Disease Management Approaches For *Cannabis sativa* L.

by

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Abstract

Cannabis (*Cannabis sativa* L.) plants are affected by many pathogens, including *Golovinomyces* spp., *Fusarium oxysporum* and *Pythium* spp. which cause severe losses to producers. Currently, there are a limited number of commercially available products to manage these diseases but data about their comparative efficacy is lacking. Therefore, the efficacy of various management options for these diseases was tested. The most effective treatments that significantly ($P < 0.05$) reduced powdery mildew were Luna Privilege SC (fluopyram), Regalia® Maxx, MilStop®, Rhapsody ASO™, neem oil, and Stargus®. Daily exposure of plants to UV-C light significantly reduced disease (by 45.2%). The most effective treatments for *F. oxysporum* management were Lalstop, Rootshield, Asperello and Stargus, which provided a significant reduction (30 to 56.3%) in mean disease severity 14 days post-inoculation. Lalstop and Rootshield were also effective at significantly reducing *P. myriotylum*.

Keywords: *Cannabis sativa*; biological control; disease management; powdery mildew; *Fusarium oxysporum*; *Pythium myriotylum*

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1.1. Cannabis Botany and Brief History

1.1.1. Botany and Anatomy

Cannabis (*Cannabis sativa* L.) is an annual flowering herb belonging to the *Cannabaceae* family. Cannabis plants are erect, apically dominant and can grow to heights of 5 to 6m, although most plants are typically closer to 2 to 3m in height (Raman et al. 2017). Stems of plants are cylindrical and green with a woody or hollow interior. Branching occurs in an alternate or opposite pattern from the stems. Leaves are green, palmate and typically consist of between 3 to 9 lobes. The margins of leaves are serrated (Farag and Kayser 2017). The length and width of leaflets may vary considerably, with different leaf morphologies often being associated with different classifications of cultivars ("indica" and "sativa").

Cannabis plants are naturally dioecious, although monoecious hemp cultivars have become more prevalent in recent years. Until flowering begins the sex of plants can be hard to visually determine. Male flowers are pale green, grow singularly or in clusters, and consist of five tepals and five stamens at the end of a thin drooping pedicel (Farag and Kayser 2017). From these hanging male flowers prodigious amounts of pollen can be produced and carried to female plants via the wind (Small and Naraine 2016). Female flowers consist of an ovary and style from which feathery hair like stigmas protrude. These flowers occur in clusters at the apex of inflorescences, which are borne in pairs on short stalks at the axils of branches (Raman et al. 2017). If female flowers are pollinated, an achene containing a single seed with a hard, brown, mottled shell is produced (Farag and Kayser 2017).

Female flowers, especially of drug type cultivars, are notable for their dense covering of trichomes. However, most aerial parts of a cannabis plant are covered in trichomes, just to a much lesser extent (Farag and Kayser 2017). Cannabis plants produce a range of glandular and non-glandular trichomes, although the focus is often on glandular trichomes (capitate-stalked or capitate-sessile) due to the metabolites they produce (Happyana et al. 2013). Within trichomes precursors to cannabinoids or cannabinoids, such as tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG) and over 100 other phytocannabinoids are synthesized and stored (Radwan et al. 2017). Terpenoids, the compounds that give cannabis its distinct odor, are also

secreted in trichomes. Over 200 different terpenoids have been isolated from cannabis including limonene, pinene, myrcene and caryophyllene. (Grassi and McPartland 2017).

1.1.2. Geographic Distribution, Uses and Classifications

Cannabis is thought to originate from western or central Asia, although the exact area it is indigenous to is unclear. Part of the reason this is not known is because cannabis has been utilized, domesticated, and subsequently spread, by humans for over 6000 years (Small 2017). From its origin, cannabis spread to other parts of Asia, Africa, Europe and eventually to North and South America. Humans throughout these areas utilized cannabis in a variety of ways, in turn selecting and breeding for those desired traits. These traits include seed, fiber (hemp) or THC production for medical, recreational or spiritual uses (cannabis, marijuana). Hemp, cannabis and other classifications of *C. sativa* will be discussed in more detail later in this introduction.

Hemp plants may be grown for their valuable bast fibers, the phloem fibers that ring the woody xylem core of the plant (Small 2017). Cannabis cultivation for fiber was introduced to western Asia, Egypt and Europe between 1000 to 2000 BC, with cultivation subsequently becoming widespread in Europe (Small 2017). From these fibers, textiles, clothing, cordage, paper and other products can be made. Although the demand for hemp fiber has decreased due to the development of other natural and synthetic fibers, it is still a significant crop throughout Europe and Asia (Small 2017).

Cannabis plants may also be grown for the seeds that they produce, which historically were considered a staple grain in areas like China (Small 2017). These seeds may be eaten whole or processed to make peanut butter or tofu-like foods. The oil from the seeds has also been used to make soaps, paints and varnishes, and for a period was even utilized as a low-cost alternative to whale oil for lighting lamps. Recently, there is renewed interest in the possible pharmaceutical use of cannabis seeds or compounds they contain, in addition to an increased focus on their potential nutritive properties or health benefits. This has in part lead to an increase in production of cannabis plants for edible and industrial oilseed, particularly in countries such as Canada and the United States. (Small 2017).

Due to the cannabinoids and terpenes they produce cannabis plants are also utilized as medicine, for religious ceremonies and recreationally. There is evidence that cannabis was used as medicine in many major civilizations of the ancient world including Assyria, Rome, Greece

and the Islamic Empire (Mechoulam and Parker 2013; Small 2017). Historically and in the modern day, cannabis has been used for the treatment of arthritis, tetanus, epilepsy, migraines, inflammation, insomnia, loss of appetite, glaucoma and other ailments (Russo 2017; Small 2017). In many parts of the world, such as India, Afghanistan and parts of South Asia cannabis has also played a role in social and spiritual life. To this day cannabis use continues to be a facet of religions including Hinduism and Rastafarianism (Hasan 1975; Small 2017; Waldstein 2020).

For thousands of years cannabis has been used as a recreational drug, yet attitudes towards cannabis use continue to vary greatly by culture. Historically, cannabis use has been more common or socially acceptable in parts of Asia, Africa or the Middle East. Elsewhere it has generally remained relatively unpopular, or its use has been stigmatized and associated with rural, poor minority communities (Hasan 1975; Small 2017). In the 20th century cannabis use gained popularity in America and Canada especially among artistic and hippy communities, with the rise of the counterculture of the 1950s and 60s. In tandem laws regulating cannabis were created. As of 1923, cannabis was criminalized under the Canadian Schedule of the Opium and Narcotics Act. Its inclusion in subsequent laws such as the Narcotics Control Act of 1961 furthered penalties for use and production of cannabis, as it was now formerly considered a narcotic (Green and Miller 1975). In America similarly strict stances were taken towards cannabis, with the Marihuana Tax Act (1937) and the Controlled Substances Act of 1970. This made cannabis illegal and subsequently considered a schedule I drug (a drug with a high potential for abuse and no accepted medical use) (Zuardi 2006). Over time there has been a slow shift in public opinion and drug policy, allowing for legal medical and recreational use of cannabis in Canada (in 2001 and 2018, respectively) (Fischer et al. 2015; Crépault 2018). However, in the United States cannabis continues to be federally classified as a schedule I drug, despite an increasing number of States allowing for legal, medicinal, or recreational use.

As *C. sativa* has been utilized by humans in these diverse ways the need for distinctions between plants cultivated for different uses has arisen. Legally in Canada industrial hemp plants are considered to be plants that have less than .3% THC in their leaves and flowers, whereas plants with THC values higher than this are considered "cannabis" (Government of Canada 2018). The language used to describe cannabis plants used for medical or recreational purposes is typically unreliable and informal. Strains of cannabis are often described as "sativa" (narrow leaflets, tall, late maturation, descendants from Asia, Africa or South America), "indica" (broad leaflets, shorter, early maturation, descendants from Afghanistan or India), "hybrid" or some mix of terms such as "indica dominant hybrid" (McPartland 2017). These classifications also attempt

to describe the effects of different strains with sativa strains being a cerebral stimulating "head" high and indica strains being a relaxing "body" high (Hazekamp 2016). Due to the extensive breeding of cannabis plants and the unregulated movement of germplasm to and from areas such as Afghanistan, the Netherlands, America, Africa and South America, since roughly the 1970s almost all cannabis plants are in essence "hybrids", with few actual landrace strains still existing (McPartland 2017). This vernacular is also not related to the formal taxonomic classifications of cannabis as *C. sativa* or *C. indica*.

Confusion around cannabis strain names and classifications is only made worse by the largely unregulated and illicit nature of the market (Hazekamp and Fischedick 2011). Over one thousand cannabis strains exist and strain names are regularly changed, counterfeited and are generally unreliable (McPartland 2017). In response to the confusion and unreliability around cannabis strain names, genetics and their effects when consumed, there is a shift towards other classification systems. Research in these areas is ongoing but it centers on comparing and identifying cannabis strains based on their genetic sequences or the cannabinoids and terpenoids they produce (chemovar) (Hazekamp and Fischedick 2011; Hazekamp 2016; McPartland 2017).

1.2. Cannabis Cultivation

1.2.1. Propagation and Vegetative Growth

The use of vegetative cuttings, or "clones", is the standard method for cannabis propagation. Cuttings are taken from stock (mother) plants of the desired variety, dipped in a rooting compound (gel or powder containing phytohormones such as indole-acetic acid) and placed in a growing media such as coco coir, rockwool, peat or soil (Punja 2021). Clones are kept in a high humidity environment for 10-14 days, under 18-24 hr lighting to facilitate rooting. Cannabis may also be propagated in aeroponic systems but this is not common. Once the cuttings are rooted they are transferred to a larger volume of growing media and allowed to grow vegetatively. Plants are kept in a vegetative state by exposing them to a photoperiod of approximately 16-24 hr of light. The length of time that plants are kept in this state varies depending on the production system and crop plan specific to the producer, as well as the genetics of the plant. It is most common to grow plants vegetatively for between 14-28 days after transplant.

Mother plants are also maintained in a vegetative state so that producers have a constant supply of cuttings. As cannabis is commercially grown for its medicinal and recreational uses, high levels of cannabinoids and terpenes are desired; Therefore, only female plants are maintained as stock plants. Stock plants may be used to produce clones for a prolonged period of time, with some producers keeping mother plants for upward of a year. However, mother plants may accumulate diseases over time, which they may pass on to cuttings taken from them (Punja 2021). Other abiotic factors and stresses on the plant may also cause them to gradually lose their vigour, reducing the health of cuttings taken from them.

Cannabis plants may be produced from seed, although due to the inherent phenotypic variability in plants grown from seed, this is not common on a large scale. Cannabis plants that flower based on the age of the plant rather than the photoperiod, known as autoflowers, are grown from seed. However, the commercial large-scale use of autoflowering varieties is not widespread, especially for indoor or greenhouse production. It is possible to produce "feminized" cannabis seeds (seeds that only produce female plants) by treating flowering female plants with compounds such as silver thiosulphate, although the use of feminized seeds is still not a prevalent approach for cannabis propagation on a commercial scale. When producers are looking for a new variety, or "pheno-hunting", they may grow cannabis plants from seed and select new stock plants based on the desired characteristics.

1.2.2. Flowering and Harvest

Flowering is induced when plants receive at least 12 hr of uninterrupted darkness. Producers may manipulate the amount of light plants receive with black out curtains in greenhouses or tarps for smaller hoop style greenhouses in order to flower plants regardless of the amount of light being produced at that time of the year. Supplemental lighting is also commonly used during the parts of the year when the growing conditions are not optimal. For producers of indoor cannabis lights are simply turned on or off to manipulate the photoperiod plants receive.

Cannabis plants are flowered in this photoperiod for approximately 7-9 weeks, depending on the variety and the crop plan of the producer. Flowers may be assessed for maturity based on the appearance of their stigmas, which transition from white to brown or orange colour typically, and the appearance of trichomes. As different metabolites accumulate in trichomes their color shifts from clear, to milky to amber. Producers may also test THC levels as plants reach maturity and adjust the date of harvest based on this data.

1.2.3. Drying and Processing

Plants are harvested by hand by cutting them at the crown. Whole plants may then be hang dried, or branches may be removed from the plants with the flowers intact and dried. Drying rooms for cannabis are specifically designed and are required to have adequate airflow and maintain specific ranges of temperature, humidity and even CO_2 as drying progresses. When flowers are dried to the desired moisture content they are then trimmed (referred to as a dry trimming) and packaged. Conversely, plants may also be trimmed when they are fresh (wet trimming) and then dried, depending on the producer. Additional time for "curing" may also be allotted when flowers are dried and trimmed. Curing is a prolonged drying process where moisture is more slowly removed from flowers in a controlled environment. This is done in order to increase the quality (flavor, smell, etc.) of the flowers. Cannabis flowers may be sold whole, in prerolled joints (prerolls), or further processed to make edibles or extracts.

1.3. Production Systems

Cannabis may be grown in a variety of production systems, including controlled environments such as greenhouses or indoor facilities, or outdoor. However, the latter is less prevalent in Canada. Controlled environment cannabis production makes extensive use of HVAC systems, fans, supplemental lighting and automated irrigation systems to optimize plant growth and yields. Cannabis may be grown in soil mixes, although this is less common than coco coir or rockwool growing medias and is often only utilized by organic producers of cannabis in Canada.

1.4. Pathogens of Cannabis

Numerous pathogens cause disease on cannabis, and as cannabis research and cultivation increase new diseases are emerging (Punja 2021). Cannabis roots and crowns, stems, foliage and flowers, both pre and postharvest, may all be infected by pathogens. The most common and damaging pathogens include fungal pathogens such as *Fusarium oxysporum* (Punja et al. 2018; Punja 2021), that causes root and crown rot and damping off on cuttings, *Golovinomyces* spp., which causes powdery mildew on foliage (Scott and Punja 2020; Punja 2021) and *Botrytis cinerea*, which causes bud rot and post-harvest decay (Punja 2021). Oomycetes, namely *Pythium* spp., are also significant pathogens on cannabis, causing root and crown rot (Punja et al. 2018; Punja 2021). Bacterial species as well as viruses and viroids are less common pathogens but may still cause considerable damage to cannabis crops (Punja 2021). Cannabis grown in all of the

previously mentioned production environments (indoor, outdoor, greenhouse) may be affected by these diseases. The disease cycle, symptoms and signs, and key management strategies for each of the most prominent pathogens is discussed in the following chapter in addition to information about emerging pathogens.

1.5. Research Objectives

Cannabis producers in Canada have access to a limited number of registered biological control products or reduced risk chemicals for disease control on cannabis, and data about the comparative efficacy of these products on cannabis is lacking. Additionally, information about the diagnosis and management of diseases on cannabis can at times be informal and inadequate. Addressing these areas may assist producers in managing and understanding diseases.

The aims of this research were to:

1. Summarize the current diagnosis and management strategies of prominent and emerging pathogens of cannabis.

2. Evaluate the efficacy of different management strategies for powdery mildew on cannabis. These include biological controls, reduced risk chemicals, ultraviolet light and genetic resistance.

3. Evaluate the efficacy of biological controls at managing *Fusarium* and *Pythium* diseases on cannabis.

Chapter 2. Management of Diseases on Cannabis in Controlled Environment Production

2.1. Introduction

Plant pathogens have been impactful to humans throughout history, damaging and destroying crops and causing hunger, malnutrition, and starvation. Some diseases have caused people to uproot their families in search of other work or food. Initially, blighted and diseased crops were seen as punishment from wrathful or unhappy Gods and ancient Romans and other peoples sought to manage plant diseases by appeasing deities with sacrifices and prayer (Agrios 2005). Eventually, through advances in science and inventions, such as the microscope, scientists were able to determine that microscopic organisms were responsible for these diseases. As a result, several hundred thousand plant diseases have been described to date. Management approaches for plant diseases have also shifted over time. Approaches have progressed from the use of compounds like Bordeaux mixture (lime and sulfur) to control downy mildew of grapes, and sulfur or copper to manage blights, to our modern synthetic chemicals, as well as biological and cultural control methods (Agrios 2005). Even with modern diagnostic tools and management strategies, plant diseases still cause hardship and upwards of $200 billion in crop losses worldwide every year.

In this chapter, general diagnostic approaches and management strategies for plant diseases will be discussed. How these management strategies apply to the most prominent diseases of cannabis produced in controlled environments (*Fusarium*, *Pythium*, powdery mildew and bud rot) and the biology of the pathogens that cause these diseases will be the main focus. As the production of hemp and cannabis increases throughout the world, new diseases are emerging or less common diseases are spreading (Punja 2021). Being able to identify and manage these new potential threats will also be discussed to assist growers.

2.2. Plant Diseases and Their Diagnosis

2.2.1. Definition

The definition used for disease is “a change from a plant’s normal development and appearance caused by a living (biotic) entity that reproduces and spreads to adjacent plants i.e., it is infectious”. This definition applies to most pathogens, including fungi, bacteria, viruses, viroids

and nematodes. Other definitions of disease may encompass nonliving (abiotic) factors, such as nutritional deficiencies, physical or mechanical damage, drought, overwatering, light damage or any persistent damage to the plant i.e., they are noninfectious and do not spread. Determining whether a plant is affected by a disease or an abiotic cause is the first step in the diagnosis of a problem. Improper diagnosis, and subsequently inadequate treatment methods, can be costly in terms of time, money (labour, cost of products, etc.) and the potential loss of yield and quality of the crop.

2.2.2. Diagnosis

To properly manage any plant disease affecting a crop, one must first identify what the problem is. Accurate diagnosis of a disease requires the investigation and consideration of many interacting factors, such as what signs and symptoms are displayed on plants in the affected areas, potential patterns of symptoms that may be present, the various biotic factors present at the time, as well as the interplay of abiotic and environmental influences.

The problem should be described in terms of specific symptoms on the affected plant. Symptoms are defined as the visible manifestation, both externally and internally, of the effects of a disease. Examples of symptoms include yellowing of the foliage (chlorosis), wilting of the plant, stunted growth, rotting of roots, and darkening of internal tissues. It should also be determined where symptoms occur on a plant, and whether they occur on all tissues or only on a specific part of the plant. For example, chlorosis may occur only on new growth, only on older leaves, or over the entire plant.

In addition to symptoms, signs of plant disease can be used in diagnosis. Signs are the observable physical presence of a pathogen and are most commonly seen in fungi. Examples include conidia (spores), mycelium, and reproductive structures such as pycnidia or sclerotia. Signs are generally characteristic of a particular pathogen and therefore are useful for the diagnosis of a disease.

The pattern of symptom appearance on affected plants may also be indicative of whether it is cause by a biotic vs. abiotic factor. Are the affected plants found throughout the growing area or only in one particular location? If all plants along a wall or in a particular tray of cuttings are affected, it could be indicative of an abiotic cause. Uniform damage on a plant, or over a larger number of plants, is typically associated with abiotic factors. Damage that is random or unevenly

distributed on the plant is more likely to be caused by a pathogen or a biotic factor. If the problem appears to be spreading to other plants, it is likely to be caused by a biotic factor. These differences are summarized in Table 2.1.

Table 2.1 A comparison of abiotic and biotic symptoms on plants

Abiotic Factors	Biotic Factors
Uniform symptoms.	Symptoms appear uneven.
Symptoms observed in a uniform or discrete area.	Affected plants are spread throughout the area.
Symptoms do not spread or progress.	Symptoms progress and may spread to neighbouring plants or growing areas.
No signs associated with pathogens are observed.	Signs associated with pathogens are observed.

2.2.3. Abiotic Factors

Abiotic and environmental stresses can complicate attempts to diagnose plant diseases as they can cause similar symptoms to those caused by some pathogens. For example, nutrient deficiencies that cause yellowing of leaves, such as nitrogen or magnesium deficiency, may appear similar to chlorosis caused by a pathogen such as *Fusarium*. The effects of overwatering may appear similar to symptoms of root rot caused by *Pythium*. Environmental and abiotic factors that can affect plant health include extremes of pH of the growing medium, nutrient excesses and deficiencies, improper drainage, lack of water or overwatering, extremes of temperature and humidity, improper light levels, physical damage from severe pruning or girdling from trellises. All of these need to be considered when a diagnosis is being made. In addition, extraneous materials (sprays, fertilizer, etc.) applied to plants should be considered with regard to potential for damage to plants if applied incorrectly or at a higher dose than recommended. If other crops are grown nearby, drift from sprays applied to those crops should also be considered as a potential source of damage or contamination to your plants.

The characteristics of the cannabis strain (genotype) being grown, and its normal appearance at that stage in its growth, should be known. Knowing the features of a healthy plant allows for more accurate comparisons to be made to the suspected symptoms on diseased plants. Chlorosis and leaf drop are normal at the end of the plant's growth cycle, cuttings may wilt if not hardened off, and some strains may appear phenotypically differently from others but that does not suggest they may be affected by a pathogen.

Confirmation of disease diagnosis is important and can be provided by a commercial testing lab, at a university diagnostic lab or by qualified consultants. Growers are advised to seek the help of someone who is proficient in disease diagnosis. This person should conduct a site visit if possible as it is often very difficult to diagnose an issue by phone or other correspondence, especially without access to samples to examine. Growers may also consult previous descriptions of these types of problems from reliable sources that can be found on the Internet or in institutional libraries to try and associate the symptoms seen with previously reported symptoms of the suspected disease.

2.3. How Disease Develops

2.3.1. Disease Development

Many factors are required for the development of a disease. The first is a susceptible host plant. The vigour of the plant, the age of the plant, conditions under which the plant is growing, and genetic composition all play a role in determining whether or not it may be susceptible to disease. The second requirement is the presence of a pathogenic organism (fungi, bacteria, virus, etc.), which also determines the incidence and severity of disease. The third requirement is an environment that is conducive to the development of a pathogen. High humidity, leaf wetness and an anaerobic or overwatered root zone are examples of environments that are conducive to the development of some pathogens. Conversely, factors such as extreme temperatures and dryness may inhibit plant disease by reducing pathogen growth, even if the plant is susceptible to infection (Agrios 2005). The environment may also play a role in the growth, reproduction and dispersal of the pathogen, as well as affect the susceptibility of the host plant.

2.3.2. Disease Triangle

The interactions between a plant, a pathogen, and the environment can be described in a disease triangle (Fig. 2.1). The disease triangle is a fundamental concept in plant disease development, which illustrates all three elements that must be present in order for a disease to develop. However, even when all requirements of the disease triangle are present, disease may fail to develop due to inappropriate timing. Therefore, “time” may be considered as the fourth element of this model, creating a disease tetrahedron, or disease pyramid. The pathogen, the host and a conducive environment must exist together for a certain period of time in order for disease to develop. The length of time during which these three elements occur together also plays a role

in the severity and incidence of disease. For example, increasing wetness duration on flowers and fruits on strawberries can increase the incidence of *Botrytis* infection. A similar phenomenon may also apply to cannabis and bud rot development.

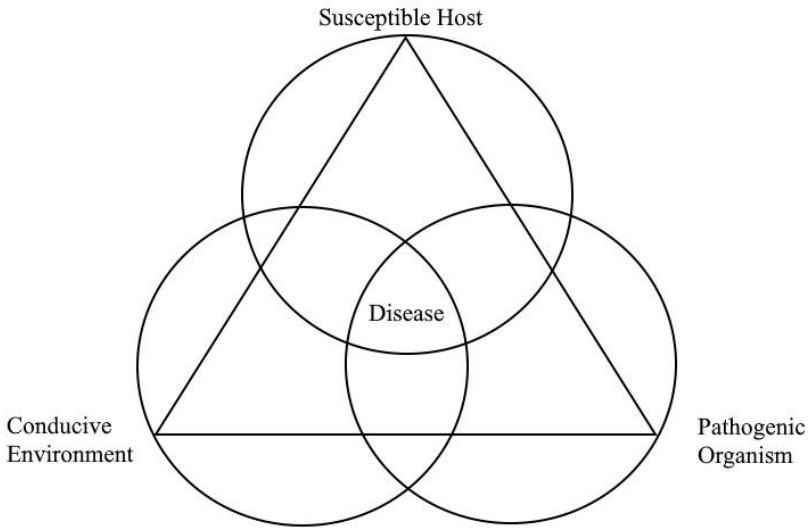

Figure 2.1 The disease triangle, highlighting the three main factors necessary for the development of disease.

By altering any individual component of the disease triangle, the incidence or severity of the disease could be altered (Agrios 2005). Therefore, disease management practices by growers should be targeted to disrupt one or more components of the disease triangle. Planting a less susceptible host genotype, altering the humidity in the growing environment, spraying a product that reduces spore production by a pathogen, are all examples of disruption of one aspect of the disease triangle that can reduce disease development.

2.3.3. The Disease Cycle

Diseases progress over time according to a series of specific events is termed the "disease cycle" (Fig. 2.2). The events that occur in succession which result in the development of disease include the following:

1) Infection of the host by a pathogen (from primary inoculum) that establishes itself in the plant tissues. Symptoms may be apparent here.

2) Colonization of the tissues as the pathogen grows and develops on the host. Symptoms will be present.

3) Reproduction to produce more inoculum (secondary inoculum) that can re-initiate infection.

4) Spread of the pathogen, sometimes in several cycles during one growing season.

5) Survival of the pathogen.

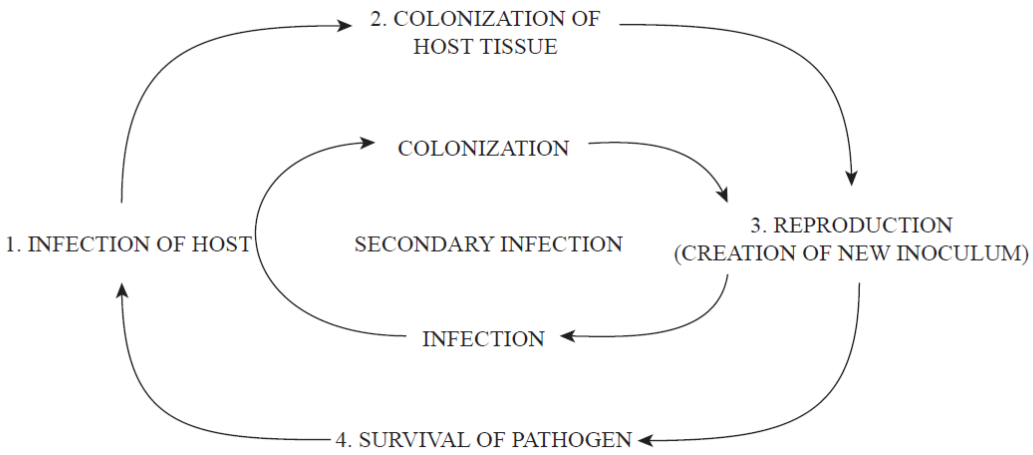

Figure 2.2 A general disease cycle, showing the steps of Infection, Colonization, Reproduction and Survival and the possibility of a secondary disease cycle.

Knowledge of the disease triangle, disease cycles, and the various biotic and abiotic factors that influence them, can be used to identify opportunities to disrupt the cycle, leading to disease management. These types of general management approaches include exclusion or avoidance, eradication, protection and resistance (genetic and induced). These approaches are discussed in more detail below.

2.4. Disease Management Principles

2.4.1. Exclusion

In this disease management strategy, the aim is to prevent the introduction of the pathogen into the growing environment. For example, exclusion can be achieved by placing a quarantine on any plant material before it is allowed to enter the facility in an area separated from the main growing environment. The plants are examined regularly for signs or symptoms of disease, or pests, before they are released. The use of pathogen or pest free plant material is

another important aspect of exclusion. Producers should verify from their source of cuttings or seeds that they were produced in an environment free of pathogens. This is particularly important as at the present time, as there are no government enforced certification requirements for propagated cannabis plants. Testing services could be used to ensure the status of plants with regard to presence of pathogens before they are introduced into a facility. Other examples of exclusion of pathogens from the growing environment include proper sanitation of equipment entering the area where plants are being grown, implementation of footbaths, and ensuring workers move through rooms considered to be most "clean" to least "clean". These should be placed at all points of potential entry into a growing facility. The use of HEPA filters and UV lights can also preclude introduction of pathogen spores into a clean facility.

2.4.2. Avoidance

This strategy aims to prevent establishment of a pathogen by creating an environment that is not conducive to disease development. For example, cultural control methods are practices that attempt to alter the growing environment to prevent infection by the pathogen. Improved drainage of soil, growing susceptible varieties only when disease pressure is low, minimizing excess nutrients, pruning plants to improve air circulation and reduce areas of high humidity, and providing proper storage conditions post-harvest are examples of cultural control methods that can reduce disease development and spread.

2.4.3. Eradication

The principle of eradication is based on eliminating or reducing the amount of the pathogen even after it has entered the growing environment, but before it spreads and becomes established. This can be achieved through cultural control methods, such as removal of infected plant material or entire plants, cleaning of benches and other surfaces during a growing cycle and between cycles, and thorough sanitization of growing rooms. Physical control methods to eradicate a pathogen include treatment of seeds with disinfectants or hot water, pasteurization of irrigation water, sterilization of growing media, and cleaning of all equipment. Tissue culture methods utilizing meristem tissue can be used to eradicate a virus from stock plants but it has not been widely used yet in cannabis.

2.4.4. Protection

Protective measures are aimed to protect the plants before they become infected, through the application of a treatment that can kill or severely reduce growth of the pathogen eg. fungicide sprays, drenches, dusts. An example of a protective measure that can be used on cannabis in Canada are sulfur-based products to manage powdery mildew. When fungal spores come in contact with sulphur-treated leaves or flower tissues, they do not germinate, thus disrupting the disease cycle. Some products may act to eradicate a pathogen as well as protect against future infection. One example of this is potassium bicarbonate products (MilStop, Armicarb) as they may eradicate a pathogen through their direct fungitoxic characteristics, as well as offer some degree of protection as they reduce the development of fungal mycelium and spores on plant tissues by altering their pH and osmotic pressure for a time. Traditional chemical fungicides are not registered for use on cannabis currently but are a form of eradication or protection used in other crops.

The application of biological control agents can also provide protection against infection by root-infecting pathogens and foliage or flower-infecting pathogens. Biological control agents (living organisms formulated in commercial products for control of pathogens or insect pests) can be used to reduce the level of pathogen inoculum in soils or on plants when applied as drenches or sprays, respectively. These microbes compete with the pathogen for resources, by colonizing the soil or tissues before pathogens are able to, or in some cases they will directly parasitize and destroy pathogens.

2.4.5. Genetic Resistance

The use of plant varieties that are resistant or tolerant to one or more diseases is a key strategy in disease management. A resistant variety is able to prevent the pathogen from infecting or reduces its growth and infection through the use of a genetically determined component, such as an enzyme or toxin, resulting in little or no infection. A tolerant variety is still susceptible to disease, but when infected, it will show less damage and still yield well. A variety is said to be immune if it never becomes diseased. At the other extreme, a susceptible variety allows infection to proceed to where the symptoms become severe and the plant could be killed. In most agricultural crops, the use of resistant varieties can be inexpensive, effective and safe. They can reduce both crop losses from disease as well as reduce disease management costs.

In cannabis, detailed information on resistant varieties is scarce and is based mostly on grower observations. However, research is beginning to identify the genetic basis of these traits, with the first cannabis resistance gene against powdery mildew (PM1), as well as potential powdery mildew susceptibility genes, being identified (Mihalyov & Garfinkel 2021; Pépin et al. 2021). The experience of growers suggests there are differences among strains in susceptibility to a number of important diseases, including powdery mildew and *Botrytis* bud rot. However, insufficient knowledge of the source(s) of strains and their genetic background can make it difficult to confirm if resistance genes are present. Genetics derived from known breeders or seedbanks, or cuttings originating from a reliable source, should be used to manage diseases. Further characterization of the basis of the observed resistance is ongoing and efforts to develop new, resistant strains of cannabis are in progress. The evaluation of land races and strains from diverse geographical origins should prove to be useful in the search for resistance genes.

2.4.6. Induced Resistance

The basal resistance that plants have against a range of pathogens can be induced through a variety of methods. For example, plant hormones such as salicylic acid can induce a series of defense responses following application that can lead to systemic acquired resistance. This form of resistance is finite in its activity but can provide protection from infection over several weeks after treatment is made. Some biocontrol agents or plant extracts are reported to be able to induce resistance by inducing expression of proteins that can enhance defense against diseases, provided they are applied in advance of the onset of infection. These defenses are typically not long lasting and their importance in disease management in cannabis needs to be researched.

