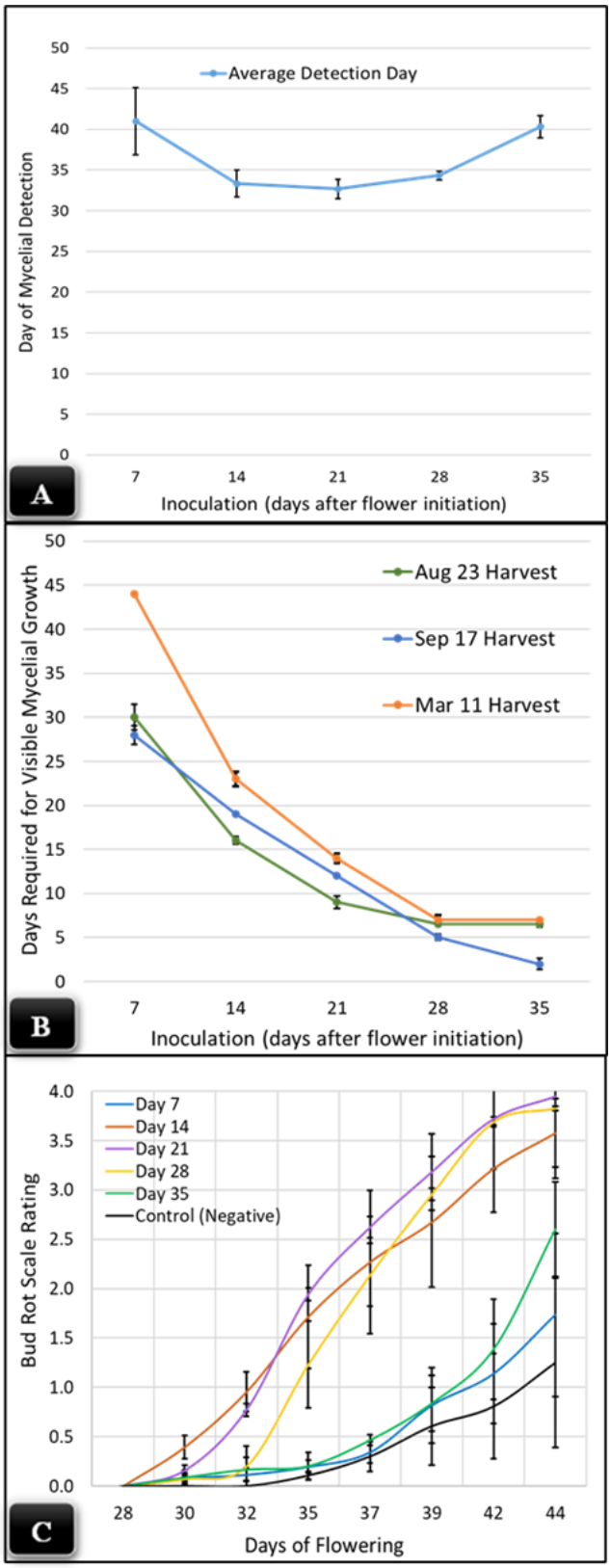


Figure 30. The disease assessments made over the 7-week flowering period resulting from artificial inoculation with spores of *B. cinerea* onto

plants of ‘OG’ at various times during the flowering period.
(A) The average time (days in the flowering period) at which mycelial growth within inflorescences was observed as a function of time of inoculation during the flowering period. Inoculations were made at the five times shown. (B) The average number of days required for mycelial growth to appear post-inoculation when inoculations were made at five different times during the flowering period. The results from three trials with different harvest dates are shown. (C) Bud rot disease severity ratings at various times during the flowering period as a function of the time of inoculation with spores of *B. cinerea*. Standard error bars reflect the variance from three trials, two with five replicate plants and one with three replicate plants per experimental group.

A close-up image of a 5-week -old inflorescence of genotype ‘OG’ shows the developing stigmas protruding outwards (Figure 31A). A longitudinal section made through the inflorescence revealed cavities that formed between flower clusters that could provide a conducive environment for *B. cinerea* development (Figure 31B). As well, some stigmas had begun to senesce, as revealed by a visible surface browning. Observations of pathogen development on the inflorescences revealed two initial symptoms. The first symptom was necrosis (browning) that developed on the petioles of the bract leaves surrounding the inflorescence (Figure 31C). Alternatively, visible growth of mycelium on stigmatic tissues was observed first (Figure 31D). Regardless, *B. cinerea* infection invariably led to the destruction of the inner portions of the inflorescence within the cavities, a symptom often not visible externally without prying open the inflorescence (Figure 31E). This was subsequently followed by the necrosis and decay of the external portions of the inflorescence (Figure 31F). Under suitable environmental conditions, the progression of infection could occur within 3-7 days but frequently was only visible if the inflorescences were forced open to reveal the internal decay (Figure 31G-I).

Figure 31. The infection stages of *B. cinerea* on cannabis inflorescences. (A) A maturing inflorescence with fresh stigmas (arrow) to which *B. cinerea* spores will adhere. (B) A dissected inflorescence showing the hidden cavities and crevices between flower clusters where *B. cinerea* infections are often found to develop. (C) Bract leaves in the centre of the inflorescence (arrow) exhibiting early-stage necrosis. (D) The infection of stigmatic tissues causing visible necrosis (arrow). (E) Bud rot development beginning in the centre of the inflorescence (arrow). (F) Advanced bud rot development visible on the entire inflorescence surface. (G-I) Bud rot development on the inner tissues of the inflorescence exposed by excising or prying apart adjacent portions of the inflorescence.

4.4.3. Effect of Genotype and Seasonal Harvest date on Bud Rot Severity

When two cannabis genotypes ('BC,' 'SC') were compared for bud rot severity from natural infection over a 6-month period, the overall disease was greatest from July to October and lowest during June and November (Figure 32A). Genotype 'BC' was significantly more susceptible to bud rot than genotype 'SC' during each month of observation. An analysis of outdoor absolute humidity and temperature measurements taken over 12 months, which coincided with these disease observations, showed that late June to late October had the highest overall temperatures and absolute humidity, with the greatest fluctuations in these parameters. The daily absolute humidity during June-October ranged from 11 to 17 g/m³, with an average of 14 g/m³, and daily temperatures ranged from 15 to 29°C, with an average of 22°C (Figure 32B). This late June to late October period, which had the greatest range and fluctuations in absolute humidity and temperature, coincided with the highest bud rot development in the greenhouse. As a result, the term "*Botrytis* susceptibility period" was used to denote the period of highest disease development in cannabis grown under greenhouse conditions in this specific geographical location of Delta, British Columbia.

A comparison of the susceptibility of nine cannabis genotypes to bud rot incidence, measured as the total weight of infected inflorescences removed during several compartmental harvests in July, showed significant differences among genotypes. Some, like 'BC,' 'CD,' and 'SC,' displayed much higher disease incidence compared to other genotypes under comparable growing conditions (Figure 32C).

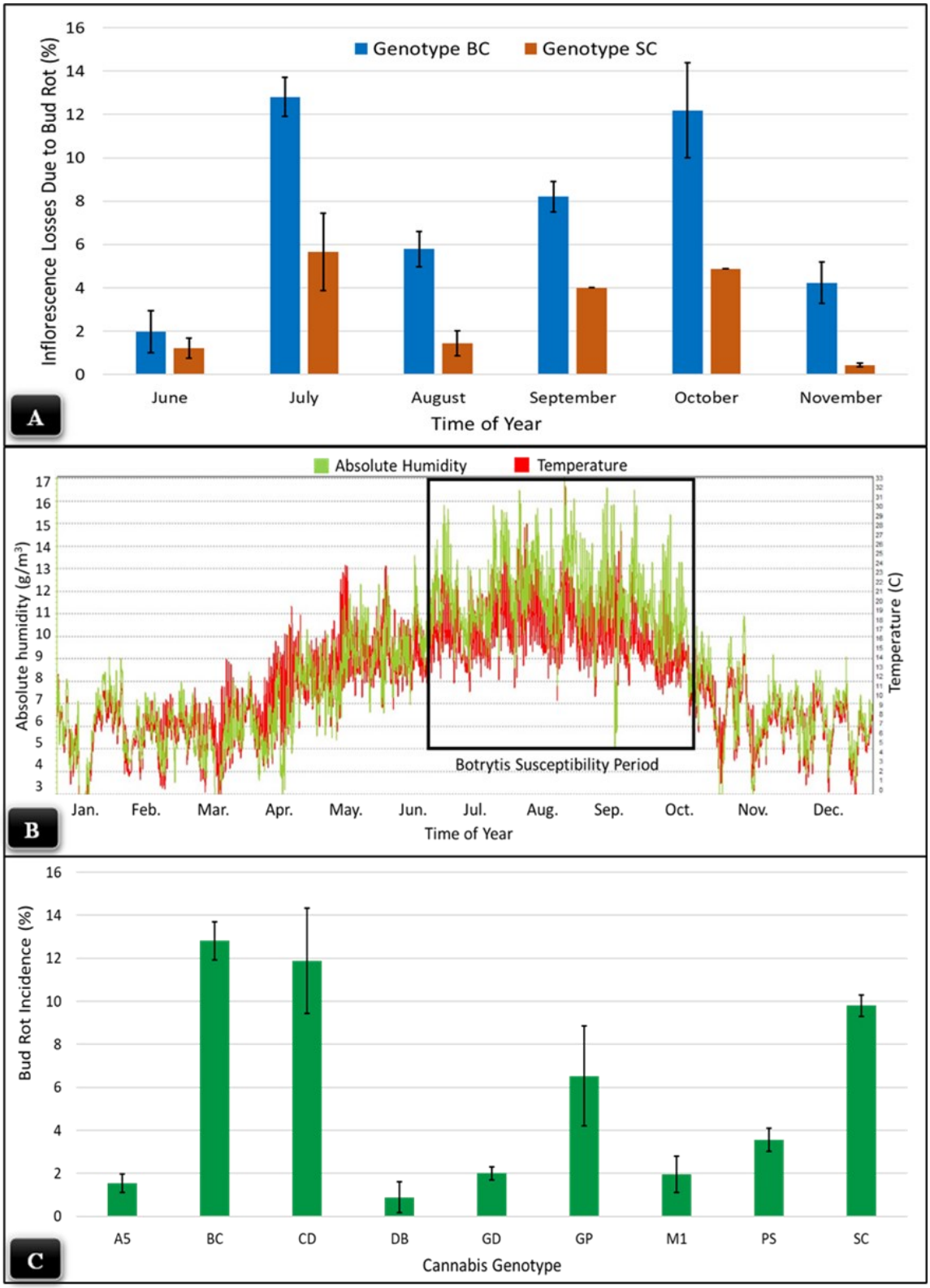


Figure 32. The effect of cannabis genotype and seasonal harvest date on the severity of bud rot. (A) The incidence of bud rot resulting from natural infections in the greenhouse on two cannabis genotypes ('BC', 'SC') at

monthly harvests conducted from June to November. (B) Measurements of absolute humidity and temperature made at monthly intervals outside the greenhouse and overlaid with the data in Figure 32A show the '*Botrytis* susceptibility period' where most infection was observed. (C) The response of nine genotypes of cannabis to bud rot resulting from natural infections at harvests made during July. There were significant differences in susceptibility among the genotypes tested.

4.4.4. Measuring Temperature and Relative Humidity Within Inflorescences

Using a handheld psychrometer to measure the microclimate within cannabis inflorescences, it was observed that relative humidity within the inflorescences was consistently higher than in the surrounding greenhouse environment from day 28 to day 49 of the 7-week flowering period (Figure 33A). Daily fluctuations in relative humidity and temperature were also significantly greater in the ambient environment compared to within the flower clusters. The relative humidity range within inflorescences was 60-87% compared to 34-76% in the ambient environment, while the temperature range was 19.5-30.2°C within the tissues versus 17.6-25.5°C outside (Figure 33B). On average, the difference in relative humidity between the flower tissues and the greenhouse environment was 15.4%, and the average temperature difference was 2.5°C (Figure 33C). These microclimatic differences between the flower tissues and the ambient environment were relatively consistent throughout the flowering period from day 28 to day 49.

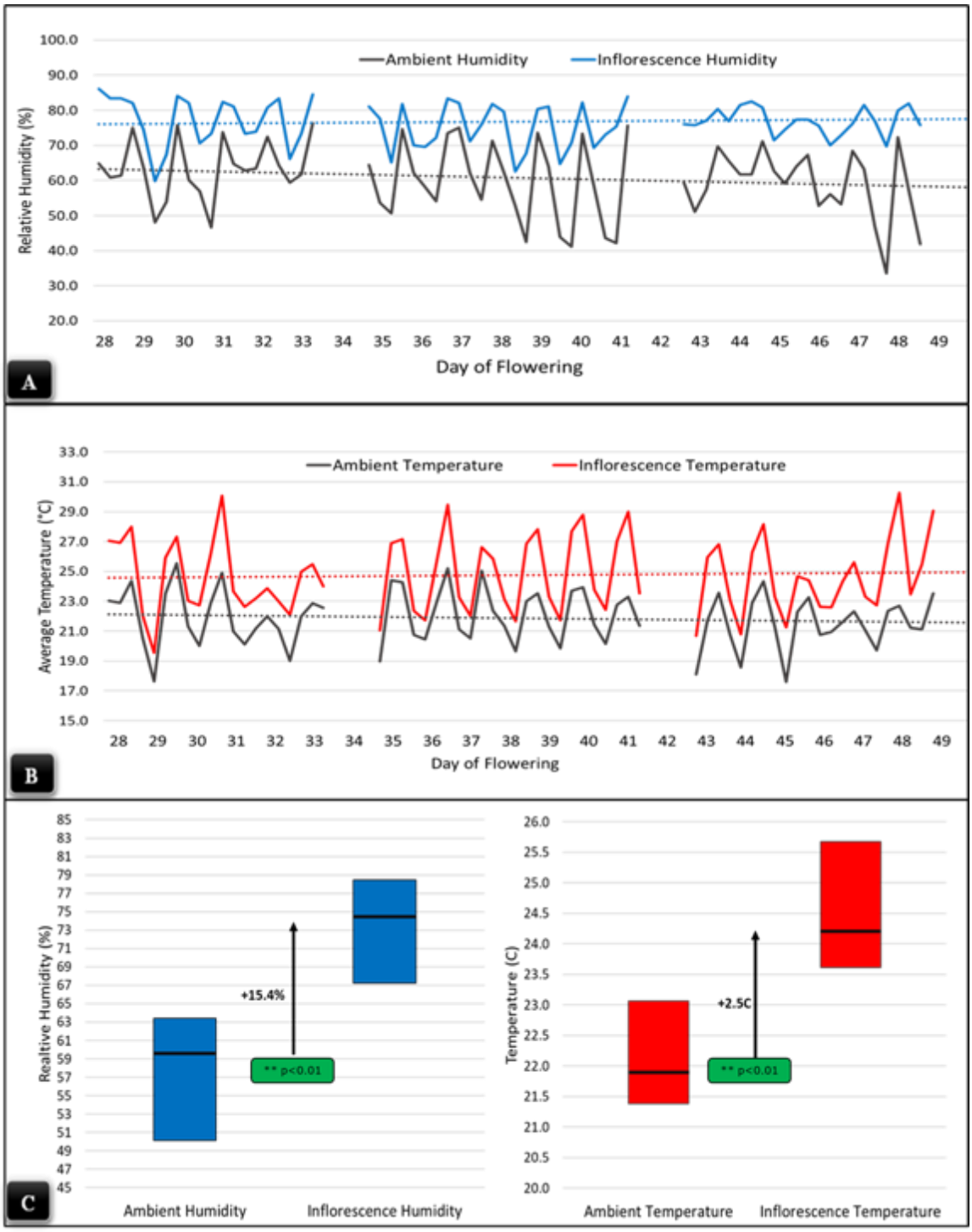


Figure 33. A comparison of microclimates within cannabis inflorescences and the ambient environment. Measurements were made using a hand-held psychrometer, four times a day, and data were plotted from day 28 to day 49 of the flowering period. (A) Relative humidity (%). (B) Average temperature. (C) The average differences in microclimates between inflorescences and ambient conditions are shown.

An infrared camera (FLIR E8 model) was used to assess the surface temperatures of different parts of the cannabis plant. Inflorescence surface temperatures were significantly higher than those of the surrounding foliage, and cannabis genotypes varied significantly in surface temperatures. For instance, the average inflorescence surface temperature was 34.6°C for 'LB,' 39.3°C for 'PD,' and 41.4°C for 'DO', when measured at 1:00 p.m. on a sunny day in August (Supplementary Figure B.1. A-C). The maximum inflorescence temperature difference between these genotypes was 6.8°C. The heat buildup within the inflorescences of 'DO' was rapidly lost within 30 min of placing the blackout curtains to provide the 12-hour dark period required by cannabis plants to flower. The temperature dropped from ~36°C to ~24°C (Supplementary Figure B.1. D, E), corresponding with a decrease in the ambient greenhouse temperature measured by the climate control system. When bud rot-infected inflorescences were compared to healthy inflorescences of genotype 'PD' using the infrared camera, no detectable differences in surface temperatures were observed (unpublished data).

4.4.5. Effect of Enhanced Air Circulation on Bud Rot Development

Enhanced air circulation provided by fans installed over cannabis plants in the greenhouse significantly reduced the relative humidity within the inflorescences, as measured using the psychrometer, by an average of 11.6% ($p < 0.01$). This also significantly reduced the incidence of bud rot by an average of 81.2% ($p < 0.05$, $p < 0.01$, $p < 0.01$) (Figure 34). The plants that were nearest to the fans exhibited minor drying damage on the tips of foliar and bract leaves.

