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The identification of Oomycetes associated with plum orchards in the Western Cape Province, South Africa

By

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DECLARATION

I, Mateka Patience Modiba, declare that the thesis/dissertation, which I hereby submit for the degree Master of Science at the University of Pretoria, is my own work and has not been submitted by me for a degree at this or any other tertiary institution.

Signature: .....

Date: .....

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PREFACE

Plum trees in the Western Cape Province have shown symptoms of trunk cankers and gummosis, which has led to their slow decline, which negatively affects the plum production industry. It has been suggested that this could be due to the prolonged drought in combination with pathogenic agents such as nematodes, oomycetes and *Pseudomonas syringae*. Oomycetes diseases have a large economic impact on agricultural crops, and results in huge annual losses to crop production globally. This thesis is based on determining if oomycetes are associated with infected plum trees in the Western Cape Province of South Africa. This thesis is presented as three independent chapters.

Chapter 1 will review previous literature on diseases of fruit crops caused by various species of oomycetes and *Pseudomonas syringae* pv. *syringae*. This chapter will also include sections on how the disease triangle and climate change influence disease development in stone fruit trees.

Chapter 2 will deal with field observations and sampling of symptomatic plum trees at several plum orchards located in Franschoek, Simondium and Wellington in the Western Cape Province of South Africa. Isolations will be performed by isolating oomycetes, which include species in the genera *Phytophthora*, *Pythium* and *Phytopythium* from the rhizosphere soil of apparently healthy and diseased trees using soil baiting technique, and oomycetes from infected plant material will be isolated by plating the plant material onto selective media. The oomycetes will be identified based on the amplification and sequencing of the ITS region of the ribosomal DNA, followed by phylogenetic analyses.

Chapter 3 will focus on the pathogenicity of *Phytophthora multivora* and *Phytopythium vexans* (isolated in Chapter 2) on plum seedlings using a 'sand-infestation pot trial' inside a greenhouse environment. In this study, a set of plum seedlings (Sun kiss cultivar) will be co-infected with either one of the oomycetes and *Pseudomonas syringae* pv. *syringae* to test synergism. Roots will be inoculated with either *P. vexans or P. multivora*, and *P. syringae* pv. *syringae* will be inoculated into the stems. The disease severity will be recorded six weeks after inoculation.

CHAPTER 1

Investigating plum decline within the framework of the disease triangle and climate change

1.1 Introduction

Several studies have shown that investing in agricultural research and development enhances global agricultural productivity. In South Africa, this investment, particularly in deciduous fruit research, has benefited the agricultural sector significantly (Thirtle et al, 1998). The Agricultural Research Council of South Africa's (ARC) stone fruit breeding programme, over a period of 15 years, has developed at least 300 deciduous fruit tree cultivars of which 63 are stone fruit crops (Tshabalala, 2015). Between ARC's various fruit tree-breeding programmes, the one responsible for breeding plum fruit trees dates back to the early 1940s. The programme focused on trees originating from the Japanese plum species, *Prunus salicina*. Previously, the success of this industry was jeopardized by the poor quality of plums. One of the reasons for inferior fruit quality was the inability of these cultivars to adapt to South African climatic conditions. Through this research programme, the ARC produced two cultivars, Laetitia and Songold, which contributed significantly to the success of the plum industry allowing South Africa to become internationally competitive with this agricultural resource (Tshabalala, 2015).

Plum trees are susceptible to a wide range of pathogens that includes bacteria, viruses, fungi and oomycetes. The individual disease cycles of these microorganisms are generally well understood. However, it is becoming increasingly evident that there has been an increase in sudden and large-scale losses of trees to decline, a term used to infer an unknown cause. Tree decline has been attributed to several factors, viz. climate change (increasing temperature and/or drought), catastrophic weather events and invasions by both native and exotic pathogens. In combination, the plants, pathogens and environmental variability are the contributing factors to plant decline (Grulke, 2011). Studies have shown that stressful environmental conditions such as drought can promote pathogen development in trees and plants (Thomas et al, 2002; Eastburn et al, 2011).

In the last few years, plum trees from orchards in the Western Cape Province of South Africa have shown symptoms of decline (Pienaar and Boonzaaier, 2018). It has been suggested that this is due to the prolonged drought periods, in combination with pathogenic agents such as *Pseudomonas syringae* pv. *syringae*, oomycetes species, infection by ring nematodes, fungal pathogens and phytoplasmas (Wenneker et al, 2011; Gurdeep et al, 2012). In this review, the different interacting factors of the disease triangle, together with the effect of climate change on plum decline, will be discussed.

1.2 Disease triangle

One of the fundamental principles in plant pathology is the disease triangle. It is a model proposed by Stevens (1960) that illustrates the interactions between the environment, the host and the pathogen in disease development. Over time other parameters have been added, viz. humans, vectors and time, and a pyramid or tetrahedron is used to depict the interactions between the four factors. More recently, environmental change, i.e. climate change, has been shown to induce effects on the components of the triangle (Grulke, 2011). Below is a discussion on how these factors or components may play a role in plum decline.

The three factors involved in decline are predisposing, inciting and contributing factors. Predisposing factors weaken the host's immunity resulting in the plant being unable to withstand adverse conditions (Mittler, 2000). These factors include soil as well as climatic conditions (Garrett et al, 2006). Climate change has a direct and indirect impact on soil (Dermody et al, 2007). Soil-climate models assume that one of the effects of climate change to soil is an increase in the loss of CO_2 in minerals and organic soil (Kardol et al, 2011). The loss of carbon will result in poor soil structure and stability, topsoil water holding capacity, mineral and nutrient availability and erosion (Lal, 2004).