2.5. Disease Management Practices for Cannabis Production

2.5.1. Cultural Control

- Clean plant materials. For the prevention of any potential disease, incoming plant material that may be infected should be quarantined and inspected. *Pythium* and *Fusarium* may be introduced into a growing facility on infected roots or media that plants are grown in. Powdery mildew and viral diseases can also be introduced into a growing facility on plants if not monitored. Footbaths, sanitary clothing and sanitation of tools and equipment should all be used to reduce the introduction of pathogens.

- Clean environment. *Pythium* can spread through free or standing water as well as irrigation systems, and extra care must be given to maintaining cleanliness in these areas. Drip lines, plumbing, hoses, water storage tanks, nutrient tanks and other irrigation equipment should be regularly cleaned. Untreated water sources such as rivers or ponds may also be a source of *Pythium* in the growing environment and should be avoided. Reusing growing media between cycles should be avoided as this allows for the transfer of pathogens between production cycles and into new environments. Sterilization of these media between cycles can reduce the inoculum carry-over. Air filters and purifiers eg. photocatalytic, UV or ozone, may be used to reduce the spread and intake of pathogen spores. The dehumidifiers and air filters should be regularly cleaned to reduce inoculum build-up.

- Care should be taken to thoroughly clean and sanitize all tools and equipment, as well as all surfaces in the growing environment during the turn around period after harvest. Special attention should be given to equipment used for harvesting plants and trimming buds. Trimming machines may trap plant debris internally, and as resin builds up on surfaces such as belts, spores of molds such as *Penicillium* may accumulate. Wounding from harvest and trimming may increase the incidence of infection, as these wounds provide an opportunity for contaminants to colonize flower tissues post-harvest.

- Hydrogen peroxide or peracetic acid (peroxyacetic acid) contained in ZeroTol or SaniDate 5.0, and alcohol containing products, can also be used for sanitation. Products containing dodecyl dimethyl ammonium chloride, such as Chemprocide or Kleengrow, may also be effectively used to sanitize equipment and surfaces, or in footbaths (Fig. 2.3). In our studies, when tested at concentrations of 0.4% and 1% Chemprocide completely inhibited growth of *Penicillium olsonii*, *F. oxysporum* and *B. cinerea* in liquid culture. ZeroTol was also effective at reducing the growth of these fungi, but only when used at a 1% concentration. These findings indicate both compounds have fungitoxic properties against various fungi commonly found in cannabis growing facilities. For cleaning equipment used in harvesting and trimming, other options may also be worth considering, such as food safe degreasers and steam.

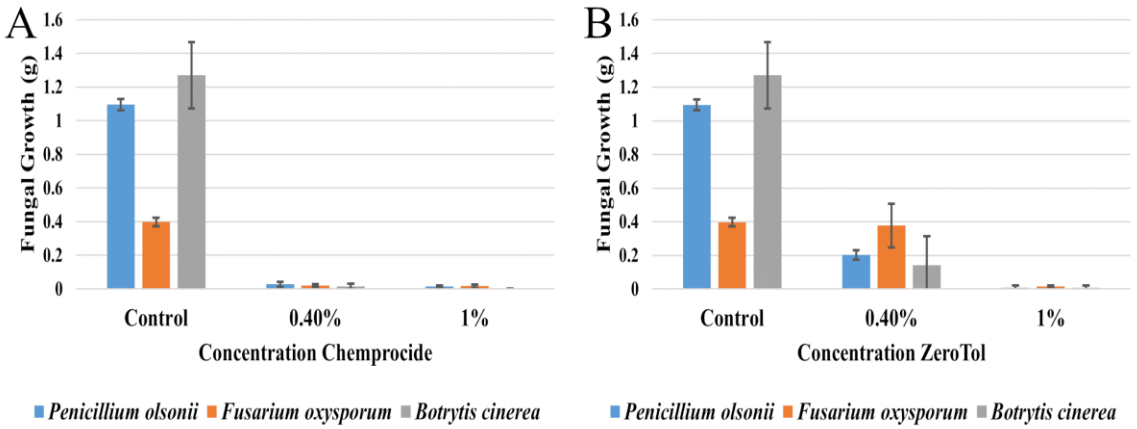

Figure 2.3 **The effects of three concentrations of (A) Chemprocide or (B) ZeroTol on the growth of *Penicillium olsonii*, *Fusarium oxysporum* and *Botrytis cinerea* in potato dextrose broth. Cultures were grown on a shaker table at 125 rpm for 7 days before being strained, dried and weighed (n=4). Error bars are 95% confidence intervals.**

- Removal of diseased plants. Removal of infected plants is important to reduce the spread of pathogens in the growing environment. Plants should be removed as soon as they show symptoms of infection by disposing of them outside the facility to reduce potential spread of inoculum. Plants affected by *Fusarium* and *Pythium* and foliage and buds infected by powdery mildew and *Botrytis* bud rot should be discarded.

- Manage irrigation. Maintaining appropriate moisture levels in the growing media or soil is key to managing root pathogens. Overwatering can encourage the spread of *Pythium* by providing free water as well as creating anaerobic conditions in the rootzone which promote infection (Punja 2021). Low spots that lead to pooling of water on the ground or on tables should be avoided. The nutrient solution should also be well aerated to reduce *Pythium* growth and infection, especially in systems like deep water culture. Well draining media will also help to reduce *Pythium* and *Fusarium* infection by reducing anaerobic conditions and increasing aeration in the rootzone. Extreme cycles of wetting and drying should be avoided as these can cause roots to die back and predispose them to infection. Compartmentalization of plants and their rootzones can reduce spread of pathogens such as *Fusarium* and *Pythium* between plants.

- Stress avoidance. Plants that are growing vigorously and not subjected to any form of stress are generally better able to tolerate or resist infection by pathogens. Forms of nutrient-related stress in the soil or nutrient solution include excessive salinity from high

salt levels, and ionic stress from high concentrations of specific ions such as Na^+, Mg^{2+} and Cl^-. These can directly reduce root growth as well as potentially cause damage or dieback of lateral roots, making them more susceptible to *Pythium* infection. Some *Pythium* species can tolerate conditions of high salinity.

Care should be taken to avoid excessive damage to roots during transplanting, and as previously mentioned, overwatering and cycles of extreme wetting and drying should also be avoided as these factors may also cause stress on plants and promote disease.

Lighting intensity should also be appropriate to the needs of the plant to avoid stress. Cuttings and younger plants growing vegetatively do not require as intense of light as plants in flower, and providing them with too much light, or too much light too quickly, may cause them to appear wilted as they turn away from the light. Leaves may also curl up or in on themselves, turn yellow and appear burnt, especially when lights are too close to the plants and plants are exposed to excessive heat.

- Limit excess nutrients. Excessive fertilization can increase the susceptibility of plants to several diseases. Surplus nitrogen can interfere with regular plant defense responses and cellular signalling, as well as limit the silica content of leaves, all of which may make tissues more vulnerable to infection. Strawberry, tomato and begonia plants have all been shown to be more susceptible to powdery mildew when given higher levels of nitrogen. Grapes fertilized with a higher rate of nitrogen were also more susceptible to *Botrytis* bunch rot. The concentration of nitrogen in a plant also plays a role in the growth and development of the pathogen, with higher levels of nitrogen resulting in increased *B. cinerea* sporulation on basil, increased powdery mildew sporulation on barley and tomato plants and the production of more virulent *B. cinerea* spores on tomato. This may result in a more rapid spread of the pathogen as it is able to produce more inoculum for subsequent infections.

  Nutrient levels also have an effect on bacterial pathogens, such as *Xanthomonas campestris* pathovars. Fertilization with nitrogen, or excess nitrogen, was found to delay the onset and severity of black rot on cabbage and leaf spot on tomato, respectively. Although it is unclear if this is a practical solution for the management of *Xanthomonas campestris* pv. *cannabis*, which has been reported to cause leaf spot on hemp, due to the

costs of inputs and potential trade offs in disease susceptibility to other pathogens and plant health.

The form of nitrogen fertilizer used, as well as the amount, have been shown to have an effect on the disease severity of pathogens such as *Fusarium* spp. as well. These factors directly affect the virulence of *Fusarium* as well as the susceptibility of the host to this pathogen (Orr & Nelson 2018).

The effects of different forms and levels of nutrients, such as nitrogen, potassium, magnesium and others, on the susceptibility of cannabis to disease has yet to be determined.

- Climate management and air movement. Relative humidity in the growing environment should be kept low (<50-60%) to reduce powdery mildew and *Botrytis* infections. Lower relative humidity is especially important later when inflorescences are mature and most susceptible to disease. The severity and incidence of powdery mildew and *Botrytis* infections have been shown to increase with increased humidity on crops such as grape and tomato.

  Relative humidity is the amount of water vapor the air is holding compared to what it can hold at a specific temperature. As the temperature of the air increases so does its capacity to hold water. When the relative humidity reaches 100%, also known as the saturation point, water can condense on plant surfaces. As relative humidity is dependent on temperature, large fluctuations in temperature should be avoided to manage humidity and condensation. For example, a growing environment which is quickly cooling or heating, such as at sunset, sunrise or when lights are turned on or off, can result in a rapid change in relative humidity and increase the possibility for condensation. This can lead to increased disease and stress on plants. Proper heating, cooling and ventilation can minimize fluctuations in temperature at critical times of the day.

  When considering relative humidity and temperature for disease management it is also important to consider the vapor pressure deficit being created, and the effect this can have on plants. The vapor pressure deficit, or VPD, is a measure of pressure created by the difference between the amount of water vapor in the air and the amount it can hold. The VPD of the growing environment can inform growers about the rate at which plants are transpiring. Too low of a VPD can cause plants to reduce transpiration, resulting in

reduced movement of water and nutrients, and increased disease. Conversely, a high VPD may also increase the rate of transpiration too much, leading to stress, drought and nutrient deficiencies. Throughout the production cycle, cannabis plants will require different VPDs to maximize their growth, and it is important that growers balance these needs with their potential effects on disease development.

Having adequate air circulation by utilizing numerous well-placed fans, pruning and trellising plants, deleafing plants and providing appropriate plant spacing can also help to create a consistent climate throughout the growing area. This will in turn help to reduce powdery mildew and *Botrytis* bud rot, and potentially other foliar diseases. Providing adequate air movement, cool temperatures and appropriate levels of humidity in drying rooms can also help to reduce post-harvest decay.

- Control insect pests. Management of insect pests (shore flies, fungus gnats, rice root aphids, etc.) is an important aspect of managing diseases in the growing environment, as these insects may be vectors for plant pathogens, enabling their spread (Scarlett et al. 2014; Willsey et al. 2017). Insects may also damage root tissues and make them more susceptible to infection, as well increase overall stress on the plants. The cannabis root aphid, for example, can potentially increase damage to roots and increase the likelihood of root pathogens such as *Fusarium* and *Pythium* infecting and causing disease.

2.5.2. Physical Control

- Pasteurization. Recirculated irrigation water is a significant way that many plant pathogens are spread. In order to eliminate these potential pathogens, water may be treated with heat, in a process known as pasteurization. This is an effective and safe method to reduce the amount of inoculum present, although the potentially large amount of energy, and subsequently high cost of treatment, may make it prohibitive on the scale required for some facilities. Generally, protocols recommend bringing water to 95° C (203° F) for 10 to 30 seconds. Research has shown that water treatment may effectively eliminate plant pathogens at lower temperatures (42-48° C) as long as the exposure to these temperatures is longer (6-12 hours). One study showed that *Fusarium oxysporum* conidia may be inactivated by heating water to 54° C for as little as 15 seconds. This may help to make pasteurization a more environmentally and economically sound practice, although it is still most likely only a realistic option for smaller producers.

- Irradiation. Treatment with ultraviolet light may be used to eradicate pathogens in irrigation water. Propagules of *Pythium*, such as zoospores, oospores and mycelium, and mycelium, conidia and chlamydospores of *F. oxysporum,* can be killed using this treatment method. The degree to which the ultraviolet light is transmitted through the water plays an important role in the dosage of radiation required to eliminate these pathogens (Majsztrik et al. 2017). Factors such as the turbidity of water can affect the transmittance of ultraviolet light. The duration of treatment required will vary with the intensity of the irradiation and the volume of water.

  Ultraviolet radiation in the form of UV-B or UV-C can also be used to manage powdery mildew on cannabis. There are several factors that can affect the efficacy of this treatment, including the intensity of the UV light plants receive, the duration of the treatment, how often treatments are applied, when treatments are applied during the day, and the amount of foliage to be treated. Typically, plants will receive one "pass" or treatment per day, with treatments during either the day or at night showing efficacy at managing powdery mildew on cucumber plants. Comparable results have also been reported by applying a higher dose of UV-C every fourth day. This approach works well at preventing new fungal infections but may have less activity on established infections. An excessive dose of UV can cause plant injury and stress, which could increase the incidence of other diseases, such as *Botrytis* gray mold. UV treatments can be made on a smaller scale using hand-held devices. Boom mounted lamps for treatments on a larger scale are also available.

  The use of gamma radiation and electron beam may be allowed to reduce post-harvest contamination on cannabis where it has received regulatory approval.

- Filtration. Pathogens may be removed from irrigation water through physical filtration (Majsztrik et al. 2017). The most common methods use either membranes of differing pore sizes or materials like sand and gravel (slow sand filtration). These methods each have their own associated costs, benefits and scalability.

2.5.3. Biological Control

There are several commercial biological control products that can be used on cannabis for disease management. The biocontrol products contain fungi such as *Trichoderma* spp.

(Rootshield, Asperello, Trianum, etc.) or *Gliocladium catenulatum* (Prestop, Lalstop). In addition, bacterial products contain *Bacillus* spp. (Rhapsody, Stargus or Double Nickel) or actinomycetes such as *Streptomyces griseoviridis* (Mycostop) (Fig. 2.4). These products contain spores of fungi or bacterial cells at a high concentration, usually in excess of 10^8 cells/ml, in a formulation that provides stability and longevity to the microbe. These biocontrol products prevent infection by *Fusarium* or *Pythium* on cuttings or rooted plants and should be applied as a drench before infection takes place.

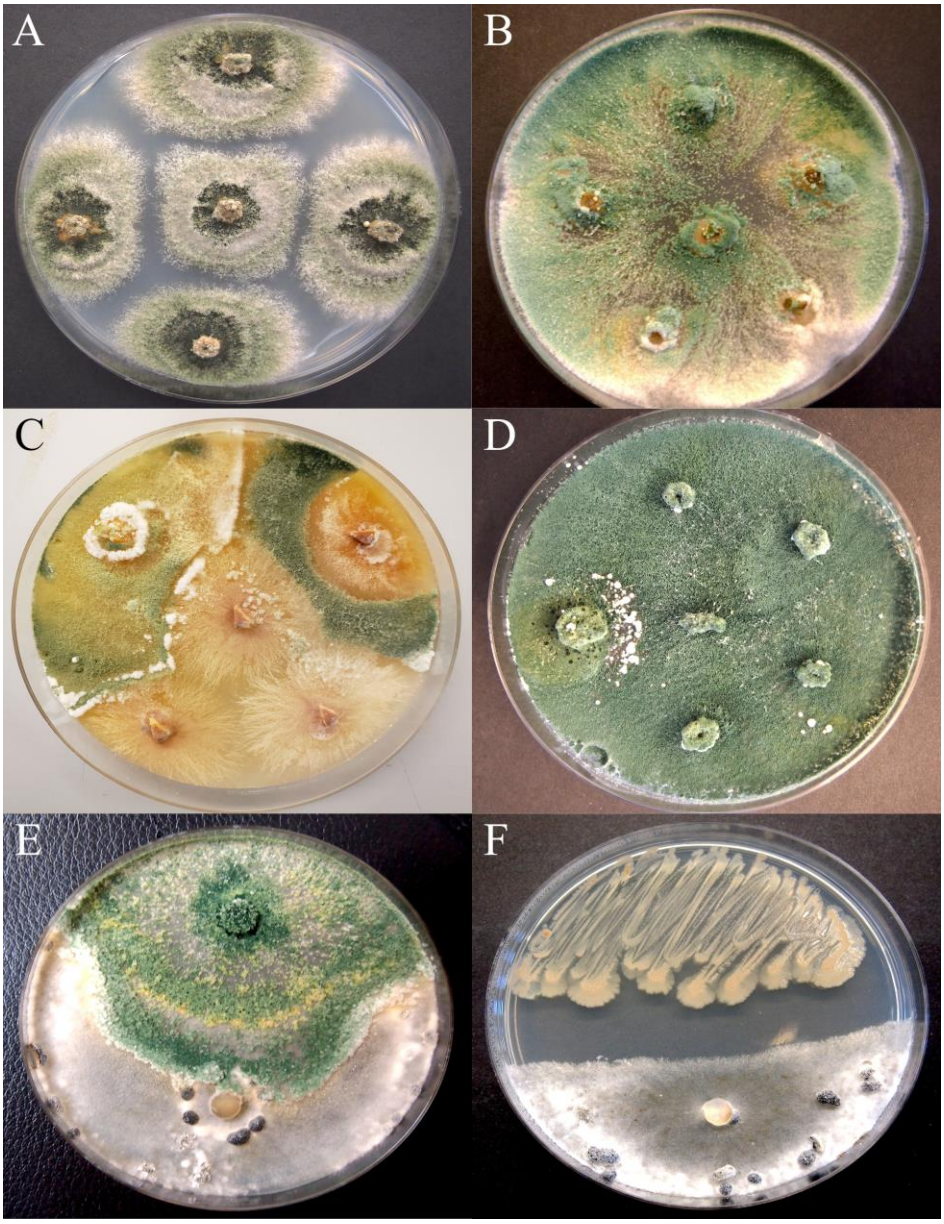

Figure 2.4 **Fungi and bacteria utilized for biological control of plant pathogens and their action against select pathogens on potato dextrose agar. (A) *Gliocladium catenulatum* (Prestop, Lalstop) growing from colonized cannabis stems. (B) *Trichoderma harzianum* from Rootshield growing from colonized cannabis stems. (C) A *Trichoderma* spp. and a *Fusarium* spp., seen at the bottom, growing from cannabis stems. (D) *Trichoderma asperellum*, from the product Asperello, growing from colonized cannabis stems. (E) *Trichoderma asperellum* overgrowing *Sclerotinia sclerotiorum* in a dual culture assay. (F) *Bacillus amyloliquefaciens* (Stargus) inhibiting the growth of *Sclerotinia sclerotiorum.* All stem tissues shown in this figure were treated with the biocontrol agent, incubated for 7 days, surface sterilized, and plated.**

Based on research conducted on many crops, these biocontrol agents can produce antibiotics or enzymes that inhibit the pathogen or may compete for space on the root system (Chatterton & Punja 2009). Fungal products based on *Trichoderma* spp. or *Gliocladium catenulatum* are also known to actively destroy (through mycoparasitism) *Pythium* and *Fusarium* hyphae. Although these biocontrol agents, especially the fungal biocontrols, are known to persist in media for extended periods of time, reapplications may still be needed. On other crops, these microorganisms have also been shown to have the added benefit of stimulating plant root and shoot growth. These bacteria and fungi have this effect as they may colonize plant tissues and interact with the plants through the production of hormones and growth enhancers.

The use of soils that naturally contain a complex of these beneficial microorganisms is another potential biological control method. These soils are often referred to as suppressive soils or "living soils", and they can reduce the growth of a pathogen and the disease it causes. These types of soils may also have the added benefit of stimulating plant growth compared to soils with less numerous or diverse microbial communities, such as sterilized soil or media like rockwool. Mature composts are a source of many of these microorganisms. Research has shown that composts may be inoculated and used as a substrate for specific biocontrol organisms such as *Trichoderma harzianum*.

The general suppressiveness of the soil seems to be associated with the overall biomass and microbial activity of the soil, whereas the ability to suppress more specific pathogens or organisms is associated with the presence and relative abundance of specific organisms. Substrate respiration, amendments added to the soil, soil physiochemistry and other abiotic conditions also alter the microbial contents and suppressiveness of soils. These approaches should be considered by organic producers.

Several biocontrol products that contain bacteria have been shown to reduce powdery mildew on crops such as cucumber, zucchini, strawberries and grapes. As with root pathogens, production of antibiotics and enzymes from the microbes in these products provides protection when they are applied prior to establishment of infection. On cannabis plants, Rhapsody or Stargus applications made at weekly intervals was shown to reduce powdery mildew development (Fig. 2.5). Actinovate SP (*Streptomyces lydicus*) was shown to have a limited effect on powdery mildew on cannabis and only at low disease pressure. The use of these biocontrol products for disease management will likely require they be applied frequently and/or used in a rotation with other products during periods when plants are most susceptible to disease.

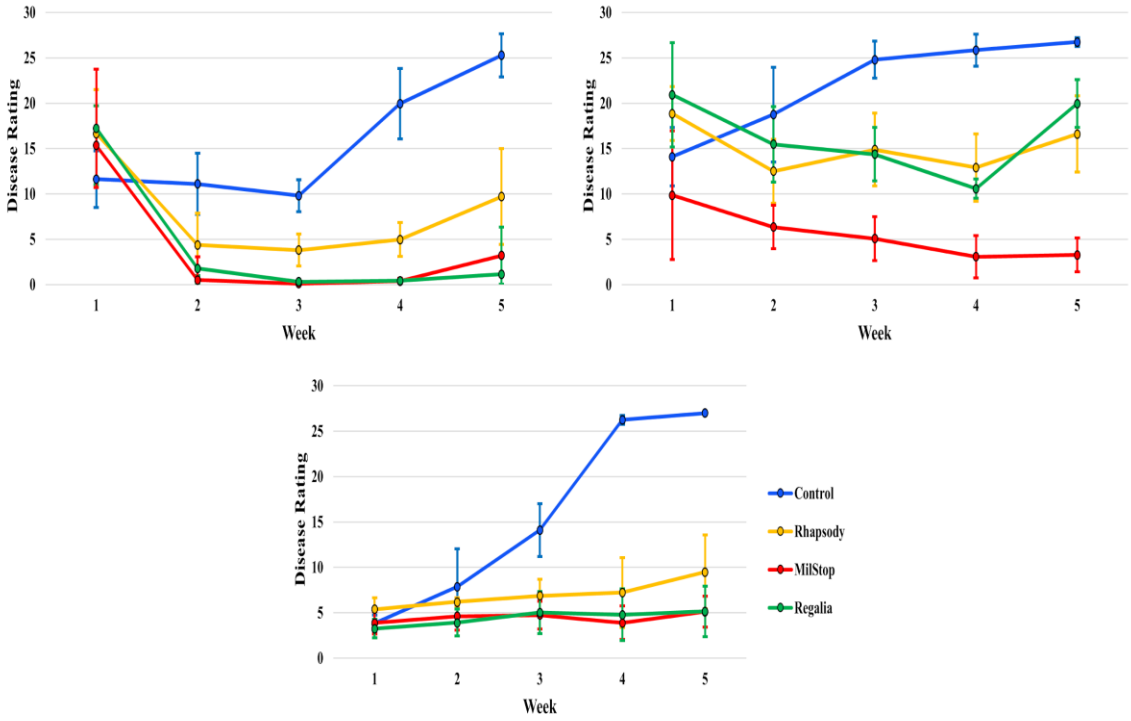

Figure 2.5 **The effects of Rhapsody ASO, Regalia Maxx and MilStop on powdery mildew disease progression under various disease pressures. Treatments were made once weekly and disease ratings were calculated by rating the 30 most diseased leaflets per plant. There were 4 replicates per treatment per trial. Error bars represent 95% confidence intervals.**

For control of *Botrytis* infection, Stargus is registered to manage bud rot for indoor and outdoor hemp production by the EPA. On cannabis, there is no data available yet to demonstrate the efficacy of these products at managing *Botrytis*.

2.5.4. Biorational Products

Products containing plant extracts such as neem oil or powder, clove oil, thyme oil, tea tree oil extract (TIMOREX GOLD), orange oil extract (PREV-AM) and others reportedly have some ability to reduce fungal growth and manage disease on other crops. Biorational products such as these still need to be evaluated for disease management on cannabis or hemp plants.

The use of plant extracts to effectively manage foliar diseases such as leaf spots and powdery mildew has been demonstrated on crops such as tomato, cucumber, pea and okra. Two effective products include Regalia Maxx (containing extracts from the giant knotweed *Reynoutria sachalinensis*) or products containing extracts from the neem tree (as oils). These products are

most effective when applied preventatively or under low disease pressure. Regalia Maxx is registered for use on cannabis and hemp in Canada while neem-based products and Regalia Maxx are approved for use on hemp in many states of the US. In other crops, Regalia Maxx has been shown to promote resistance to pathogens by increasing antimicrobial compounds or enzymes; however, the biochemical responses of cannabis plants following treatment have not been studied. Regalia Maxx has been shown to significantly reduce powdery mildew development when applied weekly (Fig. 2.5).

2.5.5. Reduced Risk Chemicals and Conventional Fungicides

In addition to the biocontrol and biorational products above, there are several other options that growers can consider as a part of their disease management strategy. These products are based on chemistries that are considered safe for plants and the environment and are described below. However, applications of these products, as well as Regalia Maxx, made during flower development can cause damage to stigmas on cannabis plants (Fig. 2.6).

- Chlorine. The addition of compounds containing chlorine to irrigation water can effectively eradicate the majority of propagules of pathogens such as *Fusarium* and *Pythium*. A concentration of 5 ppm active chlorine is recommended to reduce the spread and survival of propagules of these pathogens. Factors such as the biology of the pathogen or propagule, water quality and exposure time all affect the efficacy of chlorine.

- Potassium bicarbonate. Products such as MilStop, when applied as a foliar spray, can reduce the development and spread of powdery mildew on cannabis and other crops (Fig. 2.5) (Scott & Punja 2020). These products act by altering the pH and osmotic pressure of the surface of tissues on which they are applied, which then disrupts the growth of mycelium and spores. These products appear to have both curative and preventative effects when used to manage powdery mildew on cannabis.

Figure 2.6 Damage from foliar sprays on stigmas.

- Hydrogen peroxide. ZeroTol shows some efficacy in reducing powdery mildew on cannabis and cucumber when applied as a foliar spray (Scott & Punja 2020). The effect may be due to direct toxicity to the pathogen. On other crops, these products have been shown to play a role in inducing resistance to disease on treated plants. Data on the efficacy of hydrogen peroxide products and their mode of action is limited.

- Silicon. Foliar applications of the silicon-based product Silamol reduced powdery mildew on cannabis at low disease pressures (Scott & Punja 2020). Silicon sprays can also be effective for disease management on crops such as grape, wheat and cucumber. The use of silicon in soil or nutrient solution can also reduce powdery mildew development on crops such as rose, cucumber, zucchini and wheat. Silicon may be taken up by plants and utilized as part of the plant's defense response. Silica is deposited in plant tissues and limits powdery mildew infection. Application of silicon to the roots seems to be more effective than foliar sprays since it results in higher levels of silicon accumulation within

the plants. The use of root-applied silicon to cannabis plants for disease management has not been tested.

At the present time, applications of conventional fungicides are not approved for cannabis or hemp growers. While fungicides with active ingredients such as fludioxonil, thiabendazole, fluopyram, prothiconazole and others such as propamocarb, metalaxyl-M, cyazofamid have been shown to reduce *Fusarium*, *Pythium* and powdery mildew infections on other crops, future research may determine the safety parameters around which they could be used on cannabis and hemp crops.

2.5.6. Disease Resistance

Most agricultural crops utilize disease resistant varieties that have been developed through selective breeding. Resistance genes to pathogens such as *Fusarium* and powdery mildew have been identified and can provide stable disease reduction in other crops (Agrios 2005). In cannabis, breeding for the purpose of selecting disease resistant strains has not yet been undertaken on a scale comparable to other crops, although there exists a broad diversity of germplasm from which selections could be made. Concerted efforts are being made by cannabis and hemp producers that will begin to formally identify such sources of resistance. Screening methods using pathogens known to be of importance in specific areas of production will begin to identify the most suitable strains that combine traits of commercial interest with disease resistance traits.

2.6. Integrated Disease Management

The utilization of several different approaches for managing diseases in a cohesive plan will allow producers to implement an integrated disease management plan (IDM). This may be combined with management of insects and other pests into an integrated pest management (IPM) program. Continuous monitoring of environmental and crop variables as well as scouting for disease signs and symptoms are key to a well designed IPM strategy. This allows growers to act proactively when disease levels are low and manageable i.e., below the economic threshold (ET). The implementation and efficacy of an IPM program should be adjusted as needed based on repeated observations and consideration of good horticultural practices, the scale of the operation and costs (labour, pesticides, etc.).

Economic thresholds are based on the extent to which the disease or insect pest can potentially reduce the yield or quality of the crop if left untreated. This includes a cost-benefit analysis to establish at which point the damage caused by a pathogen or pest requires management intervention and the associated costs. The economic injury level, or EIL, is the lowest level of damage that will cause economic impact. The ET is also known as the Action Threshold, as when pest pressures reach this point, action should be taken to avoid reaching the EIL and losing crop value. Below the ET, the costs of controls exceed their possible benefits, whereas the EIL is a break-even point where the cost of control equals the benefits. For other crops, ET and EIL values have been established, whereas for cannabis, these values need to be determined by individual growers.

Factors that impact EIL and ET values include the cost of inputs (labour, pesticides, equipment), the efficacy of the inputs, and the value of the harvest. The ET value should account for the time it may take to treat the affected areas and how extensively the disease may progress during that time. The effects of diseases or pests on subsequent production cycles and the facility as a whole should be considered.

2.7. Crown Rot, Root Rot and Damping off Caused by *Fusarium* spp.

Fusarium is a prevalent and potentially devastating soilborne fungal pathogen that causes vascular wilts and crown rot on field and greenhouse crops around the world. These crops include cucumber, tomato, ornamental flowers, legumes, pulses, *Brassica* spp. and banana. On cannabis plants, *Fusarium* has been shown to be able to infect at all stages of growth, from propagation through to flowering. Affected plants develop crown and root rot symptoms and damping off on cuttings may occur. Infection of flowers by *Fusarium* has also been observed and will be discussed later.

2.7.1. Causal Agent

On cannabis, the most prevalent *Fusarium* species is *Fusarium oxysporum*, although other species such as *Fusarium proliferatum* and *Fusarium solani* can also cause similar symptoms. The mycelium, which is the vegetative form of the fungus, is visible on plants and can be white, light orange or light pink in colour. When grown on agar medium (such as potato dextrose agar, or PDA) in the laboratory, cultures of *Fusarium* tend to display

shades of purple, red, or light pink (Fig. 2.7). The spores of *Fusarium* spp. are colorless and recognizable under a microscope due to their canoe-like shape. Smaller spores called microconidia may also be present.