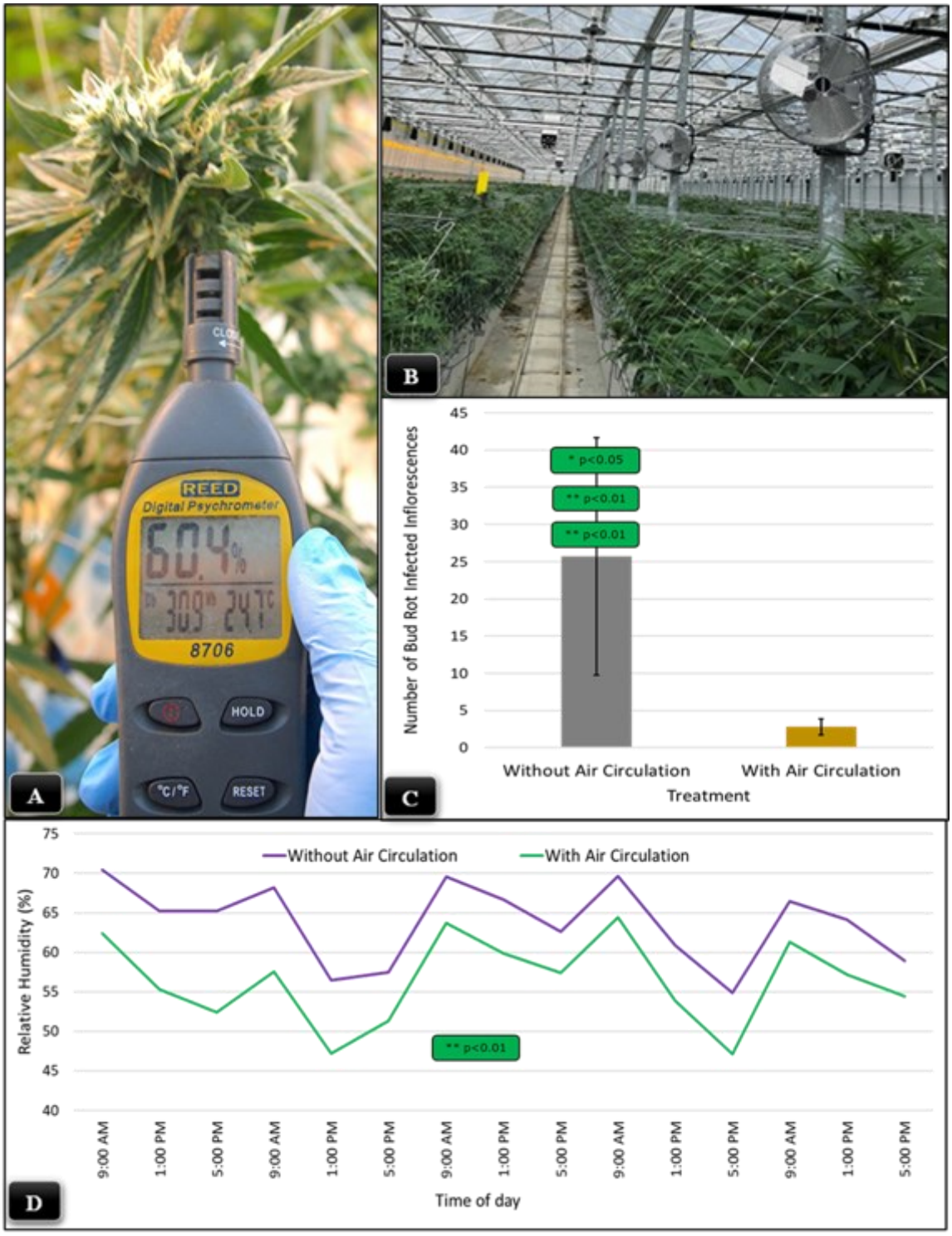


Figure 34. The effect of enhanced air circulation on bud rot development and microclimatic humidity in the inflorescence. *(*A) A Reed digital psychrometer (model 8706) used to make measurements was pressed into the centre of the inflorescence and held in place for 5 sec. (B) The placement of fans on vertical posts over the canopy of cannabis plants to enhance air circulation. (C) The comparison of bud rot development on plants without and with enhanced air circulation enabled with fans.

(D) The effect of enhanced air circulation on microclimate relative humidity within the inflorescence without and with air circulation. Measurements were made 3 times a day, and the data are presented for a 5-day period. Significantly lower relative humidity was achieved with the use of fans at all times of the day.

4.4.6. Efficacy of Biological and Reduced Risk Products in Reducing Bud Rot Development

An assessment of four commercial products to reduce *Botrytis* incidence on genotype 'BC' was made in randomized block trials conducted in three different greenhouse compartments. The disease incidence levels between these trials from natural inoculum ranged from low (Trial one, ~8 infected inflorescences), to moderate (Trial two, ~14 infected inflorescences), to high (Trial three, ~50 bud rot-infected inflorescences). The best-performing product in all three trials was Rootshield HC, which reduced bud rot incidence by an average of 61% ($p<0.01$, $p<0.01$, $p<0.05$). Applications of Regalia Maxx, Double Nickel LC, and Timorex Gold did not consistently reduce disease incidence (Figure 35). For example, applications of Timorex Gold reduced bud rot incidence under low disease pressure but not under high disease pressure, while Regalia Maxx significantly reduced bud rot under high disease pressure only.

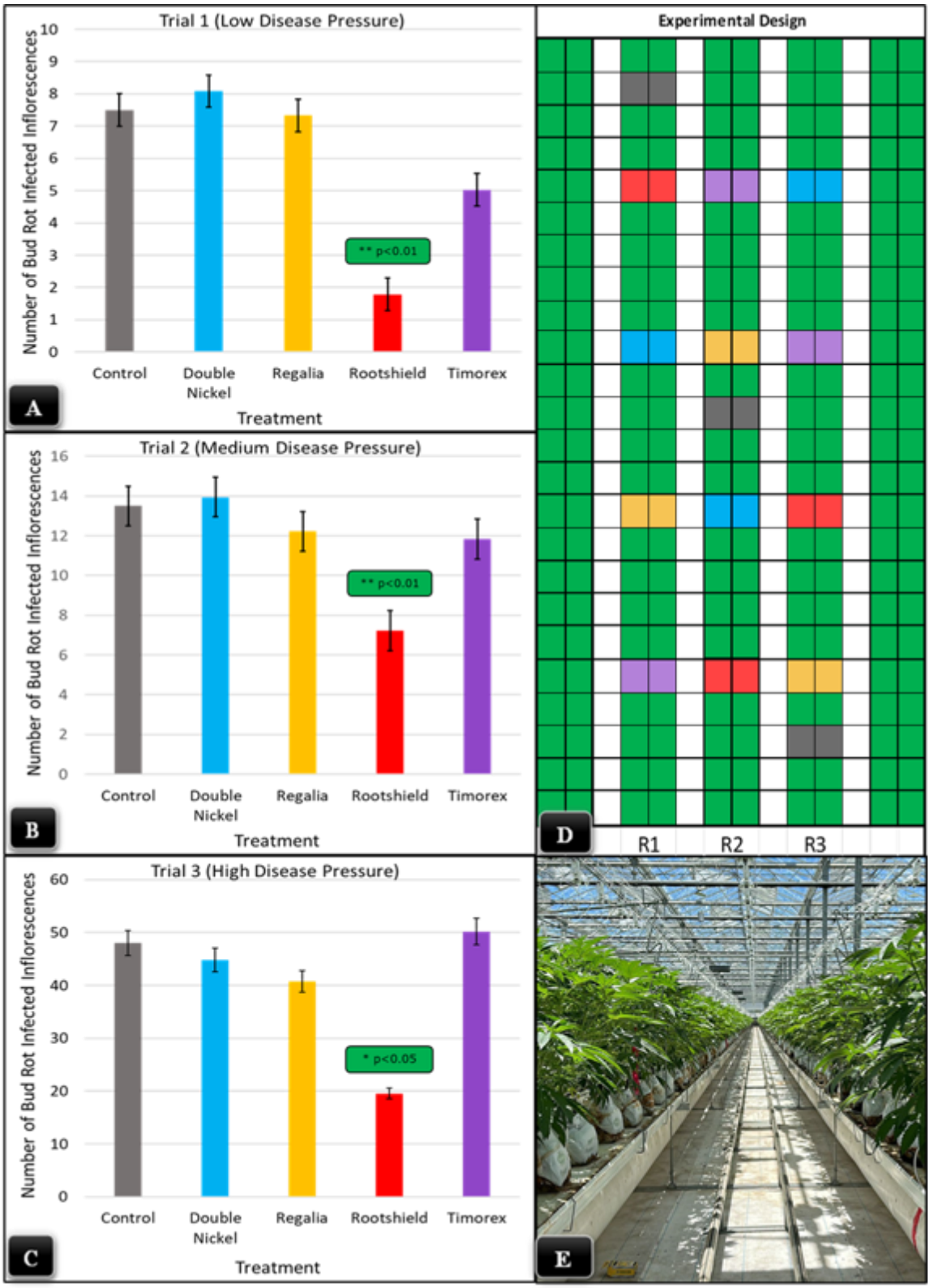


Figure 35. The evaluation of four biological and reduced-risk products for bud rot suppression in a randomized block design experiment resulting from natural infections in genotype 'BC'. (A) High disease pressure. (B) Moderate disease

pressure. (C) Low disease pressure. (D) The arrangement of treatments within the greenhouse in a randomized design is shown. Vertical boxes shaded in green represent an 'aerial' view of parallel rows of cannabis plants, each line of boxes containing 216 plants. Individual boxes contain 9 plants. There were three replicate groups of plants for each treatment done to a low, medium and high *B. cinerea* pressure growing compartment. (E) The greenhouse crop row layout seen from the ground.

A follow up large-scale trial was conducted on two cannabis genotypes in two greenhouse compartments with moderate bud rot (16 infected inflorescences) and high bud rot (45 infected inflorescences) due to *B. cinerea*. Rootshield HC applications significantly reduced disease incidence on both genotypes by 91% in the medium bud rot trial with genotype 'PD' ($p<0.05$) (Figure 36A) and by 56% in the high bud rot trial with genotype 'BC' ($p<0.01$) (Figure 36B).

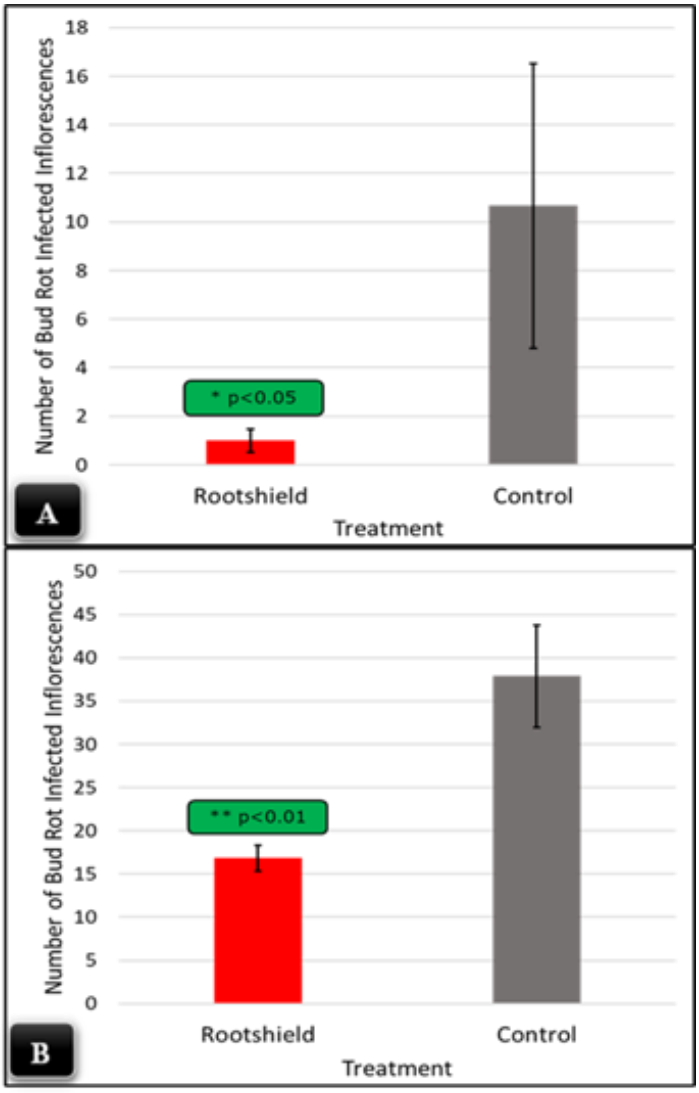


Figure 36. The evaluation of Rootshield HC for bud rot suppression in large-scale spray trials conducted on two flowering cannabis genotypes under varying disease pressures. (A) 'PD' genotype with low disease pressure. (B) 'BC' with high disease pressure.

4.5. Discussion

Distinct morphological changes were observed during inflorescence development in genotype 'OG' over the 7-week flowering period, progressing from no visible flower development under a 24-hour photoperiod to the formation of a dense compound raceme inflorescence under a 12:12-hour photoperiod. The morphological changes in developing cannabis inflorescences have been previously described (Raman et al. 2017; Small 2017; Spitzer-Rimon et al. 2019), but no attempts have been made to correlate these changes with increased susceptibility to infection by plant pathogens. The most dramatic increase in

inflorescence size occurred from the fifth week of the flowering cycle onwards (Figure 27F). By this stage, the inflorescences consisted of compact flower clusters with few air spaces, and the stigmas had changed in color from white to orange/brown, becoming enclosed within the expanding tissues (Figure 31B). This was the stage when the first visible symptoms of bud rot were observed (Figure 31C-F). We propose that the inflorescence structure at 5 weeks provided an optimal microclimate for *B. cinerea* mycelial growth due to the moister and warmer environment. Bud rot was consistently detected initially in the innermost, sheltered portions of the inflorescences before spreading outward, suggesting that infection initially occurred at these sites. Measurements of relative humidity and temperature within these inflorescences in the present study showed that they were significantly higher than the ambient environment. Punja and Ni (2021) observed that the number of bract leaves surrounding cannabis inflorescences increased as the plants approached harvest, providing suitable infection sites and creating a microclimate conducive to infection. Additionally, maturing stigmatic tissues with sticky papillae can capture *B. cinerea* spores (Figure 29C, D) (Punja 2018; Punja and Scott 2023), similar to the way they capture pollen grains (Spitzer-Rimon 2019; Punja and Holmes 2020). Williamson et al. (2007) proposed that stigmatic fluid on some plant stigmas could stimulate conidial germination. They observed *B. cinerea* hyphae growing along style tissues in raspberry and strawberry flowers to reach the ovules, a path similar to that followed by pollen tubes. Supporting these observations, *B. cinerea* often first infects floral organs in other flowering crops (Carisse 2016). In blackcurrants, *B. cinerea* infected via the style or carpels (McNicol and Williamson 1989), while in strawberries, grapes, and waxflowers, various floral organs served as initial infection sites, with infection success depending on the humidity and the specific organ inoculated (Bristow and Williamson 1986; Viret et al. 2004; Dinh et al. 2011). After penetration, *B. cinerea* may remain quiescent before causing the necrosis of leaves, flowers, or mature fruits (Carisse 2016). *B. cinerea* also infects non-floral tissues, such as leaves and fruits, penetrating undamaged tissues directly or through natural openings or wounds (Mahmoud et al. 2023). It remains unclear whether these infections occur during the day or at night when cooler, moister conditions prevail. In tomatoes, peppers, and grapevines, cooler night conditions were more favorable for *B. cinerea* infection (Morgan 1984; Park 1999; Ciliberti et al. 2015). Infrared camera measurements of inflorescence surface temperatures in the present study (Supplementary Figure B1) showed that temperatures could reach 39-41°C in the early afternoon, which would be lethal to *B. cinerea* spores (Punja and Ni 2021). Since these temperatures dropped below 24°C in the evening, which is conducive to spore germination and infection (Xu et al. 2000), it

is likely that infections on cannabis inflorescences by *B. cinerea* occur during the night-time.

In the bud rot disease progression studies conducted on genotype 'OG' following *B. cinerea* inoculation, visible mycelium development was consistently detected between days 33 and 41 of the flowering cycle, with a median detection on day 34 or week 5 (Figure 30A). The most extensive bud rot development was observed for inoculations that were made on days 14, 21, and 28 of the flowering period. We hypothesize that the microclimate within the inflorescences on day 7 of inoculation was unsuitable for infection to occur because the inflorescence was underdeveloped and lacked crevices and senescent tissues for *B. cinerea* mycelial colonization. Similarly, for inoculations performed on day 35, the inflorescences were too large and compact, limiting access to potential internal infection sites for *B. cinerea*. It is conceivable that *B. cinerea* spores applied on days 14, 21, and 28 of the flowering period remained latent until favorable microclimatic conditions allowed mycelial growth to occur, resulting in visible detection between days 33 and 41. Similar observations have been made in grape, strawberry, and rose flowers infected by *B. cinerea* (Elad 1988; Xu et al. 2000; Barnes and Shaw 2002; Keller et al. 2003). In these hosts, infection proceeded only when fruits or flowers approached maturity or when favorable environmental conditions occurred. External environmental conditions can impact the extent of bud rot development on cannabis plants, as seen by the delayed onset of infection in inoculations conducted on days 7, 14, and 21 during the trial ending in March, compared to trials ending in August and September, which fall within the "*Botrytis* susceptibility period." Our findings suggest that the fifth week of the flowering period is an appropriate time to monitor for the onset of *B. cinerea* infections.

The rate at which inflorescences develop and the final sizes achieved vary significantly among cannabis genotypes (Punja et al. 2023). This variability results in different inflorescence weights among genotypes, which correlates with their susceptibility to bud rot: larger, denser inflorescences are more susceptible to infection than smaller, less dense ones (Mahmoud et al. 2023). Other morphological differences that could impact bud rot development, including inflorescence size, have been described by Jin et al. (2021). McPartland (1996) proposed that increased water retention in large inflorescences contributed to their heightened susceptibility to bud rot. These differences could affect the ability of *B. cinerea* spores to initiate infection in different genotypes, resulting in varying disease levels at harvest, but further studies are needed to confirm this. There are no reported biochemical or physiological differences affecting the degree of cannabis genotype susceptibility to *B. cinerea*. Although Punja and Ni (2021) suggested that greater terpene production in maturing inflorescences could enhance infection by *B. cinerea*, Mahmoud et al. (2023) found that

terpene levels in harvested inflorescences of different cannabis genotypes were not correlated with increased or reduced susceptibility to *B. cinerea*. While the role of cannabis terpenes in infection remains unclear, certain terpenes and phytochemicals found in essential oils can inhibit *B. cinerea* development on various crops (Wilson et al. 1997; Fedele et al. 2020; Yong et al. 2021; Tančinová et al. 2022). Balthazar et al. (2020) identified five putative defense genes (ERF1, HEL, PAL, PR1 and PR2) that were strongly upregulated at *B. cinerea* infection sites on leaf tissues, indicating local activation of defense pathways was occurring. Similar studies need to be conducted on inflorescence tissues, the primary infection site for *B. cinerea* on cannabis.

When bud rot severity was assessed for genotypes 'BC' and 'SC' over a 6-month period under variable outdoor weather conditions, the highest disease levels were observed between July and October, with lower incidence at other times of the year (Figure 32A). This period, named the "Botrytis susceptibility period," correlated with rising outdoor absolute humidity and temperatures (Figure 32B), indicating that outdoor weather conditions can influence *B. cinerea* development within a greenhouse. The greenhouse structure is vented throughout the day during summer and fall, allowing heat and humidity to be exchanged. When nine genotypes were assessed for bud rot incidence in July, significant differences were found, with 'BC,' 'CD,' and 'SC' showing the highest susceptibility, while 'DB' and 'A5' had the lowest infection. A similar range of susceptibility levels among cannabis genotypes was reported in recent studies (Mahmoud et al. 2023; Buirs and Punja 2024), suggesting that selective breeding could potentially yield a cultivar with improved resistance to bud rot. In grapes, alfalfa, and petunias, differences in *B. cinerea* susceptibility among genotypes have also been observed (Rahman et al. 2018; Gossen and Lan 2021; Shrestha and Hausbeck 2023). The basis for these differences in host susceptibility has been elucidated for some plants. In chickpea seedlings, differences in stomatal frequency, epidermis texture, and thickness were noted between susceptible and resistant genotypes (Thakur et al. 2023). In strawberry flowers, Xiao et al. (2022) suggested that tolerance to *B. cinerea* was controlled at the transcriptome level. They found that resistant cultivars showed increased expression of genes associated with disease tolerance and decreased expression of cell wall-degrading enzymes. They also emphasized the role of calcium signaling pathways and phytohormone biosynthesis genes in pathogen resistance. In a study of grape wine cultivars, Tziros et al. (2022) showed that tolerance to *B. cinerea* rot involved a complex interaction between defense gene activation and metabolic activity, with resistant varieties exhibiting increased levels of defense compounds and enzymes. Similar studies on cannabis inflorescences could

clarify the underlying differences in susceptibility to bud rot among genotypes. In addition to morphological differences that result in larger, denser buds with increased susceptibility due to microclimatic differences, there may be differences in the levels and types of biochemical compounds that are produced, including terpenes and other volatiles. There are also likely to be differences in *B. cinerea* infection due to gene expression within the inflorescence tissues of susceptible versus tolerant genotypes, but further research at the transcriptomic level is needed to elucidate these differential responses.