Furthermore, rainfall fluctuation, such as drought periods, increases the likelihood of shrink-swell in clay soils (Bronick and Lal, 2005). The shrink-swell is a process whereby soils containing minerals absorb water causing the soil to swell, and when soil loses water, the soil begins to dry up and shrink (Dasog et al, 1988; Al-Kaisi et al, 2013). During shrinkage roots bridge the cracks and tend to snap as the crack expands, however, it is not clear to what extent this affects plant growth (Bengough et al, 2006; Whitmore and Whalley, 2009). In addition to

snapped roots, this also disturbs the foundation of the soil organic matter (Whitmore and Whalley, 2009). Climate change increases soil temperature, which is another factor that affects minerals and organic matter (Karmatar et al, 2016; Fang et al, 2019).

Inciting factors such a drought, atmospheric and soil temperature contribute to plant decline. Moderate deficiencies do not cause detectable symptoms. However, biological processes are usually the ones affected (Corcuera et al, 2004; Andersson et al, 2011; Sohar et al, 2014). Photosynthetic rate decreases leading to less accumulation of photosynthate required for plant growth and development (Tjoelker et al, 1998; Noormets et al, 2000). Extreme deficiencies can result in visible symptoms such as wilting, discoloration of leaves, rootlet mortality and ultimately tree death (Karnosky et al, 2002; Way et al, 2005). Moisture stress, for example, is known to increase the susceptibility of plants to pathogen attack by interfering with the food manufacturing process. When plants are unable to obtain essential nutrients, they become weak, and their immune becomes compromised (Lindroth et al, 1997; Wustman et al, 2001).

Contributing factors to plant decline include plant pathogens such as oomycetes and pathogenic *Pseudomonas syringae*, which usually cause visible symptoms. For instance, Phytophthora species cause root and crown rots, stem cankers or "bleeding cankers" of their host trees (Zwart and Kim, 2012; Colangelo et al, 2018). They are known to girdle the stem, which kills the phloem, leading to the interference of water and nutrient uptake subsequently killing the tree (Erwin and Ribeiro, 1996, Brown and Brasier, 2007).

A plant is diseased when its normal physiological functions are altered by abiotic and biotic factors mentioned above (Spaulding, 1958; Manion, 1991, Thomas et al, 2002; Ostry et al, 2011). The affected plant will then change in appearance or become less productive than a normal healthy plant of the same variety. In order for a plant disease to occur a host must be susceptible, pathogen virulent and the environment favorable (Nelson, 1994). Over the years, different plant hosts have been victims of decline. Despite the fact that plum trees are of economic importance in South Africa, studies on decline on this host have yet to be undertaken.

1.2.1 Host: Plum trees

Plums were domesticated in China and Europe more than 2000 years ago, and one of the predominant species in large-scale commercial plum production is the Japanese plums (*Prunus salicina*) (Klabunde et al, 2014). They were introduced in South Africa by Jan van Riebaack in 1659. There is a variety of plum cultivars produced in South Africa and Laetitia and Songold are the two most produced cultivars (Tshabalala, 2015). Plum fruits are popular due to their health properties (Vicente et al, 2009; Pennington and Fisher, 2010; Rendina et al, 2012; Nogales-Delgado et al, 2013). Temperate fruit crops like plum trees require specific temperature regimes for optimal vegetative growth and reproductive development (Srinivasan et al, 2012). Plum trees require sufficiently low temperatures during winter to enter into a dormant state. The minimum air temperature required during this period should range from 2.5o-12.5oC. Plum trees can grow well in different soil types (sandy, clay and loamy), provided they are planted at least 60 cm deep and should have a pH range of 5.5-6.5. Well-drained soil is ideal. However, plum trees are more tolerant to heavy or waterlogged soils compared to other stone fruit trees (Department of Agriculture, 2008). These growth requirements render them susceptible to climate change.

Climate change, also known as global warming, means the rise in the average surface temperatures on Earth. Climate change has caused the physical and chemical environment of the Earth to change drastically over the years, and the changes are expected to continue in the foreseeable future (Chappelka & Grulke, 2016). The primary cause of climate change is increased secondary pollutants and the release of gas emissions. Climate change affects water availability, which is of global importance more so for water-scarce countries (Dale et al, 2001). Climate change has repercussions for climate-sensitive systems such as forestry, natural resources and agriculture (Sanderman, 1996; Krupa et al, 2000; Karnosky et al, 2007). Projected changes in temperature and precipitation impacts on agricultural production, which subsequently affects the economy, leading to changes in prices due to reduced production. South Africa is a semi-arid and water-scarce country; therefore, the effects of climate change will be dramatic, and the competitiveness of agriculture will be at risk (Ogundeji & Jordaan, 2017).

Due to climate change, the two most important limiting factors of plant productivity are drought and heat stress (Garrett et al, 2006; Fahad et al, 2017). Drought is a prolonged period of deficient precipitation, which results in extensive damage to crops, and subsequently yields loss.

The Western Cape Province has experienced a persistent drought since 2014, and this condition has severely affected the agricultural sector. The Western Province has a Mediterranean climate; therefore, insufficient rainfall combined with warm temperature aggravates evapotranspiration, which leads to plant stress. The persistence of drought conditions has affected the stone fruit industry (Botai et al, 2017). Prolonged drought periods affect plant growth, development, physiology, reproduction and the overall health of a plant (Yordanov et al, 2000). Water is an important abiotic factor that limit physiological processes and ecological adaptability of plants (Fahad et al, 2017). It plays a crucial role in the transportation of nutrients from the soil to the plants, which aids in plant growth and development. Therefore, when plants are water-stressed their growth rate decreases and their immunity also becomes compromised due to starvation (Shao et al, 2005; Shao et al, 2006; Shao et al, 2008).

Another important factor that impacts plant growth is temperature. Atmospheric temperature plays a crucial role in plant growth and development, and each species has different temperature requirements (Hatfield and Prueger, 2015). Elevated temperatures are expected to become intense, frequent and prolonged in the next 30-50 years compared to recent years (Meehl et al, 2007). Elevated atmospheric temperatures result in heat shock, which subsequently affects the morphological, physiological, and biochemical processes of plants (Peng et al, 2004; Wahid et al, 2007). Heat stress may interfere with protein synthesis, inactivate vital enzymes, damage membranes and can interfere with cell division processes (Smertenko et al, 1997).

