Sustainable management approaches for fungal pathogens on vegetable crops

by

Hayley Kellam

B.Sc., Simon Fraser University, 2018

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

in the
Department of Biological Sciences
Faculty of Science

SIMON FRASER UNIVERSITY
Fall 2022

Declaration of Committee

Name:	**Hayley Kellam**
Degree:	**Master of Science**
Title:	Sustainable management approaches for fungal pathogens on vegetable crops
Committee:	**Chair: Vicki Marlatt** Associate Professor, Biological Sciences
	Zamir Punja Supervisor Professor, Biological Sciences
	Sherryl Bisgrove Committee Member Associate Professor, Biological Sciences
	Siva Sabaratnam **Examiner** Plant Pathologist BC Public Service

Abstract

Sustainable methods for the management of greenhouse pathogens such as *Fusarium* on tomato and powdery mildew on cucumber were investigated. A systematic review of calcium nutrition and its impact on diseases was performed. Calcium has been shown to impact disease development on over 40 crops and 35 pathogens. Direct inhibition of the pathogen, increase in cell wall strength and induced resistance are all modes of action that have been reported for calcium. Formulations of karanja oil (*Millettia pinnata* extract) were tested for their efficacy at reducing spore germination and mycelial growth of *Fusarium oxysporum* and in reducing disease development on greenhouse grown cucumber plants. Spore germination of *F. oxysporum* was inhibited by 100 % after 12 hr exposure when added to potato dextrose broth at a concentration of 0.1 %. The same concentration inhibited mycelial growth by 96 % after 7 days. Inconsistent *Fusarium* infection and symptom development resulted in no significant disease reduction by karanja oil. On cucumber plants, foliar sprays of the karanja formulations reduced the severity of powdery mildew infection by 73.6 %. On squash plants, foliar sprays increased plant weight by 80%. These results show the potential of karanja oil for suppressing pathogen growth in vitro and in vivo.

Keywords: *Fusarium oxysporum, Podosphaera xanthii,* powdery mildew*, Millettia pinnata,* calcium nutrition

Acknowledgements

Thank you to all those who supported me throughout my thesis:

Thanks to my supervisor, Dr. Zamir Punja for all the research and teaching opportunities you have given me during my time at SFU. Your optimism and love of both research and teaching has opened my eyes to new possibilities for my future, and I cannot thank you enough for that.

To Dr. Sherryl Bisgrove for serving on my committee. I always looked forward to updating you on my progress, and teaching alongside you each year.

To all those in the Punja lab: Janesse Holmes, Emily Betz, Cameron Scott, Li Ni, Sam Lung, Alastair Roberts, and Danielle Collyer, thank you for being so open and willing to help when projects and life threw curve balls my way.

To Terramera Inc., whose collaboration allowed me to pursue this degree, and provided me with great resources along the way.

To Ignite BC for funding this project and being such strong advocates for sustainable agricultural technology research and advancement in British Columbia.

To my family, for their support, and for not nagging me too much about how long my degree was taking to complete.

And finally, of course, thank you to my wife Claire, for joining me on this adventure. To many more.

Table of Contents

List of Tables

List of Figures

List of Acronyms

°C	Degrees celsius
ANOVA	Analysis of variable
BC	British Columbia
CFU	Colony forming units
fw	Fresh weight
g	Grams
h	Hours
HSD	Honestly significant difference test
L	Liters
M	Molar
mg	Milligrams
mL	Milliliter
mM	Millimolar
mm	Millimeter
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PDS	Phosphate buffered saline
PM	Powdery mildew
RT	Room temperature
s	Seconds
SFU	Simon Fraser University

The Tale of a Persistent Tomato: A Poem

I am a persistent tomato
Even though my label disagrees
Every *Fusarium* isolate
I disregard with ease

My weight is just as heavy
It seems as though I thrive
My stem is just as long
p is greater than 0.05

I've had many trials set before me
That have tested my determination
Here are all the things I thwarted
I'll tell you in narration

It seems like they've tried everything
All the methods that were compelling
When no one else is in the lab
They even try just yelling

My roots have been reduced
To only an inch in size
Then dipped in pure culture
To attempt to cause my demise

They've withheld light and water
To try to stress me out
But I am a persistent tomato
Resistant also to drought

I've been drenched in broth
In mycelia and in spores
And none of these effect
Any vitality scores

Maybe a different kind of broth
Could better prime it for infection
Maybe a different isolate
From a fungal bank collection

My different isolates
Have been used to seek infection
None of them can seem to bypass
My superior protection

Maybe from a wilted tomato plant
That is fresh from the field
Perhaps one of these will come
And finally make me yield

Chapter 1.

Introduction

1.1. Greenhouse production in British Columbia

The production of vegetables in greenhouse in Canada in 2020 provided a farm gate value of $1.8 billion. This production occurred on 451,808 hectares of land. The three main crops produced include tomato, cucumber and pepper, which account for 97 % of production (Statistics Canada, 2021). Two disease systems, one on tomato, the other on cucumber were investigated. Fusarium crown and root rot and wilt of tomato, caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* and f. sp. *lycopersici* (Schlecht.) Snyder & Hansen (Agrios 2005), respectively, are responsible for crop loss from the destruction of vascular tissues. The other, powdery mildew, caused by *Podosphaera xanthii* (Schlidl.) Braun & Takam, is widespread, occurring in all greenhouse producing provinces in Canada. This foliar disease can cause premature senescence of leaves and decreases in crop yield. Both diseases are of importance in Canada and new research will be required to manage their spread and damage in greenhouse crops.

1.2. Fusarium disease on tomato

Fusarium crown and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* is a disease of various crops, including of field and greenhouse tomato. This soilborne pathogen can cause great losses in yield and persist for long periods of time in the soil. Management of this pathogen relies heavily on preventative measures and use of resistant varieties, partly due to limited effective products that having been approved in Canada for its management. Great care should be taken to minimize the spread of this pathogen, especially in key tomato growing areas such as Ontario.

1.2.1. Disease Biology and Life Cycle

Fusarium oxysporum f. sp. *radicis-lycopersici* (Schlecth.) Snyder & Hansen is an ascomycete fungus within the Nectriaceae family. It is classified under the order Hypocreales, within the class Sordariomycetes. These belong to the phylum of

Ascomycota which contain fungi that typically produce non-motile sexual spores within a structure known as an ascus, although this structure has not been observed in *F. oxysporum* (Gordon 2017). The fungus is endemic to many soils and functions primarily as a saprophyte, feeding on soil debris. Some strains are pathogenic to plants, taking excessive nutrients and damaging tissues. *Fusarium oxysporum* f. sp. *radicis-lycopersici* is associated with crown and root rot symptoms, whereas *Fusarium oxysporum* f. sp. *lycopersici* causes vascular wilt. Others may be endophytic, where they live within the plant but cause no noticeable damage and may even be beneficial to its growth or defense (Lemanceau et al. 1993). Pathogenic strains can cause a variety of symptoms including discolouration, wilting, rot on the crown or roots and in some instances lead to plant death, either at an early stage of growth or on mature plants (Figure 1). When discussing the effects of *Fusarium* on different plant species, researchers often use a *forma specialis* designation for ease of communication, even though these names do not always designate a taxonomic standing (Gordon 2017).

Figure 1-1 Common symptoms of *Fusarium* infection. From left to right: an uninfected tomato plant, an infected tomato plant, and discolouration of the crown tissues on an infected tomato plant (arrow).

Infested soils may contain mycelium, macroconidia and microconidia as well as chlamydospores of the fungus. The chlamydospores are the principal, durable form that can survive in stasis for many years without a host (Nelson 1981). For germination of these spores, nutrients are required, such as sugar and amino acids released from

growing roots (Schroth and Hildebrand 1964). It has been shown that specialized strains, such as *Fusarium oxysporum* f. sp. *lycopersici g*erminate at a significantly higher rate when exposed to root exudates of their preferred host, the tomato, as compared to non-host species such as tobacco (Steinkellner et al. 2005). This sensitivity for different root exudates may account for some of the specificity of these strains. Chlamydospores germinate and spread through the soil until they reach potential infection points on the plant. Infection may begin by mycelium or germ tubes entering at wounded sites or emerging lateral root sites at the crown (Goncalez et al. 2012). Direct entry can be made with the formation of penetration pegs that allow the pathogen to break into tissues at the top of the roots or base of the stem (Boland & Kuykendall 1998). The fungus damages tissues by secreting various cell wall degrading enzymes such as polygalacturonases, pectinases, lyases and proteases (Beckman 1987). Once the fungus has entered the tissues near the crown, it spreads to the xylem vessels. Here it continues to grow until eventually it completely impedes the movement of water and nutrients to the plant, resulting in plant wilting and death (Agrios 2005). A small compact mass of hyphae called sporodochia forms on the dead plant tissues and releases conidia, which spread by wind to new soil and plants (Jarvis et al. 1988). The aerial spread of spores allows for the disease to be polycyclic, where multiple disease cycles can occur within a season (Rowe et al. 1977). Once nutrients from the plant tissues have been depleted, mycelium begins producing chlamydospores, the overwintering structures of the fungus (Smith, 2007), which can persist for long periods in the soil before re-emerging and restarting the disease cycle (Figure 2).

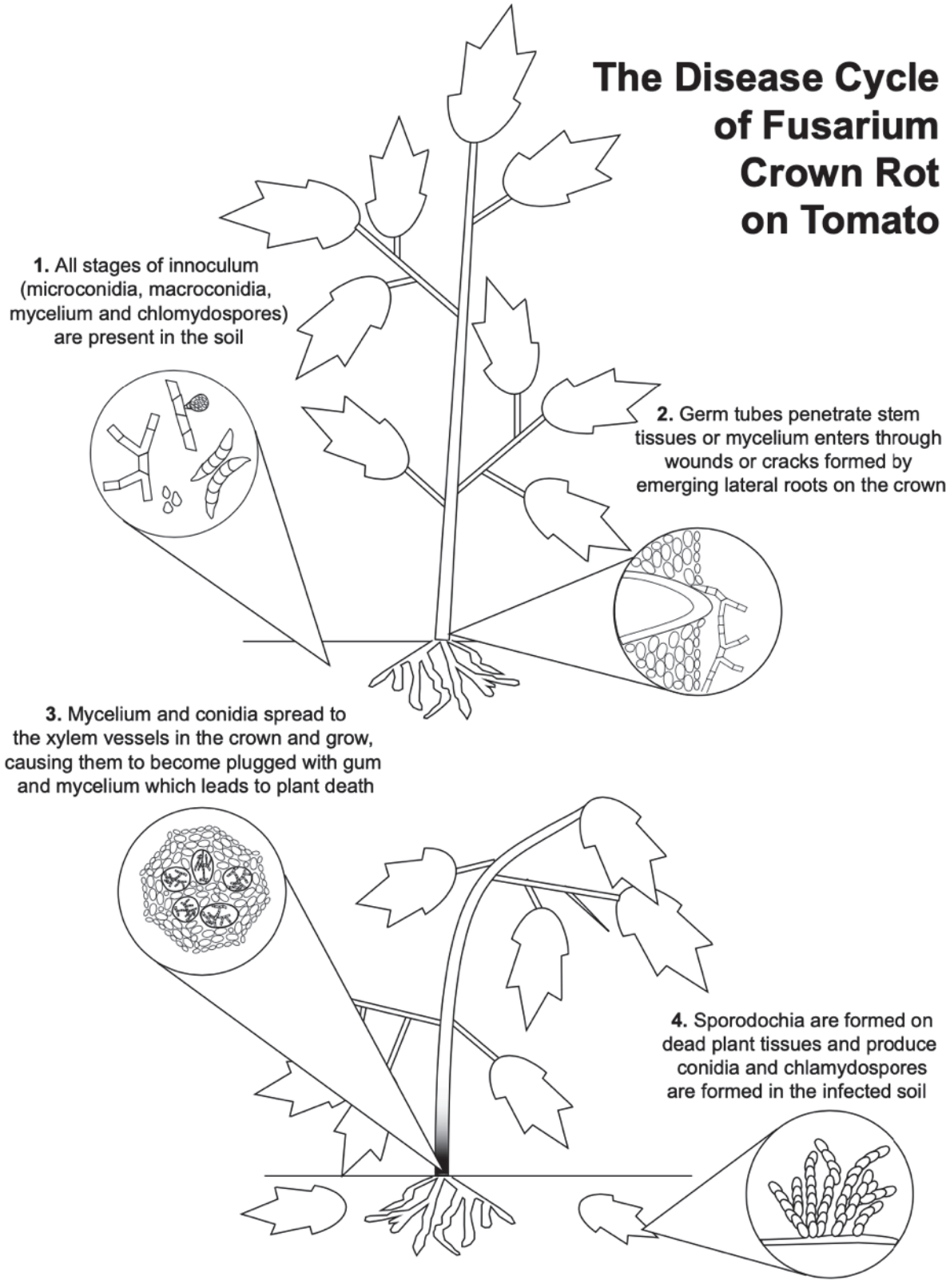


Figure 1-2 The disease cycle of Fusarium crown rot on tomato

1.2.2. Host and Environment Factors

There are many environmental factors that can influence the growth of the pathogen, the infection rate, as well as the disease severity. Tomato plants grow best with a pH from 6.0 to 6.5 (Sharma et al. 2018). These conditions are also ideal for *Fusarium* mycelium, which grows at its highest rate in a lower pH to neutral soil (6.0 – 7.0). The fungus sporulates ideally at a pH of 4.5 (Tyagi and Paudel 2014). Maximum mycelial growth rate occurs between 20 and 30 degrees Celsius (Hibar et al. 2006). It was found that disease incidence was lower (40 %) for seedlings which were root dip inoculated and grown at a higher temperature of 29 degrees Celsius as compared to 19 degrees Celsius (80 % disease incidence) (Hibar et al. 2006). Infection incidence is seen to increase with both drought stress and with humid conditions (Goncalez et al. 2012). Wounded sites can be caused by human disruption, such as pruning or animal or insect damage such as action of nematode pests in the soil. (Goncalez et al. 2012). The first instance of *Fusarium oxysporum* causing crown rot on tomato was reported in Japan in 1974 (Yamamoto et al. 1974). The first known incidence of the pathogen in Canada was reported the year after in Ontario in 1975 (Jarvis et al. 1975). As of January 2020, *F. oxysporum* has been reported in 73 countries worldwide (CABI 2020, Figure 3) with spread occurring through infected soil and plant material. *Fusarium* can disperse locally through its airborne conidia to infect nearby fields (Jarvis et al. 1998). Spores and hyphal fragments can be spread as well through abiotic means such as rain, wind and various cultivation tools and materials to nearby plants and soil (Rowe et al. 1977, Goncalez et al. 2012). These cultivation tools can include pruning shears, tractor tires, footwear, and seedling trays. Humans, animals and insects, such as *Bradysia* (fungus gnats) and *Scatella* (shore flies) can also disperse the spores and hyphae as well to new plants and soil though their natural movement within fields and greenhouses (Gillespie & Menzies 1993, Matsuda et al., 2009). The pathogen can spread longer distances via infected plants or media, such as seeds, transplants or soil (Menzies & Jarvis 1994, Jarvis 1998).

Outbreaks in greenhouses have been attributed to the reuse of infected plastic Styrofoam trays for transplanting, infected plastic stakes for drip tubes, or airborne spores that remain on the walls or poles of the greenhouse (McGovern, 1993, Toro et al., 2012, Shlevin et al., 2003). In the field, *Fusarium* can harbour within wooden stakes, and if reused they can act as an inoculum source (McGovern and Datnoff, 1992). In hydroponic systems, infection was seen to occur mostly from infected transplants

(Mihuta-Grimm et al., 1990), and plant to plant spread was limited when using rockwool media (Hartman and Fletcher, 1991). *Fusarium oxysporum* can infect plant tissues at any time in their growth stage, from seedlings to adult. It is characterized by vascular discolouration and rot of crown tissues, usually paired with root rot (Jarvis 1998). Later stages of the disease often cause wilt that precedes fruit ripening, decreasing overall tomato fruit yield.

1.2.3. Economic Significance

Total yield losses have been seen to range from 20 % to 60 % (Jarvis 1998, Rowe et al.1977) which can be devastating to tomato growers. Tomato greenhouse production in Canada totalled 15,000 acres in 2019 (Statistics Canada, 2020). In 2018, the farm-gate value of field tomatoes was $105 million, and the farm-gate value of greenhouse tomatoes was $ 60.6 million (Agriculture and Agri-Food Canada, 2019). With the severity of losses associated with the disease, farmers must take precautions to limit the chances of *Fusarium* infections. Integrated management strategies including use of best management practices, biological and chemical control can help reduce the risk, spread and severity of Fusarium crown and root rot on tomato.

1.2.4. Management

There are a variety of preventative control options available to tomato producers. These include cultural controls, choice of resistant varieties and applications of biological control agents. There are also a limited number of chemical treatment options for crops that become infected with the disease. As with most pathogens, prevention is often more effective than treatment. An integrated program should therefore highlight extensively the procedures and methods that a grower can use to minimize the risk of this and other pathogens.

Soil Health

Several nutrients have been shown to decrease disease severity including calcium, copper and nitrogen (Duffy and Defago, 1999). The method of control varies for each ion. Calcium is thought to increase cell wall strength and prevent infection from occurring. Copper ions are known to have a fungicidal effect, whereas nitrogen fertilizers are thought to lessen disease through changes in pH to the soil (Duffy and Defago,

1999). Use of ammonia nitrogen fertilizers should be avoided however, as they have been shown to increase disease incidence (Blancard 2012). Amendments with silicon have also been shown to decrease Fusarium crown rot disease in tomatoes (Huang et al. 2011). This may be due to a physical barrier being created by the sequestering of silicon in tissues or the ion may play a role in the pathways involved in induced resistance (Cai et al., 2009). Although added nutrients in the soil can induce spore germination of *Fusarium*, it can also increase the growth rate of other organisms such as bacteria that can outcompete *Fusarium* for resources (Lockwood, 1977). These bacteria may also produce fungistatic compounds that further reduce the growth of *Fusarium*. The microbial community can also be increased by the use of organic amendments to reduce *Fusarium* (Bonanomi et al. 2010).

Cultural Control

Before planting any crop, growers should consider the history of a field or greenhouse. This can allow them to consider the disease risk and potential disease pressure present in the environment. If *Fusarium* is known to have infected field soils previously, treatments should be applied to reduce or eliminate chlamydospores. One method is soil solarization, which traps the sun's energy under plastic sheets for several weeks and reduces the viability of soil borne diseases and weeds (Ajilogba and Babalola 2013). For death of the chlamydospores, soil must reach a temperature of 60 degrees Celsius for at least 30 minutes (Bollen, 1985). Solarization is less effective for *Fusarium* than other pathogens such as *Pythium* or *Sclerotium* as it is less temperature sensitive (Blancard, 2012). Therefore, it is often applied in conjunction with other control methods such as biological control (Blancard 2012). Similar results can also be obtained by allowing fields to fallow, especially during warm periods with regular ploughing. This may be effective at reducing soil borne diseases if the soil is turned regularly to expose it to the sun. The increased soil temperature of the topsoil combined with high rates of evaporation can make the soil inhospitable for fungi (Greenberg et al. 1987).

Biological soil disinfestation (BSD) can also be used, where soil is covered with organic matter (such as grass, manure or mulch), highly irrigated and sealed with tarps to create anaerobic conditions (Hewavitharana and Mazzola, 2016). These conditions are unsuitable for weeds and aerobic fungi and promote the growth of anaerobic micro-organisms. This changes the community levels of the soil and may reduce *Fusarium* and

other soil borne pathogens significantly. Tests using this method found that adding ethanol to the BSD system before covering reduced the inoculum to below a detectable level in 3 out of 4 plots (Momma et al. 2010). Although reduction of the chlamydospore viability can be accomplished by both anaerobic soil disinfestation and solarization, both these techniques may also decrease the overall soil microbiota, which may reduce the community's ability to suppress the pathogen (Gordon 2017).

There are many best management practices that can be followed to minimize the risk and spread of the disease. Moisture levels can be managed by minimizing overwatering, reducing overhead irrigation or planting in raised beds (Ajilogba and Babalola 2013). Airborne spread of the pathogen can be reduced by adding mulch to the top of soil, which can act as a physical barrier to reduce the inoculum (Ajilogba and Babalola 2013). Increasing plant spacing, reducing weeds, or separating plants entirely can reduce the spread of the disease (Ajilogba and Babalola 2013). Transplants may carry the pathogen, even with minimal or no symptoms (Hartman & Fletcher, 1991) and care should be taken to ensure plant material is clean before planting. Crop rotation has limited effectiveness due to the long-term persistence of chlamydospores in the soil as well as persistence in weed species such as clover (Goncalez et al. 2012). Trials tested several intercropping species including leek, cucumber and basil but they had no effect on the disease severity or incidence (Hage-Ahmed et al., 2013).

If a plant has been identified as infected, care should be taken to remove and destroy all infected material and residues to prevent the spread of inoculum (Ajilogba and Babalola 2013). All tools used for this process should be disinfected and sanitized along with all footwear and clothing. Farmers should remove all plant material after harvest to reduce amounts of inoculum in the soil.

Resistant Varieties

A main defence compound that can be produced by tomatoes is a-tomatine. This compound can complex with the sterols present in fungal membranes and cause ruptures of the cell (Keukens et al., 1992). Some *Fusarium oxysporum* strains, such as the forma specialis *lycopersici*, can produce the enzyme tomatinase to degrade the compound (Lairini et al., 1996). The activity of this enzyme can contribute to the virulence of the strain on tomato (Pareja-Jaime et al. 2008).

The gene-for-gene relationship is thought to be present for both the wilt causing *F. oxysporum* f. sp. *lycopersici* and the crown and root rot causing *F. oxysporum* f. sp. *radices-lycopersici* as monogenic resistance is present for both. For the three races of wilt, there is one gene that confers resistance for each, I, I-2, and I-3 for race 1, 2, and 3 respectively (Prihatna et al. 2018). A single gene, Frl, causes resistance to fusarium crown and root rot on tomato. It was introduced into the tomato from a related wild species *Solanum peruvianum* in 1963 when researchers were attempting to control tobacco mosaic virus (Alexander, 1963). The gene is located on chromosome 9 in close association with the Tm-2 gene that was selected for (Blancard, 2012). Since then, it has been introduced into multiple commercial varieties of tomato for resistance to Fusarium that are grown commercially in greenhouses. These include the determinant variety BHN871, and indeterminant varieties ‘Cauralina’, ‘Beorange’, ‘Climstar’, and ‘Bigdena’ (Table 2, Johnny’s Seed Catalogue, 2020).

Desired varieties that are not resistant to *Fusarium* infection can be produced by grafting with resistant rootstocks which have been shown to increase tolerance to the disease (Vitale et al. 2014). Resistant varieties of rootstock ‘Maxifort’ and ‘Robusta’ were discovered and are now being used by extensively by farmers (Scott & Jones 2000). It is likely however that new pathogen races will evolve, for which new resistant cultivars will need to be developed (Elmhirst 1997). Rootstock use is quite popular in Canada, the United Kingdom and in Asia where use of methyl bromide is banned. Grafting has been less popular in the United States where provisions allow farmers to still use methyl bromide fumigation for emergency exceptions which is more cost effective than grafting (King et al., 2008).

Chemical Control

Spread of the pathogen can be minimized by sanitization of container surfaces, structures and tools using a variety of disinfectants. This can include strong oxidizers that cause disruption of proteins and nucleic acids, such as bleach and hydrogen peroxide. Ethanol can also be used as it can cause protein denaturation (McDonnell and Russell, 1999). Solutions of sodium hypochlorite, the main ingredient in bleach has also been studied for its use in seed sterilization, and although it can reduce disease incidence, it does not eliminate it completely (Menzies and Jarvis, 1994). When mixed into infected soil, a 10% bleach solution was able to reduce chlamydospore

concentration of *Fusarium oxysporum* f. sp. *vasinfectum* (a pathogen of cotton) below a detectable level (Bennett et al. 2011). These methods may be effective in greenhouse production but are of limited use in field operations for treating infected soil.

Historically, field soil was sterilized by fumigation with broad-spectrum pesticides such as methyl bromide. This was applied as a toxic gas but has since been banned in most countries as it was found to contribute to ozone depletion (Larkin and Fravel 1998, Hibar et al. 2007). The only approved pesticide for suppression against *Fusarium* on tomato is Medallion (fludioxonil). Other products that have been investigated for treatment include benomyl, prochloraz, azoxystrobin, carbendazim and bromuconazole (Amini and Sidovich, 2010); however, these have not been approved for use on tomato in Canada. These pesticides operate through various mechanisms including preventing cell division, inhibiting spore germination, mycelial growth and spore production (Amini and Sidovich, 2010). Two products, Maxim 480 FS (fludioxonil), Thiram (tetramethylthiuram disulfide), have also been approved for use as seed treatments.

Biological Control

Many species have been tested for their use as biological controls for Fusarium crown and root rot on tomato. Seven are currently approved for commercial use in Canada. There are four gram-positive bacteria, *Streptomyces lydicus* De Boer strain WYEC108 which is sold as Actinovate, *Streptomyces griseoviridis* Anderson strain K61 which is sold as Mycostop, *Bacillus subtilis* strains which are sold as QST713, Minuet, Serifel and Taegro and *B. amyloliquefaciens* (Biotak). In addition, two strains of the fungus *Trichoderma harzianum* Rifai T-22 and KRL-AG2, are sold as RootShield, Bora, BW240, as well as Trianum (Ministry of Agriculture, Food and Rural Affairs, 2020, Ontario). *T. asperellum* is sold as Asperello and *Gliocladium catenulatum* is approved for use under the name Prestop. *Streptomyces* species can cause antibiosis due to production of chitinases (Anitha and Rabeeth, 2009). *T. harzianum* reduces disease by releasing phenolics, chitinases and glucanases which accumulate in the soil (El-Mohamedy et al, 2014). *Bacillus subitils* is thought to reduce disease incidence by inducing systemic resistance in plants (Loganthan et al., 2014, Kloepper et al. 2004) and producing a range of antibiotic compounds.

There are other microbial species that have been evaluated and shown to be effective for disease reduction but have not yet been approved for commercial use.

These include mycorrhizal fungi such as *Rhizophagus irregularis* (previously *Glomus intraradices*) (Datnoff 1995) as well as competing *Fusarium* species, *F. equiseti* (Corda) Sacc. (Horinouchi et al. 2008), and various *Bacillus* and *Pseudomonas* species (Zhang et al. 2015). The mechanisms of biological control for each species may vary. Some produce volatile compounds such as HCN, cyclohexanol, benzothiazol, whereas others produce hydrolytic enzymes such as b-1,3 glucanases, chitinases and proteases (Kim et al. 2008). These compounds are inhibitory towards *Fusarium* species and reduce their presence in the soil. They may also act through parasitism and predation or reduce their spread through competition for space or resources. Research has also found many plant essential oils and extracts such as thyme, allspice, fennel, garlic and mandarin orange to be effective against *Fusarium* species (Goncalez et al. 2012, Nefzi et al. 2018). Some of these compounds are antibacterial, and others, such as fennel are able to inhibit fumonisin mycotoxin production (Soliman and Badeaa 2002). None have been registered for use on tomato for *Fusarium* treatment.

1.2.5. Overall Disease Management

Integrated pest management programs are very important for diseases like Fusarium crown and root rot on tomato. Growers must implement a variety of protocols and precautions to maintain the health of their crops. The use of disinfectants, especially in greenhouse production is essential for minimizing presence and spread of the pathogen. The list of approved products for this disease is very short. Growers have limited options when it comes to biological controls, for which there are 5 approved, and pesticides, for which there is only one (Ministry of Agriculture, Food and Rural Affairs, 2020, Ontario). Despite research showing the efficacy of other agents, they have yet to go through the rigorous process of being approved for commercial applications. Farmers must therefore rely heavily on preventative control methods such as sterilization and cultural controls, which may have limited effectiveness. *Fusarium* is a persistent pest in soil due to the long-term survival of chlamydospores. This makes managing the disease in controlled settings such as greenhouses, where growing media can be completely replaced easier, unlike in the field.

Use of resistant cultivars seems to be the most effective mechanism for crop protection. There are many cultivars available commercially, and more are being produced. Just as new races of Fusarium wilt have emerged, it is likely we will one day

see new races of Fusarium crown and root rot. Research should be pursued to monitor the strains and breed new cultivars with new resistance. As well, the pest management community of researchers should work towards using nomenclature that is indicative of taxonomic standing, and not solely dependent on host range and symptoms.

The additional challenge for growers in management may be that most of these methods are preventative and therefore require financial input before pest problems may occur. Therefore, some growers may forgo preventative measures and find themselves with few options for treatment once *Fusarium* appears in their crop. Luckily, the measures taken to prevent *Fusarium* infections, such as sterilization and the use of some biological controls are effective against a broad range of pathogens and should be used as part general pest management practices.

Overall, the continuation of research into effective naming, resistance and treatment of Fusarium crown and root rot will be necessary to protect the production of tomato in Canada and around the world.

1.3. Powdery mildew disease on cucumber

Powdery mildew on cucurbits, such as cucumber (*Cucumis sativus* L.) has been recognized since the 1800s (Mondal 2020). This disease in general, characterised by white, flour-like dusty fungal growth, can be caused by a variety of pathogens, on over 10,000 plant species (Glawe 2009). George Agrios, in his textbook *Plant Pathology* described powdery mildews as “probably the most common, conspicuous, widespread and easily recognizable plant disease” (Agrios 2005).

1.3.1. Disease Biology and Life Cycle

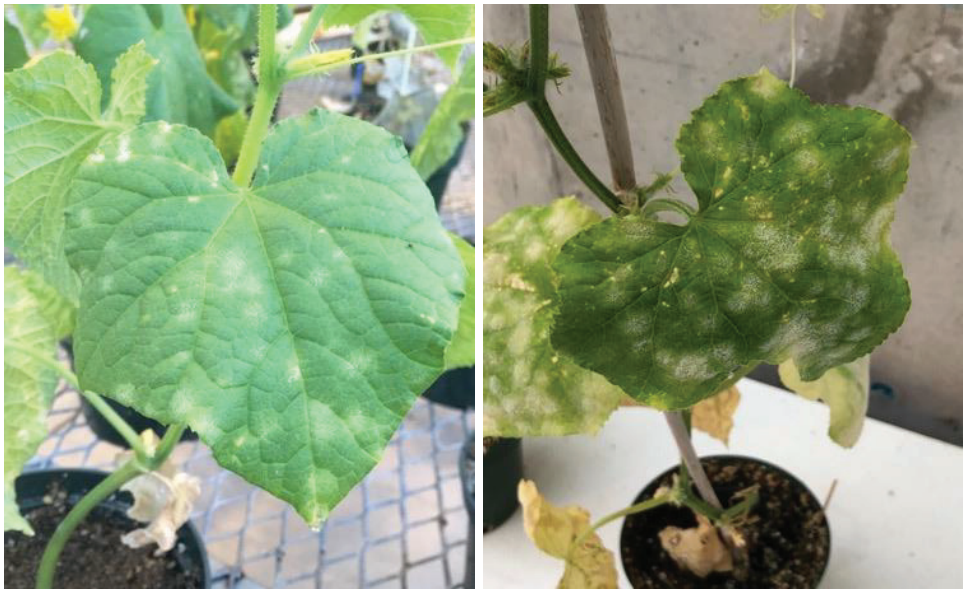


Figure 1-3 Common symptoms of powdery mildew infection on cucumber. Note white colonies on leaves.

Powdery mildew can be recognized as a white, talc-like power on the top and bottom of leaves, stems, and petioles (Keinath et al. 2017). The symptoms appear most commonly on older and shaded parts of the plant (Mondal et al. 2020). The macroscopic white colonies that appear consist of mycelia, conidiophores, and conidia (asexual spores) (Mondal et al. 2020).

The common name “powdery mildew” refers to a disease that can be caused by several pathogens. The disease is most commonly caused by *Podosphaera xanthii (*syn. *Podosphaera fuliginea and Sphaerotheca fuliginea)* and *Golovinomyces cichoracearum* (syn. *Erysiphe cichoracearum)* (Keinath et al. 2017, Mondal et al. 2020). These fungi are ascomycetes in the order Erysiphales (Horst 2013). *Podosphaera xanthii* is more widespread and prefers warmer weather. The two cannot be distinguished macroscopically (Aglave 2019) but can be differentiated by their conidia or ascospores (Horst 2013).

These fungi are obligate pathogens, requiring a living host to survive and reproduce (Keinath et al. 2017). When the spore contacts the leaf and germinates, it forms a mat of dense mycelia on the leaf and a penetration peg which enters into the leaf cells (Horst 2013). This peg extends into a haustorium inside the host cell, allowing

it to draw resources from the plant (Glawe 2009). The time from infection to symptoms can develop quickly, sometimes within 3-7 days (Keinath et al. 2017). The conidiophores form perpendicular to the leaf surface and the mycelium, producing chains of conidia (Glawe 2009). Once mature, they can spread in wind, with conidia surviving for up to 7-8 days, depending on the environmental conditions (Keinath et al. 2017). Most spores land within 2 meters, but some species have been reportedly able to spread up to 700 km away (spreading from the UK to Demark) (Glawe 2009).

The pathogen has been shown to reproduce sexually during the fall, when two different mating types contact each other. They form a fruiting body on heavily diseased plants called a chasmothecia, which contains ascospores (Keinath et al. 2017). These may appear as small dark brown to black dots on the leaves (Mondal et al. 2020). The impact of these on spreading disease is unclear, especially if plant debris is buried after field harvest, and is therefore prevented from wind dispersion of sexual spores (Keinath et al. 2017). This may play a role in allowing the fungus to overwinter, but the exact mode of re-occurrence in spring is not known (Mondal et al. 2020). The pathogen is known to have alternative hosts, including ornamental verbena, where they may also persist throughout the seasons (Keinath et al. 2017). Survival of mycelia and spores within flower buds has also been seen but are less likely to survive the low temperatures of winter (Glawe 2009).

1.3.2. Host and Environment Factors

The fungus grows best under 20 to 27 degrees Celcius but can infect with temperatures ranging from 10 to 32 degrees (Keinath et al. 2017). The ideal temperatures for various cellular processes vary, with sporulation optimized at 22 degrees, germination at 24 degrees, infection at 26 degrees (Trecate 2019). If temperatures reach 38 degrees, disease progression ceases (Aglave 2019). Low light conditions are favorable for disease development, which is why symptoms are found more commonly on shaded leaves (Aglave 2019). High humidity aids conidial survival, whereas dry conditions favor spread (Keinath et al. 2017). Presence of water on the leaves can inhibit conidial germination (Aglave 2019).

1.3.3. Economic Significance

Crop losses due to powdery mildew infection can be caused by premature senescence of the leaves, reducing the fruit yield of the plant (Keinath et al. 2017). Once leaves are covered by mycelium, they will lose their colour, changing to pale yellow, then brown, eventually shriveling and dying (Aglave 2019). The loss of colouration and integrity can then expose the leaf to further damage by the sun (Aglave 2019). Infected fruits may be undersized, deformed or may not completely ripen (Mondal et al. 2020). In British Columbia, severe infection can lead to full de-foliation by August (Ministry of Agriculture, 2022). The worldwide damage caused by powdery mildew diseases are thought to cause more losses than any other plant disease, due to their ubiquitous nature (Agrios 2005).

1.3.4. Management

Soil Composition

Soil should be monitored for levels of nutrients such as nitrogen, as some studies have shown that high fertilization may increase disease (Mondal et al. 2020). 15 mM nitrogen provided in the irrigation solution of cucumbers was seen to significantly decrease powdery mildew disease severity (Elad 2021). Treatment with 0.4 mM of phosphorous significantly increased disease severity, whereas higher concentrations (0.6mM) were comparable to the untreated control (Elad 2021). The same study also saw increases in disease severity as potassium concentration increased, and decreased severity as magnesium increased.

Cultural Control

As infection and development of mildew thrive on shaded areas of the plant, adequate spacing should be provided (Mondal et al. 2020). Weeds that may act as alternative hosts should be removed (Mondal et al. 2020). As the fungus can persist on crop debris, infected plants should be removed and destroyed (Mondal et al. 2020). Spore germination may be minimized by spraying plants with water every two to three days, but this method may increase other diseases that thrive in high humidity, such as grey mold caused by *Botrytis cinerea* (Pest Management Program, 2021).

Resistant Varieties

The use of resistant varieties is the foremost method used to manage powdery mildew (Keinath et al. 2017) with a large range of resistance amongst available varieties. Some reports indicate that resistance in cucumbers is linked to one gene (Mondal et al. 2020), whereas others have listed multiple genes impacting powdery mildew resistance (Chen et al. 2020). The resistance genes identified are thought to be involved in signalling pathways for the hypersensitive response in plants (Chen et al. 2020), with the *MLO*, *PMR* and *TCTP* gene families being the most linked with defence.

Chemical Control

Many pesticides have been used for disease management, but resistance to them has developed on multiple occasions (Keinath et al. 2017), including to benomyl and triadimefon (McGrath 2001).

Currently approved fungicides have a variety of modes of action: Group 3 fungicides including difenoconazole (Inspire and Revenue), myclobutanil (Nova), prothioconazole (Holdfast and Proline) and tetraconazole (Mettle) work by inhibiting sterol synthesis. Group 7 and 11 fungicides inhibit fungal respiration and include fluopyram (Luna and Velum), fluxapyroxad (Sercadis and Xemium), penthiopyrad (Fontelis), pydiflumetofen (Posterity) pyraclostrobin (Cabrio), and trifloxystrobin (CGA2792020), Group 19 acts on chitin synthase and includes polyoxin D zinc salt (Diplomat). Group 50 fungicides disrupt actin function and include metrafenone (Vivando) and pyriofenone (Property). Some fungicides act on multiple sites and therefore are lower risk for resistance development. These include chlorothalonil (Bravo and Echo), and folpet (Follow, Folpan, and Foltax). Some approved pesticides also have unknown modes of action, such as flutianil (Gatten) (Pest Management Regulary Agency, Pesticide Label Search, 2022).

