Pathogen growth inhibition and disease suppression on cucumber and canola plants with ActiveFlower™ (AF), a foliar nutrient spray containing boron

by

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Abstract

The effectiveness of ActiveFlower (AF), a fertilizer containing 3% boron in reducing pathogen growth and diseases on cucumber and canola plants was evaluated. In vitro, growth of *S. sclerotiorum* with AF at 0.1, 0.3 and 0.5 mL/100 mL showed pronounced inhibition at 0.5 mL/100 mL. In greenhouse experiments, the number of powdery mildew colonies on cucumber was significantly reduced by AF at the higher concentrations applied as weekly foliar sprays. On detached canola leaves, AF at 0.5 mL/100 mL and boric acid (BA) at 10 mL/L significantly reduced lesion size of *S. sclerotiorum*. Phenolic content and boron levels in foliage receiving AF applications were significantly increased. There were no significant differences in lignin. These results indicate that boron present in AF contributed to the disease suppressive effect.

Keywords: Sclerotinia stem rot; *Sclerotinia sclerotiorum*; canola; powdery mildew; *Podosphaera xanthii*; cucumber

Dedication

For my Dad and Mom. And for Feng. This work wouldn't have been completed without your unswerving support.

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Table of Contents

List of Tables

List of Figures

List of Acronyms

AF	Active Flower
AFWB	Active Flower Without Boron
CSA	Competitive Saprophytic Ability
CWDEs	Cell Wall Degrading Enzymes
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PM	Powdery Mildew
SSR	Sclerotinia Stem Rot

Chapter 1.

Introduction

1.1. Importance of canola production

Canola (*Brassica napus L.*) is a member of the Brassicaceae family (mustard family), which is the second largest oilseed crop after soybean worldwide (Djaman et al. 2018). In 2017, Canada produced the largest quantities of canola oil (18,362,000 tons), followed by China, India, Australia, Ukraine, Russia and United States (Khan et al. 2017). Canadian canola production primarily occurs in Saskatchewan, Alberta, Manitoba and the Peace River region of British Columbia. Ninety percent of the produced canola is exported as seeds, oil or meals to over 50 markets around the world. Overall canola production reached 21.3 million tonnes in 2017, contributing approximately $26.7 billion to the Canadian economy annually (Statistics Canada 2017; Canola Council of Canada 2017a). Canola oil is crushed from canola seeds, which is the third largest vegetable oil by volume in the world after palm and soybean oil (Lin et al. 2013). It is considered as one of the healthiest edible vegetable oils due to its low saturated fatty acid content (7%) (Lin et al. 2013). High levels of saturated fatty acids can increase the level of harmful cholesterol, increasing the risk of developing coronary heart disease (Lin et al. 2013). Canola oil contains substantial amounts of monounsaturated fatty acids (55%), which help control blood glucose and decrease levels of total cholesterol and low-density lipoprotein cholesterol in the human body (Lin et al. 2013). Crushing canola seeds also produces high-protein canola meal which contains abundant nutrients to feed livestock such as poultry, cattle, fish and swine. Dairy cows fed canola meal have been found to have increased milk production of up to 0.6 to 1.4 L/day (Canola Council of Canada 2017b). Canola oil has recently been used as an alternative to diesel, known as biodiesel, which can significantly reduce the impact of global warming (Ge et al. 2017).

1.2. Sclerotinia stem rot

1.2.1. Disease symptoms

Sclerotinia stem rot (SSR) or white mold caused by *Sclerotinia sclerotiorum* is recognized by the distinct white cottony mycelium on the surfaces of lesions on infected plant tissues (Heffer & Johnson 2007). The pathogen *S. sclerotiorum* can infect all parts of the host plant, including leaves, stems, petioles, roots, and reproductive organs. Symptoms first appear as water-soaked lesions with distinct margins two to three weeks after infection (Figure 1.1). Lesions enlarge irregularly and indeterminately, creating brown to grey discoloration on infected leaves and stems (Paulitz et al. 2015). As the disease progresses, patches of fluffy white mycelium form on the outside and inside of diseased tissues. The host plants wilt quickly and die prematurely once lesions have spread through-out the main stems. Infected tissues can become almost completely desiccated and bleached compared to healthy tissues (Heffer & Johnson 2007). Plants affected by *S. sclerotiorum* tend to produce fewer and smaller seeds compared to uninfected plants (Paulitz et al. 2015). In the later phases of the disease, black-colored sclerotia are commonly found on the surface of infected tissues or inside the stem.

Figure 1.1. Water-soaked lesions caused by *S. sclerotiorum* appear on canola leaves. Photo by Kurt Lindbeck. © State of New South Wales through NSW Department of Industry.

1.2.2. Identification and Biology

Sclerotinia sclerotiorum (Lib.) de Bary belongs to the family Sclerotiniaceae in the order of Helotiales. It is a filamentous ascomycete phytopathogen causing SSR, which is the most prevalent and destructive soil-borne fungal disease worldwide. This fungus is considered to be necrotrophic, as it seizes energy and nutrients mainly from dead plant cells (Heffer & Johnson 2007; Prova et al. 2018). *S. sclerotiorum* is identified by the cottony white mycelium and hard black sclerotia (Hanlin 1998; Kirk et al. 2001). Sclerotia are the survival (overwintering) structures of *S. sclerotiorum*, which are typically 2 to 5 mm in width and no more than 25 mm in length (Heffer & Johnson 2007; Prova et al. 2018). Individual sclerotium consist of a light-colored inner layer called the medulla containing fungal cells abundant in proteins and β-glucans, and a black outer protective coating containing melanin pigments which are highly resistant to degradation (Sharma

et al. 2015). Sclerotia enhance *S. sclerotiorum*'s tolerance to extreme environmental conditions, such as drought, low temperatures and heatwaves (Adams & Ayers 1979; Wang et al. 2008). In nature, sclerotia can survive in soil without plant hosts for up to 10 years under favorable environmental conditions (Heffer & Johnson 2007; Liu et al. 2017).

One to several apothecia, shaped like stalked cups, arise from a black irregular-shaped sclerotium (Figure 1.2). Apothecia are the fruiting bodies of *S. sclerotiorum* which are 5 to 15 mm in diameter and tan in color (Markell et al. 2009; Canola Council of Canada 2017c). Each sclerotium can form up to 15 apothecia from which ascospores are forcibly ejected (Canola Council of Canada 2017c). At the base of the apothecium are sacs called asci, and each ascus can produce eight ascospores (Prova et al. 2018); consequently, each apothecium can discharge up to 30 million ascospores into the air in several days (Sharma et al. 2015). Ascospores are hyaline, measuring 10.2-14.0$\mu \times$ 6.4-7.7μ in size and ellipsoid. They have smooth walls, and each ascospore has eight chromosomes (Sharma et al. 2015). The exterior of an ascospore is covered in sticky mucilage that originates from the cell wall of the asci. The mucilage assists in cementing the spore to host tissues as well as sticking spores together forming clumps (Sharma et al. 2015). Ascospores ejected from the apothecium are transported to susceptible host plants by either air currents or rain, travelling distances from a few centimetres to many kilometers (Sharma et al. 2015; Canola Council of Canada 2017a). In the laboratory, desiccated ascospores can remain viable for years if stored at low temperatures, while fresh ascospores can only survive for 5 to 21 days (depending on relative humidity) after they are discharged from the apothecia (Hunter et al. 1982; Wu 1988; Sharma et al. 2015). Aboveground parts of the plant that become infected by ascospores of *S. sclerotiorum*, will develop severe disease symptoms such as stem rot, fruit rot, flower blight and head blight (Heffer & Johnson 2007).

Figure 1.2. Mature apothecia germinated from the attached black sclerotia of *S. sclerotiorum*. Photo courtesy of Dilantha Fernando, University of Manitoba. Reprinted from Paulitz et al. (2015).

After initial infection, ascospores produce branched filamentous hyphae that are hyaline, multinucleated, thread-like, and thin walled (about 9-18 um in width) (Sharma et al. 2015; Prova et al. 2018). It is the hyphae of *S. sclerotiorum* that causes water-soaked lesions on infected plant tissues via release of toxins (oxalic acid) and cell wall degrading enzymes (CWDEs) (Heffer & Johnson 2007). Pectinolytic enzymes are also produced by *S. sclerotiorum* to macerate plant tissues which indirectly leads to cell membrane damage, eventually causing cell death (Morrall et al. 1972). The hyphae subsequently branch into a white fluffy mycelium, which, over time, aggregates itself into sclerotia. In artificial inoculation studies, both mycelium and ascospores of *S. sclerotiorum* have been used to infect host plants (Whipps et al. 2002; Zhao & Meng 2003). Mycelium is generally the less used inoculum source (compared to ascospores) due to its low competitive saprophytic ability (CSA) (Newton & Sequeira 1972).

Sclerotinia sclerotiorum can be isolated from both plant materials and soil. Generally, *S. sclerotiorum* is isolated from the host plant by collection and desiccation of sclerotia from diseased plant tissues. When sclerotia are collected from the soil, sieves with holes of 0.43-2 mm are used (Pratt 1992). To germinate sclerotia in the laboratory,

they are sterilized by CaOCl or NaOCl, sectioned and then plated on potato dextrose agar (PDA) (Pratt 1992). Steadman et al. (1994) developed a medium of PDA containing streptomycin, penicillin, pentachloronitrobenzene and bromophenol blue for the isolation of airborne ascospores of *S. sclerotiorum*.

1.2.3. Disease cycle

The sclerotia plays a central role in the disease cycle of *S. sclerotiorum* (Figure 1.3). Usually sclerotia of *S. sclerotiorum* germinate carpogenically producing apothecia; under certain conditions they germinate myceliogenically, directly infecting the susceptible host plant (Bardin & Huang 2001; Bolton et al. 2005; Heffer & Johnson 2007; Prova et al. 2018). In spring and summer, when soil is irrigated or moistened by rainfall for one to two weeks, sclerotia close to the soils surface will produce apothecia (Paulitz et al. 2015; Sharma et al. 2015). Sclerotia buried more than 5 cm below the ground remain dormant until they are brought to the surface by tilling (Canola Council of Canada 2017c). Apothecia subsequently produce ascospores which are released into the air and travel to susceptible plants. It is believed that most sclerotinia infections of canola are due to the transmission of these airborne spores (Canola Council of Canada 2017c). With prolonged periods of wetness (16-48 hr) on the leaf surface and cool to moderate temperatures (14-24°C), the likelihood of infection by ascospores is significantly increased (Heffer & Johnson 2007). Vulnerable tissues on plant hosts, such as senescent leaves and flower petals are the first areas to be infected because the ascospores lack sufficient energy to directly infect healthy tissues (Heffer & Johnson 2007). In some cases, ascospores infect plants at the seedling stage through surface wounds caused by mechanical injury or insect damage (Sharma et al. 2015). Host plants are most susceptible to infection at the flowering stage since the germination of sclerotia and production of apothecia requires prolonged wet and cool conditions which typically occur when the crop canopy is dense enough to shade the soil surface (Canola council of Canada 2017c). The fungus derives energy and nutrients from dead leaves and flower petals and the infection is spread as disease-carrying tissues fall and contact leaves and stems. Sclerotia form later inside the dead stems, which eventually fall to the ground and continue the disease cycle. Occasionally, sclerotia germinate myceliogenically by direct formation of hyphae. This form of germination is generally seen on the roots and crowns.

The hyphae penetrate decaying plant tissues and form mycelium on the surface of living tissues (Saharan & Naresh 2008). This type of germination is also favored by wet and cool soil conditions. Formation of hyphae over the flowering period can cause crown rots and basal stem cankers (Heffer & Johnson 2007).

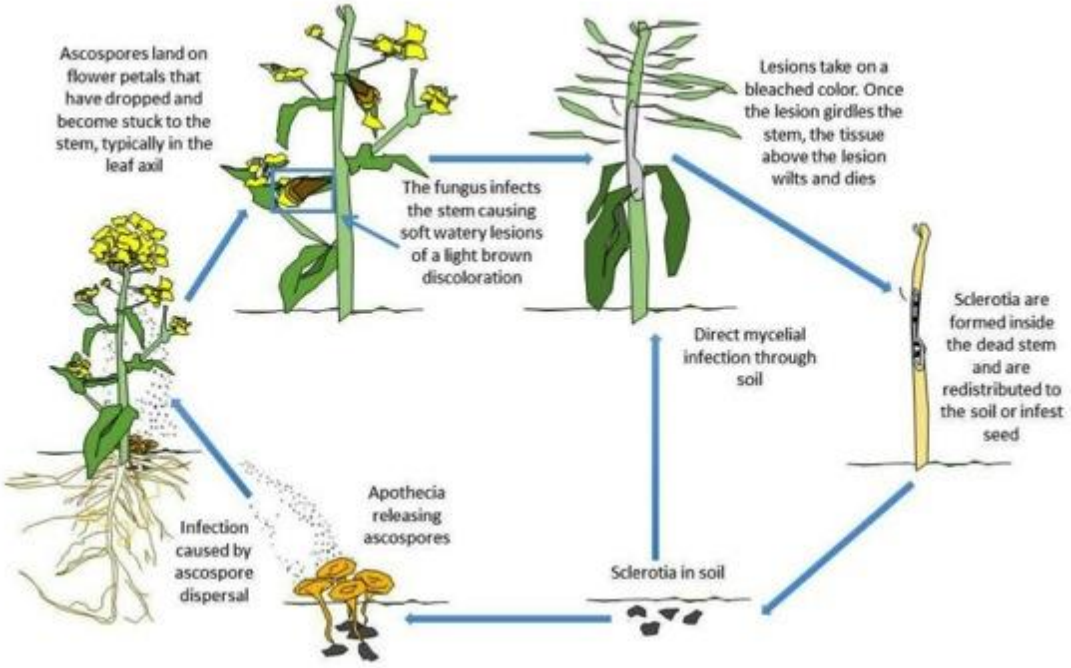

Figure 1.3. Life cycle of SSR. Figure by Tai McClellan Maaz, Washington State University. Reprinted from Paulitz et al. (2015).

1.2.4. Economic importance

Sclerotinia stem rot is one of the most destructive diseases of canola. The pathogen *S. sclerotiorum* has a broad host range, infecting over 400 plant species (Steadman et al. 1994; Kirk et al. 2001). Many economically important crops are susceptible to this pathogen, including oilseed crops (e.g. canola and sunflower) and legumes (e.g. bean, pea and soybean). *Sclerotinia sclerotiorum* mostly infects broadleaf crops (eudicots) and rarely infects monocots such as corn, barley and wheat (Paulitz et al. 2015). *Sclerotinia sclerotiorum* colonizes host tissues aggressively and can spread quickly between individual plants in the field. Infection by *S. sclerotiorum* in canola can result in reduced seed filling and an increase in lodging prior to harvest (Paulitz et al. 2015). The yield losses caused by *S. sclerotiorum* are dependent on a variety of factors,

including canopy density, site of infection, growth stage and lodging potential (Paulitz et al. 2015). When infection occurs at early stage flowering, while environmental conditions are favored by the pathogen, average yield losses can reach more than 50% in fields (Markell et al. 2009; Canola council of Canada 2017c). *S. sclerotiorum* occurs frequently in Canadian Prairies in which climates are relatively cool and moist. It has been found in semi-arid regions as irrigation contributes to the germination of sclerotia (Heffer & Johnson 2007; Sharma et al. 2015). Even in areas with dry summers, canola production was reported to be severely affected by *S. sclerotiorum* (Paulitz et al. 2015). This parasitic fungus can survive as sclerotia in the soil for a long duration, posing a high risk of infection for the following years if susceptible host plants are planted in the field. Host plants are prone to SSR during majority stages of plant growth, including seedling, vegetative and reproductive stage, as well as post-harvest storage. This disease can lead to significant losses in modern intensive cultivation as higher canopy density is likely to provide more sheltering areas for sclerotia (Heffer & Johnson 2007). Therefore, SSR could be a devastating disease once occurrence of infections becomes widespread. With the increasing occurrence of *S. sclerotiorum* worldwide, it is important to establish effective and efficient management practices for SSR.

1.2.5. Disease management

Crop rotation and fungicides are the recommended management strategies for minimizing the severity of SSR in canola (Khangura et al. 2018). Although SSR could be successfully controlled by the integration of multiple strategies, these disease-controlling applications inevitably pose a higher cost to the growers (Khangura et al. 2018).

Resistant cultivars

Currently, disease management of SSR in canola remains challenging due to a lack of resistance in most commercially grown cultivars (Khangura et al. 2018). Generally planting resistant cultivars is an inexpensive and convenient method for the management of SSR. There are rare commercial canola cultivars are highly resistant to *S. sclerotiorum*. Canola breeders have been working for a long time to develop resistant varieties to SSR since 1980, but it is extremely difficult because *S. sclerotiorum* has a vast pathogenic potential (Barker 2016). It has been reported that canola variety of

InVigor L160S bred by Bayer CropScience is tolerant to SSR (Barker 2016). Taking advantage of current biotechnological techniques may aid in producing resistant canola varieties. Recently scientists were able to transfer a gene that encodes for the enzyme oxalate oxidase, used to degrade oxalic acid, from barley into peanut. The addition of this gene lead to a higher resistance in peanut crop to infection caused by *S. minor* (Heffer & Johnson 2007).

Cultural practices

Cultural practices before seeding include sowing high-quality and clean seeds, rotating crops and using a wider row spacing. Alternating crops that are not susceptible to *S. sclerotiorum* will decrease the number of viable sclerotia in the soil over time (Heffer & Johnson 2007). Sclerotia can survive in the soil for many years and thus a minimum five-year rotation with non-susceptible crops is required to reduce the incidence of SSR (Gulya et al. 1997). Several common crops such as wheat, sorghum and corn are convenient non-SSR susceptible options for rotation (Heffer & Johnson 2007). In India, it has been shown that SSR can be successfully controlled by a series of strategies including seed treatment, burning of host crop stubble, field sanitation, deep ploughing and crop rotation with rice (Singh 2001). Crop rotation alone does not always guarantee successful control of SSR due to the wide host range of the pathogen and its ability to survive in soil even under unfavorable environmental conditions (Canola council of Canada 2017). A lack of weed control may negatively affect the performance of crop rotation as *S. sclerotiorum* infects many weeds common in the field, such as pigweed, Canada thistle, lambsquarters, ragweed, velvet leaf, snow thistle, mustard and nightshade (Heffer & Johnson 2007). Properly implemented weed control removes all possible hosts for *S. sclerotiorum* and reduces the chances of disease spread to adjacent susceptible crops from infected weeds. A balanced application of macronutrients and micronutrients is essential as it has been shown disease severity of SSR is suppressed by increasing levels of Ca, S, P and K in mustard (Singh & Tripathi 1993). In addition, canopy management plays an important role in reducing the incidence of SSR. Experimental results have shown that a wider row spacing helped reduce the incidence of SSR in legume crops compared to a narrow spacing (Heffer & Johnson 2007).

Chemical control

Chemical fungicides have been extensively applied for the control of SSR in canola (Budge & Whipps 2001). Fungicides used in seed treatment are effective in reducing disease incidence (Sharma et al. 2011). *Sclerotinia sclerotiorum* can survive as dormant mycelia in testae and cotyledons of infected seeds for over 3 years (Tu 1998). Sowing of infected seeds can result in 88-100% failure in germination (Sharma et al. 2015). Seed treatment with captan and thiophanate-methyl successfully eradicated *S. sclerotiorum* on infected seeds (Sharma et al. 2015). Foliar fungicides are widely used in fields, providing protection for crop plants that are susceptible to SSR. Applying contact or systemic fungicides during the period of flowering and senescence can provide the most effective control (Canola council of Canada 2017c). Several foliar fungicides are registered in Canada for control of SSR in canola, including Vertisan (penthiopyrad), Quash (metconazole), Astound (cyprodinil and fludioxonil), Proline 480SC (prothioconazole), Rovral Flo (iprodione) and Lance (boscalid) (Canola Council of Canada 2017c). Repeated applications of fungicides are needed if crops have a longer flowering period. The Canola Council of Canada recommends chemical fungicides are applied on petals of canola in the field at 30% flowering (Canola Council of Canada 2017c). Several dicarboximide fungicides (e.g. iprodione, vinclozolin, dimethachlon, and procymidine) can also provide disease control by limiting established lesions on infected areas, however, phytotoxicity has been previously observed following application on crop plants (Ferreira & Boley 1992). The efficacy of fungicides can be increased with the technology of the venturi nozzle which has been proven to be an effective application method as it provides the optimum pressure, further enhancing control of SSR on canola (Kutcher & Wolf 2006). The effectiveness of fungicides may vary considerably among different field crops. For example, benomyl was able to effectively control SSR on tomato at a rate of 100g/100 L, while no effect on SSR was observed on cauliflower at the same application rate (Ferreira & Boley 1992). Fungicides can be toxic and harmful to human and non-target organisms in local ecosystems. Moreover, the potential for developing resistance in *S. sclerotiorum* has become a topic of increasing concern over the past decades (Vrije et al. 2001). Cases of resistant *S. sclerotiorum* have been reported in China where foliar applications of a benzimidazole fungicide, carbendazim had previously significantly reduced the development of SSR. *S. sclerotiorum* developed resistance after frequent applications (Zhang et al. 2003; Sharma et al. 2015). Further, applying fungicides may not necessarily increase production profit due to the high cost of

fungicide and the variability of sclerotinia disease incidence in fields (Canola Council of Canada 2017c).

Micronutrients are able to suppress the development of fungal diseases in plants directly through antagonizing the pathogen or indirectly by increasing plant defense through the activation of systemic acquired resistance or inducing antagonist population in rhizosphere (Dutta et al. 2017). Interactions among plants, nutrients and pathogenic diseases are not fully understood. The responses of plant pathogens to nutrient availability are considered to be complicated because deficiency or sufficiency of nutrients regulates the physiological systems and plants in response to different pathogens differentially (Dutta et al. 2017). Disease resistance of a certain crop species to a certain pathogen can be influenced by nutrients, and some nutrients have a greater antifungal effect on plant diseases than others. For instance, the applications of mustard cake with combination of micronutrients (boron and Cu) reduced the disease infection caused by *S. sclerotiorum* ranged from 57.4 – 61.3% (Sharma et al. 2017). Soil applications of manganese fertilizers also reduced sclerotinia infections in squash (Graham and Webb 1991; Agrios 2005).