Plants are continuously exposed to drought and heat, and the combined effect of both stress factors has a dire impact than the effect of each stress alone (Dreesen et al, 2012; Rollins et al, 2013; Lipiec et al, 2013). Plants experience drought stress either when the loss of water through transpiration is extremely high or when the water supply to the roots is restricted, and often water shortage in the soil is concurrent to higher air temperatures (Anjum et al, 2011; Farooq et al, 2012, Lipiec et al, 2013). Therefore, the decrease in precipitation and rainfall, heat wave events, as well as the increase in atmospheric CO_2 levels adds complexity to the effect of drought and heat stress.

1.2.2 Pathogens

One of the three elements that stresses the host plant is a virulent pathogen (Pritchard et al 1999; von Tiedemann and Firsching, 2000). Most plant pathology studies focus on single host-single pathogen interactions. However, plants in nature interact with multiple pathogens, which creates a complex interaction (Frey-Klett et al, 2011). Therefore, plant pathology studies need to focus on these complex interactions known as co-infection, since it tends to alter the disease severity and virulence compared to when a single pathogen is involved (Kozanitas et al, 2017). The result of co-infection is reduced fitness of the host (Brown, 2015). Pathogen-pathogen interactions and host-multiple pathogen interactions may result in mutualism, coexistence, synergism or antagonism (Lamichhane and Venturi 2015). The severity of the disease on the plant is dependent on the outcome of the interactions and the host's response (Abdullah et al, 2017; Kozanitas et al, 2017; Tollenare et al, 2017).

Understanding co-infection complexes may help predict long-term dynamics of multiple disease outcomes (Abdullah et al, 2017). For instance, the outcome of co-infection complexes may be mutualism, where both pathogens are able to co-exist in peace, or competitive exclusion where one pathogen is excluded over time, or a new recombinant may emerge where one pathogen incorporates some genes obtained from another pathogen leading to large-scale epidemics (Al-Naimi et al, 2005; Friesen et al, 2006; Mordecai et al, 2016).

In cases of multiple pathogen attack, the plant's defense system is weakened even more than when a single pathogen is responsible for the disease (Lamichhane, 2015). This leads to extreme infection and consequent yield losses. Cases like this make it difficult to recognize disease symptoms on the plant. For instance, based on which pathogen we are looking for, the conditions for the disease development and establishment may differ throughout the year. Also, the presence of pathogenic species can mask that of non-pathogenic ones (Malvick & Moore, 1988).

Plants have an innate immunity that is composed of two inducible layers that defend the plant against microbial infections (Monaghan and Zipfel, 2012; Andolfo and Ercolano, 2015; Irieda et al, 2019). The first one is the pathogen-associated molecular pattern (PAMP) or pattern-triggered immunity (PTI), which detects microbial molecules, or by-products produced by microbial activity by pattern-recognition receptors on cell surfaces. Pathogens manage to successfully colonize plants by releasing their cytoplasmic effectors or apoplastic effectors into the host

(Oliveira-Garcia et al, 2015; Büttner D, 2016; Lanver et al, 2017). The second layer is the effector-triggered immunity which involves the concept of effectors by cytoplasmic nucleotide-binding, leucine-rich repeat (NB-LRR) resistance (R) proteins (Jones and Dangl, 2006; He et al, 2018).

Research on oomycetes has shown that they produce hundreds of effector proteins and they are used to target host plants at distinct sites (Birch et al, 2006; Kamoun, 2006; Tyler et al, 2006). For instance, they secrete apoplastic effectors into the plant's extracellular space, which are involved in inhibiting host enzymes, induced in response to pathogen infection (Rose et al, 2002; Tian et al, 2004; Tian et al, 2005). They may also secrete cytoplasmic effectors into plant cells where they target distinct subcellular compartments. However, cytoplasmic effector activities are still poorly understood, and more research has to be done to understand the virulence function of these proteins (Morgan and Kamoun, 2007).

Phytophthora infestans is known to produce RXLR effectors, which translocate to different subcellular locations and target various host proteins subsequently suppressing the host's immunity and promoting disease development (Whisson et al, 2016; Wang et al, 2017). PAMP innate immunity consists of various mechanisms, and stomatal closure is one of the most important ones. For instance, when a plant recognizes pathogen molecules, it will close its stomata to prevent or reduce pathogen entry. However, in the case of a suppressed immunity, the plant will be unable to close its stomata (Gudesblat et al, 2009). In addition to elevated CO_2 due to climate change, stomata remain open due to that factor as well. This then enables opportunistic pathogens such as *Pseudomonas syringae* to take advantage of the situation and gain entry. *Pseudomonas syringae* is an opportunistic pathogen and infects predisposed host plants to cause disease. It will invade the stomata, multiply, then release type III effectors, which further attacks the plant's innate immune system (Block and Alfano, 2011). Therefore, disease symptoms displayed by infected plants are as a result of a weakened immune system. Symptoms are usually observed on roots, crown and foliage.

1.2.2.1 Oomycetes associated with diseased fruit crops

Oomycetes, known as water molds, are a diverse group of organisms belonging to the Infrakingdom Chromista (Cavalier-Smith, 1981). Organisms from this group include some of the

most devastating plant pathogens of agricultural crops, such as *Phytophthora*, *Pythium* and *Phytopythium* (Kamoun et al, 2003; Herrero et al, 2011). The genus *Phytophthora* has 180 identified species to date, and some species reported in South Africa are *Phytophthora cinnamomi*, *P. multivora*, *P. cactorum*, *P. capensis*, *P. cryptogea*, *P. frigida*, and *P. alticola* (Cooke et al, 2000; Oh et al, 2013; Bose et al, 2018; Scott et al, 2019). *Phytophthora* disease symptoms include discoloration of the foliage, branch dieback and sometimes tree death, stem cankers, gummosis, crown and root rots (Erwin & Ribeiro, 1996; Werres et al, 2014).