Alternation of pesticides is recommended to minimize selection for resistant pathogens (Keinath et al. 2017). Premixes of fungicides exist, that combine these modes of action for easy use (Pscheidt 2022) including Aprovia Top (benzovindiflupyr and difenoconazole), Cyproflu (cyprodinil and fludioxonil), Elatus (benzovindiflupyr and azoxystrobin), Merivon (pyraclostrobin and fluaproxad), Miravis Duo (pydiflumetogen and difenoconazole), Pristine (boscalid and pyraclostrobin), Quadris Top (azoxystrobin

and difenoconazole), and Treoris (penthippyrad and chlorothalonil). (Pest Management Regulary Agency, Pesticide Label Search, 2022).

Some recommend the use of fungicides as a preventative application to be effective (Aglave 2019). Others recommend starting chemical control as soon as a colony appears (Mondal et al. 2020). Organic forms of sulphur, copper, mineral oil, and some other biopesticides are approved for use, with varying efficacy (Keinath et al. 2017). Sulphur has been shown to cause phytotoxicity however (Mondal et al. 2020). The British Columbia Ministry of Agriculture lists 4 products as effective for suppression that also qualify for organic production. These include Cueva (copper octanoate), Milstop and Sirocco (potassium bicarbonate), Regalia Maxx (*Reynoutria sachalinensis* extract) and Double Down, Purespray and SuffOil-X (mineral oil) (Ministry of Agriculture 2022). Essential oils have been shown to be effective when applied as a foliar spray at a concentration of up to 2.5 mL / L but become phytotoxic above this rate (Mostafa et al. 2021). Some approved plant oil based fungicides include tea tree oil (Timorex Gold) and canola oil (Doktor Doom, SuffoCoat and Vegol). Garlic powder based fungicides, Buran and Influence, are approved for suppression of powdery mildew (Pest Management Regulary Agency, Pesticide Label Search, 2022).

Biological Control

Penicillium fellutanum has been shown to inhibit powdery mildew spore germination (Srivastava and Suman 1986). Limitations in the efficacy of biological controls may be due to the higher humidity requirements of the biocontrols than of the pathogen (Aglave 2019). The British Columbia Ministry of Agriculture lists Serenade Opti, a *Bacillus subtilis* biological control product as effective for suppression in both conventional and organic production (Ministry of Agriculture 2022). Other products, Rhapsody and Green Earth, are formulated with a different strain of *B. subtilis,* QST 713. Biotak, Serifel andTaegro 2 are both approved fungicides that utilize *B. amyloliquefaciens* strains. *Streptomyces lydicus* is sold as Actinovate

1.3.5. Limitations in Management

The annually released crop profile for greenhouse cucumber in Canada lists the most important issues for each pest of interest. For powdery mildew, the top priority is the registration of new fungicides, including bio fungicides to reduce the risk of

development of resistance in the pathogen. Further recommendations outline the development of resistant cultivars and a greater understanding of cultural and environmental controls (Pest Management Program, 2021).

1.4. Organic production

Organic production limits the use of synthetic pesticides and fertilizers, as well as the use of biotechnology such as genetic modification (Library of Parliament, 2020). The practices began in the 1950s, with sales of organic produce increasing significantly over time. In 2017, the Canadian market for organic products was listed as 5.4 billion dollars (Research Institute of Organic Agriculture, 2019). As of 2016, Canada had over four thousand organic farms, with the largest percentage located in Quebec and Saskatchewan (Library of Parliament, 2020).

Due to the limitations placed on pest management in organic production systems, growers must rely on alternative means, including the use of organic fertilizers and organically sourced pesticides.

1.4.1. Mineral Nutrition

Plant nutrition has been shown to increase plant defence and reduce plant diseases (Datnoff et al. 2007). This includes both macronutrients such as nitrogen, phosphorus, potassium, and calcium, as well as micronutrients, such as iron, zinc and copper. Organic sources of these minerals may include unprocessed mined minerals, such as gypsum, clay, and limestone, or from other natural sources such as ground shells (Government of Canada, 2011).

1.4.2. Plant Extracts

“Botanical pesticides” are approved for use in an integrated pest management system, along with plant extracts, oils, and preparations (Government of Canada, 2011). The use of these products such as essential oils have shown efficacy against powdery mildew and other diseases (Mostafa 2021). New biopesticides are listed as being of research interest for new management strategies for both powdery mildew on cucumber and *Fusarium* on tomato (Pest Management Program, 2021).

1.5. Objectives

To identify sustainable approaches to disease management on tomato and cucumber, the objectives of this research were to:

1) Conduct a systematic review of the efficacy of calcium nutrition to prevent and treat plant diseases.

2) Identify modes of action of calcium in plant disease interactions reported in the literature

3) Test the efficacy of a novel biopesticide formulated as a plant extract against *Fusarium* on tomato.

4) Test the efficacy of a novel biopesticide formulated as a plant extract against powdery mildew on cucumber.

Chapter 2.

The Role of Calcium in Plant Disease

Note: This chapter is in press in: Datnoff, L. E., Elmer, W.H., & Huber, D. M. *Mineral nutrition and plant disease.* APS Press, 2022.

Calcium (Ca), a divalent alkaline cation, is the fifth most abundant element in the Earth's crust, which has an average concentration of 3.6%. Noncalcareous, highly weathered soils contain less than 1% Ca (McLean 1975), while calcareous soils may be 50% Ca carbonate or more, making their Ca contents above 10%. The Ca content of a given soil depends on its material of origin, degree of weathering, and whether or not Ca has been added by liming. The Ca in soil solution and exchangeable Ca are the main forms that can move to plant roots and be absorbed. The level of Ca in soil solution can vary, depending on the pH and nature of the soil, but it is commonly 20 to 40 mg L^{-1} (0.5 to 1.0 mM) in leached soils and 50 to 100 mg L^{-1} (1.25 to 2.5 mM) in arid soils. The two Ca-containing soil minerals with the greatest solubility are Ca sulfate and Ca carbonate. In the carbonate form, Ca occurs both alone and with magnesium in many arid soils. Exchangeable Ca is usually held more tightly in soils than potassium or magnesium, which are the two next most plentiful exchangeable cations (McLean 1975).

2.1. Effect of Ca on Soil Structure

It is commonly recognized that the characteristics of soil colloids are greatly influenced by the nature of the cations adsorbed on them. Sodium-saturated colloids cause soils to be sticky when wet and very hard when dry (Millar 1963). However, a high percentage of Ca ions on the complex results in flocculation (Baver 1928). As a result, soils in which the colloids contain a high level of Ca are usually in a high state of aggregation and are considered to be in good tilth. This condition facilitates good aeration, rapid absorption of water, and an increased capacity to hold capillary water. A good state of tilth also encourages root development and rapid emergence of seedlings as well as enhances the communities of aerobic microorganisms.

2.2. Factors Affecting Ca Influx

Several factors, especially other cations and pH, can affect the availability of Ca to plant roots. Magnesium, ammonium and soil pH have direct effect on Ca availability. Less direct effects may include tillage patterns, rotation and cover crops. (Hickman 2002). Lazaroff and Pitman (1966) found that the ratio of Ca to magnesium uptake by barley seedlings was similar to the ratio of the ions in solution when the level of Ca plus magnesium was 20 mmol L^{-1}. With higher rates of transpiration, magnesium uptake increased more than for Ca itself. Classen and Wilcox (1974) found that increasing concentrations of both ammonium and potassium in soil decreased Ca concentrations in the tissues of young corn plants, but the effect was greater for ammonium than potassium. In general, among all other cations, uptake of Ca ions is depressed most by ammonium ions. Maas (1969) found that reducing solution pH to values below 4.5 reduced Ca uptake by excised maize roots; increasing solution pH to levels above 4.5 had little effect. On the other hand, Leggett and Gilbert (1969) found no effect of solution pH on Ca uptake by excised soybean roots over the pH ranging from 3.8 to 6.5. In a 16-years tillage study, it was noticed that both reduced and no tillage resulted in increased cation exchange capacity and increased Ca fertility (Hickman 2002).

2.3. Relationship of Ca to Plant Growth

Ca is one of the most abundant basic cations in plant tissues, although its content for most crop plants is quite low in relation to potassium and chloride. The mean Ca content of plants is on the order of 1-3 kg per 100 kg of dry matter. In plants, Ca is present mainly in the leaves and stems, and less so in the seeds. For instance, the average Ca content in eight cereal grains was found to be 0.09% compared to 0.59% in the straw, although these values may vary widely depending on the quantity of available Ca in soil or nutrient solution. Loneragan et al. (1968) showed that Ca content on tomato shoots increased from 2.1 to 24.9 μM with Ca concentrations in the nutrient solution ranging from 0.8 to 1,000 μM. Other factors such as moisture conditions and the concentrations of other cations in soil, may also influence the Ca content in plant tissues. Many investigations have shown that when Ca supplies are low or the supply of some other cation is excessive, the Ca content of plants is reduced. Among all the plant's parts, leaves have the highest Ca content. This is the result of higher water movement

and water transpiration through the leaves. The Ca is relatively immobile in the plant tissues, which means that once it is located in one part of the plant, it moves slowly or not at all (Epstein 1973). This suggests that actively growing plant parts such as fruit need a constant supply of Ca, which requires maintaining adequate soil moisture.

2.4. Functions of Ca in Plant Growth

An adequate supply of Ca appears to stimulate the development of root hairs and the growth of the entire root system. The Ca is necessary for normal leaf development and tends to accumulate in the leaves and in older parts of plants such as the bark. The abundance of Ca in leaves may be due to the formation of Ca pectate in the middle lamellae of the cell walls. Other functions of Ca in the plant are described below.

2.4.1. Metabolic and physiological functions.

The cellular distribution of Ca has been studied by cellular fractionation and subsequent analysis of Ca in the cell fractions. In horse beans and yellow lupines grown in the presence of $CaCl_2$ (0.1 mol m^{-3}), at least 60% of the total Ca was associated with the cell wall, 7% with membranes, and about 33% with the soluble fraction (Rossignol et al. 1977). The third value may have been overestimated as a result of remobilization of Ca during cell fractionation, but most studies show that Ca is particularly abundant in plant cell walls (Jones and Lunt 1967; Wallace et al. 1966). One of the most recognized functions of Ca in the plant tissues is in membrane stability and maintenance of cell integrity (Legge et al. 1982). The middle lamella has the structure of a gel, mostly made up of polysaccharides such as pectic acid (α-1,4-linked polygalacturonans). The Ca ions favor gel formation in a manner similar to that in which gelling is induced in free solutions of algin or pectin. In the absence of Ca, the membranes become leaky and the solutes are lost from the cytoplasm. The plasma membrane can also be damaged if Ca is replaced from its binding sites on the exterior surface by heavy metals (Jones and Lunt 1967) or protons (Lund 1970). In physiological disorders such as blossom end rot in pepper, tomato, and watermelon (Shear 1975), the early symptoms, which often go unnoticed, appear as water-soaked lesions on the blossom end or bottom of the fruits. The affected tissues then breaks down and the area becomes sunken, dark brown or black, and leathery. Secondary microorganisms may grow on the decayed area and can

easily be mistaken for pathogens. This problem can be overcome by ensuring an adequate supply of Ca in the fruit. Rease (1996) reported that the application of $CaCl_2$ sprays or $Ca(NO_3)_2$ fertilization increased the cold-hardiness of Anjou pear and reduced the incidence of fruit disorders (pear greening and cork spot).

2.4.2. Effect of Ca on wall enzymes.

Wall-bound acid phosphatases extracted from potato tubers have been studied by Sugawara et al. (1981). The authors separated six different fractions of these enzymes; the optimum pH ranged from 4.5 to 6. When in free solution, all of these enzymes were activated by $CaCl_2$.

2.4.3. Role of Ca in signal transduction during defense response.

In plants, as in animals, many stimuli are mediated by elevation of cytosolic free Ca (Bush 1995). The involvement of Ca in responses of cell cultures or protoplasts to microbial products (elicitors) has been demonstrated (Ishihara et al. 1996; Mahady and Beecher 1994; Messiaen and Van Cutsem 1994; Siebers et al. 1990), and patch-clamp experiments have indicated that the elicitors may affect the functionality of the Ca channels in the plasma membrane (Gelli et al. 1997; Zimmermann et al. 1997). The Ca-calmodulin-dependent signaling pathway has also been found to play a significant role in conidia germination and appressoria formation of *Colletotrichum* spp. causing anthracnose on pepper (Ahn et al. 2003). The isolation of calmodulin from fungi and several species of higher plants indicated that high-affinity Ca-binding proteins are present in them (Cormier et al. 1982). These observations suggest that Ca-dependent metabolic processes in plant cells are regulated by Ca-binding proteins such as calmodulin by analogy to the known *in vitro* Ca-calmodulin-dependent functions in animal cells.

2.5. Calcium and Plant Disease

The role of Ca in the management of plant diseases is well described in the literature. Other than nitrogen, Ca is perhaps the most important nutrient in the management of diseases. In addition to agronomic benefits gained by maintaining adequate levels of Ca in plant species, numerous researchers have reported that the

application of Ca to soils, foliage, and fruit reduced the incidence and severity of several diseases of economically important crop species, as shown in Table 6.1. These reports are described in more detail below. The form of Ca applied can influence the mechanism through which Ca reduces disease development. For instance, pH-altering forms, such as lime, can influence disease through pH change, while salts containing anions, such as propionate and sorbate, can be inhibitory to pathogens by virtue of the toxicity of the anions.

2.5.1. Diseases of Cereals

Cephalosporium stripe of wheat (*Hymenula cerealis*) is greatly affected by environmental factors such as root damage from frozen soil. In two out of four years of field testing, Murray et al. (1992) found that the incidence of culms infected by *H. cerealis* decreased significantly when $Ca(OH)_2$ was added to increase soil pH from 5.1-5.3 to 6.0 and increased significantly when H_2SO_4 was added to reduce soil pH to 4.5. The relationship was linear in both years, and in the third year, there was a nearly significant (P = 0.07) linear trend for decreasing incidence of disease with increasing soil pH. By relating weather factors to disease severity, Murray et al. (1992) calculated that liming for Cephalosporium stripe would probably be most valuable in years when root wounding resulting from frozen soil was relatively minor.

Vanterpool (1940) reported that gypsum ($CaSO_4$) inhibited browning root rot of wheat caused by *Pythium arrhenomanes* and *P. tardicrescens*. Soil application of gypsum in two equal amounts of 500 kg ha^{-1} at different times significantly reduced the incidence of sheath rot on rice (*Sarocladium oryzae*) and increased yield, the result being comparable with that obtained by the application of fungicide carbendazim (Narashimhan et al. 1994).

Foliar applications of 9 mg/L of calcium silicate on rice was able to reduce rice blast disease caused by *Pyricularia oryzae* by 89.2 % with a partially resistant cultivar of rice. A resistant cultivar was also tested, and the foliar applications decrease disease by 97.9% (Ng et al. 2019).

Geetha and Shetty (2002) reported a 66% reduction of pearl millet downy mildew (*Sclerospora graminicola*), by seed treatment with $CaCl_2$, which was comparable to the

effects of the plant activator benzothiadiazole (78% reduction) and H_2O_2 (59% reduction). This study also revealed the role of Ca as a resistance inducer in the hypersensitive response of pearl millet to inoculation with *S. graminicola.*

Foliar applications of $CaCl_2$ and Ca silicate (each at 1%) were evaluated for suppression of powdery mildew (*Blumeria graminis* f. sp. *tritici*) incidence on durum wheat (De Curtis et al. 2012). Known biocontrol agents of powdery mildew, two yeasts (*Rhodosporidium kratochvilovae* and *Cryptococcus* and a yeast-like fungus (*Aureobasidium pullulans*)) were also tested alongside Ca applications. The Ca chloride did not reduce disease severity when used alone or along with a biocontrol agent. However, two Ca silicate applications made a week apart reduced disease severity by around 15 % and improved the efficacy of all three biocontrol agents.

2.5.2. Diseases of Vegetable Crops

Reports of successful suppression of carrot southern blight (*Sclerotium rolfsii*) in carrots following applications of Ca-containing compounds, which are relatively nontoxic to this fungus, suggest that Ca affected disease development by increasing host defense responses. Several Ca salts —$Ca(NO_3)_2$, $Ca(OH)_2$, $CaCl_2$, and $CaCO_3$—had no effect on the growth of mycelium and the germination and viability of sclerotia of *S. rolfsii* (Punja and Grogan 1982). However, field applications of Ca salts for controlling *S. rolfsii* had variable results, depending on disease pressure and cultivation technique. For example, when $CaSO_4$ was applied at a rate supplying Ca at 215 kg ha^{-1} to deep-plowed plots in combination with NH_4HCO_3, a 67% reduction in disease in processing carrot was obtained, whereas a 40% disease reduction was reported in disked soil with the same application rate (Punja et al. 1986). *In vitro* studies by Bateman and Beer (1965) and Punja et al. (1985) showed that the presence of Ca (as $CaCl_2$) reduced the activity of polygalacturonase on Ca pectate and on plant tissue, respectively. The presence of higher levels of Ca in carrot tissue, achieved either by vacuum infiltration of the material into tissue or by field applications of $Ca(NO_3)_2$, was reported to reduce the rate of southern blight development (Punja et al. 1986). It was proposed that increased levels of Ca in host tissues, present primarily as Ca pectate in the middle lamella of cell walls, could have rendered tissues more resistant to the action of cell wall-degrading enzymes (Punja et al. 1985). Levels of Ca in the periderm and parenchyma tissues following applications of $Ca(NO_3)_2$ and $CaSO_4$ (supplying Ca at 336 kg ha^{-1}) were significantly

higher than in tissues sampled from plots not receiving supplemental Ca (Punja et al. 1986). In sandy, coarse-textured soils with low cation exchange capacity, applications of Ca compounds were beneficial in enhancing normally low levels of Ca in the plant tissues and reducing the rate of southern blight development or its incidence.

El-Tarabily et al. (1997) reported a significant reduction in the incidence of cavity spot disease of carrots in soil infested with *Pythium coloratum* by amending the soil with 4,000 or 8,000 kg of lime, whereas amendment of the same soil with gypsum at both rates had no effect on disease incidence. The reduction in the incidence of cavity spot was related to the increase in soil pH which was associated with lime application.

The role of *Plasmodiophora brassicae* in clubroot has been recognized since the pathogen was described in 1878 (Woronin 1934) and liming of soil for the control of clubroot has been practiced for more than 200 years. Application of hydrated lime (4.5 t ha^{-1}) gave a pH of 6.7 and adequate control of clubroot in western Washington, as did lower rates of lime plus pentachloronitrobenzene (PCNB) (Anderson et al. 1976). Fletcher et al. (1982) obtained excellent disease control with limestone applied at the rate of 20 t ha^{-1} annually for two or three years in different plots. In this case, the soil pH was increased above 7.4. A single application of 5–10 t ha^{-1} in small-scale test plots in a heavily infested area provided almost complete control of clubroot of broccoli for two or three years in the Salinas Valley of California (Campbell et al. 1985). During this period, the soil pH was 6.5 or above in the limed plots. Furthermore, preplant application of lime to plots in commercial fields with scattered foci of infection effectively arrested the disease in the following crop in the Salinas Valley (Campbell et al. 1985). Thus, soil pH has come to play a dominant role in decisions about the need to lime and in evaluations of clubroot control. For example, liming may be indicated if the soil pH is less than 6.8, the general guideline used in the Salinas Valley. The desired target soil pH is often cited as 7.2 (Colhoun 1958), but this figure may not be appropriate for all soils. For example, poor control was achieved at the target pH 7.2 in one study (Wellman 1930), and good control was achieved at pH 6.7 in another study (Campbell et al. 1985). These examples provide evidence of the roles of other factors related to application (the form of lime and the need for adding lime yearly), the *P. brassicae*-host interaction as well as the inoculum density and environmental factors including temperature, light, soil moisture, and physical characteristics of the soil (Colhoun 1958). In some cases, liming is an erratic or partially effective measure and it cannot be exclusively relied on for disease

control unless lime is applied at high rates (Sherf 1976). These high rates may not be economical or may induce deficiencies of boron, iron, or manganese. Thus, liming is one of several clubroot control measures combined with host resistance (if available), chemical treatment of transplants, or soil rotation and clean seedbedswhich is supported by other studies as well. Porth et al. (2003) evaluated seven treatments, including boron, PCNB, and some other commercial nutrient formulations for managing clubroot in Chinese cabbage (*Brassica rapa* L. subsp. *pekinensis*) in Massachusetts. In this replicated experiment in commercial farms, the resistant cultivar and plants treated with Ca cyanamide showed the least disease among all the treatments.

The severity of Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*) in tomato plants decreased as the Ca concentrations in nutrient solution increased from 5 to 500 ppm (Edgington and Walker 1958). Corden (1965) and Standaert et al. (1973) also found that Ca deficiency encouraged tomato wilt development. Corden (1965) found that in tomato plants receiving a normal amount of Ca (200 µg ml^{-1}) before inoculation, with a supplementary dose of 1,000 µg ml^{-1} after inoculation, disease severity was reduced from 1.00 to 0.09, where 0 = healthy plant and 1 = severely diseased plant. The treatment also raised Ca levels in the vascular sap from 13 µg ml^{-1} in control plants to 1,081 µg ml^{-1} in plants receiving the Ca supplement. Corden (1965) suggested that the disease was reduced because Ca inhibits the activity of polygalacturonase produced by *F. oxysporum* f. sp. *lycopersici.* Jones and Woltz (1967) found in greenhouse and field experiments with soil infested with *F. oxysporum* f. sp. *lycopersici* that amending the soil with gypsum ($CaSO_4$) did not increase the soil pH and did not reduce the occurrence of Fusarium wilt. In contrast, the Ca content of tissues of plants grown in soil amended with hydrated lime ($Ca(OH)_2$) was increased, and the occurrence of wilt was reduced.

Calcium salts, including $CaCl_2$ and $Ca(NO_3)_2$, significantly reduced powdery mildew colony counts on tomato leaves in a study by Ehret et al. (2002). This study also revealed that Ca salt treatments that were effective in a single-application series were as effective as multiple applications. Although surfactant itself significantly reduced the number of mildew colonies, all combinations of Ca salts or Ca salts plus elemental S significantly reduced mildew colony counts, compared with treatment with a surfactant alone, which clearly explains the role of Ca in powdery mildew suppression on tomato foliage.

Members of the genus *Pythium* are present in agricultural and forest lands worldwide and cause serious economic losses in a broad range of hosts. Kao and Ko (1986) found that damping-off of cucumber seedlings caused by *Pythium splendens* H. Braun in artificially infested soil, decreased by about 50% following the application of lime in both greenhouse and field tests. The effect of Ca on the susceptibility of host tissue to the pathogen was also determined by sporangial inoculation of cucumber roots grown on Ca-amended and unamended soil. The incidence of infection was observed under a stereomicroscope after a three-day incubation period. Calcium reduced the incidence of necrosis on inoculated roots from 81 to 21% and reduced the average length of necrotic lesions from 20 to 8 mm.

Gray mold (*Botrytis cinerea*) severity was reduced by 35-50% in cucumber seedlings grown in commercial polyethylene tunnels fertilized with NPK containing Ca. The Ca treatments were effective under high relative humidity on two out of four sampling dates. Treatments with the fungicides diethofencarb and carbendazim reduced gray mold by 39 and 50%, respectively. In the second year, stem infection by *B. cinerea* following treatment with potassium, fungicide, or Ca was decreased by 50, 50, and 60%, respectively. A reduction in gray mold and increased yield of eggplant (*Solanum melongena* L.) were obtained by the addition of Ca to the fertilizer. Gray mold of pepper (*Capsicum annuum* L.) was reduced by 50–60% by the addition of Ca, but pepper yield was not increased (Elad et al. 1993). A single application of Ca sulfate alone or with Ca dihydrogen phosphate to glasshouse plants resulted in a reduction of gray mold in pepper and eggplant artificially inoculated with *B. cinerea* (Elad et al. 1993).

Calcium has been shown to reduce the severity of bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) on tomatoes (Berry et al. 1988). The disease severity was rated on a 5-point scale based on the degree of wilting with 0 = no signs of wilting and 5 = plants mostly wilted or dead. Tomato plants grown with 0 ppm of Ca had a disease rating of 5, whereas with 100 ppm of Ca disease severity decreased by 3.6. Higher levels of Ca (200 300 ppm) provided similar results, showing that the increase in Ca above 100 ppm had no enhanced effect on disease control.

The effect of $CaCl_2$ applications on bacterial wilt (*Ralstonia solanacearum*) development in tomato plants was determined by inoculating plants with the pathogen, followed by determining disease severity on a 4-point scale, where 0 = no symptoms and

4 = dead plants (Yamazaki and Hoshina 1995). In a cultivar of tomato that was highly susceptible to the pathogen, no Ca treatments were able to reduce symptoms, with all plants dying after 14 days. In a moderately resistant cultivar, low levels of Ca (0.4mM) had a disease index of 4 (all dead) after 20 days. Moderate levels of Ca (4.4 mM) reduced the disease index to 2.0 and high levels (20.4 mM) to nearly 0 in plants of the moderately resistant cultivar.

Tomato plants were root-dip inoculated with *Ralstonia solanacearum* and grown for 30 days (Jiang et al. 2013). Plants receiving 0.5 mM Ca provided by $Ca(NO_3)_2$ showed 100% disease severity within 14 days while 5 mM Ca reduced disease severity to 77.1 % and 25 mM had a disease severity of 56.8 %. This study also demonstrated a correlation between Ca levels in the plant and peroxidase and polyphenoloxidase activity. These enzyme levels increase during stress and may play key roles in the plant's defence system.

Pectobacterium carotovorum subsp. *carotovorum* causes soft rot on a range of crops including Chinese cabbage (*Brassica rapa* L. subsp. *Pekinensis* (Lour.) G. Olsson). Drenches and leaf sprays of 0.15 g l^{-1} Ca nitrate reduced disease severity by up to 48.5 % (da Silva Felix et al. 2016). Calcium chloride applications did not reduce the disease under the conditions tested.

2.5.3. Diseases of Legumes

The peanut plant is a unique legume, since it produces flowers above ground and pods (fruit) below the soil surface. Because of anatomical separation, few nutrients are translocated directly from the roots to the pods. During the period of pod development (nine to 12 weeks after seeding), tissues are susceptible to numerous environmental disorders, pests, and diseases. Two pathogens (*Pythium myriotylum* and *Rhizoctonia solani*) are important causes of pod rot. Hallock and Garren (1968) demonstrated that gypsum applied at high rates (1,030–3,090 kg ha^{-1}) to Virginia Bunch 46-2 reduced pod breakdown in two out of three years and increased pod yields and the percentage of sound, mature seeds. In all three years of the study, gypsum increased Ca levels in pods and reduced potassium levels. Vulnerability to pod breakdown pathogens was reduced in pods with a Ca content of 0.20% or more (Hallock and Garren 1968). Walker and Csinos (1980) reported on a three-year study of five peanut

cultivars grown in a location with a low level of soil Ca (356 kg ha^{-1}) and a location with a higher level of soil Ca (752 kg ha^{-1}) and top-dressed with gypsum (0, 560, 1,120, or 1,680 kg ha^{-1}) at early bloom. Pod rot did not occur in any cultivar receiving any of the treatments at the high-Ca location. At the low-Ca location, severe pod rot occurred in plots receiving no gypsum, but the severity decreased in all cultivars as the rate of gypsum increased. Significant differences in pod rot and agronomic characteristics were found among cultivars. Cultivars with high Ca requirements were more susceptible to pod rot than cultivars less dependent on Ca fertilization (Hallock and Garren 1968).

The availability of Ca to peanuts can affect both *Aspergillus flavus* infection and aflatoxin production by the fungus in the kernels. The Ca applications in certain soils can have a beneficial effect in lowering aflatoxin contamination, especially in Ca-deficient sandy soils (Wilson and Walker 1981). Wilson and Walker (1981) applied gypsum ($CaSO_4.2H_2O$) at rates equivalent to application of Ca at 112, 224, and 336 kg ha^{-1} and observed reduced colonization of seeds (kernels) from 7.38 to 4.06 and from 3.25 to 4.06%, respectively, and a corresponding significant reduction of aflatoxin concentration.

The development of symptoms of twin-stem abnormality of soybean, caused by a *Sclerotium* sp., was significantly reduced ($P = 0.001$) when Ca levels in aerial tissues were increased by the addition of $CaCO_3$ to the soil (Muchovej and Muchovej 1982). The addition of $MgCO_3$ to the soil enhanced the disease, providing further evidence that Ca has a role in reducing twin-stem symptoms, since Mg usually has an inhibitory effect on Ca uptake.

The addition of 0.4 mM $CaCl_2$ and/or $CaNO_3$ reduced root rot, caused by *Phytophthora sojae*, incidence in soybean plants by 50% while 4 mM of these salts totally inhibited disease development (Sugimoto et al. 2005). While the lower level of Ca (0.4 mM) stimulated pathogen growth and zoospores production *in vitro*, levels of 20 and 40 mM reduced pathogen growth, suggesting an indirect effect of Ca on increasing host resistance at the lower rate. Studies with the same host-pathogen interaction showed that Ca formate at 0.4 mM reduced disease incidence from 60 to below 5% (Sugimoto et al. 2008). Since this level stimulated mycelial growth, it was suggested that Ca application acted indirectly to enhance plant resistance. Field applications of 4 and 10 mM Ca formate reduced disease incidence from 35.9 to 10.5% and 5.3%, respectively, after 140 days of growth (Sugimoto et al. 2010). The following year, disease incidence

was reduced from 52% to 26.7 % with 4 mM Ca formate, to 21.7 % with 10 mM Ca formate and to 31.7 % with 10 mM Ca nitrate. At another site with lower initial disease incidence of 11.7 %, application of 4 mM Ca formate reduced disease to 7.5%, to 2.5 % with 10 mM Ca nitrate, and disease was eliminated completely with 10 mM Ca formate (Sugimoto et al. 2010). Mean plant height and pod yield were both increased with the addition of these Ca compounds. The stems of plants treated with 10 mM Ca formate and Ca nitrate had increased Ca content in the cambium and xylem tissues. Crystals resembling Ca oxalate were observed to form in some regions that also showed complete inhibition to fungal penetration.

Root rot, caused by *Aphanomyces euteiches*, on peas was reduced in soils supplemented with Ca (1.8×10^{-4} M g^{-1} soil) in the form of Ca carbonate or gypsum (Heyman et al. 2007). A disease severity index was used where 0 = no symptoms and 100 = dead plant. Disease severity was reduced from 80 to 11 with the application of gypsum with relatively no change in pH. While Ca carbonate applications increased the pH to 7.2, the disease severity decreased to only 73. The effect of $NaHCO_3$ (which decreases in the amount of Ca in the soil) increased disease severity from 17 to 62 and increased the pH from 7.6 to 8.7. Zoospores production was reduced the *in vitro* study when the pathogen was grown on medium containing Ca chloride at levels higher than 0.01 mM.

Precipitated calcium carbonate (PCC), also known as spent lime, was tested as a treatment for Fusarium root rot (caused by many species of *Fusarium*, including *Fusarium avenaceum*, and *F. solani* f. sp. *pisi*) on peas. Field trials were conducted to assess the effect of various rates of PPC on both pathogens (Chittem et al. 2016). Various levels of PCC were applied up to 22.5 tons per hectare. The highest Ca level reduced mean root rot severity significantly from 17.6 to 3.9% in plants infected by *F. avenaceum* and from 16.4 to 5.4% in plants infected by *F. solani* f. sp. *pisi*. This level of amendment also raised the pH from 6.8 to 7.6. Further field experiments at sites over subsequent years showed that levels of 33.8 tons per hectare were even more effective at reducing Fusarium root rot severity (Chittem et al. 2016).

2.5.4. Diseases of Fruit Trees

Infection of avocado seedlings by *Phytophthora cinnamomi* in infested soil was reduced by 71% following addition of gypsum (Messenger et al. 2000a). However, avocado seedlings grown in gypsum-amended soil and inoculated with a suspension of *P. cinnamomi* zoospores were not more resistant than seedlings grown in unamended soil. This suggests that the role of gypsum was not one of inducing host defense mechanisms in the roots but rather in some interaction with the pathogen or microbiome in the soil. In a following report, Messenger et al. (2000b), the same group of investigators showed that sporangia production by *P. cinnamomi* buried in gypsum-amended avocado soil for two days was reduced by as much as 74% in greenhouse trials. Soil extracts from gypsum-amended soil reduced *in vitro* sporangia production. Mycelium buried in unamended soil irrigated with gypsum solutions also produced fewer sporangia. These findings suggest that the mechanism by which soil amendment with gypsum reduces disease involves a direct effect on the pathogen.

Biggs et al. (1994) investigated the effects of Ca salts on the growth and pectic enzyme activity of *Leucostoma persoonii* and the colonization of peach twigs by this pathogen. Out of 20 salts tested, four significantly reduced colony diameter (Ca propionate, Ca hydroxide, Ca silicate, and Ca oxide). Calcium propionate fully suppressed lesion formation on twigs and some other salts significantly reduced lesion size. Calcium oxide, Ca silicate, and Ca propionate treatments reduced polygalacturonase activity after seven and 15 days.

Gadoury et al. (1994) reported an 80% reduction in ascospores production from cleistothecia of *Uncinula necator* on grapes in the spring when exposed to lime sulfur (Ca polysulfide) for 5 min in laboratory assays, whereas the fungicide dinocap (2,6-dinitro-4-octylphenyl crotonates and 2,4-dinitro-6-octylphenyl crotonates) took more than 1 h to reduce the number of ascospores to same level. Aqueous solutions of lime sulfur at a concentration of 120 ml L^{-1}, applied as over-the-trellis sprays at 2,800 L ha^{-1} (delivering lime sulfur at a concentration of 336 L ha^{-1}) to dormant grapevines in spring killed cleistothecia of *U. necator* on the bark of the vines and delayed the development of epidemics of powdery mildew. In vineyard trials with the *Vitis* interspecific hybrid cultivar Rosette, which is highly susceptible to powdery mildew, the severity of fruit infection was reduced from 43.1 to 14.1% in 1986, from 4.6 to 0.1% in 1988, and from 41.4 to 27.2%

in 1989 by a single eradicant treatment of lime sulfur. Treatment of the more resistant *Vitis labrusca* cultivar Concord with lime sulfur applied at the same rate reduced the severity of powdery mildew from 30.8 to 0.3% in 1988 (Gadoury et al. 1994). As the effects of Ca and S were not investigated independently, we cannot seperate the effect S may have in these results.

Phytophthora root rot, caused by *Phytophthora nicotianae*, is a serious and widespread disease of sour orange in Europe and most of the citrus-growing regions of the world. Out of 10 Ca salts evaluated for their efficacy against the pathogen *in vitro* and *in vivo,* CaO and $CaCO_3$ were found to significantly reduce mycelial weight and the production and viability of zoospores. Other Ca salts also significantly reduced the inoculum density of the pathogen in soil or root infection (Campanella et al. 2002).

Pseudothecia and ascospores of *Mycosphaerella citri* produced on infected leaf litter are a major means of dispersal of the pathogen and a major source of inoculum causing secondary infection of citrus leaves. In laboratory experiments, Mondal and Timmer (2003) observed a 90% reduction in the production of pseudothecia and ascospores after treating leaf litter with a combination of urea and $CaCO_3$ or dolomite. The rate of $CaCO_3$ or dolomite was also negatively correlated with the number of days to pseudothecia and ascospore production and the pseudothecial incidence and density. Application of $CaCO_3$ or dolomite together with urea may be useful in an integrated program for greasy spot management by reducing the inoculum source.

Calcium chloride has also been shown to be effective at treating pear trees infected with *Erwinia amylovora* and *Stemphylium vesicarium,* causing fire blight and brown spot, respectively. Pots were watered with solutions of $CaCl_2$ until their conductivity reached 8.3 mS cm^{-1}. This amendment decreased fire blight necrosis length and brown spot leaf infection 5 weeks after inoculation by 50% as compared to the control (Toselli et al. 2004).

The incidence of Xanthomonas wilt (*Xanthomonas campestris* pv. *musacearum)* in banana (*Musa* × *paradisiaca*) was reported to be reduced by increasing Ca concentration in tissue culture media from 12 mg L^{-1} to 241 mg L^{-1} (Atim et al. 2013). Disease incidence was reduced from 73 % to 40% in the variety Kayinja, and symptoms

were delayed and plant death was also delayed to 22 days instead of 14 days in the low Ca media.

Bitter rot, caused by *Colletotrichum acutatum*, in apples was reduced with $CaCl_2$ at 20 mg ml^{-1} Ca, which decreased lesion diameter from 15.1 mm to 10.6 mm (Boyd-Wilson et al 2014). The use of $CaCl_2$ also showed an additive effect with some biocontrol yeasts such as *Rhodosporidium diobovatum*, which further reduced the lesion size to 6.4 mm. Calcium carbonate did not reduce lesion size, but improved the efficacy *R. diobovatum* resulting in lesion size diameter of 8.6 mm. Since these studies did not separate the role of Cl from Ca, it is difficult to assign credit to one ion over the other.