Biological control

Biological control is a cost-effective and eco-friendly alternative for management of fungal diseases. The application of biocontrol agents typically requires stable environmental conditions to successfully parasitize *S. sclerotiorum* (Whipps 1994). Several mycoparasitic fungi, including *Gliocladium roseum*, *Fusarium* spp., *Alternaria* spp., *Penicillium* spp., *Mucor* spp., *Epicoccum* spp., and *Sporodesmium sclerotivorum* inhibited growth of *S. sclerotiorum* during *in vitro* experiments, but the effects of these fungi on the control of *S. sclerotiorum* remains uncertain for *in vivo* application (Ferreira & Boley 1992). *Coniothyrium minitans* has shown strong effectiveness on the control of *S. sclerotiorum* under field conditions (Ferreira & Boley 1992). *C. minitans* is a promising fungal biocontrol agent of *S. sclerotiorum* (Whipps & Gerlagh 1992), as it parasitizes both the mycelium and sclerotia directly (Merriman et al. 1979; Trutmann et al. 1982; Vrijie et al. 2001). Currently, using *C. minitans* as a biocontrol agent to control *S. sclerotiorum* infection can be challenging due to the decrease in efficacy of the biocontrol with increasing severity of disease. In previous greenhouse experiments, *C.*

minitans successfully controlled *S. sclerotiorum* infection on lettuce by 60% when disease incidence was at 50%; however, when the level of disease increased, the efficacy of *C. minitans* dropped quickly (Budge & Whipps 2001). *C. minitans* exists in the soil through the recovery of spores from infected sclerotia (Tribe 1957; Huang 1981; Sandys-Winsch et al. 1993). There is no evidence that *C. minitans* is naturally present in soil or organic materials or is under the protection of its own asexual fruiting bodies - pycnidia (Vrije et al. 2001). Moreover, the formation of *C. minitans* pycnidia has only been observed on plant tissues infected with *S. sclerotiorum*. *C. minitans* has been shown to reduce the infection on plants caused by *S. sclerotiorum* (Vrije et al. 2001). The colony growth of *S. sclerotiorum* was inhibited by *C. minitans*, but the rate of inhibition was highly dependent on the quality and availability of nutrients and water content when grown on PDA (Ghaffar 1972; Zazzerini and Tosi 1985; Phillips 1989; Whipps 1987; Whipps & Magan 1987). *C. minitans* produces lytic enzymes such as endo- and exo-β-1,3 glucanases and chitinases that destroy the cell wall of mycelium of *S. sclerotiorum* (Jones & Watson 1969; Jones et al. 1974). Earlier studies suggested that sclerotia isolated from different *S. sclerotiorum* exhibited different susceptibility to *C. minitans*; for example, the aberrant tan sclerotia was found to be more susceptible to *C. minitans* due to the lack of melanin in rinds when compared to the normal isolates (Huang 1983; Turner & Tribe 1976). There are two approaches for the application of *C. minitans*: soil application and spore-sprays directly onto foliage. Soil application of *C. minitans* should be conducted approximately 8 weeks before sowing or planting a crop, allowing it to spread rapidly through soil (Tribe 1957; Trutmann et al. 1980; Whipps et al. 1989; Budge & Whipps 1991) and destroy sclerotinia before they germinate within crops (Vrijie et al. 2001). A third approach involves applying fungal materials of *C. minitans* onto diseased plants or crop debris post-harvest, which must be done before plant tissues infected with sclerotia are ploughed into soil (Vrijie et al. 2001). The commercial testing of *C. minitans* as a biocontrol agent has been impeded because of the lack of efficient methods for producing adequate fungal spores for experimental trials in field and greenhouse (Vrijie et al. 2001). Solid-state fermentation is a viable option for the mass cultivation of fungal materials as *C. minitans* is able to produce more spores on solid substrates compared to liquid media (McQuilken et al. 1997**;** McQuilken & Whipps 1995).

Bacillus subtilis has great potential to be an effective biological control agent for management of SSR because it has the capacity to produce a broad range of antibiotics and anti-fungal enzymes (Cawoy et al. 2011). Results from early field experiments showed that *B. subtilis* suppressed severity of SSR in bean (Boland 1997; Tu 1997). Experimental results indicated that *B. subtilis* strain SB24 significantly reduced the incidence of SSR when applied before the infection of *S. sclerotiorum* on soybean (Zhang & Xue 2010). In North Dakota, Serenade ASO containing *B. subtilis* strain QST 2808 is a registered biocontrol product for controlling SSR in canola fields (Kandel et al. 2019). The presence of *B. subtilis* on the roots enhances plant growth and disease resistance through several mechanisms, including: 1) competition with other microbes that could cause adverse effects on the plant; 2) induction of host defense responses against pathogens; 3) and increased uptake of certain nutrients, such as nitrogen and phosphorus (Nagorska et al. 2007). *B. subtilis* cells can produce antibiotic molecules, such as iturins, surfactins and fengycins (Cawoy et al. 2014). Fengycin and iturin are capable of destroying the surface tension of cell membranes in fungi, causing the occurrence of micropores and loss of essential ions, which consequently triggers fungal cell death (Ongena & Jacques 2008; Mihalache et al. 2017). It appears that iturin is the major effective component against plant pathogens based on the size of inhibition zones on agar media (Cawoy et al. 2014). The modes of action of some bacterial strains used for biocontrol are distinct from the mechanisms described above. These strains are able to activate defense systems when host plants are attacked by pathogens, resulting in an increased level of resistance (Conrath et al. 2006). Ongena et al. (2007) reported that systemic resistance was induced by surfactin produced by strain *B. subtilis* S499.

1.3. Importance of cucumber production

Cucumber plants (*Cucumis sativus L.*) belong to the *Cucurbitaceae* family, which is believed to have originated from Northern India. It has become one of the most cultivated greenhouse vegetables among many regions, such as China, India, Russia, Canada and the United States. Greenhouse cucumber production is currently an important segment of the Canadian agricultural food industry, and its production volume reached approx. 180 million kg in 2015 (Dey et al. 2017). In Canada, greenhouse cucumber productions are primarily located in Ontario (144 million kg), British Columbia

(23 million kg), and Alberta (9 million kg) (Dey et al. 2017). Cucumber fruits have many nutritional benefits, including hydrating properties and valuable nutrients. The flesh of cucumber is rich in phytonutrients (eg. lignans, flavonoids and triterpenes) which have anti-cancer, anti-inflammatory and antioxidant benefits (Mateljan 2019). With increasing consumption of cucumber, more and more cucumbers are grown in greenhouses. The greenhouse industry uses modern technology, which provides optimal nutrients, temperature, humidity, light and moisture to maintain good plant health and productivity. Add to this potential biological control practices for diseases and insects, resulting in high quality cucumber fruits and increased production per cultivated unit area. Fungal disease, however, is still a major limiting factor in the commercial production of greenhouse cucumbers.

1.4. Powdery mildew

1.4.1. Disease symptom

Cucurbit powdery mildew caused by *Podosphaera xanthii* is characterized by easily recognizable colonies of whitish to grayish talcum-like powdery growth (Figure 1.4), largely composed of mycelia and conidia that grows on both the upper and bottom sides of the leaves, as well as on petioles and young stems of the plant (Sitterly 1978; Zitter et al. 1996). Powdery mildew fungi can spread to most parts of the plant, but it mainly appears on the surface of the leaves (Agrios 2005). The first signs of infection on plants start as circular white colonies on the bottom surface of older leaves (Nunez-Palenius et al. 2006). Infection of *Podosphaera xanthii* typically becomes evident five days after inoculation of the pathogen (Yarwood 1957). Severely infected leaves are prone to atrophy, deformity, chlorosis or yellowing (Almqvist 2012). Later on, fungal colonies may coalesce and enlarge rapidly, covering the adaxial or abaxial leaf surfaces completely under favorable environmental conditions (Perez-Garcia et al. 2009). Plants can be killed by severe infections of PM. The infections are typically not visible on cucumber fruits no matter how heavily the foliage was damaged by the mycelium (Yarwood 1957). Although PM fungi have no or rare direct effects on cucurbit fruits, lack of protective cover of foliage due to premature senescence of infected leaves will cause

fruits to be deformed, sunburnt and ripened incompletely or prematurely (Sitterly 1978; Perez-Garcia et al. 2009).

Powdery mildew can alter the staining properties of cells of their host (Corner 1935), flatten the epidermal cells, and cause an abnormal enlargement of penetrated epidermal cells (Yarwood 1957). Other histological changes include collapse of the necrotic epidermal cells and tissue under the penetrated epidermal cells (Smith 1938). Powdery mildew infection increases transpiration and respiration of the host tissues, resulting in a reduction of photosynthesis efficiency (Yarwood 1957). Research has also shown that the disease can reduce the amount of water and alkaloids, as well as increase the number of water-soluble fractions, silicon, and lime in the host plant (Yarwood 1957).

Figure 1.4. Symptoms of PM on the surface of cucumber leaf in the greenhouse.

1.4.2. Identification

At present, there are five tribes of PM genera: *Phyllactinieae, Erysipheeae, Blumeriae, Golovinomyceteae* and *Cystotheceae*. The taxonomy of PM fungi has been revised extensively due to the updates in DNA sequence data. With previous taxonomy,

PM was identified predominately according to its sexual stage, morphology of the cleistothecium and the types of appendages (Yarwood 1978). The current method for identifying PM also includes its asexual stage (Braun et al. 2002; Heffer et al. 2006). Some characteristics for identification include the type of conidia (single or in chains), the location of mycelium (epiphytic or endophytic), the number of asci in each cleistothecium and the specificity of the host (Heffer et al. 2006). Powdery mildew fungi are distinct from other fungal pathogens, they tend to produce epiphytic persistent hyphae and cause superficial damage on the above-ground parts of susceptible host plants (Heffer et al. 2006). Hyphae of *P. xanthii* are branched, flexuous to straight, 5-7 µm in width, forming white microreticular structures called mycelium (Choi et al. 2014). Conidiophores are specialized hyphae, which are straight, unbranched, 150-240 $\times$ 10-20 µm in size, producing 3 to 7 conidia in chains (Figure 1.5) (Perez-Garcia et al. 2009; Choi et al. 2014). Conidia are asexual spores of *P. xanthii*, which are hyaline, ellipsoid to ovoid or doliform in shape, around 24-40 $\times$ 5-22 µm in diameter, with cylindrical or cone-shaped fibrosin bodies (Perez-Garcia et al. 2009). The conidia of *P. xanthii* and *G. cucurbitacearum* are similar, but they can be differentiated by the presence of cylindrical or cone-shaped fibrosin bodies in conidia of *P. xanthii* (French & Schultz 2009; Martinez-Cruz et al. 2014). The PM fungi develop a specialized structure for parasitism called haustorium (Heffer et al. 2006). The haustorium of *P. xanthii* looks like a bulb jointed to the superficial secondary hypha by the haustorial neck, with the formation of tubular elongations termed lobules (Martinez-Cruz et al. 2014). The single-celled ascospores are sexual spores of *P. xanthii*, and they are produced in a sac-like ascus enclosure within a fruiting body called cleistothecium. The cleistothecia globose are dark brown or black in color and 70-100 µm in diameter, and there are eight ascopores produced in one ascus (Perez-Garcia et al. 2009).

Figure 1.5. Conidiophores and chains of conidia of *P. xanthii*. Photo courtesy of Paul Bachi, University of Kentucky Research and Education Centre, Bugwood Organization.

1.4.3. Biology and Ecology

Powdery mildew is a widespread fungal disease caused by many different species of fungi in the *Erysiphales* order of the Ascomycota phylum (McGrath 2017). Powdery mildew fungi have a broad range of hosts, infecting over 10,000 species of angiosperms across several families, including Cucurbitaceae, Asteracea, Verbenaceae, Lamiaceae, Solanaceae, and Scrophularlaceae (Glawe 2008; Perez-Garcia et al. 2009). Powdery mildew is usually easy to recognize because it forms colonies of whitish talcum-like powdery growth on both sides of leaf surfaces, as well as petioles and stems. The mycelium colonies on plant surfaces can impair photosynthesis, resulting in reduced growth, premature senescence of foliage and sometimes the death of leaves (Nunez-Palenius et al. 2006). In cucurbits, PM is primarily caused by two ectoparasitic pathogen species: *Podosphaera xanthii* (synonym *Sphaerotheca fuliginea auct. p.p.* or *Sphaerotheca fusca* or *Podosphaera fusca*) (Castag.) U. Braun & N. Shish, and

Golovinomyces cucurbitacearum (syn. *Erysiphe cichoracearum auct. p.p. or Golovinomyces cichoracearum*) (DC.) V.P. Gelyuta (Lebeda 1983; Jahn et al. 2002; Kristkova et al. 2009; Lebeda et al. 2009). *P. xanthii* and *G. cucurbitacearum* have a different geological distribution and relative occurrence across the world (Kristkova et al. 2009; Perez-Garcia et al. 2009). *P. xanthii* prevails in tropical and temperate regions, including Canada (Cerkauskas & Ferguson 2014) and the USA (Lebeda et al. 2010), while *G. cucurbitacearum* is more frequently observed in continental Europe where the climate is cooler (Miazzi et al. 2011). Such a difference in distribution may be related to the thermophily of *P. xanthii* (Miazzi et al. 2011). Another endoparasitic species of cucurbit PM is *Leveillula taurica* Arnaud, which is mainly distributed in warm and arid regions of the Mediterranean, Middle East, and South America (Kristkova et al. 2009; Beltrán-Peña et al. 2018). This species generally causes a smaller economic loss compared to the other two more influential species (Lebeda et al. 2009).

All PMs are obligate biotrophic parasites, meaning that they cannot be directly cultured in nutrient medium without living hosts (Panstruga 2003). However, they can be maintained under laboratory conditions by transferring fungal spores onto fresh tissue detached from living plant hosts. Perez-Garcia et al. (2006) suggested that PM can be stored long-term by freezing spores at -80°C. The fungi form highly specialized infection structures, known as haustoria, to absorb nutrients and water from the epidermal cells of host plant for their own growth and reproduction (Green et al. 2002). They gradually occupy the surface of leaves as mycelium and conidiophores grow (Yarwood 1957). Powdery mildew develops rapidly in plants under favorable conditions because large amounts of conidia can be produced within a short time and symptoms can appear on leaves after only 3 to 7 days of infection (McGrath 2017).

Powdery mildew can occur in cucurbits under various environmental conditions. Typically, moderate temperature, dry air conditions, low light intensity, and intensive plant cultivation favor PM growth (Yarwood 1957). The combination of climatic factors, such as temperature, relative humidity, sunlight, rainfall and wind, has a strong effect on conidial germination and transmission, mycelial growth and sporulation (Lebeda 2009). The temperature range of 20-30°C with optimum 22°C favored the conidial germination of *P. xanthii* (Nagy 1976). Conidial germination is also affected by relative humidity. Conidia of *P. xanthii* can only remain viable for 24 hours at 100% relative humidity in a saturated atmosphere, and the relative humidity favoured for conidia germination is 100%

(Nagy 1976). Although high relative humidity favors conidia germination, conidial viability is not affected by relative humidity during sporulation and conidia are able to germinate at low relative humidity (20 or 50%) (Hural 1986). The disease is generally favored by dry conditions during the processes of colonisation, sporulation and dispersal (Lebeda 2009). Jarvis et al. (2002) found that PM disease development is suppressed when water content becomes excessive on leaf surface, while, with moderate amounts of dew on leaf surfaces PM severity is increased. The highest potential of infection by *P. xanthii* occurs when the temperature is around 15°C and 65% relative humidity (Bashi & Aust 1980). The fungi are generally more active in the shade of the large overlapping cucurbits leaves, such as cucumbers and melons, than in the presence of direct sunlight (Nunez-Palenius et al. 2006).

1.4.4. Disease cycle

Cucurbit PM are polycyclic diseases that consist of asexual and sexual stages (Figure 1.6).

Asexual reproduction

All PM fungi have similar asexual life cycle. The prominent mode of reproduction for PM fungi is the dispersal of asexual conidia to susceptible host with the assistance of wind, splash from raindrops or insects (Ridout 2009). Conidia produce a short germ tube after landing on a susceptible host (Perez-Garcia et al. 2009). The terminus of germ tube is a primary differentiated appressorium where a primary haustorium forms (Perez-Garcia et al. 2009). The haustorium is responsible for the absorption of nutrients from the living plant by penetrating into the host epidermal cell (Heffer et al. 2006). The primary hypha emerges from the primary appressorium or the conidium, the secondary hypha is subsequently formed from the primary hypha branches (Perez-Garcia et al. 2009). Conidiophores arise vertically from secondary hyphae, and then several conidia are produced in chain at the tip of each conidiophore (Perez-Garcia et al. 2009). Production of conidia usually occurs within a few days after the infection. Conidia are likely to be released during midmorning to early afternoon (Glawe 2008). Eventually, the secondary hyphae are completely intertwined with conidia, forming the white mycelium visible on plant surfaces (Perez-Garcia et al. 2009).

Sexual reproduction

Cucurbit PM fungi are heterothallic, and their sexual reproduction only occurs if two hyphae of opposite mating types are present simultaneously (Figure 1.6). (Perez-Garcia et al. 2009). Ascospores are ejected from cleistothecium when environmental conditions are favorable. The majority of PM fungi are less active during the winter season. Cleistothecia are regarded as resistant structures for overwintering (Glawe 2008). There is evidence that the pathogen overwinters as mycelium in dormant buds in a number of crops and landscape plants (Glawe 2008; Yarwood 1957). Cleistothecia are considered as inoculum source of cucurbit PM, but they have been rarely found in cucurbit crops (McGrath 1994; Maia et al. 2012).

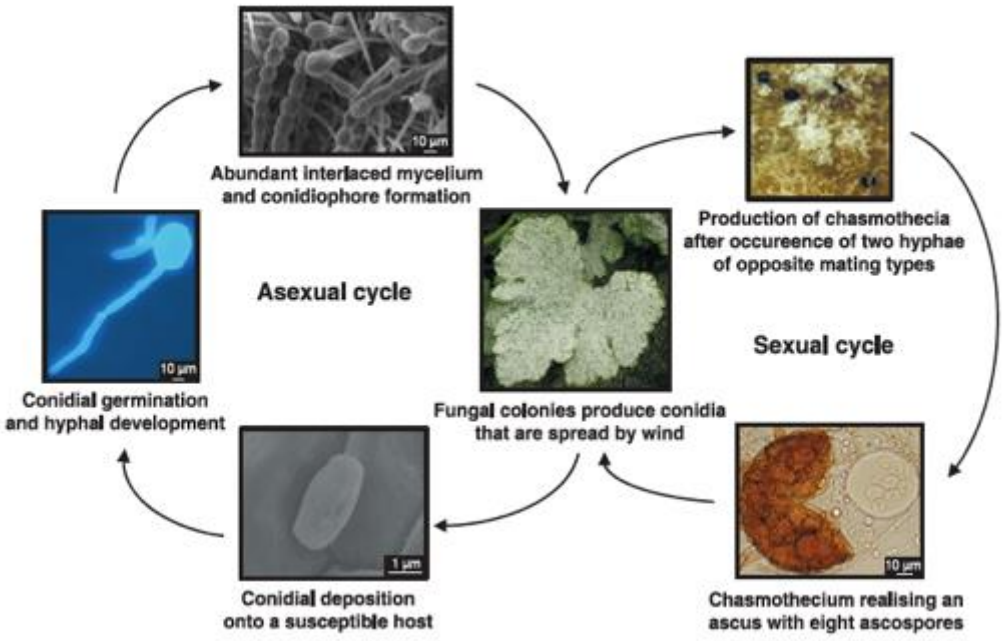

Figure 1.6. Life cycle of *P. xanthii*. Reprinted from Perez-Garcia et al. (2009).

1.4.5. Economic importance

According to the available literature, PM is one of the oldest plant diseases that has caused a profound influence on human history (Tiwari 2015). It was first described on roses by a Greek writer and gardener tracing back to 300 B.C. (Agrios 1997). *P. xanthii* has been a major causal agent of cucurbit PM in many areas of the world since

the 1960s (Jahn et al. 2002). Powdery mildew is considered to be one of the most common limiting factors for cucurbit production in many countries, such as Canada, Japan, Spain, Israel and Netherlands, where cucurbit plants are commercially grown in greenhouses at lager scale. PM poses a serious and constant threat to both field and greenhouse grown cucurbits (Romero et al. 2007a). In Canada, *P. xanthii* is the most widely dispersed and commonly recorded species causing cucurbit PM and it is currently the most important foliar disease on cucumber plants grown in greenhouses. With the rapid development of greenhouse production, cucurbit PM becomes a serious threat due to the favourable environmental conditions in greenhouses such as warm temperatures, high relative humidity, high plant density and more stable air flow, providing an ideal place for the development and spread of infection (Paulitz et al. 2001). PM has been reported to occur yearly in commercial greenhouses, leading to significant yield and economic losses in Ontario and British Colombia (Agriculture and Agri-Food Canada 2016a).

Powdery mildew infections are chronic, and often occur in plants at moderate severity. Common characteristics of chronic infections include a lack of systemic and root infections, lower rates of host plant death and slow disease development (Yarwood 1957). It has been noted that PM is not a systemic disease unless there are many independent infections appearing on one single plant (Yarwood 1957). Compared to other important groups of plant pathogens (e.g. downy mildews, viruses, rusts and root-rotting fungi), PM infections usually result in relatively less detrimental yield losses (Yarwood 1957). There are, however, documented records of disastrous losses by PM. A severe PM epidemic reduced approximately 75% yields of cucumbers in Russia in 1930 (Szembel 1930). More recently, yield losses of cucumbers caused by PM reached 30-50% in China (Shaw & Cantliffe 2003). Over 70% farm acreage of squash plants in Florida was reported to be affected by this disease (Shaw & Cantliffe 2003). As for Canada, PM has resulted in a 10-35% yield loss in many commercial cucumber greenhouses. In Ontario, the relevant loss was equivalent to $74,000 to $222,400/ha in 2010 (Cerkauskas & Ferguson 2014). Powdery mildews can reduce the quality and market value of crop products because premature leaves can cause sunburnt or incompletely ripened fruits (Zitter et al. 1996). Consequently, fruits of cucurbits do not have a marketable flavor and are more difficult to store (Pernezny et al. 2008). Infections of PM may predispose plants to other serious diseases, such as the gummy stem blight

and viruses (McGrath 2017; McGrath 1997). Thus, infection by PM may result in a higher yield loss from PM itself or increased susceptibility to other diseases.

1.4.6. Disease management

Powdery mildew is managed through a number of strategies, including selecting PM-tolerant cultivars, applying fungicides, biological agents and chemical compounds during crop production (Nunez-Palenius et al. 2006).

Resistant cultivars

In cucurbits, genetic resistance to PM plays an important role in the acquisition of better yields and crop quality, as well as a reduction of fungicide applications. Planting resistant cultivars is the most cost-efficient and simplest approach of obtaining healthy cucurbit crops without PM (Nunez-Palenius et al. 2006). Resistance to PM in cucumber was first observed in the variety Puerto Rico 37 in the USA (Morishita et al. 2003). After that, a number of resistant varieties had been developed. There are several commercially available resistant cucumber varieties, such as ‘Supremo’, ‘Marketmore’, ‘Alibi’, ‘Ashley’, ‘Supersett’ and ‘Eureka’. Some of these commercially available varieties only show partial resistance to PM (Morishita et al. 2003). Cultivating these resistant varieties can sometimes lead to reduced or delayed crop yields because they may have higher susceptibility to other serious fungal diseases, such as grey mould and gummy stem blight (Almqvist 2012). Effectiveness of disease suppression obtained from using resistant cultivars could vary as the pathogen may adapt to these cultivars in future (Lebeda et al. 2010). Mechanisms of resistance to PM are classified as pre- and post-haustorial resistance. Pre-haustorial resistance reduces the formation of haustoria through papillae (Perez-Garcia et al. 2009). Post-haustorial resistance is associated with plant cell necrosis (hypersensitive reactions), which is the main race-specific resistance to PM. The processes of this mechanism in melon plants resistant to *P. xanthii* have been described as follows. After a few hours of inoculation of pathogen, a series of physiological changes are initiated by the plants, causing a fast increase in the amount of reactive oxygen species (e.g. hydrogen peroxide and superoxide anions) in plant tissues. In response to this rapid accumulation of oxidative substances, the cell walls of

P. xanthii-infected as well as nearby cells are reinforced by an increase in lignin and callose polymers, resulting in a slower ingression of pathogen into cell and disruption of nutrient supply (Perez-Garcia et al. 2009).