Over a hundred species of *Pythium* have also been identified, and the ones that are known to cause disease in plants are *Pythium aphanidermatum*, *P. irregulare* and *P. ultimum* (Sutton et al, 2006; Ivors & Moorman, 2014; Kageyama, 2014). *Pythium* usually causes seed rot, root tip browning and rot, and seedling damping-off (Daughtrey & Benson, 2005; Sutton et al, 2006; Yang & Hong, 2016). *Phytopythium* currently includes over ten species and was recently diverged from *Pythium* (de Cock et al, 2015). The most important *Phytopythium* species are *Phytopythium helicoides* and *P. vexans*. They cause similar symptoms to that caused by *Pythium* spp. such as root damping-off and rot (Tao et al, 2011; Yang et al, 2013; Kageyama, 2014).

Although the above species are amongst the most devastating plant pathogens, limited information is known about the role they play in the development of stem cankers and gummosis on stone fruit trees. The role they play, if any, in plum disease decline is unclear and has yet to be investigated. However, the closest host to plums to be studied is cherry trees, and other studies involving fruit crops such as grapevines, citrus and avocado trees.

Oomycetes have been linked to replant and decline disease of grapevines, which causes yield and financial losses. *Phytophthora* and *Pythium* species have been reported as the most common frequently detected soil borne pathogens of grapevines in both nurseries and established grapevines (Marais, 1979; Marais, 1980). A study conducted by Spies et al (2011) investigated *Phytophthora*, *Pythium* and *Phytopythium* species associated with grapevines, and determined the pathogenicity of *Phytophthora niederhauserii* and *Phytopythium vexans* compared to that of *Phytophthora cinnamomi* and *Pythium irregulare* on resistant grapevine rootstocks. The results showed that the most common infections in grapevines were caused by *P. irregulare* (18%), *P. vexans* (16.7%), *Pythium ultimum* var. *ultimum* (15%), *P. heterothallicum* (7.3%), *P. cinnamomi* (5.1%) and *P. niederhauserii* (1.1%). The pathogenicity trial showed that *P. niederhauserii and*

P. vexans were as aggressive as the well-known grapevine pathogens *P. cinnamomi* and *P. irregulare*. Altogether, these findings showed that the common oomycetes species might induce disease on their own or in association with other pathogens and can aggravate infections already caused by these pathogens.

Another important fruit crop that is targeted by oomycetes is citrus. A study was conducted to identify the causal agent of citrus gummosis in Tunisia during 2012 and 2013. Infected trees in major citrus orchards were secreting gum from infected trunk cracks. Most studies have reported *Phytophthora nicotianae* and *P. citrophthora* as the causal agents of gummosis (Erwin & Ribeiro, 1996; Sonoda, 2000; Verniere et al, 2004, Cacciola & Di San Lio, 2008). However, *Pythium* species are abundant in the rhizosphere of diseased citrus trees and widely distributed throughout the world as plant pathogens (Maseko & Coutinho, 2002; Mostowfizadeh-Ghalamfarsa & Banihashemi, 2005).

For example, Benfradj et al (2017) believed that *Pythium* and *Phytopythium* might influence the development of gummosis in citrus and not only *Phytophthora*. They recovered *Pythium aphanidermatum, P. diclinum, P. ultimum, Phytopythium vexans,* and *P. mercurial* from the soil and diseased trunk samples. This study showed that *Pythium* species were consistently isolated from symptomatic infected citrus trees and showed that *Pythium ultimum* is the most virulent compared to the other recovered species.

Crown and root diseases have had a major economic impact on the commercial cherry industry in the United States. Whenever soil samples were examined from diseased trees, *Armillaria mellea* and *Poria ambigua* were often identified as causal agents (Proffer et al, 1987). However, if these two pathogens were not identified as causal agents, the disease was usually attributed to wet feet or sour sap. *Phytophthora* was then later suspected to be the causal agent, and it was also noticed that the highest incidences of rots usually occurred in orchards suffering from poor soil water drainage (Mircetich and Matheron, 1976). This study revealed that *Phytophthora cambivora, P. megasperma* and *P. dreschleri* were repeatedly isolated from orchards with a high incidence of tree deaths, indicating that these three species were mostly associated with diseased cherry trees.

Furthermore, Wilcox and Mircetich (1985) conducted a study to determine which other *Phytophthora* spp., other than the species mentioned above, are associated with dead and

declining cherry trees in the United States. The other *Phytophthora* species identified were, namely, *P. crypyogea*, *P. cinnamomi*, and *P. citricola*. Pathogenicity trials proved that the severity of the rots was much higher when trees were exposed to flooding compared to well-drained soil (Wilcox & Mircetich, 1985).

Cherry rootstocks that were mainly used in the past were Mazzard and *Prunus avium*, and Gisela 5 and Maxma 14 rootstocks later replaced them. These rootstocks were preferred due to their dwarfing and precocity, and ability to tolerate the Mediterranean climatic conditions. Trees in cherry orchards in Greece were experiencing crown rots, and examination of the rots identified *Phytophthora cactorum*, *P. citricola*, *P. citrophthora*, and *P. parasitica* as the causal agents of the crown rot. These findings raised concerns in choosing suitable rootstocks that will be resistant or tolerate to these pathogens.

A study was conducted evaluating the susceptibility of Gisela 5 and Maxma 14 cherry rootstocks to *Phytophthora cactorum*, *P. citrophthora*, *P. citricola* and *P. parasitica*. Two-year-old cherry trees of both rootstocks were inoculated with isolates of the above *Phytophthora* species, and all four species were found to be pathogenic to both rootstocks with similar susceptibility. When severity was compared between the four species, *P. citrophthora* and *P. parasitica* were highly virulent compared to *P. citricola*, which showed moderate virulence, and *P. cactorum*, which was the least virulent (Exadaktylou and Thomidis, 2005).