Diseases of Field Crops

Extracts from leaves of spring canola cultivars were shown to inhibit the polygalacturonase activity produced by a highly virulent isolate of the blackleg pathogen, *Leptosphaeria maculans* . Polygalacturonase-inhibitory activity present in cotyledon and leaf extracts was proven to be due to a divalent cation since it was not affected by autoclaving or protease digestion but was reduced by chelation with EDTA. Calcium levels in leaf extracts from different cultivars correlated positively with the level of polygalacturonase-inhibitory activity and the resistance of the cultivars to *L. maculans* (Annis and Goodwin 1997). In addition, Hummond and Lewis (1986) found that Ca levels, but not levels of chlorine, phosphorus, potassium, sulfur, silica, or sodium, increased when lesion expansion decreased during infection of canola stems by a highly virulent isolate of *L. maculans.*

In potato tubers, the severity of soft rot, caused by *Pectobacterium atrosepticum*, was inversely correlated with tuber Ca content (McGuire and Kelman 1984). Addition of Ca was more effective than Mg or K in preventing tissue maceration. A higher Ca concentration in the nutrient solution increased the level of tuber Ca and the amount of galacturonic acid in the cell walls (McGuire and Kelman 1986). This resulted in increased formation of Ca pectate. The distribution of Ca within potato tubers can be very irregular; the periderm region can contain up to five times as much Ca as the medullary tissue and approximately twice as much as the vascular tissue (McGuire and Kelman 1986). When $CaSO_4$ and $Ca(NO_3)_2$ were applied, Ca uptake by the potato cultivar Russet Burbank increased from 0.057 to 0.277% of dry weight in the peel and

from 0.011 to 0.063% in the medulla (McGuire and Kelman 1984). This increase in tuber Ca was correlated with a decrease in surface area decayed from 43.5 to 19.4%. Although the effectiveness of different Ca sources in enhancing tuber Ca concentration and in reducing bacterial soft rot susceptibility in a given tuber sample can vary from year to year, Tzeng et al. (1986) reported consistent and positive results from preplant applications of $CaSO_4$ to potatoes under field conditions.

Alternaria solani, the cause of early blight on potato was reduced by foliar applications of calcium chloride and calcium citrate in a gel formulation (Abou-El-Hassan et al. 2020). Plants were sprayed twice with treatments at 45 and 60 days post-planting. Disease incidence was reduced by 55.5% with calcium chloride gel sprays and by 43.0 % with calcium citrate gel.

Bacterial wilt on ginger, caused by *Ralstonia pseudosolanacearum*, was reduced by 100% with soil drenches of 4% calcium chloride (Bhai et. al. 2019). This treatment level was also able to inhibit the bacteria's growth in vitro.

2.5.5. Diseases of Tropical Crops

Ca hydroxide sprayed on scratched coffee leaves at 0.04, 0.08, or 0.1 mg cm^{-2} followed by inoculation with gemmae of *Mycena citricolor* (Rao and Tewari 1988). The number and diameter of lesions were significantly reduced, and lesions expansion was delayed at all levels, with lesion development being completely inhibited by applications of 0.1 mg cm^{-2}. Scanning electron microscopy revealed numerous Ca oxalate crystals on lesions on leaves sprayed with $Ca(OH)_2$. This suggested that the mode of action of $Ca(OH)_2$ in disease control is neutralization of oxalic acid secreted by the pathogen (Rao and Tewari 1988). Angell (1950) reported that liming of steamed soil reduced seedling blight caused by *P. ultimum* in opium poppy plants.

2.5.6. Diseases of Shade Trees

Dogwood trees are very susceptible to anthracnose caused by *Discula destructive.* Seedlings were grown in pots, to which fertilizers at 0, 50, 100, and 200% standard Ca rates were added (Holzmueller et al. 2007*)*. The pots were placed around infected trees to provide a source of inoculum, and the disease rating was determined

biweekly (0 = dead to 5 = no infection) for 6 months. A rate of 100% Ca significantly decreased anthracnose symptoms from six weeks and onwards, while neither the 50 nor 200% rates had an observable effect.

Holm oak is susceptible to *P. cinnamomi,* and Ca applications have been studied for disease reduction. In vitro, Ca oxide and Ca carbonate both reduced sporangia production by 100% and close to 100 %, respectively (Serrano et al. 2012). Calcium oxide was also very effective at reducing average radial growth after 3 days from 29 mm to 5 mm. It was hypothesized that the reduction in mycelial growth was due to the increase in pH resulting from CaO applications, not the toxicity of the ion. Calcium chloride significantly inhibited zoospore production by around 65 %. Calcium oxide, Ca carbonate, and Ca propionate amended soils all showed reduced foliar symptoms by 50% and root symptoms by 25%.

Phytophthora cinnamomi causes Phytophthora dieback in many species including the shrub *Banksia leptophylla*. Six-month-old seedlings were planted in sand with 0, 3, 10 or 30 mmol $CaSO_4.2H_2O$ kg^{-1} sand (Stasikowski et al. 2014). The sand was inoculated using wooden plugs taken from infected pine trees. Plants that received the highest Ca treatment levels along with the highest application rates of foliar phosphite had a 100 % survival rate, as compared to 53 % with only phosphite, and 8.3% with calcium alone. When $CaSO_4$ was sprayed twice, 72 hours pre-inoculation and 72 hours post-inoculation with a spore suspension of *Alternaria brassicae*, the diameter of leaf spots in the leaves of Indian mustard plants after 50 days was reduced from 7.0 (control) to 5.4, 5.1, and 3.5 with applications of 0.5, 1.0, and 1.5% $CaSO_4$, respectively (Kumar et al. 2015).

2.5.7. Diseases of Herbs and Spices

Paprika plants were grown hydroponically, and various Ca compounds were added to the nutrient solution (Yoon et al. 2010). The seedlings were inoculated with a conidial suspension of 1×10^6 conidia of *Botrytis cinerea* mL^{-1}. After a month, plants without treatment had 66.7% disease incidence, those with added Ca oxide had no symptoms, while Ca hydroxide and Ca carbonate reduced disease to 20 %. Calcium nitrate only reduced the incidence to 60 %. All of these compounds were shown to decrease mycelial growth in vitro.

Applications of foliar sprays of $CaCl_2$ (1%) every 15 days to the leaves of tea plants resulted in a 71% decrease in blister blight severity, caused by *Exobasidium vexans* (Chandra et al. 2014). Disease was evaluated based on a scale of 0 = no disease to 9 = more than 50 % leaf area infected. The plants receiving the foliar spray also had higher phenols (1000 ug $gram^{-1}$ of tissue instead of 902). The concentrations of defence enzymes were also higher, including peroxidases (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), and b-1,3 glucanase. PO and PPO are involved in the biosynthesis of lignin and other phenols, PAL is used to synthesize phenolics, phytoalexins and lignins. B-1,3 glucanase can directly act on the glucan present in fungal cell walls. Levels of nitric oxide were also elevated. This compound is thought to be a key signalling molecule in systemic acquired resistance (Chandra et a. 2014).

The effects of solid Ca amendments of various particle sizes on the population of *Ralstonia solanacerarum* was studied. The number of bacteria was found to be 1.7×10^4 CFU in soil with 1% $CaCO_3$ (0 to 1 mm particles in size) as compared to 30×10^4 CFU when 1% was applied as 5 to 7 mm particles (He et al. 2014). These larger particles had no effect on pH in the soil whereas the smallest particles increased soil pH from 6.65 to 8.03. Fertilization with a solution of 5 mM $CaCl_2$, which had no effect on pH, reduced the number of bacterial colonies by more than 50%. Sodium chloride was applied as well at the same levels, but had no effect on bacterial growth, suggesting the Ca ion was causing the reduction. Changing the pH of the soil with NaOH and HCl demonstrated that bacterial growth was significantly inhibited above 7.5 and below 5.5. Applying 1 % $CaCO_3$ (particles less than 3 mm in size) in field trials reduced disease from 23.33% to 0.83% after 60 days.

Grey mold, caused by *B. cinerea*, severity on sweet basil plants was also reduced by Ca chloride applications (Yermiyahu et al. 2006). Plants were grown with 2 mM, 4 mM or 6 mM Ca added to their irrigation systems. Cuttings were then taken and inoculated with *B. cinerea*. Those receiving higher levels of Ca showed reduced disease severity and incidence of sporulation. Sweet basil can also be affected by white mold, caused by *Sclerotinia sclerotiorum.* Amending the soil with 3.0 mM $CaCl_2$ reduced disease severity (David et al. 2019). Potassium was also found to decrease the severity of white mold, but both amendments together did not show any additive effects.

2.5.8. Diseases of Small Fruits

A study by Fang et al. (2012) evaluated the effect of pH on Fusarium wilt, caused by *Fusarium oxysporum* on strawberry plants (*Fragaria×ananassa*). Lime was used to alter the pH of soil and higher application levels were associated with lower disease severity. Disease was rated from 0 = healthy plant to 5 = plant dead. The control soil without lime had a pH of 5.2, and a shoot disease severity rating of 4.75. This was lowered to 3.0 with amendments of 0.3% lime that caused a pH change to 5.8 and disease rating was 1.75 with an application of 0.6% lime (pH of 6.7). This study did not investigate the role of Ca ions in the disease reduction but instead was investigating the changes in pH.

2 mL/L of calcium silicate was able to reduce the growth of *Phomopsis obscurans* in laboratory conditions by 72.2 % (Abd-El-Kareem et al. 2019). When 6 g/L of calcium silicate was applied as a soil treatment it was able to reduce this disease which causes leaf blight on strawberry by 73.3 %, and by 86.7 % when both a soil and foliar treatment was applied. Increases in defense enzyme activity of peroxidase, polyphenol oxidase and chitinase, was also seen in the calcium silicate treatments. Silicon is also known to have a role in plant defense, and therefore inhibitory effects cannot be attributed solely to one mineral or the other.

2.5.9. Diseases of Ornamentals

Starkey and Pedersen (1997) reported an improvement of the postharvest life of potted rose with increased levels of Ca in the nutrient solution. They also showed a negative correlation between gray mold development and increased Ca concentration in the flower. In growth chamber experiments, the amendment of the fertilizer solution with 10 or 20 mM $Ca(NO_3)_2$ greatly suppressed infection of flood-irrigated, containerized vinca seedlings in a peat-based mix by motile or encysted zoospores of *P. nicotianae* var. *parasitica* (von Broembsen and Deacon 1997). Calcium applied as either $CaCl_2$ or $Ca(NO_3)_2$ in water or Ca-free soluble fertilizer solution affected several important stages of *P. parasitica* zoospore behavior relevant to infection and disease spread. The release of zoospores from sporangia was suppressed by Ca concentrations in the range of 10 to 50 meq. These concentrations also curtailed zoospore motility; 20 meq of Ca in fertilizer solution caused all zoospores to encyst within 4 h, whereas 94% of zoospores remained

motile in an unamended solution. These results demonstrated that Ca amendments interfere with *P. parasitica* zoospore biology at multiple stages, with compounding effects on epidemiology, and that the manipulation of Ca levels in irrigation water or fertilizer solutions can contribute to the management of *P. parasitica* in recirculating irrigation systems (von Broembsen and Deacon 1997). Calcium sulfate was applied to roses before harvest to reduce the severity of gray mold caused by *B. cinerea* (Capdeville et al. 2005). The highest disease reduction was seen with the application of 10 mM or 20 mM Ca sulfate solutions that were sprayed on the plant until run-off 24 hours before harvest. Vase life was increased 30 % in uninfected flowers, and 20 % in those infected with grey mold.

Foliar sprays of calcium chloride, calcium nitrate and Ca-amino acid chelate on petunia significantly reduced the severity of blight caused by *Botrytis cinerea* (Bennett 2019). When applied at a rate of 500 mg/L, disease was reduced by 67 % on average for the three calcium sources. At a higher rate of 1250 mg/L this increased to an 85 % reduction in blight.

2.5.10. Postharvest Diseases of Tubers and Fruits

Certain physiological disorders and diseases of storage organs, such as tubers and fruits, are related to the Ca content on their tissues (Scott and Wills 1979). The Ca deficiency can result in serious economic losses in fruits such as peaches and apples. The Ca helps to regulate the metabolism of apple fruit, and its adequate concentrations maintain fruit firmness and reduce the incidence of disorders such as water core, bitter pit, and internal breakdown (Bengerth et al. 1972). Fruit ripening is generally delayed when the Ca level is increased, and the fruits maintains their quality for a longer period. In potatoes, low Ca content has been associated with physiological disorders such as internal brown spot in tubers and subapical necrosis of sprouts (Tzeng et al. 1986). Postharvest decay, caused by *Pectobacterium carotovorum*, in potatoes (McGuire and Kelman 1986) and postharvest decay caused by fungal pathogens in apples can also be effectively reduced with applications of Ca. A special type of application method is required, since the Ca concentration required for significant reduction of postharvest decay in apples and potato tubers is usually higher than what can be achieved with standard fertilization practices. While soils may contain adequate levels of Ca, most of what is taken up by plants is distributed to the leaves. Calcium sprayed directly onto

apples on the tree can increase the Ca content of the fruit, but dipping apples in solutions of Ca salt is more effective, and vacuum or pressure infiltration is superior to dipping. The absorption of Ca by apple fruit following preharvest sprays or postharvest treatments varies with the cultivar, maturity, and permeability of the peel. Although Ca applied after harvest presumably enters the fruit primarily through lenticels (Betts and Bramlage 1977), cracks in the cuticle or epidermis may also be important pathways, especially when the fruit is picked late in the season. A study of Golden Delicious fruit harvested at different maturity levels indicated a direct relationship between Ca uptake, inhibition of decay, and time of harvest (Conway and Sams 1983).

McGuire and Kelman (1984) found $CaNO_3$ to be more effective than $CaCl_2$, $CaSO_4$, or Ca gluconate in increasing the Ca content of peel tissue through pressure infiltration. Increasing the concentration of Ca in the medulla from 0.02 to 0.63% reduced the percentage of surface area decayed by *E. carotovora* subsp. *atroseptica* from 93 to 15% when tubers were held for 60 h in a mist chamber to simulate conditions that may develop in storage facilities.

In a study of the effect of three salts ($CaCl_2$, Ca propionate, and Ca silicate) on two apple bitter rot fungi (*C. gloeosporioides* and *C. acutatum*), none of the salts had any effect on conidial germination, but $CaCl_2$ and Ca propionate inhibited germ tube growth by 41 and 50% relative to the control, respectively, at Ca concentration of 1,000 mg L^{-1} (Biggs 1999). All three Ca salts reduced fungal dry weight in liquid culture. Three weekly applications of dilute Ca solutions during fruits development in the field resulted in a lower incidence of bitter rot infection than in the control. These experiments demonstrated that Ca salts had a suppressive effect on the bitter rot pathogens and could be used as part of a disease management program.

Experiments on the physiological disorder bitter pit of apple in Australia and New Zealand revealed that vacuum or pressure infiltration of $CaCl_2$ was superior to dips (Scott and Wills 1979). However, studies of bitter pit development revealed that Ca may have a secondary role in disease development (Saure 1996). The primary factor causing bitter pit was reported to be high levels of gibberellin (GA) late in the season. The elevated GA may have increased the permeability of cell membranes in the fruit adjacent to vascular bundles, thereby resulting in increased sensitivity of the fruit cells to postharvest water stress, which after harvest may have triggered a Ca deficiency,

enhancing fruit susceptibility. Externally applied growth retardants or ripening-related endogenous GA antagonists (e.g., ethylene and abscisic acid) reduced susceptibility to bitter pit, independently of Ca, by antagonizing the GA effect.

Conway et al. (1991) pressure-infiltrated apple fruits in three separate growing seasons and then inoculated them with *Glomerella cingulata*, *Penicillium expansum*, and *B. cinerea*. The Ca concentration was negatively correlated with decay caused by these pathogens in all tests. Decay caused by *G. cingulata*was reduced by 70%, decay caused by *B. cinerea* was reduced by 50%, and decay caused by *P. expansum* was reduced by 37%. Thus, Ca-induced reduction of postharvest fungal pathogens was broad in spectrum. $CaCl_2$ has also been found to be effective against the infection of apple fruits from cultivar Nittany to *Alternaria* (Biggs et al. 1993). Eight biweekly seasonal applications of $CaCl_2$ (1.27 g L^{-1}) followed by postharvest dip treatment reduced the incidence of Alternaria rot from 61% in the fruits from the control treatments to 5% in the treated fruits.

In spite of the proven efficacy of Ca salts in suppressing postharvest fungal pathogens, significant variability was reported among isolates of *B. cinerea* (Grindle 1979). This observation was supported by Chardonnet et al. (2000) in a study using three isolates of *B. cinerea,* one from decayed apple and the other two from grape. Ca infiltration reduced decay caused by all three isolates in apples, but the extent of reduction varied, with the area of decay decreasing to one-third or to one-fifth of that of an untreated control, depending on the isolate used. The amount of $CaCl_2$ providing maximum inhibition of mycelial growth and polygalacturonase activity of the different isolates varied from 1 to 4 g L^{-1} and from 1 to 16 g L^{-1}. Wisniewski et al. (1995) examined the effect of Ca and Mg salts on *B. cinerea* and *P. expansum in vitro* and on the biocontrol activity of two isolates of the yeast *Candida oleophila* Montrocher. Increasing concentrations of $CaCl_2$ (25–175 mM) resulted in decreased spore germination and germ tube growth of both pathogens, whereas $MgCl_2$ had no effect, indicating that the Ca cation rather than the Cl anion was responsible for the inhibition. The pectinolytic activity of crude enzyme obtained from the culture medium of both pathogens was also inhibited by 25-175 mM $CaCl_2$. The activity of one of the biocontrol yeasts was enhanced by the addition of 90 or 180 mM $CaCl_2$; this result was apparently due to an additive effect of the inhibition of pathogen spore germination and metabolism

by Ca and the ability of the yeast to maintain normal metabolism in the presence of toxic levels of Ca (Wisniewski et al. 1995).

Punja and Gaye (1993) obtained significant reduction in black root rot caused by *Berkleyomyces basicola* when artificially wounded and inoculated carrot roots or root slices were dipped in a 0.05 or 0.1 M solution of either Ca propionate or potassium sorbate for 2 min, compared to the standard sodium hypochlorite treatment (with chlorine at a concentration of 100 μg ml^{-1}). Treatments applied to carrot tissues within 24 h after inoculation provided a significantly higher level of disease reduction than those applied just prior to inoculation. Both Ca propionate and sodium hypochlorite were considerably more effective at low pH than at a higher pH.

The efficacy of the biocontrol agent *Pichia membranifaciens* against Rhizopus rot of nectarine was increased when spore suspensions were supplemented with 2% $CaCl_2$ (Qing and Shiping 2000). Disease incidence and lesion diameter on fruit treated with 2% $CaCl_2$ plus the yeast were significantly lower than on fruit treated with water and the yeast at low spore concentrations.

The incidence of green mold, caused by *Penicillium digitatum*, on grapefruit surface wounds was reduced by 43-52% following the application of 68 or 136 mM $CaCl_2$ (Droby et al. 1997). The efficacy of the biological control agent *P. guilliermondii* strain US-7 (10^7 cells ml^{-1}) increased almost nine-fold when suspended in a $CaCl_2$ solution in comparison to its efficacy when suspended in water.

Elevating the Ca content of peach fruits flesh by postharvest pressure infiltration with 2 and 4% $CaCl_2$ resulted in 40 and 60% less decay area, respectively, in comparison to a control, after inoculation with a conidial suspension of *Monilinia fructicola* (Conway et al. 1987). Another study revealed that several Ca salts significantly reduced the growth of *M. fructicola* on amended potato dextrose agar after seven days (Biggs et al. 1997). Fungal polygalacturonase activity was also inhibited by all salts tested, except for dibasic Ca phosphate and Ca tartrate. The greatest inhibition of polygalacturonase was observed with Ca propionate, followed by Ca sulfate, tribasic Ca phosphate, Ca gluconate, and Ca succinate. All of the salts tested reduced brown rot severity when inoculum was applied at a localized point to wounded fruits that had been dipped in a solution containing Ca at a concentration of 1,200 mg L^{-1}.

The incidence and severity of side rot of Bosc pears, caused by *Cadophora malorum*, was reduced on fruits from trees treated with $CaCl_2$ sprays during the growing season (Sugar et al. 1991). Trees were treated every two weeks beginning in early July, for a total of three applications of $CaCl_2$ at 1.2, 3.6, or 6.0 g L^{-1} of Ca. Postharvest mature fruits were wound-inoculated with different spore concentrations, followed by three months of storage at 0°C. Significant reductions in lesion area were obtained at all Ca concentrations used, in comparison to a control.

Washington et al. (1998) obtained a 41% reduction of fruit scab and an 83% reduction of leaf scab of pear by applying six to 10 sprays of 2% hydrated lime from green tip until summer. When hydrated lime was applied with flusilazole sprays, better reduction of disease incidence was obtained, which translated to an 89% increase in fruit yield as measured by crop weight, in comparison to a control. This result suggested that hydrated lime could be included in spray schedules with fungicide to reduce fungicide use in orchards.

Melon fruits from cultivar Valenciano, previously wounded, were immersed in solutions of each of four Ca salts ($CaCl_2$, $CaSO_4$, $CaCO_3$, and $Ca(NO_3)_2$) at 11% Ca. After air-drying, the fruit was inoculated with an isolate of *Paramyrothecium roridum*. Of the four salts tested, $CaCl_2$ reduced the disease by 66% in treated plants, in comparison to a control (Lima et al. 1998).

Water-soaking disorder can cause considerable economic losses in sensitive cantaloupe varieties, such as Talma. In perlite and rockwool culture, 98% of fruit showed this disorder in the pulp in Ca-deficient treatments, whereas only 16.5 and 28.7% of fruit was affected in Ca-treated (4.0 mmol L^{-1} of fertigation solution) perlite and rock wool, respectively (Madrid et al. 2004). This result indicates that Ca treatment can effectively reduce cantaloupe fruit disorders.

Brown rot, caused by *M. fructicola*, was reduced in peaches by using $CaCl_2$ applied before harvest (Elmer et al. 2007). Various spray schedules were tested, the most effective being applications of 50 ml to 100 ml, which reduced the number of fruits that showed signs of rot at the time of harvest by 50% (from over 20 % to below 10 %). Fruit from Ca treated trees also showed less incidence of rot after four weeks in storage at 2 to 4°C.

The Ca chloride dips have been shown to be effective at reducing brown rot, caused by *Monilinia laxa*, on peaches. Dipping the fruit in 2 g/L for 30 minutes reduced the severity from 36.36 % to 15.28 % (Thomidis et al. 2007). The incidence of brown rot was not significantly decreased with preharvest applications made in the field, but $CaCl_2$ showed a direct inhibitory effect on mycelial growth of *M. laxa*. The authors suggested that the results of the dip could be due to the direct toxicity of the Ca, not to increased resistance of the fruit.

Pear fruits in storage are susceptible to numerous diseases including grey mold caused by *B. cinerea* (Zhang et al. 2005). The *Cryptococcus laurentii* is reported to be a good biocontrol yeast against several fungal diseases. The activity of *C. laurentii* against *B. cinerea* on pear fruits was increased by the addition of 2% $CaCl_2$. When yeast concentration was 10^7 CFU ml^{-1}, the addition of 2% $CaCl_2$ reduced disease incidence by around 20% and lesion diameter by 5 mm.

Rot incidence in pears fruits, caused by *Alternaria alternata*, can also be reduced by $CaCl_2$ applications (Tian et al. 2006). Dipping fruit in 2% (w/v) Ca chloride before inoculation reduced disease incidence by around 20 % in fruit that had been left at 20°C for 7 days.

Lima et al. (2005) used a large number of food additives in combination with biocontrol agents to investigate their effect on *P. expansum* on stored apple fruits. The synergy factor was calculated for each interaction, which is a ratio of the expected result (if the effect was additive) as compared to the observed result. A value of 1 meant that the effect is additive, less than one is antagonistic and greater than 1 is synergistic. The effect varied for each of the biocontrols used. Calcium acetate, Ca ascorbate, Ca citrate and Ca propionate were all synergistic when treated along with *Cryptococcus laurentii* and *Aureobasidium pullulans*, but antagonistic with *Rhodotorula glutinis.* Calcium silicate was additive and $CaCl_2$ was synergistic with every biocontrol agent.

The rot of apple fruits can also be caused by *Monilinia fructigena*. Biweekly $CaCl_2$ applications (90 g L^{-1}) were applied before harvest (Holb et al. 2012). This was shown to reduce post harvest disease progress after 6 months from 11.1 % to 3.6 %. Calcium was also shown to be effective at reducing the spread of the pathogen to surrounding fruit.

The biocontrol agent *C. laurentii* is also effective at controlling blue mold, caused by *Penicillium expansum*, as well as grey mold on apple fruits (Yu et al. 2012). Combining it with Ca applications was seen to reduce disease better than either treatment alone. To investigate the effect of the timing of applications, solutions of $CaCl_2$ and yeast were applied 2 h and 24 h before inoculation with the pathogen. The 2-h treatment had no effect on disease incidence, whereas the 24-h treatment reduced the incidence of *P. expansum* by 38 % and *B. cinerea* by 61.5 % after 4 days. This shows that the increased resistance occurs when the plant has time to uptake the Ca. Peroxidase levels were seen to increase earlier in treatments with combined yeast and Ca treatments, suggesting a quicker defense response.

The *Burkholderia cepacia* is a gram-negative bacterium that can be used as a biocontrol against anthracnose, caused by *C. gloeosporioides,* in papaya. It was found that adding $CaCl_2$ and chitosan in tandem with the biocontrol agent was able to completely reduce disease severity to 0% after 18 days (Rahman et al. 2009). The components on their own were less effective, with severities of 74.6% with 0.75% chitosan of 78.8% with 1×10^9 CFU ml^{-1} of *B. cepacia*, and of 39.2% with 3.0% $CaCl_2$.

The effect of preharvest applications of $CaCl_2$ on anthracnose, caused by *Colletotrichum gloesporioides,* in papaya was also studied (Madani et al. 2014). Applying a solution of 1.5% $CaCl_2$ before harvest reduced the incidence after 21 days from 100 to 8.8%. These levels were also seen to increase Ca content in the peel, inhibit lesion development, reduce spore germination, and delay disease onset.

The Ca was found to be beneficial in improving the action of *P. membranifaciens* against anthracnose rot of loquat fruit caused by *C. acutatum* (Cao et al. 2008). Six days after fruit inoculation with 100 µL of either 2% Ca chloride, 1×10^8 CFU ml^{-1} of *P. membranifaciens* or a mixture containing both, disease incidence and lesion diameter were measured. Both Ca and the biocontrol agent independently reduced the disease but were more efficient when applied together. The same levels were tested directly against the pathogen and were observed to reduce spore germination and germ tube length in vitro, with the combination being more effective than either component alone. Chitinase and β-1-3 glucanase levels in the plant also increased along the same trends. These enzymes are indicative of increased plant defence responses. These results

indicate the treatments were inhibiting the pathogen and increasing the plant's defence to infection.

Crown rot is a disease in bananas (*Musa acuminata* AAA, cv. Grande Naine, Cavendish) caused by many fungal pathogens including *Colletotrichum musae* Arx (Bastiaanse et al. 2010). *Candida oleophila*, a strain of antagonistic yeast, can be used as a biocontrol for the disease. Calcium chloride applications were shown to improve the biocontrol efficiency of the yeast, although the Ca had no direct effect on the disease itself. Biocontrol activity, measured as the reduction of internal necrotic surfaces, of the yeast alone was 26 % and increased to 42 % with the addition of 2 % Ca chloride (w/v). Yeast reduced the lesion size the most (53 %) when both Ca was added, and the fruit was placed in modified atmosphere packaging in polyethylene bags (Bastiaanse et al. 2010).

The Ca chloride was tested for its ability to control grey mold, caused by *B. cinerea*, on table grape. A solution of 1% was applied twice, one and three months before harvest (Nigro et al. 2006). Field rot was decreased from 63.8 % to 22.5 % as compared to the control. In another trial, grapes that had received 1 % $CaCl_2$ applied 21 and 5 days before harvest were placed in storage for 1 month at 0 C followed by 5 days at 20 C. Incidence of rot in fruit treated with $CaCl_2$ was reduced by 54.8 % as compared to the control.

Preharvest applications of 1 % Ca chloride reduced postharvest incidence of grey mold on table grapes from 15 % in the control to 10% (Chervin et al. 2009). Applications were made 4 times between fruit set and harvest, then fruit were stored for 6 weeks in cold storage and placed at ambient temperature for 24 hours. Addition of 16 % ethanol alongside 1% Ca chloride reduced the instance of grey mold to 5%.

Applications of Ca EDTA were investigated for control of grey mold on table grapes (Ciccarese et al. 2012). Treatments were most effective when applied between fruits set and ripening. The foliar spray reduced the post harvest disease severity from 65.53 % to 36.99 % when applied before ripening, and to 48.73 % with a late spray after ripening. Applications before ripening resulted in higher tissue levels of Ca, resulting in increased resistance to injury in the berry. The early applications increased the plant's defense, whereas post ripening applications inhibited the pathogen directly.

A range of Ca compounds were tested for their efficacy in reducing *B. cinerea* on sweet cherries. Fruits were wounded and 20 uL of the salt (1%) was added to the wound site. After 2 hours, fruits were inoculated with 20 uL of a 5 x 10^4 spore suspension of *B. cinerea* (Ippolito et al. 2005*)*. Compared to a water control, $CaCl_2$, $CaOH_2$ and $CaNO_3$ reduced the percentage of rotten fruit. Calcium acetate, $CaCO_3$, Ca gluconate, Ca pantothenate and Ca propionate did not significantly reduce the disease. Calcium chloride had the greatest effect and was further tested for its effect together with *Aureobasidium pullulans*, a known biocontrol agent against *B. cinerea*. They were applied to the fruits after harvest separately and in tandem. The fruits were stored at 0°C for 15 days and incubated at room temperature (20°C) for 7 days before evaluation. Both treatments together showed a synergistic effect and reduced the rotted fruit by 70 %. This synergy was not seen when the same combination was applied one week before harvest, suggesting a direct effect on the pathogen. Incidence of disease was reduced by around 50 % compared to the control for all treatments (yeast alone, Ca chloride alone, or a combination) applied one week before harvest.

The Ca gluconate was investigated as a possible antifungal coating for strawberries. Immersion in a solution of 1% Ca gluconate for 5 min significantly reduced the incidence of gray mold from 91 to 61% (Hernandez-Munoz et al. 2006). The berries were also firmer suggesting disease may be reduced by increasing their mechanical resistance.

Both $CaCl_2$ and $CaSiO_3$ were applied to bean plants at a rate of 300 mg L^{-1} at 45 days after emergence to control white mold caused by *S. sclerotiorum* (Junior et al. 2010). This reduced the disease severity index from 43.3 % to 27.8 % with $CaCl_2$ and 30.4 % with $CaSiO_3$ and had no effect on yield. Treatments were tested alongside a fungicide, Flauzinam, which reduced the disease severity to 11.9 % and increased yields by 31 %.

Capsicum fruits were wounded with a 1 mm needle and dipped in various concentrations of $CaCl_2$ (100-2000 ppm) for 20 min (Lakshmesha et al. 2005). The wounds were then inoculated with 6 µL of a 10^6 conidial suspension of *Colletotrichum capsici*. Dipping the fruits in 1000 ppm $CaCl_2$ was able to control 80% of the disease incidence.

2.6. Mechanisms of Effects of Calcium on Plant Disease

2.6.1. Soil Structure

The presence of Ca in the soil can increase flocculation (Baver 1928) and, therefore, decrease the level of soil of compaction, which may reduce diseases incidence. The effect of compaction was shown in pea plants inoculated with *F. oxysporum* and *F. solani* that were planted in soils with no physical compaction (1.2 g cm^{-3}), moderate compaction (1.4 g cm^{-2}), and heavy compaction (1.6 g cm^{-3}) (Tu 1994). The root rot severity was scored from 0 (no symptoms) to 9 (81-100% diseased) was 3.2 in uncompacted soil, 4.2 in moderately compacted soil and 3.6 in heavily compacted soil.

2.6.2. Plant Structural Defense

Since Ca is a normal constituent of the cell wall and middle lamella of plants, the relationship between Ca ions and the cell wall partially explains the increased resistance to invasion by certain microorganisms induced by Ca. Calcium, being a divalent cation, has the ability to bridge two galacturonates via their carboxylate groups. In the cell wall, Ca ions bind to pectins (Conway et al. 1991), which are composed of chains of polygalacturonic acid residues with rhamnose insertions that cause marked kinks in the chain (Preston 1979). The resulting bunched configuration of the polygalacturonic acid chain allows spaces for the insertion of cations. All such spaces may be filled, since the binding of one ion causes a chain alignment that facilitates the binding of the next ion (Grant et al. 1973). Cation bridges between pectic acids or between pectic acids and other acidic polysaccharides hinder accessibility to enzymes that cause softening, produced by the host, and also to hydrolytic enzymes that cause decay produced by fungal or bacterial pathogens. Calcium ions may also affect the degradation of pectic substances by either inhibiting or stimulating the pectic enzymes, depending on the nature of the enzyme and the concentration of Ca (Atallah and Nagel 1977; Bauscher et al. 1979; Byrde and Archer 1977; Hancock and Stanghellini 1968). Calcium inhibits polygalacturonase activity at relatively low concentrations. This observation is supported by data indicating that cation-chelating agents such as EDTA or oxalic acid enhance the action of polygalacturonase on pectic substrates (Bateman and Basham 1976; Bauscher et al. 1979). In contrast, pectate lyase is stimulated by $CaCl_2$ at low concentrations (2 ×

10^{-5} to 2×10^{-3} M) (Bateman and Basham 1976). Moreover, binding of proteins to polysaccharides through phenolic acid and Ca bridges has a strengthening influence on cell walls. The mechanical strength of the cell wall, determined by cell wall extensibility, was increased by the addition of Ca (Kratzke 1988). Calcium also binds anionic groups of all membranes to form bridges between structural components, especially phospholipids and proteins, thereby maintaining selective permeability, structural integrity, and cellular compartmentalization (Blowers et al. 1988; Legge et al. 1982; Pooviah and Reddy 1987; Roux and Slocum 1982). Stress-induced leakiness of cells, whether caused by oxygen deficiency or caused by freezing injury (Kratzke 1988), can also be prevented, or reversed by Ca treatments, and respiration is greatly reduced in tissues infiltrated with Ca, compared with uninfiltrated tissues (Arteca 1982). Thus, resistance of plants to certain pathogenic microorganisms has been correlated with high Ca nutrition or high Ca content of the resistant tissues.

Conway and Sams (1984) elucidated possible mechanisms by which postharvest Ca treatment reduces decay in apples. Since Ca ions did not reduce fungal growth *in vitro* and there was no growth reduction when *P. expansum* was grown on juice extracted from Ca-treated fruit in their study, they concluded that the effect of Ca in reducing decay is indirect and that the intact fruit is necessary for this effect to be realized. A more plausible explanation of the role of Ca in reducing decay in stored fruit is that it may stabilize or strengthen cell walls of fruit, making the fruit more resistant to decay by fungal enzymes. Since Ca-treated apples remain firmer than low-Ca fruit (Bengerth et al. 1972), it is possible that Ca may prevent the pectic enzymes from destroying pectin (Faust 1974). Calcium introduced by postharvest treatment is incorporated into the cell wall in the same manner as native Ca to resist degradation by enzymes occurring naturally in the fruit as well as enzymes produced by fungal pathogens (Wieneke 1980). Although fruit in low-O_2 storage and Ca-infiltrated fruit maintain the same firmness, the latter can resist decay better. Perhaps the native pectic enzymes of the fruits are not functional or their synthesis is slowed by low O_2, whereas the chemical composition of Ca-infiltrated fruits may be changed to confer better resistance to decay by fungal enzymes.

Calcium applications were also seen to increase the firmness of fruits such as apple (Wojcik et al. 2009, Alandes et al. 2006), peach (Sohail et al. 2013), and tomato (Senevirathna and Daudasekera 2010). Peonies treated three times with 4% Ca chloride

required 18% more force break at the base of their stems than plants from the water control treatment (Li et al. 2012).

In addition to this basic mechanism of Ca effects, the element may also encourage the development of other host-specific barriers to microbial invasion or the proliferation of microbes in the host. Tzeng et al. (1986) showed that Ca fertilization resulted in an increase in the netting of potato tuber surfaces. Tubers with well-developed netting had more layers of periderm cells (an average of 13.6) than tubers with smooth skin (an average of 9.1). Periderm rupture force (measured by an Instron universal materials testing instrument) for tubers with well-developed netting was significantly higher than for smooth-skinned tubers with low Ca contents. Presumably, the thicker periderm may have increased resistance to bruising and decreased pathways for invasion by microbes such as soft rot bacteria.

2.6.3. Chemical and Enzymatic Defenses

Studies of several host-parasite systems have provided strong evidence that Ca can also play a key role in signal transduction during plant defense responses (Bush 1995; Ishihara et al. 1996; Mahady and Beecher 1994; Messiaen and Van Cutsem 1994; Zimmermann et al. 1997). Gelli et al. (1997) reported that the defense response of tomato cells against the fungus *Fulvia fulva* (syn. *Cladosporium fulvum*) was initiated by the binding of fungal elicitors to receptors at the host plasma membrane, followed by a series of biochemical reactions to cause a transient increase of cytosolic Ca levels through a specific plasma membrane Ca channel. Calcium-modulated proteins, such as calmodulin and calmodulin-like domain protein kinases, are capable of both sensing increases in the cytoplasmic concentration of free Ca and effecting changes in cellular metabolism, including the accumulation of phytoalexins. Dmitriev et al. (1996) demonstrated that Ca can act as a second messenger in the regulation of phytoalexin synthesis in onion cells cultured with a biotic elicitor derived from *B. cinerea.* Similar observations were made by Schwacke and Hager (1992) and Atkinson et al. (1990) with cultured spruce and tobacco cells, respectively.

Cellulase activity was reduced in capsicum fruits that were dipped in 1000 ppm $CaCl_2$ and inoculated with *C. capsici* (Lakshmesha et al. 2005). After 13 days in storage,

untreated infected fruits had 1100 µg ml^{-1} cellulase activity whereas the activity of this enzyme was only 350 µg ml^{-1} in the treated fruits.