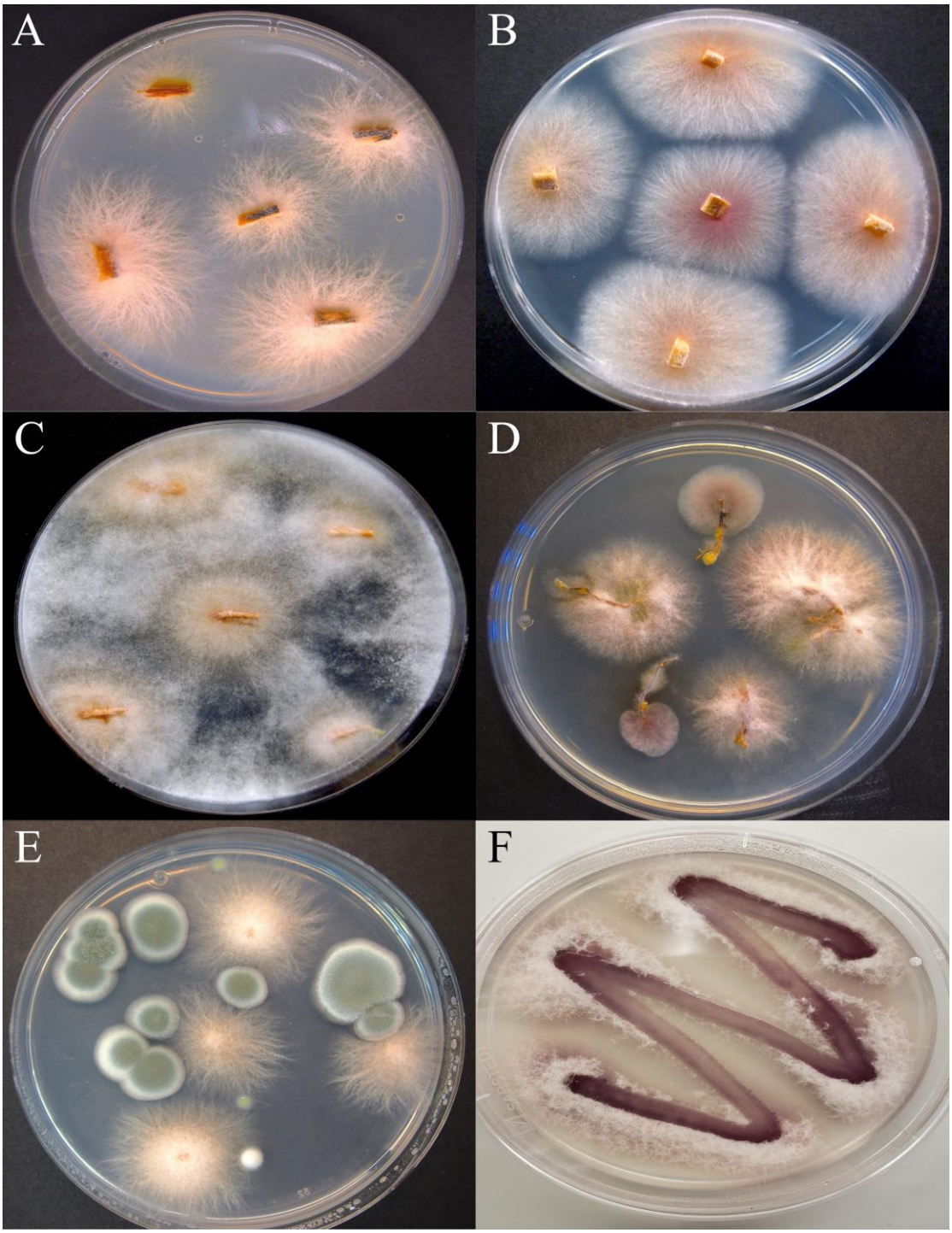

Figure 2.7 **Various species of *Fusarium* growing on potato dextrose agar medium. (A) *Fusarium oxysporum* growing from infected cannabis stems from plants sampled in vegetative growth or (B) from infected cuttings. (C) *Fusarium* and *Pythium* isolated from co-infected cannabis roots. (D) *Fusarium* and other fungi growing from infected hemp seeds. (E) A sample of the air in a propagation room showing the presence of *Fusarium oxysporum* and a *Penicillium* species. (F) A swab from a damped off cutting that produced a pure culture of *Fusarium*.**

2.7.2. Disease Cycle, Symptoms and Signs

Fusarium spp. grow best at temperatures between 28° C and 34° C, although growth at lower temperatures can occur. The fungus can be present in soil, rockwool, peat-based growing media, perlite and other substrates and grows best at pH 5-7. If soil is contaminated with *Fusarium,* the spores and mycelium can infect and colonize the developing roots of the plant (Fig. 2.8). The fungus secretes enzymes that degrade the cell walls of roots, causing root rot. Mycelium is then able to grow intercellularly (between the cells) within the roots, until it reaches the xylem vessels of the plant (Agrios 2005). Wounds from transplanting or other forms of damage eg. insect feeding injury, can increase infection by *Fusarium*. Once in the xylem vessels, the pathogen colonizes these tissues, which collapse, reducing uptake of water and nutrients. Hence, symptoms of yellowing and wilting are observed. Production of toxins by the fungus can also cause symptoms of yellowing and wilting. High temperatures during the summer can cause increased wilting of diseased plants (Punja et al. 2018).

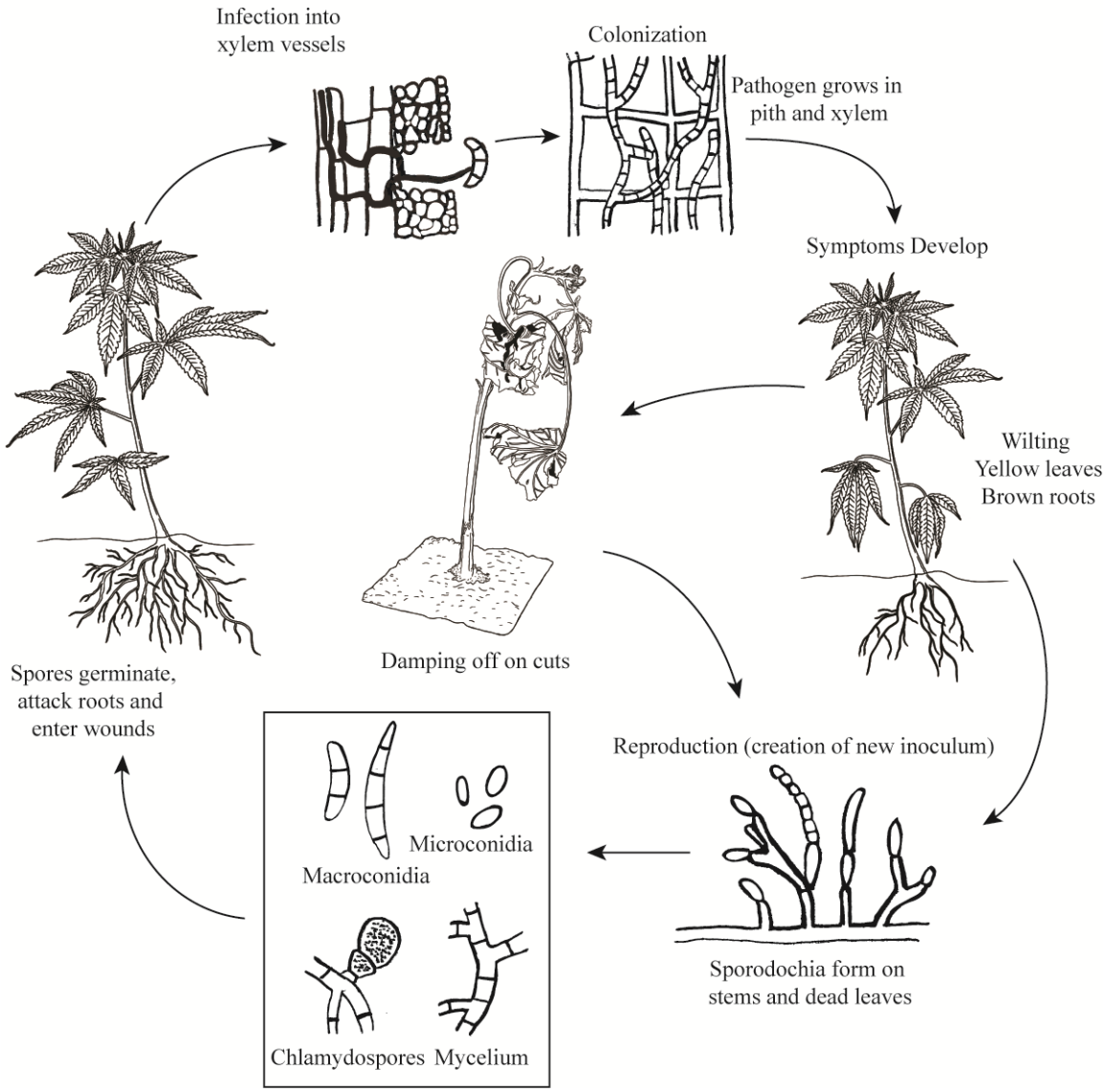

Figure 2.8 The disease cycle of *Fusarium* on cannabis (adapted from Agrios 2005).

The roots of affected plants appear brown and necrotic (root rot). In cannabis, dark sunken lesions on the crown of the plant (crown rot) also may develop. Mycelium may be visible on the infected tissues, especially at the soil line or on areas affected by crown rot. When the stems of infected plants are cut open, the pith and xylem tissues appear brown or black. Sometimes diseased plants may remain asymptomatic until plants are stressed.

Fusarium spp. can also cause damping-off on cannabis cuttings. These symptoms start as soft, dark waterlogged areas at the base of stems, which progress upward and eventually cause the cutting to collapse (Fig. 2.9). The humid conditions present during propagation of cannabis increases development of this disease. As the infection spreads, the cutting will rot, and often

times *Fusarium* mycelium can be seen growing on the affected regions. Secondary organisms such as soft rot bacteria or *Penicillium* spp. may also be present. Poor air circulation as well as overly wet media may increase the severity of damping off. Cuttings which originate from infected stock plants, even if they are free of symptoms associated with *Fusarium*, are at particular risk of developing damping off as these cuttings may already have the pathogen present inside them. This is a common way by which *Fusarium* has the ability to spread. Stock plants that have been grown for longer than approximately 6 months have a higher chance of becoming infected by *Fusarium*.


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Figure 2.9 **The signs and symptoms of *Fusarium* diseases on cannabis plants. (A, B) Damping off on cuttings caused by *Fusarium*, with visible white mycelium on the outside of the cuttings (C) Damping off affecting numerous cuttings. (D) Root and crown rot on plants in vegetative growth. (E) Stunted plants (right) next to otherwise healthy plants. (F) Chlorosis and stunting on plants in flower, with healthy plants around them. (G) Intense chlorosis throughout an infected plant. (H) Chlorosis, stunting, premature leaf drop and wilt on a mature cannabis plant. (I) A closeup highlighting the characteristic chlorosis caused by *Fusarium* infection. (J, K) Cross sections of cannabis stems showing blackened and necrotic vascular tissue.**

Spores of *Fusarium* can be spread by water, in air and on equipment. A diseased plant on a flood table or in a tray with healthy plants may release spores that spread to adjoining plants. Contaminated rooting or growing medium or soil should not be reused, as spores can survive and initiate disease in subsequent growing cycles.

Plant debris (leaves, stems) infected by *Fusarium* may also spread the pathogen to other plants. Spores and mycelium can survive on plant residues in the growing environment and in soil for several weeks and potentially initiate disease in subsequent cropping cycles. Equipment or tools, including pots, trays, domes, shears that come into contact with infected plant material, especially when there is visible mycelial growth, may spread the pathogen to other plants or other areas of the growing environment. Seeds of crops such as wheat are known to transmit *Fusarium*, although the degree to which *Fusarium* spp. are spread through cannabis seeds is not yet known.

2.7.3. Disease Management Approaches

1. Start with planting material that is free of *Fusarium*. Incoming plant material should be examined for symptoms (yellowing, wilting, root rot) and placed under quarantine and tested for the presence of the pathogen. Stock plants should be replaced approximately every 6 months to avoid a build-up of *Fusarium* internally. Suspected plants should be tested for the presence of the pathogen by a commercial testing lab.

2. Minimize introduction or spread of the pathogen through contaminated soil, plant debris, or potentially by spores on shoes or clothing of personnel and visitors. This requires that shoe covers, gloves, and footbaths with disinfectant be placed at entrances to growing rooms. Filters and UV lights in air intakes may also reduce the number of spores being brought into the facility. Equipment or tools should be cleaned with a detergent and water, as well as a disinfectant.

3. Reduce plant to plant spread. When taking cuttings from a mother plant, preparing cuttings for propagation, or pruning plants, shears should be regularly cleaned with isopropyl alcohol. Avoid movement of workers and equipment from a diseased area to a clean area. Regularly scout and remove symptomatic plants and the growing media as soon as possible and destroy them.

4. Sanitize equipment regularly. Previously used trays, domes, pots, flood tables, humidifiers, dehumidifiers, and fans should be cleaned thoroughly and regularly.

   Irrigation equipment such as drip lines or pipes may be cleaned by flushing them with hydrogen peroxide or other cleaning agents. For growers that recirculate nutrient solutions, filtration through various membrane systems or sand (slow filtration), heat treatment (pasteurization) or ultraviolet radiation may be required. Addition of chlorine-containing compounds, such as sodium hypochlorite or chlorine dioxide, to achieve levels up to 5 ppm chlorine, may reduce survival of *Fusarium*.

5. Avoid damage to roots from transplanting. Avoid excessive dry-back of the growing media or over-watering of plants. Pests such as fungus gnats, shore flies and root aphids may also create wounds on roots or act as vectors of *Fusarium*.

6. Apply biological control products such as Rootshield and Prestop as drenches at recommended rates when propagating plants. Reapplication may be beneficial later in production.

7. Provide adequate but not excessive humidity and good air movement in the propagation environment. Avoid overwatering.

2.8. Crown and Root Rot Caused by *Pythium* spp.

Pythium spp. are fungal-like organisms called oomycetes (also known as water molds). Oomycetes differ from fungi like *Botrytis cinerea* or *Fusarium oxysporum* because their cell walls contain beta glucans and cellulose rather than large amounts of chitin. These root-infecting pathogens occur world-wide and are commonly found in soil. They are favoured by wet conditions and grow over a range of temperatures, with many favoured by temperatures over 30° C in hydroponic cultivation systems. *Pythium* species cause damage to many greenhouse crops, including pepper, tomato, cucumber, and ornamentals, as well as field grown crops such as

soybean, cotton, strawberry and turfgrass. On cannabis, *Pythium* spp. cause crown rot and root rot, as well as pre and post emergence damping off on hemp.

2.8.1. Causal Agent

The *Pythium* spp. reported to infect cannabis are *Pythium myriotylum*, *Pythium dissotocum*, *Pythium ultimum* and *Pythium aphanidermatum*. When grown on PDA they produce cottony white aerial mycelium which quickly covers the plate (Fig. 2.10). Unlike *Fusarium*, they do not produce any pigment. Some species grow with a particular undulating or radiating pattern to their mycelium. Spores, if present, are produced in structures called sporangia (zoospores). Other spore types that may be produced are thick-walled oospores that allow for long-term survival.

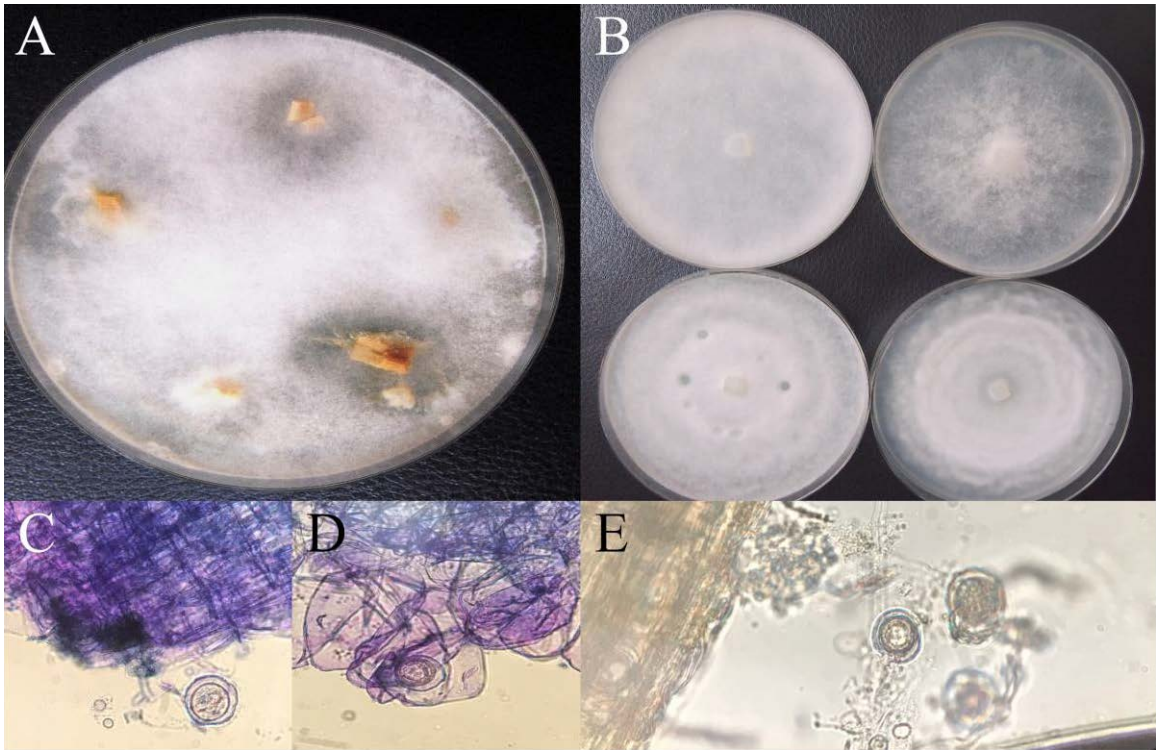


Figure 2.10 **The causal agents of *Pythium* diseases on cannabis and their biology. (A) *Pythium myriotylum* growing out of surface sterilized cannabis stem tissues. (B) Four different *Pythium* spp. isolated from infected cannabis plants. (C, D) Stained oospores produced by *Pythium myriotylum* on infected cannabis roots, as well as (E) unstained oospores and sporangia (top right structure).**

2.8.2. Disease Cycle, Symptoms and Signs

Pythium can be introduced into the growing environment on rooted cuttings, contaminated soil, water, and plant debris. Once inside the growing environment, *Pythium* begins to spread and infect the roots of plants particularly if roots are damaged during transplanting or by insect feeding (Sutton et al. 2006). Infection is achieved mainly by the zoospores, although other species of *Pythium* may rely more on structures like mycelia to spread (Fig. 2.11). Zoospores are spores that are able to actively travel through water using their flagella. These mobile spores come from germinating oospores (and the subsequent zoosporangium) and sporangium (Sutton et al. 2006). When zoospores find their way to a host root, they start the process of infection by transforming into thick celled immobile structures called cysts. These cells adhere to roots and penetrate them, allowing growth within the root tissues to begin. *Pythium* spp. are able to infect nonwounded roots as well as wounded tissues, with root tips and young root hairs being most susceptible to infection (Sutton et al. 2006).

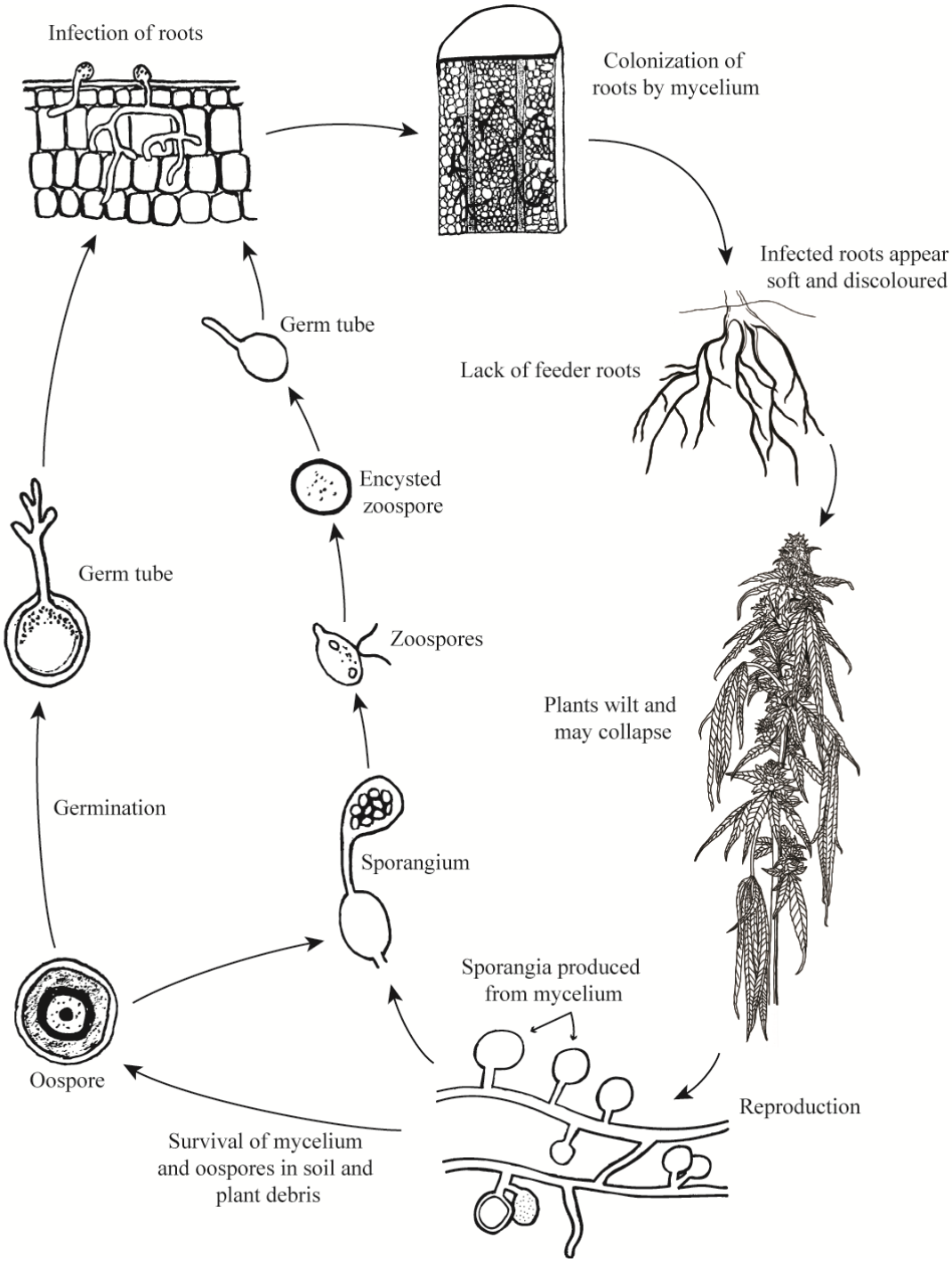


Figure 2.11 The disease cycle of *Pythium* on cannabis (adapted from Agrios 2005).

Colonization of root tissues by mycelium causes browning, with *Pythium* spreading both between and inside root cells (Agrios 2005). As the destructive necrotrophic phase of infection continues, roots may appear stubby with absence of feeder roots (Fig. 2.12). The outer region of the root, the epidermis and cortex, may slough off, leaving only the

pith and vascular bundle. The crowns of infected plants may also appear sunken and dark, which can extend several cm up the stem. The *Pythium* disease cycle includes formation of sporangia on the root surface, while oospores may be formed inside the root tissues. These propagules can spread to other plants, causing additional infections. The pathogen may also survive between growing cycles on plant debris, in growing media and in water.

Foliar symptoms will become visible as the disease progresses, especially during warm weather (Fig. 2.12). Plants infected by *Pythium* appear stunted and may begin to wilt as their root systems are destroyed. Infected plants may also appear moderately chlorotic but otherwise look healthy. Initially, wilted plants may recover but symptoms generally become more severe as the disease progresses. Wilting caused by *Pythium* occurs very rapidly, with plants drying out and dying over a few days. This is especially true in hot dry weather and on larger plants during flowering.

Figure 2.12 **Symptoms of root and crown rot on cannabis plants caused by *Pythium* species. (A, B) Root rot caused by *Pythium* species. (C) A mature cannabis plant showing symptoms of severe crown rot. (D) A healthy plant (left) compared to a plant inoculated with *Pythium myriotylum* showing root browning. (E) A healthy plant (left) compared to two plants inoculated with *Pythium myriotylum*. (F) A plant in flower starting to show symptoms of wilt and chlorosis. (G) More advanced symptoms of wilt. (H, I) Plants killed by *Pythium*.**

Damping off caused by *Pythium* is a problem on hemp if seeds are planted in media infested with the pathogen or seedlings are infected after germination from inoculum in water. If seeds germinate, they may still be killed by the pathogen before they emerge from the media. This is known as pre-emergence damping off. Seedlings may also be destroyed by *Pythium* in post-emergence damping off, as the pathogen causes root and stem tissues to collapse. Symptoms such as stunted growth, yellowing and rot near the soil line may occur on older seedlings, causing wilting and plant collapse. Damping off can cause uneven stands and a notable reduction in plant counts.

2.8.3. Disease Management Approaches

1. Starting planting material should be free of *Pythium*. Incoming plant material should be examined for symptoms and placed under quarantine and tested for the presence of the pathogen.

2. Minimize introduction or spread of the pathogen through contaminated soil, plant debris, or potentially spores on shoes or clothing of personnel and visitors. This requires that shoe covers, gloves, hair and beard nets and footbaths with disinfectant be placed at entrances to growing rooms. Equipment or tools should be cleaned with a detergent and water, as well as a disinfectant.

3. Reduce plant to plant spread by minimizing sharing of irrigation water, such as on flood tables. Avoid movement of workers and equipment from a diseased area to a clean area. Regularly scout and remove symptomatic plants and the growing media as soon as possible and destroy them.

4. Sanitize equipment regularly. Previously used trays, domes, pots, flood tables, humidifiers, dehumidifiers, and fans should be cleaned regularly.

   Irrigation equipment such as drip lines or pipes may be cleaned by flushing them with hydrogen peroxide or other cleaning agents. For growers that recirculate nutrient solutions, filtration through various membrane systems or sand (slow filtration), heat treatment (pasteurization) or ultraviolet radiation may be required. Addition of chlorine-containing compounds, such as sodium hypochlorite or chlorine dioxide, to achieve levels up to 5 ppm chlorine may reduce survival of *Pythium*.

5. Avoid damage to roots from transplanting. Pests such as fungus gnats, shore flies and root aphids may also wound roots or act as vectors of *Pythium*.

6. Avoid overwatering. This creates an anaerobic environment which is conducive to *Pythium* infection. A well draining media should also be used. Nutrient solution should be aerated, and excessive salt levels should be avoided.

7. Apply biological control products such as Rootshield and Prestop as drenches at recommended rates when propagating plants. Reapplication may be beneficial later in production.

2.9. Powdery Mildew

Powdery mildew diseases affect a large number of field and greenhouse grown crops around the world, including tomato, cucumber, pepper, rose, hops, and grapes. On cannabis, powdery mildew appears as white colonies on the upper surface of leaves, making it one of the more easily recognizable diseases. Powdery mildew occurs in all production environments and may affect plants at all stages of growth, although it will rarely kill plants it can significantly reduce their growth.

2.9.1. Causal Agent

Powdery mildew on cannabis is caused by several *Golovinomyces* species - *G. cichoracearum*, *G. ambrosiae* or *G. spadiceus.* The hop powdery mildew pathogen, *Podosphaera macularis,* can occasionally infect cannabis and hemp and cause similar symptoms but it is not considered a major pathogen.

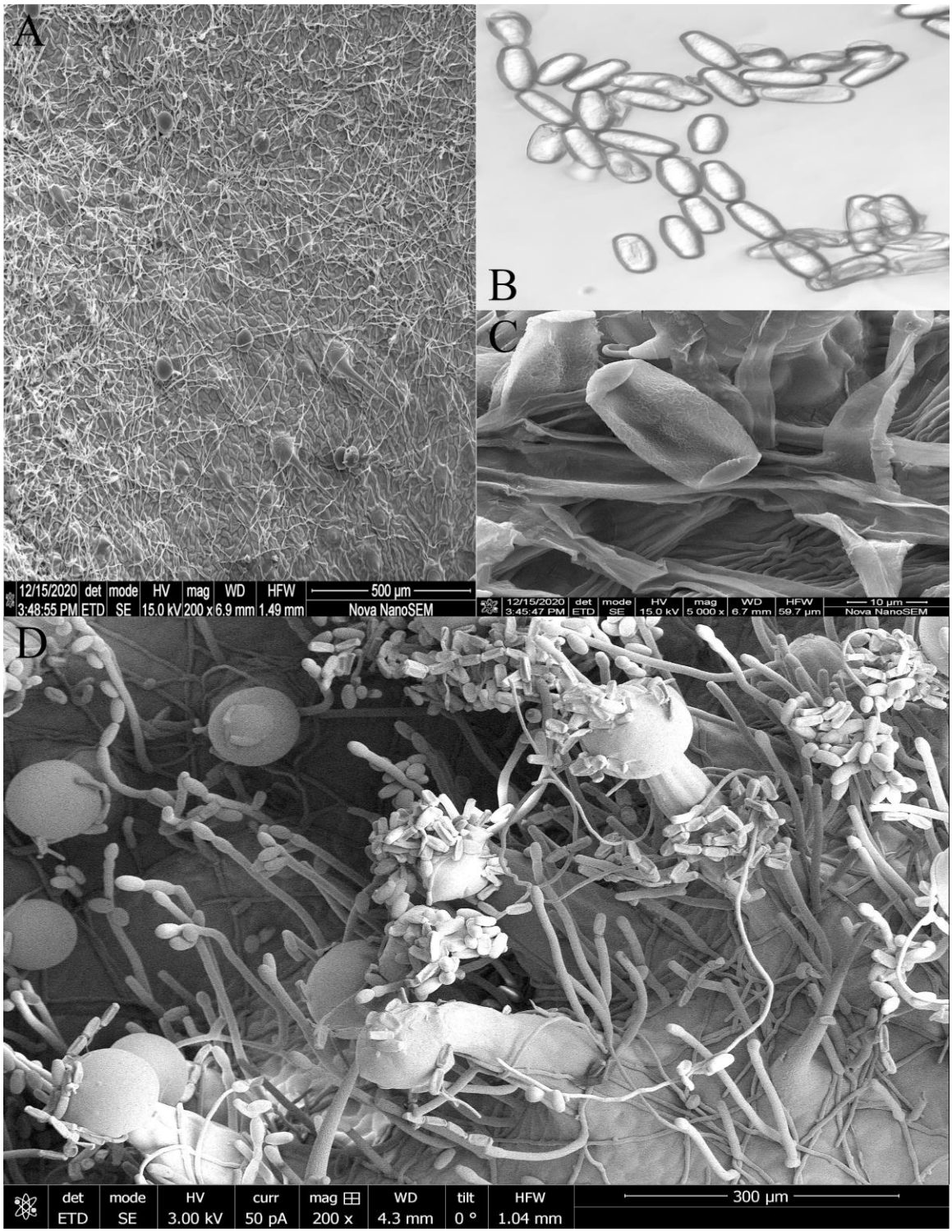


Figure 2.13 **The biology of *Golovinomyces* spp. (A) A scanning electron microscope image showing powdery mildew growing on cannabis leaves and covering trichomes. (B, C) Light microscope and scanning electron microscope images of *Golovinomyces* spp. conidia. (D) A scanning electron microscope image of powdery mildew growing over trichomes. Conidiophores can also be seen extending from the tissues.**

2.9.2. Disease Cycle, Symptoms and Signs

Powdery mildew is an obligate pathogen, which requires living tissues to grow and reproduce (Agrios 2005). Infections on cannabis plants start when spores germinate on leaves, floral tissues or less commonly on the stems of plants. These airborne spores are released from conidiophores from overwintering mycelium or neighboring infected plants (Fig. 2.13). The spores produce a germ tube which penetrates the host cells to form haustoria, structures which absorb nutrients and water from the cells without killing them (Fig. 2.14). Further mycelium and conidiophores develop as distinct white powdery patches on the upper surface (adaxial side) of leaves and release more spores. This can occur within 7 days of initial infection. Powdery mildew mycelium does not invade further into the plant from the epidermis and is not known to spread systemically within cannabis plants by infecting vascular or pith tissues. Spores adhering to the outside surface of plants can spread the disease.