1.2.2.2 Pseudomonas syringae associated with fruit crops

Pseudomonas syringae is a Gram-negative, rod-shaped bacterium and is of economic importance with a worldwide distribution (Lamichhane et al, 2014; Konavko et al., 2014). *Pseudomonas syringae* causes infections in trees and crops of over 180 species (Little et al, 1998; Agrios, 2005; Kaluzna et al, 2010; Konavko et al, 2014). In 1994, about 40 pathovars were identified and later the number increased to over 50 (Braun-Kiewnick & Sands, 2001; Höfte & De Vos, 2006; Young, 2010). Pathovar is "bacterial a strain or set of strains with the same or similar characteristics, which is differentiated at the infrasubspecific level from other strains of the same species/subspecies on the basis of pathogenicity, particularly in relation to host range" (Gonzalez et al, 2000).

The pathovar responsible for bacterial canker in stone-fruit trees is *Pseudomonas syringae* pv. *syringae* (Agrios, 1988; Shamsbakhsh & Rahimian, 1997; Bultreys & Kaluzna, 2010). *Pseudomonas syringae* pv. *Syringae* was first reported in New Zealand as the causal agent of blast of stone fruits by Dye (1954). Bacterial canker was then reported in Iran where *Ps. syringae* was detected from diseased apricot trees and later from peach trees (Bahar et al, 1985). In addition to stem canker, the infected trees displayed symptoms such as dieback, blossom blast, spur and twig blight, necrotic leaf spots, discolored leaf veins, spots on fruit and gummosis (Gotto, 1992; Hattingh & Roos, 1995; Mohammadi et al, 2001). Stem cankers often exude sap (gummosis), and as the canker enlarges, it girdles the stem, which eventually causes the death of the branch, or the entire plant (Moore, 1988; Kaluzna et al, 2010).

Pseudomonas syringae pv. *syringae* can exist in large numbers on plant surfaces without causing an infection until the plant is predisposed to factors that weaken it. This bacterium exists on the surfaces of leaves, and during rainy seasons (spring and early summer) it enters through the stomata and causes infections in developing young leaves. As the leaves mature small patches of necrosis appears on the leaves. The pathogen then infects blossoms, and lenticels leading to invasion of woody tissue resulting in canker formation. As the canker enlarges, it eventually girdles and kills the branches, which results in loss of fruit surface and eventually tree death (Kennelly, 2007).

Little et al (1998) identified and characterized different strains of *Pseudomonas syringae* pv. *syringae* that were isolated from various *Prunus* species which included almonds, peaches, plums etc. The strains isolated from leaves, flowers, branches, and dormant buds of healthy and diseased stone-fruit trees from 43 orchard sites in California between 1995 and 1996, were inoculated into Lovell peach seedlings. The results indicated that all the strains were moderate to pathogenic on the peach seedlings as the infected trees displayed symptoms of necrotic lesions and gummosis.

Similarly, Mohammadi et al (2001) observed the same results when they isolated various Iranian strains of *P. syringae* pv. *syringae* from stone fruit trees to evaluate their phenotypic properties. They tested the degree of necrosis associated with the strains on apricot leaves, immature cherry fruits and shoots. The infected apricot leaves developed water-soaked spots and necrosis; the color of the cherry fruits changed to dark brown, and the infected shoots developed chlorotic

spots, which become necrotic and dried. Overall, the results showed that the virulence of the strains was undoubtedly associated with the degree of necrosis on the cherry fruits.

1.2.3 Environment

Environmental factors have an impact on the development of plant diseases (Czembor et al, 2015; Fathi and Tari, 2016). The disease triangle clearly states that for a plant disease to occur, a susceptible plant host, a virulent pathogen, and favorable environmental conditions must interact (Agrios, 2005; Islam et al, 2017, Islam, 2018). Because of this intimate relationship, plant disease incidence and severity is expected to be significantly influenced by climate change (Ghini et al, 2008; Das et al, 2016).

The two most important environmental factors involved in plant disease development are temperature and moisture (relative humidity) (Rana & Randhawa, 2011). Temperature and moisture influence the rate of reproduction of pathogens. Climate change makes growing seasons longer, which may extend the amount of time required for reproduction and dissemination of pathogens (Dufault and De Wolf, 2006; Granke and Hausbeck, 2010; Manstretta and Ross, 2015). Temperature and relative humidity affect disease cycle events such as germination, dispersal, infection, development, establishment, survival and the reproduction rate of most pathogens (Chakraborty et al, 2008; Das et al, 2016).

Climate change modifies temperature and precipitation regimes, which usually alters the growth stage, development rate and the pathogenicity of plant pathogens (Mboup et al, 2012; Haavik et al, 2015). Climate change is expected to worsen the effects of plant pathogens (Coakley et al, 1999). Changed temperature conditions favor the overwintering of sexual propagules, which increases the evolutionary potential of a population (Tapsoba and Wilson, 1997; Pfender & Vollmer, 1999). In summary, warmer temperatures enhance pathogen fitness in terms of generation number and sexual reproduction rate, extends the amount of time available for reproduction and dissemination (Huber and Gillespie, 1992; Venette, 2009). For instance, with *Phytophthora species*, the severity and incidence of root rot in trees increases when there is a rise in winter temperatures, a shift in precipitation from summer to winter and towards heavy rainfall (Elad and Pertot, 2014).

1.3 Conclusion

Climate change affects the two most important environmental factors, temperature and moisture. During climate change there is an increase in atmospheric temperature, which subsequently reduces rainfall, resulting in drought conditions. Drought was reported to be one of the limiting factors stone fruit productions in the Western Cape. Water stress weakens the host plant by interfering with the absorption of essential nutrients required for plant growth and development. Stressed plants become more susceptible to pathogen attack. Temperature and moisture also influence pathogen occurrence and establishment. These factors alter growth and development rates of pathogens which aids in plant infection. In some cases, a single host can be infected by more than one pathogen, and this complex interaction may influence the disease severity. Studies on plant diseases have highlighted the importance of the interrelations of stress factors involved in decline. Decline is a complex phenomenon, and has been predicted that in the next coming years, there will be greater frequency of occurrence of diseases. The plum decline in South Africa could be due to the interrelation stress factors which need to be investigated. The role of *Pseudomonas syringae* pv. *syringae* causing disease in plum trees has been studied; however, the role that oomycetes play in the development of the disease has not been studied. In order to understand and develop better management strategies, the interaction of potential contributing factors has to be considered.