Pears that were treated with 2% $CaCl_2$ and infected with *A. alternata* had higher levels of *β*-1,3-glucanase, PPO, PAL, and POD than the untreated control (Tian et al. 2006). B-1,3-glucanase can hydrolyze major components of fungal cell walls, polyphenol oxidase can convert phenols into antimicrobial phenolics, peroxidase is used in the synthesis of lignin, and phenylalanine ammonia lyase can produce phenolics, phytoalexins and lignins. Increases in these four enzymes are indicative of greater plant defence responses. Higher levels of POD and PPO were also found in *R. solanacearum* infected tomatoes that had been grown in higher Ca environments (Jiang et al. 2013). Blister blight infected tea leaves treated with 1 % CaCl2 also showed higher POD, PPO, PAL, *β*-1,3-glucanase, as well as phenol activity (Chandra et al. 2014).

Lipoxygenase activity was found to be lower in apples with higher Ca content (Sharma et al. 2012). This enzyme can break down linolenic acid which may weaken the cell wall and lower plant defences.

Pectinase activity also decreased with added Ca in various plants including tobacco (He et al. 2014) and apple (Wisniewski et al. 1995). This is likely due to Ca ions binding pectin and making it less accessible to pectinase activity as described earlier.

Ethylene production was reported to increase in wheat seeds that had been grown in a nutrient solution containing higher levels of Ca (Zielińska and Michniewicz 2001) and infected with *Fusarium culmorum*. Other studies observed the opposite effect. Volpin and Elad (1991) examined rose flowers infected with *Botrytis cinerea* and reported that Ca decreased ethylene production. Post-harvest applications of Ca chloride to tomato also resulted in lower rates of ethylene production (Senevirathna and Daudesekera 2010). In both these plant-microbe interactions, higher levels of ethylene resulted in more infection.

2.6.4. Direct Antimicrobial Effects

Amending soil with Ca decreased the production of zoospores by *P. cinnamomi* (Messenger et al. 2000). Addition of 1% of either of Ca carbonate, Ca nitrate or gypsum significantly reduced the number of zoospores per mL of soil from 3,301 in the control to

980, 1,077 and 682, respectively. The amendments had no significant effect on pH, which remained between 5.5 - 6.9.

Calcium salts such as propionate and sorbate, which are frequently used as antifungal compounds against storage molds, possibly inhibit disease-causing fungi through the same mechanism, although only a few studies have assessed their potential (Al-Zaemey et al. 1993). For example, sorbic acid and its salts alter cell membranes and cell transport functions and inhibit RNA, DNA, and protein synthesis and enzymes involved in metabolic processes. Sorbic acid may also uncouple oxidative phosphorylation reactions in mitochondria (Sofos et al. 1986). Propionate is also used to reduce mold development in baked goods and bread, in grains, and in hay (Punja and Gaye 1993).

Calcium may also inhibit fungal toxins or render them inactive. Punja and Jenkins (1984a, 1984b) showed that the addition of Ca to *Sclerotium rolfsii* growth medium resulted in an observable increase in the production of insoluble crystals of Ca oxalate. The formation of these crystals resulted from the sequestering of oxalic acid produced by the fungus by exogenous Ca. Similar findings have been reported by Rao and Tewari (1988) following spraying of $Ca(OH)_2$ on coffee leaves infected with *Mycena citricolor.* The authors suggested that the mechanism of disease control is the neutralization of oxalic acid secreted by the pathogen.

The dual effects of lime in providing Ca for nutrition and in increasing soil pH, with its multiple effects on micronutrient availability, have not always been easy to separate. Numerous investigators have reported the association of an increase in soil pH with disease reduction through application of Ca-containing compounds (El-Tarabily et al. 1997; Fletcher et al. 1982; Murray et al. 1992). At higher pH, pathogenic microbes are deprived of their essential micronutrients, such as Fe and B, whereas actinomycetes and bacteria multiply very fast, and some of them may be antagonistic to pathogenic microbes.

2.6.5. Enhancement of Biological Controls

With a view to establish biological control of postharvest diseases of fruits and vegetables as a promising alternative to the use of synthetic fungicides, many successful

studies have been conducted to enhance the efficacy of biocontrol agents with Ca supplementation (Droby et al. 1997; Wisniewski et al. 1995). The mechanism by which the efficacy of biocontrol agents is improved by Ca still to be elucidated. However, it is apparent from the relevant reports that Ca may be inhibitory to conidia germination of some pathogens and affect their metabolic processes. On the other hand, biological control agents have the ability to overcome the direct effect of Ca, and their growth may be stimulated.

Another possible mechanism by which Ca may enhance the efficacy of biological control agents is by decreasing the accumulation of reactive oxygen species. Two biocontrol yeasts, *P. membranifaciens* and *Debaryomyces hansenii*, were grown in media with no Ca and with 1 and 5 mM added $CaCl_2$ (An et al. 2011). They were then placed in a hot water bath, and then their viability was determined. Both yeasts were more tolerant to heat stress when grown in Ca containing media. These treatments also had lower levels of reactive oxygen species, which would result in lower oxidative damage to the cells.

2.7. Conclusions

From the large number of published reports, it is clear that Ca plays an important role in the mineral nutrition of plant species and is able to suppress the development of many diseases. Because of the diversity of crops and their special mineral nutrition requirements as well as the diversity of pathogens groups, it is not possible to draw a general conclusion and recommendation for using Ca to protect the crops against both diseases and physiological disorders. The optimum level of Ca to ensure its efficacy for disease management will need to be established for each crop-pathogen interaction. From the published literature, it is clear that many diseases, mainly caused by *Botrytis, Colletotrichum, Penicillium*, and *Phytophthora*, have been consistently reduced by supplying Ca to plants in the different sources such as $CaCl_2$, $CaSO_4$, and $CaCO_3$ (Table 6.1). The use of Ca, particularly for the control of postharvest diseases, is receiving greater attention in view of the decreasing number of fungicides available in the market and concerns regarding their residues in food and nature. Enhancing the resistance of storage organs to decay by elevating Ca concentration in tissues enhances innate mechanisms of resistance since Ca increases their resistance against the hydrolytic enzymes by stabilizing plant cell walls and cell membranes. Applications of Ca for the

control of plant diseases would be well suited for certain crops permitting a potential reduction in the use of fungicides and improving crop quality and yields.

Table 2-1 Plant diseases reported to be reduced by the application of calcium.

Hosts	Diseases	Pathogens	Calcium sources	References
Apple	Alternaria rot	*Alternaria* spp.	Calcium chloride	Biggs et al. 1993
	Bitter rot	*Colletotrichum* sp.	Calcium chloride and calcium propionate	Biggs 1999, Boyd-Wilson et al. 2014
	Botrytis rot	*Botrytis cinerea*	Calcium chloride	Chardonnet et al. 2000, Conway et al. 1991
	Leaf spot	*Glomerella cingulata*	Calcium chloride	Conway et al. 1991
	Penicillium rot	*Penicillium expansum*	Calcium acetate, calcium ascorbate, calcium citrate, calcium propionate, calcium silicate, and calcium chloride	Lima et al. 2005, Yu et al. 2012, Conway et al. 1991
	Brown rot	*Monilinia fructigena*	Calcium chloride	Holb et al. 2012
Avocado	Root rot	*Phytophthora cinnamomi*	Calcium sulfate	Messenger et al. 2000a
Banana	Xanthomonas wilt	*Xanthomonas campestris* pv. *musacearum*	Unlisted source	Atim et al. 2013
Banksia	Phytophthora dieback	*Phytophthora cinnamomi*	Calcium sulfate	Stasikowski et al. 2014
	Crown rot	*Colletotrichum musae*	Calcium chloride	Bastiaanse et al. 2010
Basil	Grey mold	*Botrytis cinerea*	Calcium chloride	Yermiyahu et al. 2006
	White mold	*Sclerotinia sclerotiorum*	Calcium chloride	David et al. 2019
Beans	White mold	*Sclerotinia sclerotiorum*	Calcium chloride and calcium silicate	Junior et al. 2010
Broccoli	Clubroot	*Plasmodiophora brassicae*	Calcium carbonate and calcium hydroxide	Campbell et al. 1985, Anderson et al. 1976
Cabbage	Clubroot	*Plasmodiophora brassicae*	Calcium carbonate, calcium hydroxide, and calcium cyanamide	Fletcher et al. 1982, Anderson et al. 1976, Porth et al. 2003
	Soft rot	*Pectobacterium carotovorum* subsp. *carotovorum*	Calcium nitrate	Silva Felix et al. 2016
Carrot	Southern blight	*Sclerotium rolfsii*	Calcium nitrate	Punja et al. 1986
	Cavity spot	*Pythium coloratum*	Calcium oxide	El-Tarabily et al. 1997
	Black root rot	*Berkeleyomyces basicola*	Calcium propionate	Punja and Gaye 1993
Cherry	Grey mold	*Botrytis cinerea*	Calcium chloride, calcium hydroxide, and calcium nitrate	Ippolito et al. 2005
Citrus	Phytophthora root rot	*Phytophthora nicotianae*	Calcium oxide and calcium carbonate	Campanella et al. 2002
	Greasy spot	*Mycosphaerella citri*	Calcium carbonate	Mondal and Timmer 2003
Coffee	Leaf spot	*Mycena citricolor*	Calcium hydroxide	Rao and Tewari 1988
Cucumber	Root rot	*Pythium splendens*	Calcium carbonate	Kao and Ko 1986
	Gray mold	*Botrytis cinerea*	Calcium sulfate	Elad et al. 1993
Dogwood tree	Anthracnose	*Discula destructive*	Unlisted source	Holzmueller et al. 2007
Eggplant	Gray mold	*Botrytis cinerea*	Calcium sulfate	Elad et al. 1993
Ginger	Bacterial wilt	*Ralstonia pseudosolanacearum*	Calcium chloride	Bhai et al. 2019
Grapefruit	Penicillium rot	*Penicillium digitatum*	Calcium chloride	Droby et al. 1997
Grapevine	Powdery mildew	*Uncinula necator*	Calcium polysulfide	Gadoury et al. 1994

	Grey mold	*Botrytis cinerea*	Calcium chloride and calcium EDTA	Nigro et al. 2006, Chervin et al. 2009, Ciccarese et al. 2012
Holm oak	Phytophthora decline	*Phytophthora cinnamomi*	Calcium oxide, calcium carbonate, and calcium propionate	Serrano et al. 2012
Indian mustard	Blight	*Alternaria brassicae*	Calcium sulfate	Kumar et al. 2015
Loquat	Anthracnose rot	*Colletotrichum acutatum*	Calcium chloride	Cao et al. 2008
Melon	Fruit rot	*Paramyrothecium roridum*	Calcium chloride	Lima et al. 1998
Millet	Downy mildew	*Sclerospora graminicola*	Calcium chloride	Geetha and Shetty 2002
Nectarine	Rhizopus rot	*Rhizopus stolonifer*	Calcium chloride	Qing and Shiping 2000
Opium	Seedling blight	*Pythium ultimum*	Calcium carbonate	Angell 1950
Papaya	Anthracnose	*Colletotrichum gloeosporioides*	Calcium chloride	Rahman et al. 2009, Madani et al. 2014
Paprika	Grey mold	*Botrytis cinerea*	Calcium oxide, calcium hydroxide, calcium carbonate, and calcium nitrate	Yoon et al. 2010
Pea	Aphanomyces root rot	*Aphanomyces euteiches*	Calcium carbonate and calcium sulfate	Heyman et al. 2007
	Fusarium root rot	*Fusarium* sp.	Calcium carbonate	Chittem et al. 2016
Peach	Bark canker	*Leucostoma persoonia*	Calcium hydroxide, calcium propionate, calcium silicate, and calcium oxide	Biggs et al. 1994
	Brown rot	*Monilinia* sp.	Calcium chloride	Biggs et al. 1997; Conway et al. 1987, Elmer et al. 2007, Thomidis et al. 2007
Peanut	Pod rot	*Pythium myriotylum* and *Rhizoctonia solani*	Calcium sulfate	Hallock and Garren 1968, Walker and Csinos 1980
	Aflatoxin	*Aspergillus flavus*	Calcium sulfate	Wilson and Walker 1981
Pear	Side rot	*Cadophora malorum*	Calcium chloride	Sugar et al. 1991
	Scab	*Venturia pirina*	Calcium hydroxide	Washington et al. 1998
	Fire blight	*Erwinia amylovora*	Calcium chloride	Toselli et al. 2004
	Brown spot	*Stemphylium vesicarium*	Calcium chloride	Toselli et al. 2004
	Grey mold	*Botrytis cinerea*	Calcium chloride	Zhang et al. 2005
	Alternaria rot	*Alternaria alternata*	Calcium chloride	Tian et al. 2006
Pepper	Gray mold	*Botrytis cinerea*	Calcium sulfate	Elad et al. 1993
	Anthracnose	*Colletotrichum capsici*	Calcium chloride	Laksmesha et al. 2005
Petunia	Blight	*Botrytis cinerea*	Calcium chloride, calcium nitrate, calcium-amino acid chelate	Bennett 2019
Potato	Soft rot	*Pectobacterium atrosepticum*	Calcium sulfate	McGuire and Kelman 1984, 1986, Tzeng et al. 1986
	Early blight	*Alternaria solani*	Calcium chloride, calcium citrate	Abou-El-Hassan et al. 2020
Rice	Sheath rot	*Sarocladium oryzae*	Calcium sulfate	Narasimhan et al. 1994
Rose	Gray mold	*Botrytis cinerea*	Calcium chloride and calcium sulfate	Starkey and Pedersen 1997, Capdeville et al. 2005
Soybean	Twin stem	*Sclerotium* sp.	Calcium carbonate	Muchovej and Muchovej 1982
	Phytophthora root rot	*Phytophthora sojae*	Calcium chloride, calcium nitrate, and calcium formate	Sugimoto et al. 2005, Sugimoto et al. 2008, Sugimoto 2010
Strawberry	Grey mold	*Botrytis cinerea*	Calcium gluconate	Hernandez-Munoz et al. 2006
	Fusarium wilt	*Fusarium oxysporum*	Calcium carbonate	Fang et al. 2012

	Leaf blight	*Phomopsis obscurans*	Calcium silicate	Abd-El-Kareem et al. 2019
Tea	Blister blight	*Exobasidium vexans*	Calcium chloride	Chandra et al. 2014
Tobacco	Bacterial wilt	*Ralstonia solanacerarum*	Calcium carbonate and calcium chloride	He et al. 2014
Tomato	Wilt	*Fusarium oxysporum* f. sp. *lycopersici*	Calcium chloride and calcium hydroxide	Corden 1965; Edgington and Walker 1958; Jones and Woltz 1967; Standaert et al. 1973
	Powdery mildew	*Golovinomyces orontii*	Calcium chloride and calcium nitrate	Ehret et al. 2002
	Bacterial canker	*Clavibacter michiganensis* subsp. *michiganensis*	Unlisted source	Berry et al. 1988
	Bacterial wilt	*Ralstonia solanacearum*	Calcium chloride and calcium nitrate	Yamazaki and Hoshina 1995, Jiang et al. 2013
Vinca	Root rot	*Phytophthora nicotianae* var. *parasitica*	Calcium chloride and calcium nitrate	von Broembsen and Deacon 1997
Wheat	Cephalosporium stripe	*Cephalosporium gramineum*	Calcium hydroxide	Murray et al. 1992
	Browning root rot	*Pythium arrhenomanes*	Calcium sulfate	Vanterpool 1940
	Powdery mildew	*Blumeria graminis* f. sp. *tritici*	Calcium silicate	De Curtis et al. 2012

Chapter 3.

The fungicidal properties of karanja

3.1. Introduction

Greenhouse vegetable production in Canada in 2020 occurred on 1,808 hectares, providing a farm gate value of $61.6 million (Statistics Canada, 2021). Three crops, namely tomato, cucumber and pepper accounted for 97 % of the acreage. There are several diseases of importance on these crops, including Fusarium crown and root and wilt, caused by *Fusarium oxysporum* (Schlecht.) Snyder & Hansen (Agrios 2005). Infection of young plants causes damping off, and severe infections in mature plants can result in the destruction of the vascular tissues, resulting in stunting or death of the plant. A second disease of importance is powdery mildew, which occurs on all three greenhouse crops in Canada. On cucumber, the disease is caused by *Podosphaera xanthii* (Schlidl.) Braun & Takam. Both diseases are difficult to manage, with limited options available, especially for organic growers. To address the need for additional reduced-risk products for management of Fusarium and powdery mildew on tomato and cucumber plants, respectively, we assessed the efficacy of a plant-derived oil formulated product that could potentially be used in organic greenhouse production. Karanja oil formulations were tested for their ability to reduce spore germination of *F. oxysporum* and against root infection on tomato plants, as well as against powdery mildew development on cucumber plants.

Karanja oil is derived from pressed seeds of the tree *Millettia pinnata* L. Panigrahi (Family Fabaceae). This fast-growing plant has a wide temporal range. It has been found from India to Japan, and south to the northern regions of Australia. Its common name varies by region but includes Indian beech, honge, pongam, and karanj. It was previously classified under the binomials *Pongamia glabra* Vent., and *Derris indica* Lam. (Arote & Yeole 2010). The most recent interest in this tree centres around possible uses of the seeds, which can yield around 36 % oil (Pavithra et al. 2012). The oil is composed of 90 % fatty acids, including oleic acid (50 %), linoleic acid (20 %), palmitic acid (10 %), linolenic acid (5 %) and stearic acid (5 %) (Pavithra et al. 2012). The high levels of oleic acid make it a suitable candidate for biodiesel production, and much of the recent

research on karanja oil has been focused on this aspect. The oil also contains flavonoids, including karanjin and pongamol, which comprise about 1 - 5 % and 0.3 - 0.9 % of the oil, respectively (Pavithra et al. 2012, Gore & Satyamoorthy 2000). The fatty acid components of the oil have been suggested for use in production of antimicrobial drugs (Wagh et al. 2007). Karanja oil has been used traditionally as a medicinal treatment for various ailments including skin diseases (Chopade et al. 2008, Chopra et al. 2019).

Research into the ability of karanja products to reduce fungal growth in vitro has been investigated by several researchers. It was seen to inhibit the growth of fungi and oomycetes from several genera, including *Alternaria* (Kalbende & Dalal 2016, Mahantesh et al. 2017, Rex et al. 2019, Shafique et al. 2014), *Aspergillus* (Dwividel et al. 2007, Kesari et al. 2010, Mondal et al. 2012, Raju & Naik 2006, Sajid et al. 2014, Wagh et al. 2007), *Candida* (Dwivedi et al. 2007, Kesar et al. 2010, Mondal et al. 2012), *Chaetomium* (Kalbende & Dalal 2016), *Exserohilum* (Manu et al. 2017), *Fusarium* (Aishwarya & Somasekhara 2017, Hedge et al. 2017, Mondal et al. 2012, Sajid et al. 2014, Shafique et al. 2014), *Microsporum* (Rajput et al. 2021), *Mucor* (Mondal et al. 2012), *Penicillium* (Mondal et al. 2012), *Pyricularia* (Wasimofiroz et al. 2018), *Pythium* (More et al. 2017, Walters et al. 2014), *Rhizopus* (Kalbende & Dalal 2016), and *Sclerotium* (Akram et al. 2016). This research has been focused on in vitro testing of the oil against fungi directly. Recently, Bora et al. (2021) evaluated the effect of karanja water extracts against grey blight on tea caused by *Pestaliopsis*. This was the first published account of in plantae treatment with any karanja derivative and resulted in a reduction in disease incidence from 90% to 30%. No study yet has evaluated the efficacy of karanja oil-based products in plantae on cucumber or tomato plants.

This work is an initial investigation into the efficacy of karanja oil formulations as an organic pesticide for the management of the most common and widespread greenhouse vegetable diseases.

3.2. Methods

3.2.1. Treatments and pathogens

Karanja oil formulations were acquired from Terramera Inc. (Vancouver, Canada). The fungicide Pristine (boscalid and phyraclostrobin, BASF) was acquired from TerraLink Horticultural (Table 3-1, Delta, Canada). *Fusarium* species were grown at room temperature on potato dextrose agar (PDA) (Difco Laboratories). The cultures were stored on PDA slants at 4°C. The origin of each and the species are listed in Table 3-2 and were identified using PCR of the ITS1-ITS2 region along with visual characteristics. Images of each isolate growing on potato dextrose agar are shown in Figure 3-1.

Table 3-1 List of Products Tested: Formulations of karanja, fungicides, and biological controls with their active ingredients.

Code	Description	Active Ingredients
KF1	Karanja Formulation 1 (KF1)	*Millettia pinnata* extract
KF2	Karanja Formulation 2 (KF2)	*Millettia pinnata* extract
KF3	Karanja Formulation 3 (KF3)	*Millettia pinnata* extract
Pristine	Fungicide	Pyraclostrobin and boscalid
Medallion	Fungicide	Fudioxonil
Asperello	Biological control	*Trichoderma asperellum* T34
Regalia	Fungicide	*Reynoutria sachalinensis* extract

Fungal spores for germination tests were harvested from 2-week-old cultures of *Fusarium oxysporum* growing on PDA at ambient room temperatures. 1 mL of sterile deionized water was added to the surface of the colony, which was rubbed with a metal spatula to disturb the top layer and decanted off to obtain the spores. The solution was filtered to remove fragments of mycelia, and the spore suspensions were diluted to 1 x 10^6 spores per ml, calculated using a haemocytometer.

Table 3-2 The source and species of each Fusarium isolate used. Isolates were sourced internally from ongoing cannabis research, and then sourced externally for tomato isolated pathogens.

Code	Species	Source
F-1	Fusarium oxysporum	Infected cannabis plants
F-2	Fusarium oxysporum	Infected cannabis plants
F-3	Fusarium proliferatum	Infected cannabis plants
F-4	Fusarium oxysporum	Agriculture Canada, infected tomato
F-5	Fusarium oxusporum f. sp. lycopersici	Agriculture Canada, infected tomato
F-6	Fusarium oxysporum	Infected cucumber plant
F-7	Fusairum solani	Infected cannabis plant

F-8	Fusarium oxysporum	Infected tomato, community garden
F-9	Fusarium oxysporum	Infected snake plant
F-10	Fusarium oxysporum	Infected snake plant

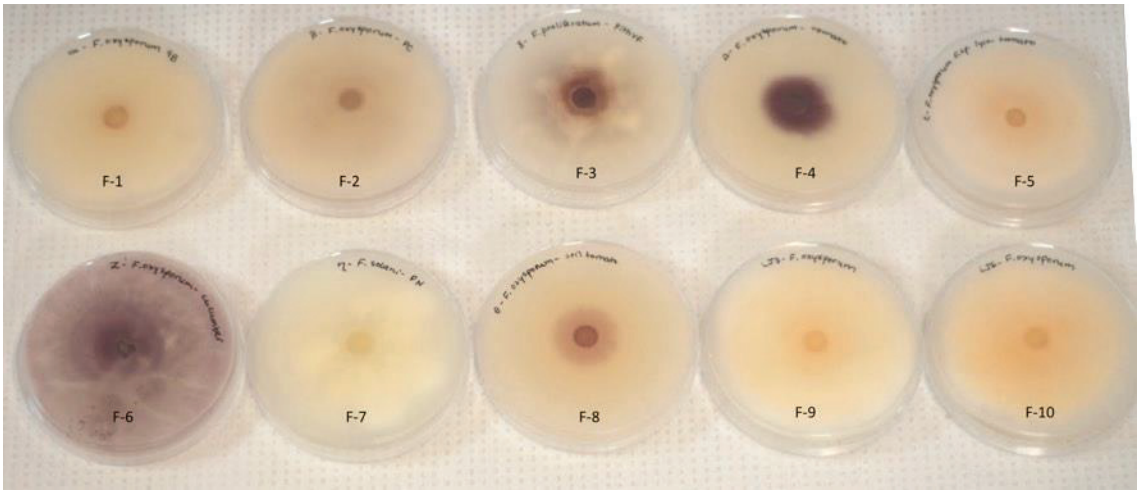


Figure 3-1 The colonies of isolates of *Fusarium* tested on tomato.

3.2.2. Efficacy of Formulations on Inhibition of Spore Germination

In a 96-well plate, 25 µL of *F. oxysporum* F-10 spore solution and 175 µL of potato dextrose broth was added to each well. For treatments, these were amended with 0.1% of karanja formulation, or 0.1% Pristine, a commercially available fungicide. A set of controls were left unamended. 12 wells were prepared per treatment type. Samples were covered by parafilm and foil and placed on a rotary shaker at 150 rpm. Four samples each were observed under a light microscope after 12 hours, another 4 samples after 24, and the rest at 36 hours. Spores were counted with a haemocytometer and those with germ tubes longer than the length of the original spore were counted as germinated.

3.2.3. Efficacy of Formulations on Inhibition of Mycelial Growth

Comparison of concentrations

Cultures of the same isolate F-10, along with an isolate of *Botrytis cinerea* isolated from cannabis were maintained at ambient temperatures on PDA plates and recultured monthly. To prepare the inoculum, a plug of mycelia was cut out of a two-week-old agar plate and placed in 100 ml of sterilized potato dextrose broth (PDB) (HiMedia Laboratories Pvt. Ltd.) in a 250 mL Erlenmeyer flask. Broths containing various

concentrations of KF1 and emulsifier (0 %, 0.5 %, 1.0%, 1.5%, 2.0%, 2.5% and 3.0%) were inoculated by adding a plug from a 2-week-old PDA plate with *F. oxysporum*. The flasks were stirred for 2 weeks on a rotary shaker at room temperature at 150 rpm. After this time, they were strained through a fine mesh filter and dried at 50°C for 48 hours in an oven. The amount of dried mycelium was then weighed and compared to the controls.

Comparison of formulations

Inoculations were prepared as above with isolate F-10. The three karanja formulations (Table 1) were added to the flasks at 0.1%, with 0.1% Pristine again being used as a positive control. Another set of flasks with pure PDB were used as a negative control. There were 4 replicates for each treatment. The flasks were placed on a rotary shaker for 1 week at 150 rpm at ambient conditions. The mycelial growth was filtered out, dried, and weighed as above.

3.2.4. Fusarium on tomato

Tomato seeds, variety “Moneymaker” from West Coast Seeds (Delta BC) were chosen for their susceptibility to Fusarium infection and used in all trials. Seeds were germinated in Sunshine Mix (TerraLink, Abbostford BC) in 72 well trays. They were covered by a humi-dome under 48W, 6400K SunBlaster T5HO lights (Langley BC). The humi-dome was removed after 7 days. When transplanted for use in experiments, plants were grown in Sunshine Mix Aggregate (TerraLink, Abbotsford BC). All fungal isolates for inoculation were prepared by inoculating PDB with a plug of mycelium and allowing the fungus to grow for 7 days on a shaker (refered to hereafter as “the inoculum”). An overview of the experiments conducted is given in Table 3-3. Each experiment is described in more detail below.

Table 3-3 An overview of the experiments conducted with *Fusarium* on tomato.

EX	Isolates Tested	Method	Age	n	Result
1	F-1	Root dip, 5 min	6	5	No significant differences
2	F-1, F-2, F-3	Root dip, 20 min	2	8	F-3 caused significant stunting
3	F-4, F-5	Root dip, 20 min	2	8	No significant differences
4	F-3	Root dip, 1-10 min	4	4	Weight decreases w/ dip time
5	F-1, F-2, F-4, F-5, F-7	Root dip, 10 min	5	6	No significant differences
6	F-3	Root dips, various	4	5	Culture caused symptoms

7	F-6	Drip and drench	4	4	No significant differences
8	F-9	Drip, drench, bath	4	3	No significant differences
9	F-8, F-10	Drip, drench	3	4	F-10 drench significant
10	F-10	Drench	5	4	No significant symptoms
11	F-10	Drench	4	6	No significant differences

Experiment 1 – *Evaluating karanja treatments*

Six-week-old tomato plants were uprooted and submerged in F-1 inoculum for 5 minutes. They were then transplanted into 500 mL pots containing 300 mL of Sunshine Mix soil (peat, lime, perlite) and moved to the greenhouse. The plants were divided into 5 treatment groups, each consisting of 5 plants. The first received no karanja treatment (control), the second was sprayed until run off with 0.5% KF1, and the last three were drenched with 50 mL of either 0.5%, 1.0% or 1.5% KF1. These treatments were reapplied weekly for 6 weeks. The plants were then uprooted, and the shoot heights and root weights were measured.

Experiment 2 – Evaluating Fusarium virulence

Two-week-old tomato plants were removed from the soil and their roots were cut to 2.5 cm in length. They were divided into 4 groups of 8 plants. Three of the groups were submerged in F-1, F-2 or F-3 inoculum for 20 minutes. The final group was submerged in sterile PDB as a non-inoculated control. The plants were transplanted into 250 mL pots and grown for 2 weeks at room temperature under lights. The dry weights of the roots and the heights of the plants were measured.

Experiment 3 – Evaluating Fusarium virulence

Experiment 2 was repeated as above but with two new tomato isolates F-4 and F-5, and a control.

Experiment 4 – Evaluating Fusarium inoculum exposure

Four-week-old tomato seedlings were removed from the soil, and their roots were cut to 2.5 cm in length. These plants were divided into 4 groups containing 4 plants each. One group was left undipped in PDB inoculum, and the rest of the groups were dipped in F-3 inoculum for 1, 5 or 10 minutes. They were grown for 3 weeks, after which the heights and wet weights were measured.

Experiment 5 – Evaluating Fusarium virulence

Five-week-old tomato plants were removed from the soil and their roots were cut to 2.5 cm in length. They were divided into 6 groups of 6 plants. Five of the groups were submerged in F-1, F-2, F-4, F-5, or F-6 inoculum for 10 minutes. The final group was submerged in sterile PDB as a non-inoculated control. The plants were transplanted into 250 mL pots and grown for 5 weeks at room temperature under lights. The dry weights of the roots and the heights of the plants were measured.

Experiment 6 – Evaluating inoculum type

Four-week-old tomato plants were uprooted and divided into four groups, each with 5 plants. The control was dipped in PDB. Another group of plants were dipped in washed mycelial suspension. The other two groups were dipped in mycelial or spore suspensions. The treatment of washed mycelia was made by pouring the culture through a fine mesh filter and washing it, then resuspending it in sterile dH_2O. Spore treatments were made by taking the filtrate and centrifuging at 4000 g for 15 minutes. It was washed 2 times with dH_2O and spun down again each time. The spores were then resuspended to their original concentration in the broth (6×10^5). All dips were done for 5 minutes. The plants were allowed to grow for 3 weeks under 16 hours of light and watered twice weekly. After this period, the plant heights and fresh weights were measured.

Experiment 7 – Evaluating Fusarium virulence

A new *Fusarium* isolate F-6, which was isolated from infected cucumber plants was grown in PDB at room temperature for 9 days on a rotary shaker. Four-week-old plants were uprooted from their germination tray and soil was washed from their roots. This process caused some root damage providing wounded areas prone to infection. Plants were replanted in 500 mL pots with Sunshine Mix 4 soil. There were 4 replicates per treatment as follows: A control dip where plants were dipped in sterile PDB for 5 minutes. A treatment dip, where they were dipped in the treatment broth for 5 minutes, and a treatment drench, where they were drenched with 30 mL of the broth at the crown. After 3 weeks, plant height and weights were measured.

Experiment 8 – Evaluating Fusarium virulence

Experiment 7 was repeated as above with the isolate F-9.

Experiment 9 – Evaluating Fusarium virulence

Experiment 7 was repeated as above with the isolates F-8 and F-10.

Experiment 10 – Evaluating Fusarium virulence

Fusarium isolate F-10 was grown in PDB at room temperature for 5 days on a rotary shaker. Five-week-old plants were transplanted in 500 mL pots with Sunshine Mix 4 soil. There were 4 replicates per treatment as follows: Control (50 mL water drench), 50 mL full strength inoculum drench, 50 mL half diluted inoculum drench, 50 mL quarter diluted inoculum drench. After 4 weeks, the plant heights and weights were measured. Cross sections of stems taken from 1 cm above the crown were photographed under a dissecting microscope. They were then surface sterilized with 5% bleach for 1 minute followed by 1 minute in 70% ethanol and rinsed 3 times with sterile distilled water. These segments were then placed on PDA for 7 days.

Experiment 11 – Evaluating karanja treatments

Tomato variety ‘Moneymaker’ was planted in JiffyMix germination soil (peat, vermiculite) in 72 cell trays and grown under 16 hours of light for 4 weeks. The plugs were transplanted into 500 mL pots with Sunshine Mix 4 soil and allowed to acclimatize for 3 days before treatments started. Plants at this stage were between 6 to 12 cm in height above the soil level. They were divided into 10 groups of 6 plants each. 20 mL of 0.01 % Asperello T34 treatment (*Trichoderma asperellum*) was applied added as a drench 48 hours before inoculation. Regalia Maxx (extract from *Reynoutria sachalinensis*/giant knotweed) was applied as a foliar spray at the recommended rate of 0.25% and sprayed until run-off 24 hours before inoculation. Karanja and Medallion fungicide (active ingredient fludioxonil) were applied as a drench to the soil until it ran through the pot at a rate of 0.30 % for karanja and 0.008 % for Medallion (label rate). One group of each treatment type was inoculated with 20 mL of *Fusarium oxysporum* F-10 in inoculum broth (1.25 x 106 CFU/mL). This was grown in PDB for 7 days at room temperature on a rotary shaker. See Table 3-4 for treatment details. Plants were grown for 4 weeks. The heights and weights of the plants were measured.

Table 3-4 An overview of the treatments, application methods, rates, replications and which groups were inoculated.

Code	Treatment	Application Method	Rate	Replicates	Inoculated?
C-I	Control	None	0.00 %	6	Yes
C-N	Control	None	0.00 %	6	No
A-I	Asperello	Drench	0.01 %	6	Yes
A-N	Asperello	Drench	0.01 %	6	No
K-I	KF2	Drench	0.30 %	6	Yes
K-N	KF2	Drench	0.30 %	6	No
R-I	Regalia	Foliar Spray	0.25 %	6	Yes
R-N	Regalia	Foliar Spray	0.25 %	6	No
M-I	Medallion	Drench	0.008 %	6	Yes
M-N	Medallion	Drench	0.008 %	6	No

Experiment 12 – Seed treatments

Tomato seeds were soaked in 0.1% karanja formulation 2 (KF2) or chitosan with control seeds being soaked in water. After 1 hr, the seeds were drained and planted in Sunshine Mix Germination Mix (TerraLink, Abbotsford BC) in 72 well trays. They were covered by a humi-dome under 48W, 6400K SunBlaster LED strip lights (Langley BC). The humi-dome was removed after 7 days. Fourteen days after sowing, the seedlings were removed from the soil and their roots were dipped in prepared F-10 *Fusarium* inoculum. These cultures were made by inoculating PDB with 1 cm plugs of 2-week-old PDA cultures of *Fusarium* and allowing them to grow for 1 week on a rotary shaker at 150 rpm. Following a 5-minute dip in the culture, the plants were re-planted in Sunshine Mix Aggregate (TerraLink, Abbostford BC) in 250 mL pots. The plants were grown for 6 weeks, and then the shoots were weighed, and their heights were measured. The experiment was repeated 4 times.

3.2.5. Powdery mildew on cucumber

Cucumber variety “Tasty Green” (from West Coast Seeds, Delta BC) was chosen for susceptibility to powdery mildew. Seeds were placed into 72 well trays with Sunshine Germination Mix and placed under a humi-dome and lights as above for 3 weeks. The seedlings were then transplanted into pots in the greenhouse in Sunshine Aggregate Mix soil in 1 L pots. They were maintained with bi-weekly 20-20-20 NPK fertilization at 1 tsp per liter (Gardenworks, Burnaby BC) and were watered twice weekly. Once the plants had 3 true leaves, they were placed around powdery mildew infected squash plants (6

plants around one squash plant 15 cm away) to allow natural inoculation to occur. Once powdery mildew colonies began to appear on the cucumbers, the plants were assigned randomly to three treatment groups, KF2 (0.5%), KF3 (0.125%) or the control (water). Treatments were applied weekly with a sprayer until run off, and repeated weekly for 4 weeks. Photos were taken weekly of the plants and using Image J (Schneider et al. 2012) the percentage of powdery mildew disease coverage was calculated.

3.2.6. Powdery mildew on squash

Organic 'Goldy' squash seeds (West Coast Seeds, Delta BC) were planted in Jiffy Mix germination soil in 72-well trays. After 3 weeks, they were transplanted into 1 L pots in the greenhouse and allowed to acclimatize for 1 week before treatment. Plants were randomly placed in proximity to squash plants infected with powdery mildew to become naturally infected. Once colonies started to appear, the plants were randomized into three treatment groups. One was sprayed weekly until runoff with a 0.3% solution of KF2, one set was drenched bi-weekly with 50 mL of a 0.3% KF2 solution, and the third was sprayed with water as a control. After 4 weeks, the wet weights of the plant shoots were measured.

3.2.7. Statistical analysis

Statistical analysis was done using RStudio Version 1.3.1093. An analysis of variance was used to determine significance between the means of treatments, and a Tukey post-hoc test was used to determine which treatments were significantly different ($p < 0.05$).

3.3. Results

3.3.1. Efficacy of Formulations on Inhibition of Spore Germination

For the first 24 hours post-inoculation, all three formulations and the commercial fungicide, Pristine, were able to significantly ($p < 0.05$) reduce the germination of spores of *Fusarium*, as seen in Figure 3-2. KF2 completely inhibited spore germination up to 36 hours and KF3 was able to reduce germination significantly from 31.3% to 13.9%. Representative images of the spores are shown in Figure 3-3.