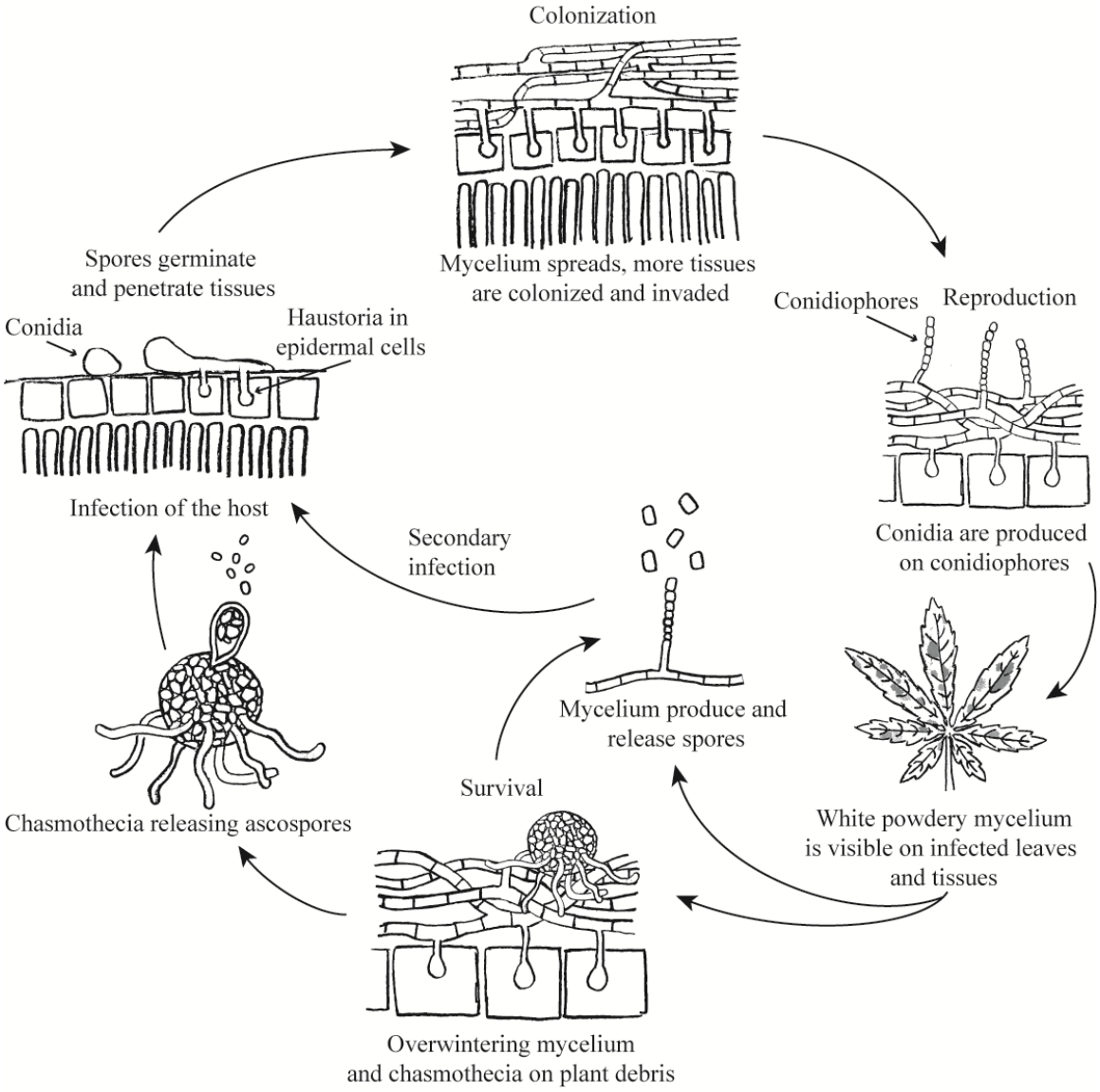


Figure 2.14 The disease cycle of Powdery Mildew on cannabis (adapted from Agrios 2005).

Generally, *Golovinomyces* species grow best in warm climates with minimal leaf moisture, but high levels of humidity encourage pathogen growth. If conditions are unfavorable, the pathogen may produce chasmothecia on leaves and plant debris. Chasmothecia of *Golovinomyces spadiceus* appear as very small brown or black dots on the undersides of leaves on hemp, although they have not been observed on cannabis plants.

Cannabis plants infected with powdery mildew may appear stunted, leaves may appear brown and drop prematurely, and flower quality and yields may decrease (Fig. 2.15). Cannabis

flower infected with powdery mildew will be of lower quality and be less marketable due to the visible growth of the fungus.

Figure 2.15 **Signs of Powdery Mildew infection on cannabis plants in different stages of growth. (A- C) Powdery mildew infection on leaves and flower tissues. (D, E) Severe powdery mildew infection.**

2.9.3. Management Approaches

1. Starting planting material should be free of powdery mildew. Incoming plant material should be examined for symptoms and placed under quarantine and tested for the presence of the pathogen.

2. Minimize introduction or spread of the pathogen through plant debris or potentially spores on shoes or clothing of personnel and visitors. This requires that shoe covers, gloves, hair and beard nets and footbaths with disinfectant be placed at entrances to growing rooms. Equipment or tools should be cleaned with a detergent and water, as well as a disinfectant. Avoid movement of workers and equipment from a diseased area to a clean area.

3. Reduce plant to plant spread through the use of air purification and filtration. Humidifiers, dehumidifiers, and fans should be cleaned regularly. Replace filters regularly.

4. Maintain relative humidity at appropriate levels (<50-60%) to limit infection. Minimize fluctuations in temperature and humidity. Ensure air movement in the growing environment with fans and convection tubing. Pruning, training and deleafing plants may help to improve air movement as well. Space plants appropriately. This will help to reduce pockets of higher humidity.

5. Remove infected leaf material. Limit the spread of inoculum by placing infected leaves in a sealed bag or container in the growing environment before removing them from the facility.

6. Apply products such as MilStop, ZeroTol or Regalia Maxx at rates and intervals as per the labels. Apply when disease pressure is low. Reapplication may be necessary. Sulfur based products, biocontrols or UV light exposure may also be used to manage powdery mildew (Scott & Punja 2020).

7. Cannabis strains that are less susceptible should be used, especially when seasonal disease pressure is high.

2.10. Bud Rot Caused by *Botrytis cinerea*

Botrytis cinerea causes diseases on a wide range of economically important crops. These include greenhouse crops such as tomato, strawberry, basil, and ornamentals such as roses, as well as field or orchard crops such as cherries, grapes, raspberries, apples and legumes. *Botrytis* affects almost all types of plant tissues and causes symptoms that include stem cankers, blossom blights, damping off, leaf spots, fruit rot and storage rot.

2.10.1. Causal Agent

On cannabis *Botrytis* primarily infects the flowers, especially in high humidity environments. This results in soft, rotten, discolored flowers and is commonly referred to as bud rot. If this disease is not managed effectively, it can quickly spread through a growing environment, resulting in severe losses. *Fusarium* species and *Sclerotinia* may also cause bud rot, although *B. cinerea* is the predominant cause of this disease. The conidia and conidiophores of *Botrytis* have a distinct and characteristic grey color, giving it the common name “grey mold” (Fig. 2.16).

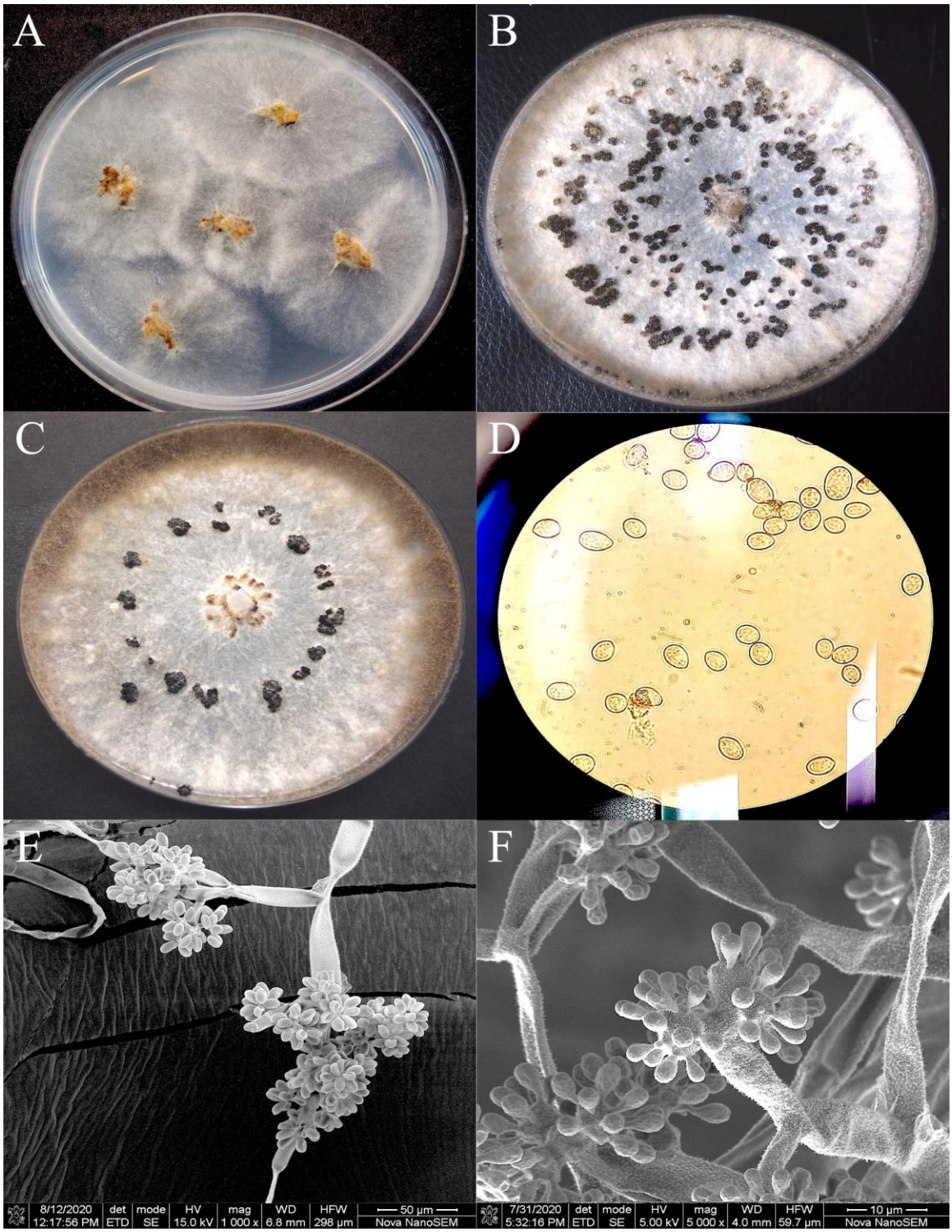


Figure 2.16 **The biology of *Botrytis cinerea*, the main cause of bud rot on cannabis. (A) *Botrytis cinerea* growing from infected flower tissues on PDA. (B) *Botrytis cinerea* on PDA producing sclerotia. (C) *Botrytis cinerea* on PDA producing sclerotia and characteristic grey conidiophores. (D) *Botrytis* spores under a microscope. (E, F) Scanning electron microscope images of *Botrytis cinerea* conidiophores.**

2.10.2. Disease Cycle and Signs

Botrytis cinerea survives as mycelium in plant debris or sclerotia (small dark hardened spheres of mycelium). From these structures, conidiophores are produced, which release spores in the growing environment. Spores are predominantly spread by air and germinate on flowers or other tissues if there is high humidity or free moisture. Overhead watering, pruning shears, workers and other equipment may also spread *Botrytis* spores.

Once spores have germinated, they penetrate into the tissues, and release enzymes which begin to degrade the cells (Fig. 2.17). Tissues may appear soft, discolored and rotten. As the pathogen continues to colonize plant tissues, it produces more spores which cause secondary disease cycles and the rapid spread of the pathogen in the growing environment. This can occur over a period of just a few days. The pathogen may also infect injured stems (injuries commonly occur from pruning or other crop work) and cause the development of stem cankers.

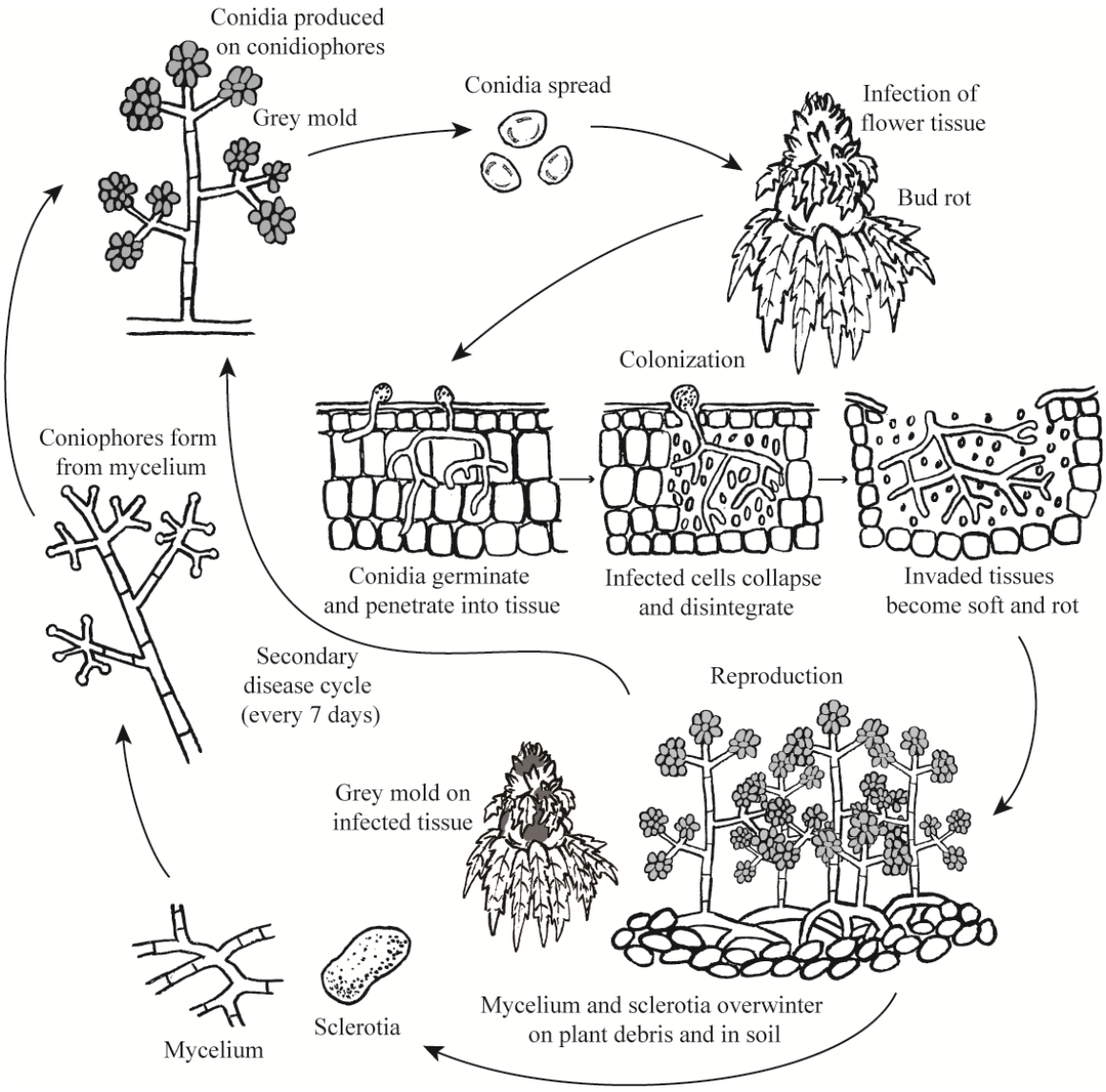


Figure 2.17 The disease cycle of *Botrytis* bud rot on cannabis (adapted from Agrios 2005).

On cannabis plants, bud rot may start on the outside of flowers, especially if wounds are present (from deleafing, etc.) or from the interiors of flowers, near the nodes where they are most dense, and humidity is highest. Flowers infected with *Botrytis* will initially appear soft and discolored before advancing to being crisp, light brown and desiccated (Fig. 2.18). Leaves near affected flowers may also appear brown and dry and can be a characteristic symptom to scout for. Grey or off-white mycelial growth may also be observed on affected flowers.

Even with consistent scouting and removal of infected buds throughout cultivation and harvest, *Botrytis* infected flowers may still be present in the post-harvest environment. These

flowers may not have signs and symptoms that are the same as infections preharvest. Mycelial growth may spread over buds placed closely together if the relative humidity and air movement in the drying room is not managed appropriately.

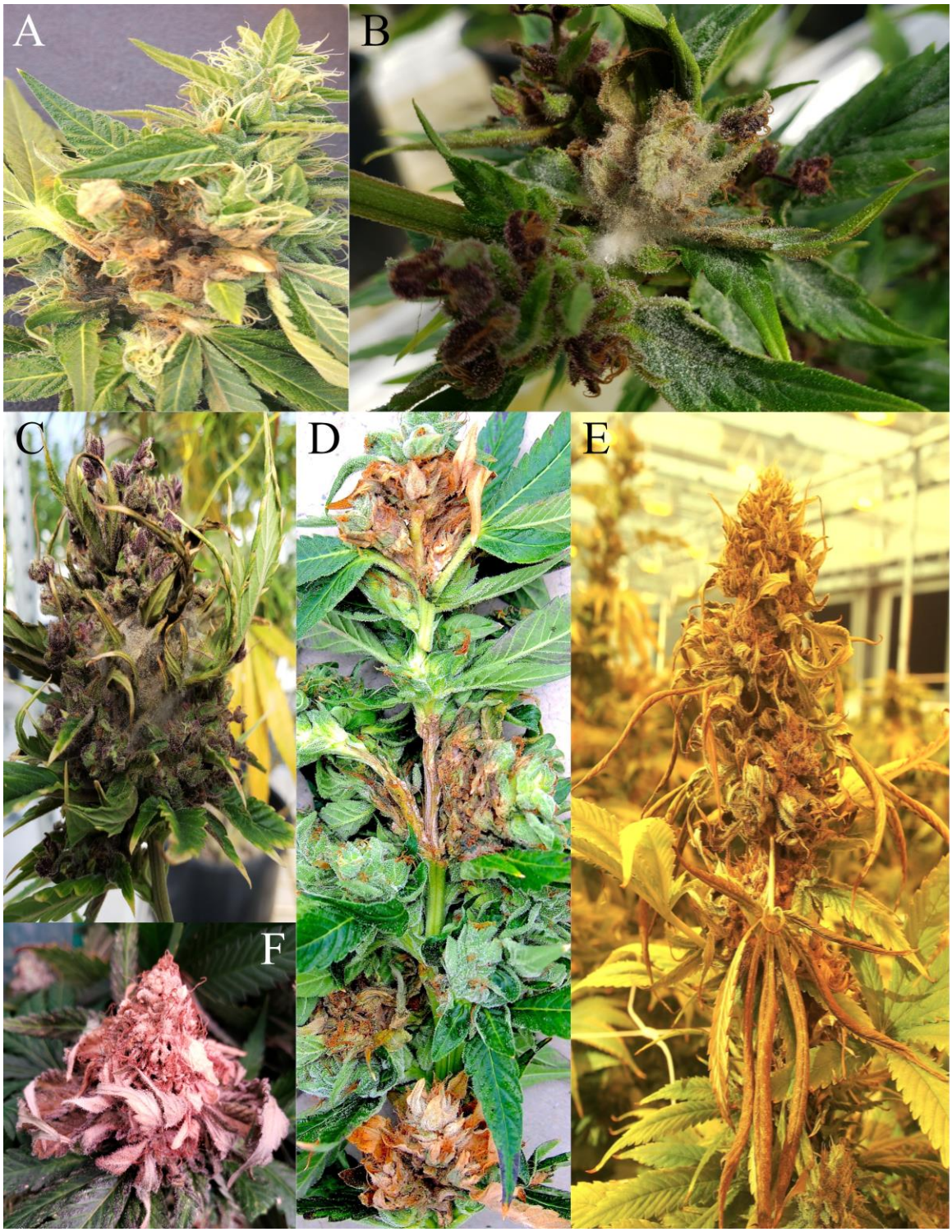


Figure 2.18 **Symptoms of bud rot on cannabis. (A) Bud rot on immature buds (B, C) Bud rot with noticeable mycelium production. (D) *Botrytis cinerea* infecting multiple sites on one branch, highlighting how infections may start in areas of dense tissue and grown outwards. (E, F) Severe bud rot causing complete destruction of affected tissues.**

2.10.3. Management Approaches

1. Minimize the introduction and spread of the pathogen in contaminated plant debris, or potentially spores on shoes or clothing of staff and visitors. This requires that shoe covers, gloves, hair and beard nets and footbaths with disinfectant be placed at entrances to growing rooms. Equipment or tools should be cleaned with a detergent and water, as well as a disinfectant. Avoid movement of workers and equipment from a diseased area to a clean area.

2. Reduce spread of spores in the growing environment through the use of air purification and filtration systems. Humidifiers, dehumidifiers, and fans should be cleaned regularly. Replace filters regularly.

3. Maintain relative humidity at levels below 50-60% to limit infection. Minimize fluctuations in temperature which can cause rapid changes in humidity and condensation on the surface of leaves and flowers. Provide air movement in the growing environment with fans and convection tubing. Pruning, training and deleafing plants may help to improve air movement as well. Space plants appropriately. This will help to reduce areas of higher humidity.

4. Remove infected flowers as soon as they appear. Limit the spread of inoculum by placing flowers in a sealed bag or container in the growing environment before removing them from the facility.

5. Select strains that are not known to produce overly dense inflorescences as they tend to trap moisture and maintain a high humidity that allows spores to germinate. Pruning and training may also help to reduce the size of flowers. Strains that are susceptible may be planted when seasonal disease pressure is low, such as during the summer.

2.11. Post-harvest Decay

2.11.1. Causal Agents

Cannabis flowers are also susceptible to rot or decay post-harvest from a variety of fungi including *Botrytis cinerea*, *Penicillium* species *Fusarium* species, *Aspergillus* species and

Cladosporium westerdijkieae, among others (Fig. 2.19) (Fig.2.20). Post-harvest decay affects yields, the quality of flowers and may cause batches to exceed the acceptable limits for total yeast and mold counts (Punja et al. 2019).

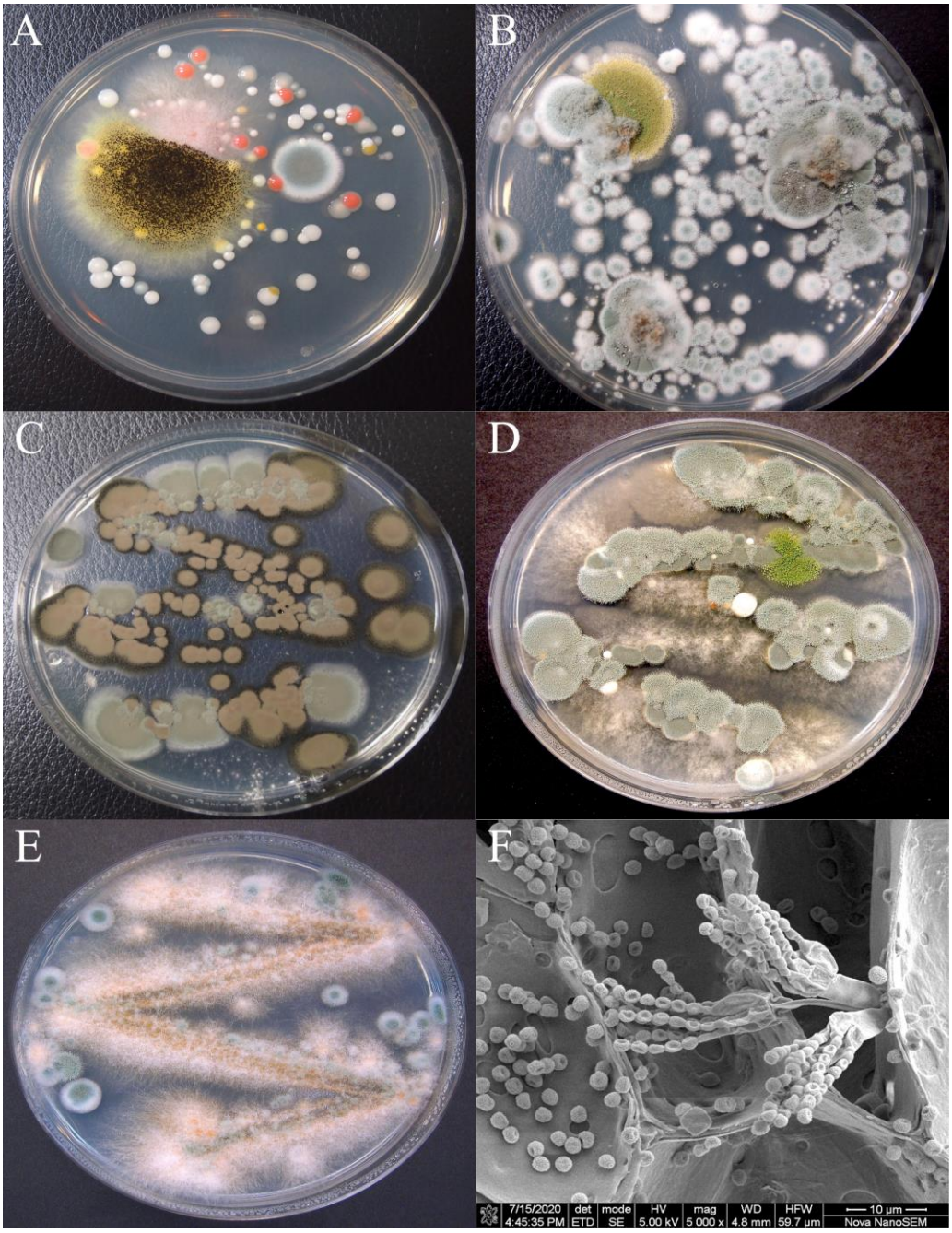


Figure 2.19 **Fungi that are found post-harvest on cannabis flowers. (A) *Aspergillus niger*, *Fusarium oxysporum*, a *Penicillium* species, bacteria and yeast on PDA from a swab of a post-harvest bud. (B) *Penicillium* and *Aspergillus flavus* growing from post-harvest flower tissues. (C) A swab of post-harvest flower showing the presence of *Penicillium* and *Cladosporium*. (D) A swab of post-harvest flower showing the presence of *Botrytis cinerea*, *Aspergillus flavus* and *Penicillium*. (E) *Fusarium* and *Penicillium* growing from a swab taken from infected post-harvest flower tissue. (F) A scanning microscope image of the conidiophores of *Penicillium*.**

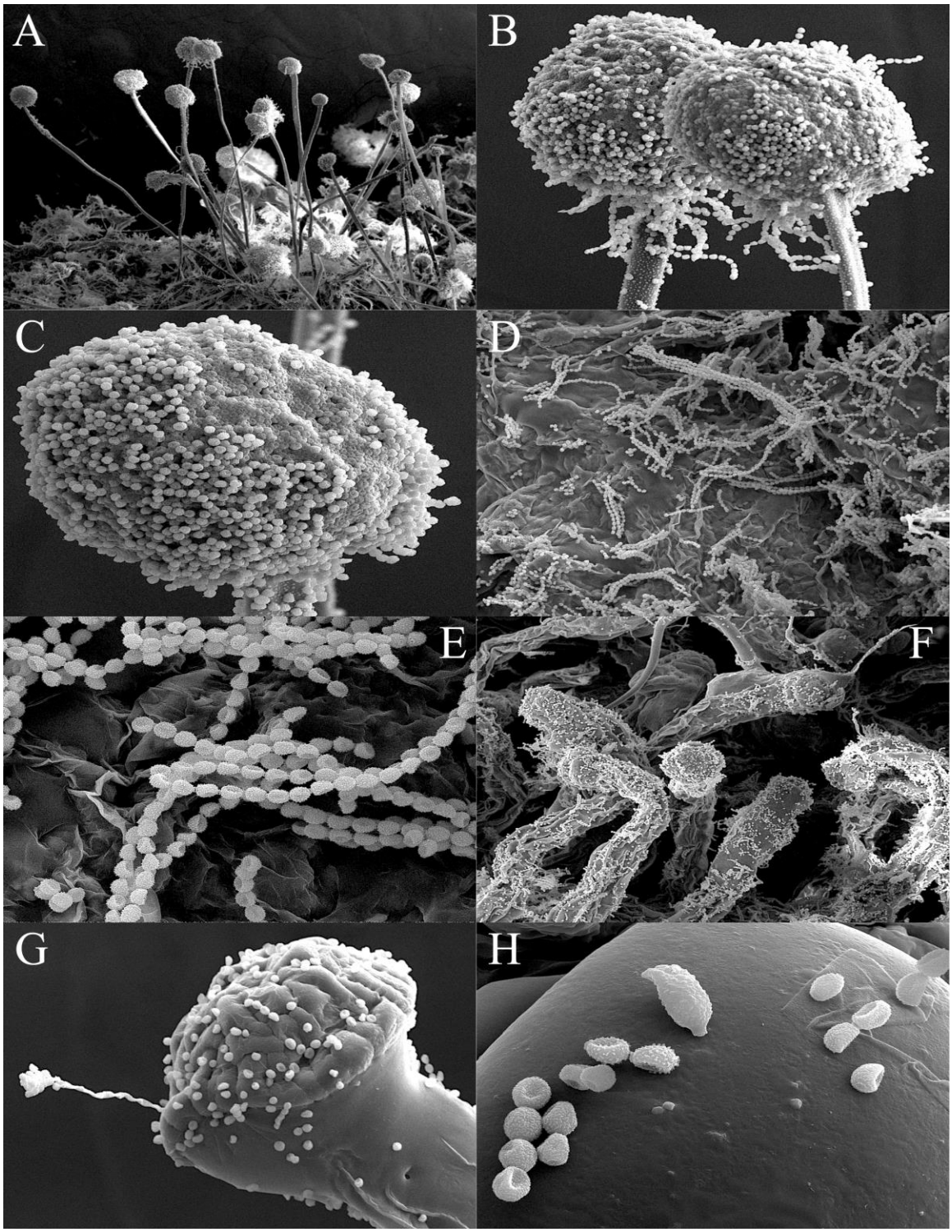


Figure 2.20 **Scanning electron microscope images of *Aspergillus* and *Penicillium* on cannabis flowers. (A - C) The spore heads of *Aspergillus* on conidiophores. (D) *Penicillium* colonizing and sporulating on flower tissues. (E) Chains of *Penicillium* spores. (F) *Penicillium* spores on trichomes. (G, H) Closeup of *Penicillium* spores on trichomes.**

2.11.2. Symptoms and Signs

Wounds from harvesting and trimming flowers, especially excessive wounding from automated trimming machines, provide openings for these fungi to colonize flower tissues. Typically, post-harvest infections may begin to be observed within 3-6 days of starting the drying period, with species such as *Penicillium, Cladosporium* and *Aspergillus* causing discoloration and decay of tissues. Small patches of white mycelium may also be observed (Fig. 2.21).

Fungi may spread during trimming and drying through the air as spores, or through contaminated equipment, tools or on the hands of workers. During the wet trim process, wounding of flowers can cause a build-up of spores that can progress to mold development during the drying process. Dry trim or hand trimming may reduce the build-up of spores. Unsanitary equipment or excessive aerial contamination and inappropriate drying conditions all contribute to post-harvest losses of cannabis.

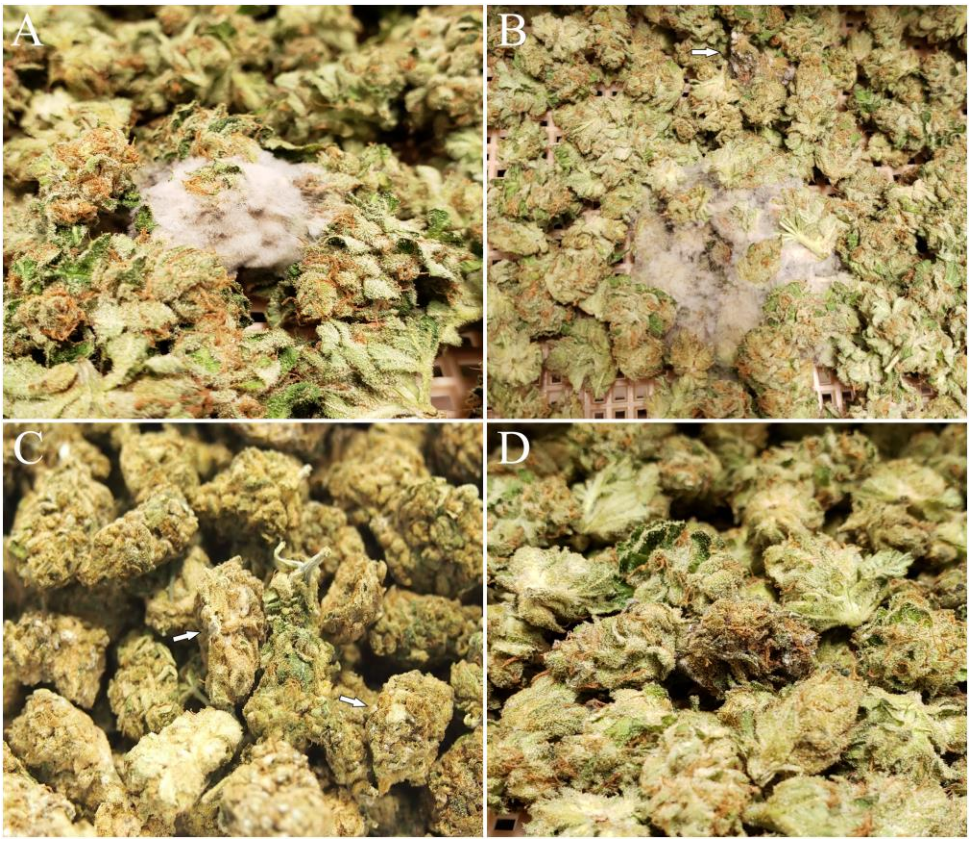


Figure 2.21 **Signs of post-harvest decay. (A) *Fusarium* growing on drying buds. (B) *Botrytis cinerea* and *Penicillium*, indicated by an arrow, growing on drying buds. (C) White spots of *Penicillium* growing on numerous drying buds. (D) *Penicillium* on a drying bud.**

2.11.3. Management Approaches

1. Harvested flowers should examined and be free of visible bud rot.

2. Minimize introduction or spread of contaminants. This requires that shoe covers, gloves, hair and beard nets and footbaths be utilized. Equipment or tools should be cleaned with a detergent and water, as well as a disinfectant. Special attention should be given to trimming machines, including regularly cleaning inside in areas where plant debris may build up. Food safe degreasers may be effective to clean areas where resin and plant debris build up.