1.4 References

Abdullah AS, Moffat CS, Lopez-Ruiz FJ, Gibberd MR, Hamblin J, Zerihun A, 2017. Host-multi-pathogen warfare: Pathogen interactions in co-infected plants. *Frontiers in plant science* **8**, 1-12.

Agrios GN, 1988. Plant Pathology. 3rd ed. Academic Press. 803 pp.

Agrios GN, 2005. Plant Pathology. P. 249-268. 5th edition. *London: Elsevier*.

Al-Kaisi MM, Elmore R, Guzman JG, Hanna HK, Hart CE et al, 2013. Drought impact on crop production and the soil environment: 2012 experiences from Iowa. *Journal of Soil and Water Conservation* **68**, 19-24.

Al-Naimi F, Garrett K, Bockus WW, 2005. Competition, facilitation, and niche differentiation in two foliar pathogens. *Oecologia* **143**, 449–457.

Andersson M, Milberg P, Bergman KO, 2011. Low pre-death growth rates of oak (*Quercus robur* L.)–Is oak death a long-term process induced by dry years? *Annuals of Forest Science* **68**, 159-168.

Andolfo G, Ercolano MR, 2015. Plant innate immunity multicomponent model. *Frontiers in Plant Science* **6**, 1-6.

Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou C M, 2011. Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. *Journal of Agronomy and Crop Science* **197**, 177–185.

Bahar M, Motjahedi H, Akhiani A, 1985. Bacterial canker disease of apricot in Efahan. Iran. *Journal of Plant Pathology* **18**, 58-68.

Benfradj N, Migliorini D, Luchi N, Santini A, Boughalleb-M'Hamdi N, 2017. Occurrence of *Pythium* and *Phytopythium* species isolated from citrus trees infected with gummosis disease in Tunisia. *Archives of Phytopathology and Plant Protection* **50**, 286-302.

Bengough AC, Bransby MF, Hans J, McKenna SJ, Roberts TJ, Valentine TA, 2006. Root responses to soil physical conditions; growth dynamics from field to cell. *Journal of Experimental Botany* **57**, 437–447.

Birch PR, Rehmany AP, Pritchard L, Kamoun S, Beynon JL, 2006. Trafficking arms: oomycete effectors enter host plant cells. *Trends in Microbiology* **14**, 8-11.

Block A, Alfano JR, 2011. Plant targets for *Pseudomonas syringae* type III effectors: virulence targets or guarded decoys? *Current Opinion in Microbiology* **14**, 39-46.

Bose T, Wingfield MJ, Roux J, Vivas M, Burgress TI, 2018. Community composition and distribution of *Phytophthora* species across adjacent native and non-native forests of South Africa. *Fungal Ecology* **36**, 17-25.

Botai CM, Botai JO, de Wit JP, Ncongwane KP, Adeola AM, 2017. Drought characteristics over Western Cape Province, South Africa. *Water* **9**, 1-16.

Bronick CJ, Lal R, 2005. Soil structure and management: a review. *Geoderma* **124**, 3-22.

Brown AV, Brasier CM, 2007. Colonization of tree xylem by *Phytophthora ramorum*, *P. kernoviae*, and other *Phytophthora species*. *Plant Pathology* **56**, 227-241.

Brown JK, 2015. Durable resistance of crops to disease: A Darwinian perspective. *Annual Review of Phytopathology* **53**, 513-539.

Bultreys A & Kaluzna M, 2010. Bacterial canker caused by *Pseudomonas syringae* on stone fruit species with special emphasis on the pathovars *syringae* and *morsprunorum* race 1 and race 2. *Journal of Plant Pathology* **92**, S1.21-S2.33.

Büttner D, 2016. Behind the lines-actions of bacterial type III effector proteins in plant cells. *FEMS Microbiology Review* **40**, 894-937.

Cacciola SO, Di San Lio GM, 2008. Management of citrus diseases caused by *Phytophthora* spp. In: Ciancia A, Mukerji KG, editors. Integrated management of diseases caused by fungi, Phyoplasma and bacteria. *Springer Netherlands*, 61-84.

Cavalier-Smith T, 1981. "Eukaryote kingdoms: seven or nine?". *Bio Systems* **14**, 461-81.

Chakraborty S, Luck J, Hollaway G, Freeman A, Norton R, Garrett KA, Percy K, 2008. Impacts of global change on diseases of agricultural crops and forest trees. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* **3**, 1-15.

Chappelka AK, Grulke NE, 2016. Disruption of the 'disease triangle' by chemical and physical environmental change. *Plant Biology* **1**, 5-12.

Coakley SM, Scherm H, Chakraborty S, 1999. Climate change and plant disease management. *Annual Review of Phytopathology* **37**, 399-426.

Colangelo M, Camarero JJ, Borghetti M, Gentilesca T, Oliva J et al, 2018. Drought and *Phytophthora* are associated with the decline of oak species in Southern Italy. *Frontier Plant Science* **9**, 1-13.

Cooke WE, Drenth A, Duncan JM, Wagels G, Brasier CM, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30**, 17-32.

Corcuera L, Camarero JJ, Gil-Pelegrín E, 2004. Effects of a severe drought on growth and wood-anatomical properties of *Quercus faginea*. *IAWA Journal* **25**, 185-204.

Czembor E, Waśkiewicz A, Piechota U, Puchta M, Czembor JH, Stępień Ł, 2015. Effect of environmental factors on *Fusarium* species and associated mycotoxins in maize grain grown in Poland. *PLOS ONE* **10**, 1-18.