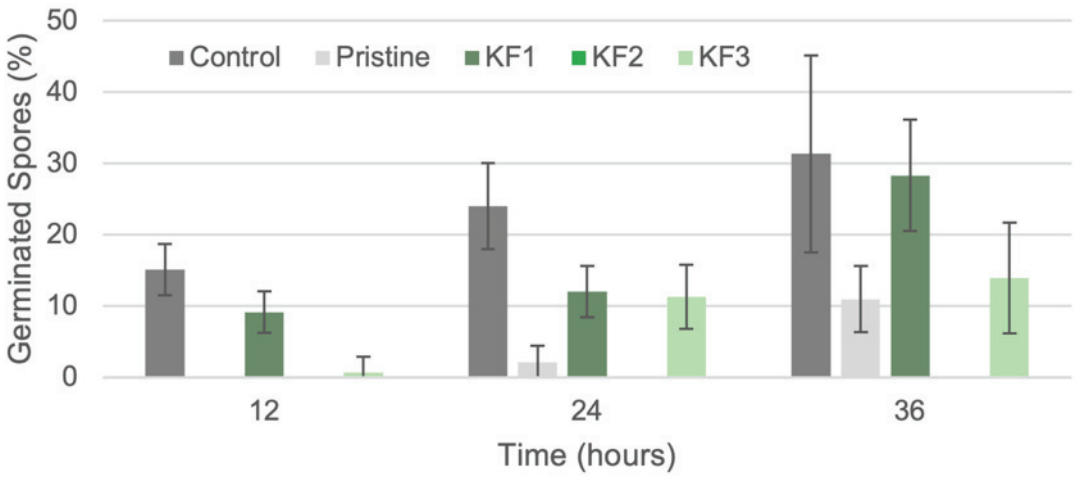


Figure 3-2 **Percent of germinated *Fusarium oxysporum* spores at 12, 24 and 36 hours (n=4, error bars show 95% confidence intervals).**

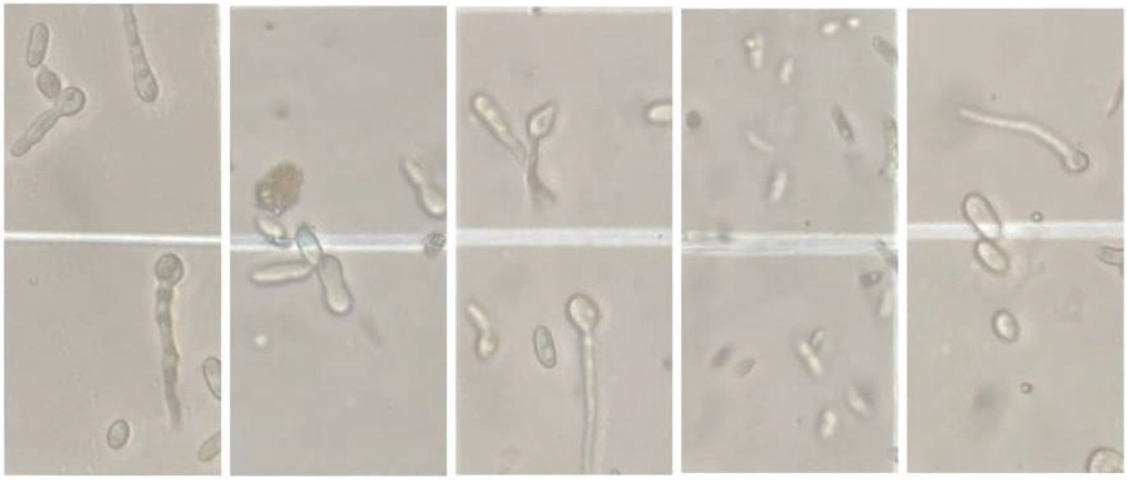


Figure 3-3 **Representative photos of spores after 36 hours. From left to right, control, Pristine, KF1, KF2, and KF3.**

3.3.2. Efficacy of Formulations on Inhibition of Mycelial Growth

Comparison of concentrations

The weight of the *Fusarium* and *Botrytis* fungal mass was increased in the higher karanja treatments (Figure 3-4). Applications of 1.5 % karanja and higher significantly increased the weight of the fungal mass ($p < 0.05$).

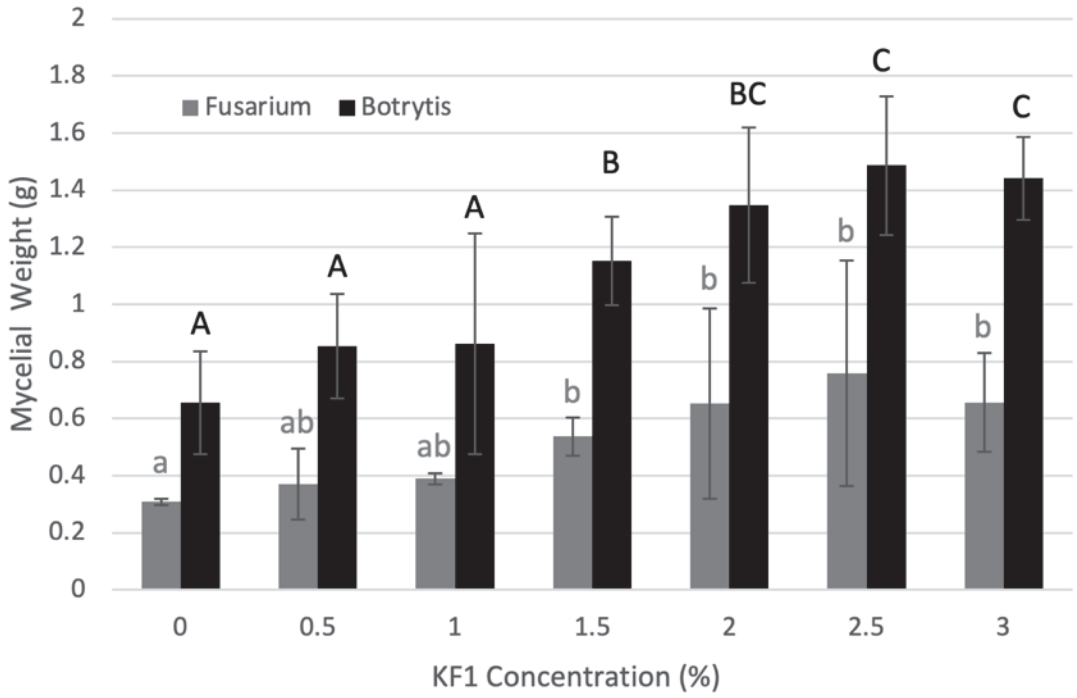


Figure 3-4 **Dry mycelial weight of *Fusarium oxysporum* after 7 days (n=3, error bars show 95% confidence intervals). Letters denote signficiant differences ($p<0.05$).**

Comparison of formulations

KF2 reduced dry mycelial weight by 96% and Pristine, the commercial fungicide, reduced mycelial weight by 73% as compared to the control (Figure 3-5). KF1 and KF3 did not show significant mycelial reduction from the control ($p<0.05$).

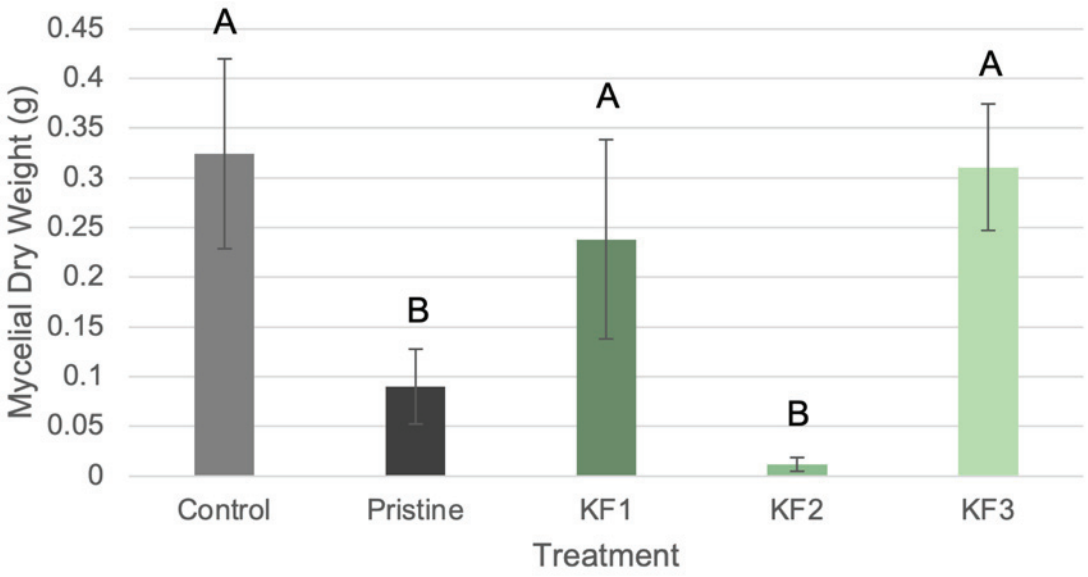


Figure 3-5 **Dry mycelial weight of *Fusarium oxysporum* after 7 days (n=4, error bars show 95% confidence intervals). Letters denote significant differences ($p<0.05$).**

3.3.3. Fusarium on tomato

Experiment 1 – Evaluating karanja treatments

There were no symptoms of *Fusarium* infection observed, although this cannot be confirmed due to a lack of an un-inoculated control. The sprayed plants were not significantly different from the control. The root weights showed that as drench concentration increased, the root mass decreased significantly, with 1.5% drenches reducing the root mass by 54.0%. (Figure 3-6).

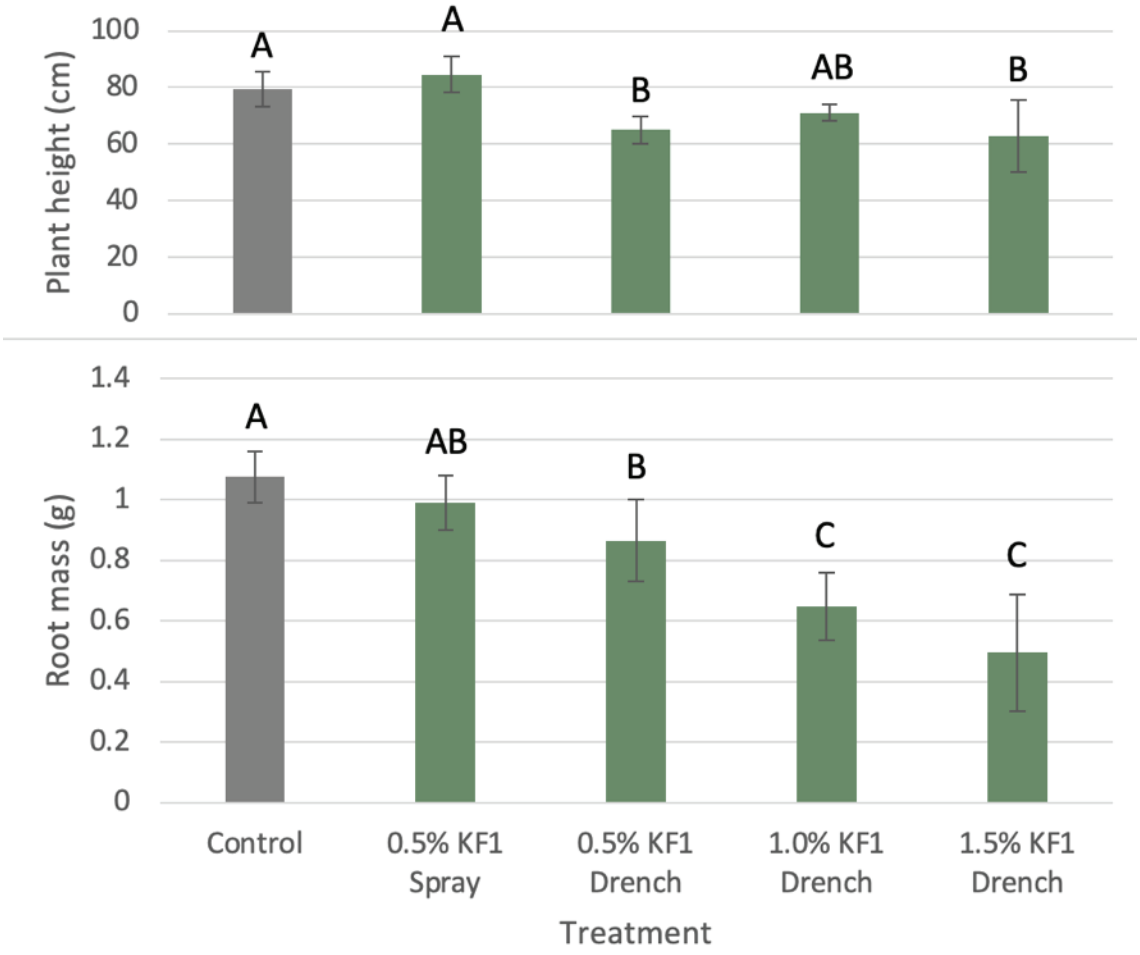


Figure 3-6 **Mean tomato heights and root masses 6 weeks after inoculation (n=5, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).**

Experiment 2 – Evaluating Fusarium virulence

The F-3 isolate significantly decreased the growth of the plants, stunting both the height and the mass of their roots (Figure 3-7, Figure 3-8). The root masses of the plants inoculated with F-3 were significantly reduced by 75%, and plant height was reduced by 49%.

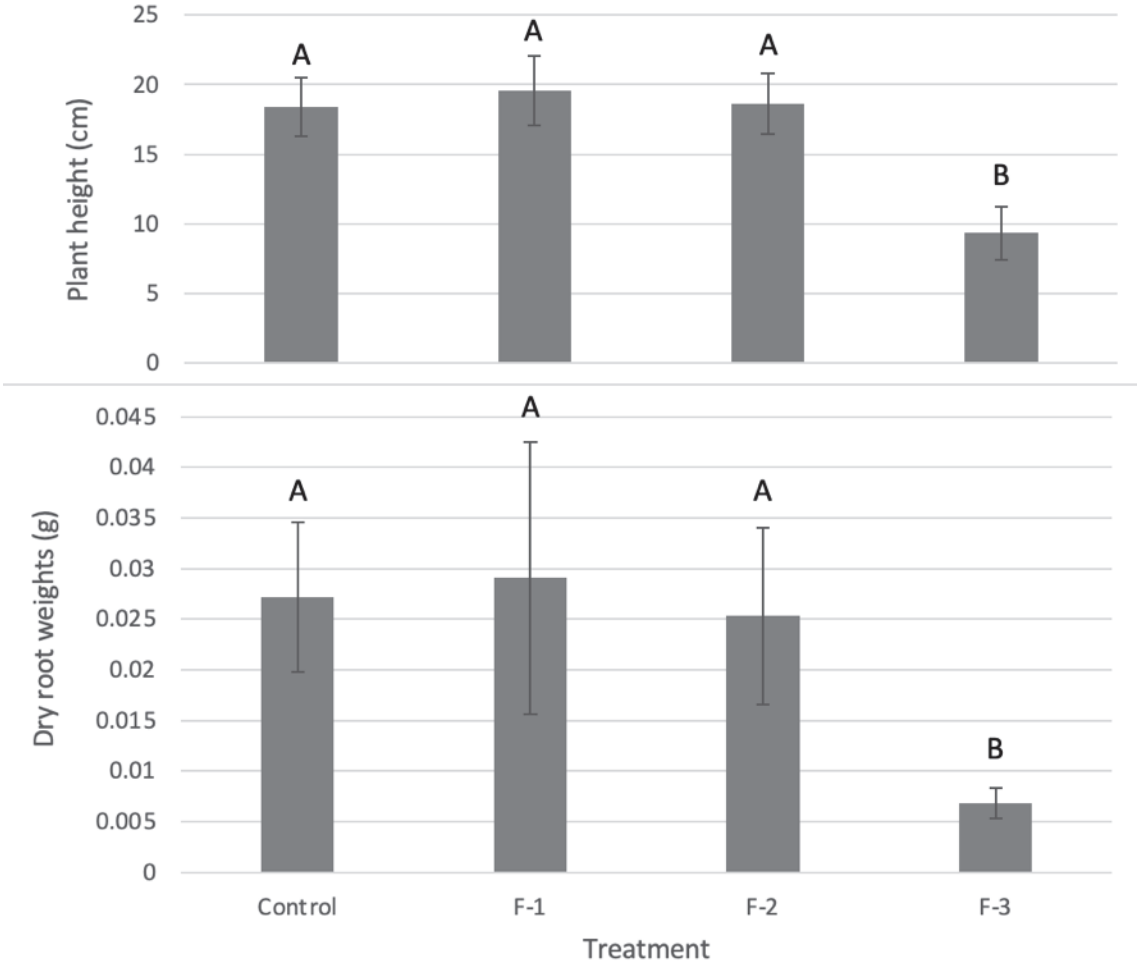


Figure 3-7 **Mean tomato heights and root masses 2 weeks after inoculation (n=8, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).**

Figure 3-8 Tomato plants at the end of Experiment 2, 2 weeks after inoculation with, from left to right, control, F-1, F-2 and F-3.

Experiment 3 – Evaluating Fusarium virulence

No significant differences in plant height or root mass were observed between the treatments (Figure 3-9).

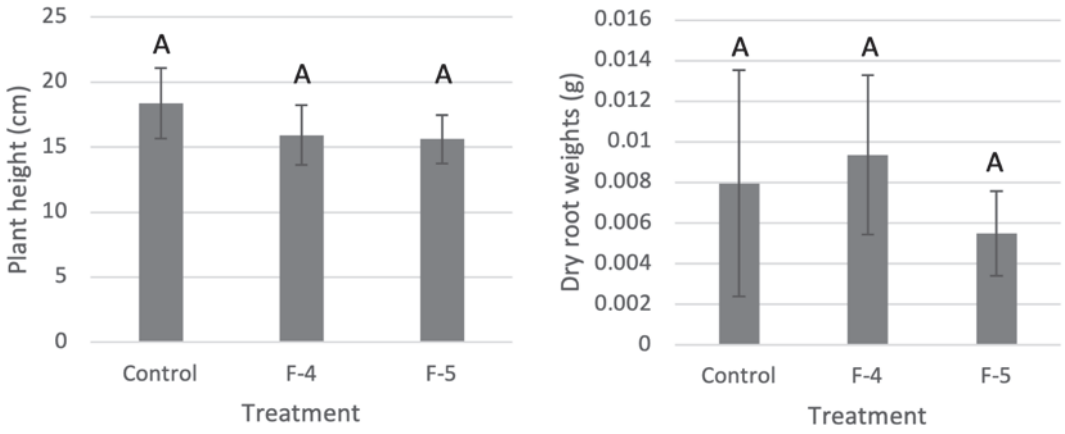


Figure 3-9 Mean tomato heights and root masses 2 weeks after inoculation (n=8, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).

Experiment 4 – Evaluating Fusarium inoculum exposure

There were significant plant height differences between inoculation times of 5 and 10 minutes, and root inhibition was seen in all F-3 inoculation times (Figure 3-10).

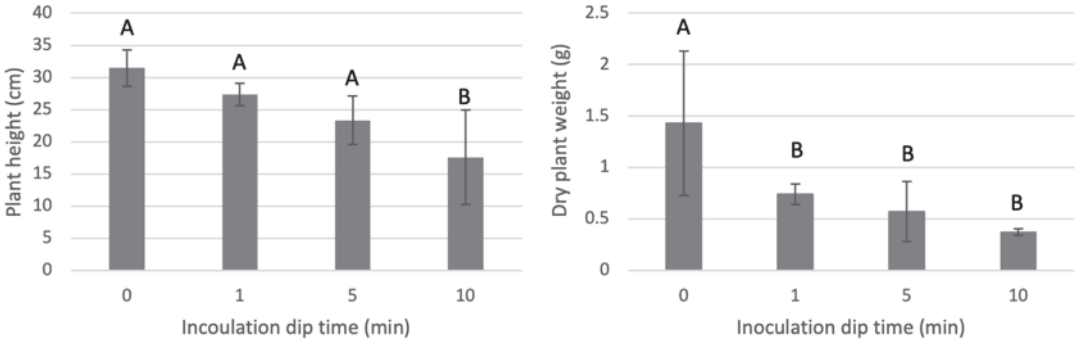


Figure 3-10 Mean tomato heights and weights 3 weeks after inoculation (n=4, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).

Experiment 5 – Evaluating Fusarium virulence

No significant differences were seen in the heights or weights of plants inoculated with any of the *Fusarium* isolates (Figure 3-11). A photo of the plants being inoculated and at the end of the trial are shown in Figure 3-12 and 3-13, respectively.

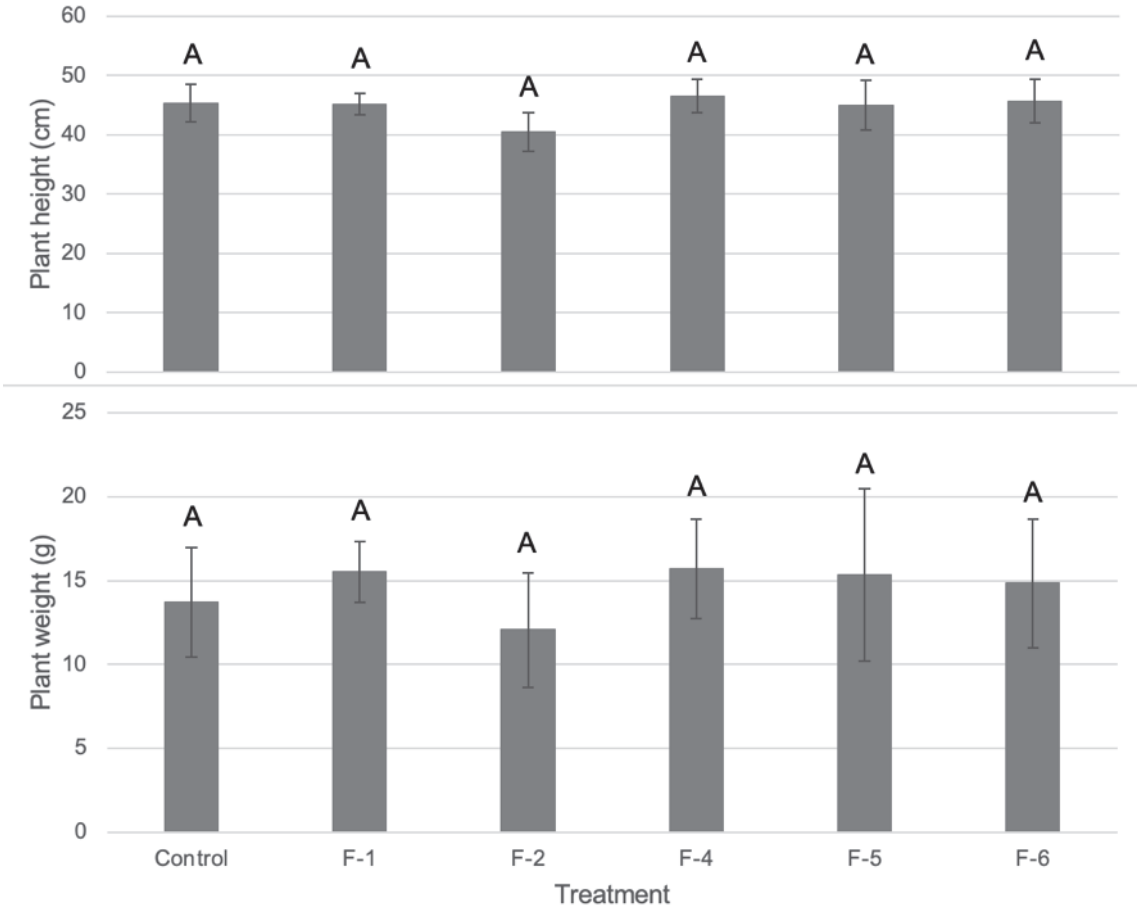


Figure 3-11 Mean tomato heights and weights 5 weeks after inoculation (n=6, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).

Figure 3-12 Root dip method, plants with cut roots were placed in Fusarium inoculum for 10 minutes before transplanting into new soil. From left to right: PDB control, F-1, F-2, F-4, F-5, and F-6.

Figure 3-13 Plants 5 weeks post inoculation. From left to right: PDB control, F-1, F-2, F-4, F-5, and F-6.

Experiment 6 – Evaluating inoculum type

The culture treatment reduced root weight by 68.2 % and plant height by 35.4 % (Figure 3-14). There was no significant difference between the control and those plants inoculated with spores or mycelia.

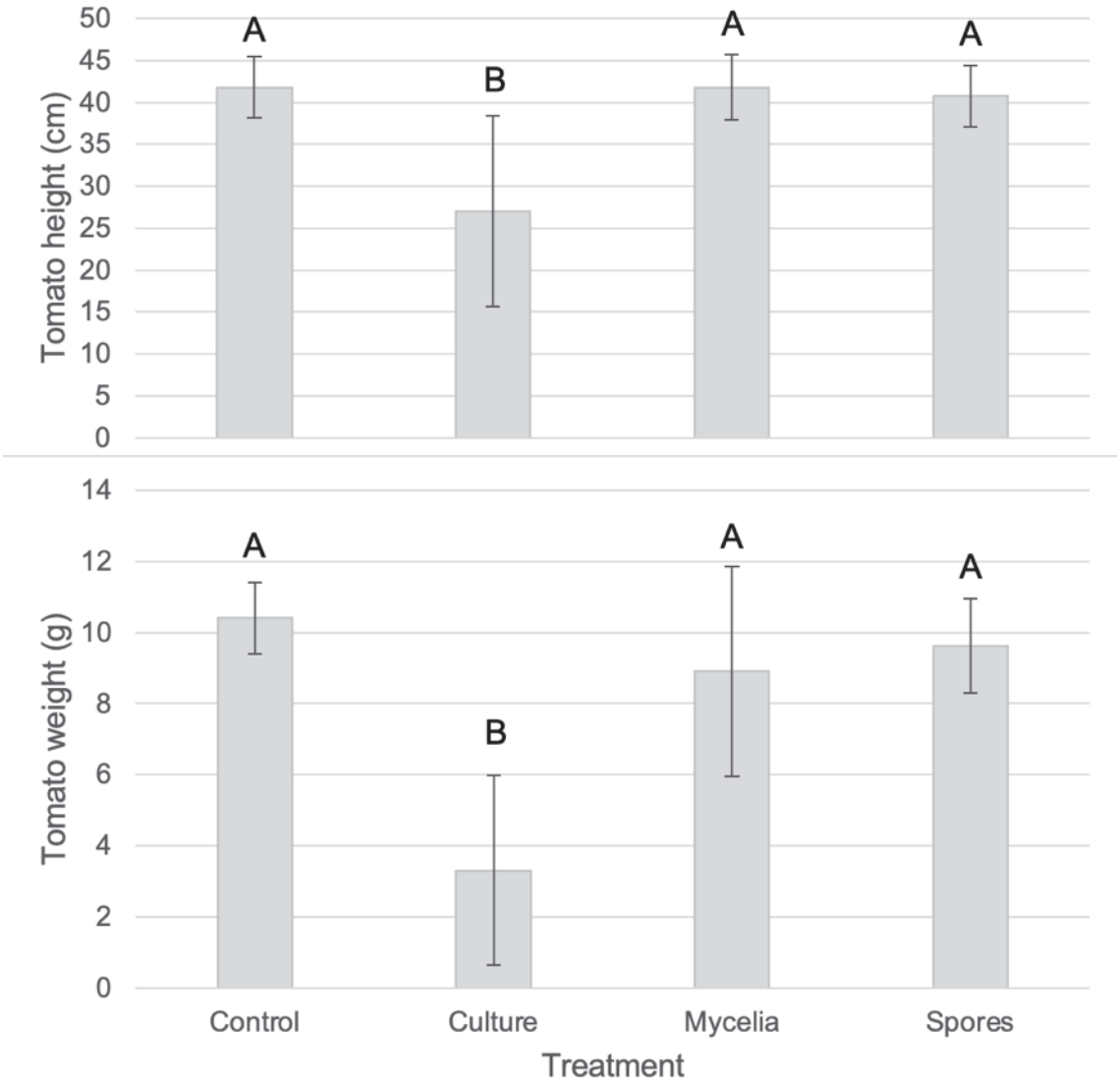


Figure 3-14 Mean tomato heights and weights 5 weeks after inoculation with culture (growth media with mycelia and spores removed), mycelia, and spores (n=5, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).

Experiment 7 – Evaluating Fusarium virulence

There was no significant difference between the control and plants treated with root drenches or dips of F-8 inoculum in terms of plant height or weight (Figure 3-15).

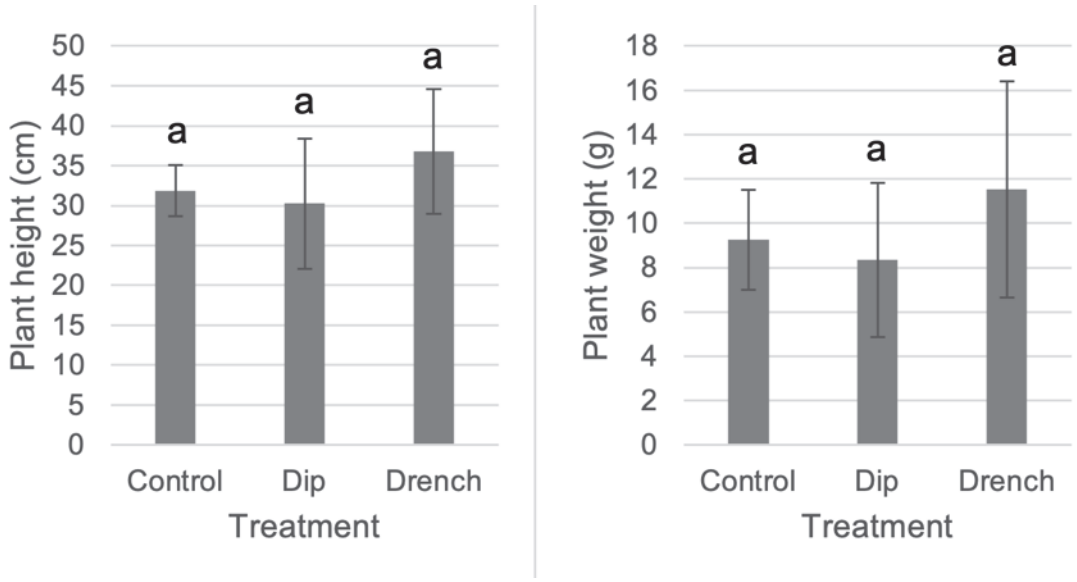


Figure 3-15 Mean tomato heights and weights 3 weeks after inoculation (n=4, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).

Experiment 8 – Evaluating Fusarium virulence

There was no significant difference between the control and plants treated with root drenches or dips of F-9 inoculum in terms of plant height or weight (Figure 3-16).

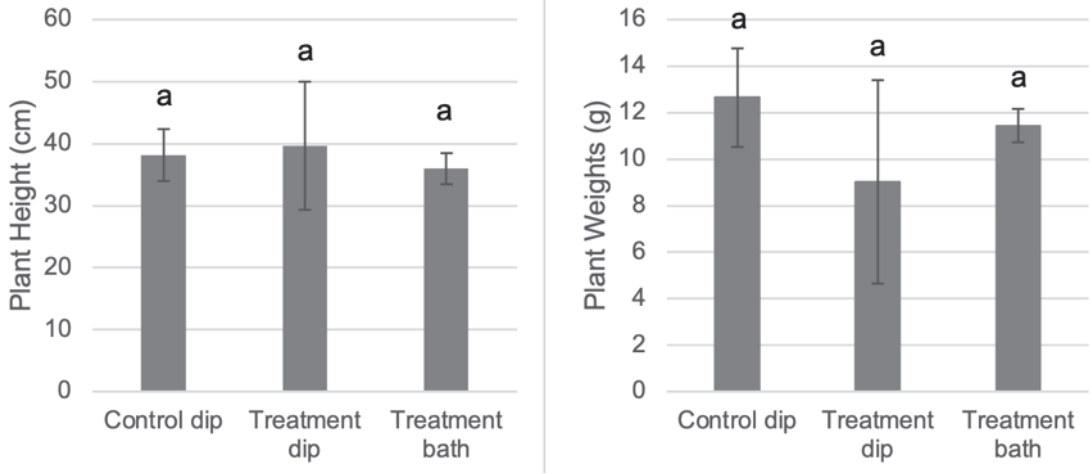


Figure 3-16 Mean tomato heights and weights 3 weeks after inoculation (n=3, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).

Experiment 9 – Evaluating Fusarium virulence

There was no significant difference between the control and plants treated with root drenches or dips of F-10 inoculum in terms of plant height or weight (Figure 3-17).

Drenches with F-10 significantly reduced both plant weight and height, whereas dips of the same inoculum did not (Figure 3-18).

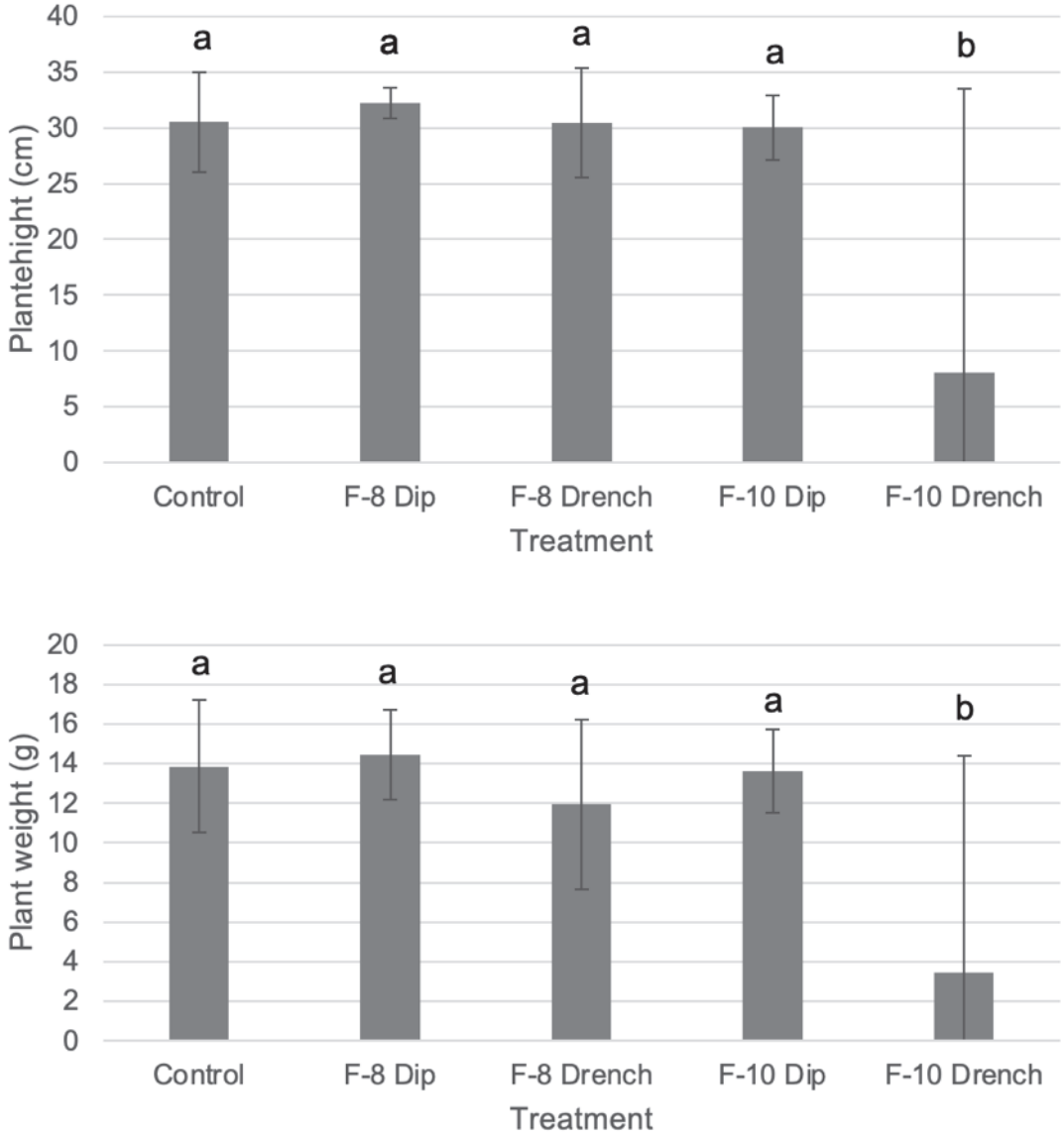


Figure 3-17 Mean tomato heights and weights 3 weeks after inoculation (n=4, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).

Figure 3-18 All treatments after two weeks. From the left (Control, F-8 dip, F-8 drench, control, F-10 dip, F-10 drench).

Experiment 10 – Evaluating Fusarium virulence

Contrary to the previous testing, inoculation with *Fusarium* F-10 did not result in significant decreases in plant height (Figure 3-19). This is likely due to the advanced age of the plants which rendered them less susceptible to infection. No significant differences were seen between plant heights and weights as compared to the control (Figure 3-20). Stem segment images were not visibly different (Figure 3-21). When plated out, *F. oxysporum* was reisolated from all treatments except the control (Figures 3-22).