3. Air filtration and purification should also be used in trimming rooms as the build-up of spores and particulate matter can be high during these operations.

4. Avoid excessive physical damage to buds during harvesting and pruning.

5. Maintain optimal humidity, temperature and airflow in the drying area. Air filtration and purification should be used in drying rooms.

6. Removed tissues that show signs of infection post-harvest.

Table 2.2 The most important pathogens currently affecting cannabis production and management options

Common Name of Disease	Pathogen(s)	Management Options
Damping-off	*Fusarium oxysporum* *Fusarium proliferatum* *Fusarium solani*	Have appropriate humidity levels Improve air circulation Apply biocontrols during propagation Remove diseased cuttings Replace stock plants regularly
Fusarium Root and Crown Rot	*Fusarium oxysporum* *Fusarium proliferatum* *Fusarium solani*	Test mother plants to ensure they are disease free Replace stock plants regularly Apply biocontrols during propagation and vegetative stages of growth Avoid injury to roots
Pythium Root and Crown Rot	*Pythium myriotylum* *Pythium dissotocum* *Pythium aphanidermatum*	Avoid overwatering Avoid injury to roots Apply biocontrols during propagation and vegetative stages of growth Treat irrigation water with UV or chlorine
Powdery Mildew	*Golovinomyces* spp.	Improve air circulation Manage temperature and humidity Prune and deleaf plants Apply products such as Regalia Maxx, MilStop, ZeroTol or others Treat plants with UV-C Remove and destroy diseased leaves Plant resistant varieties
Bud Rot	*Botrytis cinerea* *Fusarium* spp.	Improve air circulation Manage temperature and humidity Remove diseased buds Avoid varieties with dense inflorescences

Post-Harvest Decay	*Botrytis cinerea* *Penicillium* spp.	Maintain appropriate humidity, temperature, and air movement in drying rooms Avoid damage to buds during harvesting and trimming Removed infected post-harvest buds

2.12. Emerging Pathogens in Controlled Environments

In addition to the major pathogens of cannabis discussed previously, there is the potential for other pathogens reported on hemp or cannabis to become more prevalent and widespread. It is unclear to what extent diseases reported on hemp produced in the field may cause disease in indoor or greenhouse growing environments, but growers should be aware of alternate sources of inoculum from crops grown in proximity to cannabis.

2.12.1. *Sclerotinia* Hemp Canker and Bud Rot

Sclerotinia species have been reported to cause hemp canker and crown rot on hemp in California, Kentucky, New York, Alberta and New Brunswick (Bains et al. 2000; Koike et al. 2019). Major hosts of *Sclerotinia* species include soybean, canola, potato, sunflower and other vegetable crops. Symptoms of infection on hemp include wilt, browning or bleaching of foliage, and dry discolored lesions on the stem or crown of the plant. Small, black sclerotia may be present externally on lesions or internally when tissues are broken open. Cottony white mycelium may also be observed, which is a characteristic feature of *Sclerotinia* infections and gives it the common name “White Mold”. Infections on inflorescences from *Sclerotinia* can also occur on hemp and cannabis (Fig. 2.22).

Figure 2.22 **Some of the emerging and less common pathogens of cannabis and hemp. (A) Symptoms of hop latent viroid on a cannabis plant growing vegetatively. (B) An example of a mosaic type symptom commonly caused by viruses. (C, D) Infections caused by *Botrytis* and other fungi on cuttings. (E) *Botrytis* infecting the stem of a cannabis plant. (F) *Sclerotinia* growing from infected cannabis flower tissues.**

2.12.2. Southern Blight

Sclerotium rolfsii is the causal agent of southern blight on hemp and numerous other crops such as apple, peanut, potato, tomato, beans, cereals and cotton (Mersha et al. 2020). Southern blight has been reported on hemp in Kentucky, Alabama, Virginia, Tennessee and North Carolina as well as in parts of Italy.

Symptoms include wilting and yellowing of mature plants, discoloration and rot of the crown and death of plants. White mycelium can often be seen growing out from the base of infected plants and along the soil surface. Tan or off white sclerotia may also be produced on diseased tissues. Symptoms of southern blight are worsened by warm dry weather.

2.12.3. *Rhizoctonia* Root and Stem Rot

Root and stem rot, caused by *Rhizoctonia solani*, has been reported on hemp in North Carolina and Kentucky. Symptoms include root rot, necrotic lesions on the stems of plants at the soil line and wilting. *Rhizoctonia* also can reportedly cause damping off as well as web blight, which causes foliage and flowers to die back rapidly and may leave a tan or off-white mycelial webbing on affected tissues. Alternate hosts of *Rhizoctonia solani* include rice, bean, soybean, corn, cotton, wheat, turf grass and greenhouse crops such as tomato, pepper and eggplant.

2.12.4. *Neofusicoccum* Stem Canker and Dieback

Hemp plants in Italy and the United States (Arkansas) have been reported to be affected by *Neofusicoccum parvum*, which causes symptoms of leaf curl and leaf discoloration as well as stem canker and dieback on the main stem and branches. Stem cankers appeared as sunken tan lesions. White mycelium and black pycnidia were also visible on lesions. *Neofusicoccum* often caused the death of plants. These symptoms have been observed and replicated on a range of cultivars of hemp, including Carmagnola, Bejko, Cherry Wine, Cherry Blossom and Berry Blossom. This pathogen has a broad host range and also infects crops such as grape, strawberry, stone fruits and ornamental trees.

2.12.5. Fungal and Bacterial Leaf Spots

Leaf spots on hemp have been reported to be caused by fungal or bacterial pathogens belonging to the genera *Septoria*, *Bipolaris*, *Cercospora* and *Xanthomonas* (Szarka et al. 2020).

These pathogens predominantly occur in the warm and humid southeastern states of the United States, including Kentucky, Florida and North Carolina on hemp. Symptoms may appear as circular or angular spots with or without a distinct border on leaves. There may also be a yellow halo around the spots. Some species produce pycnidia on infected tissues, which appear as black flecks on the diseased area. As the disease becomes more severe, spots may coalesce. Alternate hosts of these pathogens and possible sources of inoculum include cereals and grains, as well as citrus and soybean.

2.12.6. Viruses and Viroids

Beet curly top virus (BCTV) has been reported on hemp in western Colorado (Giladi et al. 2020). Symptoms start as a fading of leaf color to a pale green, which extends from the base of the leaves to the tips. A yellow and green mosaic pattern also develops. As the disease progresses, symptoms affect the entire plant, including new growth, and leaves will take on a curled narrow appearance. The plant may appear stunted, curled and distorted. This virus has been reported to affect numerous different cultivars of hemp at various stages of growth. Alternate hosts of BCTV include sugar beet, beans, peppers, spinach and tomato. BCTV is spread only through leafhoppers.

Another virus that affects cannabis plants is lettuce chlorosis virus (LCV), which to date has been reported from Israel and Canada (Hadad et al. 2019). Affected plants have symptoms of interveinal chlorosis which can cause leaves to appear chlorotic and bright yellow throughout, partial necrosis and brittleness. Transmission of LCV was confirmed to be through *Bemisia tabaci* whiteflies and not through seed. Vegetative cuttings taken from infected mother plants showed symptoms that were often more severe than the symptoms observed on the mother plants. This particular isolate of LCV, currently named LCV-Can, was confirmed to infect two different varieties of lettuce as well as rose periwinkle. Other plants susceptible to LCV include lettuce, beets, tobacco and weeds such as shepherd's purse and hemlock.

Hop latent viroid was first reported on cannabis plants in California and has spread to growing regions in North America and likely elsewhere (Bektas et al. 2019; Warren et al. 2019). A viroid, unlike a virus, does not have a protective outer coating (a capsid or envelope) and are only RNA. Affected plants may have chlorotic and distorted leaves, brittle stems, an abnormal or stunted pattern of growth and reduced yield (Fig. 2.22). Flowers may also "dud", as the viroid can cause a reduction in flower development. Infected plants may appear asymptomatic for prolonged

periods of time before showing symptoms. Hop latent viroid is known to spread mechanically and through seed. It is believed to have originated from hop plants where it causes no symptoms. The extent of damage caused by HPLv is currently unknown but it is of concern to the industry at large.

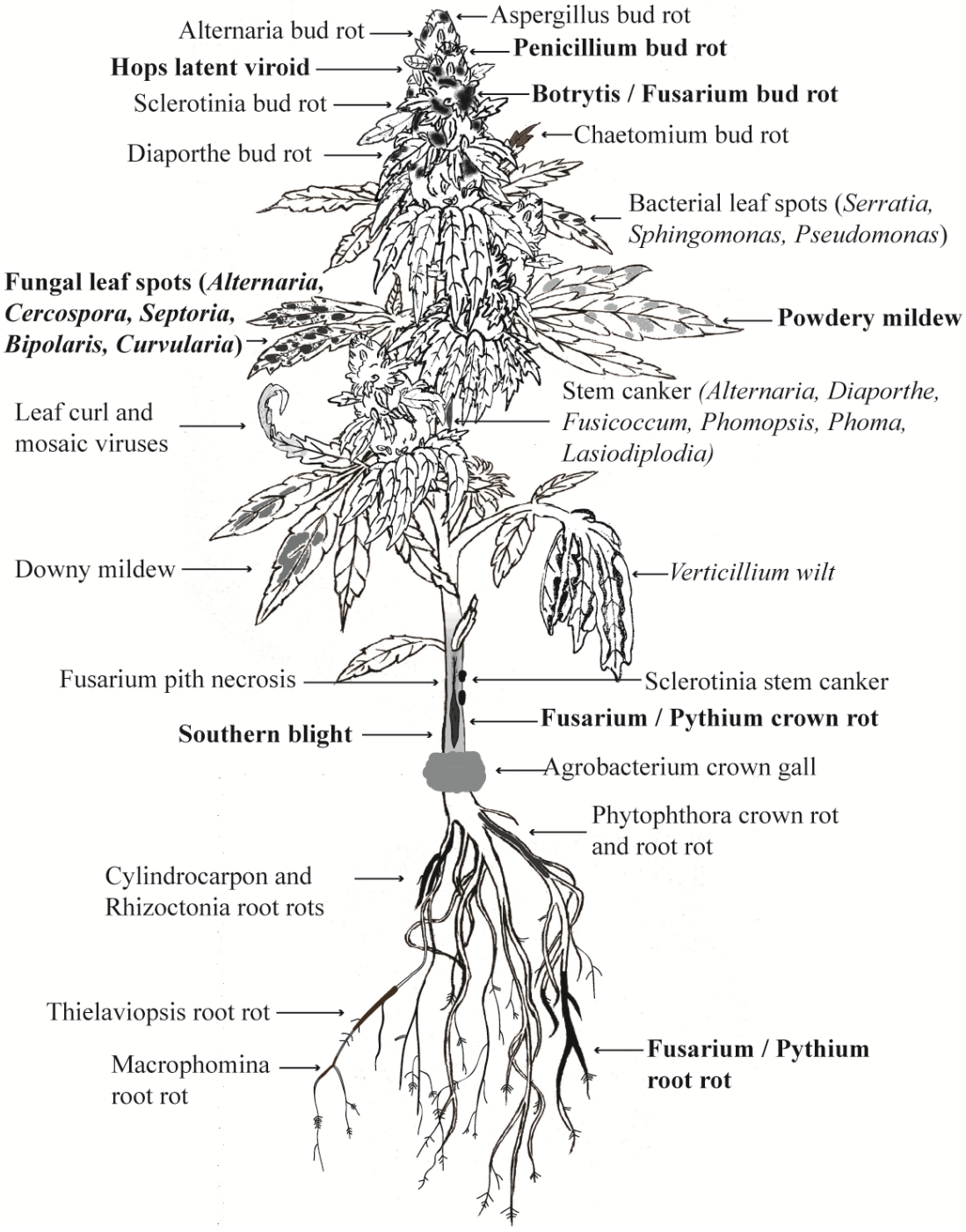


Figure 2.23 **The currently recognized pathogens on cannabis and hemp plants. Pathogens shown in bold are the most damaging. (Punja 2021)**

Chapter 3. Evaluation of Disease Management Approaches for Powdery Mildew Management on *Cannabis sativa* L. (marijuana) Plants.

3.1. Introduction

Powdery mildew diseases affect a wide range of host species and are caused by a large number of different obligate parasites belonging to several genera (Agrios 2005). On cannabis (*Cannabis sativa* L., marijuana), powdery mildew in Canada has been reported to be caused by *Golovinomyces cichoracearum sensu lato* (Pépin et al. 2018), and by closely related *Golovinomyces* species on hemp in other parts of North America (Schoener & Wang 2018; Gwinn et al. 2019; Ocamb & Pscheidt 2019; Szarka et al. 2019; Weldon et al. 2020). Powdery mildew is a prevalent disease that affects plant production in indoor controlled environments, greenhouses, and outdoor locations (Punja 2018; Punja et al. 2019). Following spore germination and infection, which can occur on leaf and stem surfaces, colonies that develop can limit photosynthesis and reduce nutrient availability to the plant, causing premature leaf drop as well as reducing overall vigor and potential yield of plants. Powdery mildew diseases, in general, have been managed by applications of fungicides and other chemical products, biological control agents, induction of disease resistance, and development of cultivars with genetic resistance (Agrios 2005).

On cannabis, powdery mildew disease management relies on cultural control methods, such as removal of diseased leaves (deleafing), maintaining relative humidity at levels not conducive for pathogen development, applications of vaporized sulphur or reduced risk chemicals such as potassium bicarbonate (MilStop®) or hydrogen peroxide (ZeroTol®) (Health Canada 2019). There are currently no synthetic chemical fungicides registered for powdery mildew control on cannabis in Canada unlike on other crops. While reduced risk chemical products including potassium bicarbonate, hydrogen peroxide and lactic acid are registered for use on cannabis in Canada (Health Canada 2019), data on their comparative efficacy is lacking. In addition, the assessment of other potential methods for disease control, including biological control agents, has not been previously conducted.

The rapid expansion of the cannabis industry in Canada requires that adequate disease control options for powdery mildew be identified for producers through an assessment of available options that do not impose additional risk to consumers and that meet Health Canada's

requirements for use (Health Canada 2019). Since strains (genotypes) of cannabis can also vary in their susceptibility to powdery mildew infection, an assessment of the levels of resistance to this disease requires further study.

In the present study, the efficacy of nine reduced risk chemical or biological control products in managing powdery mildew development on cannabis plants was evaluated (Table 3.1). These products included three biological control agents, namely Actinovate® SP (containing *Streptomyces lydicus* strain WYEC 108), Rhapsody ASOTM (containing *Bacillus subtilis* strain QST 713), and Stargus® (containing *Bacillus amyloliquefaciens* strain F727). Chemical treatments evaluated included MilStop (potassium bicarbonate), ZeroTol (hydrogen peroxide), Silamol® (orthosilicic acid), neem oil, boric acid, and the plant defense inducer Regalia® Maxx (containing an extract of *Reynoutria sachalinensis*). The fungicide Luna® Privilege SC 500 (fluopyram) was included as an industry standard for comparative purposes. Luna Privilege Greenhouse, which makes use of the same active compound, but at a higher concentration, is registered for use in Canada for the control of powdery mildew and *Botrytis* diseases on greenhouse grown cucumbers, peppers, tomatoes as well as other diseases (Bayer 2019). In addition, the efficacy of UV-C light to manage powdery mildew was also examined by daily exposure of leaves. Finally, the susceptibility of 12 strains (genotypes) of cannabis to disease development was assessed to determine their resistance to powdery mildew.

Table 3.1 Products evaluated for management of powdery mildew on cannabis plants and the average percent disease reduction values obtained over three trials compared to untreated control plants.

				Average disease reduction (%)		
Product	**Active Ingredient**	**Source**	**Rate Used**	**Trial 1**	**Trial 2**	**Trial 3**
Actinovate® SP	*Streptomyces lydicus* WYEC 108 0.037 % (w/w)	Novozymes BioAg. Saskatoon, Canada	0.38 g/L	33.26	6.73	20.56
Boric acid	2 % boron	Sigma-Aldrich. Missouri, USA	12.5 mL/L	22.23	45.32	37.7

Luna Privilege SC 500	40.98 % fluopyram	Bayer CropScience Inc. New Jersey, USA	5 ml/L	78.26	79.09	76.92
MilStop®	85 % potassium bicarbonate	BioWorks Inc. New York, USA	2.4 g/L	82.72	76.55	72.07
Neem oil	70 % cold pressed neem oil	Terramera, Inc. British Columbia, Canada	1 mL/L	80.6	59.53	70.55
Regalia Maxx	20 % extract of *Reynoutria sachalinensis*	Marrone Bio Innovations. California, USA	2.5 mL/L	80.25	32.24	71.83
Rhapsody ASO™	QST 713 dried *Bacillus subtilis*, contains a minimum of 1 x 10^9 CFU/g	Bayer CropScience Inc. New Jersey, USA	15 mL/L	55.61	35.43	71.83
Silamol®	2.5 % available silicon [Si(OH)4]	Frontline Growing Products Ontario Canada	2.4 mL/L	24.77	73.19	69.26
Stargus®	96.4 % *Bacillus amyloliquefaciens* strain F727 cells and spent fermentation media. Contains a minimum of 1 x 10^9 CFU/g	Marrone Bio Innovations. California, USA	15 mL/L	52.4	41.41	63.7
ZeroTol®	27 % hydrogen peroxide	BioSafe Systems. Connecticut, USA	10 mL/L	51.99	16.92	38.27

All products were applied as foliar sprays to plants until run-off. Applications were made weekly for four weeks. Disease was rated weekly starting before the first application. Average disease reductions were calculated from the differences in the AUDPC values between the control and the treatment specific to that trial.

3.2. Materials and Methods

3.2.1. Plant Materials and Inoculation

Vegetative cuttings (approximately 12 cm in height) of the powdery mildew susceptible strain 'Copenhagen Kush' (CPH) were obtained from stock (mother) plants maintained in an indoor growing room with 24 hr lighting and rooted in a mixture (3:1) of coco (Canna Coco) and perlite (Dutch Treat) in individual 8.5 cm^2 pots. The pots were placed under humidity domes (18 cm height dome with vents) for 10–14 days at room temperature (22–25 ^oC) in an indoor growing room with ambient lighting. To maintain high humidity during this time, the insides of the domes were misted with water every other day. After this time, the domes were removed, and plants were placed under two Sunblaster brand (Langley, British Columbia, Canada) 54 watt 6400k T5HO lights with a 24 h photoperiod. Plants were watered as needed with a solution of 1 ml/L Sensi Grow Coco pH Perfect A+B (Advanced Nutrients, West Hollywood, California, USA) and 1ml/L General Hydroponics Calimagic (General Hydroponics, Santa Rosa, California, USA) adjusted to a pH of 5.8–6.2 using Advanced Nutrients pH-Down (Advanced Nutrients, West Hollywood, California, USA). To initiate powdery mildew, initial inoculum originating from cannabis plants grown in a licenced facility in British Columbia was transferred to healthy plants by collecting spores and dusting plants. A single heavily infected plant was then placed in the center of the room and allowed to release conidia. This ensured that background levels of inoculum in the indoor room where the experiments were to be conducted were sufficient for the disease to establish naturally. Rooted plants were removed from the high humidity environment, and received one spray of MilStop to minimize any variance in the initial levels of infection. The plants were then maintained for seven days in the growing room before treatments were applied (Table 3.1).

3.2.2. Treatment Applications and Disease Assessments

Plants were arranged in groups of four per treatment, unless otherwise noted. In each experiment, two or three different treatments were compared to a control group. Products to be tested were prepared on the day of application and sprayed onto plants using a pressurized hand held spray bottle to runoff. At the start of the experiment (week 1), plants were assessed for powdery mildew infection, after which they received the appropriate treatment. Disease was assessed on the 30 most diseased leaflets (assessed visually) per group by placing them into one of five categories, based on the percent coverage of mildew (1–20 %, 21–40 %, 41–60 %, 61–80

%, or 81–100 %). To calculate a disease rating score, the number of leaflets in each category was multiplied by a factor of 0.1, 0.3, 0.5, 0.7, or 0.9, respectively (with these numbers representing the midpoint of each category). Plants received four weekly sprays in total and five disease assessments were made over the span of five weeks, each at weekly intervals. Luna Privilege SC 500 (Bayer Cropscience, Research Triangle Park, North Carolina, USA) was only applied once, at the beginning of the experiment. Each experiment was repeated three times for each treatment (referred to as trials).

3.2.3. Data Analysis

The average disease rating score for each treatment per week was plotted over the duration of the experiment to obtain a disease progress curve for each treatment in each of the trials. The results from each trial are presented separately as there were differences in the initial levels of infection on the plants as well as the overall disease pressure during the trial (see Table 3.2). To statistically show these differences between trials, the disease ratings were compared by ANOVA followed post hoc by Tukey's HSD test at $P < 0.05$ and are presented in Table 3.2. To test for significant differences between treatments within each trial, the data were also subjected to ANOVA followed post hoc by Tukey's HSD test at $P < 0.05$ and are presented in Table 3.2. Finally, area under the disease progress curve (AUDPC) was calculated from the disease progress curves (Simko & Piepho 2012) and compared for differences among treatments within each trial by ANOVA followed post hoc by Tukey's HSD test at $P < 0.05$. These results are presented in Figs. 3.3-3.6.

Table 3.2 **Comparisons of final (week 5) disease rating scores in response to different treatments to manage powdery mildew on cannabis plants. Comparisons were made between trials and between treatments within a trial.**

Treatment	Trial 1	Trial 2	Trial 3
Regalia®	1.15 a A	19.95 b A	5.15 a A
Rhapsody™	9.7 a BC	16.6 a A	9.5 a A
MilStop®	3.2 a AC	3.2 a B	5.1 a A
Control 1	**25.28 a D**	**26.8 a C**	**27 a B**
ZeroTol®	7.18 a A	19.8 b A	12.25 ab A
Actinovate®	13.55 a A	22.67 b A	21.55 b B
Control 2	**22.15 a B**	**26.1 ab A**	**26.85 b C**

Boric acid	21.4 a A	18.55 a A	20.1 a A
Silamol®	16.95 a A	3.28 b B	10.08 c B
Control 3	**27 a B**	**26.35 b C**	**27 a C**
Stargus®	7.08 a A	12.55 b A	4.13 a A
Neem	1.95 a B	8.1 b B	3.65 ab A
Luna	1.75 ab B	3.7 a C	0.78 b A
Control 4	**22.05 a C**	**25 a D**	**20.8 b B**
CleanLight™	11.74 a A	3.68 b A	—
Control 5	**23.9 a B**	**26.34 b B**	**—**

The average disease severity scores for each treatment per trial are shown at week 5. Significant differences between trials of the same treatment are indicated by a lowercase letter, while uppercase letters signify significant differences between different treatments in the same trial. There were four replicate plants per trial except in the case of the CleanLight trials (n = 8) and the second trial of ZeroTol, Actinovate and their respective control (n = 3). No comparisons are made between treatments that are not grouped together. Different letters represent significant ($P < 0.05$) differences, as determined through ANOVA with Tukey's post hoc test.

3.2.4. UV Treatments for Powdery Mildew Control

Plants of strain CPH were grown as previously described and arranged in groups of 8, with 8 untreated (control) plants per trial. The treated plants received daily exposure to UV-C light from a hand-held CleanLight™ Pro (Honselersdijk, NL) unit over a period of four weeks. Plants were treated for 3–5 s per day (equal to 3–6 mJ/cm^2 of radiation, as per the manufacturer) by moving the unit uniformly over and around the plant at a distance of 5 cm away. Treated and control plants were rated for disease as previously described, with the 30 most diseased leaflets being assessed at weekly intervals five times over the duration of the trial. Disease rating scores were calculated for treated and control plants each week and graphed, and AUDPC values were calculated as previously described. This experiment was conducted two times. Data are presented separately for each trial (Fig. 3.7). The disease rating scores and AUDPC values were compared for significant differences between treated and control plants using ANOVA followed post hoc by Tukey's test at $P < 0.05$ (Fig. 3.7).

3.2.5. Scanning Electron Microscopy

To compare the extent of powdery mildew development (mycelial growth and sporulation) between control plants and those receiving specific treatments, leaf samples ca. 0.5 cm^2 in size were prepared for scanning electron microscopy as described by Punja (2018). Leaves were obtained from untreated (control) plants with young and old powdery mildew colonies, as

well as from plants receiving four weekly treatments of Regalia, Rhapsody, or MilStop. In addition, leaves from plants exposed to CleanLight (daily exposure for 28 days) were included. The extent of mycelial growth of powdery mildew and presence of sporulation were visually compared for control and treated leaves.

3.2.6. Effect of Regalia Maxx on Chlorophyll 'a' and 'b' Levels

On plants receiving four applications of Regalia Maxx, it was observed that leaves appeared darker green when compared to control plants. Therefore, methanol extraction and spectrophotometric analysis of chlorophyll *a* and *b* in leaf samples was performed according to Sumanta et al. (2014). Two trials were conducted — one in an indoor growing room and the other under greenhouse conditions. Leaf samples were collected from approximately mid-height after the plants had received 4 applications, with 3–5 replicate samples. Mean values for chlorophyll *a* and *b* from the Regalia Maxx treated plants and the untreated control plants were compared for significant differences using ANOVA followed post hoc by Tukey's test at $P < 0.05$. Data for the two trials are presented separately.

3.2.7. Screening of Strains for Susceptibility to Powdery Mildew

Stock plants of 12 cannabis strains grown in an indoor room were used as a source of cuttings. The plants received two weekly sprays of MilStop (BioWorks, Victory, New York, USA) to reduce powdery mildew infection, after which cuttings were taken and rooted as described previously. The cuttings were dusted with powdery mildew spores from fresh infected leaf tissue before being placed under the humidity dome. Two visibly infected plants of strain CPH were also placed under the domes to provide an additional source of inoculum. In each trial, there were two groups of four plants arranged around two diseased plants. Cuttings were left under the domes for a period of two weeks, during which the inside of the domes was misted on alternate days to maintain high humidity. At the end of two weeks, individual plants were rated for disease by placing them into one of five categories based on the percentage coverage of powdery mildew on the leaves, as described previously. The average disease score per strain was determined and graphed. Significant differences between the disease scores were determined using ANOVA followed post hoc by Tukey's test at $P < 0.05$. The experiment was conducted twice.

3.3. Results

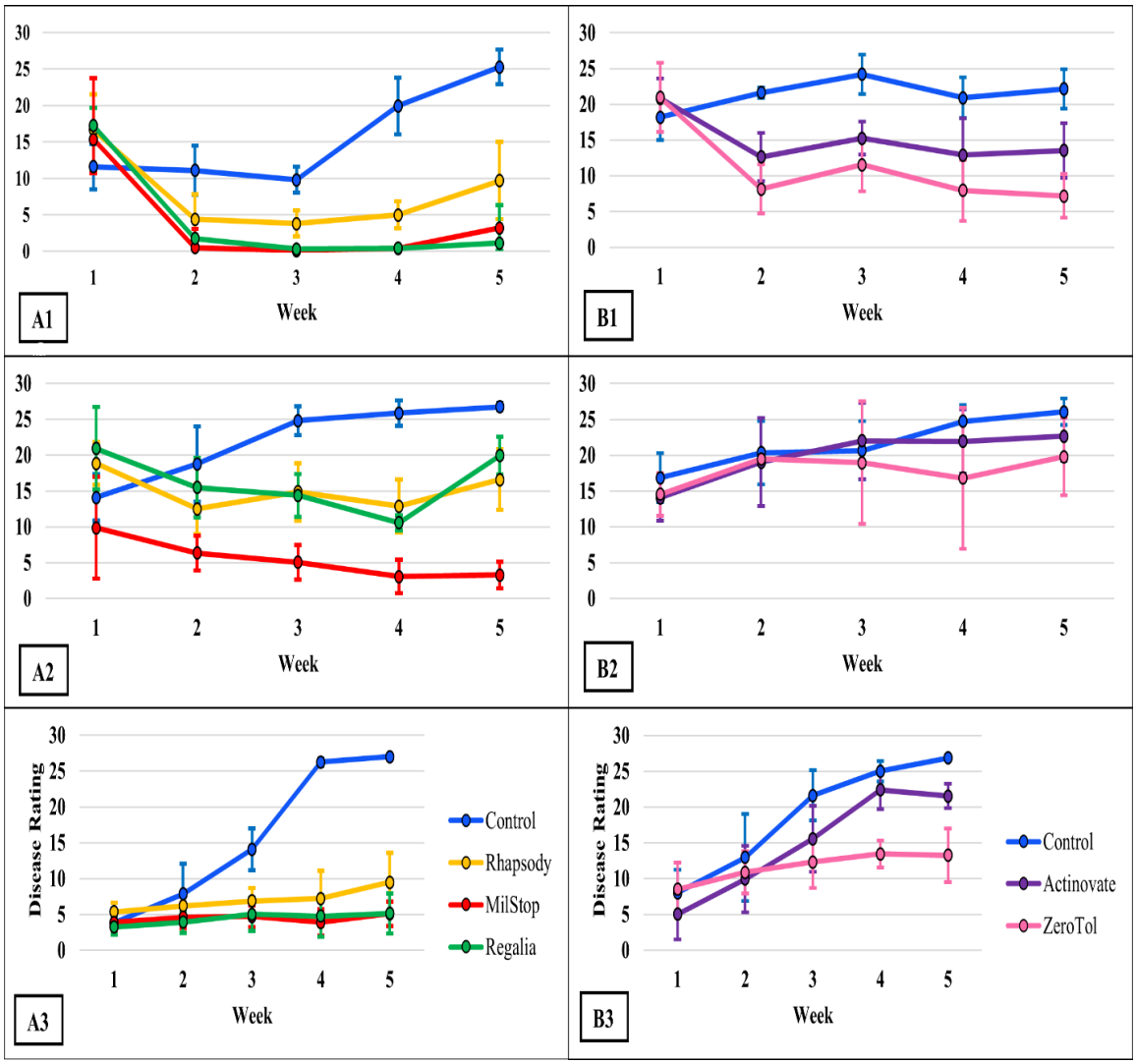
3.3.1. Development of the Powdery Mildew Fungus

The symptoms of powdery mildew infection on cannabis leaves were quite apparent as white mycelial growth and sporulation of the pathogen on the upper surface of leaves on plants in the growing room (Fig. 1A). Under the scanning electron microscope, a cross-section through a leaf showed mycelial growth on the epidermal surface (Fig. 1B), and sporulation was abundant on the leaf surface (Fig. 1C). The production of spores in chains is a characteristic of the genus *Golovinomyces* (Pepin et al. 2018).

Figure 3.1 **Development of powdery mildew on cannabis leaves. (1A) Initial infections are visible as white colonies on the upper surface of leaves. (1B) Scanning electron microscopic image through a cross section of a diseased leaf showing powdery mildew mycelium growing over the surface of epidermal cells. The underlying cells of the epidermis and mesophyll layer, and the lower epidermis, can be seen in this section. (1C) Abundant spore production from an older powdery mildew colony on the leaf surface.**

3.3.2. Treatment Applications and Disease Assessments

The foliar treatments applied to manage powdery mildew had different levels of efficacy when compared to the untreated controls. The results from each of the three trials conducted for each product showed some variation between trials, which was attributed to differing levels of initial infection and/or disease pressure during the trials. The disease progress curves for each trial for each product tested are shown in Fig. 3.2. They show the varying rates and levels of disease development over time. A comparison of final, week 5, disease rating scores in response to different treatment applications to manage powdery mildew on cannabis plants is presented in Table 3.2. Statistical comparisons were made between trials and between treatments within a trial. The statistical analysis showed that disease levels on control plants in each of the experiments, i.e., Control 1 to Control 5 values, were in the range of 20.8 to 27, and thus showed fairly consistent final disease levels in all trials. However, in a number of treatments, the data from different trials were significantly different. For example, in trial 2, the values for disease ratings were significantly different for Regalia, ZeroTol, Actinovate, Stargus, and neem when compared to the values for trials 1 and 3 (Table 3.2). The week five disease rating scores, however, showed that most treatments significantly reduced the development of powdery mildew in all three trials, except for ZeroTol, boric acid and Actinovate (Table 3.2), which were the poorest performing products.