When the relative humidity and temperature within inflorescences were compared to ambient conditions, it was observed that inflorescences trap heat and accumulate higher internal humidity. Temperature and relative humidity are known to influence *B. cinerea* sporulation and germination in many crops. Thomas et al. (1988) demonstrated that these variables, along with wind speed, significantly impacted aerial mycelium development and conidia production. They found that optimal mycelial growth on grape berries occurred at 21°C with 94% humidity and no wind. Conidia production was highest under these conditions with slight wind, but mycelium did not develop at 69% humidity with wind. Gossen and Lan (2021) showed that temperature and wetness duration significantly influenced *B. cinerea* infection on alfalfa flowers, with the highest infection occurring at 20°C. Prolonged surface wetness intensified infection, especially between 15°C and 20°C. Ciliberti et al. (2015) observed similar results in grapevines, where infection incidence, mycelial growth, and conidial germination were highest at 20°C and significantly lower at both 5°C and 30°C. These patterns were consistent across different grapevine growth stages. The mycelial growth of *B. cinerea* isolates from cannabis on agar medium was shown to be optimal at 20°C and completely inhibited at 30°C (Punja and Ni 2021).

In the present study, enhancing air circulation using fans resulted in lower relative humidity within inflorescence tissues, significantly reducing bud rot incidence (Figure 34C). Enhanced air circulation can alter the leaf boundary layer, reducing localized relative humidity and limiting spore germination and mycelial development (Kuroyanagi et al. 2014; Yasutake et al. 2015). These findings support the common practice of increasing air circulation to manage *B. cinerea* and other pathogens in greenhouse crops (Morgan 1984; Elad 2016; Baptista et al. 2012). However, continuous air circulation can be challenging to maintain in large greenhouses and may negatively affect plant growth by increasing water stress. Our observations confirm the importance of maintaining low relative humidity to reduce cannabis inflorescence infections by *B. cinerea* under greenhouse conditions.

Climate models have been created to understand the relationship between *B.*

cinerea spore production and climatic conditions. In field strawberries, Xu et al. (2000) created a model which showed that low daytime vapor deficits (high absolute humidity) and warm night-time temperatures (up to 25°C) contributed to increased conidia levels and greater fruit rot incidence. They also monitored field strawberries during the flowering period and found a correlation between elevated conidia numbers and increased temperature and relative humidity. Martínez-Bracero et al. (2022) monitored *B. cinerea* spores in Ireland and showed that atmospheric spores peaked during late summer and were influenced by humidity, rainfall, and specific wind directions. Körner et al. (2014) reported that in greenhouse gerberas, *B. cinerea* spore germination was a function of average spore density and greenhouse microclimate. In our study, we did not monitor conidial levels in the greenhouse, assuming that *B. cinerea* inoculum levels were sufficiently high during periods of elevated bud rot incidence. However, spore monitoring studies within and outside the greenhouse may reveal periods when *B. cinerea* spore levels are highest and how environmental conditions and seasonal differences affect inoculum levels. Our results indicate that both genotype and environmental conditions influence the susceptibility of cannabis inflorescences to bud rot development. Identifying a "*Botrytis* susceptibility period" allows growers to develop a specific timeframe to implement preventative measures, particularly for more susceptible genotypes. Growers can also avoid planting highly susceptible genotypes from July to October or reduce planting density during this period.

Biological control agents and reduced-risk products, including those evaluated in this study, have been developed to manage *B. cinerea* on various crops (Wilson et al. 1997; Nicot et al. 2016; Bika et al. 2020; Roca-Couso 2021; Shrestha and Hausbeck 2021; Esquivel-Cervantes et al. 2022). In the context of cannabis, only a limited number of products are approved for use against *B. cinerea*. In addition to the products tested here, the following are listed on the Health Canada Pesticide Label Search Registry website (https://pr-rp.hc-sc.gc.ca/ls-re/index-eng.php): Actinovate SP (*Streptomyces lydicus* WYEC 108) by Novoenzymes (Bagsvaerd, Denmark); Prestop / Prestop WG (*Gliocladium catenulatum* strain J1446) by Lallemand Plant Care (Kurjenkellontie, Finland); LifeGard WG (*Bacillus mycoides* isolate J) by Certis Biologicals (Columbia, Maryland); and Zerotol (hydrogen peroxide) by BioSafe Systems L.L.C. (East Hartford, Connecticut). Prestop, LifeGard, and Zerotol were tested by Buirs and Punja (2024) during greenhouse cannabis bud rot suppression trials but showed less promising results, so they were not included in this study.

Two microbial biocontrol agents were tested in this study: *Bacillus amyloliquefaciens* in Double Nickel LC and *Trichoderma harzianum* in Rootshield HC. When three applications

of Double Nickel LC were made at 7-day intervals starting on day 14 of the flowering period, no significant reduction in *B. cinerea* severity was observed in small-scale trials. However, Buirs and Punja (2024) found that Double Nickel, when applied following the methods used in the large-scale trials in the present study, significantly reduced disease incidence in one out of two trials. This product may still hold potential for evaluation on different genotypes, in spray rotations, during different flowering stages, or for root zone treatments. No previous studies have been conducted with this biocontrol agent on cannabis crops.

Bacillus amyloliquefaciens is a naturally occurring soil-dwelling and root-colonizing bacterial species that has been successfully applied to plant canopies to suppress *B. cinerea* on peppers, melons, pears, lilies, and rapeseed (Nicot et al. 2011). When applied to the phyllosphere, *B. amyloliquefaciens* can suppress *B. cinerea* by competing for exogenous nutrients and rapidly colonizing niches that *B. cinerea* would otherwise occupy (Canada Pest Management Regulatory Agency, 2015). Antagonism is another mechanism of *B. amyloliquefaciens*, which operates primarily through its production of antifungal compounds such as bacillomycin D and fengycin (Koumoutsi et al. 2004). These compounds are produced at higher concentrations in the presence of specific pathogens, demonstrating a responsive detection and suppression mechanism by this agent (Cawoy et al. 2015). A significant portion of the *B. amyloliquefaciens* genome is dedicated to antibiotic production, with one strain found to allocate over 9% to this function, which is significantly higher than other closely related bacterial species (Chowdhury et al. 2015). Applying *B. amyloliquefaciens* to the root zone has been shown to induce systemic resistance (ISR) against various pathogens and to enhance plant development in a range of plants. Root zone colonization triggers the release of numerous lipopeptides (e.g., surfactin) and volatile organic compounds (e.g., 2,3-butanediol), which reduce disease and promote plant growth by affecting plant signaling pathways like the jasmonic acid signaling pathway (Kloepper et al. 2004; Chowdhury et al. 2015). This *B. amyloliquefaciens*-induced ISR has proven effective against *B. cinerea*, with the plant species being the most significant variable (Sarosh et al. 2009; Salvatierra-Martinez et al. 2018; Zhou et al. 2020). The optimal conditions for *B. amyloliquefaciens* development are a temperature of 37°C and a pH of 5-7 (Gotor-Vila et al. 2017).

Trichoderma harzianum is another naturally occurring rhizosphere species that has been successfully used to suppress *B. cinerea* on numerous crops (Nicot et al. 2011; Nicot, et al., 2016), including tomato (Lee et al. 2006), bean, pepper, tobacco (Meyer et al. 1998), cucumber (Elad et al. 1993), and grapevine (Latorre et al. 1997). Preventive applications of *T. harzianum* reduced gray mold on cucumber plants (Elad et al. 1993) and fruit rot on

strawberry plants (Freeman et al. 2004) under commercial conditions, with results comparable to those obtained with conventional fungicides.

Suppression of *B. cinerea* by *T. harzianum* occurs through competition, antagonism (antibiosis), and hyper-parasitism (mycoparasitism) (Harman et al. 2004; Vos et al. 2015). Competition involves the consumption of exogenous nutrients by *T. harzianum* in the phyllosphere, rendering them unavailable to *B. cinerea* for spore germination. In antagonism situations, *T. harzianum* interferes with pathogenic processes (e.g., reducing pectolytic enzyme activity) and producing antibiotics (e.g., trichozianins A1 and B1) (Elad 1996). In hyper-parasitism situations, *T. harzianum* will physically coil around *B. cinerea* hyphae, followed by the secretion of extracellular proteins like Th-L-AAO (Cheng et al. 2012).

When applied to the root zone, *T. harzianum* can also induce plant immune responses that prime the host plant against pathogens (Harman et al. 2004; Hermosa et al. 2013; Nawrocka and Małolepsza 2013; Vos et al. 2015; Rodrigues et al. 2023). This application suppresses *B. cinerea* in plant canopies by inducing biochemical changes that activate the plant's defense system (Zimand et al. 1996; Meyer et al. 1998; Martínez-Medina et al. 2013). Besides acting as a biocontrol agent, *T. harzianum* promotes plant development by forming a symbiotic relationship with the root zone (rhizosphere competence) (Harman 2000), often resulting in increased root and shoot growth (Yedidia et al. 2001; Chacón 2007) and increased yield. These effects depend on the strain of *T. harzianum* and the targeted crop (Harman 2011). The optimal environment for *T. harzianum* development is a low (~1 kPa) vapor pressure deficit (VPD) microclimate (Elad 1996), with temperatures around 30°C (Gams and Bisset 1998).

In the present study, multiple applications of Rootshield made at 7-day intervals starting 4 weeks before symptom appearance significantly reduced *B. cinerea* incidence in both small-scale and large-scale trials. The efficacy of this biocontrol agent may vary due to seasonal climatic differences, which can influence disease pressure. Punja and Ni (2021) reported a significant reduction in disease severity and increased colonization and sporulation of the biocontrol agents *T. asperellum* and *Gliocladium catenulatum* (Prestop) on detached cannabis inflorescences in vitro. In contrast, Kirkby et al. (2023) observed no significant reduction in *B. cinerea* lesion development on detached hemp leaves following *T. harzianum* treatment, possibly due to less favorable conditions for the biocontrol agent to establish on leaves compared to inflorescences. For optimal efficacy, *T. harzianum* spores need to establish within inflorescences and colonize the stigmas and bract tissues, which are infection sites for *B. cinerea*. Hjeljord et al. (2001) demonstrated that *T. harzianum* was able to colonize

newly opened strawberry flowers and those with dehisced petals more effectively than *B. cinerea*, providing greater protection against infection. The biocontrol agent also colonized the stamens and stigmas of strawberry flowers. Although the extent to which *T. harzianum* can colonize pistillate flowers in cannabis is unknown, it likely occurs extensively to provide the level of protection against *B. cinerea* observed in this study. Scanning electron microscopic studies of *G. catenulatum* on detached cannabis inflorescences showed that the biocontrol agent colonized and extensively sporulated within 5 days of application (Punja and Ni 2021).

The ability of *T. harzianum* to colonize and establish in cannabis flowers was unexpected, as it is primarily found in the plant rhizosphere, where root exudates and a stable environment favor survival (Andrews 1992). Despite this, some strains of *T. harzianum* occur naturally in the phyllosphere of crops such as cucumber and tomato (Mónaco et al. 2009; Sawant 2014). The survival of *Trichoderma* spp. in the phylloplane varies depending on the crop, age of the plant organ, humidity and temperature conditions, and the nutrient regime under which *Trichoderma* was introduced (Elad and Kirshner 1992, 1993). Foliar applications of *T. harzianum* have been shown to disrupt microbial communities in the phyllosphere, suppressing not only *B. cinerea* but also other microbes on strawberry plants (Sylla et al. 2013) and cannabis plants (Buirs and Punja 2024). Applying *Trichoderma* to flowering cannabis plants may increase the total colony-forming units in harvested inflorescences, which could increase the risk of failing mandatory Health Canada yeast and mold tests (Punja 2021c; Punja et al. 2023). High microbial counts exceeding the limit of 50,000 cfu/g can be reduced using irradiation such as gamma or electron beam to sterilize batches (Hazekamp 2016; Jerushalmi et al. 2020b; Majumdar et al. 2023), but the cost of these methods can be prohibitive for many cannabis producers.

Two additional non-microbial reduced-risk products evaluated in this study—Timorex Gold and Regalia Maxx—are formulated from extracts derived from *Melaleuca alternifolia* (tea tree) and *Reynoutria sachalinensis* (giant knotweed), respectively (Abbey et al. 2019). *Melaleuca alternifolia*, a perennial shrub originating from Australia, produces tea tree oil, which has demonstrated antifungal activity against *B. cinerea* in several studies (Bishop and Reagan 1998; Cheng and Shao 2011; Nicot et al. 2016; Shao et al. 2013). This extract shows broad-spectrum activity against *B. cinerea*, offering multi-target fungicidal effects either prophylactically or curatively. It inhibits conidia germination and mycelial growth (Nicot et al. 2016). Tea tree oil primarily works by altering *B. cinerea* membrane permeability through changes in fatty acid composition and electrical conductivity, causing leakage or coagulation of cytoplasmic material (Shao et al. 2013). This effect is attributed to components in the

extract such as terpinen-4-ol, alpha-terpineol, terpinolene, and 1,8-cineole, which are synergistic in their antifungal activity (Yu et al. 2015).

Foliar applications of *R. sachalinensis* have also been shown to suppress *B. cinerea* on various crops with moderate to high efficacy (Schilder et al. 2002; Schmitt 2001; Schmitt et al., 2002), although specific research on its mode of action is limited. As an active ingredient in reduced-risk products, *R. sachalinensis* triggers induced systemic resistance (ISR) through the absorption of its active compounds (e.g., emodin) into plants. This leads to an increase in defensive compounds (e.g., phenolics) that inhibit *B. cinerea* (Daayf 2000; Nicot et al. 2016). The extract also enhances enzyme activity (e.g., chitinase) in various plants (Schneider and Ullrich 1994), providing translaminar protection by inducing papillae formation and lignification of cell walls (Nicot et al. 2016). These changes prime plants to rapidly defend against pathogenic attacks. However, *R. sachalinensis* does not seem to affect *B. cinerea* conidial germination or mycelial growth (Schmitt 2001). Uniquely, *R. sachalinensis* has been found to increase chlorophyll levels in some crops (Schmitt 2001; Scott and Punja 2021) and to delay leaf senescence in cucumber plants (Schmitt 2001). When applied to different *Begonia* cultivars, it caused various physiological effects, including compact growth, expedited flower induction, increased flower size, and red-variegated leaves. In cyclamen cultivars, stem elongation was observed (Schmitt 2001). These "side effects" could potentially benefit cannabis beyond disease protection.

In conclusion, this study identified the stages of cannabis inflorescence development which are most vulnerable to *B. cinerea* infection, revealing that inflorescences in the fifth week of flowering are particularly susceptible. Inoculation timing significantly influenced disease progression, with the most severe disease occurring from inoculations made at days 14, 21, and 28 of the flowering period. This information provides guidance for the optimal timing of suppression strategies. The study also highlighted the impact of environmental factors, particularly temperature and humidity, on infection progression, establishing a "*Botrytis* susceptibility period" from July to October, when disease pressure was highest, and identifying a bud rot microclimate risk range of approximately 60-87% relative humidity and 19.5-30.2°C within inflorescences. Among the management approaches identified, the discovery of significant differences in bud rot susceptibility between cannabis genotypes highlights the importance of selecting or breeding resistant genotypes. Enhanced air circulation was found to be a highly effective disease reduction strategy, which reduced relative humidity within inflorescences and significantly suppressed *B. cinerea* infection. Applications of the biocontrol agent *T. harzianum*, the active ingredient in Rootshield HC, also

effectively suppressed bud rot. Additionally, other biocontrol and reduced-risk products, including Double Nickel LC, Timorex Gold, and Regalia Maxx, demonstrated varying levels of efficacy. Integrating these strategies with other pest management approaches could yield promising results in managing bud rot. Overall, this study provides growers and researchers with a foundational resource for understanding the epidemiology of *B. cinerea* and integrating proven strategies into greenhouse-cultivated cannabis operations to suppress this destructive pathogen.

Chapter 5. Discussion & Concluding Remarks

The cultivation of *Cannabis sativa* in Canadian greenhouse environments poses significant challenges due to the plants' susceptibility to various pathogens. To address this issue, we developed a comprehensive Integrated Disease Management (IDM) framework that focused on managing key pathogens such as *Botrytis cinerea* (bud rot), *Fusarium* spp. (root rot), *Pythium* spp., powdery mildew (*Golovinomyces ambrosiae*), and Hop Latent Viroid (HLVd). This IDM protocol is tailored to the unique challenges of greenhouse-grown cannabis and combines cultural, environmental, genetic, and biological strategies to mitigate disease threats across all stages of the production cycle.

The early pathogen detection of pathogens and precise timing of interventions were crucial factors in creating a practical and commercially applicable IDM framework. For instance, artificial spore inoculations of *B. cinerea* conducted at 14, 21, or 28 days into the 49-day flowering period resulted in the highest disease development compared to inoculations at 7 or 35 days. Visible mycelial growth within inflorescences was observed at 33–41 days, regardless of inoculation time. These findings illustrate the importance of timing treatment during the critical window before *B. cinerea* becomes established.

Genotype selection emerged as a crucial factor in the effectiveness of the IDM framework. We observed significant variability among cannabis genotypes in their susceptibility to various pathogens. Certain genotypes, such as 'LB', exhibited greater resistance to bud rot, powdery mildew, hop latent viroid, and root disease. Depending on the genotype, the incidence of bud rot ranged from 1% to as much as 13%. This finding reinforces the idea that genotype selection can serve as an important line of defense against cannabis diseases. Inherent resistance or tolerance to pathogens in specific genotypes has the potential to reduce reliance on external interventions and to improve the sustainability of disease management practices. The genetic variability observed also demonstrates the importance of breeding programs that focus on enhancing disease resistance, as resistance research in cannabis has been limited. While current breeding efforts have primarily prioritized maximizing yields and THC content, incorporating disease-resistance traits into breeding objectives could significantly reduce pathogen pressures and improve overall plant health.