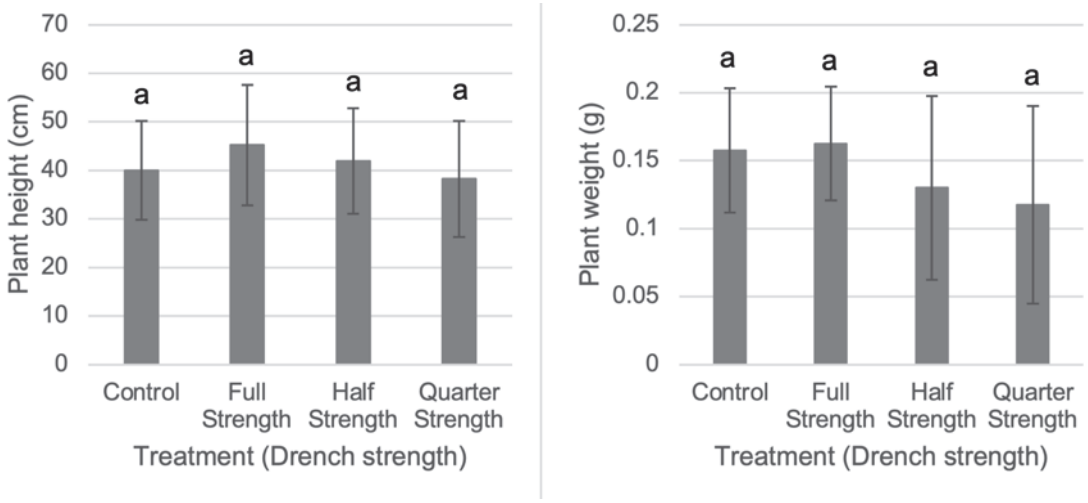


Figure 3-19 Mean tomato heights and root dry weights 4 weeks after inoculation (n=4, error bars show 95% confidence intervals). Letters denote signficant differences ($p<0.05$).

Figure 3-20 **Plants after 4 weeks: Control (top left), full strength inoculation (top right), half strength inoculation (bottom left) and quarter strength inoculation (bottom right). No significant differences were seen in heights of the plants.**

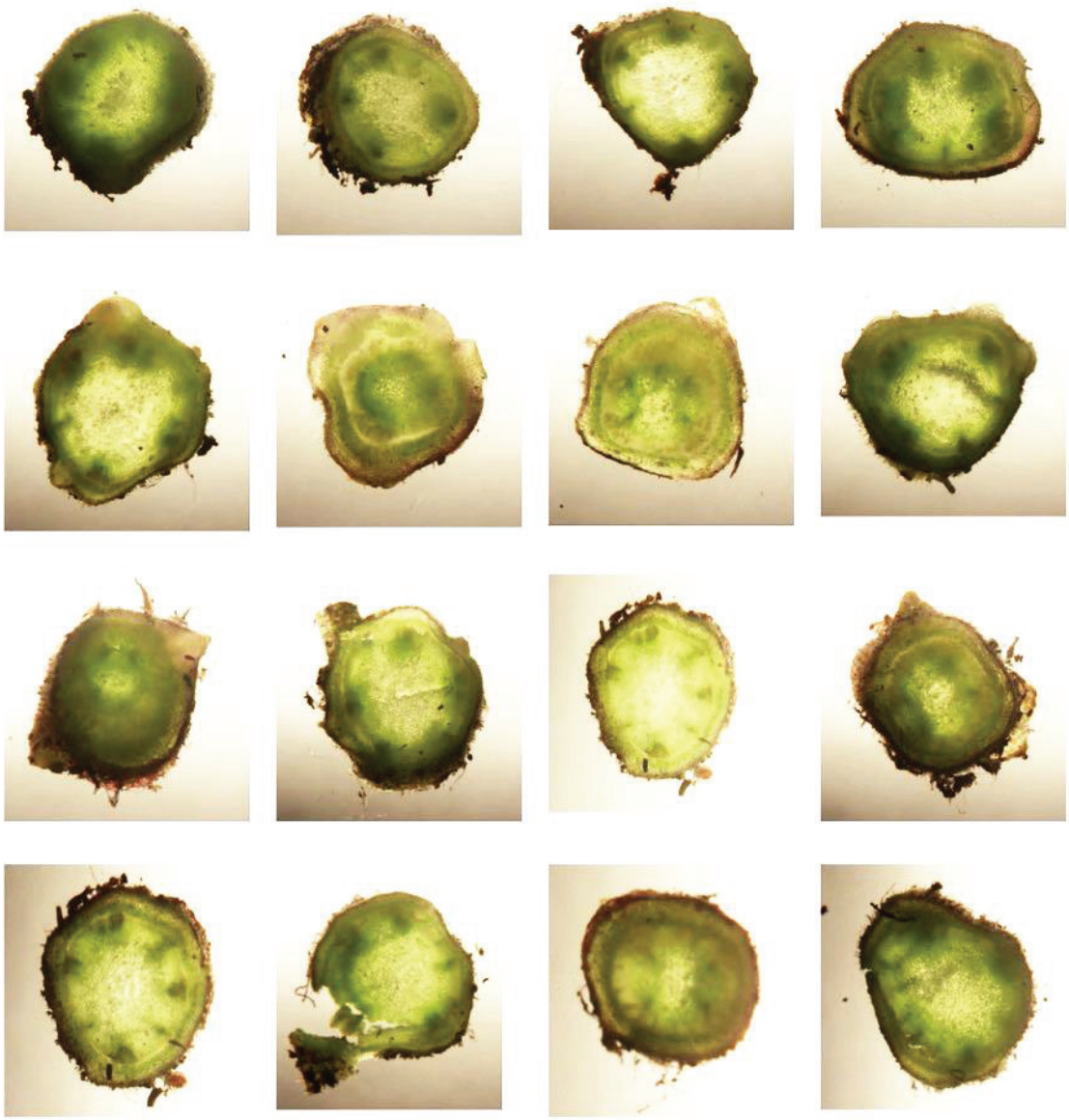


Figure 3-21 **Cross sections of stems: Control (top row), full strength inoculation (second row), half strength inoculation (third row) and quarter strength inoculation (bottom row)**

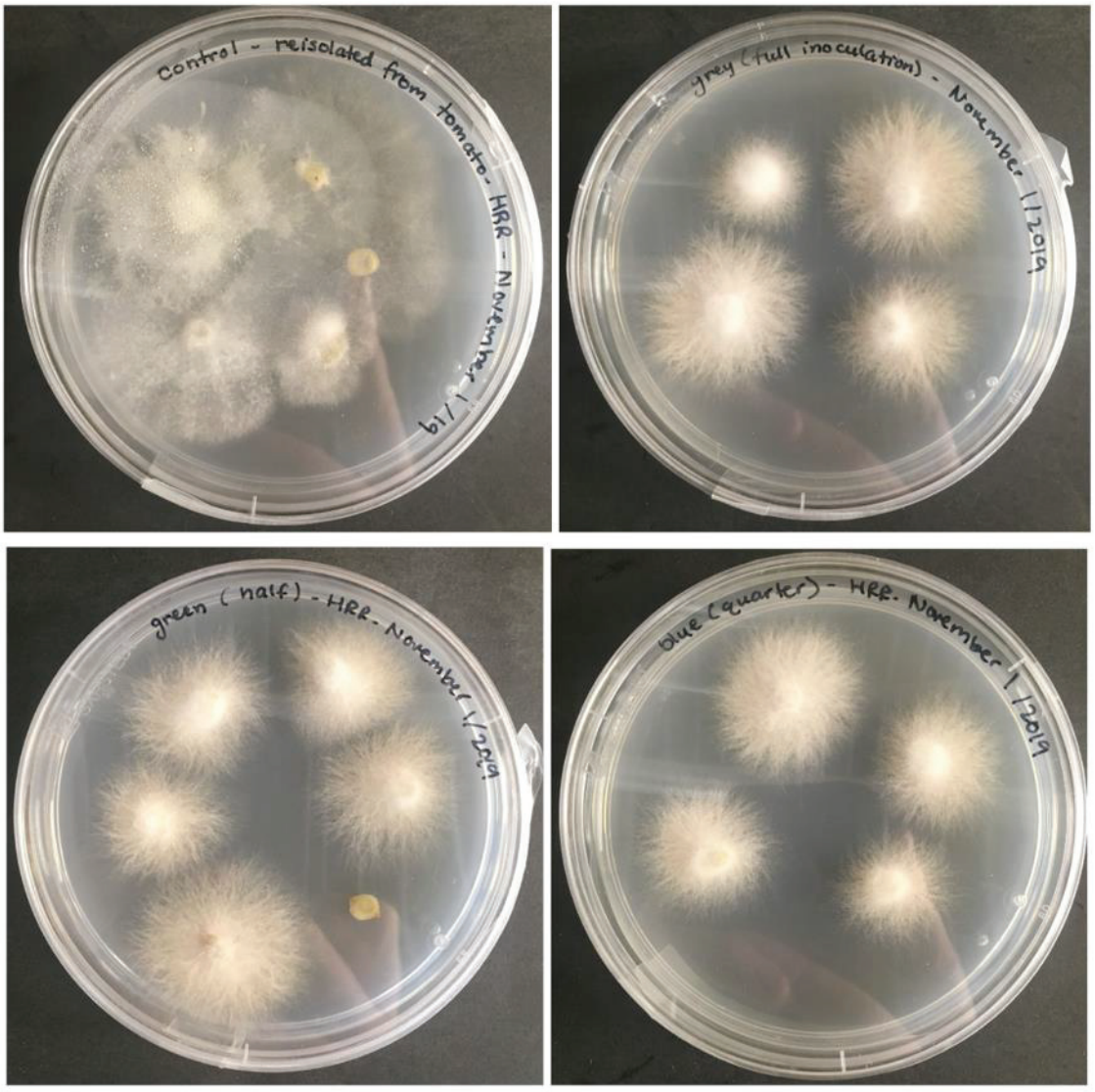


Figure 3-22 **Results of re-isolation from stem tissues: Control (top left), full strength inoculation (top right), half strength inoculation (bottom left) and quarter strength inoculation (bottom right). *Fusarium* was reisolated from all the inoculation treatments but was not present in the control. Other unidentified fungi were present in the control tissues.**

Experiment 11 – Evaluating karanja treatments

No disease symptoms were seen during this time, though some chlorosis did occur, likely due to nutritional stress as plants were not fertilized (Figure 3-23). This was done to stress the plant to promote infection. Although no infection occurred, karanja treatments had decreased wet weight (Figure 3-24).

Figure 3-23 Uninfected tomato plants (image on the left): from left to right (untreated, Asperillo, karanja, Regalia, Medallion) Infected tomato plants (Image on the right): from left to right (untreated, Asperillo, karanja, Regalia, Medallion)

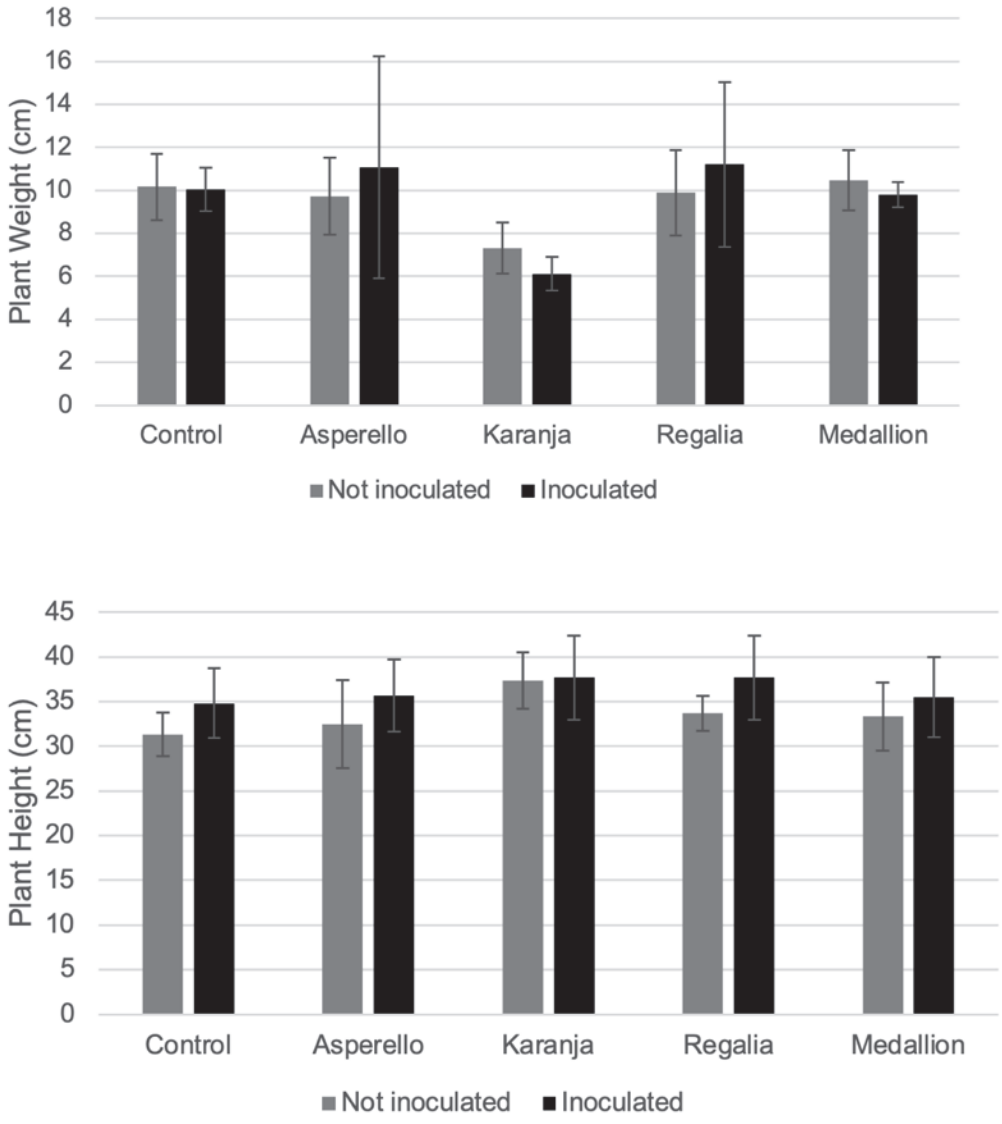


Figure 3-24 Mean tomato heights and weights (n=6, error bars show 95% confidence intervals).

Experiment 12 – Seed treatments

Results varied largely between all four trials (Figure 3-25), with trial 1 showing a significant reduction of plant growth in the F-10 inoculated, untreated seeds. Trial 2 and 4 had no significant differences between treatments, and Trial 3 showed all inoculated seeds, regardless of pre-treatment, with reduced plant growth.

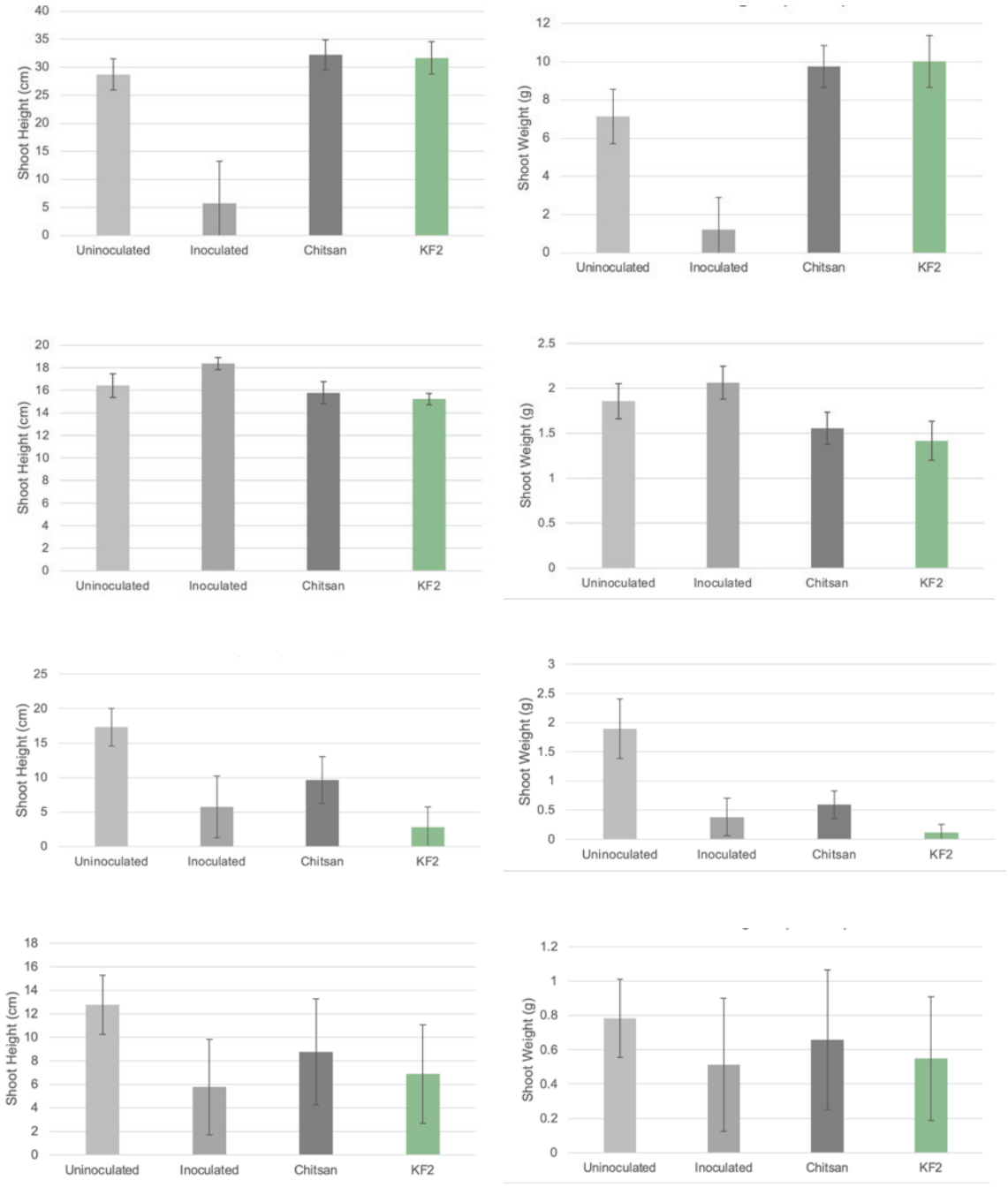


Figure 3-25 Shoot height and shoot weight results of four trials of seed pre-treatment, all at 0.01% (n=6, error bars show 95% confidence intervals).

3.3.4. Powdery mildew on cucumber

No significant differences were seen between treatments 1 week after the first spray. After 2 weeks, those plants sprayed with KF2 had significantly less percent coverage of powdery mildew on cucumber leaves as compared to the water control ($p < 0.05$, Figure 3-26). This treatment, however, also caused phytotoxicity on the leaves as seen in Figure 3-27. The same significance was seen in week 3. There was no significance seen between treatments in the final week of testing (week 4).

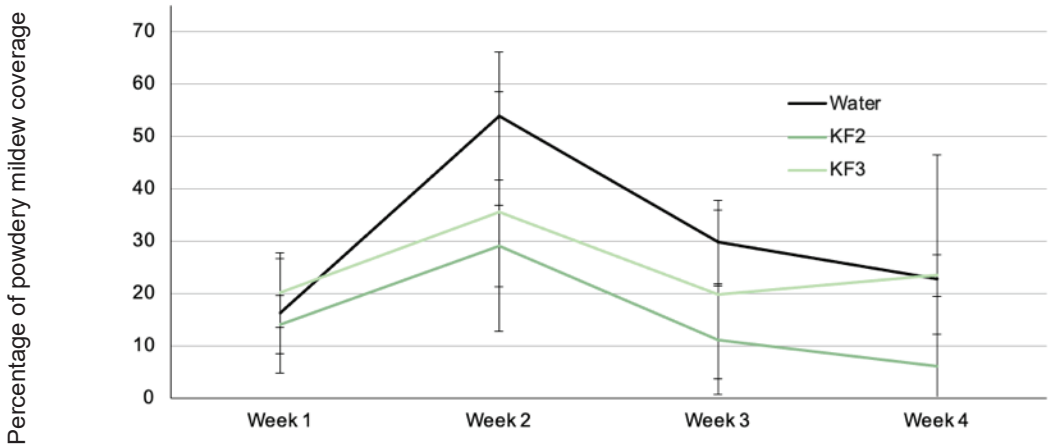


Figure 3-26 Percentage of powdery mildew leaf coverage after 10 weeks of growth, 5 weeks post inoculation (n=4, error bars show 95% confidence intervals)

Figure 3-27 Representative leaves at the end of treatments, from left to right, KF2, KF3, control.

3.3.5. Powdery mildew on squash

The foliar application treatments of KF2 at 2 weeks showed less colonies than the controls or the drenches, which do not appear to be significantly different. Examples of disease severity on leaves is shown in Figure 3-28. After 4 weeks, the control and drench plants were significantly stunted (Figure 3-29). The weight of the drench treatment was significantly reduced (from 17.5 g to 7.6 g), and the spray treatment was significantly heavier(31.5 g).

Figure 3-28 Representative leaves from each treatment, from left to right, control, KF2 drench, and KF2 spray after 2 weeks.

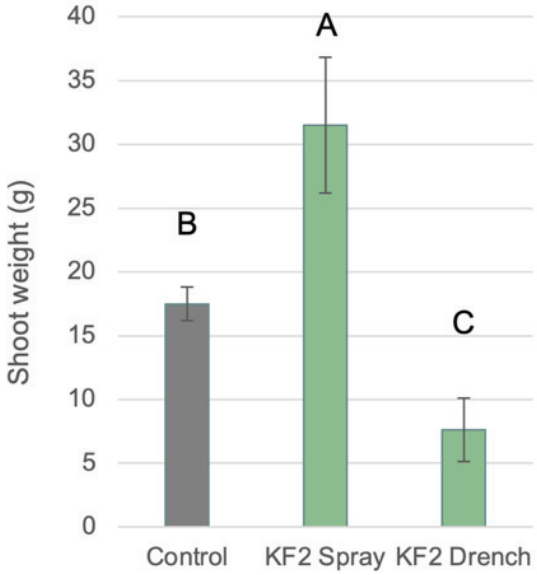


Figure 3-29 Weight of squash shoots (n=8, error bars show 95% confidence intervals). Letters denote signficant differences (p<0.05).

3.4. Discussion

For the in vitro tests, the karanja formulation KF2 was the most effective at reducing both spore germination and mycelial growth, matching or outperforming the commercial fungicide treatment. KF1 and KF3 did not show significant reduction of mycelial growth or of spore germination after 36 hours. This indicates that the preparation and formulation of karanja treatments do have a significant impact on their efficacy. Mycelial growth experiments run with higher concentrations of KF1 against *Fusarium* and *Botrytis* showed increased final weight of the filtrate. This is perhaps due to the oil remaining behind in the filtration process, as it may have adhered to the mycelia present, causing an increase in weight that did not correlate solely with the mycelial weight present.

Due to lack of controls containing individual formulation ingredients, the mode of action cannot be determined. Previous research in vitro has linked the efficacy of karanja preparations to flavonoids present in the extracts (Sharma et al. 2011). Many flavonoid compounds have been shown to have anti-fungal or anti-bacterial activity (Treutter 2005). The most widely investigated flavonoid found in karanja oil is karanjin, which has been shown to have anti-oxidative effects and can scavenge reactive oxygen species (Singh et al. 2021, Noor 2020). Pongamol is the second most investigated flavonoid and is also shown to have antioxidant activity (Jahan et al. 2021). The benefits of flavonoids in a pathogen system are two-fold, both antagonistic and protective. The compounds can be directly anti-fungal, inhibiting cell division as well as disrupting the fungal cell wall (Aboody and Mickmaray 2020). The presence of antioxidants could also protect plants from reactive oxygen species produced by the pathogen during infection (Gessler et al. 2007).

In planta results showed a decrease in percent coverage of powdery mildew on cucumber plants sprayed with KF2. The decrease in colony coverage of the leaf seen in the control as time progressed was likely due to heavily infected leaves dying off. Foliar sprays on squash protected the plant against significant powdery mildew damage, resulting in a higher plant weight. The significance of the damage to the leaves was such that percent coverage could not be determined as with cucumber, and so plant weight was measured instead. Drenches with the same concentration, however, resulted in significant phytotoxicity. This toxicity with drenches was also seen on tomato plants, with

dry weights decreasing as the percentage of KF1 concentration in drenches increased. Phytotoxicity of plant essential oils has been reported for many plant extracts and is thought to be due to the presence of monoterpenoids (Adb-El Gawad et al. 2020). Phytotoxicity with essential oils has been seen with foliar applications of clove oil on Hostas at 10% (w/v) but not with lower concentrations of 0.116% (McDonnell et al. 2015). Drenches of clove oil on tomato caused significant inhibition of germination and stunting of transplants at levels as low as 0.2% (Meyer et al. 2008).

Finding an isolate of *Fusarium* capable of producing consistent symptoms on tomato proved difficult. Isolate F-3 (*F. proliferatum* from cannabis) showed aggressive action against tomatoes, killing all 8 replicates. To ensure that the symptoms seen were due to infection, and not due to toxicity from any of the metabolites produced while the fungus grew in the broth, tomatoes were dipped in the culture with spores and mycelia removed, and in spore and mycelia water suspensions that had been washed clean of metabolites. Only the plants dipped in the culture lacking the spores or mycelia showed significant stunting. The toxicity was therefore induced by the culture filtrate, devoid of any live spores or mycelia and was therefore not appropriate for use in further fungal testing. Isolate F-10 showed promise, killing all but one replicate. The age of plants when inoculated with F-10 was found to be important, as older plants did not show symptoms. When tissue was taken from the crown of these tomatoes and surface sterilized at the end of the experiment, F*usarium* was re-isolated, suggesting the fungus is infecting the plants, but not showing significant symptoms in older plants during the timeframe tested.

Results from the seed pre-treatment with KF2 varied, which some trials showing symptoms induced by the pathogen, and others not. This variation in pathogenicity could be due to the age of the isolate, varying light or water conditions, or other human related variation. No significant results can therefore be deduced from these highly variable trials.

Further trials will be required to determine which components of the oil are responsible for the fungicidal effects seen, as well as through which mechanisms they act. Testing of plants for increases in total phenolics did not yield significant results, but investigation into other defence compounds or defence enzymes may elucidate some induced resistance mechanisms. Rates of application must also be investigated to limit

any phototoxic effects seen at higher levels. Karanja oil could prove to be a useful as an organic pesticide for reduction of fungal diseases. With further research, both the efficacy and the mechanism of Karanja in fungal plant-pathogen interactions can be determined. Whether the oil functions through direct fungicidal or fungistatic effects on the fungus, through upregulation of the plant's natural defence system, or both will be key in determining the practical usage of this compound.

Chapter 4.

Conclusions and future research

4.1. Calcium nutrition

Calcium has been shown to be an import nutrient for plant defence. Many modes of action have been described, impacting the pathogen, environment, and the plant host. Direct pathogen inhibition can be caused by inhibition of spore germination or enzyme action. The environment can be impacted by certain forms of calcium that are able to impact soil pH, making it less conducive to disease. The main mode of action, however, is due to calcium increasing plant's natural defence systems. Calcium ions cause an increase in plant cell wall strength, and a decrease in the rate of enzymatic degradation of pectin. Increased hypersensitive responses have also been attributed to the increase in free calcium which plants use in defence signalling. Overall, calcium has been demonstrated to be an important factor for controlling disease development on over 40 crops, against 35 different pathogens, and therefore should be considered as a useful tool in integrated sustainable pest management systems.

4.2. Karanja formulations

The use of karanja formulations as a biopesticide has mostly been investigated through in vitro methods. Except for a single study published in 2021, no research on in plantae systems have been reported. Spore germination and mycelial growth of *Fusarium* was inhibited in vitro by a karanja formulation, KF2. Studies on powdery mildew on cucumber showed that treatment with the formulations decreased percentage coverage of the disease. No consistent results were seen with *Fusarium* due to a lack of suitable virulent isolate, but drenches with the products were seen to be phytotoxic. The degree of inhibition both in vitro and on cucumber was seen to be impacted by the formulation of karanja, and thus further testing with appropriate controls for additives would need to be run before exact mode of actions can be determined.

Although the efficacy of karanja oil formulations have shown some promising results, more research will be required before the practical applications of it can be

determined. Investigations into other pathogen systems beyond those tested here should be done. The mode of action of these karanja formulations should be determined. Possible future tests could include analysis of defence enzyme activities, such as peroxidases and phenylalanine lyase. Further work would be needed to determine rates of application that do not show any signs of phytotoxicity. Long term field or greenhouse experiments would also be necessary to confirm the usefulness of this product in the hands of producers.

References

Abd-El Gawad, El Gendy, A. E.-N. G., Assaeed, A. M., Al-Rowaily, S. L., Alharthi, A. S., Mohamed, T. A., Nassar, M. I., Dewir, Y. H., & Elshamy, A. I. (2020). Phytotoxic Effects of Plant Essential Oils: A Systematic Review and Structure-Activity Relationship Based on Chemometric Analyses. *Plants (Basel)*, *10*(1), 36.

Abd-El-Kareem, F., Elshahawy, E., and Abd-Elgawad, M, M, M. (2019) Management of strawberry leaf blight disease caused by *Phomopsis obscurans* using silicate salts under field conditions. Bulletin of the National Research Centre 43:1-6.

Aboody, & Mickymaray, S. (2020). Anti-Fungal Efficacy and Mechanisms of Flavonoids. Antibiotics (Basel), 9(2), 45.

Abou-El-Hassan, S., Metwaly, H, A., and Ali, A, M. (2020). Efficiency of potassium and calcium compounds in gel formula to control early blight disease, improve productivity, and shelf life of potato. Egypt. J. Agric. Res. 98:154-168.

Aglave. (2019). Cucurbits. In *Handbook of Plant Disease Identification and Management* (1st ed., pp. 251–406). CRC Press.

Agriculture and Agri-Food Canada, Crop and Horticulture Division (2019) Statistical Overview of the Canadian Vegetable Industry.

Agriculture and Agri-Food Canada, Crops and Horticulture Division (2021) Statistical Overview of the Greenhouse Vegetable Industry 2020.

Agriculture and Agri-Food Canada, Pest Management Program (2021a) Crop Profile for Greenhouse Cucumber in Canada, 2020.

Agriculture and Agri-Food Canada, Pest Management Program (2021b) Crop Profile for Greenhouse Tomato in Canada, 2020.

Agrios, George N. (2005) Plant pathology. 5th Edition. San Diego: Academic Press.

Ahn, I.-P., Uhm, K.-H., Kim, S., and Lee, Y.-H. (2003). Signaling pathways involved in preinfection development of *Colletotrichum gloeosporioides, C. coccodes,* and *C. dematium* pathogenic on red pepper. Physiol. Mol. Plant Pathol. 63:281–289.

Aishwarya, D.S., & Somasekhara Y.M. (2017). Evaluation of botanicals, fungicides, and bio-agents against Pigeon Pea wilt (Fusarium oxysporum f. sp. udum). International Journal of Advanced Life Sciences, 10(4), 439–447.

Ajilogba, C, F., and O. Babalola (2013) Integrated Management Strategies for Tomato Fusarium Wilt. Biocontrol Science 18(3), 117-127.

Akram, A., Shaheen, I., Akhund, S., Nayyar, B. G., & Seerat, W. (2016). 19. In vitro antifungal activity of Pongamia pinnata against collar rot pathogen (Sclerotium rolfsii) of Chickpea. Pure and Applied Biology (PAB), 5(3), 520-528.

Al-Zaemey, A. B., Magan, N., and Thompson, A. K. (1993). Studies on the effect of fruit-coating polymers and organic acids on growth of *Colletotrichum musae in vitro* and on postharvest control of anthracnose of bananas. Mycol. Res. 97:1463–1468.

Alandes, L., Hernando, I., Quiles, A., Pérez-Munuera, I., & Lluch, M. A. (2006). Cell wall stability of fresh-cut fuji apples treated with calcium lactate. J. Food Sci. 71:615-620.

Alexander, L.J. (1963) Transfer of a dominant type of resistance to the four known Ohio pathogenic strains of tobacco mosaic virus (TMV), from *Lycopersicon peruvianum to L. esculentum*. Phytopathology 53, 869.

Amini, J., and D, F, Sidovich. (2010). The effects of fungicides on *Fusarium oxysporum f. sp. lycopersici* associated with Fusarium wilt of tomato J. Plant Prot. Res., 50, 172-178.

An, B., Li, B., Qin, G., & Tian, S. (2012). Exogenous calcium improves viability of biocontrol yeasts under heat stress by reducing ROS accumulation and oxidative damage of cellular protein. Curr. Microbiol. 65:122–127.

Anderson, W. C., Gabrielson, R. L., Haglund, W. A., and Baker, A. S. (1976). Clubroot control in crucifers with hydrated lime and PCNB. Plant Dis. Rep. 60:561–565.

Angell, H. R. (1950). Seedling blight: II. Soil in relation to seedling blight of opium poppy and peas. Aust. J. Agric. Res. 1:132–140.

Anitha, A., and M, Rabeeth (2009) Control of Fusarium wilt of tomato by bioformulation of *Streptomyces griseus* in Green house condition Afr. J. Basic Appl. Sci., 1, 9-14.

Annis, S. L., and Goodwin, P. H. (1997). Inhibition of polygalacturonase activity produced by *Leptosphaeria maculans* by leaf extracts of canola and its relationship to calcium. Can. J. Plant Pathol. 19:1–7.

Arteca, R. (1982). Infiltration and movement of ^{45}Ca into potatoes. HortScience 17:757–758.

Atallah, M. T., and Nagel, C. W. (1977). The role of calcium ions in the activity of an endo-pectic-acid lyase on oligogalacturonides. J. Food Biochem. 1:185–206.

Atim, M., Beed, F., Tusiime, G., Tripathi, L., and van Asten, P. (2013). High potassium, calcium, and nitrogen application reduce susceptibility to banana *Xanthomonas* wilt caused by *Xanthomonas campestris pv. musacearum*. Plant Dis. 97:123-130.

Atkinson, M. M., Keppler, L. D., Orlandi, E. W., Baker, C. J., and Mischke, C. F. (1990). Involvement of plasma membrane calcium influx in bacterial induction of the K^+/H^+ and hypersensitive responses in tobacco. Plant Physiol. 92:215–221.

Bastiaanse, H., de Lapeyre de Bellaire, L., Lassois, L., Misson, C., & Jijakli, M. H. (2010). Integrated control of crown rot of banana with *Candida oleophila* strain O, calcium chloride and modified atmosphere packaging. Biol. Control 53:100–107.

Bateman, D. F., and Basham, H. G. (1976). Degradation of plant cell walls and membranes by microbial enzymes. Pages 316–355 in: Encyclopedia of Plant Physiology. Vol. 4, Physiological Plant Pathology. R. Heitefuss and P. H. Williams, eds. Springer-Verlag, New York.

Bateman, D. F., and Beer, S. V. (1965). Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii.* Phytopathology 55:204–211.

Bauscher, R. W., Hudson, J. M., and Adams, J. R. (1979). Inhibition of polygalacturonase softening of cucumber pickles by calcium chloride. J. Food Sci. 44:1786–1787.

Baver, L. D. (1928). The relation of exchangeable cations to the physical properties of soils. J. Am. Soc. Agron. 20:921–941.

Beckman, C.H. (1987) *The Nature of Wilt Diseases of Plant*. St. Paul: APS Press.

Bengerth, F., Diley, D. R., and Dewey, D. H. (1972). Effect of post-harvest calcium treatments on internal breakdown and respiration of apple fruits. J. Am. Soc. Hortic. Sci. 97:679–682.

Bennett, K, L. (2019). The effects of calcium on botrytis blight of pentunia flowers. MS Thesis. Clemsons University. 2019.

Bennett, R, S., O'Neill, W., Smith, L., and R, B, Hutmacher. (2011) Activity of commercial detergents against conidia and chlamydospores of Fusarium oxysporum f. sp. vasinfectum J. Cotton Sci., 15, 162-169.

Berry, S.Z., Madumadu, G.G. & Uddin, M.R. (1988). Effect of calcium and nitrogen on bacterial canker disease of tomato. Plant Soil. 112:113-120.

Betts, H. A., and Bramlage, W. J. (1977). Uptake of calcium by apples from post-harvest dips in calcium chloride solutions. J. Am. Soc. Hortic. Sci. 102:785–788.

Bhai, R, S., Prameela, T, P., Vincy, K., Biju, C, N., Srinivasan, V., and Babu, K, N. (2019). Soil solarization and amelioration with calcium chloride or *Bacillus licheniformis* - an effective integrated strategy for the management of bacterial wilt of ginger incited by *Ralstonia pseudosolanacearum.* Eur J Plant Pathol 154:903-917.

Biggs, A. R. (1999). Effects of calcium salts on apple bitter rot caused by two *Colletotrichum* spp. Plant Dis. 83:1001–1005.

Biggs, A. R., Ingle, M., and Solihati, W. D. (1993). Control of *Alternaria* infection of fruit of apple cultivar Nittany with calcium chloride and fungicides. Plant Dis. 77:976–980.

Biggs, A. R., El-Kholi, M. M., and El-Neshawy, S. M. (1994). Effect of calcium salts on growth, pectic enzyme activity, and colonization of peach twigs by *Leucostoma persoonii.* Plant Dis. 78:886–890.

Biggs, A. R., El-Kholi, M. M., El-Neshawy, S., and Nickerson, R. (1997). Effects of calcium salts on growth, polygalacturonase activity, and infection of peach fruit by *Monilinia fructicola.* Plant Dis. 81:399–403.

Blancard, D. (2012) Tomato Disease: Identification, Biologu and Control: A colour Handbook, Second Edition. CRC Press.

Blowers, D. P., Boss, W. F., and Trewavas, A. J. (1988). Rapid changes in plasma membrane protein phosphorylation during initiation of cell wall digestion. Plant Physiol. 86:505–509.

Boland, G.J. and L, D, Kuykendall. (1998) Plant-Microbe Interactions and Biological Control. New York: Marcel Dekker, 442.

Bollen, G. J. (1985). Lethal temperatures of soil fungi. In Ecology and management of soil-borne plant pathogens, 191-193.

Bonanomi, G., Antignani, V., Capodilupo, M., and F, Scala. (2010) Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. Soil Biol. Biochem. 42, 136–44.

Bora, P., Bora, L. C., Bhuyan R.P. (2021) Evaluation of some botanicals and microbial bioformulations against grey blight disease of tea (Camellia sinensis). Indian Council of Agricultural Research.