Dale VH, Joyce LA, McNulty S, Neilson RP, Payres M, Flannigan MD, Hanson PJ, Irland LC, Lugo AE, Peterson CJ, Simberloff D, Swanson FJ, Stocks BJ, Wotton BM, 2001. Climate change and forest disturbances. *Bioscience* **51**, 723-734.

Das T, Majumdar MHD, Devi RKT, Rajesh T, 2016. Climate change impacts on plant diseases. *SAARC Journal of Agriculture* **14**, 200-209.

Dasog GS, Action DF, Mermut AR, De Jong E, 1988. Shrink-swell potential and cracking in clay soils of Saskatchewan. *Canadian Journal of Soil Science* **68**, 251-260.

Daughtrey ML, Benson DM, 2005. Principles of plant health management for ornamental plants. *Annual Review of Phytopathology* **43**, 141-169.

de Cock AWAM, Lodhi AM, Rintoul TL et al, 2015. *Phytopythium*: molecular phylogeny and systematics. *Persoonia* **34**, 25-39.

Department of agriculture, 2008. Plums. *Department of Agriculture: South Africa*, 1-2.

Dermody O, Weltzin JF, Engel EC et al, 2007. How do elevated [CO_2], warming, and reduced precipitation interact to affect soil moisture and LAI in an old field ecosystem? *Plant Soil* **301**, 255-266.

Dreesen PE, De Boeck HJ. Janssens IA, Nijs I, 2012. Summer heat and drought extremes trigger unexpected changes in productivity of a temperate annual/biannual plant community. *Environmental and Experimental Botany* **79**, 21-30.

Dufault NS, De Wolf ED, Lipps PE, Madden LV, 2006. Role of temperature and moisture in the production and maturation of *Gibberella zeae* perithecia. *Plant Disease* **90**, 637-644.

Eastburn DM, McElrone AJ, Bilgin DD, 2011. Influence of atmospheric and climatic change on plant–pathogen interactions. *Plant Pathology* **60**, 54-69.

Elad Y, Pertot I, 2014. Climate change impacts on plant pathogens and plant diseases. *Journal of Crop Improvement* **28**, 99-139.

Erwin DC, Ribeiro OK, 1996. *Phytophthora* disease worldwide. *Phytopathology*, 1-562.

Exadaktylou E, Thomidis T, 2005. Susceptibility of Gisela 5 and Maxma 14 cherry rootstocks to four *Phytophthora* species. *Scientia Horticulturae* **106**, 125-128.

Fahad S, Bajwa AA, Nazir U, Anjum SA, 2017. Crop production under drought and heat stress: Plant responses and management options. *Frontier in Plant Science* **8**, 1-16.

Fang X, Luo S, Lyu S, 2019. Observed soil temperature trends associated with climate change in the Tibetan Plateau, 1960–2014. *Theoretical and Applied Climatology* **135**, 169-181.

Farooq M, Hussain M, Wahid A, Siddique KHM, 2012. Drought stress in plants: An overview. In: Plant Responses to drought stress (Ed. R. Aroca), *Springers Press, Berlin-Heidelberg*.

Fathi A, Tari DB, 2016. Effect of drought stress and its mechanism in plants. *International Journal of Life Sciences* **10**, 1-6.

Fathi A, Tari DB, 2016. Effects of drought stress and its mechanism in plants. *International Journal of Life Sciences* **10**, 1-6.

Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A, 2011. Bacterial-fungal interactions: Hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology and Molecular Biology Reviews* 75, 583-609.

Friesen TL, Stukenbrock EH, Liu Z, Meinhardt S, Ling H, Faris JD, et al. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. *Nature Genetics* **38**, 953-956.

Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE, 2000. *Annual Review of Phytopathology* **44**, 489-509.

Ghini R, Hamada E, Bettiol W, 2008. Climate change and plant diseases. *Scientia Agricola 65*, 98-107.

Gonzalez AJ, Landeras E, Mendoza MC, 2000. Pathovars of *Pseudomonas syringae* Causing Bacterial Brown Spot and Halo Blight in *Phaseolus vulgaris* L. Are Distinguishable by Ribotyping. *Applied and Environmental Microbiology* **66**, 850-854.

Goto M, 1992.Fundamentals of Bacterial plant pathology. *Academics Press, Inc. New York*. 342 pp.

Granke LL, Hausbeck MK, 2010. Effects of temperature, humidity, and wounding on development of *Phytophthora* rot of cucumber fruit. *Plant Disease* **94**, 1417-1424.

Grulke NE, 2011. The nexus of host and pathogen phenology: understanding the disease triangle with climate change. *New Phytologist*, **189**, 8-11.

Gudesblat GE, Torres PS, Vojnov AA, 2009. Stomata and pathogens: Warfare at the gates. *Plant Signaling Behavior* **4**, 1114-1116.

Gurdeep S, Virk S, Nagal A, 2012. Citrus diseases caused by *Phytophthora* species. *GERF Bulletin of Biosciences* **3**, 18-27.

Haavik LJ, Billings SA, Guldin JM, Stephen FM, 2015. Emergent insects, pathogens and drought shape changing patterns in oak decline in North America and Europe. *America and Europe. Forest Ecology and Management* **354**, 190-205.

Hatfield JL, Prueger JH, 2015. Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes* 10, 4-10.

Hattingh, MJ & Roos IMM, 1995. Bacterial canker. In: “Compendium of stone fruit diseases.” (Eds): Ogawa JM, Zehr EI, Bird GW, Ritxhie DF & Uymoto JK, The American Phytopathology Society. Press, St. Paul MN, USA, pp. 48-50.

He Q, Naqvi S, McLellan H, Boevink PC, Champouret N, Hein I, Birch PRJ, 2018. Plant pathogen effector utilizes host susceptibility factor NRL1 to degrade the immune regulator SWAP70. *Proceedings of the National Academy of Sciences* **115**, E7834-E7843.