Cultural control

Monitoring and early detection of disease symptoms in plants is the first and most important step in the management of PM. Several cultural practices can be used as the first step to control the PM. Appropriate cultural practices include planting cucurbits in well-ventilated locations where sunlight is present, placing cucurbits away from plants infected with PM, removing and destroying heavily infected leaves to decrease inoculum levels (Li 2012). Overcrowding of crops will cause a rapid spread of PM among plants; hence, the avoidance of overcrowding is highly recommended to control PM. Another effective approach to be considered is to avoid irrigating the foliage of infected host plant because splashed water helps the dispersal of PM conidia to other healthy nearby plants. Crop rotation as a prevention measure efficiently controls many important diseases, but it is ineffective in the control of PM because conidia are able to survive and germinate even under low relative humidity (Gay et al. 1985). Fast growing succulent tissues are more susceptible to PM; thus, for these plants, it is important to avoid applying excessive nitrogen fertilizers in late summer (Newman & Pottorff 2013).

Fungicides

Applying fungicides is still the primary method for controlling PM all over the world (Hollomon & Wheeler 2002). Fungicides are most effective when applied with cultural controls (Schwartz & Gent 2007). Fungicides have to be applied as early as possible to prevent PM since most fungicides used for the control of PM are preventative (Li 2012; Nunez-Palenius et al. 2006). There are some registered fungicides for the control of cucurbit PM, including systemic fungicides, myclobutanil (Nova®), azoxystrobin (Quadris®), trifloxystrobin (Flint®) and contact fungicides such aschlorothalonil (Li 2012). Contact fungicides are multi-site inhibitors; therefore, there is less potential for the development of resistance to the fungicide (McGrath 2001). Contact fungicides are used less frequently to manage PM because they are unable to prevent infection on the lower surfaces of leaves, resulting in a lower efficacy (Lebeda et al. 2010). As for non-systemic fungicides, sulfur and copper are considered as natural fungicides for disease suppression of PM. Sulfur (Microthiol Disperss®) decreases the

severity of cucurbit PM, and target fungi currently haven't developed resistance to sulfur (Nunez-Palenius et al. 2006). However, there is evidence that suggests that sulfur cannot control PM if the disease severity is extremely high in squash crops (Nunez-Palenius et al. 2006). Sulfur may cause phytotoxicity seen as scorch on the leaves under warm weather conditions, especially in muskmelons and honeydews (Nunez-Palenius et al. 2006). Copper salts (Kocide®) are less phytotoxic than sulfur so it is recommended for the control of cucurbit PM (Zitter et al. 1996). Fungicide is by far the most effective control method, but PM may potentially develop resistance to fungicides over repeated applications (Brown 2002). In 1967, it was first found that PM fungi developed resistance to fungicides in the USA (Schroeder & Provvidenti 1969). After that, the possibility of developing fungicide resistance in cucurbit PM is prevalent worldwide. Major cucurbit PM species (*P. xanthii* and *G. cichoracearum*) have already developed resistance to fungicides (McGrath 2001; Sedlakova & Lebeda 2008). Cucurbits PM already has resistance to some fungicide classes, such as Qo inhibitors, organophosphates, methyl benzimidazole carbamates, quinoxalines, sterol demethylation inhibitors and morpholines (McGrath 2001). Since PM has the potential to develop resistance to fungicides, it is recommended to alternate fungicide in order to maintain the effectiveness on preventing PM pathogens (Li 2012).

Non-fungicidal chemicals

There are some biorational alternatives with low phytotoxicity that have been employed in greenhouses to reduce the occurrence of PM in cucurbit crops, including natural and mineral oils, unpasteurized milk, and silicon and salts of monovalent cations. Silicon was used as an additive to nutrient liquid to enhance plant growth, but it also can delay the PM development by the reinforcement of host plant's defence system in cucumber plants (Schuerger & Hammer 2003). Solidified silicon lies below the leaf surface to enhance the resistance to penetration by pathogens. Unpasteurized milk as a natural product has been applied on cucumber plants for effectively suppressing the incidences of *P. xanthii* (Bettiol 1999). The defense mechanism of milk against PM has not been clearly elucidated yet; it is possibly due to the presence of amino acids, salts or an antimicrobial agent (Nunez-Palenius et al. 2006). Neem oil obtained from the seeds of neem tree (*Azadirachta indica*) can be applied to prevent severe PM outbreak in cucumber. However, neem oil should be used with caution considering its potential harmful impact on bees and beneficial predators (Nunez-Palenius et al. 2006).

Vegetable oils and soap are used to manage PM they act on contact, directly affecting the growth of PM fungi on leaf surfaces (Almqvist 2012). Some vegetable oils, such as sunflower oil, soybean oil and corn oil, are effective on controlling PM of cucumber plants (Du et al. 2010). Salts of monovalent cations have been found to reduce the PM severity, such as potassium (Milstop®), mono-potassium phosphate, sodium and ammonium bicarbonate. Furthermore, cucumber PM can be controlled by garlic extracts when susceptible plants are treated at an early stage of infection (Almqvist 2012).

Biological control

Many filamentous fungi, yeasts and bacteria have been tested to control cucurbit PM, but only a few are proven to be effective in reducing the development of the disease. There are some biological products which have been registered for the control of cucurbits PM. AQ10® biological fungicide was specifically developed for PM. The active ingredient of AQ10® is a mycoparasite *Ampelomyces quisqualis* Ces. (Nunez-Palenius et al. 2006). This biocontrol agent is able to parasitize PM fungi, blocking their nutritional absorption to limit the number of haustoria (Romero et al. 2003; Paulitz & Belanger 2001). As a consequence, the growth of fungi is inhibited and size of the colony on leaf surfaces is reduced (Romero et al. 2003). Moreover, these mycoparasites cause a reduction in the number of conidia and conidiophores, and reproductive structures; therefore, the spread of disease is impeded (Romero et al. 2007a). AQ10® is most effective as a preventative treatment when applied with a mineral oil or silicone surfactant in the morning or at night (Schwartz & Gent 2007). However, AQ10® cannot be applied along with conventional fungicides such as sulfur, EBDCs and strobilurins (Schwartz & Gent 2007). Mycotal® contains *Lecanicillium lecanii* which is a commercial entomopathogenic and mycoparasitic fungi used for biological control of PM. Like *Ampelomyces quisqualis*, this micro-organism uses a similar mechanism of action and is able to provide sufficient control of PM when the density of the pathogen is moderate in the greenhouse (Paulitz & Belanger 2001). A study has demonstrated that mycoparasites tend to be more effective with a high (> 80%) relative humidity (Romero et al. 2007a). Since a higher relative humidity is required for the survival of biocontrol agents, many biocontrol agents cannot be used to control PM in drier environments (Nunez-Palenius et al. 2006).

Serenade® containing a species of bacteria (*Bacillus subtilis*) is another biological control product. *Bacillus subtilis* is believed to be a safe biocontrol agent for a number of fungal diseases. These bacteria colonize PM fungi quickly and efficiently and produce a large number of endospores so that they can be stored for a long time and formulated effectively as products (Shoda 2000; Reva et al. 2004). They produce several lipopeptide antibiotics, such as iturin and fengycin lipopeptides, which prevent the infection of PM fungi on leaves (Romero et al. 2004; Romero et al. 2007c; Nunez-Palenius et al. 2006). Conidia of PM will fail to germinate in the presence of these lipopeptides as they have the ability to attack biological membranes of target cells and induce injury in ultrastructure and morphology (Romero et al. 2007b). High relative humidity is also required for *B. subtilis* to produce and secrete more antifungal compounds for the suppression of PM (Romero et al. 2007a).

At present, Sporodex®, biocontrol made from a yeast-like fungus *Sporothrix flocculosa* (syn. *Pseudozyma flocculosa*) is likely the most successful biocontrol product which can protect cucumber plants grown under greenhouse conditions against PM fungi (Paulitz & Belanger 2001). Research has shown that although the efficacy of Sporodex® is not better than the fungicide applications, cucumber yields have been increased by about 15% after treatments (Paulitz & Belanger 2001). *S. flocculosa* exerts antifungal properties by producing fatty acid related substances which induce plasmolysis in infected host cells at various growth stages (Hajlaoui et al. 1992). It is unlikely for PM fungi to develop resistance to antibiotics produced by *S. flocculosa* because these antibiotics degrade quickly in the fields (Paulitz & Belanger 2001). The conidia of *S. flocculosa* are formulated as wettable powder for using in greenhouses. The disadvantage of this type of formulation is the difficulty in dissolving the residues left on the leaves which can cause a reduction in crop quality (Paulitz & Belanger 2001).

1.5. Active Flower

Active Flower™ (AF) is a fertilizer manufactured by Active AgriScience (http://activeagriscience.com/) that provides nutrients, polyamines and organic acids that support and enhance plant fertility. It improves pollen hydration, germination, pollen tube growth and viability, and encourages bee foraging activity which increases fertilization. In

the field, 1 L of AF is mixed with 20 L water for spray application. Concentrations of AF at 0.1, 0.3 and 0.5 ml/100 ml were used in this study for greenhouse application. Many growers have reported that AF can increase the yields of crops, such as canola, corn, soybean, peas, and wheat, which may be due to the increased frequency of pollination induced by AF applications. According to the minimum analysis, AF contains 3% boron (B) plus 8: 4: 12 of N: P: K. Boron is a micronutrient that is necessary for the growth and health of all the crops (Pizzorno 2015), which is related to translocation of sugar, rate of water absorption, and development of leaves, stems and flowers (Blevins and Lukaszewski 1998). Boron deficiency can cause cell walls to swell and split, resulting in a weakened physical barrier to fungal pathogens. It is believed that boron can reduce the severity of many diseases due to its important role in (1) cell wall formation and stability, (2) the maintenance of structural and functional integrity of biological membranes, and (3) the metabolism of phenolics or lignin (Brown et al. 2002; Dordas 2008). It was observed that boron treatments (borax at 500 and 750 ppm) inhibited mycelial growth of *Fusarium oxysporum* f. sp. *cubense* and the addition of borax at 500 ppm increased the sporulation capacity of *Trichoderma viride* (Sanjeev & Eswaran 2008). Boron can be used as effective means of disease management of soil borne plant pathogens. For example, boron application (0.05, 0.5 and 2.5 mg/L) reduced the disease severity of bacterial wilt caused by *Ralstonia solanacearum* in tomato (Jiang et al, 2016).

Chapter 2.

Effects of a foliar fertilizer containing boron on development of Sclerotinia stem rot (*Sclerotinia sclerotiorum*) on canola (*Brassica napus* L.)

Abstract

Canola (*Brassica napus* L.) is grown extensively in Canada and Sclerotinia stem rot (*Sclerotinia sclerotiorum* Lib. De Bary) is one of the most destructive fungal diseases on this crop. A foliar fertilizer containing 3% boron plus 8:4:12 of N: P: K [Active Flower™ (AF)] was evaluated and assessed its effects in reducing disease severity. Concentrations of AF at 0, 0.1, 0.3 and 0.5 mL/100 mL were first tested for inhibition of mycelial growth of *S. sclerotiorum* in potato dextrose broth. Growth inhibition was pronounced only at 0.5 mL/100 mL. Boric acid (BA), an important component of AF, was also tested against fungal growth at 10 mL/L, and no significant effect was found. Foliar applications of AF at 0.1, 0.3 and 0.5 mL/100 mL were made weekly to canola plants 'Westar' grown under greenhouse conditions. Foliar treatments were also made with an AF formulation that did not contain boron at the same concentrations as AF, and BA was applied at 10 mL/L as a separate treatment. After four applications, total nutrient levels and specific enzyme activities were measured in treated and control leaf tissues. Applications of AF at 0.5 mL/100 mL and BA at 10 mL/L enhanced boron levels in leaves by five-fold and three-fold, respectively, compared to the untreated control, while other nutrient levels were unchanged. Mycelial development and lesion size of *S. sclerotiorum* on detached leaves was significantly ($P<0.05$) reduced by these treatments. Levels of phenolic compounds in leaves treated with 0.5 mL/100 mL AF were enhanced by 2-fold compared to the control. There were no significant differences ($P=0.05$) in leaf lignin content, peroxidase or polyphenoloxidase enzyme activities between the control and AF (0.5 mL/100 mL) treatment. These results suggest that enhanced boron levels in canola leaves were associated with a suppressive effect on disease due to *S. sclerotiorum*.

Keywords

Sclerotinia stem rot; *Sclerotinia sclerotiorum*; Canola; Fungal disease; Boron; Foliar fertilizer; Active Flower™; Phenolic compounds

2.1. Introduction

Canola (*Brassica napus L.*) belongs to the *Brassica* genus and is a valuable seed crop in many countries throughout the world. Germany, France, China, Canada and India produced the largest quantities of canola in 2014. In Canada, canola production was reported to be 21.3 million tonnes in 2017 (Statistics Canada 2017), with a total economic value to the Canadian economy of $ 26.7 billion (Canola Council of Canada 2017a). The primary production areas for canola are Saskatchewan, Alberta, Manitoba and the Peace River region of British Columbia. Canada exports 90% of its canola as seeds, oil or meal to over 50 markets around the world. Fungal diseases are a serious threat to crop yield. Sclerotinia stem rot (SSR) (*Sclerotinia sclerotiorum* Lib. De Bary) is one of the most destructive fungal diseases of canola. Symptoms occur first as water-soaked, light brown coloured lesions on the leaves or stems 2 to 3 weeks after inoculation, usually from ascospore produced from overwintering sclerotia, and lesions expand and become greyish white (Canola Council of Canada 2017c). The plant wilts quickly and dies when lesions spread onto the main stems. Yield losses caused by SSR are particularly significant when infections occur at the early stage of flowering under humid conditions (Jurke et al. 2013). Disease management is challenging due to a lack of genetic resistance to SSR in most commercially grown canola cultivars (Khangura et al. 2018). Crop rotations and fungicides are the recommended strategies for management of SSR in canola (Khangura et al. 2018) but can be costly for growers. Alternative approaches to manage SSR need to be researched.

Active Flower™ (AF) is a foliar fertilizer (http://activeagriscience.com/) that contains nutrients (3% boron plus 8: 4: 12 of N: P: K) and a polyamine complex that is used to enhance flowering, pollination and fertilization of crops such as canola, corn,

soybean, peas and wheat. Yield increases of 5-12% have been reported following application of AF (Active AgriScience 2017). Boron is a micronutrient that plays an essential role in the growth and development of plants (Zhang et al. 2014). Canola requires a high level of boron, but excessive boron may result in phytotoxicity on the margins of older leaves (Deora et al. 2011). Boron deficiency may also delay growth, flowering and pollen germination (Stangoulis et al. 2001). Plants often take up boron in the form of boric acid (BA) (Camacho-Cristobal et al. 2008). Boron was used as an addition in disease management due to soil borne plant pathogens. The regulation mechanism of boron on reducing tomato bacterial wilt caused by *Ralstonia solanacearum* was due to the increases in the concentrations of Ca and boron, rate and amount of H2O2 accumulation, as well as the enzyme activities of peroxidase (POD) and polyphenol oxidase (PPO) in tomato plants (Jiang et al. 2016).

Since fungal diseases, including SSR, are a major concern on canola crops, and given the reported yield increases due to AF applications, we hypothesized that AF may have a secondary effect in reducing fungal infection. The objectives of this research were: 1) to measure growth reduction of *S. sclerotiorum* in potato dextrose broth with varying concentrations of AF at 0.1, 0.3 and 0.5 mL per 100 mL, and BA at a concentration of 10 ml per L; 2) to determine the effects of AF on disease development on canola plants; 3) to measure physiological changes in plants in response to AF, including phenolic compounds, nutrient levels and activities of POD and PPO.

2.2. Materials and methods

2.2.1. Evaluation of AF and BA on *S. sclerotiorum* growth in vitro

An isolate of *S. sclerotiorum* was obtained from infected canola seedlings and was grown on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) at ambient room temperature (21-23℃). After two weeks, a 1-cm-diameter plug taken from the actively growing margin of the colony was transferred into a 250 ml flask containing 100 ml of potato dextrose broth (PDB) (HiMedia Laboratories Pvt. Ltd.). Concentrations of AF of 0.1, 0.3 and 0.5 mL/100 mL were added into cooled, autoclaved PDB to test the effects on *S. sclerotiorum* growth. PDA could not be used as AF addition resulted in a

gel formation and the agar would not solidify. Boric acid (manufactured by ActiveAgriScience Inc.) was tested at rate of 10 mL/L in PDB as well. Flasks were placed on a shaker at 125 rpm at room temperature. After two weeks, the mycelium was filtered out through a filter net and dried in an oven at 50 ℃ for 48 hours. The dry weights were compared to controls that were not amended. There were five replicates used for each treatment and the experiment was conducted twice.

2.2.2. Canola plant treatments

Canola seed 'Westar' was sown in 'Sunshine Mix' (TerraLink Inc. Abbotsford, BC) in a research greenhouse located on the Burnaby mountain campus, Simon Fraser University. The greenhouse temperature fluctuated within the range of 22 to 35 ℃ (June-September) and relative humidity was 60-80%. Supplemental light was provided from incandescent lamps during cloudy or rainy days. At three weeks after seeding, individual plants were transplanted to 3.8 L pots and grown in the greenhouse for an additional three weeks. These plants were treated with AF at 0.1, 0.3, 0.5 mL per 100 mL. A hand-held spray bottle was used to apply AF on the lowest four to five leaves to run-off. Water was sprayed as the control. There were five replicate canola plants for each treatment. Plants were fertilized once every two weeks using Tout usage soluble fertilizer (20-20-20) (Premier Tech Home and Garden Inc. Rivière-du-Loup, Québec). In a separate experiment, treatments of AF formulated without B (provided by Active AgriScience Inc.) at the same concentrations as AF, and BA at 10 mL/L were also applied on canola plants. After receiving four applications, more than 20 leaves from each treatment were harvested and used for *in vitro* inoculation with *S. sclerotiorum*. The AF experiment was conducted twice, each during June to September of 2016 and 2018, and the ActiveFlower Without Boron (AFWB) and BA experiments were conducted twice during June to October of 2018.

Nutrient levels in canola leaves were determined through analysis conducted by a commercial lab (A&L Canada Laboratories Inc., London, ON). There were three replicate leaves harvested for each treatment for nutrient analysis. Lignin levels were also determined through analysis conducted from each treatment using the acid

detergent lignin (ADL) method with sulfuric acid (72% by weight) (Ankom Technology 2016). The analyses were conducted twice using different samples.

2.2.3. Canola leaf inoculation with *S. sclerotiorum*

Leaves receiving four applications were harvested, which were all of a similar size. Detached canola leaves were placed in plastic containers lined with moist paper towels. In the first trial, a 1-cm-diameter mycelial plug from a 2-week-old *S. sclerotiorum* culture was placed in the center of each leaf. In the second trial, *S. sclerotiorum* was grown in PDB for one week and 0.1 mL of mycelium was inoculated on the center of each leaf using a pipette. Boxes containing leaves were placed at ambient laboratory conditions. Lesion size was measured in two directions and averaged 5 to 7 days after inoculation with *S. sclerotiorum*. The areas of the lesion were not determined as lesions sometimes developed irregularly on leaves. Five replicate leaves were used for each treatment in both trials.

2.2.4. Phenolic levels in canola leaves

AF treated and control canola leaves were harvested from plants and dried at room temperature for 48 hours. Major veins were removed with a razor and the leaf fragments were cut into small pieces. Samples of 0.1 g were soaked in 50 ml boiling distilled water, left to stand for 5 min, and then filtered through 90 mm filter papers (Whatman UK) into 100 ml flasks and left to cool for 20 min. The total phenolic content of the canola leaves was determined by the modified Folin-Ciocalteu method (Tahirović et al., 2014). Briefly, 0.2 mL of extract of each treatment was added into test tube cuvettes. Next, 1 mL Folin-Ciocalteu's phenol reagent (Sigma USA) was added into the solution and left to sit for 10 min. A volume of 0.8 mL 7.5% Na_2CO_3 was added and left for 30 min at room temperature. Gallic acid (GA) (Sigma USA) and distilled water were added into cuvettes to make concentrations of 0, 20, 40, 60, 80, and 100 mg/L GA for the standard curve. The absorbance was measured at 743 nm wavelength by the use of a spectrophotometer (ThermoSpectronic, UVG 1210140). The total phenolic levels were

calculated as mg of gallic acid equivalents by using an equation obtained from the gallic acid calibration curve. There were 7 replicates for each treatment.

2.2.5. Enzyme activity assays of canola leaves

Sample preparation

Plant crude extract was prepared from 0.5 g canola leaf samples (AF treated and control) for enzyme activity assays. The sample was ground with 3 mL 0.2 M borate buffer and 3 mL 10% polyvinylpyrrolidone (PVP) on ice and the homogenate was added to 10 mL borate buffer. After that, the solution was centrifuged at 10,000 rpm for 20 min at 4 ℃. The supernatant was transferred to 15 mL tubes (Falcon) for enzyme activity assays. Pyrogallol solution (0.05 M) was made by adding 6.3 g pyrogallol in 1 L distilled water for enzyme analysis. The peroxidase (POD) and polyphenol oxidase (PPO) enzymatic activity assays were described previously by Liu et al. (2017). There were six replicate samples for each treatment.

Peroxidase (POD) activity

Plant crude extract (0.1 mL), 1 mL pyrogallol solution (0.05 M), and 3.9 mL phosphate buffer (pH 7.8) were mixed in 15 mL Falcon tubes using Vortex Genies 2. The tubes were left to react in a water bath at 37 ℃ for 10 min. 1 μL 0.08% H_2O_2 was added into the solution before measuring the absorbance at 470 nm (A_{470}) continuously for 30 min. The POD enzyme activity was calculated as $(\Delta A_{470} \times V_t)$ / $(FW \times V_s \times 0.01 \times t)$. ΔA_{470} was the change of the absorbance of 470 nm, V_t was the volume of plant crude extract, FW was the fresh weight, V_s was total reaction volume, t was the reaction time (min).

Polyphenol oxidase (PPO) activity

Plant crude extract (0.1 mL), 1 mL pyrogallol solution (0.05 M), and 3.9 mL phosphate buffer (pH 7.8) were mixed in 15 mL tubes using Vortex Genies 2. The reaction was conducted in a water bath at 37 ℃ for 5 min. After that, the absorbance change of solution was recorded at 525 nm (A_{525}) continuously for 30 min. PPO enzymatic activity was calculated as $(\Delta A_{525} \times V_t)$ / $(Wp \times V_s \times 0.01 \times t)$. ΔA_{525} was the

change of the absorbance of 470 nm, V_t was the volume of plant crude extract, W_p was the weight of total protein (mg in 100 uL of sample), V_s was total reaction volume, t was the reaction time (min).

2.2.6. Statistical analysis

Statistical analysis for the data from all experiments was conducted using Statistical Analysis System software (Version 13; JMP; SAS Institute, Inc.). Significant differences among treatment means were separated using Tukey-Kramer post hoc test when Analysis of Variance results indicated there were significant differences among the control and treatment factors. The homogeneity of variance between two experiments was tested, no statistical difference was detected, and thus data from two experiments were combined for analysis.