Cultural and environmental controls served as highly accessible tools for reducing pathogen pressure. Adjusting key environmental parameters—such as

humidity, temperature, and plant density—played a significant role in controlling pathogen proliferation. Our observations indicated that within 49-day-old inflorescences, humidity and temperature were 15.4% and 2.5°C higher, respectively, than ambient conditions. Enhanced air circulation using strategically placed fans effectively reduced the risk of bud rot by mitigating microclimatic conditions conducive to *Botrytis cinerea* growth. Specifically, enhanced air circulation reduced bud rot incidence by 66–92%. This intervention was particularly impactful during the high-humidity months of June to November, when disease incidence was highest, ranging from 1% to 13% on inflorescences depending on the genotype evaluated. Furthermore, adjusting planting density and considering seasonal plantings are additional steps that can help mitigate pathogen pressures at vulnerable growth stages when more easily adjustable climate control measures are insufficient. For example, scheduling flowering periods outside the high-humidity months of September and October could reduce bud rot incidence.

The application of biological control agents (BCAs) was another impactful component of our IDM strategy. Early introduction of biological root-zone agents—*Trichoderma harzianum* (Rootshield), *Trichoderma asperellum* (Asperello), and *Gliocladium catenulatum* (Prestop)—were found to significantly suppress root pathogens like *Fusarium* and *Pythium* spp. Additionally, *Trichoderma harzianum* (Rootshield HC) demonstrated significant efficacy in reducing bud rot caused by *Botrytis cinerea*. Preventive applications of *T. harzianum* at weeks 2, 3, and 4 of the flowering stage reduced disease by 47–91% under moderate and high disease pressures. The success of this BCA lies in its ability to colonize inflorescence tissues competitively, thereby reducing opportunities for pathogen establishment and proliferation. Other BCAs, such as *Bacillus amyloliquefaciens* (Double Nickel LC), *Bacillus mycoides* (LifeGard), *Gliocladium catenulatum* (Prestop), and the reduced-risk product Regalia Maxx, provided varying levels of disease reduction. Treatments with these agents showed that biological products could be integrated into disease management programs but that their efficacy varied with environmental conditions and the timing of applications. This variability underscores the importance of precisely aligning BCA applications with plant growth stages, environmental conditions, and pathogen pressures to achieve optimal results.Future research in cannabis integrated disease management should prioritize several key areas to enhance plant health, yield, and disease control. Evaluating endophytes and microbial antagonists would likely be of significant value, as certain bacterial and fungal species show promise in inhibiting pathogens and promoting plant

growth through a variety of mechanisms. The identification of the full profile of beneficial and pathogenic endophytes in greenhouse-cultivated cannabis plants via sequencing studies will aid this approach. Preliminary research has been performed, but more extensive studies examining the locations these endophytes inhabit in the plant and how they spread would be valuable. Supplementary amendments of endophytes determined to be beneficial is an area of research that has proven fruitful and deserves further exploration. Another area of research that could present significant value would be investigating genotypic differences—including morphological, biochemical, and transcriptomic factors—which may lead to insights that guide the breeding of resistant cultivars. Advancing tissue culture techniques would help growers produce pathogen-free plants more economically and would be of particular importance to aid efforts to eliminate viruses and viroids from commercial cannabis operations, but scaling up these methods remains a challenge. Researching the colonization patterns of biocontrol agents like *Trichoderma harzianum* in cannabis flowers could influence how it is applied to cannabis crops, optimizing its efficacy. Investigating supplementation with nutrients like copper, silicon, and calcium could suppress diseases by strengthening plant defenses or inhibiting pathogen development, but optimal formulations and application methods need further research. Incorporating advanced technologies such as AI-powered disease detection platforms, infrared imaging for early stress identification, and electronic nose systems to detect pathogen-induced volatile compounds could revolutionize disease monitoring. However, these technologies require refinement to accurately distinguish between similar stress symptoms, and the economics of using these at a commercial scale are unclear. Additionally, exploring the potential benefits of induced plant defense responses via the application of natural compounds or beneficial microbes presents a promising strategy for enhancing disease resistance. Overall, these research directions offer the opportunity to deepen the understanding of the cannabis pathosystem and improve the integrated management strategies available for cannabis producers.

This thesis presents a robust integrated disease management (IDM) framework for cultivating *Cannabis sativa*, combining preventive measures, cultural and environmental controls, genotype selection, and biological control agents to effectively suppress the most significant greenhouse cannabis pathogens. Pre-emptive actions like selecting disease-tolerant genotypes and implementing strategic cultural practices, coupled with the use of biological agents and environmental modifications, have shown

promising results in minimizing disease incidence. Exploring alternative strategies—including beneficial endophytes, tissue culture techniques, nutrient supplementation, and technology-aided scouting—offers additional avenues for enhancing plant health and resilience. Broad adoption of these integrated strategies could provide significant economic and environmental benefits, but ongoing research and regulatory support are essential to refine the IDM framework and meet evolving challenges. With continued innovation and technological integration, this IDM approach could serve as a foundation for sustainable, high-quality cannabis production worldwide, enabling producers to enhance yield and quality while minimizing costs and environmental impacts.

References

Abbey, J., Percival, D., Abbey, L., Asiedu, S. K., Prithiviraj, B., & Schilder, A. (2019). Biofungicides as an alternative to synthetic fungicides for the control of grey mould (*Botrytis cinerea*)—Prospects and challenges. *Biocontrol Science and Technology*, *29*(3), 207–228. https://doi.org/10.1080/09583157.2018.1548574

Abdu-Allah, G. A. M., & Abo-Elyousr, K. A. M. (2017). Effect of certain plant extracts and fungicides against powdery mildew disease of grapevines in Upper Egypt. *Archives of Phytopathology and Plant Protection*, *50*(19-20), 957–969. https://doi.org/10.1080/03235408.2017.1407471

Abel, E. L. (1980). *Marihuana: The first twelve thousand years*. Springer Science & Business Media.

Adhikary, D., Kulkarni, M., El-Mezawy, A., Mobini, S., Elhiti, M., Gjuric, R., Ray, A., Polowick, P., Slaski, J. J., & Jones, M. P. (2021). Medical cannabis and industrial hemp tissue culture: Present status and future potential. *Frontiers in Plant Science*, *12*, Article 627240. https://doi.org/10.3389/fpls.2021.627240

Adkar-Purushothama, C. R., Sano, T., & Perreault, J.-P. (2023). Hop latent viroid: A hidden threat to the cannabis industry. *Viruses*, *15*(3), Article 681. https://doi.org/10.3390/v15030681

Ahmed, M. F. A. (2018). Evaluation of some biocontrol agents to control Thompson Seedless grapevine powdery mildew disease. *Egyptian Journal of Biological Pest Control*, *28*, Article 93. https://doi.org/10.1186/s41938-018-0098-0

Ahrens, A., Llewellyn, D., & Zheng, Y. (2023). Is twelve hours really the optimum photoperiod for promoting flowering in indoor-grown cultivars of *Cannabis sativa*? *Plants*, *12*(14), Article 2605. https://doi.org/10.3390/plants12082605

Ajouz, S., Walker, A. S., Fabre, F., Leroux, P., Nicot, P. C., & Bardin, M. (2010). Variability of *Botrytis cinerea* sensitivity to pyrrolnitrin, an antibiotic produced by biological control agents. *BioControl*, *56*(3), 353–363. https://doi.org/10.1007/s10526-010-9333-7

Akinrinlola, R. J., & Hansen, Z. R. (2023). Efficacy of organic fungicides against hemp powdery mildew caused by *Golovinomyces ambrosiae* in a greenhouse in Tennessee. *Plant Disease*, *107*(6), 1867–1873. https://doi.org/10.1094/PDIS-09-22-2094-RE

Aleksić, G., Milićević, Z., Kuzmanović, S., Starović, M., Stevanović, M., Delibašić, G., & Živković, S. (2019). Efficacy of copper citrate in grapevine disease control. *Pesticidi i fitomedicina*, *34*(2), 103–109. https://doi.org/10.2298/PIF1902103A

Anagnostis, A., Tagarakis, A. C., Asiminari, G., Papageorgiou, E., Kateris, D., Moshou, D., & Bochtis, D. (2021). A deep learning approach for anthracnose infected trees classification in walnut orchards. *Computers and Electronics in Agriculture*, *182*, 105998. https://doi.org/10.1016/j.compag.2021.105998

André, C., Hausman, J. F., & Guerriero, G. (2016). *Cannabis sativa*: The plant of the thousand and one molecules. *Frontiers in Plant Science*, *7*, Article 19. https://doi.org/10.3389/fpls.2016.00019

Andrews, J. H. (1992). Biological control in the phyllosphere. *Annual Review of Phytopathology*, *30*, 603–605. https://doi.org/10.1146/annurev.py.30.090192.003131

Atallah, O. O., Yassin, S. M., & Verchot, J. (2024). New insights into hop latent viroid detection, infectivity, host range, and transmission. *Viruses*, *16*, Article 30. https://doi.org/10.3390/v16010030

Avila-Adame, C., Tan, E., Campbell, B., Huang, H., Fernandez, L., Koivunen, M., & Marrone, P. (2008). MOI-106: A new alternative for controlling fungal plant pathogens in ornamentals and edible crops. *Phytopathology*, *98*, S16.

Awasthi, L. P. (2021). *Biopesticides in organic farming: Recent advances*. CRC Press.

Balthazar, C., Cantin, G., Novinscak, A., Joly, D. L., & Filion, M. (2020). Expression of putative defense responses in cannabis primed by *Pseudomonas* and/or *Bacillus* strains and infected by *Botrytis cinerea*. *Frontiers in Plant Science*, *11*, Article 572112. https://doi.org/10.3389/fpls.2020.572112

Balthazar, C., Novinscak, A., Cantin, G., Joly, D. L., & Filion, M. (2022). Biocontrol activity of *Bacillus* spp. and *Pseudomonas* spp. against *Botrytis cinerea* and other cannabis fungal pathogens. *Phytopathology*, *112*, 549–560. https://doi.org/10.1094/PHYTO-03-21-0128-R

Baptista, F. J., Bailey, B. J., & Meneses, J. F. (2012). Effect of nocturnal ventilation on the occurrence of *Botrytis cinerea* in Mediterranean unheated tomato greenhouses. *Crop Protection*, *32*, 144–149. https://doi.org/10.1016/j.cropro.2011.11.005

Barcaccia, G., Palumbo, F., Scariolo, F., Vannozzi, A., Borin, M., & Bona, S. (2020). Potentials and challenges of genomics for breeding *Cannabis* cultivars. *Frontiers in Plant Science*, *11*, Article 573299. https://doi.org/10.3389/fpls.2020.573299

Barnes, S. E., & Shaw, M. W. (2002). Factors affecting symptom production by latent *Botrytis cinerea* in *Primula* × *polyantha*. *Plant Pathology*, *51*(6), 746–754. https://doi.org/10.1046/j.1365-3059.2002.00761.x

Bassolino, L., Fulvio, F., Cerrato, A., Citti, C., Cannazza, G., Capriotti, A. L., Alberti, I., Terracciano, I., Pecchioni, N., & Paris, R. (2024). Metabolic characterization and transcriptional profiling of polyphenols in *Cannabis sativa* L. inflorescences with different chemical phenotypes. *Planta*, *260*(4), Article 76. https://doi.org/10.1007/s00425-024-04505-z

Beever, R. E., & Weeds, P. L. (2007). Taxonomy and genetic variation of *Botrytis* and *Botryotinia*. In Y. Elad, B. Williamson, P. Tudzynski, N. Delen. (Eds.), *Botrytis: Biology, pathology and control* (pp. 29–52). Springer Netherlands. https://doi.org/10.1007/978-1-4020-2626-3_3

Berdugo, C. A., Zito, R., Paulus, S., & Mahlein, A. K. (2014). Fusion of sensor data for the detection and differentiation of plant diseases in cucumber. *Plant Pathology*, *63*(6), 1344–1356. https://doi.org/10.1111/ppa.12219

Bhojwani, S. S., & Dantu, P. K. (2013). *Plant tissue culture: An introductory text*. Springer. https://doi.org/10.1007/978-81-322-1026-9

Bika, R., Palmer, C., Alexander, L., & Baysal-Gurel, F. (2020). Comparative performance of reduced-risk fungicides and biorational products in management of postharvest *Botrytis* blight on bigleaf hydrangea cut flowers. *HortTechnology*, *30*(6), 659–669. https://doi.org/10.21273/HORTTECH04656-20

Birch, N., & Glare, T. R. (Eds.). (2020). *Biopesticides for sustainable agriculture*. Burleigh Dodds Science Publishing.

Bishop C. D., & Reagan J. (1998). Control of the storage pathogen *Botrytis cinerea* on Dutch white cabbage (*Brassica oleracea* var. *capitata*) by the essential oil of *Melaleuca alternifolia*. *Journal of Essential Oil Research*, *10*(1), 57–60. https://doi.org/10.1080/10412905.1998.9700838

Blanco de Santos, B., & Romero, F. (2006). Relationship between concentrations of *Botrytis cinerea* conidia in air, environmental conditions, and the incidence of grey mould in strawberry flowers and fruits. *European Journal of Plant Pathology*, *114*(4), 415–425. https://doi.org/10.1007/s10658-006-0007-3.

Blanco-Ulate, B., Morales-Cruz, A., Amrine, K. H., Labavitch, J. M., Powell, A. T., & Cantu, D. (2014). Genome-wide transcriptional profiling of *Botrytis cinerea* genes targeting plant cell walls during infections of different hosts. *Frontiers in Plant Science*, *5*, Article 435. https://doi.org/10.3389/fpls.2014.00435

Borgatta, J., Ma, C., Hudson-Smith, N., Elmer, W., Plaza Pérez, C. D., De La Torre-Roche, R., Zuverza-Mena, N., Haynes, C. L., White, J. C., & Hamers, R. J. (2018). Copper based nanomaterials suppress root fungal disease in watermelon (*Citrullus lanatus*): Role of particle morphology, composition and dissolution behavior. *ACS Sustainable Chemistry & Engineering*, *6*, 14847–14856. https://doi.org/10.1021/acssuschemeng.8b04171

Bridgen, M. P. (2016). Using ultraviolet-C (UV-C) irradiation on greenhouse ornamental plants for growth regulation. *Acta Horticulturae*, *1134*, 49–56. https://doi.org/10.17660/ActaHortic.2016.1134.6

Bristow, P. R., McNicol, R. J., & Williamson, B. (1986). Infection of strawberry flowers by *Botrytis cinerea* and its relevance to grey mould development. *Annals of Applied Biology*, *109*(3), 545–554. https://doi.org/10.1111/j.1744-7348.1986.tb03211.x

Buirs, L., & Punja, Z. K. (2024). Integrated management of pathogens and microbes in *Cannabis sativa* L. (Cannabis) under greenhouse conditions. *Plants*, *13*(6), 786. https://doi.org/10.3390/plants13060786

Bulger, M., Ellis, M., & Madden, L. (1987). Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. *Phytopathology*, *77*(8), 1225–1230. https://doi.org/10.1094/Phyto-77-1225

Busby, P. E., Ridout, M., & Newcombe, G. (2016). Fungal endophytes: Modifiers of plant disease. *Plant Molecular Biology*, *90*(6), 645–655. https://doi.org/10.1007/s11103-016-0473-1

Canada Pest Management Regulatory Agency. (2015). *Bacillus amyloliquefaciens* strain D747. Government of Canada. https://publications.gc.ca/site/eng/9.806503/publication.html

Caplan, D., Matzneller, P., & Gutierrez, J. D. (2022). Harvest and post-harvest. In Y. Zheng (Ed.), *Handbook of cannabis production in controlled environments* (pp. 292–310). CRC Press.

Carisse, O. (2016). Epidemiology and aerobiology of *Botrytis* spp. In S. Fillinger & Y. Elad (Eds.), *Botrytis: The fungus, the pathogen and its management in agricultural systems* (pp. 127–148). Springer International.

Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P., & Ongena, M. (2015). Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. *Microbial Biotechnology*, *8*(2), 281–295. https://doi.org/10.1111/1751-7915.12238

Cellini, A., Blasioli, S., Biondi, E., Bertaccini, A., Braschi, I., & Spinelli, F. (2017). Potential applications and limitations of electronic nose devices for plant disease diagnosis. *Sensors*, *17*, 2596. https://doi.org/10.3390/s17112596

Chacón, M. R., Rodríguez-Galán, O., Benítez, T., Sousa, S., Rey, M., Llobell, A., & Delgado-Jarana, J. (2007). Microscopic and transcriptome analyses of early

colonization of tomato roots by *Trichoderma harzianum*. *International Microbiology*, *10*(1), 19–27. https://doi.org/10.2436/20.1501.01.4

Chandra, S., Lata, H., ElSohly, M. A., Walker, L. A., & Potter, D. (2017a). Cannabis cultivation: Methodological issues for obtaining medical-grade product. *Epilepsy & Behavior*, *70*(Pt. B), 302–312. https://doi.org/10.1016/j.yebeh.2016.11.029

Chandra, S., Lata, H., Khan, I. A., & ElSohly, M. A. (2017b). *Cannabis sativa* L.: Botany and horticulture. In S. Chandra, H. Lata, & M. A. ElSohly (Eds.), *Cannabis sativa L.: Botany and biotechnology* (pp. 79–100). Springer International.

Charles, M., Goulet, A., & Arul, J. (2008). Physiological basis of UV-C induced resistance to *Botrytis cinerea* in tomato fruit IV. Biochemical modification of structural barriers. *Postharvest Biology and Technology*, *47*(1), 41–53. https://doi.org/10.1016/j.postharvbio.2007.05.019

Cheng, C., Yang, C., & Peng, K. (2012). Antagonism of *Trichoderma harzianum* ETS 323 on *Botrytis cinerea* mycelium in culture conditions. *Phytopathology*, *102*(11), 1054–1063. https://doi.org/10.1094/PHYTO-11-11-0315

Cheng, S., & Shao, X. (2011, May). In vivo antifungal activities of the tea tree oil vapor against *Botrytis cinerea*. In *Proceedings of the 2011 International Conference on New Technology of Agricultural* (pp. 949–951). Ningbo University.

Chou, M. C., & Preece, T. F. (1968). The effect of pollen grains on infections caused by *Botrytis cinerea* Fr. *Annals of Applied Biology*, *62*(1), 11–22. https://doi.org/10.1111/j.1744-7348.1968.tb03846.x

Chowdhury, S. P., Hartmann, A., Gao, X., & Borriss, R. (2015). Biocontrol mechanism by root-associated *Bacillus amyloliquefaciens* FZB42—a review. *Frontiers in Microbiology*, *6*, 780. https://doi.org/10.3389/fmicb.2015.00780

Ciliberti, N., Fermaud, M., Languasco, L., & Rossi, V. (2015). Influence of fungal strain, temperature, and wetness duration on infection of grapevine inflorescences and young berry clusters by *Botrytis cinerea*. *Phytopathology*, *105*(3), 325–333. https://doi.org/10.1094/PHYTO-05-14-0152-R

Clarke, R. C., & Merlin, M. D. (2013). *Cannabis: Evolution and ethnobotany*. University of California Press.