A1
B1
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Rhapsody
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Actinovate
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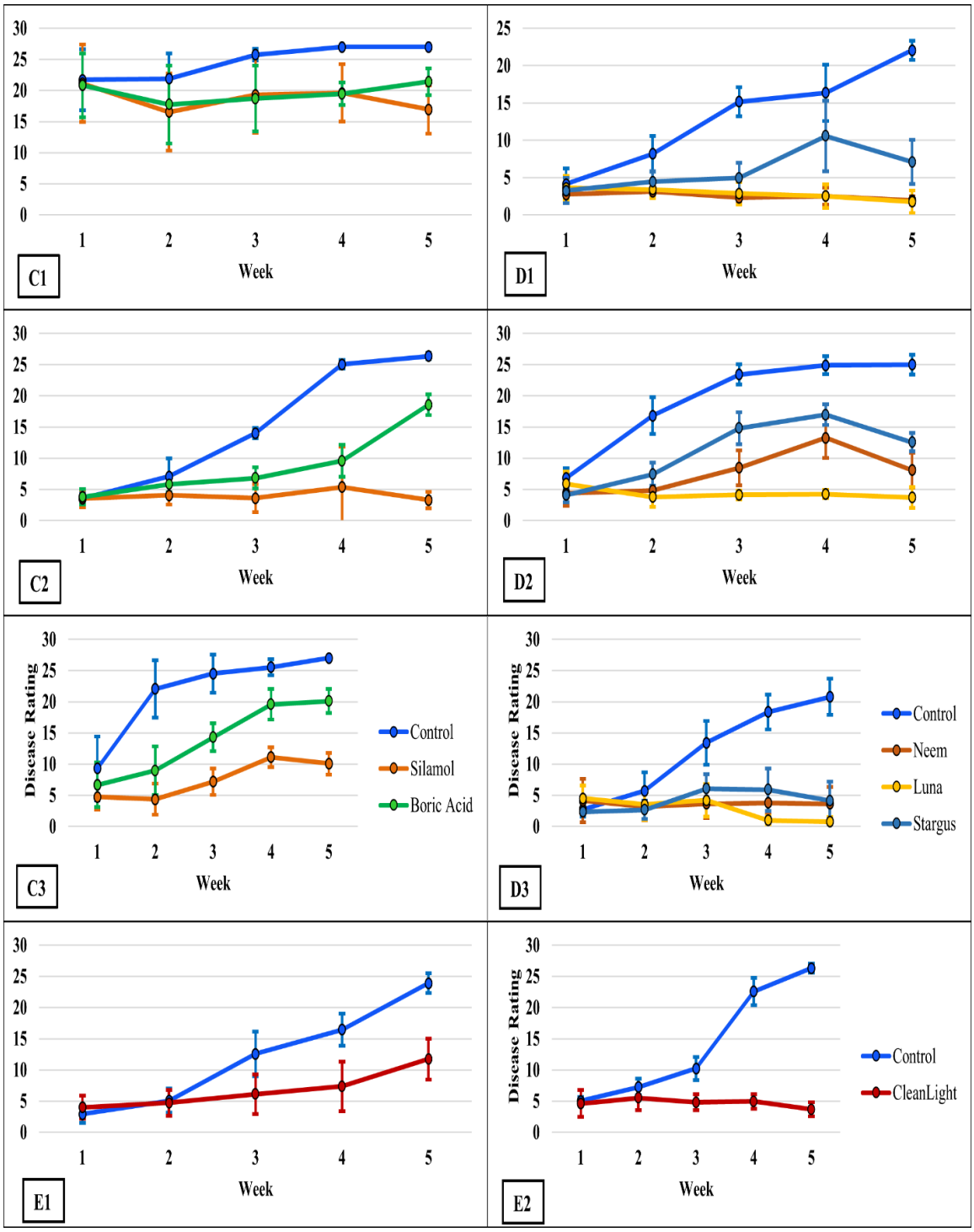
C1
Week
D1
Week
C2
Week
D2
Week
C3
Disease Rating
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Control
Silamol
Boric Acid
D3
Disease Rating
Week
Control
Neem
Luna
Stargus
E1
Week
E2
Disease Rating
Week
Control
CleanLight

Figure 3.2 **The disease progress curves for powdery mildew development on cannabis plants when treated with Regalia Maxx, Rhapsody ASO, MilStop® (A1-3), ZeroTol®, Actinovate® (B1-3), boric acid, Silamol® (C1-3), Stargus®, neem oil, Luna Privilege SC 500 (D1-3), CleanLight™ UV-C (E1-2), and their respective untreated controls. Data represents the average disease score for each treatment in each week. There were four replicate plants per trial except in the case of the CleanLight trials (n = 8) and the second trial of ZeroTol, Actinovate and their respective control (n = 3). Error bars are 95 % confidence intervals.**

3.3.3. Product Efficacy Based on Area Under the Disease Progress Curve

The AUDPC values were calculated from the disease progress curves and are presented in Figs. 3.3–3.7. The most effective products, which provided significant ($P < 0.05$) reductions in AUDPC values consistently in all three trials were: Luna Privilege SC 500; MilStop; neem oil; Regalia Maxx; Rhapsody ASO; and Stargus. These products also provided average disease reductions of over 50 % (Table 3.1) for the majority of the trials. Products showing significantly reduced AUDPC values in two out of three trials were ZeroTol, boric acid, and Silamol, although these products often reduced disease by less than 50 % in most of the trials (Table 3.1), with the exception of Silamol. Actinovate only provided significant disease control in one of the three trials. Plants receiving daily exposure to CleanLight had significantly ($P < 0.05$) lower AUDPC values in both trials, when compared to their appropriate controls (Fig. 3.7). The AUDPC values and the week five disease rating scores, therefore, provided similar conclusions on the efficacy of the products tested. The percentage reduction in disease levels for each of the products in each trial based on AUDPC values is summarized in Table 3.1. These values were derived by comparison of AUDPC values for the treatments with the untreated control used in each trial.

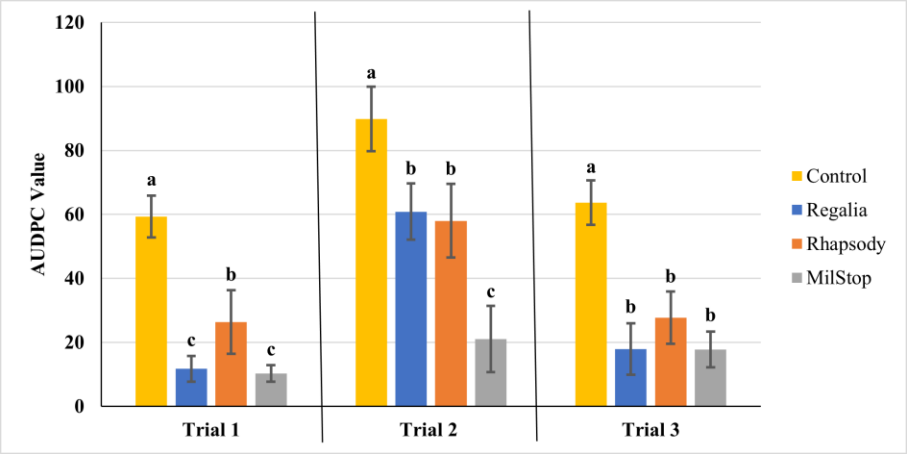

Figure 3.3 **Effect of four weekly applications of Regalia Maxx, Rhapsody ASO and MilStop® on development of powdery mildew on cannabis plants compared to a nontreated control. Data represent areas under the disease progress curves (AUDPC) from three repeated trials, each with four replicate plants. Error bars are 95 % confidence intervals. Letters above the error bars represent significant differences in the AUDPC values of the treatments, as determined through ANOVA and Tukey's post hoc test (P < 0.05).**

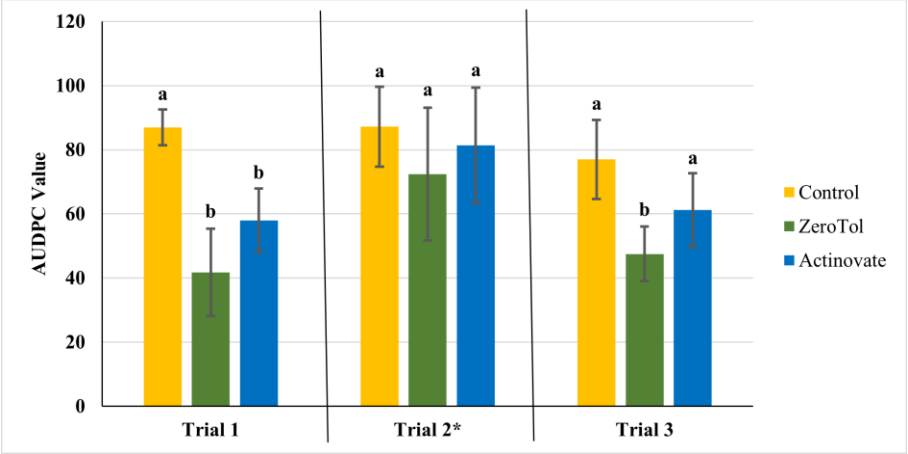

Figure 3.4 **Effect of four weekly applications of ZeroTol® and Actinovate® on development of powdery mildew on cannabis plants compared to a nontreated control. Data represent areas under the disease progress curves (AUDPC) from three repeated trials, each with four replicate plants (except in the case of trial 2 where an asterisk denotes three replicate plants were used instead of four). Error bars are 95 % confidence intervals. Letters above the error bars represent significant differences in the AUDPC values of the treatments, as determined through ANOVA and Tukey's post hoc test (*P* < 0.05).**

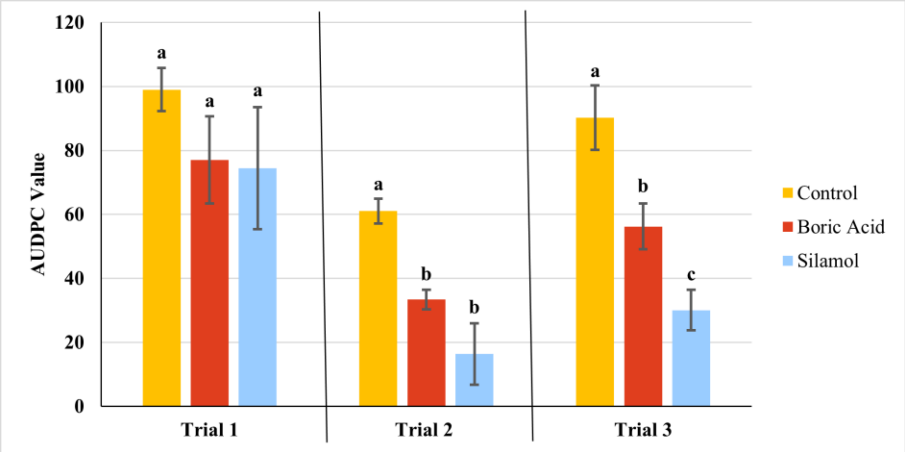

Figure 3.5 **Effect of four weekly applications of boric acid and Silamol® on development of powdery mildew on cannabis plants compared to a non-treated control. Data represent areas under the disease progress curves (AUDPC) from three repeated trials, each with four replicate plants. Error bars are 95 % confidence intervals. Letters above the error bars represent significant differences in the AUDPC values of the treatments, as determined through ANOVA and Tukey's post hoc test ($P < 0.05$).**

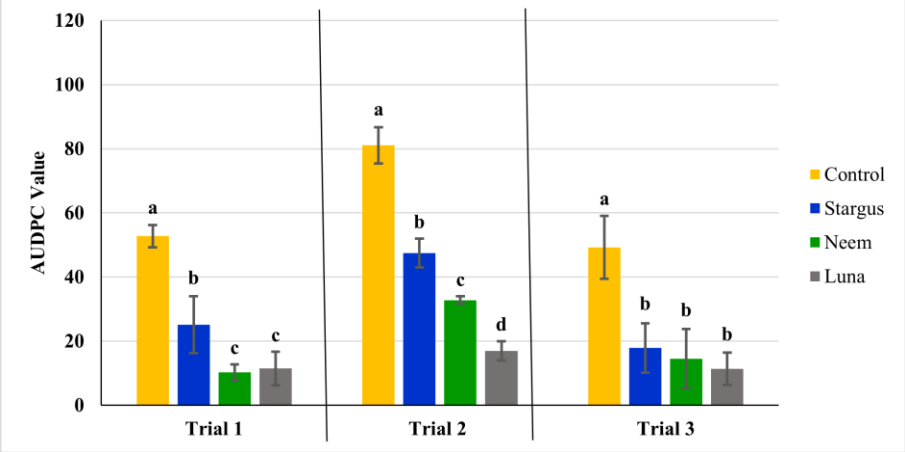

Figure 3.6 **Effect of four weekly applications of Stargus®, neem oil and Luna Privilege SC 500 on development of powdery mildew on cannabis plants. Data represent areas under the disease progress curves (AUDPC) from three repeated trials, each with four replicate plants. Error bars are 95 % confidence intervals. Letters above the error bars represent significant differences in the AUDPC values of the treatments, as determined through ANOVA and Tukey's post hoc test ($P < 0.05$).**

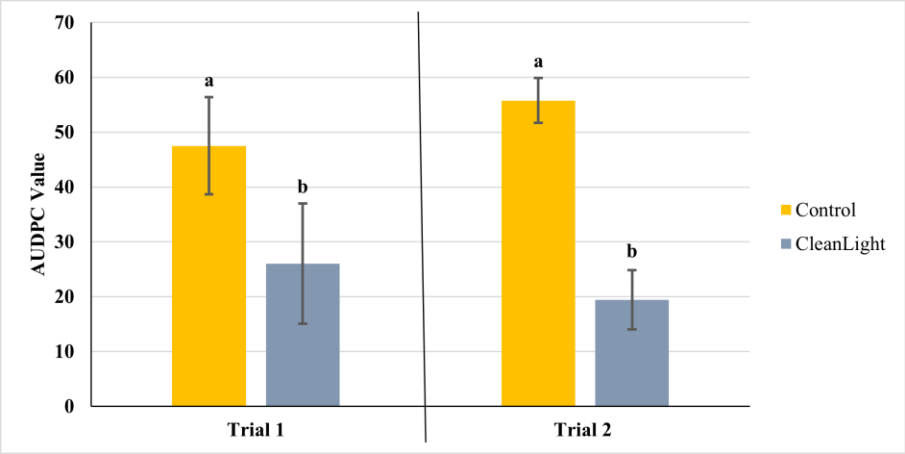

Figure 3.7 **Effect of daily exposure of cannabis leaves to UV-C light on powdery mildew development compared to a nontreated control. Data represent areas under the disease progress curves (AUDPC) from two repeated trials, each with eight replicate plants. Error bars are 95 % confidence intervals. Letters above the error bars represent significant differences in the AUDPC values of the treatments, as determined through ANOVA and Tukey's post hoc test ($P < 0.05$).**

3.3.4. Visual Representation of Product Efficacy

Representative leaves were sampled from plants in each of the treatments and photographed to show the differences in product efficacy (Fig. 3.8). While the leaves were from different experiments, they were selected to represent the disease rating values shown in Table 3.2. When compared to the untreated control leaves, treatments with CleanLight, Luna, Regalia Maxx and MilStop showed the lowest disease severity and incidence of powdery mildew, and leaves from treatments such as neem oil, Stargus, Rhapsody and ZeroTol also showed a visible reduction in disease development. By comparison, leaves treated with Actinovate and boric acid had the highest infection levels (Fig. 3.8).

Figure 3.8 **A representation of the comparative efficacy of 11 treatments at managing powdery mildew development on leaves of cannabis strain 'Copenhagen Kush'. (A) Untreated control. (B) CleanLight Pro Unit. (C) Luna SC Privilege 500. (D) Regalia Maxx. (E) MilStop. (F) Neem oil. (G) Stargus. H) Rhapsody ASO. (I) ZeroTol. (J) Silamol. (K) Boric acid. (L) Actinovate. Representative leaves were sampled from plants in each of the treatments and photographed to show the differences in product efficacy.**

3.3.5. Scanning Electron Microscopy

Scanning electron microscopic images showing growth of the powdery mildew pathogen on leaf surfaces of untreated cannabis plants were compared to those following applications of disease management products. Weekly applications of three products (Rhapsody, Regalia, MilStop) or daily exposure of plants to UV-C light were made. On control leaves, mycelium from a young developing colony was observed ramifying over the surface (Fig. 3.9A), followed by more extensive mycelial proliferation and spore production in a mature colony on an untreated leaf (Fig. 3.9B). Following four applications of Rhapsody, the extent of mycelial proliferation was noticeably reduced (Fig. 3.9C), while some sporulation was evident. On leaves treated with Regalia Maxx, there was an absence of mycelial growth on the leaf surface, and trichomes could be seen (Fig. 3.9D). Following applications of MilStop, mycelial growth was sparse and hyphae and conidia appeared to be plasmolyzed (Fig. 3.9E). Exposure of leaves to UV light also revealed a total absence of mycelial growth (Fig. 3.9F). These observations show that there were obvious differences in pathogen development following various treatments when compared to the control and lend support to the disease measurement values reported above.

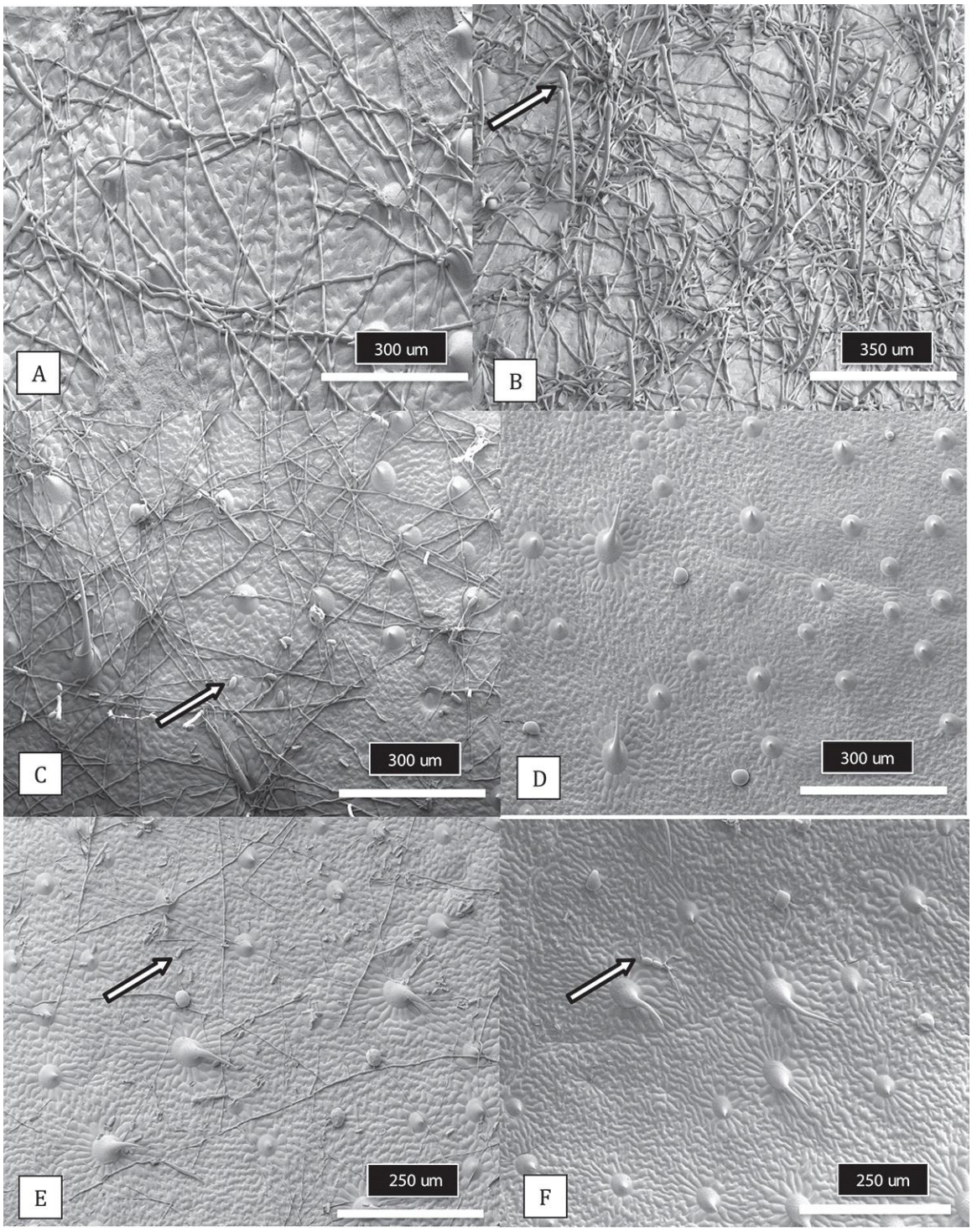
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Figure 3.9 **Scanning electron microscopic images showing growth of the powdery mildew pathogen on leaf surfaces of untreated cannabis plants or following applications of disease management products. Weekly applications of three products or daily exposure of plants to UV-C light were made as indicated. Samples were collected after four applications of products or following a 28-day exposure to UV-C light. (A) Mycelium of a young developing colony on an untreated leaf. (B) Mycelium and spore production (arrow) of a mature colony on an untreated leaf. (C) Reduced mycelial growth on the leaf surface, with some sporulation (arrow) following applications of Rhapsody. (D) Total absence of mycelial growth on the leaf surface following applications of Regalia®. Hair-like projections that are visible are the trichomes. (E) Sparse mycelial growth and collapsed conidia (arrow) following applications of MilStop®. (F) Total absence of mycelial growth following daily exposure to UV-C light. Arrow shows conidia attempting to germinate.**

3.3.6. Effect of Regalia Maxx on Chlorophyll 'a' and 'b' Levels

Samples of leaves from plants receiving 4 applications of Regalia Maxx and grown in an indoor environment had mean chlorophyll *a* levels that were significantly ($P < 0.05$) higher than leaves from untreated plants, while there was no significant difference in chlorophyll *b* levels between treated and untreated plants (Fig. 3.10A). The chlorophyll *a*/*b* ratios for untreated and Regalia Maxx treated plants were 0.8 and 1.4, respectively. On plants grown in the greenhouse, there were significant ($P < 0.05$) differences between the mean levels of both chlorophyll *a* and chlorophyll *b* in Regalia treated leaves compared to untreated leaves (Fig. 3.10B). The chlorophyll *a*/*b* ratios for untreated and Regalia Maxx treated plants were 2.67 and 2.61, respectively. The total chlorophyll content in leaves was about three-fold higher in plants grown in the greenhouse compared with the indoor environment, likely due to differences in light intensity levels and growing conditions.

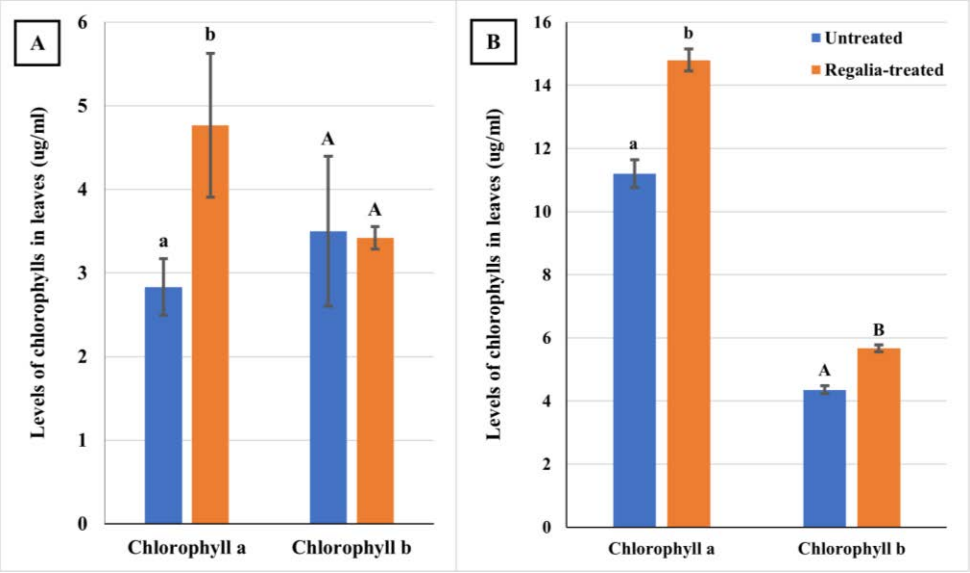

Figure 3.10 **Effect of four weekly applications of Regalia Maxx on mean chlorophyll a and b levels in leaves of: (A) indoor grown plants (n = 3) and (B) greenhouse grown plants (n = 5) compared to their respective controls. Levels of chlorophyll a were significantly increased in both trials, while chlorophyll b was increased in the greenhouse trial. Error bars are 95 % confidence intervals. Letters above the error bars represent significant differences in chlorophyll levels, as determined through ANOVA and Tukey's post hoc test ($P < 0.05$).**

3.3.7. Screening of Strains for Susceptibility to Powdery Mildew

The disease scores for 12 cannabis strains are shown in Fig. 3.11A. Seven of the strains had significantly lower ($P < 0.05$) disease scores compared to the five highly susceptible strains, suggesting they were resistant to infection. A comparison of disease development on leaves selected from strains 'Space Queen', 'Pennywise', and 'Sweet Durga Mata' is shown in Fig. 3.11B.

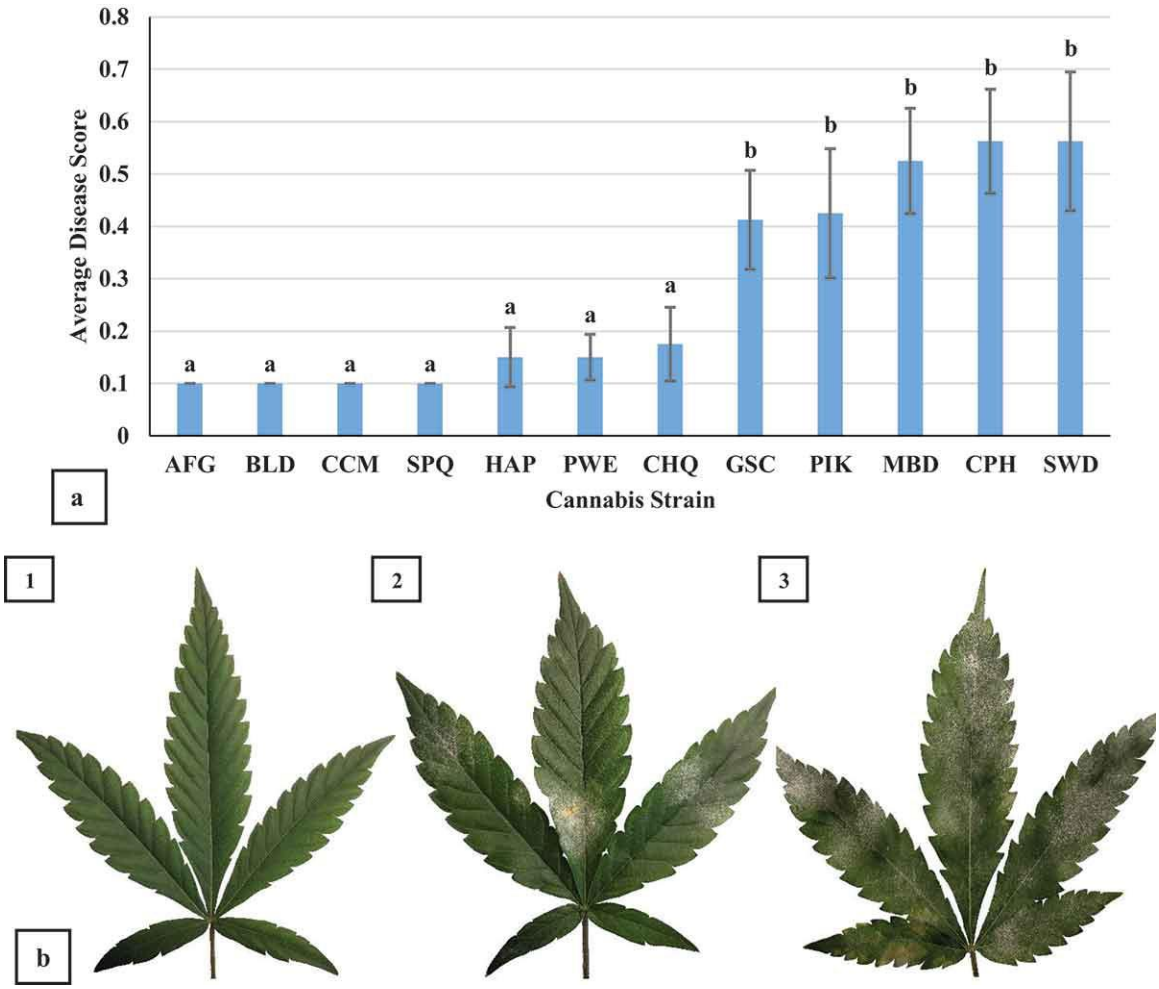

Figure 3.11 **(A) Response of 12 different strains (genotypes) of cannabis plants to powdery mildew infection. Mean disease score values were calculated for infected plants of each strain to evaluate their susceptibility to powdery mildew two weeks after pathogen introduction. Error bars are 95 % confidence intervals. Letters above the error bars represent significant differences in the average disease severity scores of the treatments, as determined through ANOVA and Tukey's post hoc test ($P < 0.05$). The strain abbreviations are: AFG = 'Afghani'; BLD = 'Blue Deity'; CCM = 'Critical Cali Mist'; SPQ = 'Space Queen'; HAP = 'Hash Plant'; PWE = 'Pennywise'; CHQ = 'Cheese Quake'; GSC = 'Girl Scout Cookies'; PIK = 'Pink Kush'; MBD = 'Moby Dick'; CPH = 'Copenhagen Kush'; and SWD = 'Sweet Durga Mata'. (B) Comparison of powdery mildew development on leaves of three strains of cannabis. Representative leaves were sampled to illustrate a range of susceptibility to disease. (1) 'Space Queen'. (2) 'Pennywise'. (3) 'Sweet Durga Mata'.**

3.4. Discussion

Powdery mildew occurs on cannabis (marijuana) and hemp plants grown indoors, in greenhouses and in outdoor environments and is a difficult disease to manage because of the rapid rate at which the pathogen can spread and a lack of available fungicides that are used to manage powdery mildew diseases on other crops (Agrios 2005). The spread of inoculum within a cannabis production facility from diseased plants is from the release of air-borne spores that are produced in large numbers (Punja 2018) as well as by infected plant materials that growers may move within and between different production facilities and from which diseased cuttings may be taken. In the present study, introduction of a diseased plant within the growing room where experiments were conducted resulted in sufficient spread and consistent disease development on control plants in repeated trials over time.

The taxonomic placement of the cannabis powdery mildew pathogen was confirmed to be in the genus *Golovinomyces* (formerly *Erysiphe*) by Pépin et al. (2018). In that study, and in subsequent work by other researchers from the United States, *Golovinomyces* has been shown to be the cause of powdery mildew on hemp (Schoener & Wang 2018; Gwinn et al. 2019; Ocamb & Pscheidt 2019; Szarka et al. 2019; Weldon et al. 2020). In these published studies, the ITS1–ITS4 region of rDNA was used to identify the pathogen. Based on the homology to sequences previously deposited in GenBank®, three species with 100 % sequence homology to each other, have been proposed to infect cannabis and hemp plants: *G. ambrosiae*, *G. spadiceus*, and *G. chicoracearum*. Further distinction among these species will require additional molecular markers other than ITS and phylogenetic analysis.