2.3. Results

2.3.1. Evaluation of AF and BA on *S. sclerotiorum* growth in vitro

To determine whether enhanced boron application may have an effect on susceptibility of canola leaves to *S. sclerotiorum*, 3 treatments - AF, AF without boron (AFWB), and boric acid (BA) - were each assessed for mycelial growth inhibition in vitro as well as lesion development on detached leaves following treatment with these compounds. The most pronounced growth inhibition of *S. sclerotiorum* was observed with 0.5 mL/100 mL of AF compared to the control (Figure 2.1A). There was some mycelial growth inhibition with AF at 0.1 and 0.3 mL/100 mL, but no obviously observable differences were found in comparison to the control (Figure 2.1B). However, AF at 0.5 mL/100 mL inhibited mycelial growth by more than 90% when compared to the control (Figure 2.1C). AFWB at 0.1 and 0.3 mL/100 mL did not reduce mycelial growth and in some cases increased the dry weights of *S. sclerotiorum* (data not shown). AFWB at 0.5 mL/100 mL reduced dry weights by around 70% (data not shown). Boric acid was also tested for growth inhibition of *S. sclerotiorum* at 10 mL/L. Results showed that there

were no significant differences (P=0.05) between the control and BA treatments at these concentrations (data not shown).

2.3.2. Nutrient levels in leaves

Plant analysis reported the levels of several important nutrients in leaves, including Nitrogen, Nitrate Nitrogen, Sulfur, Phosphorus, Potassium, Magnesium, Calcium, Sodium, Boron, Zinc, Manganese, Iron, Copper, Aluminum and Chloride. Results showed that no obvious change was found in total nutrient levels between AF treated leaves and the control except for boron and Cu. The level of boron in canola leaves treated with AF was increased, especially at 0.5 mL/100 mL, by five-fold compared to the control (Figure 2.2). Control plants had boron levels within the normal range (55 ppm), and plants receiving AF at 0.5 ml/100 ml had boron levels in excess of 270 ppm in leaves. Phytotoxic symptoms appeared on the margins of some older leaves treated with AF at 0.5 mL/100 mL as chlorotic and necrotic patches. In addition, plants treated with BA at 10 ml/L showed significantly higher boron concentrations (158 ppm) than control plants (48 ppm) (Figure 2.2).

2.3.3. Disease severity

Lesions of *S. sclerotiorum* developed as early as 2-3 days after inoculation of detached canola leaves. After 5-7 days, symptoms appeared as water-soaked, light brown necrotic areas. Control leaves had extensive disease symptoms with the largest lesion size at 9.27 cm (Figure 2.3A) with grayish white mycelium developing on the surface of leaves. Treatment with AF at 0.1 mL/100 mL did not reduce the disease severity on leaves compared to the control (Figure 2.3B). Lesion size was reduced with AF at 0.3 mL/100 mL, which had an average lesion size of 8.2 cm (Figure 2.3C). The smallest lesion size (6.84 cm) was observed on canola leaves treated with AF at 0.5 mL/100 mL (Figure 2.3D), which is a significant reduction compared to the control (Figure 2.3E). At this rate, phytotoxicity was observed on the edges of some leaves. There were no obvious differences in lesion size between the control and AFWB-treated

leaves (Figure 2.4). In addition, development of *S. sclerotiorum* was reduced on leaves treated with BA at 10 mL/L in comparison to control leaves. In the first trial with BA, *S. sclerotiorum* had little growth 4 days post inoculation on BA treated leaves (Figure 2.5B), whereas it developed quickly and grew grayish white mycelium on the leaf surface of the control leaves (Figure 2.5A). BA at 10 mL/L significantly reduced the lesion size by 57% compared to the control 5 days after inoculation with *S. sclerotiorum* (Table 2.1). In the second trial with BA, the development of *S. sclerotiorum* was significantly suppressed on BA-treated leaves as well (Table 2.1), while the lesion size was 5.5 cm after 5 days (Figure 2.5D), which was larger than the lesion size (3.96 cm) obtained from the first experiment. The lesion size was significantly reduced in BA treatments in the second trial (Table 2.1).

2.3.4. Phenolic compounds

Boron applications can induce several secondary metabolites with high toxicity to pathogens, including phenolic compounds. Phenolic compounds were measured by the absorbance of 740 nm. A positive linear relationship between phenolic compound levels and AF applications was observed. While AF treatment at 0.1mL/100 mL did not show a significant increase from the control, treatments of AF at 0.3 and 0.5 mL/100 mL were higher than the control. AF at 0.5 mL/100 mL (18.95 mg/gm leaf tissue) significantly increased the levels of phenolic compounds by 2-fold compared to the control (9.30 mg/gm leaf tissue) (Figure 2.6).

2.3.5. Lignin levels

Lignin acts as a preformed, passive defense in plants, as it thickens the cell wall to build a physical barrier against invasion of pathogens. However, there were no significant differences between control and AF treated leaves. AF treatments at these three concentrations had no effect on lignin formation in canola leaves. Lignin levels in AF treated leaves ranged from 1.48 to 1.73% (Figure 2.7). Leaves treated with AFWB contained higher levels of lignin, ranging from 0.93 to 4.92%

2.3.6. Changes in POD and PPO activity

Previous studies indicated that POD and PPO have potential roles in plant defense systems against the invasion of pathogens. Our results showed that POD activity did not increase in leaves with AF treatments as there were no significant differences between the control and AF treatments for POD activity in canola leaves (Figure 2.8). Also, no significant increases in PPO activity in canola leaves were observed in AF treatments when compared to the control (Figure 2.9).

2.4. Discussion

Active Flower™ (AF) is a foliar-applied fertilizer mixture that contains 3% boron plus N: P: K at 8: 4: 12 and a polyamine complex. Mycelial results showed that AFWB had a similar trend as AF in inhibiting mycelial growth of *S. sclerotiorum* at 0.5 mL/100 mL, but it appeared that AF had a greater antifungal effect than AFWB. Since AF is a complex mixture of several different ingredients [including EDTA, copper (Cu), zinc (Zn), manganese (Mn)], the fungitoxicity observed at 0.5 mL/100 mL (150 ppm boron) is likely due to the presence of other components. The low in vitro fungitoxicity of boric acid is supported by previous studies. Concentrations of 100 to 1000 mg/L of BA did not suppress mycelial growth of four species within the Botryosphaeriaceae (Pitt et al. 2012). However, in *Eutypa lata*, boric acid reduced radial growth and ascospore germination by 50% on PDA at 125 and 475 ppm, equivalent to 22 and 83 ppm boron (Rolshausen & Gubler 2005). The inhibitory effects of boron on mycelium growth may vary in different fungi, which could be due to the differences in sensitivity to boron.

Phytotoxicity was observed on older canola foliage as burning of tips or edges on plants treated with AF or AFWB at 0.5 mL/100 mL. Boron accumulation increases with leaf age, with the potential to cause more damage (Deora et al. 2011). Symptoms of boron phytotoxicity were reported on canola leaves at a much lower rate under greenhouse conditions than in the field (Deora et al. 2011). Karamanos et al. (2002) reported that boron at 5.1 kg/ha did not cause phytotoxicity on canola grown in western

Canada. Our results showed that boron levels were significantly increased in canola tissues after four foliar applications of AF and BA. Treatments of AF reduced disease severity of sclerotinia white mold by up to 26% on detached canola leaves compared to the control. Meanwhile, BA at 10 mL/L limited the development sclerotinia white mold by around 39-57% on detached canola leaves in comparison to the control.

Previous research has shown that boron, when applied as boric acid or in other forms, reduced disease development in many different host-pathogen interactions. When applied as boric acid, reduction in *Fusarium solani* on beans, *Blumeria graminis* on wheat, and *Verticillium albo-atrum* on cotton and tomato plants, was reported (Dordas 2008). The efficacy of boron in disease suppression on *Brassicaceae* crops, either in controlled environment conditions or in the field, has also been demonstrated (Nott et al. 1999; Ruaro et al. 2009). These included reduced fungal root colonization and lower disease development on Chinese cabbage (Nott et al. 1999) and reduced development of clubroot caused by *P. brassicae* on canola plants following drench applications of BoronMax (8.1% B complexed with plant carbohydrates), Boron (10% B as H_3BO_3), and Sloubor (20.5% B as $Na_2B_8O_{13}4H_2O$) (Deora et al. 2011). A rate of 4 kg/ha B effectively reduced the severity of clubroot by 64% compared to the control in field trials (Deora et al. 2011). The severity of late blight on tomato (*Phytophthora infestans*) was reduced by boron treatment, potentially through systemic acquired resistance (Frenkel et al. 2010). Two B-based products (Biopaste and Bioshield) containing 5% BA significantly reduced Eutypa dieback (*E. lata*) on grapevines by 78% to 98% in field trials (Rolshausen & Gubler 2005) and the boron-containing products Biopaste and Gelseal, as well as BA, provided effective control of *E. lata* when applied to the pruning wound on grapevines (Sosnowski et al. 2010; Pitt et al. 2012). On corn, foliar applications of boron at 2.24 kg/ha reduced severity of gray leaf spot compared to the non-treated control in field experiments (Kaur & Nelson 2015). Similarly, foliar application of boron (as H_3BO_3, at 6.25 mg/l of boron) decreased the number of leaf lesions and severity of tan spot caused by *Drechslera tritici-repentis* in winter durum wheat, especially at the booting stage, because B was involved in physiological and biochemical effects in plants (Simoglou & Dordas 2006).

The severity of fungal diseases may be reduced by boron since it plays an important role in cell wall formation and stability, maintenance of structural and functional integrity of biological membranes, and production of phenolic compounds or lignin

(Brown et al. 2002; Dordas 2008; Elmer 2015). A majority of boron in the cell wall cross-links with pectin polymers to increase cell wall stability and rigidity (Loomis & Durst 1992; Liew et al. 2012). It was reported that boron enhanced the level of phenolic compounds in plants (Frenkel et al. 2010). As critical components of the cell wall, phenolic compounds are involved in various biological functions, such as providing mechanical support and strength, mediation of growth and morphogenesis, as well as stress- or pathogen- induced responses (Moalemi et al. 2012). Rapid accumulation of phenolic compounds and lignin is the first step in the defense mechanism of plants in response to fungal infection to suppress the invasion of a pathogen (Matern & Kneusel 1988; Liew et al. 2012). In our study, levels of phenolic compounds were increased in AF treated canola leaves, which could explain the reduced disease levels as these compounds are fungitoxic. Moalemi et al. (2012) reported that phenolic compounds were significantly increased under excess boron (1 mM provided as H_3BO_3) in the cells of marshmallow (*Althaea officinalis* L.) in comparison to the control (0.01 mM). It was found that an increase in tannic acids (defense response chemicals) was most obvious among wall-bound phenolic compounds with boron (1 mM) (Moalemi et al. 2012). It is likely that boron can bind to tannic acids when there is a high level of B in cells (Moalemi et al. 2012). Liu et al. (2017) observed phenolic compounds were highly enhanced in response to *S. sclerotiorum* infection in the resistant sunflower variety Fengkuiza No.1. Another study suggested that the synthesis of phenolic compounds was associated with the constitutive resistance against *Botrytis cinerea* in leaves of kiwifruit (Wurms et al. 2003). Our results indicated that phenolic compounds produced by canola plants potentially provide protection against the invasion of *S. sclerotiorum*, which may be induced by the applications of AF.

Plants are able to synthesize lignin de novo in response to pathogen attack (Vermerris & Nicholson 2006). Applications of boron were able to improve lignin formation in rice (Dobermann & Fairhurst 2000). However, in our study, foliar applications of AF containing 3% boron did not increase the level of lignin in foliage when plants were grown under greenhouse conditions. A recent study revealed that lignin modification in alfalfa (*Medicago sativa*) did not increase foliar disease resistance (Samac et al. 2018).

Plants can produce several antioxidant enzymes in response to invasion by pathogens (Malencic et al. 2010). Liu et al. (2017) found activity of POD was increased

in sunflower in response to infection by *S. sclerotiorum*. In our study, however, there were no significant increases in activity of POD in AF treated canola leaves. In addition, Liu et al.'s (2017) findings showed that the activity of PPO was suppressed, and PPO might not be critical in defensive mechanisms of the plant against *S. sclerotiorum* in sunflower. Our results support these findings as PPO activity was not increased in comparison with the control when leaves were treated with AF applications, but the disease development of *S. sclerotiorum* was suppressed by AF on detached leaves.

Potentially, applications of AF containing 3% boron offer an alternative approach to management of SSR on canola plants. Field application rates are recommended at 1 L AF with 20 L water per acre, equating to 5 mL AF per 100 mL water, which is 10-fold higher than the AF concentrations (0.5 mL/100 mL) used in the greenhouse. Boron is an important component in AF which while reducing disease development on detached leaves did not inhibit mycelial growth in liquid culture. This suggests an indirect effect in increasing plant defense. Future research is also required to evaluate efficacy of AF and BA on the inhibition of disease severity in a long-term experiment in the field.

Acknowledgments

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Table

Table 2.1. Effect of boric acid on *S. sclerotiorum* development on detached canola leaves.

Treatment	Lesion size caused by *S. sclerotiorum*[a] (cm)	
	Experiment 1[b]	Experiment 2[b]
Water	9.29 a	9.04 a
BA-10	3.96 b	5.50 b

[a]*S. sclerotiorum* development was rated on cultivar 'Westar' after four weekly applications of boric acid (10 mL/L) or water were made. Lesion size was rated on six leaves randomly selected from 10 plants in 2 groups (n=6). Means followed by a different letter are significantly different according to Tukey's HSD test ($P \leq 0.05$).
[b]Experiment 1 was conducted during June to July 2018 and Experiment 2 was conducted during August to September 2018.

Figures


a
b

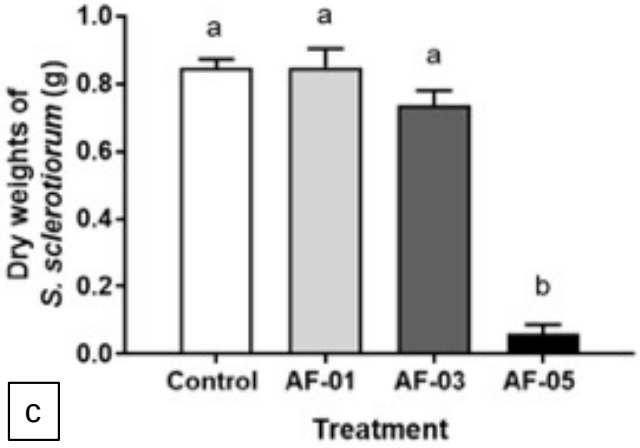
1.0
0.8
0.6
0.4
0.2
0.0
Dry weights of S. sclerotiorum (g)
a
a
a
b
Control
AF-01
AF-03
AF-05
Treatment
c

Figure 2.1. **Effect of AF at 0.1, 0.3 and 0.5 mL/100 mL on mycelium growth of *S. sclerotiorum* after two weeks incubation in potato dextrose broth (PDB). (a) *S. sclerotiorum* growth in PDB with AF at 0.5 mL/100 mL (right) and the non-treated control (left). (b) Mycelium was filtered out and dried in weighing boat. Treatments from left to right are non-treated control, AF at 0.1 mL/100 mL, AF at 0.3 mL/100 mL and AF at 0.5 mL/100 mL. (c) Dry weights of *S. sclerotiorum* in treatments of AF at 0.1, 0.3 and 0.5 mL/100 mL and the Control. Data were combined from two experiments. Vaules are means ± S.E., n=10 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different (p<0.05).**

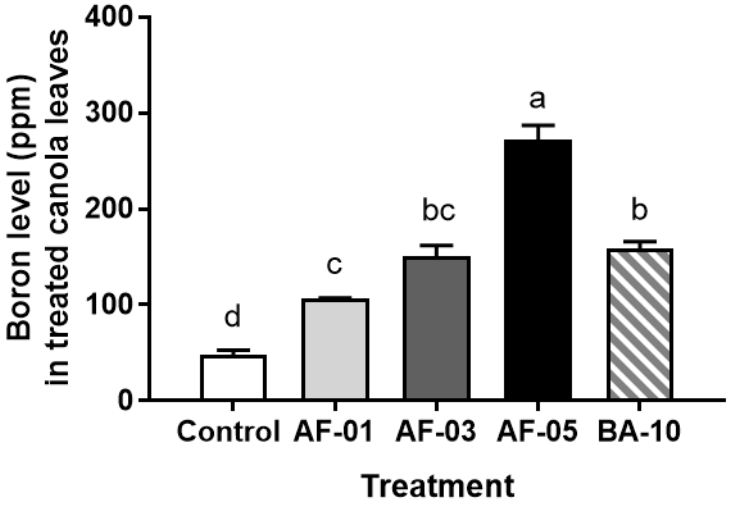

Figure 2.2. **Boron levels in canola leaves treated with 4 applications of AF at 0.1, 0.3 and 0.5 mL/100 mL, and BA at 10 mL/L compared to the control. Data were combined from two experiments. Values are means ± S.E., n=6 for all groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different (p<0.05).**

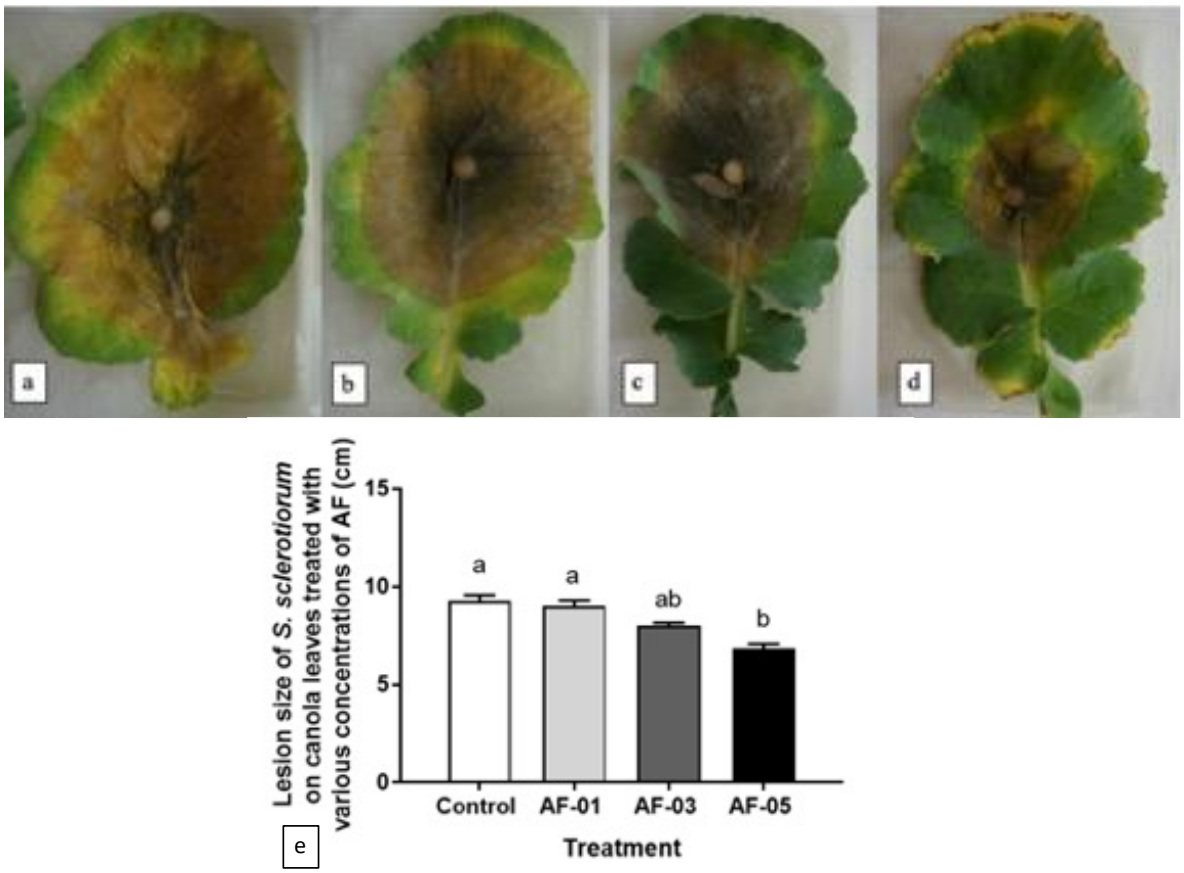

Figure 2.3. **Lesion size caused by *S. sclerotiorum* on detached canola leaves. (a) Water-treated control. (b) AF at 0.1 mL/100 mL. (c) AF at 0.3 mL/100 mL. (d) AF at 0.5 mL/100 mL. (e) Lesion size of *S. sclerotiorum* on canola leaves treated with various concentrations of AF compared to the control. Data were combined from two experiments. Values are means ± S.E., n=11 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different ($p<0.05$).**

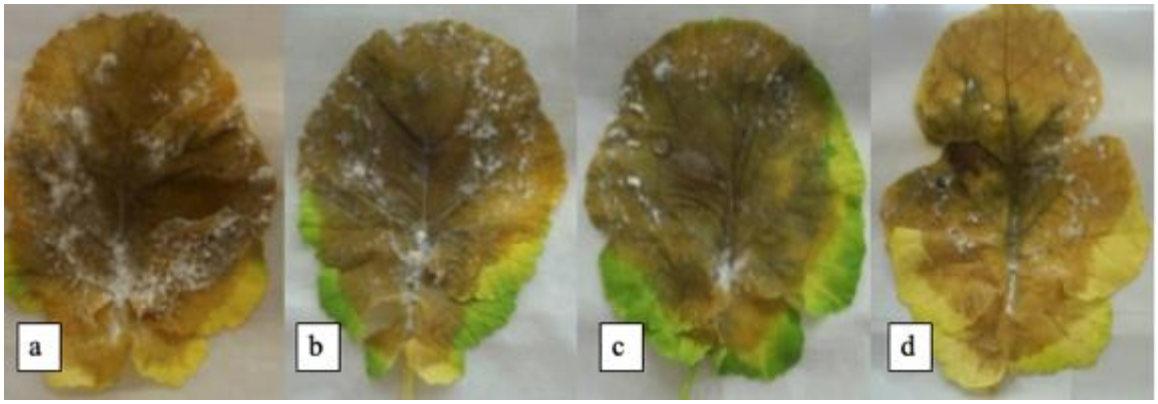

Figure 2.4. **Lesion size caused by *S. sclerotiorum* on AFWB-treated canola leaves. (a) Water-treated control. (b) AFWB at 0.1 mL/100 mL. (c) AFWB at 0.3 mL/100 mL. (d) AFWB at 0.5 mL/100 mL.**

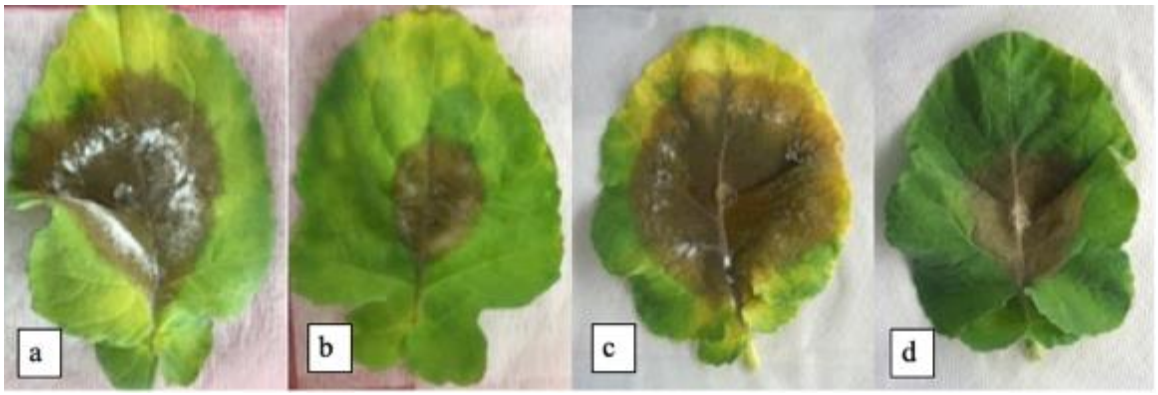

Figure 2.5. **Lesion size of *S. sclerotiorum* on BA-treated canola leaves. (a) Experiment 1 – water-treated control. (b) Experiment 1 – BA at 10 mL/L. (c) Experiment 2 – water-treated control. (d) Experiment 2 – BA at 10 mL/L.**