Coertze, S., & Holz, G. (1999). Surface colonization, penetration, and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of *Botrytis cinerea*. *Plant Disease*, *83*(10), 917–924. https://doi.org/10.1094/PDIS.1999.83.10.917

Comeau, D., Balthazar, C., Novinscak, A., Bouhamdani, N., Joly, D. L., & Filion, M. (2021). Interactions between *Bacillus* spp., *Pseudomonas* spp., and *Cannabis sativa* promote plant growth. *Frontiers in Microbiology*, *12*, 715758. https://doi.org/10.3389/fmicb.2021.715758

Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, *19*, 29–37. https://doi.org/10.1016/j.jare.2019.03.004

Daayf, F., Ongena, M., Boulanger, R., El Hadrami, I., & Bélanger, R. R. (2000). Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew infected plants with extracts of *Reynoutria sachalinensis*. *Journal of Chemical Ecology*, *26*(7), 1579–1593. https://doi.org/10.1023/A:1005578510954

Darras, A. I., Demopoulos, V., Bali, I., & Tiniakou, C. (2012). Photomorphogenic reactions in geranium stimulated by brief exposures of ultraviolet-C irradiation. *Plant Growth Regulation*, *68*, 343–350. https://doi.org/10.1007/s00344-011-9453-z

Datnoff, E. L., Elmer, W. H., & Rodrigues, F. A. (2023). *Mineral Nutrition and Plant Disease* (2nd ed.). American Phytopathological Society.

Dik, A. J. & Albajes, R. (2000). Principles of Epidemiology, Population Biology, Damage Relationships and Integrated Control of Diseases and Pests. In R. Albajes, M. L. Gullino, J. C. van Lenteren, & Y. Elad (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 69-81). Kluwer Academic Publishers. https://doi.org/10.1007/0-306-47585-5

De Jesús Romo-Paz, F., Folgado, R., Delgado-Aceves, L., Zamora-Natera, J. F., & Portillo, L. (2021). Tissue culture of *Physalis angulata* L. (Solanaceae): Techniques for micropropagation and germplasm long-term preservation. *Plant Cell, Tissue and Organ Culture*, *144*, 73–78. https://doi.org/10.1007/s11240-020-01822-2

De Silva, N. I., Brooks, S., Lumyong, S., & Hyde, K. (2019). Use of endophytes as biocontrol agents. *Fungal Biology Reviews*, *33*, 133–148. https://doi.org/10.1007/s13225-019-00405-y

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. (2012). The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, *13*(4), 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Dewey, F. M., & Grant-Downton, R. (2015). Botrytis biology, detection and quantification. In S. Fillinger & Y. Elad (Eds.), *Botrytis – the fungus, the pathogen and its management in agricultural systems* (pp. 17–34). Springer International. https://doi.org/10.1007/978-3-319-23371-0_2

Dhillon, G. S., Hukkeri, S., Nightingale, D., & Callaway, J. (2022). Evaluation of different techniques to decontaminate medical cannabis and their effect on cannabinoid content. *ACS Agricultural Science & Technology*, *2*(9), 1126–1133. https://doi.org/10.1021/acssagcy.2c00312

Dinh, S. Q., Joyce, D. C., Irving, D. E., & Wearing, A. H. (2011). Histology of waxflower (*Chamelaucium* spp.) flower infection by *Botrytis cinerea*. *Plant Pathology*, *60*(2), 278–287. https://doi.org/10.1111/j.1365-3059.2010.02347.x

Dixon, E., Leonberger, K., Amsden, B., Szarka, D., Munir, M., Payee, W., Datnoff, L., Tubana, B., & Gauthier, N. (2022). Suppression of hemp powdery mildew using root-applied silicon. *Plant Health Progress*, *23*(3), 260–264. https://doi.org/10.1094/PHP-01-22-0005-SC

Dumigan, C. R., & Deyholos, M. K. (2022). Cannabis seedlings inherit seed-borne bioactive and anti-fungal endophytic bacilli. *Plants*, *11*(20), Article 2127. https://doi.org/10.3390/plants11202127

Elad, Y., & Kirshner, B. (1992). Establishment of an active *Trichoderma* population in the phylloplane and its effect on grey mould (*Botrytis cinerea*). *Phytoparasitica*, *20*(1), 137–141. https://doi.org/10.1007/BF02980425

Elad, Y., & Kirshner, B. (1993). Survival in the phylloplane of an introduced biocontrol agent (*Trichoderma harzianum*) and populations of the plant pathogen *Botrytis cinerea* as modified by abiotic conditions. *Phytoparasitica*, *21*(4), 303–313. https://doi.org/10.1007/BF02981048

Elad, Y., Zimand, G., Zaqs, Y., Zuriel, S., & Chet, I. (1993). Use of *Trichoderma harzianum* in combination or alternation with fungicides to control cucumber grey mould (*Botrytis cinerea*) under commercial greenhouse conditions. *Plant Pathology*, *42*(3), 324–332. https://doi.org/10.1111/j.1365-3059.1993.tb01508.x

Elad, Y. (1988). Latent infection of *Botrytis cinerea* in rose flowers and combined chemical and physiological control of the disease. *Crop Protection*, *7*(6), 361–366. https://doi.org/10.1016/0261-2194(88)90003-8

Elad, Y. (2016). Cultural and Integrated Control of Botrytis spp. In: Fillinger S, Elad Y, editors. *Botrytis* - the Fungus, the Pathogen and Its Management in Agricultural Systems. Cham (Switzerland): Springer International. p. 149–164.

Elad, Y., Pertot, I., Cotes Prado, A. M., & Stewart, A. (2015). Plant hosts of *Botrytis* spp. In S. Fillinger & Y. Elad (Eds.), *Botrytis – the fungus, the pathogen and its management in agricultural systems* (pp. 413–486). Springer International Publishing. https://doi.org/10.1007/978-3-319-23371-0_20

Elad, Y. (1996). Mechanisms involved in the biological control of *Botrytis cinerea*-incited diseases. *European Journal of Plant Pathology*, *102*(8), 719–732. https://doi.org/10.1007/BF01877146

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection*, *19*(8–10), 709–714. https://doi.org/10.1016/S0261-2194(00)00094-6

Elad, Y., & Volpin, H. (1993). Reduced development of grey mould (*Botrytis cinerea*) in bean and tomato plants by calcium nutrition. *Journal of Phytopathology*, *139*(2), 146–156. https://doi.org/10.1111/j.1439-0434.1993.tb01410.x

Eljounaidi, K., Lee, S. K., & Bae, H. (2016). Bacterial endophytes as potential biocontrol agents of vascular wilt diseases—Review and future prospects. *Biological Control*, *103*, 62–68. https://doi.org/10.1016/j.biocontrol.2016.07.013

Elmer, W. H., & White, J. C. (2016). The future of nanotechnology in plant pathology. *Annual Review of Phytopathology*, *54*, 409–429. https://doi.org/10.1146/annurev-phyto-080615-100056

ElSohly, M. A., & Slade, D. (2005). Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sciences*, *78*(5), 539–548. https://doi.org/10.1016/j.lfs.2005.09.011

ElSohly, M. A., Radwan, M. M., Gul, W., Chandra, S., & Galal, A. (2017). Phytochemistry of *Cannabis sativa* L. In M. A. ElSohly (Ed.), *Phytocannabinoids* (Vol. 103, pp. 1–36). Springer International Publishing. https://doi.org/10.1007/978-3-319-45541-9_1

Esawy, A. A., Elsharkawy, M. M., Omara, R. I., Khalifa, M. A. F., Fadel, F. M., & El-Naggar, M. M. (2021). Biological control of *Golovinomyces cichoracearum*, the causal pathogen of sunflower powdery mildew. *Egyptian Journal of Biological Pest Control*, *31*, Article 133. https://doi.org/10.1186/s41938-021-00479-2

Esquivel-Cervantes, L. F., Tlapal-Bolaños, B., Tovar-Pedraza, J. M., Pérez-Hernández, O., Leyva-Mir, S. G., & Camacho-Tapia, M. (2022). Efficacy of biorational products for managing diseases of tomato in greenhouse production. *Plants*, *11*, Article 1638. https://doi.org/10.3390/plants11101638

Fadiji, A. E., & Babalola, O. O. (2020). Exploring the potentialities of beneficial endophytes for improved plant growth. *Saudi Journal of Biological Sciences*, *27*, 3622–3633. https://doi.org/10.1016/j.sjbs.2020.04.037

Farag, S., & Kayser, O. (2017). The cannabis plant: Botanical aspects. In V. R. Preedy (Ed.), *Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis and Treatment* (pp. 3–11). Elsevier.

Fedele, G., González-Domínguez, E., Si Ammour, M., Languasco, L., & Rossi, V. (2020). Reduction of *Botrytis cinerea* colonization of and sporulation on bunch trash. *Plant Disease*, *104*(3), 808–816. https://doi.org/10.1094/PDIS-08-19-1593-RE

Feng, H., Gonzalez Viejo, C., Vaghefi, N., Taylor, P. W. J., Tongson, E., & Fuentes, S. (2022). Early detection of *Fusarium oxysporum* infection in processing tomatoes (*Solanum lycopersicum*) and pathogen–soil interactions using a low-cost portable electronic nose and machine learning modeling. *Sensors*, *22*, Article 8645. https://doi.org/10.3390/s22228645

Fermaud, M., & Le Menn, R. (1992). Transmission of *Botrytis cinerea* to grapes by grape berry moth larvae. *Phytopathology*, *82*, 1393–1398.

Fleming, H., Chamberlain, Z., Zager, J. J., & Lange, B. M. (2023). Controlled environments for cannabis cultivation to support “omics” research studies and production. *Methods in Enzymology*, *680*, 353–380. https://doi.org/10.1016/bs.mie.2022.07.028

Fleming, M. P., & Clarke, R. C. (1998). Physical evidence for the antiquity of *Cannabis sativa* L. (Cannabaceae). *Journal of the International Hemp Association*, *5*(2), 80–92.

Flemming, C. A., & Trevors, J. T. (1989). Copper toxicity and chemistry in the environment: A review. *Water, Air, & Soil Pollution*, *44*, 143–158. https://doi.org/10.1007/BF00163319

Fountas, S., Malounas, I., Athanasakos, L., Avgoustakis, I., & Espejo-Garcia, B. (2022). AI-assisted vision for agricultural robots. *AgriEngineering*, *4*, 674–694. https://doi.org/10.3390/agriengineering4030043

Fraceto, L. F., Grillo, R., de Medeiros, G. A., Scognamiglio, V., Rea, G., & Bartolucci, C. (2016). Nanotechnology in agriculture: Which innovation potential does it have? *Frontiers in Environmental Science*, *4*, Article 20. https://doi.org/10.3389/fenvs.2016.00020

Freeman, S., Minz, D., Kolesnik, I., Barbul, O., Zveibil, A., Maymon, M., Nitzani, Y., Kirshner, B., Rav-David, D., & Bilu, A. (2004). *Trichoderma* biocontrol of *Colletotrichum acutatum* and *Botrytis cinerea* and survival in strawberry. *European Journal of Plant Pathology*, 110(4), 361–370. https://doi.org/10.1023/B:EJPP.0000021057.93305.d9

Freires, A. L. A., Figueiredo, F. R. A., Alves, T. R. C., Barroso, K. A., da Silva, I. V. P., Silva, J. L. S., de Almeida Nogueira, G., Melo, N. J. A., Júnior, R. S., Negreiros, A. M. P. (2022). Alternative products in the management of powdery mildew (*Podosphaera xanthii*) in melon. *Tropical Plant Pathology*, 47(5), 608–617.

Frink, S., Marjanovic, O., Tran, P., Wang, Y., Guo, W., Encarnacion, N., Alcantara, D., Moezzi, B., & Vrdoljak, G. (2022). Use of X-ray irradiation for inactivation of *Aspergillus* in cannabis flower. *PLOS ONE*, 17(11), e0277649. https://doi.org/10.1371/journal.pone.0277649

Gabler, F., Smilanick, J., Mansour, M., Ramming, D., & Mackey, B. (2003). Correlations of morphological, anatomical, and chemical features of grape berries with tolerance to *Botrytis cinerea*. *Phytopathology*, 93(10), 1263–1273. https://doi.org/10.1094/PHYTO.2003.93.10.1263

Gabriele, M., Vitali, F., Chelucci, E., & Chiellini, C. (2022). Characterization of the cultivable endophytic bacterial community of seeds and sprouts of *Cannabis sativa* L. and perspectives for the application as biostimulants. *Microorganisms*, 10(10), 1742. https://doi.org/10.3390/microorganisms10101742

Gams, W., & Bissett, J. (1998). Morphology and identification of *Trichoderma*. In G. E. Harman & C. P. Kubicek (Eds.), *Trichoderma and Gliocladium* (pp. 3–34). Taylor & Francis Group.

Garfinkel, A. R. (2020). Three *Botrytis* species found causing gray mold on industrial hemp (*Cannabis sativa*) in Oregon. *Plant Disease*, 104(7), 2026. https://doi.org/10.1094/PDIS-01-20-0055-PDN

Gautam, A. K., Kant, M., & Thakur, Y. (2013). Isolation of endophytic fungi from *Cannabis sativa* and study their antifungal potential. *Archives of Phytopathology and Plant Protection*, 46(6), 627–635. https://doi.org/10.1080/03235408.2012.749696

Glare, T. R., Caradus, J., Gelernter, W., Jackson, T. A., Keyhani, N., Köhl, J., Marrone, P., Morin, L., & Stewart, A. (2012). Have biopesticides come of age? *Trends in Biotechnology*, 30(5), 250–258. https://doi.org/10.1016/j.tibtech.2012.01.003

Gossen, B. D., & Lan, Z. (2021). Wetness duration, temperature, and cultivar affect floral infection by *Botrytis cinerea* in alfalfa. *Canadian Journal of Plant Pathology*, 43(6), 812–819. https://doi.org/10.1080/07060661.2021.1922940

Gotor-Vila, A., Teixidó, N., Sisquella, M., Torres, R., & Usall, J. (2017). Biological characterization of the biocontrol agent *Bacillus amyloliquefaciens* CPA-8: The effect of temperature, pH and water activity on growth, susceptibility to antibiotics and detection of enterotoxic genes. *Current Microbiology*, 74(9), 1089–1099. https://doi.org/10.1007/s00284-017-1289-8

Grassi, G., & McPartland, J. M. (2017). Chemical and morphological phenotypes in breeding of *Cannabis sativa* L. In S. Chandra, H. Lata, & M. A. ElSohly (Eds.), *Cannabis sativa* L. – Botany and Biotechnology (pp. 137–161). Springer International Publishing.

Grof, C. P. L. (2018). Cannabis, from plant to pill. *British Journal of Clinical Pharmacology*, 84(6), 2463–2467. https://doi.org/10.1111/bcp.13618

Gupta, R., Keppanan, R., Leibman-Markus, M., Rav-David, D., Elad, Y., Ment, D., & Bar, M. (2022). The entomopathogenic fungi *Metarhizium brunneum* and *Beauveria*

bassiana promote systemic immunity and confer resistance to a broad range of pests and pathogens in tomato. *Phytopathology*, 112(7), 784–793. https://doi.org/10.1094/PHYTO-03-21-0080-R

Guzmán-Guzmán, P., Kumar, A., de Los Santos-Villalobos, S., Parra-Cota, F. I., Orozco-Mosqueda, M. D. C., Fadiji, A. E., Hyder, S., Babalola, O. O., & Santoyo, G. (2023). Trichoderma species: Our best fungal allies in the biocontrol of plant diseases—A review. *Plants*, *12*(3), Article 432. https://doi.org/10.3390/plants12030432

Gwinn, K. D., Leung, M. C. K., Stephens, A. B., & Punja, Z. K. (2023). Fungal and mycotoxin contaminants in cannabis and hemp flowers: Implications for consumer health and directions for further research. *Frontiers in Microbiology*, *14*, Article 1278189. https://doi.org/10.3389/fmicb.2023.1278189

Hannusch, D. J., & Boland, G. J. (1996). Interactions of air temperature, relative humidity and biological control agents on grey mold of bean. *European Journal of Plant Pathology*, *102*(2), 133–142. https://doi.org/10.1007/BF01877100

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). Trichoderma species—Opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, *2*(1), 43–56. https://doi.org/10.1038/nrmicro797

Harman, G. E. (2000). Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Disease*, *84*(4), 377–393. https://doi.org/10.1094/PDIS.2000.84.4.377

Harman, G. E. (2011). Trichoderma—Not just for biocontrol anymore. *Phytoparasitica*, *39*(2), 103–108. https://doi.org/10.1007/s12600-011-0151-y

Hathaway, A. D., & McCann, C. J. S. (2022). *The High North*. University of British Columbia Press.

Hazarika, S., Choudhury, R., Saikia, S., & Sarma, U. (2020). Pathogen detection in Khasi mandarin orange using serological and electronic nose diagnostic technique. *International Journal of Innovative Technology and Exploring Engineering*, *9*(4), 2981–2985. https://doi.org/10.35940/ijitee.D2077.029420

Hazekamp, A., Tejkalová, K., & Papadimitriou, S. (2016). Cannabis: From cultivar to chemovar II—A metabolomics approach to cannabis classification. *Cannabis and Cannabinoid Research*, *1*, 202–215. https://doi.org/10.1089/can.2016.0017

Hazekamp, A. (2016). Evaluating the effects of gamma-irradiation for decontamination of medicinal cannabis. *Frontiers in Pharmacology*, *7*, Article 108. https://doi.org/10.3389/fphar.2016.00108

Health Canada. (2018). *The Cannabis Act*. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/laws-regulations.html

Health Canada. (2024a, March). *Cannabis licensed area and employment: Inventory, sales and licensed area*. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/research-data/market/licensed-area.html

Health Canada. (2024b, September 27). *Licensed cultivators, processors and sellers of cannabis under the Cannabis Act*. https://www.canada.ca/en/health-canada/services/drugs-medication/cannabis/industry-licensees-applicants/licensed-cultivators-processors-sellers.html

Hermosa, R., Rubio, M. B., Cardoza, R. E., Nicolás, C., Monte, E., & Gutiérrez, S. (2013). The contribution of *Trichoderma* to balancing the costs of plant growth and defense. *International Microbiology*, *16*(2), 69–80. https://doi.org/10.2436/20.1501.01.181

Hillig, K. W. (2005). Genetic evidence for speciation in *Cannabis* (Cannabaceae). *Genetic Resources and Crop Evolution*, *52*(2), 161–180. https://doi.org/10.1007/s10722-003-4452-y

Hjeljord, L. G., Stensvand, A., & Tronsmo, A. (2001). Antagonism of nutrient-activated conidia of *Trichoderma harzianum* (atroviride) P1 against *Botrytis cinerea*. *Phytopathology*, *91*(12), 1172–1180. https://doi.org/10.1094/PHYTO.2001.91.12.1172

Holmes, J. E., Lung, S., Collyer, D., & Punja, Z. K. (2021). Variables affecting shoot growth and plantlet recovery in tissue cultures of drug-type *Cannabis sativa* L.