Currently, there are several products registered for use on cannabis in Canada to manage powdery mildew. These include wettable and vaporized sulphur, MilStop (potassium bicarbonate), ZeroTol (hydrogen peroxide), Regalia Maxx (an extract of giant knotweed), and Lacto-San (lactic acid) (Health Canada 2019.) Actinovate was also previously registered but has since been deregistered. Comparative efficacy studies utilizing these products for powdery mildew control on cannabis have not been previously conducted. Our results confirm that MilStop and Regalia Maxx were the most effective at reducing powdery mildew infection and spread when applied weekly. Biocontrol products such as Rhapsody ASO and Stargus (both containing *Bacillus* spp.) also suppressed powdery mildew to some degree. Lower efficacy was observed with other products tested, such as boric acid, Silamol, ZeroTol, and Actinovate. While the fungicide Luna Privilege SC 500 was the most effective product in reducing disease

development, it is not registered for use on cannabis in Canada or in the United States and was included in this study for comparative purposes. In a recent study, Betz & Punja (2020) demonstrated that Regalia Maxx effectively controlled powdery mildew development on wasabi plants (caused by *Erysiphe cruciferarum*), while Rhapsody also reduced disease progression to a lesser extent and Actinovate was not effective. On cucumber plants, Ni & Punja (2020b) demonstrated that weekly applications of Rhapsody reduced powdery mildew development (caused by *Podosphaeria xanthii*) to the same extent as the fungicide Luna Privilege SC 500.

The active compound in Luna Privilege SC 500 (fluopyram) is a systemic fungicide that has been shown to control both powdery mildew and leaf spot on tart cherry (Proffer et al. 2012), with preventative and moderately curative effects on gray mould caused by *Botrytis cinerea* on strawberry (Veloukas & Karaoglanidis 2012) and on table grapes (Vitale et al. 2016). One application of Luna Privilege SC 500 significantly reduced powdery mildew development on treated cannabis plants in all three trials in this study. It could be an effective treatment to manage powdery mildew on stock (mother) plants to prevent subsequent spread of the pathogen during vegetative propagation. Product registration for use on cannabis is still required in Canada.

Regalia Maxx is a formulated extract of giant knotweed (*Reynoutria sachaliensis*) that has been shown to induce systemic resistance in plants, resulting in the production and accumulation of phytoalexins, phenolic compounds (Daayf et al. 1997, 2000) and flavonoids (Fofana et al. 2002; McNally et al. 2003). Increased activity of pathogenesis-related proteins such as glucanase, chitinase, and peroxidase has also been reported (Schneider & Ullrich 1994). Regalia was previously shown to be effective in reducing powdery mildew development on a number of crops, including cucumber (Fofana et al. 2002; Rur et al. 2018), tomato (Konstantinidou-Doltsinis et al. 2006), squash (Zhang et al. 2016), and wasabi (Betz & Punja 2020). When tested on cannabis, Regalia Maxx reduced powdery mildew development in all three trials, showing efficacy under a range of disease pressures. The mechanism of action may include production of the previously mentioned biochemical compounds, but this requires further study. Scanning electron microscopic observations showed that Regalia-treated leaves had no visible development of mycelium on the leaf surface. This may be the result of a combination of fungitoxic and induced resistance mechanisms.

It was observed that applications of Regalia Maxx caused a dark green appearance on leaves and significantly increased chlorophyll *a* levels were observed although untreated and Regalia Maxx treated plants had similar chlorophyll *a*/*b* ratios. The enhanced chlorophyll levels

may provide a benefit to the plants, as powdery mildew infection is known to decrease photosynthesis levels in infected leaf tissues (Gordon & Duniway 1982; Balkema-Boomstra & Mastebroek 1995).

Formulated potassium bicarbonate products, including MilStop and Armicarb®, act as fungitoxic compounds through a range of mechanisms, including altering the pH of leaf and fruit surfaces and osmotic pressure, which in turn inhibit the development of fungal mycelium and spores (Mitre et al. 2018). Potassium bicarbonate products have been reported to be effective at controlling apple scab and powdery mildew on apples (Jamar et al. 2008; Mitre et al. 2018) and significantly reduced the incidence and severity of powdery mildew on gooseberry (Wenneker 2016). Moyer & Peres (2018) demonstrated that when applied to gerbera daisies to control powdery mildew, MilStop was effective at reducing disease, and depending on the cultivar of daisy, it was comparable to treatments of systemic fungicides. Scanning electron microscopic observations of MilStop treated cannabis leaves showed significantly reduced mycelial development compared to the control, with some evidence of plasmolyzed cells that may be the result of osmotic or pH changes.

Two *Bacillus* spp. products were tested to evaluate their efficacy at managing powdery mildew on cannabis plants. These were Rhapsody ASO, which contains *Bacillus subtilis* strain QST 713, and Stargus, which contains *Bacillus amyloliquefaciens* strain 727. *Bacillus* spp. are known to produce many antifungal compounds, including the lipopeptides iturins and fengycins (Romero et al. 2007; Siahmoshteh et al. 2018; Ni & Punja 2019), proteases (Takami et al. 1989; Degering et al. 2010), and siderophores (Shoda 2000), which act collectively to reduce pathogen development. Additionally, application of these bacteria induces host resistance that can suppress subsequent infection by pathogens (Ongena et al. 2005; Lahlali et al. 2013) as well as enhance plant growth (Kloepper et al. 2004; Pérez-García et al. 2011). *Bacillus* based products have also been shown to be effective in managing powdery mildew, particularly when plants are treated preventatively, or if it is used in combination with systemic fungicides (Keinath & DuBose 2004; Romero et al. 2007; Gilardi et al. 2008; Elmhirst et al. 2011; Ni & Punja 2019b). Scanning electron microscopic observations of treated cannabis leaves showed that Rhapsody applications reduced mycelial growth to some extent, but visible development of the pathogen still occurred and spores were produced.

Neem oil from the evergreen tree *Azadirachta indica* is known to contain an array of antifungal compounds, with the triterpenoids present acting in a synergistic or additive manner in

order to achieve their antifungal effects (Govindachari et al. 1998). When neem oil fractions were purified and single triterpenoids were tested against a range of common plant pathogens, such as *Fusarium oxysporum* and *Alternaria tenuis,* there was a loss of effect (Govindachari et al. 1998). When applied to whole plants, neem products had a significant effect on reducing powdery mildew disease development on rose (Pasini et al. 1997), pea (Patil et al. 2017; Vivek et al. 2017; Deshmukh et al. 2018), okra (Moharam & Obiadalla 2012), and other crops. Our results are consistent with previously published reports that show that neem oil or neem extracts are effective at reducing powdery mildew development.

The use of ultraviolet light (UV-C and UV-B) to reduce pathogen and mold development in agricultural production has been previously described, including for management of post-harvest decay of strawberries, potatoes, and tomatoes (Liu et al. 1993; Stevens et al. 1999; Nigro et al. 2000). As well, UV light has been used for management of powdery mildew on crops such as roses, strawberries, cucumbers, and tomatoes (Willocquet et al. 1996; Suthaparan et al. 2012; Suthaparan et al. 2014; Suthaparan et al. 2016) and as a seed treatment to reduce fungal infection (Siddiqui et al. 2011). The CleanLight Pro unit, which uses UV-C light, was effective at reducing powdery mildew development in both trials conducted, with few powdery mildew colonies developing once treatment was started. Previous studies suggest that the efficacy of UV light in managing diseases is due to both its direct germicidal activity as well as its indirect ability to induce defence responses in plants, resulting in increased levels of phenolic compounds and pathogenesis-related proteins (Douillet-Breuil et al. 1999; Bonomelli et al. 2004; Hijnen et al. 2006). Scanning electron microscopic observations of treated cannabis leaves showed that mildew spores had not germinated, and mycelial growth was inhibited, suggesting a direct toxic effect. The application of UV-C light could be done on a larger scale in current cannabis production facilities through the use of mounted booms or in-row units which are available commercially and utilized for other crops (Gadoury 2019).

The use of boron to manage diseases as a soil amendment, as well as a foliar application, has been previously investigated. Boron applied to soil was reported to significantly reduce the development of clubroot on canola in field trials (Deora et al. 2011). However, when applied as a foliar treatment in the form of boric acid to field grown potatoes, it did not have a significant effect on late blight and was ineffective against *Phytophthora infestans* growth based on ED_{50} data (Frenkel et al. 2010). Boric acid as a foliar treatment decreased the severity of late blight in potted greenhouse tomatoes, both on leaves that were treated and in distal leaves, possibly through the induction of phenolic compounds and peroxidase enzymes (Frenkel et al. 2010).

Boric acid as a foliar spray also significantly reduced the number of lesions per flag leaf when used to treat tan spot on wheat (Simoglou & Dordas 2006). Canola plants treated with boric acid had twice the levels of boron in leaves compared to control plants and development of *Sclerotinia sclerotiorum* was significantly reduced (Ni & Punja 2020a). In our study, although applications of boric acid provided a significant reduction in disease in two out of three trials, the low average disease reduction in all three trials (less than 50 %) indicates it is not an effective option for powdery mildew management.

Silicon is known to be effective at managing powdery mildew when applied as an amendment to soil or in nutrient solution as well as a foliar spray (Bowen et al. 1992; Schuerger & Hammer 2003; Guével et al. 2007; Shetty et al. 2011). When applied to the roots, soluble silicon products resulted in increased silica deposition in leaves, especially in the apoplast of epidermal cells as well as induction of host defences. This host response included silica deposition around areas of appressoria penetration, resulting in a reduction in haustoria formation, and an increase in the production of phenolic compounds (Menzies et al. 1991; Samuels et al. 199l; Shetty et al. 2011). When applied as a foliar treatment, plants accumulated considerably lower levels of silicon, suggesting that management of disease from foliar silica products is due to a direct interaction with the pathogen rather than through a plant mediated mode of action (Guével et al. 2007). It is possible that the efficacy of Silamol is partially due to the low pH of the solution, with the rate used in our trials having a pH of 3.2. In two of three trials, Silamol significantly reduced powdery mildew development on cannabis plants when applied as a foliar spray. Silamol was not effective in the first trial due to the higher initial disease levels, suggesting that it is most effective when used as a preventative treatment under low disease pressure. The efficacy of silicon applied as a fertilizer to the roots of cannabis plants on powdery mildew suppression has not been investigated.

There is a limited amount of research on the efficacy of hydrogen peroxide, present in products such as ZeroTol, for disease management. Hydrogen peroxide has been shown to have some efficacy at reducing powdery mildew development on cucumber plants (Hafez et al. 2008) and for post-harvest reduction of *Botrytis cinerea* infection on white pepper fruits (Hafez 2010) as well as inducing resistance to downy mildew in pearl millet (Geetha & Shetty 2002). Hydrogen peroxide may work through two modes of action — it is known to have biocidal effects and may act as an inducer to trigger the hypersensitive response and signal transduction, inducing genes involved in systemic acquired resistance (Allan & Fluhr 1997; Alvarez et al. 1998; Kuźniak & Urbanek 2000; Hückelhoven & Kogel 2003). Our results showed that ZeroTol was partially

effective in reducing powdery mildew development on cannabis plants but was not as effective as some of the other treatments described above.

Previous research has shown Actinovate AG to be effective at reducing powdery mildew disease severity compared to unsprayed controls, but it was ineffective compared to a water control on grape (Janousek et al. 2009), and compared to both water and unsprayed treatments, it was ineffective at lowering disease incidence. On squash and cantaloupe, Actinovate alone was not found to reduce powdery mildew severity compared to a water control (Zhang et al. 2011). The efficacy of Actinovate is most likely due to antibiotic and chitinase production by *Streptomyces* spp. as well as induction of plant defenses following application (Conn et al. 2008; Vurukonda et al. 2018). However, in the present study, Actinovate did not provide significant disease reduction.

Genetic resistance has been one of the most effective approaches for powdery mildew management on a wide range of crops. The genetic basis of powdery mildew resistance is in part based on R-genes, known to be present in crops such as tomato, cucumber, wheat, hops, melon, and numerous others (Haung et al. 2000; Bai et al. 2003; Sakata et al. 2006; Cao et al. 2011; Ning et al. 2014; Wolfenbarger et al. 2014). The mechanistic basis for resistance of plant tissues to powdery mildew infection is complex, and numerous changes in gene expression and biochemical compounds are described (Qiu et al. 2015). Among the 12 cannabis strains that were tested for their susceptibility to powdery mildew in this study, it was observed that seven showed partial or complete resistance to infection. This qualitative rating may suggest that one major gene for resistance is present. Differing levels of susceptibility to powdery mildew infection have also been observed among hemp cultivars (Cala et al. 2019). The basis for this resistance in cannabis and hemp is not yet known. The isolation and cloning of R-genes in the *C. sativa* genome to powdery mildew resistance is needed to enhance breeding of cannabis strains with disease resistance.

This research represents an initial investigation into the management of powdery mildew on cannabis. Further research is needed to determine how potential adjustments to the rates of these products may affect their efficacy, the possible benefits to using products in tandem (alternating Regalia Maxx and MilStop rather than just using MilStop, for example), as well as the effects of spraying these products at different intervals, not just weekly. In addition, further research is needed to establish modes of action of some of the most effective products described in this study.

3.5. Funding

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Chapter 4. Biological Control of *Fusarium oxysporum* Causing Damping-Off and *Pythium myriotylum* Causing Root and Crown Rot on Cannabis (*Cannabis sativa* L.) Plants

4.1. Introduction

Root rot, crown rot and damping off diseases caused by species of *Fusarium* and *Pythium* affect numerous crops throughout the world (Agrios 2005). Cannabis (*Cannabis sativa* L.) is reported to be affected by multiple species of *Fusarium* and *Pythium*, including *F. oxysporum*, *F. solani*, *F. proliferatum*, *P. myriotylum*, *P. dissotocum* and *P. aphanidermatum* (Punja and Rodriquez 2018; Punja et al. 2018; Punja 2020a; Punja 2020b). These pathogens infect cannabis plants grown indoors as well as in greenhouse and field production. Symptoms of *Fusarium* infection on plants include stunting, chlorosis, wilting, root rot and crown rot (Punja 2020b). *Fusarium* can also cause damping-off on cannabis cuttings, characterized by symptoms of soft, discolored regions on stems (Punja 2020b). Infections can advance rapidly, causing cuttings to collapse and die. *Pythium* infection on cannabis plants causes root and crown rot, stunting, chlorosis, wilting and death (Punja and Rodriquez 2018). These symptoms may be especially severe under conditions of extreme heat, which can cause plants to rapidly wilt and die, resulting in considerable losses for producers.

Current management practices for *Fusarium* and *Pythium* species rely on cultural control methods, such as avoiding overwatering, utilizing well draining growing media, sanitizing tools and equipment, and ensuring plant materials and irrigation sources are free of pathogen inoculum. There are no traditional fungicides currently registered by the Pest Management Regulatory Agency in Canada to manage these pathogens. However, several biological control products are registered for management of root and crown rot caused by *Fusarium* and *Pythium*. These include products containing fungi such as *Trichoderma* spp. (Rootshield®, Asperello T34 and Trianum), *Gliocladium catenulatum* (Lalstop®) or actinobacteria such as *Streptomyces lydicus* (Actinovate® SP). No *Bacillus* spp. are currently registered to manage either of these pathogens on cannabis. Since the efficacy, growth and survival of biological control agents are known to be affected by environmental factors (temperature, moisture, nutrients), application timing (preventative vs. curative), the presence of other microbes, the target pathogen and intrinsic factors of the biocontrol agent itself, comparisons made under identical experimental conditions are required

for an assessment of efficacy (Bae and Knudsen 2005; Bonaterra et al. 2007; Bardin et al. 2015; Fedele et al. 2019). At the present time, data demonstrating the comparative efficacy of these products to manage *Fusarium* and *Pythium* in cannabis production is lacking.

The objective of this study was to evaluate the efficacy of five biological control products in reducing infection of cuttings by *F. oxysporum* as well as root rot caused by *P. myriotylum* on cannabis plants. These products were Asperello (*Trichoderma asperellum*), Rootshield Plus WP (*Trichoderma harzianum* and *Trichoderma virens*), Lalstop (*Gliocladium catenulatum,* Prestop) and Stargus (*Bacillus amyloliquefaciens*). Rhapsody ASO (*Bacillus subtilis*) was also tested , but only against *F. oxysporum*. The extent to which the biocontrol agents colonized cuttings internally was also studied to evaluate the endophytic capability of *Trichoderma* spp. and *G. catenulatum.*

4.2. Materials and Methods

4.2.1. Pathogen Isolation

Symptoms of damping-off on cuttings caused by *Fusarium* spp. include chlorosis, wilting and soft and discolored stem tissues, resulting in collapse and death (Fig. 4.1A, B). Mycelium may develop on the affected stems (Fig. 4.1C). Symptoms on more mature plants include stunting, yellowing and occasionally wilting (Punja 2020b). To recover *Fusarium* from these tissues, affected cuttings and roots were surface sterilized by immersing tissues in a 10% bleach solution (Javex, containing 6.25% NaOCl) for 30 s followed by a 30 s rinse in sterile distilled water. Tissues were dried on sterile paper towels and small segments were plated onto potato dextrose agar containing streptomycin sulfate at 140 mg L^{-1} (PDA+S). Petri dishes were incubated under ambient laboratory conditions (temperature range of 21-24^{o} C with fluorescent lighting) for 7-10 days and colonies resembling *Fusarium* were transferred to fresh PDA+S. They were identified based on the morphology of colonies and spores, followed by PCR of the ITS1 and ITS4 region of rDNA as described by Punja et al. (2018). For recovery of *Pythium* spp., root samples from plants with symptoms of stunting, chlorosis and root and crown rot (Fig. 4.2) were surface sterilized as described above and colonies resembling *Pythium* spp. were subcultured and identified using the ITS primers as described above. All cultures were maintained on PDA+S.

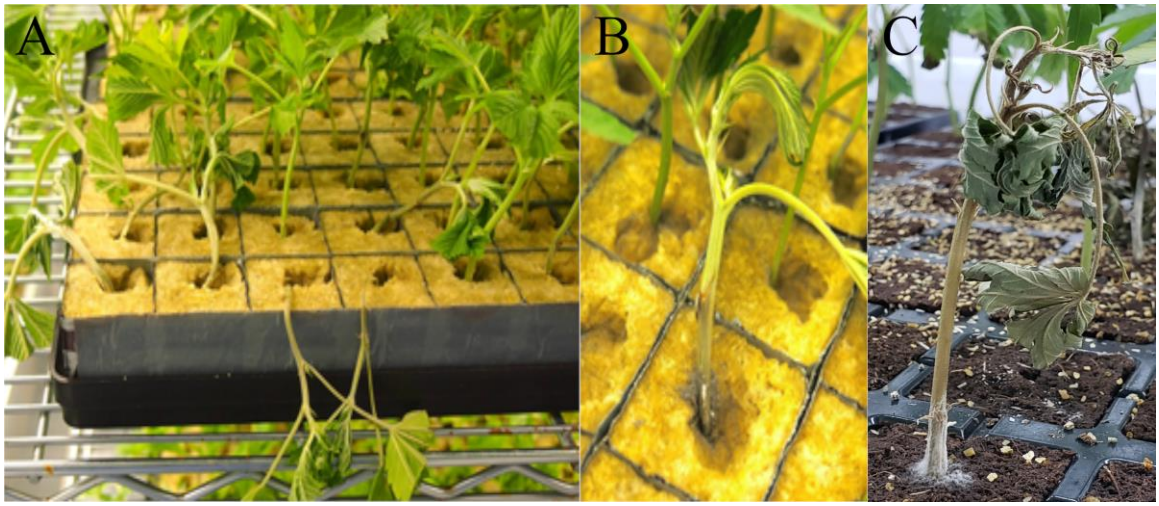

Figure 4.1 **Symptoms of damping off on cannabis cuttings caused by *Fusarium oxysporum* grown in rockwool cubes (A, B) and in peat medium (C).**

Figure 4.2 **Symptoms of *Pythium* infection on cannabis plants. (A) Brown necrotic roots seen on the underside of a coco coir block. Plants were in the vegetative stage of growth.**

4.2.2. Inoculum Production

A mycelial plug of *F. oxysporum* was added to 100 mL of potato dextrose broth (PDB) in 250 mL Erlenmeyer flask and cultures were shaken at 150 rpm for 7 days under ambient laboratory conditions. The contents of the flask were mixed with autoclaved deionized water (1:1, v/v) and blended for 20 sec in a Waring blender. To determine the concentration of *F. oxysporum* inoculum, serial dilutions were made and plated onto PDA+S plates. After 5-7 days, colonies of *Fusarium* were identified based on their morphology and counted. The average concentration of inoculum for the three trials was 7.8 x 10^7 cfu/mL. *Pythium* inoculum was produced by growing cultures in 150 mL of half-strength PDB in 250 mL Erlenmeyer flasks at 150 rpm for 7 days. Cultures were blended for 20 sec in a Waring blender before use. To determine the concentration of *P. myriotylum* inoculum, serial dilutions were made and plated onto PDA+S plates. After 5-7 days, colonies of *Pythium* were identified based on their morphology and counted. The average concentration of inoculum used was 3.25 x 10^4 cfu/mL.

4.2.3. *Fusarium* Inoculation and Disease Assessment

The cannabis strain 'White Rhino' was selected as it is susceptible to *Fusarium* infection. Stock plants were used as a source of vegetative cuttings. These cuttings were approximately 10-15 cm in height and were inserted into 4 cm Grodan rockwool blocks arranged inside plastic containers, with 6 cuttings per container. Treatments included Rootshield WP Plus, Asperello, Lalstop, Stargus and Rhapsody ASO and were prepared as per the rates indicated in Table 4.1. Suspensions (500 mL) of each biocontrol agent were added to the plastic containers and left for 30 min, after which the excess liquid was drained, and containers were placed on a plastic tray and covered with a dome to maintain high humidity. Controls received an equal volume of sterile distilled water. The trays were placed in a growth chamber (Conviron Adaptis, Winnipeg, MB) set at 24o C under a 24-hr photoperiod provided by 24 watt 6400 k T5HO lamps (Sunblaster, Langley, BC) for 48 hr. After this period, *Fusarium* inoculum was applied with a pipette to deliver 2 mL at the base of each cutting. The trays with domes were returned to the growth chamber. Cuttings were assessed for disease symptoms after 7 and 14 days. Ratings were made using a scale of 0-4, with 0 = no symptoms, 1 = minor chlorosis, 2 = moderate chlorosis of leaves and/or slight wilting 3 = obvious wilting and/or extensive chlorosis, visible mycelial growth up stem of cutting, 4 = extreme wilting, chlorosis and/or necrosis. The experiment was conducted three times for a total of 18 cuttings per treatment. The disease severity ratings for each treatment were averaged across the three trials, for each time of assessment. The data were analyzed using

ANOVA followed by a *post hoc* Tukey's HSD test at $P < 0.05$ to determine if there were significant differences.

4.2.4. *Pythium* Inoculation, Disease Assessment and Plant Growth Measurements

For experiments involving *Pythium*, the cannabis strain 'Island Honey' was used. Cuttings were dipped in Remo Roots (Maple Ridge, BC) rooting hormone and placed in a T24 Turboklone (Reno, Nevada, USA) aeroponic cloning system provided with a humidity dome that was misted with water. The Turboklone was placed under two 54 watt 6400 k T5HO Sunblaster lights with a 24-hr photoperiod. The reservoir of the Turboklone contained Rapid Start (General Hydroponics, Santa Rosa, California, USA) at a rate of 0.25 ml/L and approximately 7.5 L of tap water adjusted to a pH of 5.8-6.2 using Advanced Nutrients pH-Down (Advanced Nutrients. West Hollywood, CA, USA). When cuttings had rooted and hardened off (approximately 14 days after being placed in the Turboklone units), they were potted in a 3:1 mix of coco (Canna Coco) and perlite (Dutch Treat) in 8.5 cm^2 pots. Plants were placed in trays, with each treatment having its own tray, and were watered with a nutrient solution containing 1 ml/L Sensi Grow Coco pH Perfect A+B (Advanced Nutrients, West Hollywood, CA, USA) and 1 mL/L General Hydroponics Calimagic (General Hydroponics, Santa Rosa, CA, USA) adjusted to a pH of 5.8–6.2 using Advanced Nutrients pH-Down prior to addition of the biocontrol treatments. Subsequently, plants were watered as needed with the same solution. The biocontrol treatments included Rootshield Plus WP, Asperello, Lalstop and Stargus (Table 4.1). Each plant received 25 mL of the biocontrol agent, with control groups receiving an equal volume of water. Treatments were applied 7 days in advance of the addition of *P. myriotylum* inoculum, with the exception of one of the Stargus treatment groups, where the biocontrol was applied 2 days (Stargus 2d) before the addition of *Pythium* rather than 7 days prior (Stargus 7d). After treatment, all plants were placed inside a growth chamber (Conviron Adaptis, A1000 model) set at 30° C with a 24-hr photoperiod provided by four 21w 4100k bulbs After one week, a scalpel was used to wound the roots of the plants, by inserting it into the growing medium approximately 3 cm away from the stem and penetrating 2-3 cm deep. A volume of 20 mL of the blended *Pythium* inoculum was poured into each pot, with the exception of the negative control.

Plants were assessed for disease severity at 7, 14 and 18 days after inoculation, using a scale of 0-5, where 0 = no symptoms, 1 = minor chlorosis, 2 = moderate chlorosis, plants appear stunted, 3 = severe chlorosis, plants appear stunted and/or wilted, 4 = extreme chlorosis, stunting

and/or or wilting is pronounced, 5 = plants wilted and dead. In addition, at each assessment time, the height of each plant was measured. When the trial concluded at 18 DPI, the shoots of plants were removed from the roots by cutting them at the crown and weighed. Excess growing medium was removed from roots by shaking them by hand and then rinsing thoroughly in water. The length of each root mass was then measured from where roots started on the stem to where the majority of roots ended. Roots were left on paper towel to air dry for approximately 30 min and then weighed. The experiment was repeated 3 times, to provide 18 plants per treatment. All disease severity scores and plant growth measurements (shoot height, shoot weight, root length and root weight) were averaged for each of the treatments and time points. To determine statistical differences between these means, values were compared using ANOVA followed by a *post hoc* Tukey's HSD test at $P < 0.05$.

4.2.5. Endophytic Colonization of Cuttings

Cuttings of strain 'Moby Dick' or 'White Rhino' were inserted into 4 cm Grodan rockwool blocks arranged in a plastic container and 500 mL of Rootshield, Asperello or Prestop was added (as per rates in Table 4.1). After 30 min, the excess solution was poured out and the containers were placed onto individual trays, covered by a humidity dome, and placed in a Conviron Adaptis growth chamber set at 24° C. Control cuttings received an equal volume of water. Plants received 24 hr of light provided by a Sunblaster brand 24 watt 6400 k T5HO lamp. After 2 or 7 days, cuttings were removed and their foliage was trimmed. The stems were surface sterilized as previously described. Each stem was then sectioned into three segments (0-5, 5-10 and 10-15 cm) and two pieces of tissue from each section were plated on PDA+S. The recovery of fungal colonies was recorded after 7 days of incubation under ambient laboratory conditions.

The trial was conducted twice per time point (2 and 7 DPI) and per cannabis strain utilized, for a total of 24 samples (12 plants) per segment for each treatment. To test for statistical significance between the mean percent recovery values of different treatments in the same segment (i.e. all of the treatments in the 0-5 cm segment), means were compared using ANOVA followed by a *post hoc* Tukey's HSD test at $P < 0.05$. Comparisons were made using a repeated measures ANOVA followed by a *post hoc* Tukey's HSD test at $P < 0.05$ to test for differences in mean percent recovery of endophytic fungi of the same treatment in different segments (Rootshield colonization at 0-5 cm compared to 5-10 cm, for example). The cannabis strains were analyzed separately, and all comparisons were made within the same time point (2 DPI or 7 DPI).

Table 4.1 Products evaluated for management of *F. oxysporum* causing damping-off and *P. myriotylum* causing root and crown rot on cannabis.

Product	Active Ingredient	Source	Rate Used	Application Method
Asperello®	*Trichoderma asperellum* strain T34.	Biobest Ltd. Ontario, Canada	1 g/L	Drench
Lalstop® (Prestop®)	*Gliocladium catenulatum* strain J1446.	Danstar Ferment AG. Zug, Switzerland.	5g/L	Drench
Rhapsody ASO™	QST 713 *Bacillus subtilis*.	Bayer CropScience Inc. New Jersey, USA	15 mL/L	Drench
Rootshield® Plus WP	*Trichoderma harzianum* strain KRL-AG2. *Trichoderma virens* strain G-41.	BioWorks Inc. New York, USA	.45g/L	Drench
Stargus®	96.4 % *Bacillus amyloliquefaciens* strain F727 cells and spent fermentation media.	Marrone Bio Innovations. California, USA	15 mL/L	Drench

4.3. Results

4.3.1. *Fusarium* Inoculation and Disease Assessment

The mean disease severity values due to *Fusarium* infection for all biocontrol treatments except Rhapsody were significantly ($P < 0.05$) lower than the inoculated control at 7 days after inoculation (Fig. 4.3A). At 14 days, Lastop treated cuttings had the lowest disease rating while Rootshield, Asperello and Stargus provided intermediate, but still significant, protection (Fig. 4.3B). Cuttings treated with the biocontrol agents appeared greener and more vigorous compared to those inoculated with *Fusarium* only, although chlorosis and symptoms on foliage were still evident on all cuttings, especially as the experiment progressed (Fig. 4.4). Mycelial growth and the incidence of lesions on the stems of cuttings treated with Lalstop, Rootshield and Asperello

was also reduced compared to the control cuttings, where mycelium grew up the stems, resulting in the death.