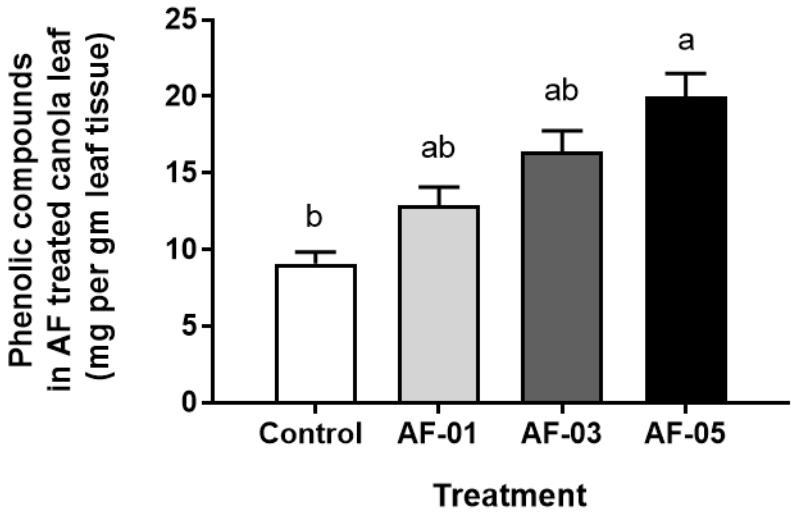

Figure 2.6. **Levels of phenolic compounds in canola leaves treated with four applications of Control and AF at 0.1, 0.3 and 0.5 mL/100 mL. Values are means ± S.E., n=7 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different ($p<0.05$).**

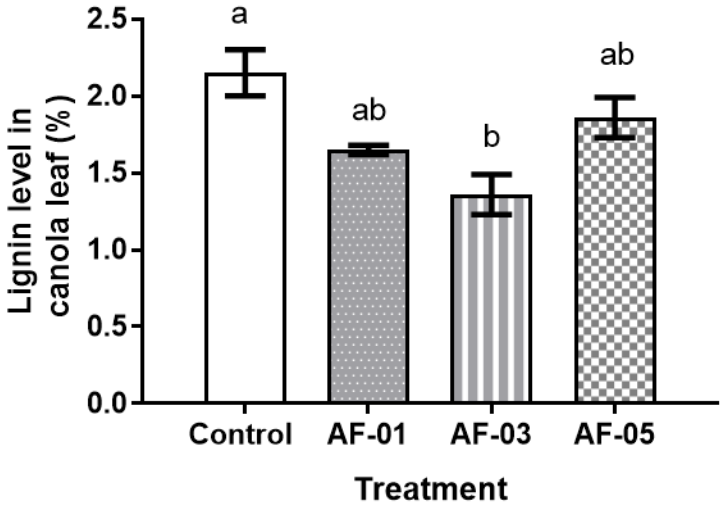

Figure 2.7. **Levels of lignin in canola leaves treated with four applications of Control and AF at 0.1, 0.3 and 0.5 mL/100 mL. Values are means ± S.E., n=4 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different ($p<0.05$).**

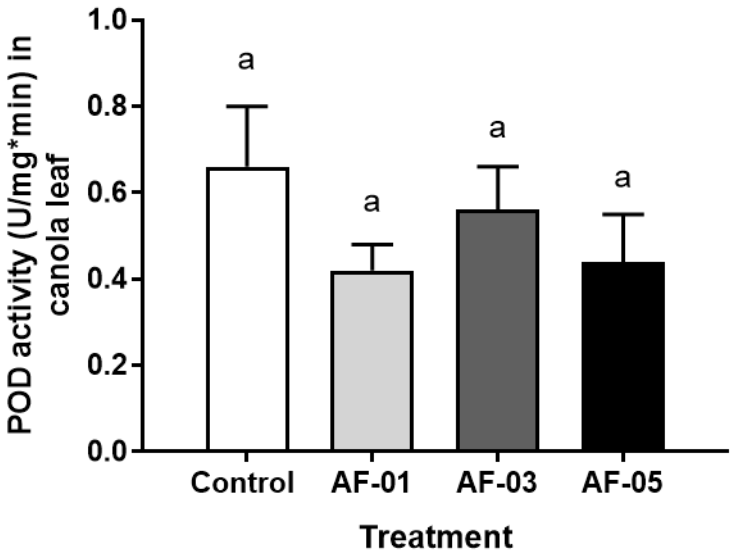

Figure 2.8. **The enzymatic activity of POD in canola leaves treated with four applications of Control and AF at 0.1, 0.3 and 0.5 mL/100 mL. Values are means ± S.E., n=6 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different ($p<0.05$).**

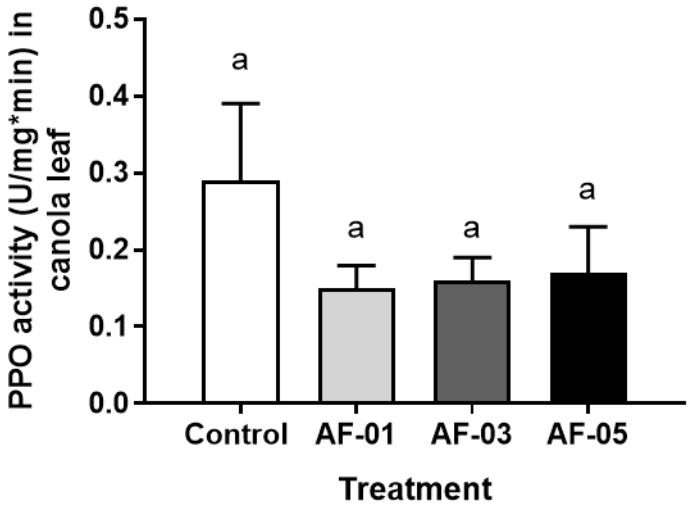

Figure 2.9. **The enzymatic activity of PPO in canola leaves treated with four applications of Control and AF at 0.1, 0.3 and 0.5 mL/100 mL. Values are means ± S.E., n=6 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different ($p<0.05$).**

Chapter 3.

Evaluation of Active Flower, a foliar fertilizer containing boron on the disease development of powdery mildew (*Podosphaera xanthii*) on greenhouse cucumber plants (*Cucumis sativus L.*)

Abstract

Powdery mildew (PM), caused by *Podosphaera xanthii,* is a widespread fungal disease on greenhouse cucumber and poses a serious threat to crop production in many countries, including Canada. The efficacy of ActiveFlower (AF), a foliar fertilizer containing 8: 4: 12 of N: P: K plus 3% boron, to manage PM was evaluated in this study. Applications of AF at 0.1, 0.3 and 0.5 mL/100 mL were weekly applied to cucumber 'Tasty Green' plants grown under greenhouse conditions. Water was applied as the control. The number of mildew colonies on cucumber leaves was significantly reduced by AF at 0.3 and 0.5 mL/100 mL when applied at the onset of infection. Results showed that phenolic content, B levels in foliage receiving AF were significantly increased. In a second trial, disease severity was also reduced by AF treatments. After the first application, PM was suppressed significantly (P=0.0003) by AF at 0.5 mL/100 mL compared to the control. However, after three applications of AF, only AF at 0.3 mL/100 mL significantly reduced the disease severity by 30% in comparison to the control. Results from enzyme analysis of treated leaves showed that there were no significant differences between control and AF treatments in peroxidase (POD) and polyphenoxidase (PPO). These findings suggest that foliar fertilizer of AF containing B can be applied to reduce the severity of PM under low to moderate disease pressure in greenhouses.

3.1. Introduction

Cucumber plants (*Cucumis sativus L.*) belong to the *Cucurbitaceae* family, which is believed to have originated from India. Greenhouse cucumber production is an important segment of the Canadian agriculture food industry and is popular in many areas of the world, such as China, India, Russia and the United States. In 2015, greenhouse cucumber was one of the primary greenhouse vegetable crops grown annually in Canada with about 180 million kg of production (Dey et al. 2017). The major greenhouse cucumber production areas are mainly in Ontario (144 million kg), British Columbia (23 million kg), and Alberta (9 million kg) (Dey et al. 2017). The farm gate value of greenhouse cucumbers in Canada was $334 million in 2016 (Agriculture and Agri-Food Canada 2016b). Greenhouse industry uses modern technologies, which provides optimal nutrient, temperature, humidity, light and moisture to maintain good plant health and productivity, as well as potential biological control practices for diseases and insects, resulting in high quality cucumber fruits and increased production per cultivated unit area. However, fungal disease still is a major limiting factor during commercial production of greenhouse cucumbers. In British Colombia, PM (*Podosphaera xanthii*) is widespread and yearly occurrence in greenhouse cucumber production, can cause severe yield and economic losses (Agriculture and Agri-Food Canada 2014). In cucurbits, PM is caused by three pathogens: *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea auct. p.p.*), *Golovinomyces cucurbitacearum* (syn. *Erysiphe cichoracearum auct. p.p.*), and *Golovinomyces orontii* (syn. *Erysiphe cichoracearum auct. p.p.*) (Jahn et al. 2002), with the first species being more widely dispersed than the other two. Roberts & Kucharek (2005) reported that the incidence of outbreaks of PM has increased in recent years. Powdery mildew forms obvious colonies of whitish and powdery mycelium on the leaf surface, stems and petioles of plants, which affects plant photosynthesis, resulting in reductions in plant growth, premature foliage loss and consequently significant yield losses (Nunez-Palenius et al. 2006). There is a negative linear association between disease severity and yield loss for cucumber plants (Dik & Albajes 1999). Powdery mildew develops fast under favorable conditions because many conidia can be produced within a short time and symptoms can appear on leaves after only 3 to 7 days of infection in the plant (McGrath 2017). Powdery mildew can occur in cucurbits in a wide range of environmental conditions. There are many environmental factors that may have impacts on the development of the disease such as temperature,

relative humidity and light. Jarvis et al. (2002) pointed out that the severity of the disease will be increased with dew on leaf surfaces, but it can adversely affect the development of this disease when the water content is excessive on the leaf surface. On the contrary, PM can get worse with relative low humidity when there is a high inoculum concentration with spores on surrounding plants (Nunez-Palenius et al. 2006).

Powdery mildew is managed through a number of strategies. These include selecting PM-tolerant cultivars, and applying fungicides, biological agents and chemical compounds (Nunez-Palenius et al. 2006). The management of PM in cucumber primarily relies on the use of resistant varieties and the application of fungicide (Morishita et al. 2003). Although many resistant varieties were found, it has been reported that the ability of these resistant varieties to reduce PM may not complete (Zijlstra & Groot 1992). Repeated applications of fungicides work effectively for the control of PM in cucurbit crops, but the fungi can develop resistance which reduces the efficiency of fungicides (Brown 2002). As for the use of biological control agents, a higher relative humidity is required for their survival than PM, so many biological control agents cannot control PM in drier environmental conditions (Nunez-Palenius et al. 2006). Consequently, it is necessary to search other alternatives to involve in the integrated disease management of PM.

Active Flower™ (AF) is a chemical fertilizer manufactured by Active AgriScience (http://activeagriscience.com/) that provides nutrients, polyamines and organic acids that support and enhance plant fertility. It improves pollen hydration, germination, pollen tube growth and viability, and encourages bee foraging activity which increases fertilization. Many growers have reported that AF can increase the yields of crops, such as canola, corn, soybean, peas, wheat, which may be due to the increased frequency of pollination induced by AF applications. According to the minimum analysis, AF contains 3% boron plus 8: 4: 12 of N: P: K. Boron is a micronutrient that is necessary for the growth and health of all crops (Blevins & Lukaszewski 1998; Pizzorno 2015). The effect of boron on PM has been discovered in barley since 1930 (Eaton 1930). However, little research has been done in recent years. It has been reported that boron can affect the tolerance mechanisms in plants responding to pathogens through influencing the plant physiology and biochemistry (Simoglou & Dordas 2006). Since PM is a major concern on cucumber crops, and given the reported yield increases due to AF applications, we hypothesized that AF may have the potential to reduce fungal infection. The objective of

the present study was to determine the effects of foliar applied AF containing boron at three concentrations (0.1, 0.3 and 0.5 mL/100 mL) on the development of PM caused by *P. xanthii* in greenhouse cucumber.

3.2. Methods and materials

3.2.1. Experimental design

The experiments were conducted in a research greenhouse located on the Burnaby mountain campus, Simon Fraser University during August to October 2016 (trial 1) and June to September 2018 (trial 2). Cucumber seeds 'Tasty Green' (West Coast Seeds Inc. Vancouver, BC) were germinated on moist filter paper in Petri dishes for a week and then transferred to greenhouse potting medium 'Sunshine Mix' (TerraLink Inc. Abbotsford, BC) and grown for 4 weeks. The average daytime temperatures ranged from 22 to 35 ℃ in the greenhouse and relative humidity was 60-80%. Supplemental light was provided from incandescent lamps during cloudy or rainy days. A hand spray bottle was used to spray AF on the lowest four to five leaves to run-off. Treatments of AF at 0.1, 0.3 and 0.5 mL per 100 mL were made to the lower four leaves to run-off. Applications were repeated weekly for a total of three applications. There were 5 replicate plants for each treatment and water was sprayed as a control. To endure mildew infection, in trial 1, infected cucumber leaves were cut into small pieces (1×1 cm) to include at least one colony of PM. Plants to be inoculated were misted with a small amount of water before the infected leaf pieces were placed onto each of three leaves. All cucumber plants were placed inside a plastic tent to provide high relative humidity (90-100%) for 72 h, after which the plastic was removed. Counting of PM colonies was conducted five days after the second and third applications of AF from four lower leaves. For trial 2, squash plants infected with PM were used as a source of inoculum since PM fungi develop rapidly on squash leaves. Spores were dusted from heavily infected squash leaves onto cucumber leaves. Plants were placed inside a humid chamber as before. Colonies of PM were counted before the first AF application and then subsequently five days after each AF treatment. A randomized complete block design (RCBD) was used in two experiments.

Nutrient levels in cucumber leaves were determined through analysis conducted by a commercial lab (A&L Canada Laboratories Inc., London, ON) after four applications of AF. There were three replicate leaves harvested for each treatment for nutrient analysis and experiments were repeated for three times.

3.2.2. Phenolic levels in cucumber leaves

AF treated and control cucumber leaves were harvested from plants and dried at room temperature for 48 hours. Major veins were removed with a razor and the leaf fragments were cut into small pieces. Samples of 0.1 g were soaked in 50 ml boiling distilled water, left to stand for 5 min, and then filtered through 90 mm filter papers (Whatman UK) into 100 ml flasks and left to cool for 20 min. The total phenolic content of the cucumber leaves was determined by the modified Folin-Ciocalteu method (Tahirović et al. 2014). Briefly, 0.2 mL of extract of each treatment was added into test tube cuvettes. Next, 1 mL Folin-Ciocalteu's phenol reagent (Sigma USA) was added into the solution and left to sit for 10 min. A volume of 0.8 mL 7.5% Na_2CO_3 was added and left for 30 min at room temperature. Gallic acid (GA) (Sigma USA) and distilled water were added into cuvettes to make concentrations of 0, 20, 40, 60, 80, and 100 mg/L GA for the standard curve. The absorbance was measured at 743 nm wavelength by the use of a spectrophotometer (ThermoSpectronic, UVG 1210140). The total phenolic levels were calculated as mg of gallic acid equivalents by using an equation obtained from the gallic acid calibration curve. There were 7 replicates for each treatment.

3.2.3. Enzyme activity assays of canola leaves

Sample preparation

Plant crude extract was prepared from 0.5 g cucumber leaf samples (AF treated and control) for enzyme activity assays. The sample was ground with 3 mL 0.2 M borate buffer and 3 mL 10% polyvinylpyrrolidone (PVP) on ice and the homogenate was added to 10 mL borate buffer. After that, the solution was centrifuged at 10,000 rpm for 20 min at 4 ℃. The supernatant was transferred to 15 mL tubes (Falcon) for enzyme activity

assays. Pyrogallol solution (0.05 M) was made by adding 6.3 g pyrogallol in 1 L distilled water for enzyme analysis. The peroxidase (POD) and polyphenol oxidase (PPO) enzymatic activity assays were described previously by Liu et al. (2017). There were six replicate samples for each treatment.

Peroxidase (POD) activity

Plant crude extract (0.1 mL), 1 mL pyrogallol solution (0.05 M), and 3.9 mL phosphate buffer (PB) (pH 7.8) were mixed in 15 mL Falcon tubes using Vortex Genies 2. The tubes were left to react in a water bath at 37 ℃ for 10 min. 1 μL 0.08% H_2O_2 was added into the solution before measuring the absorbance at 470 nm (A_{470}) continuously for 30 min. The POD enzyme activity was calculated as $(\Delta A_{470} \times V_t) / (FW \times V_s \times 0.01 \times t)$. ΔA_{470} was the change of the absorbance of 470 nm, V_t was the volume of plant crude extract, FW was the fresh weight, V_s was total reaction volume, t was the reaction time (min).

Polyphenol oxidase (PPO) activity

Plant crude extract (0.1 mL), 1 mL pyrogallol solution (0.05 M), and 3.9 mL phosphate buffer (PB) (pH 7.8) were mixed in 15 mL tubes using Vortex Genies 2. The reaction was conducted in a water bath at 37 ℃ for 5 min. After that, the absorbance change of solution was recorded at 525 nm (A_{525}) continuously for 30 min. PPO enzymatic activity was calculated as $(\Delta A_{525} \times V_t) / (Wp \times V_s \times 0.01 \times t)$. ΔA_{525} was the change of the absorbance of 470 nm, V_t was the volume of plant crude extract, W_p was the weight of total protein (mg in 100 uL of sample), V_s was total reaction volume, t was the reaction time (min).

3.2.4. Statistical analysis

Statistical analysis for the data from all experiments was conducted using Statistical Analysis System software (Version 13; JMP; SAS Institute, Inc.). Data from

two experiments were analyzed separately. Significant differences among treatment means were separated using Tukey-Kramer post hoc test when ANOVA results indicated there were significant differences among the control and treatment factors.

3.3. Results

3.3.1. Effects of AF on development of powdery mildew

Disease severity was rated as the number of powdery colonies developing on the four bottom leaves of the plants. The highest disease incidence (around 200 colonies/leaf) was observed on leaves receiving three weekly applications of water (Figure 3.1A). Treatment with AF at 0.3 and 0.5 mL/100 mL reduced the number of powdery colonies to approximately 50 when compared to the control (Table 3.1). Some phytotoxicity was observed on the margin of leaves treated with AF at 0.5 ml/100 mL (Figure 3.1D). In a second trial, disease severity was also reduced by AF treatments. Powdery mildew developed more rapidly on both treated and non-treated leaves in trial 2 than trial 1. At the beginning of trial 2, all cucumber plants were infected with PM at a similar level, and there was no significant difference among plants on the number of powdery colonies before AF applications. After the first application, disease severity on cucumber leaves was suppressed significantly (P=0.0003) by AF at a rate of 0.5 mL/100 mL. Application of AF at 0.5 mL/100 mL reduced the number of colonies by around 65% compared to the control receiving water only on week 1 (Table 3.2). On control plants, PM covered the surface of leaves, but only a few colonies appeared on leaves treated with AF at 0.5 mL/100 mL after the first application (Figure 3.2). However, after three applications of AF, only AF at 0.3 mL/100 mL significantly reduced the disease severity by 30% in comparison to the control (Table 3.2). The ability of AF in suppressing development of PM was lower when there was a higher disease level. AF affects how quickly the infections proceeds without eliminating it.

Nutrient analysis showed that AF application increased boron levels in cucumber leaves. Plants receiving AF at a rate of 0.5 mL/100 mL significantly (P=0.0353) increased B levels by about 6-fold compared to the control (Table 3.3). AF at 0.1 and 0.3

mL/100 mL also increased boron levels in cucumber leaves; while, there were no significant differences among control and AF treatments at these two concentrations.

3.3.2. Phenolic compounds in cucumber leaves

Boron applications can induce several secondary metabolites with high toxicity to pathogens, such as phenolic compounds. All cucumber leaves treated with AF applications had different levels of phenolics from the control leaves without AF applications. However, there were no significant differences among cucumber leaves with AF treatments. Cucumber leaves treated with AF at 0.3 ml/100 ml had the highest concentration of phenolic compounds at 16.42 mg per gm leaf tissue. The levels of phenolic compounds in control leaves were much lower than cucumber leaves with AF treatments (Figure 3.3).

3.3.3. Changes of POD and PPO

Results showed that cucumber leaves treated with AF at 0.1 ml/100 ml had the highest concentration of POD (Figure 3.4) and PPO (Figure 3.5). However, there were no significant differences between control and AF treatments.

3.4. Discussion

The management of PM could be difficult because the fungus *P. xanthii* potentially develops fungicide resistance quickly. Micronutrients such as boron may play an important role in reducing disease severity and could be an alternative to be used in agriculture. In the present study, applications of AF at higher concentrations suppressed the development of PM under low to moderate disease pressure. However, efficacy of AF against PM was lower under high disease pressure. Generally, 1 L of AF is mixed with 20 L water for foliar application in the field (www.activeagriscience.com). Concentrations of AF applied at 0.1, 0.3 and 0.5 ml/100 ml in this study in the

greenhouse are much lower than field rate applications. AF contains high levels of boron at 3%. Boron is an essential micronutrient for growth of plants and may be involved in disease suppression due to it's role in cell wall formation and stability, the maintenance of structural and functional integrity of biological membranes, and the movement of sugar into growing parts of plants (Stangoulis & Graham 2007; Dordas 2008). Our results showed that cucumber leaves treated with AF at a rate of 0.5 mL/100 mL had significantly higher boron levels than control leaves treated with water only. Schutte (1967) found that the fungi develop more rapidly in boron deficient wheat plants, resulting in the disease severity in boron deficient plants several times higher than that in boron sufficient plants. Abundant boron in cucumber leaves may provide protection for plants against PM fungi. Previous studies reported that boron suppressed many important pathogenic diseases including clubroot (*Plasmodiophora brassicae*) in crucifers, damping off (*Fusarium solani*) in bean, Verticillium wilt (*Verticillium albo-atrum*) in tomato, PM (*Blumeria graminis*) in wheat and tomato leaf curl virus in tomato (Marschner 1995; Simoglou & Dordas 2006). In additional, experimental results showed that foliar treatments of B (as H_3BO_3, at 6.25 mg/L of boron) decreased the number of lesions and severity of tan spot in wheat and was involved in physiological and biochemical effects in plants (Simoglou & Dordas 2006). In addition, Mertely et al. (2005) indicated that the biofungicide product of Prevam containing boron, orange oil and organic surfactants effectively suppressed PM development in strawberries.