Frontiers in Plant Science, *12*, Article 732344. https://doi.org/10.3389/fpls.2021.732344

Holz, G., Coertze, S., & Williamson, B. (2007). The ecology of *Botrytis* on plant surfaces. In Y. Elad, B. Williamson, P. Tudzynski, & N. Delen (Eds.), *Botrytis: Biology, Pathology and Control* (pp. 9–27). Springer. https://doi.org/10.1007/978-1-4020-2626-3

Hsiang, T., & Chastagner, G. A. (1992). Production and viability of sclerotia from fungicide-resistant and fungicide-sensitive isolates of *Botrytis cinerea*, *B. elliptica*, and *B. tulipae*. *Plant Pathology*, 41(5), 600–605. https://doi.org/10.1111/j.1365-3059.1992.tb02459.x

International Commission on Microbiological Specifications for Foods. (2018). *Microorganisms in foods 7: Microbiological testing in food safety management* (2nd ed.). Springer. https://doi.org/10.1007/978-3-319-11903-5

Isenegger, D. A., Ford, R., & Taylor, P. W. J. (2011). Disease reaction of chickpea (*Cicer* spp.) genotypes to Botrytis grey mould (*Botrytis cinerea*). *Australasian Plant Pathology*, 40(6), 583–590. https://doi.org/10.1007/s13313-011-0081-7

Islam, W., Tayyab, M., Khalil, F., Hua, Z., Huang, Z., & Chen, H. Y. H. (2020). Silicon-mediated plant defense against pathogens and insect pests. *Pesticide Biochemistry and Physiology*, 168, 104641. https://doi.org/10.1016/j.pestbp.2020.104641

Janisiewicz, W. J., Takeda, F., Nichols, B., Glenn, D. M., Jurick II, W. M., & Camp, M. J. (2016). Use of low-dose UV-C irradiation to control powdery mildew caused by *Podosphaera aphanis* on strawberry plants. *Canadian Journal of Plant Pathology*, 38, 430–439. https://doi.org/10.4141/cjpp-16-020

Jerushalmi, S., Maymon, M., Dombrovsky, A., & Freeman, S. (2020a). Fungal pathogens affecting the production and quality of medical cannabis in Israel. *Plants*, 9(7), Article 882. https://doi.org/10.3390/plants9070882

Jerushalmi, S., Maymon, M., Dombrovsky, A., & Freeman, S. (2020b). Effects of cold plasma, gamma and e-beam irradiations on reduction of fungal colony forming

unit levels in medical cannabis inflorescences. *Journal of Cannabis Research*, 2, Article 12. https://doi.org/10.1186/s42238-020-00028-6

Jin, D., Henry, P., Shan, J., & Chen, J. (2021). Identification of phenotypic characteristics in three chemotype categories in the genus *Cannabis*. *HortScience*, 56(4), 481–490. https://doi.org/10.21273/HORTSCI15607-20

Jones, M., & Monthony, A. S. (2022). Cannabis propagation. In Y. Zheng (Ed.), *Handbook of cannabis production in controlled environments* (pp. 41–90). CRC Press.

Kanto, T., Miyoshi, A., Ogawa, T., Maekawa, K., & Aino, M. (2004). Suppressive effect of potassium silicate on powdery mildew of strawberry in hydroponics. *Journal of General Plant Pathology*, 70(3), 207–211. https://doi.org/10.1007/s10327-004-0117-8

Kanwar, J., Kaul, M. K., Naruka, I. S., & Singh, P. P. (2019). In vitro micrografting technique in sweet orange (*Citrus sinensis* cv. Blood Red) to produce virus-free plants. *Indian Journal of Agricultural Sciences*, 89(4), 494–499. https://doi.org/10.56093/ijas.v89i3.87595

Karlsson, M., Atanasova, L., Jensen, D. F., & Zeilinger, S. (2017). Necrotrophic mycoparasites and their genomes. *Microbiology Spectrum*, 4(6). https://doi.org/10.1128/microbiolspec.FUNK-0020-2016

Keller, M., Viret, O., & Cole, F. M. (2003). *Botrytis cinerea* infection in grape flowers: Defense reaction, latency, and disease expression. *Phytopathology*, 93(3), 316–323. https://doi.org/10.1094/PHYTO.2003.93.3.316

Kirkby, K., Roser, S., & Plett, K. (2023). Using detached industrial hemp leaf inoculation assays to screen for varietal susceptibility and product efficacy on *Botrytis cinerea*. *Plants*, 12(18), Article 3278. https://doi.org/10.3390/plants12183278

Kloepper, J., Ryu, C.-M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*, *94*(11), 1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259

Köhl, J. (2004). Biological control of *Botrytis spp.* by *Ulocladium atrum* through competitive colonisation of necrotic tissues. Plant Research International B.V. Retrieved from https://edepot.wur.nl/40774

Konstantinidou, P. C., Gilardi, G., Gard, B., & Gullino, M. L. (2022). Vegetable and herb disease management in protected culture. In W. H. Elmer, M. McGrath, R. J. McGovern (Eds.), *Handbook of vegetable and herb diseases* (pp. 1–50). Springer. https://doi.org/10.1007/978-3-030-35512-8_6-1

Konstantinidou-Doltsinis, S., & Schmit, A. (1998). Impact of treatment with plant extracts from *Reynoutria sachalinensis* (F. Schmidt) Nakai on intensity of powdery mildew severity and yield in cucumber under high disease pressure. *Crop Protection*, *17*, 649–656. https://doi.org/10.1016/S0261-2194(98)00066-0

Körner, O., Holst, N., & de Visser, P. (2014). A model-based decision support tool for grey mould prediction. *Acta Horticulturae*, *1037*, 569–574. https://doi.org/10.17660/ActaHortic.2014.1037.71

Koumoutsi, A., Chen, X.-H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Vater, J., & Borriss, R. (2004). Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive cyclic lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. *Journal of Bacteriology*, *186*(4), 1084–1096. https://doi.org/10.1128/JB.186.4.1084-1096.2004

Kozhar, O., & Peever, T. L. (2018). How does *Botrytis cinerea* infect red raspberry? *Phytopathology*, *108*(11), 1287–1298. https://doi.org/10.1094/PHYTO-01-18-0016-R

Kruidhof, H. M., & Elmer, W. H. (2020). Cultural methods for greenhouse pest and disease management. In M. Gullino, R. Albajes, & P. Nicot (Eds.), *Integrated pest and disease management in greenhouse crops* (pp. 285–330). Springer. https://doi.org/10.1007/978-3-030-22304-5_10

Kuroyanagi, T., Yoshikoshi, H., Kinoshita, T., & Kawashima, H. (2014). Use of air circulation to reduce wet leaves under high humidity conditions. *Environmental Control in Biology*, *51*(4), 215–220. https://doi.org/10.2525/ecb.51.215

Kusari, P., Kusari, S., Spiteller, M., & Kayser, O. (2013). Endophytic fungi harbored in *Cannabis sativa* L.: Diversity and potential as biocontrol agents against host plant-specific phytopathogens. *Fungal Diversity*, *60*(1), 137–151. https://doi.org/10.1007/s13225-012-0216-3

Kusari, P., Kusari, S., Lamshöft, M., Sezgin, S., Spiteller, M., & Kayser, O. (2014). Quorum quenching is an antivirulence strategy employed by endophytic bacteria. *Applied Microbiology and Biotechnology*, *98*, 7173–7183. https://doi.org/10.1007/s00253-014-5807-3

Lamichhane, J. R., Osdaghi, E., Behlau, F., Köhl, J., Jones, J. B., & Aubertot, J. N. (2018). Thirteen decades of antimicrobial copper compounds applied in agriculture: A review. *Agronomy for Sustainable Development*, *38*, Article 28. https://doi.org/10.1007/s13593-017-0477-7

Laothawornkitkul, J., Moore, J. P., Taylor, J. E., Possell, M., Gibson, T. D., Hewitt, C. N., & Paul, N. D. (2008). Discrimination of plant volatile signatures by an electronic nose: A potential technology for plant pest and disease monitoring. *Environmental Science & Technology*, *42*, 8433–8439. https://doi.org/10.1021/es801738s

Latorre, B. A., Agosín, E., San Martín, R., & Vásquez, G. S. (1997). Effectiveness of conidia of *Trichoderma harzianum* produced by liquid fermentation against *Botrytis* bunch rot of table grape in Chile. *Crop Protection*, 16(3), 209–214. https://doi.org/10.1016/S0261-2194(96)00102-0

Lee, S. K., Shon, H. B., Kim, G. G., & Chung, Y. R. (2006). Enhancement of biological control of *Botrytis cinerea* on cucumber by foliar sprays and bed potting mixes of *Trichoderma harzianum* YC459 and its application on tomato in the greenhouse. *The Plant Pathology Journal*, 22(3), 283–288. https://doi.org/10.5423/PPJ.2006.22.3.283

Li, H. L. (1973). An archaeological and historical account of cannabis in China. *Economic Botany*, 28(4), 437–448. https://doi.org/10.1007/BF02862859

Liaghat, S., Mansor, S., Ehsani, R., Shafri, H. Z. M., Meon, S., & Sankaran, S. (2014). Mid-infrared spectroscopy for early detection of basal stem rot disease in oil

palm. *Computers and Electronics in Agriculture*, 101, 48–54. https://doi.org/10.1016/j.compag.2013.12.012

Liang, Y., Sun, W., Si, J., & Römheld, V. (2005). Effects of foliar- and root-applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. *Plant Pathology*, 54, 678–685. https://doi.org/10.1111/j.1365-3059.2005.01246.x

Lindenthal, M., Steiner, U., Dehne, H. W., & Oerke, E. C. (2005). Effect of downy mildew development on transpiration of cucumber leaves visualized by digital infrared thermography. *Phytopathology*, 95(3), 233–240. https://doi.org/10.1094/PHYTO-95-0233

Lindow, S. E., & Brandl, M. T. (2003). Microbiology of the phyllosphere. *Applied and Environmental Microbiology*, 69(4), 1875–1883. https://doi.org/10.1128/AEM.69.4.1875-1883.2003

Liu, B., Davies, K., & Hall, A. (2020). Silicon builds resilience in strawberry plants against both strawberry powdery mildew *Podosphaera aphanis* and two-spotted spider mites *Tetranychus urticae*. *PLOS ONE*, 15(10), Article e0241151. https://doi.org/10.1371/journal.pone.0241151

Long, T., Wagner, M., Demske, D., Leipe, C., & Tarasov, P. E. (2017). Cannabis in Eurasia: Origin of human use and Bronze Age trans-continental connections. *Vegetation History and Archaeobotany*, 26(2), 245–258. https://doi.org/10.1007/s00334-016-0579-6

Lyu, D., Backer, R., Robinson, W. G., & Smith, D. L. (2019). Plant growth-promoting rhizobacteria for cannabis production: Yield, cannabinoid profile, and disease resistance. *Frontiers in Microbiology*, 10, Article 1761. https://doi.org/10.3389/fmicb.2019.01761

Mahamad, S., Wadsworth, E., Rynard, V., Goodman, S., & Hammond, D. (2020). Availability, retail price, and potency of legal and illegal cannabis in Canada after recreational cannabis legalization. *Drug and Alcohol Review*, 39(4), 337–346. https://doi.org/10.1111/dar.13069

Mahmoud, M., Ben Rejeb, I., Punja, Z. K., Buirs, L., & Jabaji, S. (2023). Understanding bud rot development, caused by *Botrytis cinerea*, on cannabis (*Cannabis sativa* L.) plants grown under greenhouse conditions. *Botany*, 101(7), 231–243. https://doi.org/10.1139/cjb-2022-0139

Mahmud, M. S., Zaman, Q. U., Esau, T. J., Chang, Y. K., Price, G. W., & Prithiviraj, B. (2020). Real-time detection of strawberry powdery mildew disease using a mobile machine vision system. *Agronomy*, 10(8), Article 1027. https://doi.org/10.3390/agronomy10081027

Majumdar, C. G., ElSohly, M. A., Ibrahim, E. A., Elhendawy, M. A., Stanford, D., Chandra, S., Wanas, A. S., & Radwan, M. M. (2023). Effect of gamma irradiation on cannabinoid, terpene, and moisture content of cannabis biomass. *Molecules*, 28(20), Article 7710. https://doi.org/10.3390/molecules28207710

Makarichian, A., Chayjan, R. A., Ahmadi, E., & Zafari, D. (2022). Early detection and classification of fungal infection in garlic (*Allium sativum*) using electronic nose. *Computers and Electronics in Agriculture*, 192, Article 106575. https://doi.org/10.1016/j.compag.2021.106575

Margaritopoulou, T., Toufexi, E., Kizis, D., Balayiannis, G., Anagnostopoulos, C., Theocharis, A., Rempelos, L., Troyanos, Y., Leifert, C., & Markellou, E. (2020). *Reynoutria sachalinensis* extract elicits SA-dependent defense responses in courgette genotypes against powdery mildew caused by *Podosphaera xanthii*. *Scientific Reports*, *10*, Article 3354. https://doi.org/10.1038/s41598-020-67890-z

Martínez-Bracero, M., Markey, E., Clancy, J. H., Sodeau, J., & O'Connor, D. J. (2022). First long-time airborne fungal spores study in Dublin, Ireland (1978–1980). *Atmosphere*, *13*(2), Article 313. https://doi.org/10.3390/atmos13020313

Martínez-Medina, A., Fernández, I., Sánchez-Guzmán, M. J., Jung, S. C., Pascual, J. A., & Pozo, M. J. (2013). Deciphering the hormonal signalling network behind the systemic resistance induced by *Trichoderma harzianum* in tomato. *Frontiers in Plant Science*, *4*, Article 206. https://doi.org/10.3389/fpls.2013.00206

Masmoudi Ben Khedher, S., Kamoun, A., Zouari, N., Tounsi, S., & Trigui, M. (2017). Combinatorial effect of mutagenesis and medium component optimization on

Bacillus amyloliquefaciens antifungal activity and efficacy in eradicating *Botrytis cinerea*. *Microbiological Research*, *197*, 29–38. https://doi.org/10.1016/j.micres.2017.01.001

Matzneller, P., Gutierrez, J. D., & Caplan, D. (2022). Canopy management. In Y. Zheng (Ed.), *Handbook of cannabis production in controlled environments* (pp. 109–125). CRC Press.

Mayton, H., Amirkhani, M., Loos, M., Johnson, B., Fike, J., Johnson, C., Myers, K., Starr, J., Bergstrom, G. C., & Taylor, A. (2022). Evaluation of industrial hemp seed treatments for management of damping-off for enhanced stand establishment. *Agriculture*, *12*, Article 591. https://doi.org/10.3390/agriculture12040591

McNicol, R. J., & Williamson, B. (1989). Systemic infection of black currant flowers by *Botrytis cinerea* and its possible involvement in premature abscission of fruits. *Annals of Applied Biology*, *114*(2), 243–254. https://doi.org/10.1111/j.1744-7348.1989.tb02101.x

McPartland, J. M. (1996). A review of *Cannabis* diseases. *Journal of the International Hemp Association*, *3*(1), 19–23.

McPartland, J. M. (2018). *Cannabis* systematics at the levels of family, genus, and species. *Cannabis and Cannabinoid Research*, *3*, 203–212. https://doi.org/10.1089/can.2018.0039

McPartland, J. M. (2017). *Cannabis sativa* and *Cannabis indica* versus “Sativa” and “Indica”. In S. Chandra, H. Lata, & M. A. ElSohly (Eds.), *Cannabis sativa L. – Botany and Biotechnology* (pp. 101–122). Springer International Publishing.

Mercier, J., & Wilson, C. L. (1994). Colonization of apple wounds by naturally occurring microflora and introduced *Candida oleophila* and their effect on infection by *Botrytis cinerea* during storage. *Biological Control*, *4*(2), 138–144. https://doi.org/10.1006/bcon.1994.1022

Meyer, G. D., Bigirimana, J., Elad, Y., & Höfte, M. (1998). Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *European Journal of Plant Pathology*, *104*(3), 279–286. https://doi.org/10.1023/A:1008628806616

Mihalyov, P. D., & Garfinkel, A. R. (2021). Discovery and genetic mapping of PM1, a powdery mildew resistance gene in *Cannabis sativa* L. *Frontiers in Agronomy*, *3*, Article 720215. https://doi.org/10.3389/fagro.2021.720215

Mmbaga, M. T., & Sauve, R. J. (2004). Management of powdery mildew in flowering dogwood in the field with biorational and conventional fungicides. *Canadian Journal of Plant Science*, *84*, 837–844. https://doi.org/10.4141/P03-104

Mohammad-Razdari, A., Rousseau, D., Bakhshipour, A., Taylor, S., Poveda, J., & Kiani, H. (2022). Recent advances in e-monitoring of plant diseases. *Biosensors and Bioelectronics*, *201*, Article 113953. https://doi.org/10.1016/j.bios.2021.113953

Mónaco, C., Dal Bello, G., Rollán, M. C., Ronco, L., Lampugnani, G., Arteta, N., Abramoff, C., Aprea, A., Larran, S., & Stocco, M. (2009). Biological control of *Botrytis cinerea* on tomato using naturally occurring fungal antagonists. *Archives of Phytopathology and Plant Protection*, 42(8), 729–737. https://doi.org/10.1080/03235400701390646

Monthony, A. S., Page, S. R., Hesami, M., & Jones, A. M. P. (2021). The past, present and future of *Cannabis sativa* tissue culture. *Plants*, 10(1), 185. https://doi.org/10.3390/plants10060185

Morelli, M., Bahar, O., Papadopoulou, K. K., Hopkins, D. L., & Obradović, A. (2020). Editorial: Role of endophytes in plant health and defense against pathogens. *Frontiers in Plant Science*, 11, 1312. https://doi.org/10.3389/fpls.2020.01312

Morgan, W. M. (1984). The effect of night temperature and glasshouse ventilation on the incidence of *Botrytis cinerea* in a late-planted tomato crop. *Crop Protection*, 3(2), 243–251. https://doi.org/10.1016/0261-2194(84)90058-9

Munkvold, G. P., & Gullino, M. L. (2020). Seed and propagative material. In M. Gullino, R. Albajes, & P. Nicot (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 331–354). Springer. https://doi.org/10.1007/978-3-030-22304-5_11

Nawrocka, J., & Małolepsza, U. (2013). Diversity in plant systemic resistance induced by *Trichoderma*. *Biological Control*, 67(2), 149–156. https://doi.org/10.1016/j.biocontrol.2013.07.005

Nehra, N. S., & Kartha, K. K. (1994). Meristem and shoot tip culture: Requirements and applications. In I. K. Vasil & T. A. Thorpe (Eds.), *Plant Cell and Tissue Culture* (pp. 37–70). Springer. https://doi.org/10.1007/978-94-017-2681-8_3

Newman, S., Roll, M., & Harkrader, R. (1999). Naturally occurring compounds for controlling powdery mildew of greenhouse roses. *HortScience*, 34(4), 686–689. https://doi.org/10.21273/HORTSCI.34.4.686

Nicot, P. C., Decognet, V., Fruit, L., Bardin, M., & Trottin-Caudal, Y. (2002). Combined effect of microclimate and dose of application on the efficacy of biocontrol agents for the protection of pruning wounds on tomatoes against *Botrytis cinerea*. *IOBC/WPRS Bulletin*, 25(10), 73–76.