Boyd-Wilson, K. S. H., Butler, R. C., Alspach, P. A., Everett, K. R., Pushparajah, I. P. S., & Walter, M. (2014). Compounds alone and in combination with yeasts to control *Colletotrichum acutatum* in apples. Australasian Plant Pathol. 43:703–714.

Bush, D. S. (1995). Calcium regulation in plant cells and its role in signaling. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46:95–122.

Byrde, R. J. W., and Archer, S. A. (1977). Host inhibition or modification of extracellular enzymes of pathogens. Pages 213–246 in: Cell Wall Biochemistry Related to Specificity in Host-Plant Pathogen Interactions. B. Solheim and J. Raa, eds. Columbia University Press, New York.

CABI (2020) Invasice Species Compendium: Fusarium oxysporum. Wallingford, UK: CABI

Cai, K., Gao, D., Chen, J., and S, Luo. (2009) Probing the mechanisms of silicon-mediated pathogen resistance. Plant Sig Behav 4, 1–3.

Campanella, V., Ippolito, A., and Nigro, F. (2002). Activity of calcium salts in controlling Phytophthora root rot of citrus. Crop Prot. 21:751–756.

Campbell, R. N., Greathead, A. S., Myers, D. F., and de Boer, G. J. (1985). Factors related to control of clubroot of crucifers in the Salinas Valley of California. Phytopathology 75:665–670.

Cao, S., Zheng, Y., Tang, S., & Wang, K. (2008). Improved control of anthracnose rot in loquat fruit by a combination treatment of *Pichia membranifaciens* with CaCl2. Int. J. Food Microbiol. 126:216–220.

Capdeville, G. De, Maffia, L. A., Finger, F. L., & Batista, U. G. (2005). Pre-harvest calcium sulfate applications affect vase life and severity of gray mold in cut roses. Scientia Horticult. 103:329–338.

Chandra, S., Chakraborty, N., Chakraborty, A., Rai, R., Bera, B., & Acharya, K. (2014). Archives of Phytopathology and Plant Protection Induction of defence response against blister blight by calcium chloride in tea. Arch. Phytopathol. Plant Prot. 5408:1–10.

Chardonnet, C. O., Sams, C. E., Trigiano, R. N., and Conway, W. S. (2000). Variability of three isolates of *Botrytis cinerea* affects the inhibitory effects of calcium on this fungus. Phytopathology 90:769–774.

Chen, Yu, G., Wang, X., Meng, X., & Lv, C. (2020). Genetics and Resistance Mechanism of the Cucumber (Cucumis sativus L.) Against Powdery Mildew. *Journal of Plant Growth Regulation*, *40*(1), 147–153.

Chervin, C., Lavigne, D., & Westercamp, P. (2009). Reduction of gray mold development in table grapes by preharvest sprays with ethanol and calcium chloride. Postharvest Biol. Technol. 54:115–117.

Chittem, K., & Khan, M. F. R. (2016). Efficacy of precipitated calcium carbonate in managing *Fusarium* root rot of field pea. Phytoparasitica 295–303.

Chopade, V. V, Tankar, A. N., Pande, V. V, Tekade, A. R., Gowekar, N. M., Bhandari, S. R., & Khandake, S. N. (2008). Pongamia pinnata: Phytochemical constituents, Traditional uses and Pharmacological properties: A review. International Journal of Green Pharmacy , 2 (1), 72–75.

Chopra, R.N., Nayar, S.L., Chopra, I. . (1956). Glossary of Indian Medicinal Plants. CSIR,(New Delhi, India), 186–187.

Ciccarese, A., Stellacci, A. M., Gentilesco, G., & Rubino, P. (2013). Effectiveness of pre- and post-veraison calcium applications to control decay and maintain table grape fruit quality during storage. Postharvest Biol. Technol. 75:135–141.

Classen, M. E., and Wilcox, G. E. (1974). Comparative reduction of calcium and magnesium composition of corn tissue by NH_4-N and K fertilization. Agron. J. 66:521–522.

Colhoun, J. (1958). Club root disease of crucifers caused by *Plasmodiophora brassicae* Woron. Phytopathol. Pap. 3. Commonwealth Mycological Institute, Kew, England.

Conway, W. S., and Sams, C. E. (1983). Calcium infiltration of Golden Delicious apples and its effect on decay. Phytopathology 73:1068–1071.

Conway, W. S., and Sams, C. E. (1984). Possible mechanisms by which postharvest calcium treatment reduces decay in apples. Phytopathology 74:208–210.

Conway, W. S., Greene, G. M., II, and Hickey, K. D. (1987). Effects of preharvest and postharvest calcium treatments of peaches on decay caused by *Monilinia fructicola.* Plant Dis. 71:1084–1086.

Conway, W. S., Sams, C. E., Abbott, J. A., and Bruton, B. D. (1991). Postharvest calcium treatment of apple fruit to provide broad-spectrum protection against postharvest pathogens. Plant Dis. 75:620–622.

Corden, M. E. (1965). Influence of calcium nutrition on Fusarium wilt of tomato and polygalacturonase activity. Phytopathology 55:222–224.

Cormier, M. J., Jarrett, H. W., and Charbonneau, H. C. (1982). Role of Ca^{2+}-calmodulin in the metabolic regulation in plants. Pages 125–139 in: Calmodulin and Intracellular Ca^{++} Receptors. S. Kakiuchi, H. Hidaka, and A. R. Means, eds. Plenum Press, New York.

Datnoff, L, E., Nemec, S., and K, Pernezny. (1995) Biological control of Fusarium crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biol Cont 5, 427–431.

Datnoff, L. E, Elmber, W. H., & Huber, D. M. (2007) Mineral nutrition and plant disease. American Phytopathological Society (APS Press)

David, D. R., Yermiyahu, U., Fogel, M., Faingold, I., & Elad, Y. (2019). Plant nutrition for management of white mold in sweet basil. Phytoparasitica 47:99–115.

De Curtis, F., De Cicco, V., & Lima, G. (2012). Efficacy of biocontrol yeasts combined with calcium silicate or sulphur for controlling durum wheat powdery mildew and increasing grain yield components. Field Crops Res. 134: 36–46.

Dmitriev, A., Djatsok, J., and Grodzinsky, D. 1996. The role of Ca^{2+} in elicitation of phytoalexin synthesis in cell culture of onion (*Allium cepa* L.). Plant Cell Rep. 15:945–948.

Droby, S., Wisniewski, M. E., Cohen, L., Weiss, B., Touitou, D., Eilam, Y., and Chalutz, E. 1997. Influence of $CaCl_2$ on *Penicillium digitatum,* grapefruit peel tissue, and biocontrol activity of *Pichia guilliermondii.* Phytopathology 87:310–315.

Duffy, B. K., and G, Défago. (1999). Macro-and microelement fertilizers influence the severity of Fusarium crown and root rot of tomato in a soilless production system. *HortScience*, *34*(2), 287-291.

Dwivedi, D., Dwivedi, M., Malviya, S., & Singh, V. (2017). Evaluation of wound healing, anti-microbial and antioxidant potential of Pongamia pinnata in wistar rats. Journal of Traditional and Complementary Medicine, 7(1), 79–85.

Edgington, L. V., and Walker, J. C. (1958). Influence of calcium and boron nutrition on development of Fusarium wilt of tomato. Phytopathology 48:324–326.

Ehret, D. L., Menzies, J. G., Bogdanoff, C., Utkhede, R. S., and Frey, B. (2002). Foliar applications of fertilizer salts inhibit powdery mildew on tomato. Can. J. Plant Pathol. 24:437–444.

El-Mohamedy, R, S, R., Abdel-Kareem, F., Jabnoun-Khiareddine, H., and M, Daami-Remadi (2014) Chitosan and *Trichoderma harzianum* as fungicide alternatives for controlling Fusarium crown and root rot of tomato. Tunis. J. Plant Prot., 9, 31-43.

El-Tarabily, K. A., Hardy, G. E. St. J., McKay, A. G., and Sivasithamparam, K. (1997). Amendment of soil with lime or gypsum and its effect on cavity spot disease of carrots (*Daucus carota* L.) caused by *Pythium coloratum.* Aust. J. Exp. Agric. 37:265–270.

Elad, Y., Yunis, H., and Volpin, H. (1993). Effect of nutrition on susceptibility of cucumber, eggplant, and pepper crops to *Botrytis cinerea.* Can. J. Bot. 71:602–608.

Elad, Barnea, D., Rav-David, D., & Yermiyahu, U. (2021). Nutrient Status of Cucumber Plants Affects Powdery Mildew (Podosphaera xanthii). *Plants (Basel)*, *10*(10), 2216.

Elmer, P. A. G., Spiers, T. M., & Wood, P. N. (2007). Effects of pre-harvest foliar calcium sprays on fruit calcium levels and brown rot of peaches. Crop Prot. 26:11–18.

Elmhirst, J, F. (1997) A new strain of fusarium crown and root rot on greenhouse tomatoes. Canadian Journal of Plant Pathology 19, 231–2.

Epstein, E. (1973). Flow in the phloem and the immobility of calcium and boron: A new hypothesis in support of an old one. Experientia 29:133–136.

Fang, X., You, M. P., & Barbetti, M. J. (2012). Reduced severity and impact of *Fusarium* wilt on strawberry by manipulation of soil pH, soil organic amendments and crop rotation. Eur. J. Plant Pathol. 134:619–629.

Faust, M. (1974). The role of calcium in the respiratory mechanism and senescence of apples. Pages 87–92 in: Facteurs et Regulation de Maturation des Fruits. Int. Colloq. CNRS 238.

Fletcher, J. T., Hims, M. J., Archer, F. C., and Brown, A. (1982). Effects of adding calcium and sodium salts to field soils on the incidence of clubroot. Ann. Appl. Biol. 100:245–251.

Gadoury, D. M., Pearson, R. C., Riegel, D. G., Seem, R. C., Becker, C. M., and Pscheidt, J. W. (1994). Reduction of powdery mildew and other diseases by over-the trellis applications of lime sulfur to dormant grapevines. Plant Dis. 78:83–87.

Geetha, H. M., and Shetty, H. S. (2002). Induction of resistance in pearl millet against downy mildew disease caused by *Sclerospora graminicola* using benzothiadiazole, calcium chloride and hydrogen peroxide - A comparative evaluation. Crop Prot. 21:601–610.

Gelli, A., Higgins, V. J., and Blumwald, E. (1997). Activation of plant plasma membrane Ca^{2+}-permeable channels by race-specific fungal elicitors. Plant Physiol. 113:269–279.

Gessler, Aver'yanov, A. A., & Belozerskaya, T. A. (2007). Reactive oxygen species in regulation of fungal development. *Biochemistry (Moscow)*, *72*(10), 1091–1109.

Gillespie, D, R., and J, G, Menzies. (1993) Fungus gnats vector *Fusarium oxysporum f. sp. radicis-lycopersici.* Ann Appl Biol 123, 539–544.

Glawe, D. A (2008). The Powdery Mildews : A Review of the World's Most Familiar (Yet Poorly Known) Plant Pathogens. *Annual Review of Phytopathology*, *46*(1), 27–51.

Goncalez, E., Aparecido, C, C., and J, D, Felicio. Epidemiology, environmental sources and prevention. In Rios, T. F., & Ortega, E. R. (Eds.). (2012). *Fusarium:* Epidemiology, environmental sources and prevention.

Gordon, T, R., and R, D, Martyn. (1997). The evolutionary biology of *Fusarium oxysporum.* Annu. Rev. Phytopathol. 35, 111–28.

Gordon, T, R. (2017) Fusarium oxysporum and the Fusarium Wilt Syndrome. Annu. Rev. Phytopathol. 55, 23-39.

Gore, V. K., & Satyamoorthy, P. (2000). Determination of pongamol and karanjin in karanja oil by reverse phase HPLC. Analytical Letters, 33(2), 337–346.

Government of Canada (2011) Organic Production Systems Permitted Substances Lists. ICS 67.040. National Standard of Canada, Canadian General Standards Board.

Grant, G. T., Morris, E. R., Rees, D. A., Smith, P. J. C., and Thom, D. (1973). Biological interactions between polysaccharides and divalent cations: The egg-box model. FEBS Lett. 32:195–198.

Greenberger, A., Yogev, A., and J, Katan. (1987). Induced suppressiveness in solarized soils. Phytopathology 77, 1663–67.

Grindle, M. (1979). Phenotypic difference between natural and induced variants of *Botrytis cinerea.* J. Gen. Microbiol. 111:109–120.

Hage-Ahmed, K., Krammer, J., and S, Steinkellner (2013) The intercropping partner affects arbuscular mycorrhizal fungi and *Fusarium oxysporum f. sp. lycopersici* interactions in tomato Mycorrhiza, 23, 543-550.

Hallock, D. L., and Garren, K. H. (1968). Pod breakdown, yield, and grade of Virginia type peanuts as affected by Ca, Mg, and K sulfates. Agron. J. 60:253–257.

Hancock, J. G., and Stanghellini, M. E. (1968.) Calcium localization in *Hypomyces*-infected squash hypocotyls and effect of Ca on pectate lyase activity and tissue maceration. Can. J. Bot. 46:405–409.

Hartman, J, R., and J, T, Fletcher. (1991) Fusarium crown and root rot of tomatoes in the UK. Plant Pathology 40, 85–92.

He, K., Yang, S. Y., Li, H., Wang, H., & Li, Z. L. (2014.) Effects of calcium carbonate on the survival of *Ralstonia solanacearum* in soil and control of tobacco bacterial wilt. Eur. J. Plant Pathol. 140:665–675.

Hegde, K. T., Veeraghanti, K. S., & Kavita Hegde, C. T. (2017). Efficacy of bio-agents, botanicals and fungicides against Fusarium oxysporum f. sp. dianthi causing wilt of carnation. International Journal of Chemical Studies IJCS, 5(56), 139–142.

Hewavitharana, S, S., and Mazzola, M. (2016). Carbon source–dependent effects of anaerobic soil disinfestation on soil microbiome and suppression of *Rhizoctonia solani* AG-5 and *Pratylenchus penetrans*. Phytopathology 106, 1015–28.

Hernández-Muñoz, P., Almenar, E., Ocio, M. J., & Gavara, R. (2006) Effect of calcium dips and chitosan coatings on postharvest life of strawberries (*Fragaria x ananassa*). Postharvest Biol. Technol. 39:247–253.

Heyman, F., Lindahl, B., Persson, L., Wikström, M., & Stenlid, J. (2007). Calcium concentrations of soil affect suppressiveness against *Aphanomyces* root rot of pea. Soil Biol. Biochem. 39:2222–2229.

Hibar, K., Daami-Remadi, M., Jabnoun-Khiareddine, H., and M, El Mahjoub (2006) Temperature effect on mycelial growth and on disease incidence of *Fusarium oxysporum f. sp. radices-lycopersici*. Plant Pathology Journal 5(2), 233-238.

Hibar K, Daami-Remadi M, Ayed F, and M, El-Mahjoub (2007) Fusarium crown and root rot of tomato and its chemical control. Int J Agric Res 2, 687–695.

Hickman, M.V. (2002). Long-term tillage and crop rotation effects on soil chemical and mineral properties. J. Plant Nutrit. 25:1457-1470.

Holb, I. J., Balla, B., Vámos, A., & Gáll, J. M. (2012). Influence of preharvest calcium applications, fruit injury, and storage atmospheres on postharvest brown rot of apple. Postharvest Biol. Technol. 67:29–36.

Holzmeller, E. J., Jose, S., & Jenkins, M. A. (2007). Influence of calcium, potassium, and magnesium on *Cornus florida* L. density and resistance to dogwood anthracnose. Plant Soil. 290:189-199.

Horinouchi, H., Katsuyama, N., Taguchi, Y., and M, Hyakumachi. (2008) Control of Fusarium crown and root rot of tomato in a soil system by combination of a plant growth-promoting fungus, *Fusarium equiseti,* and biodegradable pots. Crop Protect 27, 859–864.

Horst. (2013). *Westcott's Plant Disease Handbook by R. Kenneth Horst.* (8th ed. 2013.). Springer Netherlands : Imprint: Springer.

Huang, C., Roberts, P., and L, Datnoff. (2011). Silicon Suppresses Fusarium Crown and Root Rot of Tomato: Silicon Suppresses Fusarium Crown and Root Rot of Tomato. Journal of Phytopathology, 159(7-8), 546-554.

Hummond, R. E., and Lewis, B. G. (1986). Ultrastructural studies of the limitation of lesions caused by *Leptosphaeria maculans* in stems of *Brassica napus* var. *oleifera.* Physiol. Mol. Plant Pathol. 28:251–265.

Ippolito, A., Schena, L., Pentimone, I., & Nigro, F. (2005). Control of postharvest rots of sweet cherries by pre- and postharvest applications of *Aureobasidium pullulans* in combination with calcium chloride or sodium bicarbonate. Postharvest Biol. Technol. 36:245–252.

Ishihara, A., Miyagawa, H., Kuwahara, Y., Ueno, T., and Mayama, S. (1996). Involvement of Ca^{2+} ion in phytoalexin induction in oats. Plant Sci. 115:9–16.

Jahan, Mahmud, M. H., Khan, Z., Alam, A., Khalil, A. A., Rauf, A., Tareq, A. M., Nainu, F., Tareq, S. M., Emran, T. B., Khan, M., Khan, I. N., Wilairatana, P., & Mubarak, M. S. (2021). Health promoting benefits of pongamol: An overview. *Biomedicine & Pharmacotherapy*, *142*, 112109–112109.

Jarvis, W.R., Thorpe, H.J. and B, H, MacNeill. (1975) A foot and root rot disease of tomato caused by *Fusarium oxysporum*. Can Plant Dis Surv 55, 25–26.

Jarvis W.R., and R, A, Shoemaker. (1988) Taxonomic status of Fusarium oxysporum causing foot and root rot of tomato. Phytopathology 68, 1679–1680.

Jarvis, W.R. (1998) Fusarium crown and root rot of tomatoes. Phytoprotection 69, 49-64.

Jiang, J. F., Li, J. G., & Dong, Y. H. (2013). Effect of calcium nutrition on resistance of tomato against bacterial wilt induced by *Ralstonia solanacearum.* Eur. J. Plant Pathol. 136: 547–555.

Johnny's Seed Catalogue (2020), johnnyyseeds.com, Accessed April 2020.

Jones, J. P., and Woltz, S. S. (1967). Fusarium wilt (race 2) of tomato: Effect of lime and micronutrient soil amendments on disease development. Plant Dis. Rep. 51:645–648.

Jones, R. G. W., and Lunt, O. R. (1967). The function of calcium in plants. Bot. Rev. 33:407–426.

Júnior, T, J, P., F. Vieira, R., Teixeira, H., & Eustáquio S. Carneiro, J. (2010). Foliar application of calcium chloride and calcium silicate decreases white mold intensity on dry beans. Tropical Plant Pathol. 34:171–174.

Kalbende, S. P., & Dalal, L. P. (2016). Effect of plant extracts on book deteriorated fungal species. Natural Product Research, 1–9.

Kao, C. W., and Ko, W. H. (1986). The role of calcium and microorganisms in suppression of cucumber damping-off caused by *Pythium splendens* in a Hawaiian soil. Phytopathology 76:221–225.

Keinath, Wintermantel, Zitter, Keinath, Anthony Perry, Wintermantel, William M., & Zitter, T. A. (2017). *Compendium of cucurbit diseases and pests / edited by Anthony P. Keinath, William M. Wintermantel, Thomas A. Zitter.* (Second edition.). APS Press.

Kesari, V., Das, A., & Rangan, L. (2010). Physico-chemical characterization and antimicrobial activity from seed oil of Pongamia pinnata, a potential biofuel crop. Biomass and Bioenergy , 34 (1), 108–115.

Keukens, E, A, J., Devrije, T., Fabrie, C, H, J, P., Demel, R, A., Jongen, W, M, F., and B, Dekruijff. (1992) Dual specificity of sterol-mediated glycoalkaloid induced membrane disruption. *Biochim. Biophys. Acta*, 1110, 127-136.

King, S, R., Davis, A, R., Liu, W., and A, Levi. (2008) Grafting for disease resistance HortScience, 43(6), 855-862.

Kim, Y.C., Jung, H. and K, Y, Kim. (2008) An effective biocontrol bioformulation against phytophthora blight of pepper using growth mixtures of combined chitinolytic bacteria under different field conditions. Eur J Plant Pathol 120, 373–382.

Kloepper, J. W., Ryu, C. M., and S, Zhang. (2004) Induced Systemic Resistance and Promotion of Plant Growth by Bacillus sp. Phytopathol., 94, 1259-66.

Kratzke, M. G. (1988). Study of mechanism of calcium uptake by potato tubers and cellular properties affecting soft rot. Ph.D. Thesis. University of Wisconsin, Madison.

Kumar, A., Kumar, S., Kumar, R., Chand, G. and Kolte, S.J. (2015). In vitro and in vivo evaluation of eco-friendly chemicals on alternaria blight disease (*Alternaria brassicae*) management and yield attributes in Indian mustard (*Brassica juncea*). J. Appl. Nat. Sci. 7:43-51.

Lairini, K., Perez, Espinosa, A., Pineda, M. and M, RuizRubio. (1996) Purification and characterization of tomatinase from *Fusarium oxysporum* f. sp. *lycopersici*. *Appl. Environ. Microbiol*. 62, 1604-1609.

Lakshmesha, K. K., Lakshmidevi, N., & Mallikarjuna Aradhya, S. (2005). Inhibition of cell wall degrading cellulase enzyme by the incitant of anthracnose disease caused by *Colletotrichum capsici* on capsicum fruit. Arch. Phytopathol. Plant Protect. 38:295–302.

Larkin, R. P., and D, R, Fravel. (1998) Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. Plant Dis., 82, 1022-1028.

Lazaroff, N., and Pitman, M. G. (1966). Calcium and magnesium uptake by barley seedlings. Aust. J. Biol. Sci. 19:991–1005.

Legge, R. L., Thompson, J. E., Baker, J. E., and Lieberman, M. (1982). The effect of calcium on the fluidity of phase properties of microsomal membranes isolated from postclimacteric Golden Delicious apples. Plant Cell Physiol. 23:161–169.

Leggett, J. E., and Gilbert, W. A. (1969). Magnesium uptake by soybeans. Plant Physiol. 44:1182–1186.

Lemanceau, P., Bakker, P.A.H.M., De Kogel, W.J., Alabouvette, C. and B, Schippers. (1993) Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogen *Fusarium oxysporum f. sp. dianthi*. Appl. Environ. Microbiol. 59, 74-82.

Li, C., Tao, J., Zhao, D., You, C., & Ge, J. (2012). Effect of calcium sprays on mechanical strength and cell wall fractions of herbaceous peony (*Paeonia lactiflora* pall.) inflorescence stems. Int. J. Mol. Sci. 13:4704–4713.

Lima, G. S. de A., Assunção, I. P., and Oliveira, S. M. A. de. (1998). Effect of treatment of melon fruits (*Cucumis melo* L.) with different calcium sources on rot caused by *Myrothecium roridum.* Summa Phytopathol. 24:276–279.

Lima, G., Spina, A. M., Castoria, R., de Curtis, F., & de Cicco, V. (2005). Integration of biocontrol agents and food-grade additives for enhancing protection of stored apples from *Penicillium expansum.* J. Food Prot. 68:2100–2106.

Lockwood, J, L. (1977) Fungistasis in soils. Biol. Rev. 52, 1-43.

Loganathan, M., Garg, R., Venkataravanappa, V., Saha, S., and A, B, Rai (2014). Plant growth promoting rhizobacteria (PGPR) induces resistance against Fusarium wilt and improves lycopene content and texture in tomato. Afr. J. Microbiol. Res., 8(11), 1105-1111.

Loneragan, J. F., Snowball, K., and Simmons, W. J. (1968). Response of plants to calcium concentration in solution culture. Aust. J. Agric. Res. 19:845–857.

Lund, J. F. (1970). The effect of calcium and its relation to several cations in soybean root growth. Soil Sci. Soc. Am. Proc. 34:456–459.

Maas, E. V. (1969). Calcium uptake by excised maize roots and interactions with alkali cations. Plant Physiol. 44:985–989.

Madani, B., Muda Mohamed, M. T., Biggs, A. R., Kadir, J., Awang, Y., Tayebimeigooni, A., & Shojaei, T. R. (2013). Effect of pre-harvest calcium chloride applications on fruit calcium level and post-harvest anthracnose disease of papaya. Crop Prot. 55:55–60.

Madrid, R., Valverde, M., Alcolea, V., and Romojaro, F. (2004). Influence of calcium nutrition on water soaking disorder during ripening of cantaloupe melon. Sci. Hortic. 101:69–79.

Mahady, G. B., and Beecher, C. W. W. (1994). Elicitor-stimulated benzophenanthridine alkaloid biosynthesis in bloodroot suspension cultures is mediated by calcium. Phytochemistry 37:415–419.

Matsuda, Y., Nonomura, T., and H, Toyoda. Involvement of the Shore Fly *Scatella stagnalis* (Diptera: Ephydridae) in Expansion of Fusarium Crown and Root Rot Disease from Diseased to Healthy Tomato Seedlings in Greenhouse Hydroponics, 42, 237-242.

McDonnell, G., and A, D, Russell. (1999) Antiseptics and disinfectants: activity, action and resistance Clin. Microbiol. Rev., 12(1), 147-179.

McDonnell, Yoo, J., Patel, K., Rios, L., Hollingsworth, R., Millar, J. & Paine, T. (2015). Can essential oils be used as novel drench treatments for the eggs and juveniles of the pest snail Cornu aspersum in potted plants? Journal of Pest Science, 89(2), 549-555.

McGovern, R, J., and L, E, Datnoff (1992) Fusarium crown and root rot of tomato: reevaluation of management strategies C.S. Vavrina (Ed.), Fla. Tom. Instit. Proc., Vegetable Crops Special Series, SS HOS 1, University of Florida-IFAS 75-82.

McGovern, R, J., Vavrina, C, S., & L, A, MacKay. (1993). The effect of transplant tray type and tomato cultivar on the incidence of Fusarium crown and root rot in tomato transplants. Proc. Fla. State Hortic. Soc., 106, 173-175.

McGrath (2001). Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. *Plant Disease*, *85*(3), 236–245.

McGuire, R. G., and Kelman, A. (1984). Reduced severity of Erwinia soft rot in potato tubers with increased calcium content. Phytopathology 74:1250–1256.

McGuire, R. G., and Kelman, A. (1986). Calcium in potato tuber cell walls in relation to tissue maceration by *Erwinia carotovora* pv. *atroseptica.* Phytopathology 76:401–406.

McLean, E. O. (1975). Calcium levels and availabilities in soils. Commun. Soil Sci. Plant Anal. 6:219–232.

Messenger, B. J., Menge, J. A., and Pond, E. (2000a). Effects of gypsum soil amendments on avocado growth, soil drainage, and resistance to *Phytophthora cinnamomi.* Plant Dis. 84:612–616.

Messenger, B. J., Menge, J. A., and Pond, E. (2000b). Effects of gypsum on zoospores and sporangia of *Phytophthora cinnamomi* in field soil. Plant Dis. 84:617–621.

Messiaen, J., and Van Cutsem, P. (1994). Pectic signal transduction in carrot cells: Membrane, cytosolic and nuclear responses induced by oligogalacturonides. Plant Cell Physiol. 35:677–689.

Menzies, J, G., and W, R, Jarvis. (1994) The infestation of tomato seed by *Fusarium oxysporum f. sp. radicis-lycopersici.* Plant Pathol 43, 378-386.

Meyer, Lakshman, D.K., Zasada, I. A., Vinyard, B. T., & Chitwood, D. J. (2008). Phytotoxicity of clove oil to vegetable crop seedlings and nematotoxicity to root-knot nematodes. *HortTechology,* 18(4), 631-638.

Millar, C. E. (1963). Calcium and magnesium. Pages 194–208 in: Soil Fertility. John Wiley and Sons, New York.

Mihuta-Grimm, I., Erb, W, A., and R, C, Rowe. (1990). Fusarium crown rot of tomato in rock wool systems: sources of inoculum and disease management with benomyl Plant Dis., 74, 996-1002.

Ministry of Agriculture, Food and Rural Affairs (2020). Crop Protection Guide for Greenhouse Vegetable 2020–2021 Ontario.

Ministry of Agriculture, Food and Rural Affairs (2022). *Cucurbits*. Province of British Columbia. Retrieved July 12, 2022, from https://www2.gov.bc.ca/gov/content/industry/agriservice-bc/production-guides/vegetables/cucurbits.

Momma, N., Momma, M., and Y, Kobara. (2010). Biological soil disinfestation using ethanol: Effect on Fusarium oxysporum f. sp. lycopersici and soil microorganisms. Journal of General Plant Pathology. 76, 336-344.

Mondal, S. N., and Timmer, L. W. (2003). Effect of urea, $CaCO_3$, and dolomite on pseudothecial development and ascospore production of *Mycosphaerella citri.* Plant Dis. 87:478–483.

Mondal, Mondal, K., & Islam, N. (2014). Antifungal Potentials of Derris indica (LAM.) Bennet Extractives. *Journal of Life and Earth Science*, *7*, 61–65.

Mondal, Mondal, C. K., & Mondal, P. (2020). Diseases of Cucurbits and Their Management. In *Stresses of Cucurbits: Current Status and Management* (pp. 115–222). Springer Singapore.

More, Y. D., Gade, R. M., & Shitole, A. V. (2017). Evaluation of antifungal activities of extracts of aegle marmelos, syzygium cumini and pongamia pinnata against pythium debaryanum. Indian Journal of Pharmaceutical Sciences, 79(3), 377–384.

Mostafa, Hashem, M., Alshehri, A. M., Alamri, S., Eid, E. M., Ziedan, E.-S. H. E., & Alrumman, S. A. (2021). Effective Management of Cucumber Powdery Mildew with Essential Oils. *Agriculture (Basel)*, *11*(11), 1177.

Muchovej, R. M., and Muchovej, J. J. (1982). Calcium suppression of *Sclerotium* induced twin stem abnormality of soybean. Soil Sci. 134:181–185.

Murray, T. D., Walter, C. C., and Anderegg, J. C. (1992). Control of Cephalosporium stripe of winter wheat by liming. Plant Dis. 76:282–286.

Narashimhan, V., Ramadoss, N., Sridhar, V., and Kareem, A. A. (1994). Using gypsum to manage sheath rot in rice. Int. Rice Res. Notes 19:27–28.

Nefzi, A., Aydi Ben Abdallah, R., Jabnoun-Khiareddine, H., Ammar, N., Medimagh-Saïdana, S., Haouala, R., & M, Daami-Remadi. (2018). Management of Fusarium Crown and Root Rot of tomato by *Solanum linnaeanum* L. extracts. Scientia Horticulturae, 238, 204-214.

Nelson, P, E. (1981) Life cycle and epidemiology of *Fusarium oxysporum*. In Fungal Wilt Diseases of Plants. ed. ME Mace, AA Bell, CH Beckman, pp. 51–80. New York: Academic. 640.

Ng, L, C., Hafiz, E, S., Sariam, O., and Razi, R, I, M. (2019). The effect of calcium silicate as foliar application on aerobic rice blast disease development. Eur J Plant Pathol. 153:533-543.

Nigro, F., Schena, L., Ligorio, A., Pentimone, I., Ippolito, A., & Salerno, M. G. (2006). Control of table grape storage rots by pre-harvest applications of salts. Postharvest Biol. Technol. 42:142–149.

Noor, A.A.M., Othman, A.N.N., Lum, P.T., Mani, S., Shaikh, M. F., & Sekar, M.(2020). Molecules of Interest – Karanjin – A Review. Pharmacogn K. 12(4): 938-945.

Pareja,Jaime, Y., Roncero, M, I, G., and M, C, Ruiz-Roldan. (2008) Tomatinas from *Fusarium oxysporum* f. sp. *lycopersici* is required for full virulence on tomato plants. *Mol. Plant–Microbe Interact*. 21, 728-736.

Pavithra, H. R., Gowda, B., Kumar, K. R., Prasanna, K. T., & Shivanna, M. B. (2012). Oil, fatty acid profile and karanjin content in developing pongamia pinnata (L.) pierre seeds. JAOCS, Journal of the American Oil Chemists' Society, 89(12), 2237–2244.

Pest Management Program, Agriculture and Agri-Food Canada (2021). Crop Profile for Greenhouse Cucumber in Canada, 2020.

Pooviah, B. W., and Reddy, A. S. N. (1987). Calcium messenger system in plants. CRC Crit. Rev. Plant Sci. 6:47–103.

Porth, G., Mangan, F., Wick, R., and Autio, W. (2003). Evaluation of management strategies for clubroot disease of *Brassica* crops. New England Vegetable and Fuit Conf. http://www.nevbc.org/sessions_03/leafy_greens_herbs/evaluation_management_strategies_clubroot_disease_brassica.pdf

Preston, R. D. (1979). Polysaccharide conformation and cell wall function. Annu. Rev. Plant Physiol. 30:55–78.

Prihatna, C., Barbetti, M, J., and S, J, Barker (2018) A novel tomato fusarium wilt tolerance gene. Front. Microbiol. Plant Microbe Interactions, Open Access.

Pscheidt, J.W., and Ocamb, C.M. (2022) 2022 Pacific Northwest Plant Disease Management Handbook. Oregon State University

Punja, Z. K., and Gaye, M.-M. (1993). Influence of postharvest handling practices and dip treatments on development of black root rot on fresh market carrots. Plant Dis. 77:989–995.

Punja, Z. K., and Grogan, R. G. 1982. Effects of inorganic salts, carbonate-bicarbonate anions, ammonia, and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii.* Phytopathology 72:635–639.

Punja, Z. K., and Jenkins, S. F. 1984a. Influence of medium composition on mycelial growth and oxalic acid production in *Sclerotium rolfsii.* Mycologia 76:947–950.

Punja, Z. K., and Jenkins, S. F. 1984b. Light and scanning electron microscopic observations of calcium oxalate crystals produced during growth of *Sclerotium rolfsii* in culture and infected tissue. Can. J. Bot. 62:2028–2032.

Punja, Z. K., Huang, J.-S., and Jenkins, S. F. 1985. Relationship of mycelial growth and production of oxalic acid and cell wall degrading enzymes to virulence in *Sclerotium rolfsii.* Can. J. Plant Pathol. 7:109–117.

Punja, Z. K., Carter, J. D., Campbell, G. M., and Rossell, E. L. 1986. Effects of calcium and nitrogen fertilizers, fungicides, and tillage practices on incidence of *Sclerotium rolfsii* on processing carrots. Plant Dis. 70:819–824.

Qing, F., and Shiping, T. 2000. Postharvest biological control of Rhizopus rot of nectarine fruits by *Pichia membranefaciens.* Plant Dis. 84:1212–1216.

Rahman, M. A., T. M. M Mahmud, J. Kadir, R. A. R. and M. M. B. 2009. Enhancing the Efficacy of *Burkholderia cepacia* B23 with calcium chloride and chitosan to control anthracnose of papaya during storage. Plant Pathol. 25:361–368.

Rajput, M., Bithel, N., & Vijayakumar, S. (2021). Antimicrobial, antibiofilm, antioxidant, anticancer, and phytochemical composition of the seed extract of Pongamia pinnata. Archives of Microbiology, 1-20

Raju, K., & Naik, M. K. (2006). Effect of pre-harvest spray of fungicides and botanicals on storage diseases of onion. 59(2), 133–141.

Rao, D. V., and Tewari, J. P. 1988. Suppression of the symptoms of American leaf spot of coffee with calcium hydroxide. Plant Dis. 72:688–690.

Rease, J. T. 1996. Calcium nutrition affects cold hardiness, yield, and fruit disorders of apple and pear trees. J. Plant Nutr. 19:1131–1155.

Research Institute of Organic Agriculure (2019). The World of Organic Agriculure: Statistics and Emerging Trends 2019.

Rex, B., Prabhu, S., & J. S. Kumar (2019). Antifungal efficacies of plant extracts against *Alternaria solani* (Ellis and Martin) jones and grout under *in vitro* conditions. Annals of Phytomedicine. 8(1):1-5.

Rossignol, M., Lamant, D., Salsac, L., and Heller, R. 1977. Calcium fixation by the roots of calcicole and calcifuge plants: The importance of membrane systems and their lipid composition. Pages 483–490 in: Transmembrane Ionic Exchange in Plants. M. Thellier, A. Monnier, M. Demarty, and J. Dainty, eds. CNRS, Paris, and Editions Universitaires, Rouen, France.

Roux, S. J., and Slocum, R. C. 1982. Role of calcium in mediating cellular functions important for growth and development in higher plants. Pages 409–451 in: Calcium and Cell Function. W. Y. Cheung, ed. Academic Press, New York.

Rowe, R, C., Farley, J, D., and D, L, Coplin. (1977) Airborne spore dispersal and recolonization of steamed soil by *Fusarium oxysporum* in tomato greenhouses. Phytopathology 67, 1513-1517.

Sajid, Z. I., Anwar, F., Shabir, G., Rasul, G., Alkharfy, K. M., & Gilani, A. H. (2012). Antioxidant, antimicrobial properties and phenolics of different solvent extracts from bark, leaves and seeds of Pongamia pinnata (L.) pierre. Molecules, 17(4).

Saure, M. C. 1996. Reassessment of the role of calcium in development of bitter pit in apple. Aust. J. Plant Physiol. 23:237–243.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675.

Schroth, M, N., and D, C, Hildebrand. (1964) Influence of plant exudates on root infecting fungi. Annu. Rev. Phytopathol. 2, 101-32.

Schwacke, R., and Hager, A. 1992. Fungal elicitors induce a transient release of active oxygen species from cultured spruce cells that is dependent on Ca^{2+} and protein-kinase activity. Planta 187:136–141.

Scott, K. J., and Wills, R. B. H. 1979. Effects of vacuum and pressure infiltration of calcium chloride and storage temperature on the incidence of bitter pit and low temperature breakdown of apples. Aust. J. Agric. Res. 30:917–928.