Boron may not directly inhibit fungal growth (Frenkel et al. 2010; Ni & Punja 2019). Our in *vitro* results showed that boron has a weak effect on fungal growth in PDB (Ni & Punja 2019), which was similarly found by Frenkel et al. in *vitro* experiments (2010). The suppression of PM development in cucumber plants may be due to the systemic acquired resistance which could be induced by the applications of boron (Simoglou & Dordas 2006). Foliar applications of boron (in the form of H_3BO_3) protected cucumber plants against PM because boron has the ability to release Ca^{2+} cations from the cell wall, resulting in induction of systemic acquired resistance (Reuveni et al. 1997; Reuveni & Reuveni 1998). It was reported that boron applications activate systemic acquired resistance in tomato and potato to reduce the infection caused by *Phytophthora infestans* (Frenkel et al. 2010). On the other hand, Paveley et al. (1997) demonstrated that the fungicides reduce the disease severity because they increase the time for photosynthesis by delaying the loss of green leaf area. It is obviously that PM fungi

colonize the surfaces of leaves rapidly, thereby severely affecting photosynthesis in plants. It seems that applications of AF delay the spread of pathogen, which allows plants to undergo more photosynthesis than control plants. In addition, high levels of boron in plants inhibit the glycolysis pathway to suppress the fungal growth of *Penicillium chrysogenum* and *Saccharomyces cerevisae* (Bowen & Gauch 1966). The activity of aldolase could be inhibited by high levels of B, which negatively affect the ability of fungi to use carbohydrates for maintaining metabolic process (Bowen & Gauch 1966; Rolshausen & Gubler 2005).

Our results indicated that high levels of phenolic compounds produced by cucumber plants potentially provide protection against PM, which may be induced by the applications of AF. Applications of boron can enhance the level of phenolic compounds in plants (Camacho-Cristobal et al. 2008; Frenkel et al. 2010). As critical components of the cell wall, phenolic compounds are involved in various biological functions, such as providing mechanical support and strength, mediation of growth and morphogenesis, as well as stress- or pathogen- induced responses (Moalemi et al. 2012). Rapid accumulation of phenolic compounds and lignin is the first step in the defense mechanism of plants in response to fungal infection to suppress the invasion of a pathogen (Matern & Kneusel 1988; Liew et al. 2012). Furthermore, plants can produce several antioxidant enzymes in response to invasion by pathogens (Malencic et al. 2010). However, according to our results from enzyme analysis, no significant differences were found between control and AF-treated leaves in POD and PPO.

It was observed that high levels of B cause phytotoxicity on plants (Ben-Gal & Shani 2003; Yermiyahu et al. 2006). For example, boron applications resulted in a reduction in the severity of early blight (*Alternaria solani*) in potato plants; meanwhile, phytotoxicity was found on leaves (Frenkel et al. 2010). Similarly, we also found that AF at 0.5 mL/100 mL with high levels of boron was phytotoxic, causing leaf margin necrosis on cucumber plants. Cucumber appeared to be more sensitive to AF than canola (Ni & Punja 2019). Thus, further studies are required to determine the optimum level of boron for the control of PM on greenhouse cucumbers, preventing the damage caused by phytotoxicity.

The findings from this study suggest that the foliar fertilizer AF has a potential to be involved in the integrated disease management of PM in greenhouse cucumber, and

the disease suppression may be closely associated with the enhanced boron levels in leaves. It seems that boron is unable to inhibit fungi growth directly, but it may suppress the disease severity by the induction of secondary metabolites. However, the efficacy of AF on disease control may be affected by disease severity. Additional studies are required to find out the optimum concentration of boron to limit the negative effects due to phytotoxicity.

Acknowledgments

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Tables

Table 3.1. Powdery mildew development on cucumber leaves treated with three concentrations of AF in trial 1.

Treatments	Number of powdery colonies
Control	199 a
AF-01	132 b
AF-03	49 c
Af-05	49 c

Trial 1 was conducted during June to October 2016. Data are the average of 20 replicate leaves in each treatment. There are five cucumber plants in each treatment.
Applications were made weekly 3 times at the onset of mildew development. Plants were rated after three AF applications.
Means followed by a different letter are significantly different at P=0.05 according to Tukey's HSD test.

Table 3.2. Number of PM colonies on cucumber plants after three applications of Active Flower at 0.1, 0.3 and 0.5 mL/100 mL in trial 2.

Treatment	Week 0	Week 1	Week 2	Week 3
Control (Water)	35.4 a	114.5 a	135.16 a	157.92 a
AF-01	28.35 a	80.25 ab	105.32 ab	139.12 ab
AF-03	35.8 a	55.0 bc	87.88 b	110.32 b
AF-05	28.0 a	40.9 c	89.24 b	123.76 ab

Trial 2 was conducted during June to September 2018. Number of powdery colonies were counted before the first foliar AF treatments on week 0. Data are the average of 20 replicate leaves in each treatment. There are five cucumber plants in each treatment. Applications were made weekly 3 times at the onset of mildew development. Plants were rated weekly. Means followed by a different letter are significantly different at P=0.05 according to Tukey's HSD test.

Table 3.3. Boron levels in cucumber leaves after four applications of Active Flower at 0.1, 0.3 and 0.5 mL/100 mL.

Treatments	B level (ppm)
Control (Water)	19 b
AF-01	38.67 ab
AF-03	90.67 ab
Af-05	128.67 a

Boron levels were determined in treated cucumber leaves when receiving four applications of AF. Data are the average of 9 replicate leaves in each treatment. Means followed by a different letter are significant at P=0.05 according to Tukey's HSD test.

Figures

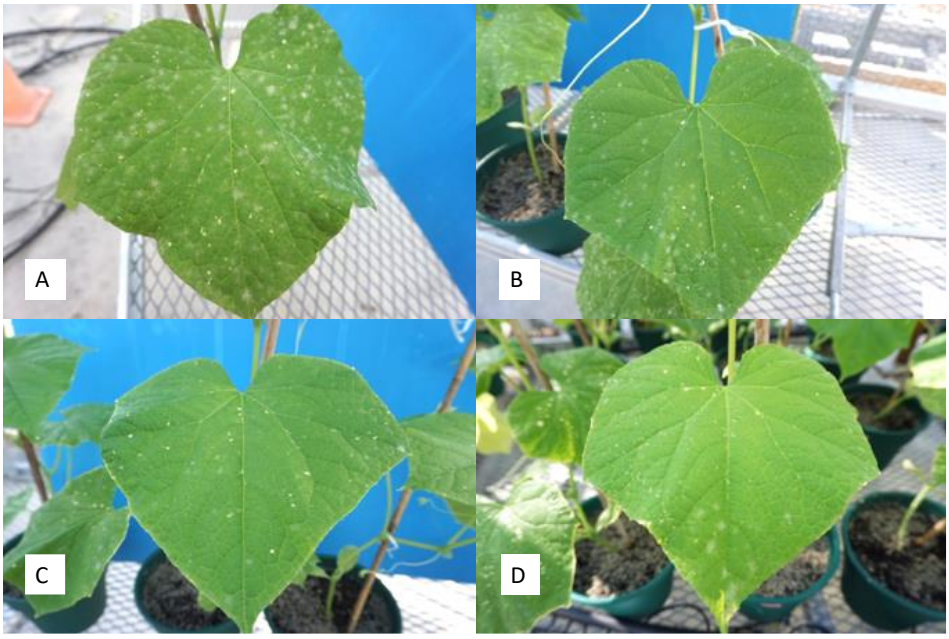

Figure 3.1. **Comparison of Control (A) and AF-treated leaves at 0.1 (B), 0.3 (C) and 0.5 (D) mL/100 mL after three applications.**

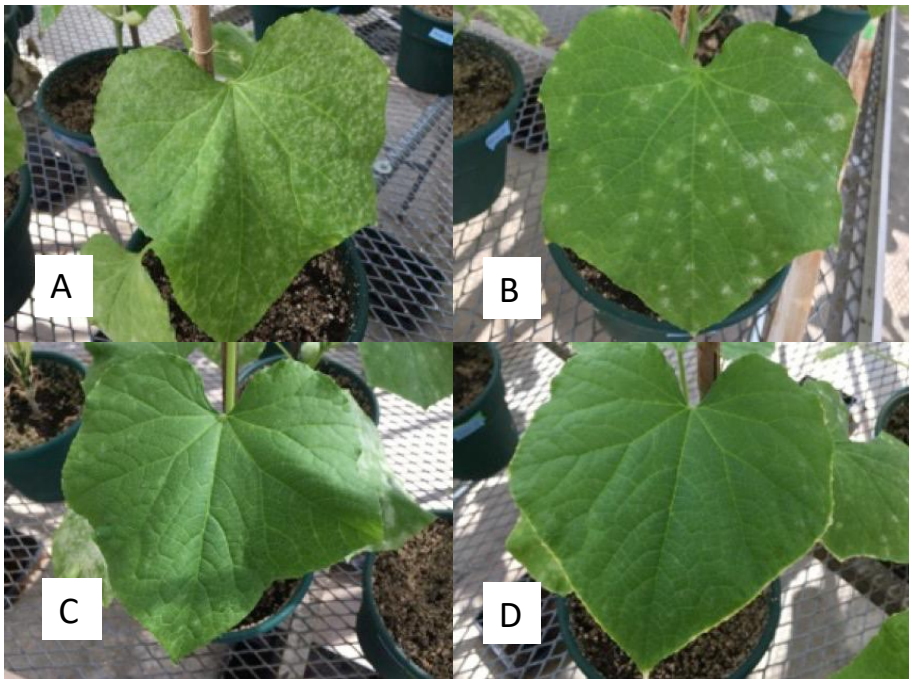

Figure 3.2. **Comparison of Control (A) and AF-treated leaves at 0.1 (B), 0.3 (C) and 0.5 (D) mL/100 mL after one application.**

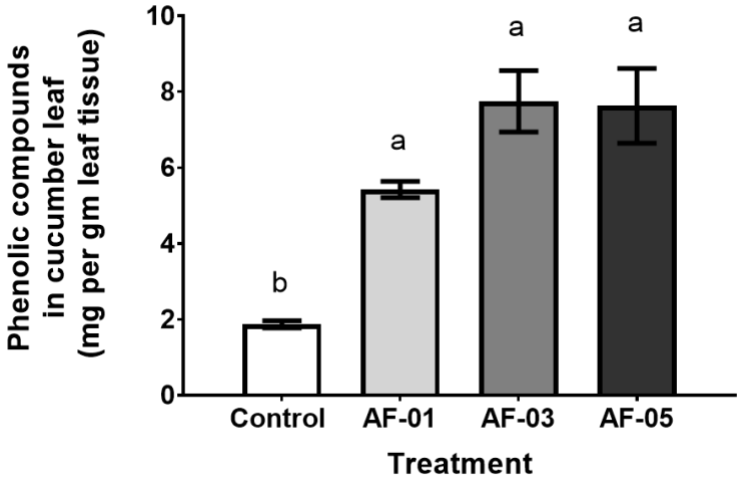

Figure 3.3 **Levels of phenolic compounds in cucumber leaves treated with four applications of Control and AF at 0.1, 0.3 and 0.5 mL/100 mL. Values are means ± S.E., n=6 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different ($p<0.05$).**

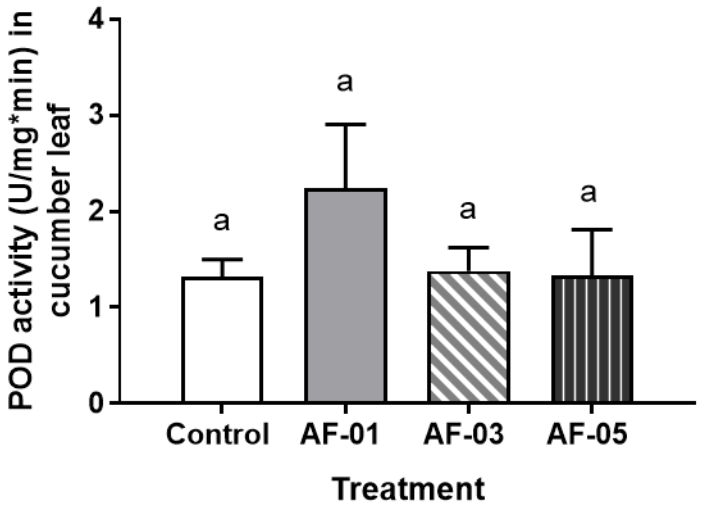

Figure 3.4. **The enzymatic activity of POD in cucumber leaves treated with four applications of Control and AF at 0.1, 0.3 and 0.5 mL/100 mL. Values are means ± S.E., n=6 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different (p<0.05).**

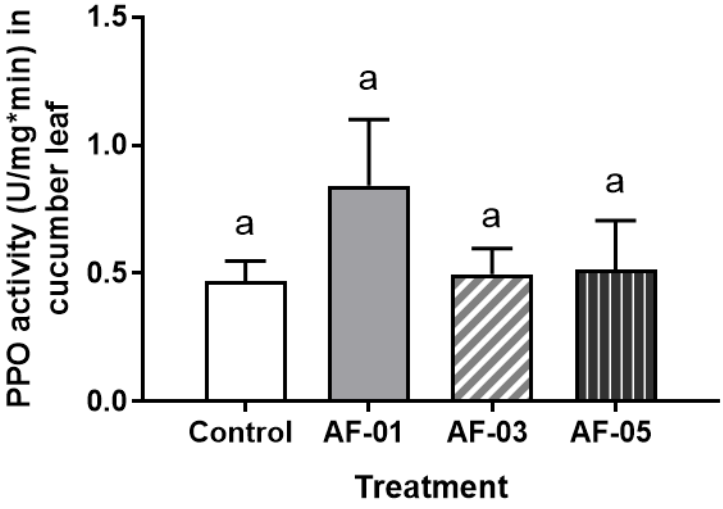

Figure 3.5. **The enzymatic activity of PPO in cucumber leaves treated with four applications of Control and AF at 0.1, 0.3 and 0.5 mL/100 mL. Values are means ± S.E., n=6 for all four groups. One-factor ANOVA and Tukey's multiple comparison test were used to test the effects of different WSF concentrations and exposure length. Values that do not share a common letter are statistically different (p<0.05).**

Chapter 4.

Discussion

This is the discussion for all of the chapters. To evaluate the effectiveness of the foliar fertilizer of Active Flower (AF) in inhibition of fungal growth, I measured mycelial growth of 5 fungi in PDB. Mycelial growth of *S. sclerotiorum, Botrytis cinerea, Fusarium oxysporum*, *Pythium dissotocum, Phoma lingam and Thielaviopsis basicola* was inhibited by AF and AFWB at a relatively high concentration, but it appeared that AF had a greater antifungal effect than AFWB. As an important component of AF, boric acid (BA) did not inhibit mycelial growth in liquid culture. It suggests that boron seems not to act directly on fungal growth. Since AF is a complex mixture of several different ingredients, the fungitoxicity observed at 0.5 mL/100 mL is likely due to the presence of other components.

The efficiency of AF in the reduction of development of Sclerotinia stem rot (SSR) was tested by the inoculation of AF-treated canola leaves with *S. sclerotiorum* under a controlled environment. The disease was suppressed on detached canola leaves treated with AF at a rate of 0.5 mL/100 mL. The role of boron in suppression of disease development also was shown in previous research for various economically important crops. In addition, application of AF at a rate of 0.5 mL/100 mL reduced the development of powdery mildew (PM) on cucumber leaves under low to moderate disease pressure. However, efficacy of AF on disease control of PM may be affected by disease severity because PM was not completely suppressed under high disease pressure.

Boron level were significantly enhanced in AF-treated canola and cucumber leaves. Application of BA also increased B level in canola leaves. The severity of fungal diseases may be associated with boron since it plays an important role in cell wall formation and stability, maintenance of structural and functional integrity of biological membranes, and production of phenolic compounds or lignin (Brown et al. 2002; Dordas 2008; Elmer 2015). Boron applications can induce several secondary metabolites with high toxicity to pathogens, including phenolic compounds (Ruiz et al. 1998; Camacho-Cristobal et al. 2008). Phenolic compounds can directly inhibit fungal growth and sporulation by suppressing the development of hyphae or binding the enzymes

produced by fungi (Delrio et al., 2004). In this study, levels of phenolic compounds were highly enhanced in canola and cucumber leaves treated with AF or BA applications, which could explain the reduced disease levels as these compounds are fungitoxic. Similarly, phenolic compounds were highly enhanced in response to *S. sclerotiorum* infection in resistant sunflower (Liu et al. 2017). Severity of PM on leaves was reduced in cucumber cultivars when the levels of phenolic compounds were significantly increased (Daayf, et al., 2000). Our results indicated that phenolic compounds produced by canola and cucumber plants potentially provide protection against fungal pathogens, which may be induced by the application of AF.

The suppression of PM development in cucumber plants may be due to the systemic acquired resistance which could be induced by the application of B (Simoglou & Dordas 2006). Foliar applications of BA protected cucumber plants against PM because boron has the ability to release Ca^{2+} cations from the cell wall, resulting in induction of systemic acquired resistance (Reuveni et al. 1997; Reuveni & Reuveni 1998). On the other hand, the fungicides reduce the disease severity because they increase photosynthesis by delaying the loss of green leaf area (Paveley et al. 1997). It is known that PM fungi cover the surfaces of leaf rapidly, thereby severely affecting photosynthesis in plants. It seems that applications of AF delay the spread of PM fungi, which allows plants to do more photosynthesis than control plants.

Applications of B were able to improve lignin formation in rice (Dobermann & Fairhurst 2000). However, in canola, foliar applications of AF containing 3% boron did not increase lignin levels in foliage. A recent study revealed that lignin modification in alfalfa did not increase foliar disease resistance (Samac et al. 2018). According to our results, lignin may not play an important role in increasing disease resistance to SSR in canola.

In enzyme analysis, there were no significant increases in the activities of POD and PPO in AF-treated canola and cucumber leaves. It was reported that B application can induce the production of POD that has high toxicity to pathogens (Ruiz et al. 1998; Camacho-Cristobal et al. 2008). However, it seems that application of AF cannot induce the enzymatic activity of POD or the level of B in AF was too low to induce these activities. The activity of PPO might not play a critical role in defensive mechanisms of

the plant against *S. sclerotiorum* in sunflower (Liu et al. 2017). Our results also suggest that the level of PPO was not increased in the plant in response to SSR or PM.

High levels of boron are likely to cause phytotoxicity on plants (Ben-Gal & Shani 2003; Yermiyahu et al. 2006). In present study, phytotoxicity was observed on older canola foliage as burning of tips or edges on some leaves treated with AF at a rate of 0.5 mL/100 mL (150 ppm boron). Cucumber appeared to be more sensitive to AF than canola. An obvious leaf margin necrosis was observed on cucumber leaves treated with AF at the same concentration. Symptoms of boron phytotoxicity were reported on canola leaves at a much lower rate under greenhouse conditions than in the field (Deora et al. 2011). It suggests that the level of boron needs to be reduced when applied to greenhouse crops for disease management. The optimum level of boron used in reducing disease incidence and severity may vary depending on the susceptibility of crops.

Application of AF containing 3% boron potentially offers an alternative approach for the integrated management of SSR on canola plants and PM on cucumber plants. AF treatments were found to inhibit fungal growth by measuring the dry weights of fungi. Boron in the form of BA is an important component of AF which did not inhibit mycelial growth in liquid culture, whereas it probably has an indirect effect in enhancing plant defense by induction of secondary metabolites. The levels of phenolic compounds were enhanced in AF-treated leaves, which could increase the resistance of canola against the invasion of *S. sclerotiorum*. In the present study, the levels of POD and PPO were not increased by AF applications. The resistance mechanism of crop leaves also involves other enzymes responding to pathogen invasion. For example, phenylalanine ammonialyase (PAL) is an important enzyme in the metabolism of phenylpropanoid compound, which regulates the biosynthesis of flavonoids to increase crop resistance (He et al. 2018). More experiments could be conducted to determine if the activity of PAL is highly induced in AF-treated leaves. Other research experiments that could be conducted include testing the activities of pathogenesis-related proteins (PRs) in leaves, such as chitinase and β-1,3-glucanase (He et al. 2018). These PRs participate in the resistance mechanism of crops under environmental stress to inhibit fungal growth and improve disease resistance (He et al. 2018). The assessment of PM severity could be conducted by taking digital images of treated leaf and calculating the “white” areas of mildew infection compared to the background “gray” representing healthy areas using

ImageJ software (https://imagej.net/Welcome) instead of counting the number of PM colonies. Further studies are required to confirm the mechanism of boron in disease suppression following foliar application in canola. In the present study, efficacy of boron was assessed only for detached canola leaves receiving four applications of BA under greenhouse environments. Our results indicate that application of boron has potential as a component of the management of SSR of canola. Research is still needed to evaluate the efficacy of boron in disease suppression on leaves that are attached to live plant. Further research is also required to determine the optimum level of boron for the control of fungal disease of different crops, preventing the damage on leaves caused by phytotoxicity. The concentrations of AF used in fields are much higher than the concentration used in the greenhouse. The efficacy of AF in reducing disease development in canola fields is unknown. Therefore, a long-term field experiment is required to evaluate the efficacy of boron in the suppression of disease severity. Besides, more experiments could be conducted to determine the effectiveness of boron on other economically important crops for disease management, such as sunflowers, soybeans, and squash.

References

Active AgriScience, 2017. Active Flower case studies. Available at: http://activeagriscience.com/case-studies/active-flower

Adams, P.B., Ayers, W.A., 1979. Ecology of *Sclerotinia* species. *Phytopathology*, 69, 896-898.

Agriculture and Agri-Food Canada, 2014. Crop profile for greenhouse cucumber in Canada, 2014. Available at: http://publications.gc.ca/collections/collection_2016/aac-aafc/A118-10-15-2014-eng.pdf

Agriculture and Agri-Food Canada, 2015. Canadian canola. Available at: http://www.agr.gc.ca/eng/industry-markets-and-trade/buying-canadian-food-products/canadian-canola/?id=1426170869200

Agriculture and Agri-Food Canada, 2016a. Crop profile for greenhouse cucumber in Canada, 2014. Available at: http://publications.gc.ca/collections/collection_2016/aac-aafc/A118-10-15-2014-eng.pdf

Agriculture and Agri-Food Canada, 2016b. Statistical overview of the Canadian greenhouse vegetable Industry, 2016. Available at: http://www.agr.gc.ca/eng/industry-markets-and-trade/canadian-agri-food-sector-intelligence/horticulture/horticulture-sector-reports/statistical-overview-of-the-canadian-greenhouse-vegetable-industry-2016/?id=1511888129369

Agrios, G. N. 1997. Plant Pathology. 4th ed. Academic Press, San Diego, CA.

Agrios, G.N., 2005. Plant Pathology. Fifth edition. Burlington: Elsevier Academic Press.

Almqvist, A., 2012. Biological control of powdery mildew in greenhouse produced cucumber. Available at: https://stud.epsilon.slu.se/5163/1/almqvist_a-c_130103.pdf

Ankom technology, 2016. Method 8 – determining acid detergent lignin in beakers. Available at: https://www.ankom.com/sites/default/files/document-files/Method_8_Lignin_in_beakers.pdf

Bardin, S.D., Huang, H.C., 2001. Research on biology and control of sclerotinia diseases in Canada. *Canadian Journal of Plant Pathology*, 23, 88-98.

Barker, B., 2016. Sclerotinia stem rot in review. Available at: https://www.topcropmanager.com/sclerotinia-stem-rot-in-review-18571/

Bashi, E., Aust, H.J., 1980. Quality of spores produced in cucumber powdery mildew compensates for their quantity. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 87, 594–599.