Nicot, P. C., Stewart, A., Bardin, M., & Elad, Y. (2016). Biological control and biopesticide suppression of *Botrytis*-incited diseases. In S. Fillinger & Y. Elad (Eds.), *Botrytis: The Fungus, the Pathogen and Its Management in Agricultural Systems* (pp. 165–187). Springer International. https://doi.org/10.1007/978-3-319-23371-0_9

Nicot, P. C., Bardin, M., Alabouvette, C., Köhl, J., & Ruocco, M. (2011). Potential of biological control based on published research. 1. Protection against plant pathogens of selected crops. In *Classical and Augmentative Biological Control Against Diseases and Pests: Critical Status Analysis and Review of Factors Influencing Their Success*. IOBC - International Organisation for Biological and Integrated Control of Noxious Animals and Plants.

Gullino, M. L., Albajes, R., & Nicot, P. C. (Eds.). (2020). *Integrated Pest and Disease Management in Greenhouse Crops*. Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5

Pan, L., Zhang, W., Zhu, N., Mao, S., & Tu, K. (2014). Early detection and classification of pathogenic fungal disease in post-harvest strawberry fruit by electronic nose

and gas chromatography–mass spectrometry. *Food Research International*, 62, 162–168. https://doi.org/10.1016/j.foodres.2014.02.020

Park, S. H., Lee, J. T., Chung, S.-O., & Kim, H.-K. (1999). Forecasting the pepper gray mold rot to predict the initial infection by *Botrytis cinerea* in greenhouse conditions. *Plant Pathology Journal*, 15(4), 158–161.

Pereira Francisco, V., Cerny, M., Valentin, R., Milone-Delacourt, F., Paillard, A., & Alignan, M. (2024). Development of GC–MS coupled to GC–FID method for the quantification of cannabis terpenes and terpenoids: Application to the analysis of five commercial varieties of medicinal cannabis. *Journal of Chromatography: Analytical Technologies in the Biomedical and Life Sciences*, 1247, 124316. https://doi.org/10.1016/j.jchromb.2024.124316

Poland, J., Balint-Kurti, P., Wisser, R., Pratt, R., & Nelson, R. (2009). Shades of gray: The world of quantitative disease resistance. *Trends in Plant Science*, *14*(1), 21–29. https://doi.org/10.1016/j.tplants.2008.10.006

Punja, Z. K. (2018). Flower and foliage-infecting pathogens of marijuana (*Cannabis sativa* L.) plants. *Canadian Journal of Plant Pathology*, *40*(3), 514–527. https://doi.org/10.1080/07060661.2018.1535467

Punja, Z. K. (2021a). Emerging diseases of *Cannabis sativa* and sustainable management. *Pest Management Science*, *77*(12), 3857–3870. https://doi.org/10.1002/ps.6307

Punja, Z. K. (2021b). Epidemiology of *Fusarium oxysporum* causing root and crown rot of cannabis (*Cannabis sativa* L.) plants in commercial greenhouse production. *Canadian Journal of Plant Pathology*, *43*(2), 216–235. https://doi.org/10.1080/07060661.2020.1788165

Punja, Z. K. (2021c). The diverse mycoflora present on dried cannabis (*Cannabis sativa* L., marijuana) inflorescences in commercial production. *Canadian Journal of Plant Pathology*, *43*(1), 88–100. https://doi.org/10.1080/07060661.2020.1758959

Punja, Z. K., & Ni, L. (2021). The bud rot pathogens infecting cannabis (*Cannabis sativa* L., marijuana) inflorescences: Symptomology, species identification,

pathogenicity, and biological control. *Canadian Journal of Plant Pathology*, *43*(6), 827–854. https://doi.org/10.1080/07060661.2021.1936650

Punja, Z. K., & Rodriguez, G. (2018). *Fusarium* and *Pythium* species infecting roots of hydroponically grown marijuana (*Cannabis sativa* L.) plants. *Canadian Journal of Plant Pathology*, *40*(4), 498–513. https://doi.org/10.1080/07060661.2018.1535466

Punja, Z. K., & Scott, C. (2023). Organically grown *Cannabis sativa* (L.) plants contain a diverse range of culturable epiphytic and endophytic fungi in inflorescences and stem tissues. *Botany*, *101*(7), 255–269. https://doi.org/10.1139/cjb-2022-0116

Punja, Z. K., Holmes, J. E. (2020). Hermaphroditism in Marijuana (*Cannabis sativa* L.) Inflorescences – Impact on Floral Morphology, Seed Formation, Progeny Sex Ratios, and Genetic Variation. Frontiers in Plant Science, 11:718–718. https://doi.org/10.3389/fpls.2020.00718

Punja, Z. K., Collyer, D., Scott, C., Lung, S., & Sutton, D. (2019). Pathogens and molds affecting production and quality of *Cannabis sativa* L. *Frontiers in Plant Science*, *10*, Article 1120. https://doi.org/10.3389/fpls.2019.01120

Punja, Z. K., Ni, L., Lung, S., & Buirs, L. (2023). Total yeast and mold levels in high THC-containing cannabis (*Cannabis sativa* L.) inflorescences are influenced by genotype, environment, and pre- and post-harvest handling practices. *Frontiers in Microbiology*, *14*, Article 1192035. https://doi.org/10.3389/fmicb.2023.1192035

Punja, Z. K., Ni, L., & Roberts, A. (2021). The *Fusarium solani* species complex infecting cannabis (*Cannabis sativa* L., marijuana) plants and a first report of *Fusarium* (*Cylindrocarpon*) *lichenicola* causing root and crown rot. *Canadian Journal of Plant Pathology*, *43*(4), 567–581. https://doi.org/10.1080/07060661.2020.1866672

Punja, Z. K., & Scott, C. (2022). Management of diseases on cannabis in controlled environment production. In Y. Zheng (Ed.), *Handbook of cannabis production in controlled environments* (pp. 216–252). CRC Press.

Punja, Z. K., Scott, C., & Lung, S. (2022). Several *Pythium* species cause crown and root rot on cannabis (*Cannabis sativa* L.) plants grown under commercial greenhouse conditions. *Canadian Journal of Plant Pathology*, *44*(1), 66–81. https://doi.org/10.1080/07060661.2021.1954695

Punja, Z. K., Wang, K., Lung, S., & Buirs, L. (2024). Symptomology, prevalence, and impact of hop latent viroid on greenhouse-grown cannabis (*Cannabis sativa* L.) plants in Canada. *Canadian Journal of Plant Pathology*, *46*(2), 174–197. https://doi.org/10.1080/07060661.2023.2279184

Radwan, M. M., Wanas, A. S., Chandra, S., & ElSohly, M. A. (2017). Natural cannabinoids of cannabis and methods of analysis. In S. Chandra, H. Lata, & M. A. ElSohly (Eds.), *Cannabis sativa* L.—*Botany and biotechnology* (pp. 161–183). Springer International Publishing.

Rahman, M. U., Hanif, M., Wan, R., Hou, X., Ahmad, B., & Wang, X. (2018). Screening *Vitis* genotypes for responses to *Botrytis cinerea* and evaluation of antioxidant enzymes, reactive oxygen species and jasmonic acid in resistant and susceptible hosts. *Molecules*, *24*(1), Article 5. https://doi.org/10.3390/molecules24010005

Raman, V., Lata, H., Chandra, S., Khan, I. A., & ElSohly, M. A. (2017). Morpho-anatomy of marijuana (*Cannabis sativa* L.). In S. Chandra, H. Lata, & M. A. ElSohly (Eds.), *Cannabis sativa L. – Botany and Biotechnology* (pp. 123–136). Springer International Publishing.

Rao, C. K. (2014). *Adoption of tissue culture in horticulture: A study of banana-growing farmers from a South-Indian state*. Cambridge Scholars Publishing.

Razdan, V. K., & Sabitha, M. (2009). Integrated disease management: Concepts and practices. In R. Peshin & A. K. Dhawan (Eds.), *Integrated Pest Management* (pp. 369–389). Springer. https://doi.org/10.1007/978-1-4020-8992-3_15

Raziq, F., & Fox, R. T. V. (2004). The effect of carrier substrate, dose rate and time of application on biocontrol efficacy of fungal antagonists against *Armillaria* root rot of strawberry plants. *Biological Agriculture & Horticulture*, *22*(2), 157–172. https://doi.org/10.1080/01448765.2004.9754998

Reeh, K. W., & Cutler, G. C. (2013). Laboratory efficacy and fungicide compatibility of *Clonostachys rosea* against *Botrytis* blight on lowbush blueberry. *Canadian Journal of Plant Science*, *93*(4), 639–642. https://doi.org/10.4141/cjps2012-306

Reekie, H., & Punja, Z. K. (2023). Calcium and plant disease. In E. L. Datnoff, W. H. Elmer, & F. A. Rodrigues (Eds.), *Mineral Nutrition and Plant Disease* (2nd ed., chapter 6). American Phytopathological Society.

Roberts, A. J., & Punja, Z. K. (2022). Pathogenicity of seedborne *Alternaria* and *Stemphylium* species and stem-infecting *Neofusicoccum* and *Lasiodiplodia* species to cannabis. *Canadian Journal of Plant Pathology*, *44*, 250–269. https://doi.org/10.4141/cjpp-2022-015

Roca-Couso, R., Flores-Félix, J. D., & Rivas, R. (2021). Mechanisms of action of microbial biocontrol agents against *Botrytis cinerea*. *Journal of Fungi*, *7*(12), Article 1045. https://doi.org/10.3390/jof7121045

Rodrigues, A. O., May De Mio, L. L., & Soccol, C. R. (2023). *Trichoderma* as a powerful fungal disease control agent for a more sustainable and healthy agriculture: Recent studies and molecular insights. *Planta*, *257*(2), Article 31. https://doi.org/10.1007/s00425-022-04053-4

Rosslenbroich, H., & Stuebler, D. (2000). *Botrytis cinerea*—history of chemical control and novel fungicides for its management. *Crop Protection*, *19*(8), 557–561. https://doi.org/10.1016/S0261-2194(00)00072-7

Rotolo, C., De Miccolis Angelini, R. M., Dongiovanni, C., Pollastro, S., Fumarola, G., Di Carolo, M., Perrelli, D., Natale, P., & Faretra, F. (2018). Use of biocontrol agents and botanicals in integrated management of *Botrytis cinerea* in table grape vineyards. *Pest Management Science*, *74*(3), 715–725. https://doi.org/10.1002/ps.4767

Russo, E. B. (2007). History of cannabis and its preparations in saga, science, and sobriquet. *Chemistry & Biodiversity*, *4*(8), 1614–1648. https://doi.org/10.1002/cbdv.200790144

Russo, E. B. (2011). Taming THC: Potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacology*, *163*(7), 1344–1364. https://doi.org/10.1111/j.1476-5381.2011.01238.x

Russo, E., & Guy, G. W. (2006). A tale of two cannabinoids: The therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Medical Hypotheses*, *66*(2), 234–246. https://doi.org/10.1016/j.mehy.2005.08.026

Sakr, N. (2016). Silicon control of bacterial and viral diseases in plants. *Journal of Plant Protection Research*, *56*, 331–336. https://doi.org/10.1515/jppr-2016-0052

Salvatierra-Martinez, R., Arancibia, W., Araya, M., Aguilera, S., Olalde, V., Bravo, J., & Stoll, A. (2018). Colonization ability as an indicator of enhanced biocontrol capacity—An example using two *Bacillus amyloliquefaciens* strains and *Botrytis cinerea* infection of tomatoes. *Journal of Phytopathology*, *166*(9), 601–612. https://doi.org/10.1111/jph.12718

Samuels, A. L., Glass, A. D. M., Ehret, D. L., & Menzies, J. G. (1991). Distribution of silicon in cucumber leaves during infection by the powdery mildew fungus (*Sphaerotheca fuliginea*). *Canadian Journal of Botany*, *69*, 140–146. https://doi.org/10.1139/b91-020

Sankaran, S., Mishra, A., Ehsani, R., & Davis, C. (2010). A review of advanced techniques for detecting plant diseases. *Computers and Electronics in Agriculture*, *72*, 1–13. https://doi.org/10.1016/j.compag.2010.03.001

Sanzani, S. M., Schena, L., De Cicco, V., & Ippolito, A. (2012). Early detection of *Botrytis cinerea* latent infections as a tool to improve postharvest quality of table grapes. *Postharvest Biology and Technology*, *68*, 64–71. https://doi.org/10.1016/j.postharvbio.2012.02.003

Sarosh, B. R., Danielsson, J., & Meijer, J. (2009). Transcript profiling of oilseed rape (*Brassica napus*) primed for biocontrol differentiates genes involved in microbial interactions with beneficial *Bacillus amyloliquefaciens* from pathogenic *Botrytis cinerea*. *Plant Molecular Biology*, *70*(1–2), 31–45. https://doi.org/10.1007/s11103-009-9455-4

Sawant, I. S. (2014). *Trichoderma–foliar pathogen interactions*. *Open Mycology Journal*, *8*, 58–70. https://doi.org/10.2174/1874437001408010058

Schilder, A., Gillet, J., Sysak, R., & Wise, J. (2002, February 4–7). Evaluation of environmentally friendly products for control of fungal diseases of grapes. In *Proceedings of the 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing and Viticulture* (pp. 163–167).

Schmitt, A., Kunz, S., Nandi, S., Seddon, B., & Ernst, A. (2002, January 31–February 2). Use of *Reynoutria sachalinensis* plant extracts, clay preparations and *Brevibacillus brevis* against fungal diseases of grape berries. In *Proceedings of the 10th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing and Viticulture* (pp. 146–151).

Schmitt, A. (2001, April 26–28). Induced responses by plant extracts from *Reynoutria sachalinensis*: A case study. In *Proceedings of the Study Group: Induced Resistance in Plants Against Insects and Diseases* (pp. 83–88).

Schneider, S., & Ullrich, W. R. (1994). Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. *Physiological and Molecular Plant Pathology*, *45*(4), 291–304. https://doi.org/10.1016/S0885-5765(05)80060-8

Scott, C., & Punja, Z. K. (2020). Evaluation of disease management approaches for powdery mildew on *Cannabis sativa* L. (marijuana) plants. *Canadian Journal of Plant Pathology*, *43*(3), 394–412. https://doi.org/10.1080/07060661.2020.1836026

Scott, M., Rani, M., Samsatly, J., Charron, J.-B., & Jabaji, S. (2018). Endophytes of industrial hemp (*Cannabis sativa* L.) cultivars: Identification of culturable bacteria and fungi in leaves, petioles, and seeds. *Canadian Journal of Microbiology*, *64*(10), 664–680. https://doi.org/10.1139/cjm-2018-0108

Scott, C., & Punja, Z. (2022). Management of diseases on cannabis in controlled environment production. In Y. Zheng (Ed.), *Handbook of Cannabis Production in*

Controlled Environments (1st ed., pp. 213–252). CRC Press. https://doi.org/10.1201/9781003150442-8

Scott, C., & Punja, Z. K. (2023). Biological control of *Fusarium oxysporum* causing damping-off and *Pythium myriotylum* causing root and crown rot on cannabis (*Cannabis sativa* L.) plants. *Canadian Journal of Plant Pathology*, 45, 238–252. https://doi.org/10.4141/cjpp-2023-002

Scott, C., Punja, Z. K., & Sabaratnam, S. (2021). *Diseases of cannabis in British Columbia*. BC Ministry of Agriculture. Retrieved from https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/agriculture-and-seafood/animal-and-crops/plant-health/disease-factsheets/specialty-crops/diseases_of_cannabis_in_british_columbia.pdf

Scott, M., Rani, M., Samsatly, J., Charron, J.-B., & Jabaji, S. (2018). Endophytes of industrial hemp (*Cannabis sativa* L.) cultivars: Identification of culturable bacteria and fungi in leaves, petioles, and seeds. *Canadian Journal of Microbiology*, 64, 664–680. https://doi.org/10.1139/cjm-2017-0391

Shakeel, Q., Bajwa, R. T., Rashid, I., Aslam, H. U., Iftikhar, Y., Mubeen, M., Li, G., & Wu, M. (2022). Concepts and applications of infrared thermography for plant disease measurement. In I. U. Haq & S. Ijaz (Eds.), *Trends in Plant Disease Assessment* (pp. 109–125). Springer.

Shao, C., Wang, H., Yu, D., & Mungai, C. (2013). The possible mechanism of antifungal action of tea tree oil on *Botrytis cinerea*. *Journal of Applied Microbiology*, 114(6), 1642–1649. https://doi.org/10.1111/jam.12193

Sharma, A. K., & Sharma, P. (Eds.). (2020). *Trichoderma—Host-pathogen interactions and applications*. Springer Nature.

Sharma, N., Sharma, L., & Singh, B. P. (2019). Propagation. In R. M. Sharma, R. Yamdagni, A. K. Dubey, & V. Pandey (Eds.), *Strawberries* (pp. 179–192). CRC Press. https://doi.org/10.1201/b21441-197

Shen, Y., Borgatta, J., Ma, C., Elmer, W., Hamers, R. J., & White, J. C. (2020). Copper nanomaterial morphology and composition control foliar transfer through the

cuticle and mediate resistance to root fungal disease in tomato (*Solanum lycopersicum*). *Journal of Agricultural and Food Chemistry*, 68, 11327–11338. https://doi.org/10.1021/acs.jafc.0c04546

Shetty, R., Jensen, B., Shelton, D., Jørgensen, K., Pedas, P., & Jørgensen, H. J. L. (2021). Site-specific, silicon-induced structural and molecular defence responses against powdery mildew infection in roses. *Pest Management Science*, 77, 4545–4554. https://doi.org/10.1002/ps.6493

Shetty, R., Jensen, B., Shetty, N. P., Hansen, M., Hansen, C. W., Starkey, K. R., & Jørgensen, H. J. L. (2012). Silicon-induced resistance against powdery mildew of roses caused by *Podosphaera pannosa*. *Plant Pathology*, 61, 120–131. https://doi.org/10.1111/j.1365-3059.2011.02493.x

Shrestha, S., & Hausbeck, M. K. (2021). Evaluation of geranium cultivars and biorational products to control *Botrytis* blight in the greenhouse. *Plant Health Progress*, 22(4), 474–482. https://doi.org/10.1094/PHP-01-21-0010-RS

Shrestha, S., & Hausbeck, M. K. (2023). Management of *Botrytis cinerea* in petunia using cultivar tolerance and biorational products. *Plant Health Progress*, 24(3), 312–319. https://doi.org/10.1094/PHP-11-22-0118-RS

Shtienberg, D., Elad, Y., Niv, A., Nitzani, Y., & Kirshner, B. (1998). Significance of leaf infection by *Botrytis cinerea* in stem rotting of tomatoes grown in non-heated greenhouses. *European Journal of Plant Pathology*, 104, 753–763. https://doi.org/10.1023/A:1008690925443

Singh, V., Adil, S., & Quraishi, A. (2022). Elimination of BBTV via a systemic in vitro electrotherapy approach. *Journal of Virological Methods*, 300, 114367. https://doi.org/10.1016/j.jviromet.2021.114367

Sirangelo, T. M. (2023). NLR- and MLO-based resistance mechanisms against powdery mildew in *Cannabis sativa*. *Plants*, 13, 105. https://doi.org/10.3390/plants13010105

Sirangelo, T. M., Ludlow, R. A., & Spadafora, N. D. (2023). Molecular mechanisms underlying potential pathogen resistance in *Cannabis sativa*. *Plants*, *12*(9), 2764. https://doi.org/10.3390/plants12152764

Slack, S. A., & Tufford, L. A. (1995). Meristem culture for virus elimination. In O. L. Gamborg & G. C. Phillips (Eds.), *Plant cell, tissue and organ culture* (pp. 117–128). Springer. https://doi.org/10.1007/978-3-642-79048-5_10

Small, E. (2015). Evolution and classification of *Cannabis sativa* (marijuana, hemp) in relation to human utilization. *The Botanical Review*, *81*, 189–294. https://doi.org/10.1007/s12229-015-9157-3

Small, E. (2016). *Cannabis: A complete guide* (1st ed.). CRC Press. https://doi.org/10.1201/9781315367583

Small, E. (2017). Classification of *Cannabis sativa* L. in relation to agricultural, biotechnological, medical and recreational utilization. In S. Chandra, H. Lata, & M. A. ElSohly (Eds.), *Cannabis sativa* L. – botany and biotechnology (pp. 1–63). Springer International Publishing.