Scott, J, W., and J, P, Jones. (2000) Tomato breeding lines resistant to fusarium crown and root rot. Hortscience 35, 1183-4.

Senevirathna, P., & Daundasekera, W. 2010. Effect of postharvest calcium chloride vacuum infiltration on the shelf life and quality of tomato (cv. 'Thilina'). Ceylon J. Sci., Biol. Sci. 39:35.

Serrano, M. S., de Vita, P., Fernández-Rebollo, P., & Sánchez Hernández, M. E. 2012. Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex.* Eur. J. Plant Pathol. 132:271–279.

Shafique, S., Akram, W., Anjum, T., Ahmad, A., & Shafique, S. (2014). Comparative studies on phytochemistry, antibacterial and antifungal properties of Alstonia scholaris and Millettia pinnata. Australasian Plant Disease Notes, 9(1).

Sharma, N., Acharya, S., Kumar, K., Singh, N., & Chaursia O, P. (2018). Hydroponics as an advanced technique for vegetable production: An overview. Journal of Soil and Water Conservation, 17(4): 364-371.

Sharma, A., Tyagi, S., Nag, R., Chatureverdi, A., & Nag, T. N. (2011). Antimicrobial activity and cellular toxicirt of flavonoid extracts from *Pongamia pinnata* and *Vitiex negundo.* Romanian Biotechnology Letters, 16(4): 9396-6400.

Sharma, R. R., Pal, R. K., Singh, D., Singh, J., Dhiman, M. R., Rana, M. R. 2012. Relationships between storage disorders and fruit calcium contents, lipoxygenase activity, and rates of ethylene evolution and respiration in 'Royal Delicious' apple. J. Hort. Sci. Biotechnol. 87:367-373

Shear, C. B. 1975. Calcium related disorders of fruits and vegetables. HortScience 10:361–365.

Sherf, A. F. 1976. Clubroot of cabbage, cauliflower, and broccoli. Cornell Univ. Coop. Ext. Leafl.

Shlevin, E., Saguy, L, S., Mahrer, Y., and J, Katan. Modeling the survival of two soilborne pathogens under dry structural solarization. Phytopathology, 93, 1247-1257.

Siebers, B., Gräf, P., and Weiler, E. W. 1990. Calcium fluxes across the plasma membrane of *Commelina communis* L. assayed in a cell-free system. Plant Physiol. 93:940–947.

Singh, Bhatt, G., Gujre, N., Mitra, S., Swaminathan, R., Limaye, A. ., & Rangan, L. (2021). Karanjin. *Phytochemistry (Oxford)*, *183*, 112641.

Silva Felix, C. L., Lima Ramos Mariano, R., Souza, E. B., Silva Felix, K. C., & Oliveira, W. J. 2016. Calcium-mediated reduction of soft rot disease in Chinese cabbage. Eur. J. Plant Pathol. 147:73–84.

Smith, S. (2007). An overview of ecological and habitat aspects in the genus Fusarium with special emphasis on the soil-borne pathogenic forms. Plant Pathol. Bull. 16, 97-120.

Sohail, M., Khalil, S. A., Afridi, S. R., Khan, R. U., Ahmad, T., & Ullah, F. 2013. Enhancing post harvest storage life of peach fruits using calcium chloride. J. Chemical Soc. Pak. 35:109–113.

Sofos, J. N., Pierson, M. D., Blocher, J. C., and Busta, F. F. 1986. Mode of action of sorbic acid on bacterial cells and spores. Int. J. Food Microbiol. 3:1–18.

Soliman, K, M., and R, I, Badeaa. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food and Chemical Toxicology, 40, 1669-1675.

Srivastava SL, Suman B (1986) Inhibitory effect of some phylloplane fungi on powdery mildew disease development. Indian Phytopathol 39:83–86.

Standaert, J. Y., Myttenaere, C., and Meyer, J. A. 1973. Influence of sodium/calcium ratios and ionic strength of the nutrient solution on Fusarium wilt of tomato. Plant Sci. Lett. 1:413–420.

Starkey, K. R., and Pedersen, A. R. 1997. Increased levels of calcium in the nutrient solution improves the postharvest life of potted roses. J. Am. Soc. Hortic. Sci. 122:863–868.

Stasikowski, P. M., Mccomb, J. A., Scott, P., & Paap, T. 2014. Calcium sulphate soil treatments augment the survival of phosphite-sprayed *Banksia leptophylla* infected with *Phytophthora cinnamomi.* Australasian Plant Pathol. 43:369–379.

Statistics Canada (2020) Area, production and farm gate value of marketed vegetables. Table: 32-10-0365-01. Ottawa. Accessed April 2020

Statistics Canada (2021) Certified organic fruit and vegetable production estimates 2020, The Daily, Wednesday, July 14

Steinkellner, S., Mammerler, R., and H, Vierheilig (2005) Microconidia germination of the tomato pathogen *Fusarium oxysporum* in the presence of root exudates. J. Plant Interact. 1, 23-30.

Sugar, D., Powers, K. A., and Hilton, R. J. 1991. Enhanced resistance to side rot in pears treated with calcium chloride during the growing season. Plant Dis. 75:212–214.

Sugawara, S., Inamoto, Y., and Ushijima, M. 1981. Resolution and some properties of acid phosphatase isozyme bound to the cell wall of potato tubers. Agric. Biol. Chem. 45:1763–1773.

Sugimoto, T., Aimo, M., Sugimoto, M., & Watanabe, K. 2005. Reduction of phytophthora stem rot disease on soybeans by the application of $CaCl_2$ and $Ca(NO_3)_2$. J. Phytopathol. 153:536-543.

Sugimoto, T., Watanabe, K., Yoshida, S., Aino, M., Irie, K., Matoh, T., and Biggs, A. R. 2008. Select calcium compounds reduce the severity of *Phytophthora* stem rot of soybean. Plant Dis. 92:1559-1565.

Sugimoto, T., Watanabe, K., Yoshida, S., Aino, M., Furiki, M., Shiono, M., Matoh, T., and Biggs, A. R. 2010. Field application of calcium to reduce *Phytophthora* stem rot of soybean, and calcium distribution in plants. Plant Dis. 94:812-819.

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.

Thomidis, T., Sotiropoulos, T., Karagiannidis, N., Tsipouridis, C., Papadakis, I., & Almaliotis, D. 2007. Efficacy of three calcium products for control of peach brown rot. HortTechnology 17:234-237

Tian, S., Wan, Y., Qin, G., & Xu, Y. 2006. Induction of defense responses against Alternaria rot by different elicitors in harvested pear fruit. Appl. Microbiol. Biotechnol. 70:729–734.

Toselli, M., Marangoni, B., Malaguti, D., Sorrenti, G., Bazzi, C., & Collina, M. 2004. Use of soil-applied calcium chloride to reduce fire blight and brown spot susceptibility of pear. Acta Horticult. 638:301–305.

Toro, E., Hochmuth, R, C., Belibasis, E., and C, L, Harmon. (2012). Reducing Fusarium spp. inoculum in irrigation systems: a sanitation case study in greenhouse-grown tomatoes Proc. Fla. Hortic. Soc., 125, 209-211.

Trecate, Sedláková, B., Mieslerová, B., Manstretta, V., Rossi, V., & Lebeda, A. (2019). Effect of temperature on infection and development of powdery mildew on cucumber. *Plant Pathology*, *68*(6), 1165–1178.

Treutter. (2005). Significance of Flavonoids in Plant Resistance and Enhancement of Their Biosynthesis. *Plant Biology (Stuttgart, Germany)*, *7*(6), 581–591.

Tu, J. C. 2012. Effects of soil compaction, temperature, and moisture on the development of the *Fusarium* root rot complex of pea in southwestern Ontario. Phytoprotection 75:125-131.

Tyagi, S., and R, Paudel (2014) Effect of different pH on the growth and sporulation of *Fusarium oxysporum:* The causal organism of wilt disease of Tomato. International Journal of Basic and Applied Biology. 2(1), 103-106.

Tzeng, K. C., Kelman, A., Simmon, K. E., and Kelling, K. A. 1986. Relationship of calcium nutrition to internal brown spot of potato tubers and sub-apical necrosis of sprouts. Am. Potato J. 63:87–97.

Vanterpool, T. C. 1940. Studies on browning root rot of cereals: VI. Further contributions on the effects of various soil amendments on the incidence of the disease in wheat. Can J. Res. Sect. C 18:240–257.

Yamamoto, I., Komada, H., Kuniyau, K., Saito, M., and A, Ezuka. (1974) A new race of *Fusarium oxysporum f. Sp. lycopersici* inducing root rot of tomato. Proc. Kansai Plant Prot. Soc. 16, 17-29.

Volpin, H., & Elad, Y. 1991. Influence of calcium nutrition on susceptibility of rose flowers to *Botrytis* blight. Phytopathology 81:1390-1394.

von Broembsen, S. L., and Deacon, J. W. 1997. Calcium interference with zoospore biology and infectivity of *Phytophthora parasitica* in nutrient irrigation solutions. Phytopathology 87:522–528.

Wagh, P., Rai, M., Deshmukh, S. K., & Durate, M. C. T. (2007). Bio-activity of oils of Trigonella foenum-graecum and Pongamia pinnata. African Journal of Biotechnology, 6(13), 1592–1596.

Walker, M. E., and Csinos, A. S. 1980. Effect of gypsum on yield, grade, and incidence of pod rot in five peanut cultivars. Peanut Sci. 7:109–113.

Wallace, A., Frolich, E., and Lunt, O. R. 1966. Calcium requirements of higher plants. Nature (London) 209:634.

Walters, D., Raynor, L., Mitchell, A., Walker, R., & Walker, K. (2004). Antifungal activities of four fatty acids against Plant Pathogenic fungi. Mycopathologia, 157(1), 87–90.

Washington, W. S., Villalta, O., and Appleby, M. 1998. Control of pear scab with hydrated lime alone or in schedules with other fungicide sprays. Crop Prot. 17:569–580.

Wasimfiroz, R.M., Hosagoudar, G.N., & Narayanaswamy, H. (2018). Evaluation of fungicides, bio-agents and plant extracts against Pyricularia oryzae . 6 (2), 1875–1879.

Wellman, F. L. 1930. Clubroot of crucifers. U.S. Dep. Agric. Tech. Bull. 181.

Wieneke, J. 1980. Autoradiographic localization of ^{45}Ca and ^{36}Cl after postharvest dipping of apple fruits. Pages 334–336 in: Mineral Nutrition of Fruit Trees. D. Atkinson, J. E. Jackson, R. O. Sharples, and W. M. Waller, eds. Butterworths, London.

Wilson, D. M., and Walker, M. E. 1981. Calcium potential aflatoxin foe. Southeast. Peanut Farmer 19:6B.

Wisniewski, M. E., Droby, S., Chalutz, E., and Elam, Y. 1995. Effects of Ca^{2+} and Mg^{2+} on *Botrytis cinerea* and *Penicillium expansum in vitro* and on the biocontrol activity of *Candida oleophila.* Plant Pathol. 44:1016–1024.

Wójcik, P., Gubbuk, H., Akgül, H., Gunes, E., Ucgun, K., Koçal, H., & Kucukyumuk, C. 2009. Effect of autumn calcium spray at a high rate on Granny Smith apple quality and storability. J. Plant Nutr. 33:46-56.

Woronin, M. S. 1934. *Plasmodiophora brassicae:* The cause of cabbage hernia. C. Chupp, transl. Phytopathol. Classics No. 4. American Phytopathological Society, St. Paul, Minn.

Yamamoto, I., Komada, H., Kuniyau, K., Saito, M., and A, Ezuka. (1974) A new race of *Fusarium oxysporum f. Sp. lycopersici* inducing root rot of tomato. Proc. Kansai Plant Prot. Soc. 16, 17-29.

Yamazaki, H., & Hoshina, T. 1995. Calcium nutrition affects resistance of tomato seedlings to bacterial wilt. HortScience. 30:91–93.

Yermiyahu, U., Shamai, I., Peleg, R., Dudai, N., & Shtienberg, D. 2006. Reduction of *Botrytis cinerea* sporulation in sweet basil by altering the concentrations of nitrogen and calcium in the irrigation solution. Plant Pathol. 55:544–552.

Yoon, C. S., Rog Yeoung, Y., & Sup Kim, B. 2010. The suppressive effects of calcium compounds against *Botrytis cinerea* in paprika. Kor. J. Hort. Sci. Technol. 28:1072–1077.

Yu, T., Yu, C., Lu, H., Zunun, M., Chen, F., Zhou, T., Sheng, K., & Zheng, X. 2012. Effect of *Cryptococcus laurentii* and calcium chloride on control of *Penicillium expansum* and *Botrytis cinerea* infections in pear fruit. Biol. Control. 61:169–175.

Zhang, H., Zheng, X., Fu, C., & Xi, Y. 2005. Postharvest biological control of gray mold rot of pear with *Cryptococcus laurentii.* Postharvest Biol. Technolo. 35:79–86.

Zielihska, M., & Michniewicz, M. 2001. The effect of calcium on the production of ethylene and abscisic acid by fungus *Fusarium culmorum* and by wheat seedlings infected with that pathogen. Acta Physiol. Plant. 23:79–85.

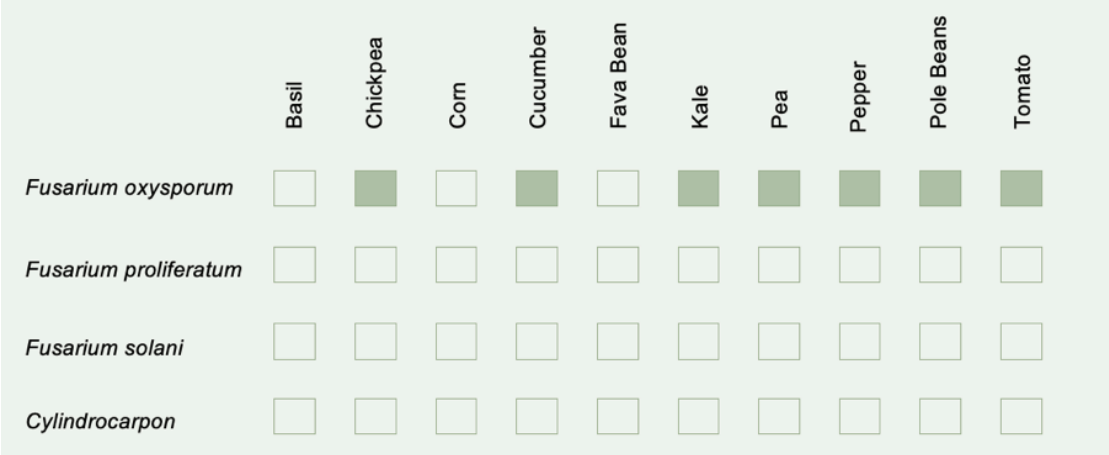
Zimmermann, S., Nürnberger, T., Frachisse, J.-M., Wirtz, W., Guern, J., Hedrich, R., and Scheel, D. 1997. Receptor-mediated activation of a plant Ca^{2+}-permeable ion channel involved in pathogen defense. Proc. Natl. Acad. Sci. USA 94:2751–2755.

Appendix. Host Range Study of *Fusarium*

Host Range Study of *Fusarium* Species Isolated from Cannabis

Four isolates of fungi - *Fusarium oxysporum, Fusarium proliferatum, Fusarium solani* and a C*ylindrocarpon sp.* from cannabis were tested for their ability to cause stunting on 10 common crops. The host range of these newly isolated pathogens was investigated to determine possible alternative hosts for the pathogen.

Inoculum was prepared in PDB and allowed to grow in solution on a shaker at room temperature at 150 rpm for one week. Meanwhile, plants were grown in germination mix soil in 72 well tray until they formed 2 true leaves, which took 2 to 4 weeks depending on the plant (seeds were purchased from West Coast Seeds, Delta BC). After this time, plants were uprooted, and their roots were reduced by 50% by cutting with scissors, and then emersed in the inoculum for 5 minutes. Control plants were dipped in pure sterile PDB, one had their roots cut along with the others, another did not. They were transplanted into 200 mL of propagation mix in 250 mL pots in the greenhouse and allowed to grow for 5 weeks. There were 5 replicates per treatment, and 2 to 3 trials of each were run. After this time the heights, dry and wet weights were measured. These were found to be similar, so only heights are reported below for each. Significant differences between means were calculated with ANOVA, followed by a Tukey HSD Post-Hoc test. No stunting was caused by *F. solani, Cylindrocarpon or F. proliferatum*. Those crops impacted in at least two trials by *F. oxysporum* include bean, chickpea, cucumber, kale, pea, pepper, and tomato. Fava bean, basil and corn were unaffected in all trials.



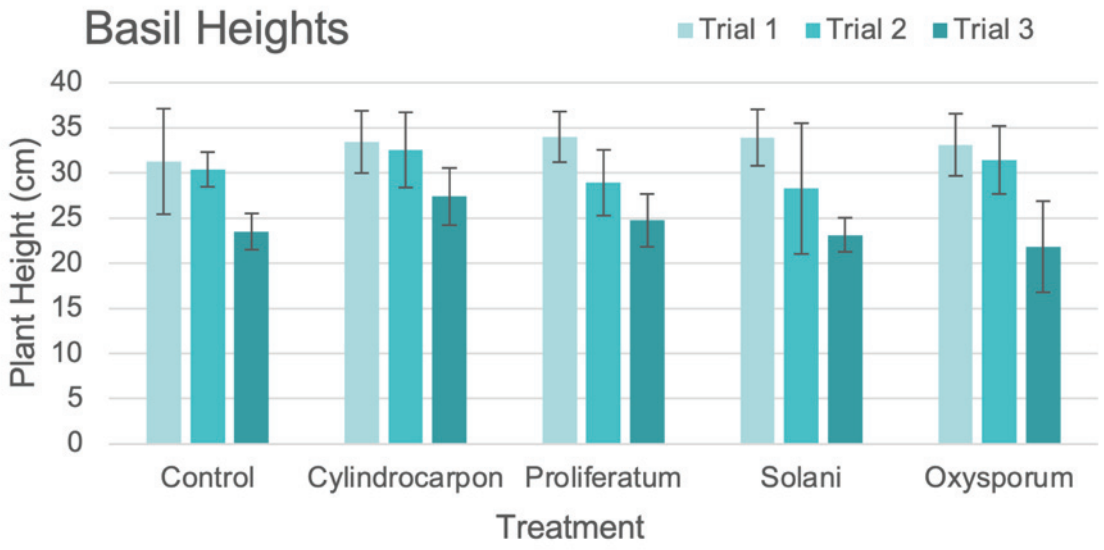


Figure A1: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

Figure A2: **Representative basil plants, from left to right, control, C*ylindrocarpon sp.*, *Fusarium proliferatum*, *Fusarium solani* and *Fusarium oxysporum* inoculated plants.**

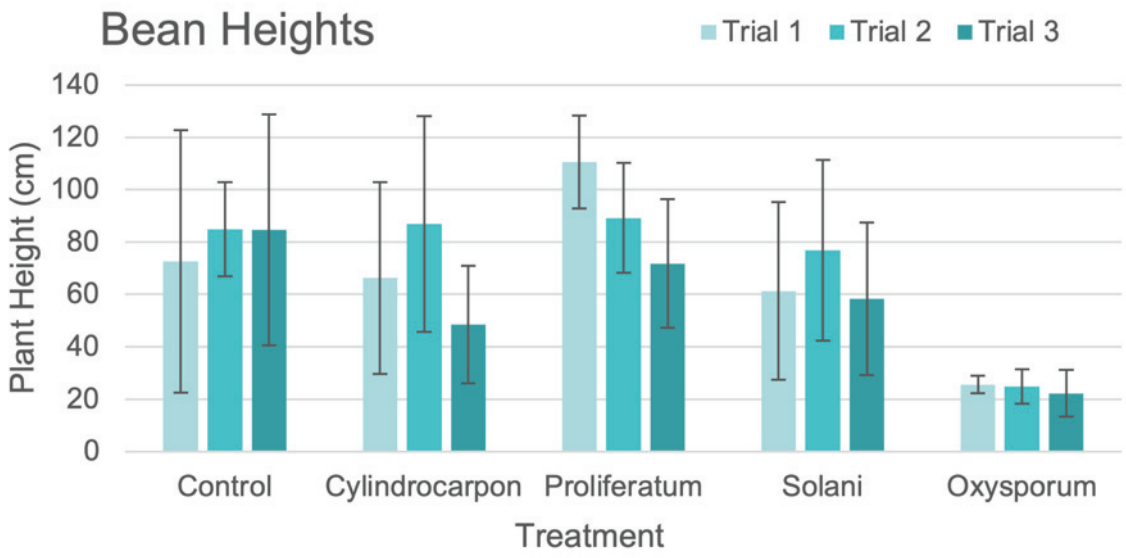


Figure A3: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

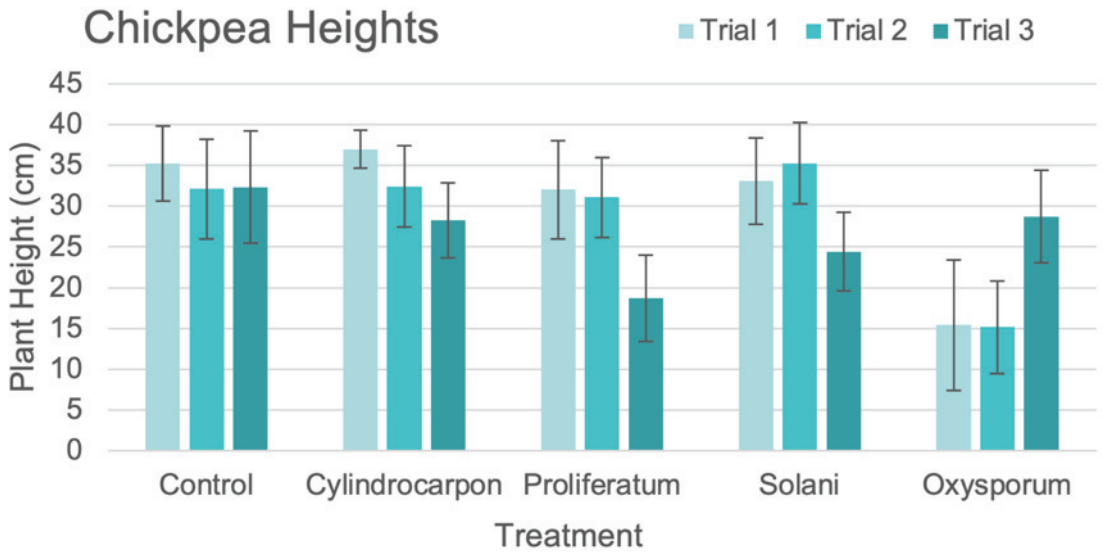


Figure A4: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

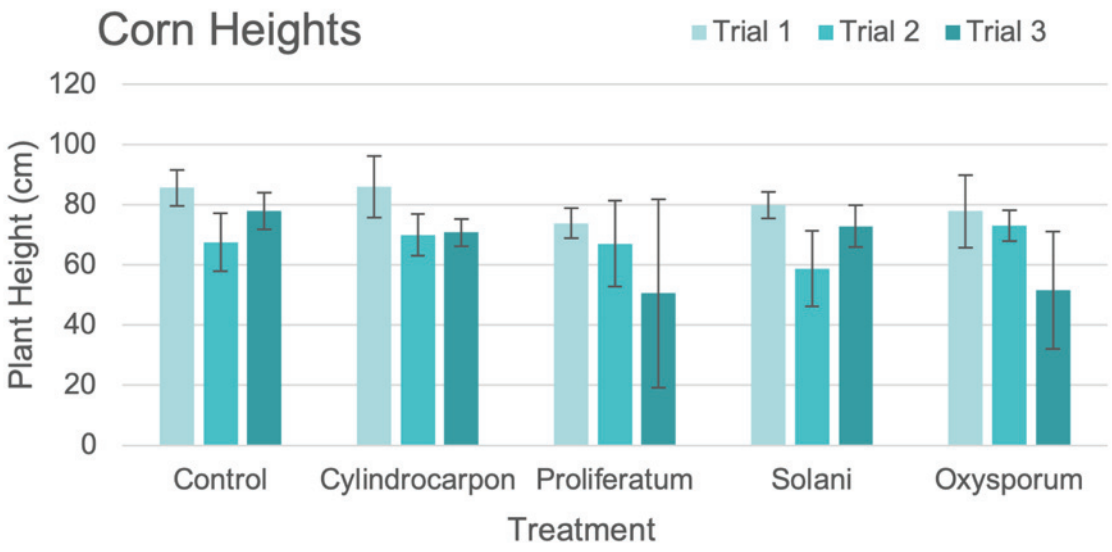


Figure A5: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

Figure A6: **Representative corn plants, from left to right, control, *Cylindrocarpon sp.*, *Fusarium proliferatum*, *Fusarium solani* and *Fusarium oxysporum* inoculated plants.**

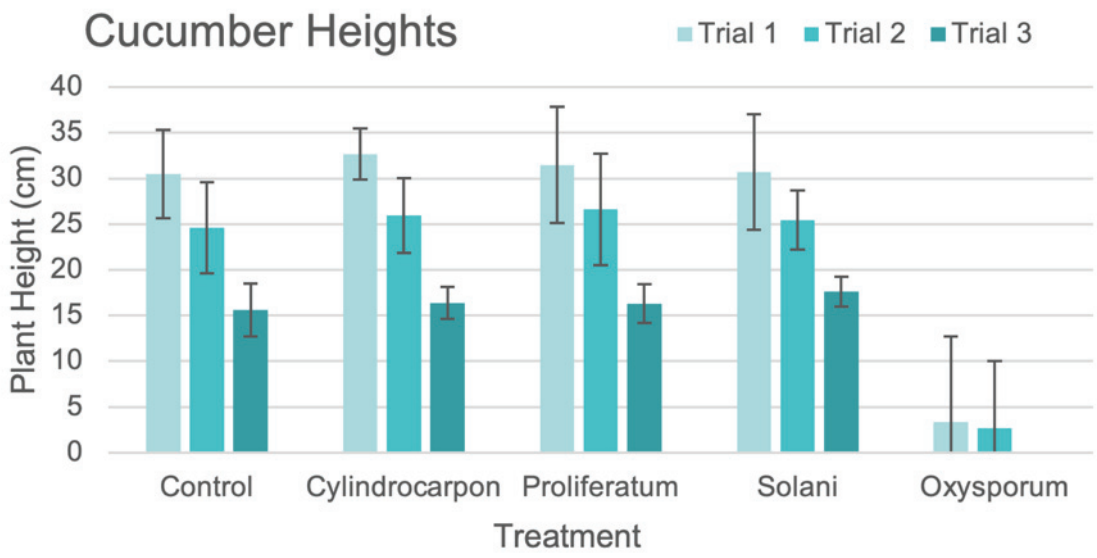


Figure A7: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

Figure A8: **Representative cucumber plants, from left to right, control, *Cylindrocarpon sp.*, *Fusarium proliferatum*, *Fusarium solani* and *Fusarium oxysporum* inoculated plants.**

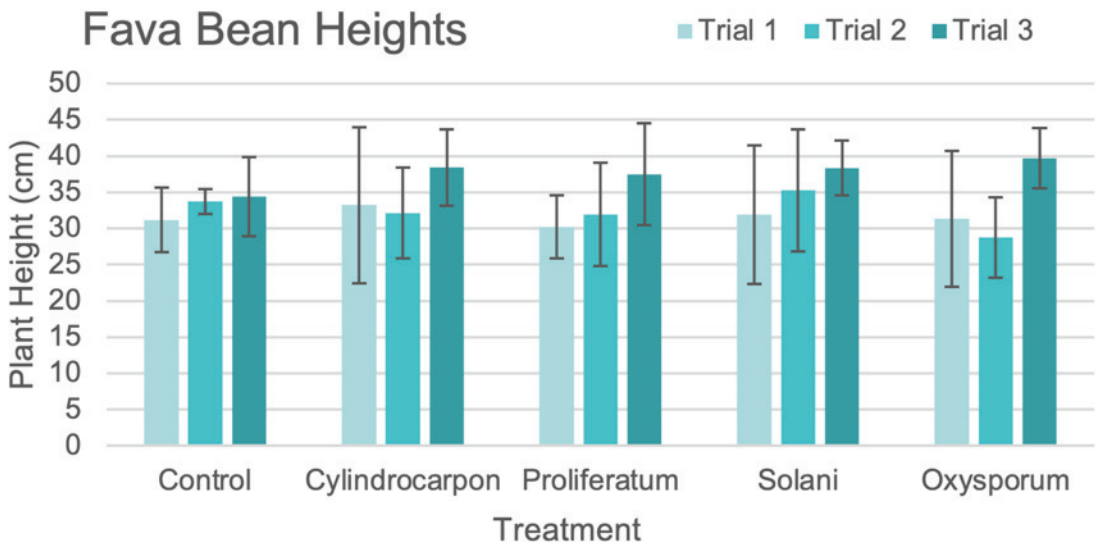


Figure A9: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

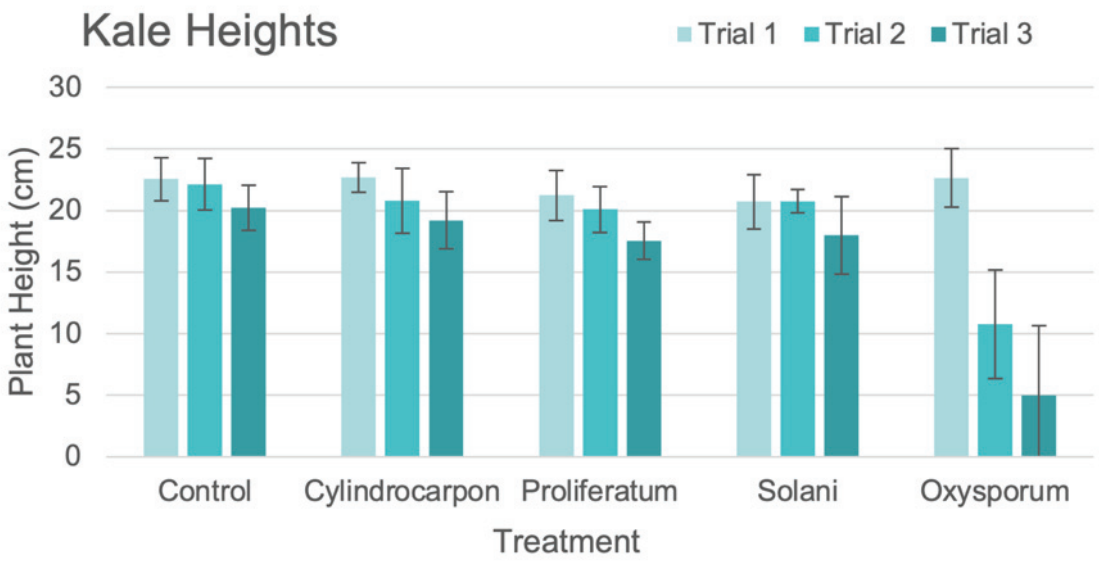


Figure A10: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

Figure A11: **Representative kale plants, from left to right, control, *Cylindrocarpon sp.*, *Fusarium proliferatum*, *Fusarium solani* and *Fusarium oxysporum* inoculated plants.**

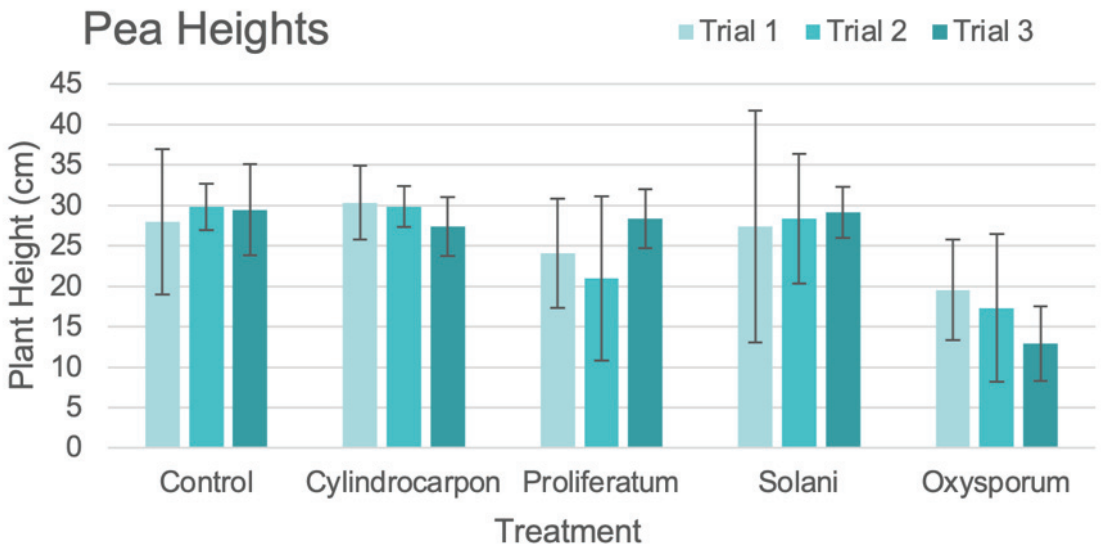


Figure A12: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

Figure A13: **Representative pea plants, from left to right, control, C*ylindrocarpon sp.*, *Fusarium proliferatum*, *Fusarium solani* and *Fusarium oxysporum* inoculated plants.**

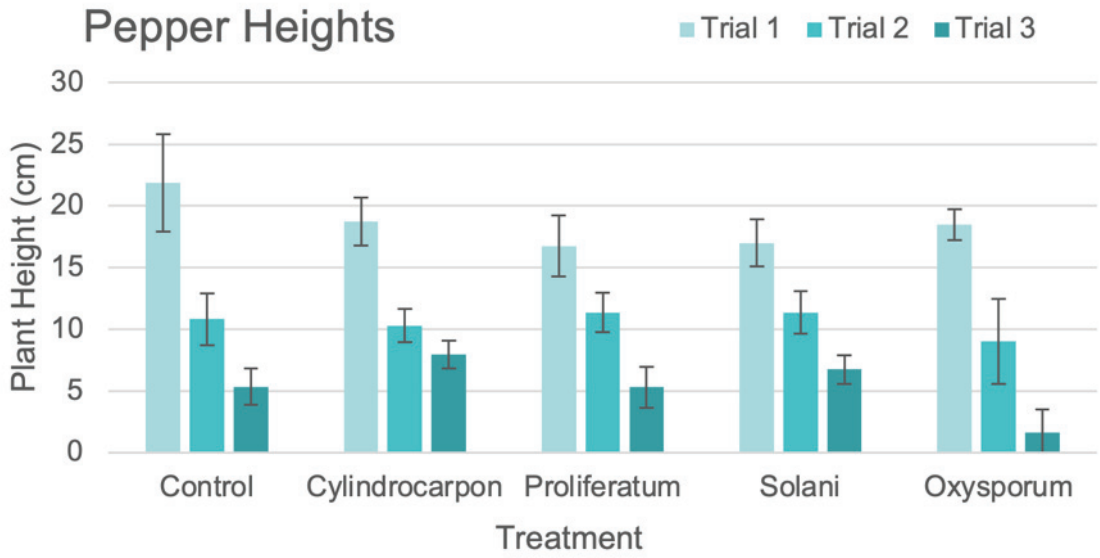


Figure A14: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

Figure A15: **Representative pepper plants, from left to right, control, *Cylindrocarpon sp.*, *Fusarium proliferatum*, *Fusarium solani* and *Fusarium oxysporum* inoculated plants.**

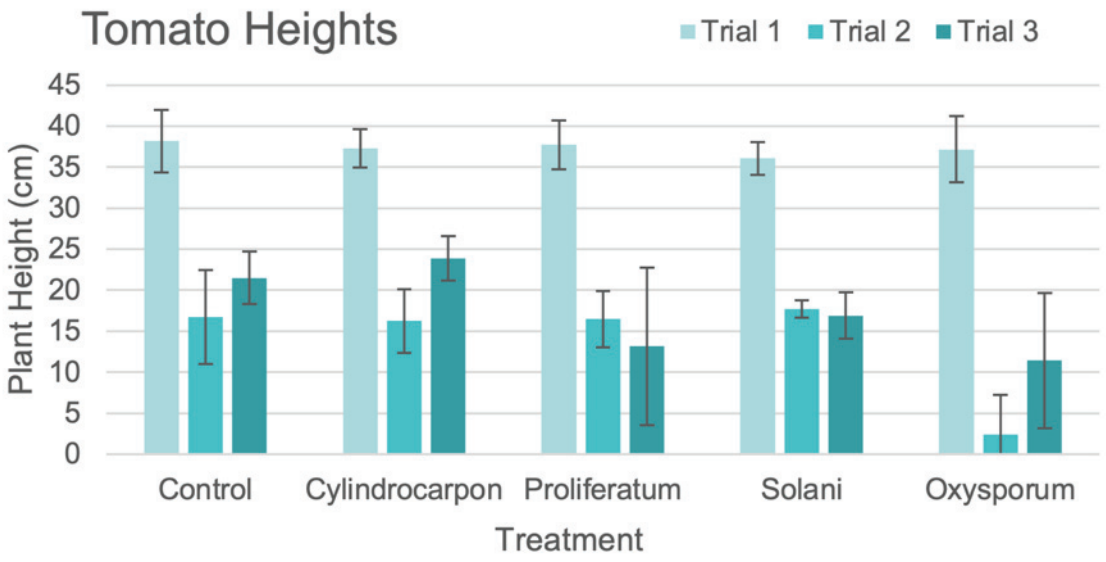


Figure A16: **Mean plant heights over three trials, with error bars denoting 95% confidence intervals (n=5).**

Figure A17: **Representative tomato plants, from left to right, control, *Cylindrocarpon sp.*, *Fusarium proliferatum*, *Fusarium solani* and *Fusarium oxysporum* inoculated plants.**