Ben-Gal, A., Shani, U., 2003. Water use and yield of tomatoes under limited water and excess boron. *Plant and Soil*, 256, 179-86.

Beltrán-Peña, H., Solano-Báez, A.R., Apodaca-Sánchez, M.Á., Camacho-Tapia, M., Félix-Gastélum, R., Tovar-Pedraza, J.M., 2018. First report of Leveillula taurica causing powdery mildew on cucumber in Mexico. *Journal of Plant Pathology*, 100, 353–353.

Bettiol, W., 1999. Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginea*) in greenhouse conditions. *Crop Protection*, 18, 489-492.

Blevins, D.G., Lukaszewski, K.M., 1998. Boron in plant structure and function. *Annual Review Plant Physiology Plant Molecular Biology,* 49, 481-500.

Boland, G.J., 1997. Stability analysis for evaluating the influence of environment on chemical and biological control of white mold (*Sclerotinia sclerotiorum*) of bean. *Biological Control*, 9, 7-14.

Bolton, M.D., Thomma, B.P., Nelson, B.D., 2005. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Molecular Plant Pathol*ogy, 7, 1-16.

Bowen, J.E., Gauch, H.G., 1966. Nonessentiality of boron in fungi and the nature of its toxicity 1. *Plant Physiology*, 41, 319-324.

Braun, U., Cook, R.T.A., Inman, A.J., Shin, H.D., 2002. The taxonomy of the powdery mildew fungi. Pages 13-55 in: The powdery mildews: A Comprehensive Treatise, vol. 1. In: Belanger, R. R. Bushnell, W.R., Dik A.J., Carver, T.L.W. edition. *American Phytopathological Society Press*, Saint Paul, MN.

Brown, J., 2002. Comparative genetics of avirulence and fungicide resistance in the powdery mildew fungi. In Bélanger, R., Bushnell, W.R., Dik, A.J., Carver, T.L.W. edition. The powdery mildews. A Comprehensive Treatise. APS Press, St. Paul, Minnesota, pp. 56-65.

Brown, P.H., Bellaloui, N., Wimmer, M.A., Bassil, E.S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F., Romheld, V., 2002. Boron in plant biology. *Plant Biology*, 4, 205-223.

Budge, S.P., Whipps, J.M., 1991. Glasshouse trials of *Coniothyrium minitans* and Trichoderma species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. *Plant Pathology*, 40, 59-66.

Budge, S.P., Whipps, J.M., 2001. Potential for integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. *Phytopathology*, 91, 2.

Camacho-Cristobal, J.J., Rexach, J., Gonzalez-Fontes, A., 2008. Boron in plants: deficiency and toxicity. J. *Integrative Plant Biology*, 50, 1247-1255.

Canola Council of Canada, 2017a. Canola now worth $26.7 billion to Canadian economy. Available at: https://www.canolacouncil.org/news/canola-now-worth-$267-billion-to-canadian-economy/

Canola Council of Canada, 2017b. Canola meal research shows an increase in milk production and feed cost saving. Available at: https://www.canolacouncil.org/news/canola-meal-research-shows-an-increase-in-milk-production-and-feed-cost-savings/

Canola Council of Canada, 2017c. Sclerotinia stem rot. Available at: https://www.canolacouncil.org/canola-encyclopedia/diseases/sclerotinia-stem-rot/

Cawoy, H., Bettiol, W., Fickers, P., Ongena, M., 2011. *Bacillus*-based biological control of plant diseases. In pesticides in the modern world - pesticides use and management. Available at: https://www.intechopen.com/books/pesticides-in-the-modern-world-pesticides-use-and-management/bacillus-based-biological-control-of-plant-diseases

Cawoy, H., Debois, D., Franzil, L., Pauw, E.D., Thonart, P., Ongena, M., 2014. Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. *Micro Biotechnology*, 8, 281-295.

Cerkauskas, R.F., Ferguson, G., 2014. Management of powdery mildew (*Podosphaera xanthii*) on greenhouse cucumber in Ontario. *Canadian Journal of Plant Pathology*, 36, 22-37.

Choi, I.Y., Cheong, S.S., Joa, J.H., Cho, S.E., Shin, H.D., 2014. First report of powdery mildew caused by *Podosphaera xanthii* on *Sechium edule* in Korea. *Plant Disease*, 99, 162–162.

Conrath, U., Beckers, G.J.M., Flors, V., Garcia-Agustin, P., Jakab, G., Mauch, F., Newman, M.A., Pieterse, C.M.J., Poinssot, B., Pozo, M.J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., Mauch-Mani, B., Prime, A., 2006. Priming: Getting ready for battle. *Molecular Plant-Microbe Interactions*, 19,1062-1071

Corner, E.J.H., 1935. Observations on resistance to powdery mildews. *New Phytologist*, 34, 180-200.

Delrio, J.A., Gomez, P., Baidez, A., Fuster, M.D., Ortuno, A., Frias, V., 2004. Phenolic compounds have a role in the defence mechanism protecting grapevine against the fungi involved in petri disease. *Phytopathologia Mediterranea*, 43, 87-94.

Deora, A., Gossen, B.D., Walley, F., McDonald, M.R., 2011. Boron reduces development of clubroot in canola. *Canadian Journal of Plant Pathology*, 33, 475-484.

Dey, D., Calpas, J., Morton, D., Dyck, D., 2017. Commercial greenhouse vegetable production. Government of Alberta agriculture and forestry. Available at: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex1443

Dik, A., Albajes, R., 1999. Principles of epidemiology, population biology, damage relationships and integrated control of diseases and pests. In Albajes, R, L Gullino, J, van Lenteren, Y, Elad, edition. Integrated pest and disease management in greenhouse crops. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 69-81.

Djaman, K., O'Neill, M., Owen, C., Smeal, D., West, M., Begay, D., Angadi, S., Koudahe, K., Allen, S., Lombard, K., 2018. Seed yield and water productivity of irrigated winter canola (*Brassica napus* L.) under semiarid climate and high elevation. *Agronomy*, 8, 90.

Dobermann, A., Fairhurst, T., 2000. Rice - nutrient disorder and nutrient management. 1st Edition. Los Baños, Laguna, IRRI and Singapore, Potash & Phosphate Institute.

Dordas, C., 2008. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agronomy Sustainable Development*, 28, 33-46.

Du, X-l., Xing, G-y., Ren, A-z., Zhao, P-b., 2010. Control effect of vegetable oil on cucumber powdery mildew. *Plant Disease and Pests*, 1, 43-46.

Dutta, S., Ghosh, P., Ghorai, A., Roy, M.D., Das, S., 2017. Micronutrients and Plant Disease Suppression. *Fertilizers and Environment News*, 3, 4-8.

Eaton, F.M., 1930. The effect of boron on powdery mildew and spot blotch of barley. *Phytopathology*, 20, 967-972.

Elmer, W.H., 2015. Using mineral nutrition to suppress plant diseases. Fact sheet. The Connecticut Agricultural Experiment Station. Department of Plant Pathology and Ecology. New Haven. Available at: www.ct.gov/caes.

Ferreira, S.A., Boley, R.A., 1992. *Sclerotinia sclerotiorum*. Available at: http://www.extento.hawaii.edu/kbase/crop/type/s_scler.htm

French, R., Schultz, D., 2009. Powdery mildew of cucurbits. Texas AgriLife Extension Service. A&M Plant Pathology and Microbiology. Available at: http://amarillo.tamu.edu/files/2010/11/PowderyMildewCucurbits.pdf

Frenkel, O., Yermiyahu, U., Forbes, G.A., Fry, W.E., Shtienberg, D., 2010. Restriction of potato and tomato late blight development by sub-phytotoxic concentrations of boron. *Plant Pathology*. 59, 626-633.

Gay, J.L., Martin, M., Ball, E., 1985. The impermeability of powdery mildew conidia and their germination in arid environments. *Plant Pathology*, 34, 353-362.

Ge, J.C., Ki Yoon, S., Choi, N., 2017. Using canola oil biodiesel as an alternative fuel in diesel engines: A review. *Applied Science*, 7, 881.

Ghaffar, A., 1972. Some observations on the parasitism of *Coniothyrium minitans* on the sclerotia of *Sclerotinia sclerotiorum*. *Pakistan Journal of Botany*, 4, 85-87.

Glawe, D.A., 2008. The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. *Annual Review Phytopathology*, 46, 27-51.

Graham, D.R., Webb, M.J., 1991. Micronutrients and disease resistance and tolerance in plants. In: Mortvedt, J.J., Cox, F.R., Shuman, L.M. and Welch R.M. (Eds.), Micronutrients in Agriculture, 2nd ed. Soil Science Society of America Inc., Madison,Wisconsin, USA, pp. 329–370.

Green, J.R., Carver, T.L.W., Gurr, S.J., 2002. The formation and function of infection feeding structures. In Bélanger, R.R., Bushnell, W.R., Dik, A.J., Carver, T.L.W., edition. The powdery mildews. A Comprehensive Treatise. Saint Paul, MN: APS Press. pp. 66-82.

Gulya, T., Rashid, K.Y., Masirevic, S.M., 1997. Sunflower diseases. In: Schneiter, A.A. edition. Sunflower Technology and Production. American Society of Agronomy, Crop Science Society of American, Soil Science Society of America, Madison, Wisconsin. Pp. 263-379.

Hajlaoui, M.R., Benhamou, N., Belanger, R.R., 1992. Cytochemical study of the antagonistic activity of *Sporothrix flocculosa* on rose powdery mildew, *Sphaerotheca pannosa var. rosae*. *Phytopathology*, 82, 583-589.

Hanlin, R.T., 1998. Combined keys to lllustrated genera of Ascomycetes volumes I & II. APS Press. St. Paul, MN.

Heffer, V., Johnson, K.B., Powelson, M.L., Shishkoff, N., 2006. Identification of powdery mildew fungi anno. The Plant Health Instructor. Available at: https://www.apsnet.org/edcenter/disandpath/fungalasco/labexercises/Pages/PowderyMildew.aspx

Heffer, L.V., Johnson, K.B., 2007. White mold (Sclerotinia). The Plant Health Instructor. Available at: https://www.apsnet.org/edcenter/disandpath/fungalasco/pdlessons/Pages/WhiteMold.aspx

He, Y., Li, X., Zhan, F., Xie, C., Zu, Y., Li, Y., Yue, M., 2018. Resistance-related physiological response of rice leaves to the compound stress of enhanced UV-B radiation and *Magnaporthe oryzae*. *Journal of Plant Interactions*, 13, 321–328.

Hollomon, W.D., Wheeler, E.I., 2002. Controlling powdery mildews with chemistry. In Belanger, R.R., Bushnell, R.W., Dik, J.A., Carver, W.L.T. edition. The powdery mildews. A Comprehensive Treatise. 249-255, APS Press, ISBN 0-89054-291-0, St. Paul, MN, USA.

Huang, H.C., 1981. Distribution of *Coniothyrium minitans* in Manitoba sunflower fields. *Canadian Journal of Plant Pathology*, 3, 219-222.

Huang, H.C., 1983. Pathogenicity and survival of the tan-sclerotia strain of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology*, 5, 245-247.

Hunter, J.E., Steadman, J.R., Cigna, J.A., 1982. Preservation of ascospores of *Sclerotinia sclerotiorum* on membrane filters. *Phytopathology*, 72, 650-652.

Hural, K., 1986. Influence of atmospheric humidity on sporulation of *Sphaerotheca pannosa* (Wallr.) Lev. var. *rosae* Wor. Master's Thesis. Available at: https://ir.library.oregonstate.edu/downloads/td96k612c

Jahn, M., Munger, H.M., McCreight, J.D., 2002. Breeding cucurbit crops for powdery mildew resistance. In Bélanger, R.R., Bushnell, W.R., Dik, A.J., Carver, T.L.W. edition. The powdery mildews. A Comprehensive Treatise. The American Phytopathological Society, St. Paul, Minnesota, pp. 239-248.

Jarvis, W., Gubler, W.G., Grove, G.G., 2002. Epidemiology of powdery mildews in agricultural ecosystems. In Belanger, R., Bushnell, W.R., Dik, A.J., Carver, T.L.W. edition. The powdery mildews. A Comprehensive Treatise. The American Phytopathological Society, St. Paul, Minnesota, pp. 169-199.

Jiang, J.F., Wan, X., Li, J.G., Dong, Y.H., 2016. Effect of boron nutrition on resistance response of tomato against Bacterial wilt caused by *Ralstonia solanacearum*. *Journal of Plant Pathology*, 98, 117-122.

Jones, D., Gordon, A.H., Bacon, J.S.D., 1974. Co-operative action by endo- and exo-β-(1–3)-glucanases from parasitic fungi in the degradation of cell-wall glucans of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Biochemical Journal*, 140, 47-55.

Jones, D., Watson, D., 1969. Parasitism and lysis by soil fungi of *Sclerotinia sclerotiorum* (Lib.) de Bary, a phytopathogenic fungus. *Nature*, 224, 287-288.

Jurke, C., Phillips, K., Senko, S., Holmes-Saltzman, A., Orchard, D., Gabert, K., Sekulic, G., 2013. Moisture raises Sclerotinia stem rot risk. Canola council of Canada. Available at: https://www.canolacouncil.org/news/moisture-raises-sclerotinia-stem-rot-risk/

Kandel, H., Lubenow, L., Keene, C., Knodel, J.J., 2019. Canola production field guide. Published in cooperation with and support from the Northern canola grower association. Available at: https://www.ag.ndsu.edu/publications/crops/canola-production-field-guide#section-64

Karamanos, R.E., Goh, T.B., Stonehouse, T.A., 2002. Canola response to boron in Canadian prairie soils. *Canadian Journal of Plant Science*, 83, 249–259.

Kaur, G., Nelson, K.A., 2015. Effect of foliar boron fertilization of fine textured soil on corn yields. *Agronomy*, 5, 1-18.

Khangura, R., Beard, C., Hills, A., 2018. Managing sclerotinia stem rot in canola. Agriculture and Food. Department of primary industries and regional development. Government of Western Australia. Available at: https://www.agric.wa.gov.au/canola/managing-sclerotinia-stem-rot canola?page=0%2C0

Khan, S., Anwar, S., Kuai, J., Ullah, S., Fahad, S., Zhou, G., 2017. Optimization of nitrogen rate and planting density for improving yield, nitrogen use efficiency, and lodging resistance in oilseed rape. *Frontiers Plant Science*, 8, 532.

Kirk, P.M., Cannon, P., David, J., Stalpers, J., 2001. Ainsworth and Bisby's Dictionary of the Fungi. 9th edition. CABI Publishing; Wallingford, UK. pp. 655.

Kristkova, E., Lebeda, A., Sedlakov., B., 2009. Species spectra, distribution and host range of cucurbit powdery mildews in the Czech Republic, and in some other European and Middle Eastern countries. *Phytoparasitica*, 37, 337-350.

Kutcher, H.R., Wolf, T.M., 2006. Low-drift fungicide application technology for sclerotinia stem rot control in canola. *Crop Protection*, 25, 640-646.

Lebeda, A., 1983. The genera and species spectrum of cucumber powdery mildew in Czechoslovakia. *Phytopathology Zeitschrift*, 108, 71-79.

Lebeda, A., Sedláková, B., Křístková, E., Vysoudil, M., 2010. Long-lasting changes in the species spectrum of cucurbit powdery mildew in the Czech Republic – influence of air temperature changes or random effect? *Plant Protection Science*, 45, S41–S47.

Lebeda, A., MgGrath, M.T., Sedlakova, B., 2010. Fungicide Resistance in Cucurbit Powdery Mildew Fungi, Fungicides, In Carisse, O. edition. InTech, Available at: http://www.intechopen.com/books/fungicides/fungicide-resistance-in-cucurbit-powdery-mildew-fungi

Li, Y.H., 2012. Powdery mildew of cucurbits. The Connecticut Agricultural Experiment Station. Department of plant pathology and ecology. Available at: http://www.ct.gov/caes/lib/caes/documents/publications/fact_sheets/plant_pathology_and_ecology/powdery_mildew_of_cucurbits_10-31-12.pdf

Liew, Y.A., Syed Omar, S.R., Husni, M.H.A., Zainal, A.M.A., Nur Ashikin, P.A., 2012. Effects of foliar applied copper and boron on fungal diseases and rice yield on cultivar MR219. *Pertanika Journal of Tropical Agricultural Science*, 35, 339-349.

Lin, L., Allemekinders, H., Dansby, A., Campbell, L., Durance-Tod, S., Berger, A., Jones, P.J., 2013. Evidence of health benefits of canola oil. *Nutrition Review*, 71, 370–385.

Liu, J., Zhang, Y., Meng, Q., Shi, F., Ma, L., Li, Y., 2017. Physiological and biochemical responses in sunflower leaves infected by *Sclerotinia sclerotiorum*. *Physiological and Molecular Plant Pathology*, 100, 41-48.

Loomis, W.D., Durst, R.W., 1992. Chemistry and biology of boron. *BioFactors*, 3, 229-239.

Maia, G.S., 2012. Isolation, Identification and characterization of cucurbit powdery mildew in North Central Florida. Master's thesis defense. Available at: http://ufdc.ufl.edu/UFE0044229/00001

Malencic, D., Kiprovski, B., Popvic, M., Prvulovic, D., Miladinovic, J., Djordjevic, V., 2010. Changes in antioxidant systems in soybean as affected by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Plant Physiology Biochemistry*, 48, 903-908.

Markell, S., Kandel, H., del Rio, L., Halley, S., Olson, L., Mathew, F., Hanson, B., Lamey, A., 2009. Sclerotinia of Canola. North Dakota State University Extension Publication. pp.1410.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, second edition. Academic Press, London, pp. 889.

Martínez-Cruz, J., Romero, D., Dávila, J.C., Pérez-García, A., 2014. The *Podosphaera xanthii* haustorium, the fungal Trojan horse of cucurbit-powdery mildew interactions. *Fungal Genetics and Biology*, 71, 21–31.

Mateljan, G., 2019. Cucumbers. The world's healthiest foods. Available at: http://www.whfoods.com/genpage.php?tname=foodspice&dbid=42

Matern, U., Knuesel, R.E., 1988. Phenolic compounds in plant disease resistance. *Phytoparasitica*, 16, 153-170.

McGrath, M.T., 1994. Heterothallism in *Sphaerotheca fuliginea*. *Mycologia*, 86, 517-523.

McGrath, M.T., 1997. Powdery mildew of cucurbits. Cooperative Extension, Cornell University. Online publication. pp. 732.30. Available at: https://ecommons.cornell.edu/bitstream/handle/1813/43293/powdery-mildew-cukes-FS-NYSIPM.pdf?sequence=1

McGrath, M.T., 2001. Fungicide resistance in cucurbit powdery mildew: experiences and challenges. Plant Disease, 85, 236-245, ISSN 0191-2917.

McGrath, M.T., 2017. Powdery mildew of Cucurbits. Cooperative extension. Available at: http://vegetablemdonline.ppath.cornell.edu/factsheets/Cucurbits_PM.htm

McQuilken, M.P., Budge, S.P., Whipps, J.M., 1997. Production, survival and evaluation of liquid culture produced inocula of *Coniothyrium minitans* against *Sclerotinia sclerotiorum*. *Biocontrol Science Technology*, 7, 23-36.

McQuilken, M.P., Whipps, J.M., 1995. Production, survival and evaluation of solid-substrate inocula of Coniothyrium minitans against *Sclerotinia sclerotiorum*. *European Journal of Plant Pathology*, 101, 101-110.

Merriman, P.R., Pywell, M., Harrison, G., Nancarrow, J., 1979. Survival of sclerotia of *Sclerotinia sclerotiorum* and effects of cultivation practices on disease. *Soil Biology Biochemistry*, 11, 567-570.

Mertely, J., Seiji, T., Torres, C., Peres, N.A., 2005. Evaluation of fungicides to control powdery mildew on annual strawberry. APS Press. St. Paul MN 61: SMF009.

Miazzi, M., Laguardia, C., Faretra, F., 2011. Variation in *Podosphaera xanthii* on Cucurbits in Southern Italy: Variation in *Podosphaera xanthii*. *Journal of Phytopathology*, 159, 538–545.

Michalak, A., 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal Environmental Studies*, 15, 523-530.

Mihalache, G., Balaes, T., Gostin, I., Stefan, M., 2017. Lipopeptides produced by *Bacillus subtilis* as new biocontrol products against *fusariosis* in ornamental plants. *Environmental Science and Pollution Research*, 25, 29784-29793.

Moalemi, R., Aghdasi, M., Ghanati, F., 2012. Induction of phenolic compounds is affected by boron supply in Marshmallow (*Althaea officinalis* L.) cells. *Progress Biological Science*, 1, 68-75.

Morishita, M., Sugiyama, K., Saito, T., Sakata, Y., 2003. Powdery mildew resistance in cucumber. JARQ 37, 7-14. Available at: https://doi.org/10.6090/jarq.37.7

Morrall, R.A.A., Duczek, L.J., Sheard, J.W., 1972. Variation and correlations within and between morphology, pathogenicity, and pectolytic enzyme activity in sclerotinia from Saskatchewan. *Canadian Journal of Botany*, 50, 767-786.

Moyer, C., 2007. Evaluation of silicon and biofungicide products for manafing powdery mildew caused by *Podosphaera fusca* in Gerbera daisy (Gerbera jamesonii). University of Florida. Available at: http://ufdc.ufl.edu/UFE0021376/00001

Nagorska, K., Bikowski, M., Obuchowski, M., 2007. Multicellular behavior and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. *Acta Biochimica Polonica*, 54, 495-508.

Nagy, G.S., 1976. Studies on powdery mildews of cucurbits II. Life cycle and epidemiology of *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*. *Acta Phytopathologica Academiae Scientiarum Hungaricae*, 11: 205–210.

Newman, S., Pottorff, L.P., 2013. Powdery mildews. Colorado State University Extension. Fact sheet No. 2.902.

Newton, H.C., Sequeira, L., 1972. Ascospores as the primary infective propagule of *Sclerotinia sclerotiorum* in Wisconsin. *Plant Disease*, 56, 789-802.

Ni, L., Punja, Z.K., 2019. Effects of a foliar fertilizer containing boron on development of Sclerotinia stem rot (*Sclerotinia sclerotiorum*) on canola (*Brassica napus* L.) leaves. *Journal of Phytopathology*, In Press.

Nott, H., Falloon, R., Cheah, L.H., 1999. Clubroot control from safe chemicals. N.Z. Commer. Grower 54, 24-26.

Nunez-Palenius, H.G., Hopkins, D., Cantliffe, D.J., 2006. Powdery mildew of cucurbits in Florida. University of Florida. IFAS Extension. Available at: http://edis.ifas.ufl.edu

Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J.L., Thonart, P., 2007. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environmental Microbiology*, 9, 1084-1090.

Ongena, M., Jacques, P., 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiology*, 16, 115-125.

Panstruga, R., 2003. Establishing compatibility between plants and obligate biotrophic pathogens. *Current opinion in Plant Biology*, 6, 320-326.

Paulitz, T.C., Belanger, R.R., 2001. Biological control in greenhouse systems. *Annual Review of Phytopathology*, 39, 103-133.

Paulitz, T., Schroeder, K., Beard, T.L., 2015. Sclerotinia stem rot or white mold of canola. Washington State University. Available at: http://cru.cahe.wsu.edu/CEPublications/FS188E/FS188E.pdf

Paveley, N.D., Lockley, K.D., Sylvester-Bradley, R., Thomas, J., 1997. Determination of fungicide spray decisions for wheat. *Pesticide Science*, 49, 379-388.