Sonneveld, C., & Voogt, W. (2009). Nutrient management in substrate systems. In C. Sonneveld & W. Voogt (Eds.), *Plant nutrition of greenhouse crops* (pp. 277–312). Springer. https://doi.org/10.1007/978-90-481-2532-6_13

Spitzer-Rimon, B., Duchin, S., Bernstein, N., & Kamenetsky, R. (2019). Architecture and florogenesis in female *Cannabis sativa* plants. *Frontiers in Plant Science*, *10*, 350. https://doi.org/10.3389/fpls.2019.00350

Stack, G. M., Cala, A. R., Carlson, C. H., Quade, M. A., Marrero-González, M. I., Wilkerson, D. G., Gentner, D. R., Crawford, J. L., Philippe, G., Rose, J. C., Viands D. R., Smart D., & Smart L. (2021). Season-long characterization of high-cannabinoid hemp (*Cannabis sativa* L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. *GCB Bioenergy*, *13*(3), 546–561. https://doi.org/10.1111/gcbb.12793

Stack, G. M., Cala, A. R., Quade, M. A., Toth, J. A., Monserrate, L. A., Wilkerson, D. G., Carlson, C. H., Mamerto, A., Michael, T. P., Crawford, S., Smart D., & Smart L.

(2024). Genetic mapping, identification, and characterization of a candidate susceptibility gene for powdery mildew in *Cannabis sativa* L. *Molecular Plant-Microbe Interactions*, *37*(1), 51–61. https://doi.org/10.1094/MPMI-04-23-0043-R

Stasiak, M., & Dixon, M. (2022). Growing facilities and environmental control. In Y. Zheng (Ed.), *Handbook of cannabis production in controlled environments* (1st ed., pp. 13–40). CRC Press. https://doi.org/10.1201/9781003150442-2

Statistics Canada. (2023, October 16). *Research to insights: Cannabis in Canada*. https://www150.statcan.gc.ca/n1/pub/11-631-x/11-631-x2023006-eng.htm

Sun, Y., Wang, J., & Cheng, S. (2018). Early diagnosis of *Botrytis cinerea* infestation of tomato plant by electronic nose. *Applied Engineering in Agriculture*, *34*, 667–674. https://doi.org/10.13031/aea.12748

Sylla, J., Alsanius, B. W., Krüger, E., Reineke, A., Strohmeier, S., & Wohanka, W. (2013). Leaf microbiota of strawberries as affected by biological control agents. *Phytopathology*, *103*(10), 1001–1011. https://doi.org/10.1094/PHYTO-01-13-0014-R

Sylla, J., Alsanius, B. W., Krüger, E., & Wohanka, W. (2015). Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. *European Journal of Plant Pathology*, *143*(3), 461–471. https://doi.org/10.1007/s10658-015-0698-4

Taghinasab, M., & Jabaji, S. (2020). Cannabis microbiome and the role of endophytes in modulating the production of secondary metabolites: An overview. *Microorganisms*, *8*(8), 355. https://doi.org/10.3390/microorganisms8080355

Tančinová, D., Mašková, Z., Mendelová, A., Foltinová, D., Barboráková, Z., & Medo, J. (2022). Antifungal activities of essential oils in vapor phase against *Botrytis cinerea* and their potential to control postharvest strawberry gray mold. *Foods*, 11(19), Article 2945. https://doi.org/10.3390/foods11192945

Thakur, R., Devi, R., Lal, M. K., Tiwari, R. K., Sharma, S., & Kumar, R. (2023). Morphological, ultrastructural, and molecular variations in susceptible and

resistant genotypes of chickpea infected with *Botrytis* grey mould. *PeerJ*, 11, e15134. https://doi.org/10.7717/peerj.15134

Thomas, C. S., Marois, J. J., & English, J. T. (1988). The effects of wind speed, temperature, and relative humidity on development of aerial mycelium and conidia of *Botrytis cinerea* on grape. *Phytopathology*, 78(3), 260–265. https://doi.org/10.1094/Phyto-78-260

Tian, S., Qin, G., Li, B., & Ma, W. (2016). *Botrytis cinerea* infection-induced host responses in postharvest fruits: Insights into mechanisms of infection and defense. *Critical Reviews in Plant Sciences*, 35(6), 496–509. https://doi.org/10.1080/07352689.2016.1261074

Traversari, S., Cacini, S., Galieni, A., Nesi, B., Nicastro, N., & Pane, C. (2021). Precision agriculture digital technologies for sustainable fungal disease management of ornamental plants. *Sustainability*, 13, 3707. https://doi.org/10.3390/su13073707

Tripathi, R., Tewari, R., Singh, K. P., Keswani, C., Minkina, T., Srivastava, A. K., De Corato, U., & Sansinenea, E. (2022). Plant mineral nutrition and disease resistance: A significant linkage for sustainable crop protection. *Frontiers in Plant Science*, 13, Article 883970. https://doi.org/10.3389/fpls.2022.883970

Tucci, M., Ruocco, M., De Masi, L., De Palma, M., & Lorito, M. (2011). The beneficial effect of *Trichoderma* spp. on tomato is modulated by the plant genotype. *Molecular Plant Pathology*, 12(4), 341–354. https://doi.org/10.1111/j.1364-3703.2010.00674.x

Tziros, G. T., Ainalidou, A., Samaras, A., Kollaros, M., Karamanoli, K., Menkissoglu-Spiroudi, U., & Karaoglanidis, G. S. (2022). Differences in defence-related gene expression and metabolite accumulation reveal insights into the resistance of Greek grape wine cultivars to Botrytis bunch rot. *OENO One*, 56(2), 111–123. https://doi.org/10.20870/oeno-one.2022.56.2.5451

Urban, L., Charles, F., de Miranda, M. R. A., & Aarrouf, J. (2016). Understanding the physiological effects of UV-C light and exploiting its agronomic potential before and after harvest. *Plant Physiology and Biochemistry*, 105, 1–11. https://doi.org/10.1016/j.plaphy.2016.08.008

Urbasch, I. (1983). On the genesis and germination of chlamydospores of *Botrytis cinerea*. *Journal of Phytopathology*, 108, 54–60.

Vagelas, I., Papadimos, A., & Lykas, C. (2021). Pre-symptomatic disease detection in the vine, chrysanthemum, and rose leaves with a low-cost infrared sensor. *Agronomy*, 11, 1682. https://doi.org/10.3390/agronomy11091682

van Kan, J. A. L. (2006). Licensed to kill: The lifestyle of a necrotrophic plant pathogen. *Trends in Plant Science*, 11(5), 247–253. https://doi.org/10.1016/j.tplants.2006.03.005

van Lenteren, J. C., & Nicot, P. C. (2020). Integrated pest management methods and considerations concerning implementation in greenhouses. In M. L. Gullino, R. Albajes, & P. C. Nicot (Eds.), *Integrated pest and disease management in greenhouse crops* (pp. 177–193). Springer. https://doi.org/10.1007/978-3-030-22304-5_6

Veloso, J., & van Kan, J. A. L. (2018). Many shades of grey in *Botrytis*–host plant interactions. *Trends in Plant Science*, 23(7), 613–622. https://doi.org/10.1016/j.tplants.2018.05.014

Viret, O., Keller, M., Jaudzems, V. G., & Cole, F. M. (2004). *Botrytis cinerea* infection of grape flowers: Light and electron microscopical studies of infection sites. *Phytopathology*, 94(8), 850–857. https://doi.org/10.1094/PHYTO.2004.94.8.850

Volpin, H., & Elad, Y. (1991). Influence of calcium nutrition on susceptibility of rose flowers to *Botrytis* blight. *Phytopathology*, *81*, 1390-1394. https://doi.org/10.1094/Phyto-81-1390

Vos, C. M. F., De Cremer, K., Cammue, B. P. A., & De Coninck, B. (2015). Toolbox of *Trichoderma* spp. in the biocontrol of *Botrytis cinerea* disease. *Molecular Plant Pathology*, *16*, 400–412. https://doi.org/10.1111/mpp.12189

Wang, Q., Cuellar, W. J., Rajamäki, M.-L., Hirata, Y., & Valkonen, J. P. (2008). Combined thermotherapy and cryotherapy for efficient virus eradication: Relation of virus distribution, subcellular changes, cell survival and viral RNA degradation

in shoot tips. *Molecular Plant Pathology*, *9*, 237–250. https://doi.org/10.1111/j.1364-3703.2007.00446.x

Wang, S. (2021). *Diagnosing hemp and cannabis crop diseases*. CAB International. https://doi.org/10.1079/9781789246070.0000

Watkinson, S. C., Boddy, L., & Money, N. P. (Eds.). (2016). *The fungi* (3rd ed.). Academic Press.

Weiberg, A., Wang, M., Lin, F.-M., Zhao, H., Zhang, Z., Kaloshian, I., Huang, H.-D., & Jin, H. (2013). Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science*, *342*(6154), 118–123. https://doi.org/10.1126/science.1239705

Whipps, J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, *52*(357), 487–511. https://doi.org/10.1093/jexbot/52.3.487

Williamson, B., McNicol, R. J., & Dolan, A. (1987). The effect of inoculating flowers and developing fruits with *Botrytis cinerea* on post-harvest grey mould of red raspberry. *Annals of Applied Biology*, *111*(2), 285–294. https://doi.org/10.1111/j.1744-7348.1987.tb01454.x

Williamson, B., Tudzynski, B., Tudzynski, P., & Van Kan, J. A. L. (2007). *Botrytis cinerea*: The cause of grey mould disease. *Molecular Plant Pathology*, *8*(5), 561–580. https://doi.org/10.1111/j.1364-3703.2007.00417.x

Wilson, C. L., Solar, J., El Ghaouth, A., & Wisniewski, M. (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Disease*, *81*(2), 204–210. https://doi.org/10.1094/PDIS.1997.81.2.204

Wilson, A. D. (2018). Applications of electronic-nose technologies for noninvasive early detection of plant, animal and human diseases. *Chemosensors*, *6*(4), Article 45. https://doi.org/10.3390/chemosensors6040045

Wong, M.-Y., Saidi, N. B., Vadamalai, G., Teh, C. Y., & Zulperi, D. (2019). Effect of bioformulations on the biocontrol efficacy, microbial viability and storage stability

of a consortium of biocontrol agents against *Fusarium* wilt of banana. *Journal of Applied Microbiology*, *127*(2), 544–555. https://doi.org/10.1111/jam.14310

Wood, R. K. S. (1951). The control of diseases of lettuce by the use of antagonistic organisms I: The control of *Botrytis cinerea* Pers. *Annals of Applied Biology*, *38*(1), 203–216. https://doi.org/10.1111/j.1744-7348.1951.tb07797.x

Xiao, G., Zhang, Q., Zeng, X., Chen, X., Liu, S., & Han, Y. (2022). Deciphering the molecular signatures associated with tolerance to *Botrytis cinerea* in strawberry flower by comparative and dynamic transcriptome analysis. *Frontiers in Plant Science*, *13*, Article 888939. https://doi.org/10.3389/fpls.2022.888939

Xu, X., Harris, D. C., & Berrie, A. M. (2000). Modeling infection of strawberry flowers by *Botrytis cinerea* using field data. *Phytopathology*, *90*(12), 1367–1374. https://doi.org/10.1094/PHYTO.2000.90.12.1367

Xu, H., Zhu, S., Ying, Y., & Jiang, H. (2006). Early detection of plant disease using infrared thermal imaging. In *Infrared Components and Their Applications* (Vol. 6381, pp. 638110–638117). SPIE. https://doi.org/10.1117/12.685534

Yasutake, D., Yu, X., Asano, T., Ishikawa, M., Mori, M., Kitano, M., & Ishikawa, K. (2015). Control of greenhouse humidity and airflow with fogging and circulation systems and its effect on leaf conductance in cucumber plants. *Environmental Control in Biology*, *52*(2), 101–105. https://doi.org/10.2525/ecb.52.101

Yedidia, I., Srivastva, A. K., Kapulnik, Y., & Chet, I. (2001). Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant and Soil*, *235*(2), 235–242. https://doi.org/10.1023/A:1011990013955

Yermiyahu, U., Shamai, I., Peleg, R., Dudai, N., & Shtienberg, D. (2006). Reduction of *Botrytis cinerea* sporulation in sweet basil by altering the concentrations of nitrogen and calcium in the irrigation solution. *Plant Pathology*, *55*, 544–552. https://doi.org/10.1111/j.1365-3059.2006.01388.x

Yong, S. H., Song, H. J., Park, D. J., Kim, D. H., Park, K. B., & Choi, M. S. (2021). Chemical compositions and antifungal activity against *Botrytis cinerea* of the

essential oils from the leaves of three conifer species. *Forest Science and Technology*, *17*(4), 169–179. https://doi.org/10.1080/21580103.2021.1976683

Yu, W., Wang, J., Shao, X., Xu, F., & Wang, H. (2015). Antifungal modes of action of tea tree oil and its two characteristic components against *Botrytis cinerea*. *Journal of Applied Microbiology*, *119*(5), 1253–1262. https://doi.org/10.1111/jam.12939

Yu, X., Li, B., Fu, Y., Jiang, D., Ghabrial, S. A., Li, G., Peng, Y., Xie, J., Cheng, J., Huang, J., & Yi, X. (2010). A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus. *Proceedings of the National Academy of Sciences*, *107*(18), 8387–8392. https://doi.org/10.1073/pnas.0913535107

Zapata, C. J., Miller, C. J., & Smith, R. H. (1995). An *in vitro* procedure to eradicate potato viruses X, Y, and S from Russet Norkotah and two of its strains. *In Vitro Cellular & Developmental Biology – Plant*, *31*, 153–159. https://doi.org/10.1007/BF02036346

Zhang, Q., Chen, X., Guo, H., Trindade, L. M., Salentijn, E. J., Guo, R., Guo, M., Xu, Y., & Yang, M. (2018). Latitudinal adaptation and genetic insights into the origins of *Cannabis sativa* L. *Frontiers in Plant Science*, *9*, Article 1876. https://doi.org/10.3389/fpls.2018.01876

Zhang, X., & Nuss, D. L. (2016). Engineering super mycovirus donors: Redefining the limits of mycovirus transmission to *Botrytis cinerea*. *PLoS Pathogens*, *12*(11), e1005677. https://doi.org/10.1371/journal.ppat.1005677

Zheng, Y. (2022a). Introduction. In Y. Zheng (Ed.), *Handbook of Cannabis Production in Controlled Environments* (pp. 1–11). Taylor & Francis Group.

Zheng, Y. (2022b). Rootzone management in cannabis production. In Y. Zheng (Ed.), *Handbook of Cannabis Production in Controlled Environments* (pp. 163–188). Taylor & Francis Group.

Zhou, F., Xu, M., Chen, X., Qiu, J., Wang, F., Yan, R., Wang, J., Zhao, S., Xin, X., & Chen, L. (2020). Application of antagonist *Bacillus amyloliquefaciens* NCPSJ7

against *Botrytis cinerea* in postharvest Red Globe grapes. *Food Science & Nutrition*, *8*(3), 1499–1508. https://doi.org/10.1002/fsn3.1434

Zhou, X., & Zeng, Z. (2021). The role of CRISPR/Cas system in plant resistance to biotic stress. *Frontiers in Plant Science*, *12*, Article 717404. https://doi.org/10.3389/fpls.2021.717404

Zimand, G., Elad, Y., & Chet, I. (1996). Effect of *Trichoderma harzianum* on *Botrytis cinerea* pathogenicity. *Phytopathology*, *86*(11), 1255–1260. https://doi.org/10.1094/Phyto-86-1255

Appendix Supplementary Materials

Supplemental Table A.1. Greenhouse cannabis cultivation conditions during early flowering (weeks 1 - 2) and late flowering (weeks 3 - 7).

Parameter	Early Flowering [a]	Late Flowering [b]
Temperature	22 - 25 C	22 - 25 C
Relative Humidity	65 - 75 %	55 - 65 %
Absolute Humidity	16 - 18 g/m^3	12 - 16 g/m^3
Humidity Deficit	10 - 11 g/m^3	8 - 9 g/m^3
Light Intensity	1500 - 1700 $\mu mol/m^2/s$	1500 - 1700 $\mu mol/m^2/s$
Daily Total Light	12 - 34 mol/m^2	12 - 34 mol/m^2
Air Circulation	14 - 15 $m^3/m^2/h$	15 - 16 $m^3/m^2/h$
Daily Total Water	0.5 - 1.5 L / plant	2 - 4 L / plant
Water EC	Feed: 2.2 - 2.8 / Drain: 5 - 6	Feed: 2.2 - 2.8 / Drain: 5 - 6
Water pH	Feed: 5.5 - 6 / Drain: 6 - 7	Feed: 5.5 - 6 / Drain: 6 - 7

[a] Early flowering was defined as weeks 1 - 3 of flowering
[b] Late flowering was defined as weeks 4 - 7 of flowering

Supplemental Figure B.1. Images of Infrared-derived surface temperatures of inflorescences of several different cannabis genotypes. The temperatures indicated are those generated by the IR measuring device that correlates emitted fluorescence to temperature visualized with colour gradients, dark blue indicating the 'coolest' surface in the image and light yellow indicating the 'warmest' surface. A) Inflorescence of 'LB'. B) Inflorescence of 'OG'. C) Inflorescence of 'DO'. D) Wide-angle image of a 'DO' crop, 30 minutes before blackout initiation. E) Wide-angle image of a 'DO' crop, 30 minutes after blackout initiation. The addition of the blackout curtain reduced the surface temperature of the inflorescence significantly.