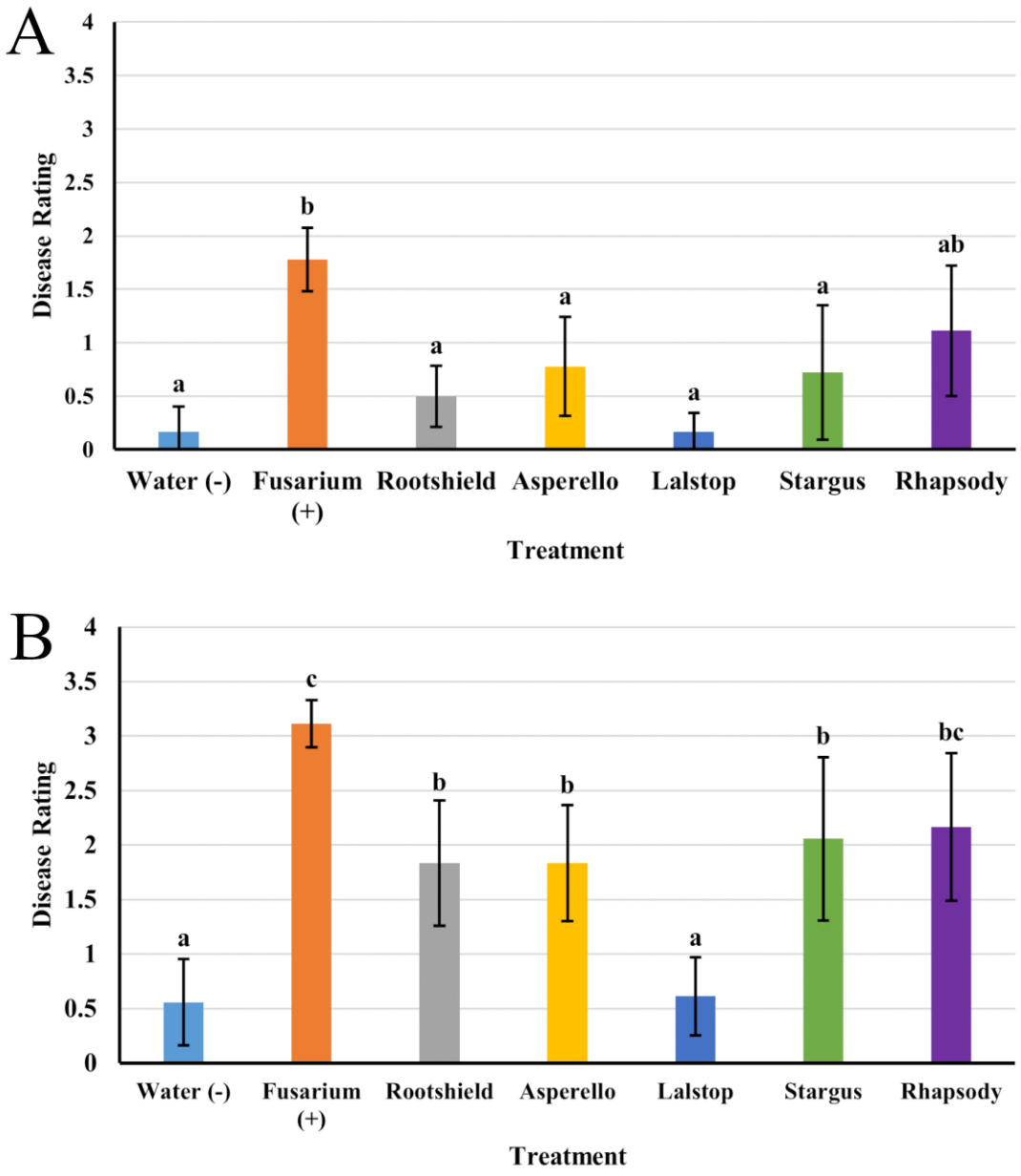

Figure 4.3 **Efficacy of five biological control agents in reducing disease severity on cannabis cuttings due to *F. oxysporum*. Disease was assessed at 7 days (A) and 14 days (B) after inoculation. Data are the means from three experiments with 6 plants per treatment (n=18). Different letters above each bar denote significant differences in the disease severity ratings determined through ANOVA and Tukey's post hoc test ($P < 0.05$). Error bars represent 95% confidence intervals.**

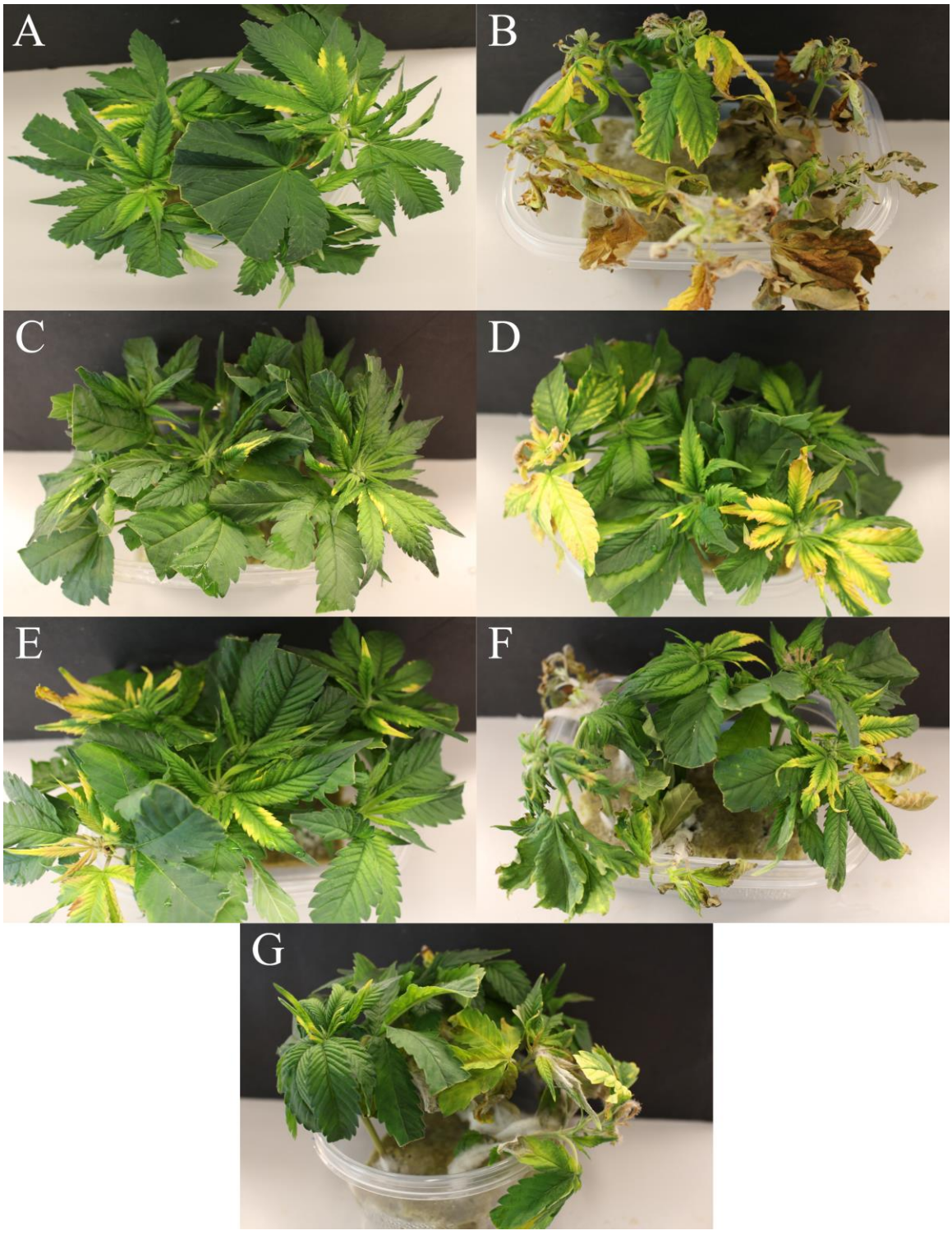
A
B
C
D
E
F
G

Figure 4.4 **The appearance of cannabis stem cuttings following treatment with five biological control agents applied 48 hr prior to inoculation with *F. oxysporum*. A) Noninoculated control. Some yellowing can be seen due to senescence. B) *Fusarium* inoculated cuttings showing colonization of stems and necrosis and yellowing and mycelial growth of the pathogen. C) Lalstop treatment appearing similar to the noninoculated control. D) Rootshield treatment showing some yellowing of the foliage due to *Fusarium*. E) Asperello treatment showing some yellowing of leaves. (F) Stargus treatment showing yellowing, necrosis and mycelial growth of the pathogen. (G) Rhapsody treatment showing necrosis and extensive mycelial growth.**

4.3.2. *Pythium* Inoculation and Disease Management

The application of biocontrol drenches had varying levels of efficacy against *Pythium* root rot. Stargus applied at 2 days prior to *Pythium* inoculation provided the greatest reduction in disease rated at 7 days (Fig. 4.5). At 14 days, none of the biocontrol treatments had a mean disease severity rating that was significantly lower than the *Pythium* control. However, at 18 DPI, Lalstop and Rootshield treatments significantly reduced mean disease severity while the remaining treatments (Stargus 2d, Stargus 7d and Asperello) did not. Plants treated with Lalstop, Rootshield or Asperello had fewer symptoms of chlorosis or wilting and generally appeared more vigorous at 18 days. When the roots of these plants were examined, they had fewer lesions, more feeder roots and the root volume appeared larger. By comparison, roots of the control plants were necrotic, with fewer feeder roots and reduced volume (Fig. 4.6). There was no significant effect on plant height (Table 4.2) or root length (Table 4.3) for any treatment, except for Lalstop, which had mean fresh root weights (Table 4.3) that were equivalent to those of the uninoculated control.

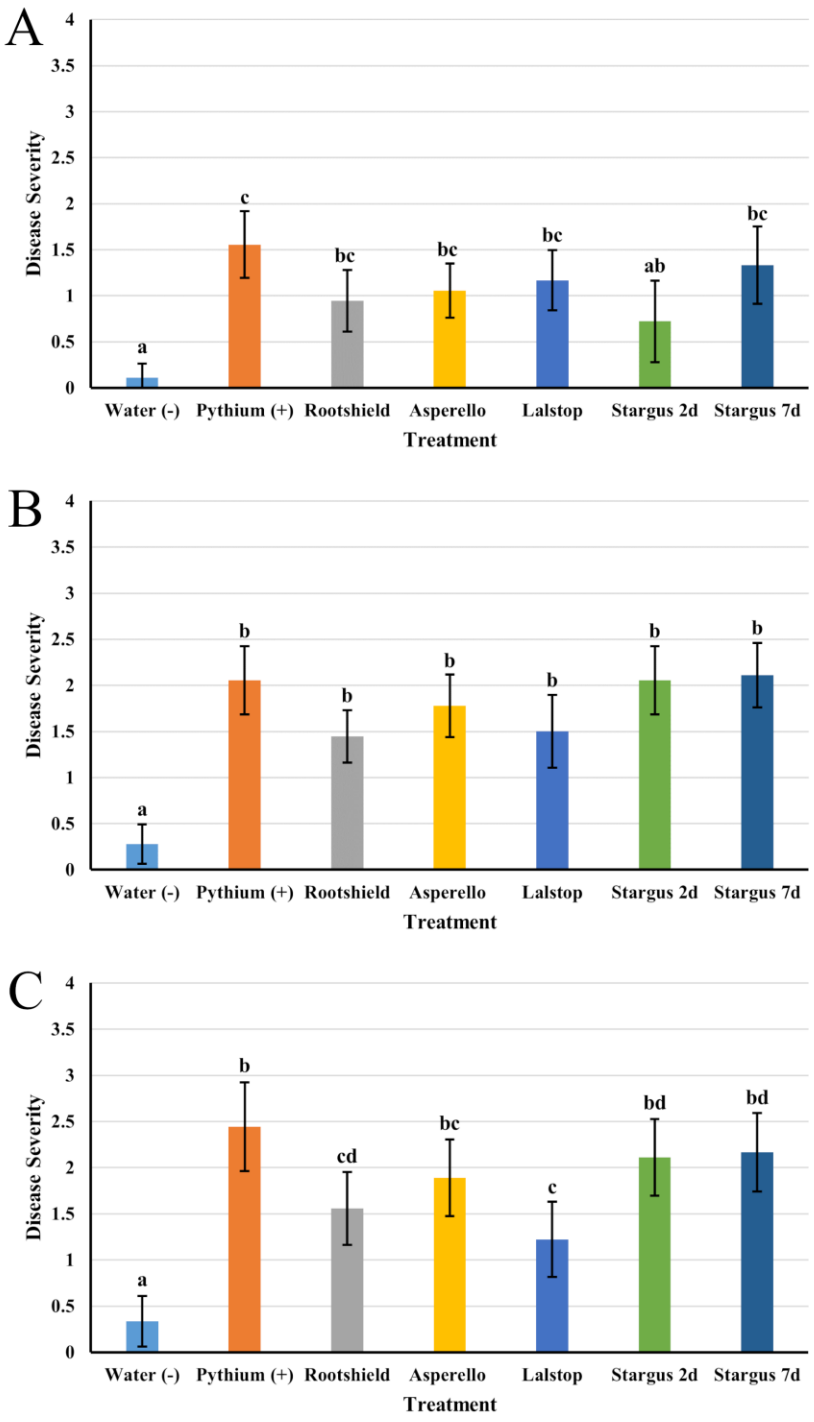

Figure 4.5 **Efficacy of four biological control agents in reducing disease severity on cannabis cuttings due to *Pythium myriotylum*. Disease was assessed at 7 days (A), 14 days (B) and 18 days (C) after inoculation. Data are the means from three experiments with 6 plants per treatment (n=18). Different letters above each bar denote significant differences in the disease severity ratings determined through ANOVA and Tukey's post hoc test ($P < 0.05$). Error bars represent 95% confidence intervals.**

Figure 4.6 **Symptoms of disease caused by *Pythium myriotylum* on cannabis plants with and without application of biocontrol agents. A) Lalstop was applied 7 days before inoculation with *Pythium*. Disease was rated after 18 days of incubation at 30 C. The plant on the left is the pathogen only treatment, the plant on the right received Lalstop. (B) Rootshield was applied 7 days before inoculation with *Pythium*. The root system on the left is from the biocontrol treated plant, the one on the right received the pathogen only. Roots were removed from the pots 18 days after inoculation with *Pythium*.**

Table 4.2 **The effect of four biological control agents applied to cannabis plants 7 days prior to inoculation with *Pythium myriotylum*. Shoot height and fresh weight are shown.**

Treatment	Shoot height (cm)			Shoot fresh weight (g)
	Day 7	Day 14	Day 18	
Noninoculated Control	16.5 ± 1.4 a	34.7 ± 1.8 a	37.4 ± 1.4 a	14.4 ± 1.9 a
P. myriotylum (P)	16.7 ± 1.1 a	29.2 ± 2.2 b	31.5 ± 2.2 bc	11.1 ± 1.8 ab
Asperello + P	15.2 ± 1.1 a	26.6 ± 2.3 b	30.4 ± 2.7 bc	10.9 ± 2.2 ab
Lalstop (Prestop) + P	15.9 ± 1.4 a	28.7 ± 1.6 b	33.5 ± 2.0 ab	13.7 ± 2.2 ab
Rootshield Plus WP + P	15.1 ± 1.3 a	28.4 ± 2.1 b	32.4 ± 2.5 bc	11.8 ± 2.0 ab
Stargus + P (2d)	14.4 ± 1.6 a	25.2 ± 1.8 b	28 ± 2.0 c	9.11 ± 1.6 b
Stargus + P (7d)	16.1 ± 1.8 a	29.1 ± 3.0 b	32.0 ± 2.9 bc	12.1 ± 2.5 ab

Plant heights were measured throughout the experiment while shoot fresh weight was assessed at the end of the experiment. Data are the means from three experiments, each with 6 replications per treatment. Means within a column followed by a different letter indicate significant differences between treatments in the shoot height or weight as determined by ANOVA and Tukey's post hoc test ($P < 0.05$).

Table 4.3 The effect of four biological control agents applied to cannabis plants 7 days prior to inoculation with *Pythium myriotylum*. Root length and fresh weight are shown.

Treatment	Root Length (cm)	Root Fresh Weight (g)
NoninoculatedControl	23.3 ± 2.0 a	14.0 ± 3.1 a
P. myriotylum (P)	19.4 ± 2.8 a	4.2 ± 1.0 b
Asperello + P	20.3 ± 4.2 a	7.0 ± 2.0 b
Lalstop (Prestop) + P	22.5 ± 1.8 a	10.7 ± 1.7 a
Rootshield Plus WP + P	20.2 ± 2.3 a	7.1 ± 1.5 b
Stargus + P (2d)	17.7 ± 3.0 a	6.2 ± 2.1 b
Stargus + P (7d)	19.1 ± 2.3 a	6.8 ± 1.3 b

Root length and root fresh weight were measured at the end of the experiment. Data are the means from three experiments, each with 6 replications per treatment. Means within the column followed by a different letter indicate significant differences in the root length or root fresh weight values of the treatments, as determined through ANOVA and Tukey's post hoc test ($P < 0.05$).

4.3.3. Endophytic Colonization of Cuttings

Stem segments of 'Moby Dick' and 'White Rhino' were colonized by *G. catenulatum* and *Trichoderma* spp. at 0-5 and 5-10 cm distances after 2 and 7 days (Fig. 4.7) (Fig. 4.8). However, colonization was only significant in the 0-5 cm segments at either time.

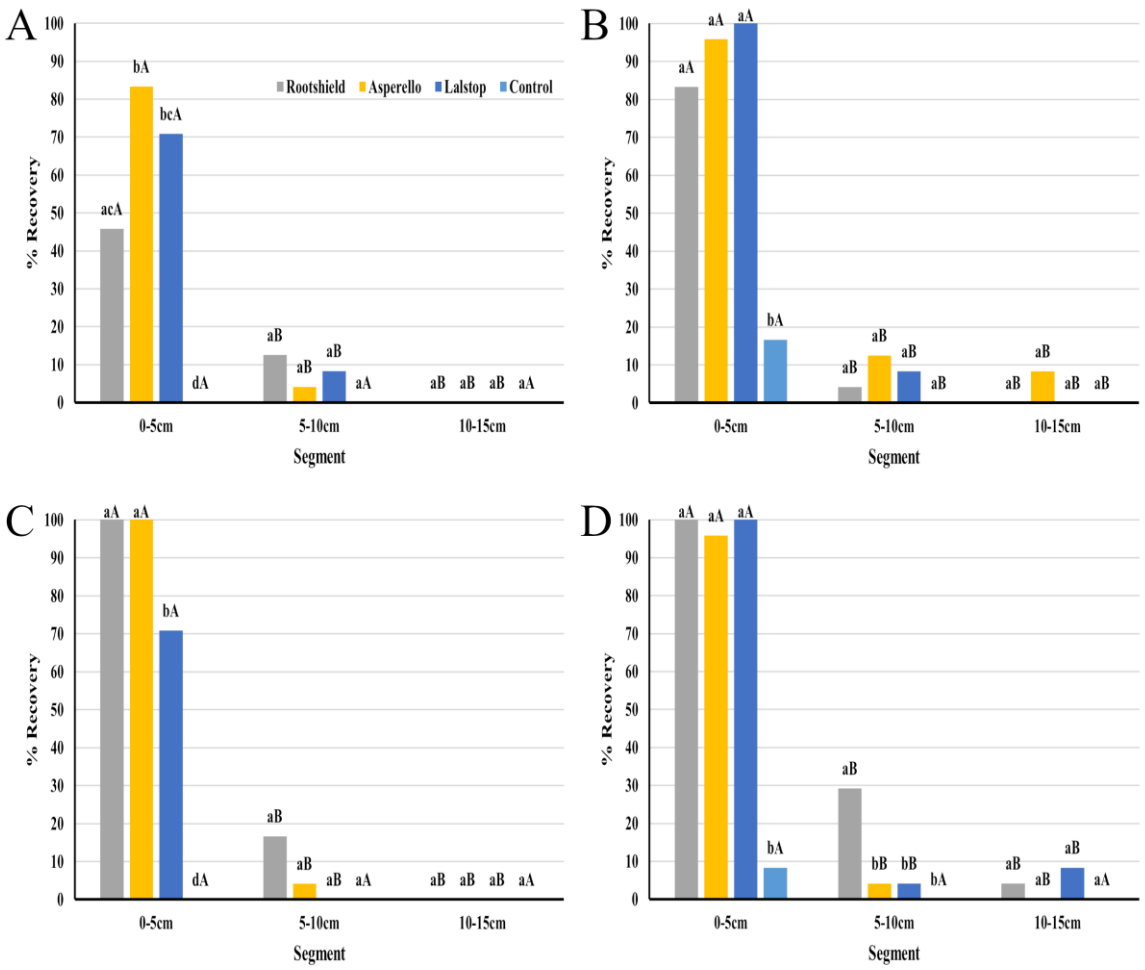

Figure 4.7 **The percent recovery of three biological agents following application to cannabis cuttings as a drench treatment to rockwool followed by incubation for 2 or 7 days. The surface-sterilized stem pieces were divided into 5 cm long segments and plated onto PDA. Percent recovery after 2 days and 7 days are shown for strains Moby Dick (A, B) and White Rhino (C, D), respectively. Data are the means from two experiments, with 6 plants per treatment per experiment. Different letters above each bar denote significant differences in the percent recovery of biocontrol agents as determined through ANOVA and Tukey's post hoc test ($P < 0.05$). Error bars represent 95% confidence intervals.**

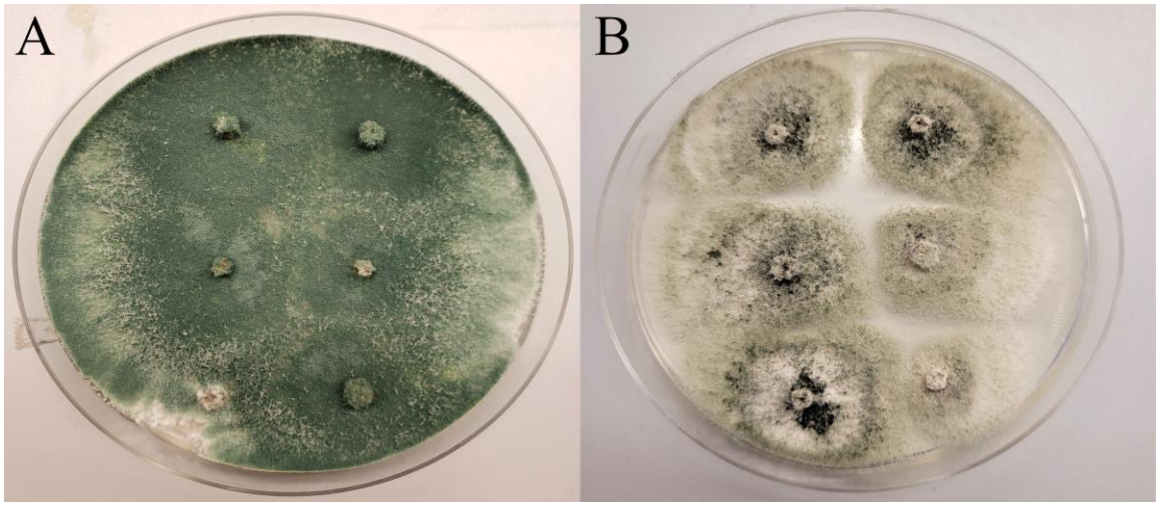

Figure 4.8 **Recovery of two biocontrol agents following application to cannabis stem cuttings as a drench treatment to rockwool followed by incubation for 7 days. The surface sterilized stem pieces from the 0-5 cm distance were plated onto PDA. (A) *Trichoderma harzianum* growing from Rootshield treated cuttings. (B) *Gliocladium catenulatum* growing from Lalstop treated cuttings.**

4.4. Discussion

Damping-off, as well as root and crown rot caused by *Fusarium* and *Pythium* spp., have been reported to occur in several regions of Canada and the US where cannabis and hemp are grown (Punja et al. 2018; Punja and Rodriquez 2018; Gauthier et al. 2019). Currently, producers have limited options for disease management since traditional fungicides are not registered for use on either crop. The Pest Management Regulatory Agency has approved the use of several biological control products in Canada, including Asperello T34, Rootshield Plus WP, Lalstop and Trianum. Our results demonstrated that Lalstop and Rootshield provided the greatest reduction in disease symptoms caused by *Fusarium* and *Pythium* when they were applied 48 hr or 7 days, respectively, prior to pathogen exposure. Asperello and Stargus were also effective in reducing *Fusarium* but were less effective against *Pythium*.

On cannabis cuttings, the inoculum causing initial infection may originate from the environment in which the cuttings are rooted, which is kept warm (24-27^{o} C) and humid (80-90% relative humidity). Spores of *F. oxysporum* have been detected in the air in the rooting rooms, as well as in recirculating water (Punja 2020b). In addition, cuttings may have incipient infections originating from the stock plants, where *F. oxysporum* was shown to be transmitted internally and asymptomatically, likely in the pith and xylem tissues (Punja 2020b). Therefore, biocontrol

agents applied to cuttings should be able to colonize the cut surface, the emerging roots, as well as internally in the pith tissues. The biocontrol agents were applied 48 hr prior to pathogen inoculation to ensure this colonization had occurred. Endophytic colonization, defined as recovery following surface-sterilization of tissues, was observed to occur in the 0-10 cm stem segments of cuttings by *Gliocladium* and *Trichoderma* spp., suggesting that could be one of ways in which disease was reduced i.e., through competitive exclusion of the pathogen.

The active ingredient in Lalstop (previously known as Prestop) is *G. catenulatum* (*Clonostachys rosea* f. *catenulata*) strain J1446 . On greenhouse cucumbers this biocontrol agent was shown to significantly reduce root and stem rot disease severity and seedling mortality caused by *F. oxysporum* f. sp. *radicis-cucumerinum* when applied as a drench 24 hr or 48 hr prior to inoculation with the pathogen (Rose et al. 2003). In addition, on spinach plants, applications of *G. catenulatum* controlled *F. oxysporum* f. sp. *spinaciae* wilt as well as *Fusarium* wilt caused by *F. foetens* on Hiemlais begonia (Tian and Zheng 2013). *Fusarium* crown and root rot on greenhouse peppers, caused by *F. oxysporum,* and other *Fusarium* diseases, were also reduced by preventative Prestop applications (Cummings et al. 2009; Cerkauskas 2017). In the present study, treatment with Lalstop 48 hr prior to pathogen inoculation significantly reduced disease severity on cannabis cuttings due to *F. oxysporum* at 7 and 14 DPI.

Gliocladium catenulatum was also shown to be effective against *Pythium* diseases. Damping-off on American ginseng seedlings, caused by *P. ultimum* and *Fusarium* spp., was significantly reduced when Prestop Mix was used as a seed treatment and as a drench to beds (Rahman and Punja 2007). In both growth chamber and greenhouse trials, Prestop or Prestop Mix drenches significantly reduced *P. aphanidermatum* development on cucumber, lowering percent plant mortality compared to plants treated with the pathogen only (Punja and Yip 2003). In the present study, Lalstop significantly reduced disease severity due to *P. myriotylum* on plants at 18 DPI. Lalstop treated plants also had average fresh root weights that were not significantly different from the uninoculated control.

The rhizocompetence and efficacy of *G. catenulatum* against *Fusarium* and *Pythium* is reported to be due to multiple mechanisms, including production of enzymes such as β-1,3-glucanases that inhibit pathogen growth, mycoparasitic activity, and enhanced rhizosphere and root colonization (McQuilken et al. 2001; Syama et al. 2008; Syama and Punja 2009). Endophytic colonization of cacao and cucumber by *G. catenulatum* was shown to occur (Rubini et al. 2005; Syama et al. 2008).

Trichoderma spp., including those present in Rootshield (*T. harzianum* strain KRL-AG2 and *T. virens* strain G-41) and Asperello (*T. asperellum* strain T34) have been reported to reduce a number of diseases, including *Fusarium* wilt on tomato (*F. oxysporum* f. sp. *lycopersici*) (Larkin and Fravel 1998; Taghdi et al. 2015; Hasan et al. 2020), cucumber (*F. oxysporum* f. sp. *cucumerinum*) (Li et al. 2019) and on field and greenhouse grown strawberry (*F. solani*) (Pastrana et al. 2016). Additionally, diseases such as damping off on pea, tomato, lettuce and root rot of cucumber, tomato and lettuce caused by *Pythium* spp. have been shown to be suppressed by applications of *Trichoderma* spp. (Lynch et al. 1991; Wolffhechel and Jensen 1992; Naseby et al. 2000; Utkhede et al. 2000; Jayaraj et al. 2006; Elshahawy and El-Mohamedy 2019). In the present study, Rootshield and Asperello reduced disease caused by *F. oxysporum* at 7 and 14 DPI, and Rootshield also reduced disease caused by *P. myriotylum*.

Similarly, to *G. catenulatum*, *Trichoderma* spp. are known to act as biocontrol agents through direct interactions with pathogens, such as the secretion of enzymes, antibiosis, mycoparasitism and competition for resources, as well as through interactions and associations with plants. These interactions may include colonization of root tissues internally and externally (Harman et al. 2004). This may in turn induce local or systemic resistance to pathogens and increase plant growth and tolerance to abiotic stresses (Benhamou and Chet 1997; Green et al. 2001; Harman et al. 2004; Druzhinina et al. 2011; Mukherjee et al. 2012; Contreras-Cornejo et al. 2016). The mechanisms by which disease development was reduced in cannabis cuttings is unknown but may involve a number of the above mechanisms.

Bacillus subtilis and *B. amyloliquefaciens* are reported to reduce root rot and damping off caused by soilborne pathogens, including *Fusarium* and *Pythium* spp. (Abdelzaher 2003; Jayaraj et al. 2005; Zouari et al. 2016; Shahzad et al. 2017; Han et al. 2019). In the present study, when applied as a drench to rockwool blocks, *B. amyloliquefaciens* significantly reduced disease severity by *F. oxysporum* at 7 and 14 DPI, but *B. subtilis* had no effect. For *Pythium* management, there was no observable effect due to applications of *B. amyloliquefaciens*. It is unclear why *B. amyloliquefaciens* was not effective against *Pythium* based on the efficacy of *Bacillus* spp. in other previously mentioned studies. *Bacillus* spp. have been reported to colonize plants endophytically, in turn promoting their growth and suppressing disease through a range of mechanisms (Struz et al. 2000; Tan et al. 2013; Zouari et al. 2016; Shahzad et al. 2017). These bacteria may colonize and benefit cannabis plants in a similar manner, but this has yet to be demonstrated.

Further research is needed to evaluate the efficacy of these biocontrol products on a larger scale, over longer periods of time and in a range of different production systems. The effect of repeated applications of these biocontrol products later in the production cycle and the extent of survival of biocontrol agents in the growing media also needs to be evaluated. In the present study, the ability of several biocontrol agents to colonize cannabis stem tissues endophytically was demonstrated. The extent to which they may colonize cannabis tissues over a longer period of time, as well as their potential growth promoting and pathogen suppressing effects, require further research. Our results support the current registration of several biocontrol products for use on cannabis to manage these pathogens.

4.5. Funding

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Chapter 5. Discussion and Future Research

Cannabis plants are affected by numerous diseases which can cause producers significant crop losses. These diseases include powdery mildew caused by *Golovinomyces* spp. and damping-off, as well as root and crown rot caused by *Fusarium* and *Pythium* spp. Currently, there are several products registered for use on cannabis in Canada to manage these diseases. However, comparative efficacy studies utilizing these products for disease control on cannabis have not been previously conducted. Our results confirm that MilStop and Regalia Maxx were the most effective at reducing powdery mildew infection and spread when applied weekly. Biocontrol products such as Rhapsody ASO and Stargus (both containing *Bacillus* spp.) also suppressed powdery mildew to some degree. Lower efficacy was observed with other registered products tested, such as ZeroTol, and Actinovate. Our results also demonstrated that the use of ultraviolet radiation (UV-C) or genetic resistance are effective management strategies for powdery mildew.

The Pest Management Regulatory Agency has approved the use of several biological control products in Canada, including Asperello T34, Rootshield Plus WP, Lalstop and Trianum. Our results demonstrated that Lalstop and Rootshield provided the greatest reduction in disease symptoms caused by *Fusarium* and *Pythium* when they were applied 48 hr or 7 days, respectively, prior to pathogen exposure. Asperello and Stargus were also effective in reducing *Fusarium* but were less effective against *Pythium*. By integrating accurate disease diagnosis, cultural controls the previously evaluated biological controls or reduced risk chemicals producers may effectively manage cannabis diseases.

This research is a preliminary evaluation of the currently available management options for powdery mildew, and *Fusarium* and *Pythium* diseases on cannabis. In order to thoroughly evaluate the efficacy and practicality of these approaches, further research should take place on a larger scale, over entire production cycles, with different timings of treatments and under field or greenhouse conditions. The effect that different production systems and growing medias have on the efficacy of these strategies, especially biocontrol products applied as drenches, requires further work as well. Additional attention should be spent investigating the mechanisms through which these biocontrol agents act. Although in some cases the mode of action of these biocontrols have been demonstrated on other crops, very limited if any work has been done to understand how they may interact in a pathosystem involving cannabis. This includes the ability of biocontrol agents to colonize cannabis tissues internally, the effect this has on disease

management and the different factors that may affect this colonization (strain, environmental factors, the presence of other microbes, etc.).

Whether or not the application of these microbes negatively impacts post-harvest yeast and mold counts, and strategies for post-harvest remediation of cannabis buds, must be evaluated. Electron beam or gamma radiation are currently the standard methods employed for post-harvest treatment of cannabis buds, but these options may not be available or appeal to all producers. This is due to their high costs, potentially undetermined effects on the quality of cannabis buds (smell, taste, appearance, etc.), and that they may not be compatible with organic production systems. Subsequently, the effects of ozone, chlorine dioxide gas or other forms of radiation treatment must be assessed.

Bud rot caused by *Botrytis cinerea*, and to a lesser extent other pathogens, is also a significant concern for producers. Although in Canada there are a limited number of products registered to manage *Botrytis* on cannabis, such as fungal and bacterial biocontrols, data about the comparative efficacy of these products is lacking. Preliminary work may investigate the effects of these products on a smaller scale or on flower tissues removed from plants, but subsequent work should consider the effects of differing production systems, the efficacy of preventative or curative applications and the role that different genotypes may play, in terms of genetic resistance and cannabis flower morphology. However, evaluating these management strategies may prove more challenging than assessing the efficacy of products against other diseases, as flowering cannabis plants are required.

Lastly, the genetic resistance of cannabis varieties should also be evaluated in further detail, for both powdery mildew and other diseases including *B. cinerea*, *Fusarium* spp. and *Pythium* spp. Identifying varieties that are resistant to disease, and the molecular basis of this resistance, may allow for breeding of cannabis strains with resistance to one or more of these pathogens. The genetic basis of resistance has been determined on other crops and may provide a starting point for this investigation on cannabis. Landrace cannabis populations may be a particular source of interest to breeders or researchers hoping to find genetic resistance to these diseases. The genetic engineering of cannabis strains for disease resistance is also being undertaken, although there are numerous challenges still facing this work including the lack of reliable tissue cultures methods for regenerating transgenic cannabis.

Chapter 6. References

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