Pernezny, K., Maynard, D., Stall, W.M., 2008. Powdery mildew of vegetables. Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciecne, University of Florida. Online publication. pp.14.

Perez-Garcia, A., Mignorance, E., Rivera, M.E., Del Pin D., Romero, D., Tores, J.A., De Vicente, A., 2006. Long-term preservation of *Podosphaera fusca* using silica gel. *Journal of Phytopathology*, 154,190–192.

Perez-Garcia, A., Romero, D., Fernandez-Ortuno, D., Lopez-Ruiz, F., Vicente, A., Tores, J.A., 2009. The powdery mildew fungus *Podosphaera fusca* (synonym *Podosphaera xanthii*), a constant threat to cucurbits. *Molecular Plant Pathology*, 10, 153-160.

Phillips, A.J.L., 1989. Fungi associated with sclerotia of *Sclerotinia sclerotiorum* in South Africa and their effects on the pathogen. *Phytophylactia*, 21, 135-139.

Pitt, W.M., Sosnowski, M.R., Huang, R., Qiu, Y., Steel, C.C., Savocchia, S., 2012. Evaluation of fungicides for the management of Botryosphaeria canker of grapevines. *Plant Disease*, 96,1303-1308.

Pizzorno, L., 2015. Nothing Boring About Boron. *Integrative Medicine*, 14, 4.

Pratt, R.G*.,* 1992. *Sclerotinia*: in methods for research on soilborne phytopathogenic fungi, edited by L.L. Singleton, J.D. Mihail, and C.M. Rush. APS Press. St. Paul, MN. pp. 74-77.

Prova, A., Akanda, A.M., Islam, S., Hossain, Md.M., 2018. Characterization of *Sclerotinia sclerotiorum*, an emerging fungal pathogen causing blight in hyacinth bean (*Lablab purpureus*). *Plant Pathology Journal*, 34, 367-380.

Reuveni, M., Agapov, V., Reuveni, R., 1997. A foliar spray of micronutrient solutions induces local and systemic protection against powdery mildew (*Sphaerotheca fuliginea*) in cucumber plants. *European Journal of Plant Pathology*, 103, 581-588.

Reuveni, R., Reuveni, M., 1998. Foliar-fertilizer therapy-a concept in integrated pest management. *Crop Protection*, 17, 111-118.

Reva, O.N., Dixelius, C., Meijer, J., Priest, F.G., 2004. Taxonomic characterization and plant colonizing abilities of some bacteria related to *Bacillus amyloliquefaciens* and *Bacillus subtilis*. *FEMS Microbiology Ecology*, 48, 249-59.

Ridout, C.J., 2009. Profiles in pathogenesis and mutualism: powdery mildews. Plant Relationships, 2nd Edition. pp. 51-68.

Roberts, P., Kucharek, T., 2005. Florida plant disease management guide: watermelon. Electronic data information source of UF/IFAS extension, PDMG-V3-55. Available at: http://edis.ifas.ufl.edu/

Rolshausen, P.E., Gubler, W.D., 2005. Use of boron for the control of Eutypa dieback of grapevines. *Plant Disease*, 89, 734-738.

Romero, D., Rivera, M.E., Cazorla, F.M., de Vicente, A., Pérez-García, A., 2003. Effect of mycoparasitic fungi on the development of *Sphaerotheca fusca* in melon leaves. *Mycological Research*, 107, 64-71.

Romero, D., Pérez-García, A., Rivera, M.E., Cazorla, F.M., de Vicente, A., 2004. Isolation and evaluation of antagonistic bacteria towards the cucurbit powdery mildew fungus *Podosphaera fusca*. *Applied Microbiology and Biotechnology*, 64, 263-269.

Romero, D., de Vicente, A., Zeriouh, H., Cazorla, F.M., Fernández-Ortuño, D., Torés, J.A., and Pérez-García, A., 2007a. Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. *Plant Pathology*, 56, 976–986.

Romero, D., de Vicente, A., Olmos, J.L., Dávila, J.C., Pérez-García, A., 2007b. Effect of lipopeptides of antagonistic strains of *Bacillus subtilis* on the morphology and ultrastructure of the cucurbit fungal pathogen *Podosphaera fusca*. *Journal of Applied Microbiology*, 103, 969-976.

Romero, D., de Vicente, A., Rakotoaly, R.H., Dufour, S.E., Veening, J.W., Arrebola, E., Cazorla, F.M., Kuipers, O.P., Paquot, M., Pérez-García, A., 2007c. The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus subtilis* toward *Podosphaera fusca*. *Molecular Plant– Microbe Interactions*, 20, 430-440.

Ruaro, L., Lima Neto, V.D.C., Justiniano, P., Junior, R., 2009. Influence of boron, nitrogen sources and soil pH on the control of club root of crucifers caused by *Plasmodiophora brassicae*. *Tropical Plant Pathology*, 34, 231-238.

Ruiz, J.M., Bretones, G., Baghour, M., Ragala, L., Belakbir, A., Romero, L., 1998. Relationship between boron and phenolic metabolism in tobacco leaves. *Phytochemistry*, 48, 269-272.

Saharan, G.S., Naresh, M., 2008. Sclerotinia diseases of crop plants: biology, ecology, and diseases management. *Springer Science and Business Media* B.V. The Netherlands, pp. 485.

Samac, D.A., Ao, S., Dornbusch, M., Grev, A., Wells, S., Martinson, K., Sheaffer, C., 2018. Lignin reduction in alfalfa (*Medicago sativa*) does not affect foliar disease resistance. (Abstract). In: The 11th International Congress of Plant Pathology: Plant health in a global economy. 2018 Jul 29 – Aug 3, Boston, Massachusetts. 1160-P.

Sandys-Winsch, C., Whipps, J.M., Gerlagh, M., Kruse, M., 1993. World distribution of the sclerotial mycoparasite *Coniothyrium minitans*. *Mycological Research*, 97, 1175-1178.

Sanjeev, K.K. & Eswaran, A., 2008. Efficacy of micro nutrients on banana Fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*) and it's synergistic action with *Trichoderma viride*. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 36, 52-54.

Schroeder, W.T., Provvidenti, R., 1969. Resistance to benomyl in powdery mildew of cucurbits. *Plant Disease Report*, 53, 271-275.

Schuerger, A.C. Hammer, W., 2003. Suppression of powdery mildew on greenhouse-grown cucumber by addition of silicon to hydroponic nutrient solution Is inhibited at higher temperature. *Plant Disease*, 177-185.

Schutte, K.H., 1967. The influence of boron and copper deficiency upon infection by *Erysiphe graminis* DC the powdery Mildew in wheat var. *Kenya Plant Soil*, 27, 450-452.

Schwartz, H.F., Gent, D.H., 2007. Powdery mildew (Cucumber, Melon, Pumpkin, Squash, and Zucchini). High Plains IPM Guide, a cooperative effort of the University of Wyoming, University of Nebraska, Colorado State University and Montana State University.

Sedlakova, B., Lebeda, A., 2008. Fungicide resistance in Czech populations of cucurbit powdery mildews. *Phytoparasitica*, 36, 272-289, ISSN 0334-2123.

Sharma, P., Meena, P.D., Singh, S., Rai, P.K., 2017. Efficacy of Micro-Nutrients, Fungicides and Bio-Agents against Sclerotinia Stem Rot (*Sclerotinia sclerotiorum*) of Indian Mustard. *International Journal of Current Microbiology and Applied Sciences*, 6, 620-626.

Sharma, P., Meena, P.D., Verma, P.R., Saharan, G.S., Mehta, N., Singh, D., Kumar, A., 2015. *Sclerotinia sclerotiorum* (Lib.) de Bary causing sclerotinia rot in oilseed *Brassicas*: A review. *Journal of Oilseed Brassica,* 6, 1-44.

Sharma, P., Meena, P.D., Rai, P.K., Kumar, S. Siddiqui, S.A., 2011. Evaluation of soil amendments, botanical and fungicide against *Sclerotinia sclerotiorum* causing stem rot of Indian mustard. *Journal of Mycology and Plant Pathology*, 41: 151.

Shaw, N.L., Cantliffe, D.J., 2003. Hydroponically produced mini-cucumber with improved powdery mildew resistance. In: Proceedings of the Annual Meeting of the Florida State Horticulture Society Florida State Horticulture Science, Orlando, FL. pp. 58-62.

Shoda, M., 2000. Bacterial control of plant diseases. *Journal of Bioscience and Bioengineering*, 89, 515-21.

Simoglou, K.B., Dordas, C., 2006. Effect of foliar applied boron, manganese and zinc on tan spot in winter durum wheat. *Crop Protection*, 25, 657-663.

Singh, R., Tripathi, N.N., 1993. Pathogens and host factors responsible for severity of sclerotinia rot of Indian mustard. *Crop Research (Hisar)*, 6, 126-130.

Singh, S., 2001. Management of sclerotinia rot in Indian mustard – an integrated approach. Proc International Sclerotinia Workshop, Central Science Laboratory, York, UK, pp. 139.

Sitterly, W.P., 1978. Powdery mildew of cucurbits. In Spencer, D.M. edition. The powdery mildews, pp. 359-379. London: Academic Press.

Smith, O.F., 1938. Host-parasite relations in red clover plants resistant and susceptible to powdery mildew *Erydphe polygonl*. *Journal of Agricultural Research*, 57, 671-682.

Sosnowski, M.R., Loschiavo, A., Wicks, T., Scott, E., 2010. Sustaining grapevines infected with Eutypa dieback. SAR06/01—Final report to the grape and wine research and development corporation. Online publication. Available at: http://www.sardi.sa.gov.au/__data/assets/pdf_file/0004/133897/GWRDC_Final_Report_SAR_06_01.pdf

Stangoulis, J.C.R., Brown, P.H., Bellaloui, N., Reid, R.J., Graham, R.D., 2001. The efficiency of boron utilization in canola. *Australian Journal of Plant Physiology*, 28, 1109-1114.

Stangoulis, J.C.R, Graham, R.D., 2007. Boron and Plant Disease. In: Datnoff, L.E., Elmer, W.H., Huber, D.M., editors. Mineral Nutrition and Plant Disease. USA: The American Phytophathological Society. pp. 207-214.

Statistics Canada, 2017. Production of principal field crops, November 2017. The Daily. Released 2017-12-06. Available at: https://www150.statcan.gc.ca/n1/en/daily-quotidien/171206/dq171206a-eng.pdf?st=tN_-yHkT

Steadman, J.R., Marcinkowska, J., Rutledge, S., 1994. A semi-selective medium for isolation of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology*, 16, 68-70.

Szembel, S.J., 1930. Control of powdery mildew of cucumbers by means of disodium hydrogen ortho-arsenate. Comment. Inst. Astrachanensis ad. defensionern Plantarium 2" 21-31. In Russian. Abs. in Rev. *Applied Mycology*, 10, 500-501.

Tahirović, I., Kožljak, M., Toromanović, J., Čopra-Janićijević, A., Klepo, L., Topčagić, A., Demirović, H., 2014. Total phenolic content and antioxidant capacity in infusions of various herbal teas. *Bulletin Chemists Technologists Bosnia and Herzegovina*, 42, 51-55.

Tiwari, R.K., 2015. Taxonomy and diversity of powdery mildew fungi of cucurbits. Available at: http://krishikosh.egranth.ac.in/bitstream/1/5810021354/1/final%20pdf%20in%20cd.pdf

Tribe, H.T., 1957. On the parasitism of *Sclerotinia trifoliorum* by *Coniothyrium minitans*. *Transactions of the British Mycological Society*, 40, 489-499.

Trutmann, P., Keane, P.J., Merriman, P.R., 1980. Reduction of sclerotial inoculum of *Sclerotinia sclerotiorum* with *Coniothyrium minitans*. *Soil Biology Biochemistry*, 12, 461-465.

Trutmann, P., Keane, P.J., Merriman, P.R., 1982. Biological control of *Sclerotinia sclerotiorum* on aerial parts of plants by the hyperparasite *Coniothyrium minitans*. *Transactions of the British Mycological Society*, 78, 521-529.

Turner, G.J., Tribe H.T., 1976. On *Coniothyrium minitans* and its parasitism of *Sclerotinia* species. *Transactions of the British Mycological Society,* 66, 97-105.

Tu, J.C., 1997. Biological control of white mould in white bean using *Trichoderma viride*, *Gliocladium roseum* and *Bacillus subtilis* as protective foliar spray. *Mededelingen van de Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen, Universiteit Gent*, 62, 979-986.

Tu, J.C., 1998. The role of white mold-infected white bean (*Phaseolus vulgaris* L.) seeds in the dissemination of *Sclerotinia sclerotiorum* (Lib.) de Bary. *Journal of Phytopathology*, 121: 40-50.

Vermerris, W., Nicholson, R., 2006. Phenolic compound biochemistry. Chapter 6: The role of phenols in plant defense. Springer, Dordrecht.

Vrije, T., Antoine, N., Buitlaar, R.M., Bruckner, S., Dissevelt, M., Durand, A., Gerlagh, M., Jones, E.E., Luth, P., Oostra, J., Ravensberg, W.J., Renaund, R., Rinzema, A, Weber, F.J., Whipps, J.M., 2001. The fungal biocontrol agent *Coniothrium minitans*: production by solid-state fermentation, application and marketing. *Applied Microbiology Biotechnology*, 56, 58-68.

Wang, A., Lin, W., Chen, X., Lu, G., Zhou, J., Wang, Z., 2008. Isolation and identification of Sclerotinia stem rot causal pathogen in *Arabidopsis thaliana*. *Journal of Zhejian University Science B*, 9, 818-822.

Whipps, J.M., 1987. Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. *New Phytologist*, 107, 127-142.

Whipps, J.M., Magan, N., 1987. Effect of nutrient status and water potential of media on fungal growth and antagonist-pathogen interactions. *European Mediterranean Plant Protection Organization Bulletin*, 17, 581-591.

Whipps, J.M., Budge, S.P., Ebben, M.H., 1989. Effect of *Coniothyrium minitans* and *Trichoderma harzianum* on Sclerotinia disease of celery and lettuce in the glasshouse at a range of humidities. In: Cavalloro R, Pelerents C (eds) Integrated pest management in protected vegetable crops. (Proc CEC/IOBC Group Meet) International organization for biological and integrated control, Rotterdam, pp. 233-243.

Whipps, J.M., Gerlagh, M., 1992. Biology of *Coniothyrium minitans* and its potential for use in disease biocontrol. *Mycological Research*, 96, 897–907.

Whipps, J.M., 1994. Advances in biological control in protected crops. Brighton Crop Protection Conference, *Pests and Diseases*, 2, 1259-1264.

Whipps, J.M., Budge, S.P., McClement, S., Pink, D.A.C., 2002. A glasshouse cropping method for screening lettuce lines for resistance to *Sclerotinia sclerotiorum*. *European Journal Plant Pathology*, 108, 373-378.

Wu, H., 1998. Effects of bacteria on germination and degradation of sclerotia of *Sclerotinia sclerotiorum* (Lib) de Bary. MSc. Thesis, North Dakota State University, Fargo, ND, pp.312.

Wurms, K.V., George, M.P., Lauren, D.R., 2003. Involvement of phenolic compounds in host resistance against *Botrytis cinerea* in leaves of the two commercially important kiwifruit (*Actinidia chinensis* and *A. deliciosa*) cultivars. New Zealand Journal of Crop Horticultural Science, 31, 221-233.

Yarwood, C.E., 1957. Powdery mildews. The botanical review. Vol. XXXIII, No. 4.

Yarwood, C.E., 1978. Water stimulates *Sphaerotheca fuliginea*. *Mycologia*, 70,1 035-1039.

Yermiyahu, U., Ben-Gal, A., Sarig, P., 2006. Boron toxicity in grapevines. *Horticulture Science*, 41, 1698-1703.

Zazzerini, A., Tosi, L., 1985. Antagonistic activity of fungi isolated from sclerotia of *Sclerotinia sclerotiorum*. *Plant Pathology*, 34, 415-421.

Zhang, X.L., Sun, X.M., Zhang, G.F., 2003. Preliminary report on the monitoring of the resistance of *Sclerotinia libertinia* to carbendazim and its internal management. *Chinese Journal Pesticide Science Administration*, 24: 18-22.

Zhang, J.X., Xue, A.G., 2010. Biocontrol of sclerotinia stem rot (*Sclerotinia sclerotiorum*) of soybean using novel *Bacillus subtilis* strain SB24 under control conditions. *Plant Pathology*, 59, 382-391.

Zhang, D., Zhao, H., Shi, L., Xu, F., 2014. Physiological and genetic responses to boron deficiency in *Brassica napus*: a review. *Soil Science Plant Nutrition*, 60, 304-31.

Zhao, J., Meng, J., 2003. Detection of loci controlling seed glucosinolate content and their association with sclerotinia resistance in *Brassica napus*. *Plant Breeding*, 122, 19-23.

Zitter, T.A., Hopkins, D.L., Thomas, C.E., 1996. Compendium of cucurbit diseases. APS Press, St. Paul, Minn.

Appendix A.

Dry weight of AF-treated cucumber plants

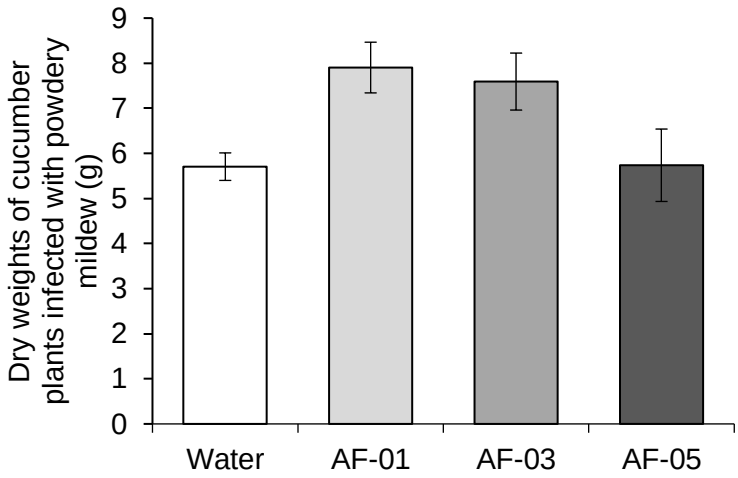

Figure A.1. Dry weights (g) of cucumber plants infected with powdery mildew after four applications of water (control) and AF at 0.1, 0.3 and 0.5 mL/100 mL.

Material and Methods: Cucumber seeds cultivar 'Tasty Green' (West Coast Seeds Ltd.) were germinated in the lab for a week on moist filter paper and then transferred to greenhouse potting medium 'Sunshine Mix' and grown for 4 weeks prior to treatments. The average greenhouse temperatures ranged from 22 to 35 ℃ during June to September 2016. Solutions of AF at 0.1, 0.5 and 0.8 ml per 100 ml were made to the lower 3 leaves to run-off. Applications were repeated weekly for a total of 4 applications. There were 5 replicate plants for each treatment and water was sprayed as a control. The average heights of the cucumber plants were measured from the soil level to the growing point of plant per week. The dry weights were determined at the end of the experiment by cutting the above-ground foliage and drying to constant weight at 50 ℃ for 48 hours.

Appendix B.

Effect of AF on fungal growth

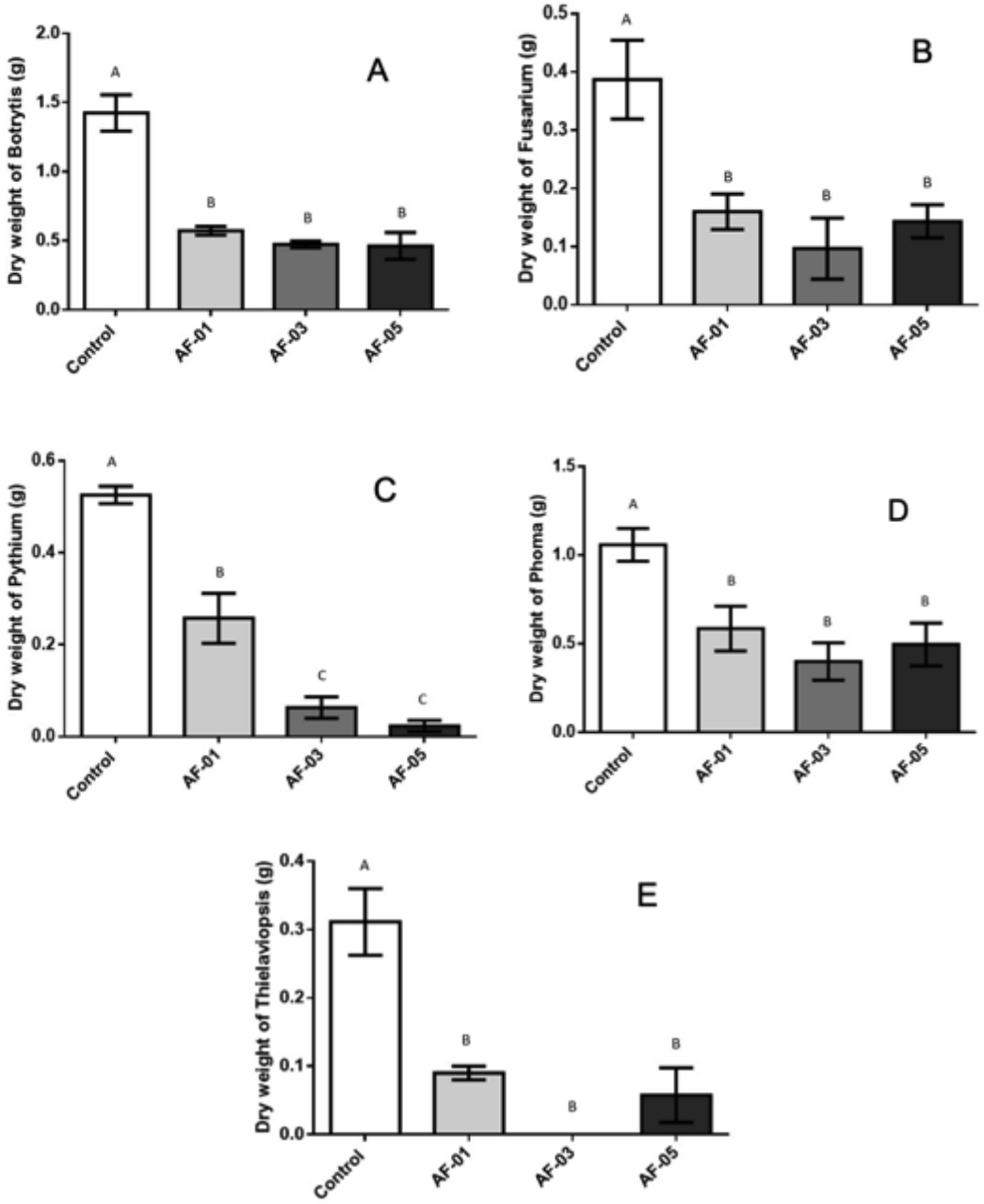

Figure B.1. Effect of AF at 0.1, 0.3 and 0.5 mL/100 mL on mycelium growth of five fungi after two-week incubation in potato dextrose broth (PDB). (A) *Botrytis cinerea,* (B) *Fusarium oxysporum*, (C) *Pythium dissotocum,* (D) *Phoma lingam and* (E) *Thielaviopsis basicola*.