Herrero ML, Toppe B, Brurberg MB, 2011. First report of *Phytophthora ramorum* causing shoot dieback on bilberry (*Vaccinium myrtillus*) in Norway. *Plant Disease* **95**, 355.

Höfte M, De Vos P, 2007. Plant pathogenic *Pseudomonas* species. *Plant-Associated Bacteria*, 507-533.

Huber L, Gillespie TJ, 1992. Modeling leaf wetness in relation to plant disease epidemiology. *Annual Review of Phytopathology* **30**, 553-577.

Irieda H, Inoue Y, Mori M, Yamada K, Oshikawa Y, Saitoh H, Uemura A, Terauchi R, Kitakura S, Kosaka A, Singkaravanit-Ogawa S, Takano Y, 2019. Conserved fungal effector suppresses PAMP-triggered immunity by targeting plant immune kinases. *Proceedings of the National Academy of Sciences* **116**, 496-505.

Islam W, 2018. Plant disease epidemiology: Disease triangle and forecasting mechanisms in highlights. *Hosts and Viruses* **5**, 7-11.

Islam W, Zhang J, Adnan M, Noman A, Zaynab M, Wu Z, 2017. Plant virus ecology: A glimpse of recent accomplishments. *Applied Ecology and Environmental Research* **15**, 691-705.

Ivors KL, Moorman GW, 2014. Oomycete plant pathogens in irrigation water. In: Hong CX, Moorman GW, Wohanka W, Buttner C, eds. Biology, detection, and management of plant pathogens in irrigation water. St Paul, MN, USA: APS Press, 57-64.

Jones JDG, Dangl JL, 2006. The plant immune system. *Nature* **444**, 323-329.

Joung JM, 2010. Taxonomy of *Pseudomonas syringae*. *Journal of Plant Pathology* **92**, S1.5-S1.14.

Kageyama K, 2014. Molecular taxonomy and its application to ecological studies of Pythium species. *Journal of General Plant Pathology* **80**, 314-326.

Kamoun S, 2003. Molecular genetics of pathogenic oomycetes. *Eukaryotic Cell* **2**, 191-199.

Kamoun S, 2006. A catalogue of the effector secretome of plant pathogenic oomycetes. *Annual Review of Phytopathology* **44**, 41-60.

Kardol P, Reynolds WN, Norby RJ, Classen AT, 2011. Climate change effects on soil microarthropod abundance and community structure. *Applied Soil Ecology* **47**, 37–44.

Karmakar R, Das I, Dutta D, Rakshit A, 2016. Potential effects of climate change on soil properties: A review. *Science International* **4**, 51-73.

Karnosky DF, Percy KE, Xiang B, Callan B, Noormets A, Mankovskas B, Hopkins A, Sober J, Jones J, Dickson RE, Isebrands JG, 2002. Interacting elevated CO_2 and tropospheric O_3 predisposes aspen (*Populus tremuloides* Michx.) to infection by rust (*Melampsora medusae f. sp. tremuloidae*). *Global Change Biology* **8**, 329–338.

Karnosky DF, Skelly JM, Percy KE, Chappelka AH, 2007. Perspectives regarding 50 years of research on effects of tropospheric ozone air pollution on US forests. *Environmental Pollution* **147**, 489–506.

Klabunde GHF, Dalbo MA, Nodari RO, 2014. DNA fingerprinting of Japanese plum (*Prunus salicina*) cultivars based on microsatellite markers. *Crop Breeding and Applied Biotechnology* **14**, 139-145.

Konavko D, Morocko-Bicevska I, Bankina B, 2014. *Pseudomonas syringae* as important pathogen of fruit trees with emphasis on plum and cherry. Research for Rural Development 1, 19-25.

Kozanitas M, Osmundson TW, Linzer R, Garbelotto M, 2017. Interspecific interactions between the Sudden Oak Death Pathogen *Phytophthora ramorum* and two sympatric *Phytophthora* species in varying ecological conditions. *Fungal Ecology* **28**, 86–96.

Krupa S, McGrath MT, Andersen CP, Booker FL, Burkey KO, Chappelka BI, et al, 2000. Ambient ozone and plant health. *Plant Disease* **85**, 4–12.

Lal R, 2004. Soil Carbon Sequestration Impacts on Global Climate Change and Food Security. *Science* **304**, 1623-1627.

Lamichhane JR, 2015. Bacterial disease of crops: Elucidation on the factors that lead to differences between field and experimental infections. *Advances in Agronomy* **134**, 227-246.

Lamichhane JR, Varvaro L, Parisi L, Auderson J, Morris CE, 2014. Disease and Frost Damage of Woody Plants Caused by *Pseudomonas syringae*: Seeing the Forest for the Trees. *Advances in Agronomy* **126**, 235-293.

Lamichhane JR, Venturi V, 2015. Synergisms between microbial pathogens in plant disease complexes: a growing trend. *Frontiers in Plant Science* **6**,385.

Lanver D, et al. 2017. *Ustilago maydis* effectors and their impact on virulence. *Nature Reviews Microbiology* **15**, 409–42.

Lindroth RL, Roth S, Kruger EL, Volin JC, Koss PA, 1997. CO_2-mediated changes in aspen chemistry: effects on gypsy moth performance and susceptibility to virus. *Global Change Biology* **3**, 279–289.

Lipiec J, Doussan C, Nolsalewicz A, Kondracka K, 2013. Effect of drought and heat stress on plant growth and yield: a review. *International Agrophysics* **27**, 463-477.

Little EL, Bostock RM, Kirkpatrick BS, 1998. Genetic characterization of *Pseudomonas syringae* pv. *syringae* strains from stone fruits in California. *Applied and Environmental microbiology*, 3818-3823.

Long HB, Sun YF, Bai C, Peng DL, 2015. First Report of the Root-Knot Nematode *Meloidogyne enterolobii* Infecting Jackfruit Tree in China. *APS Publications* **99**, 1868.

Malvick DK, Moore LW, 1998. Survival and dispersal of a marked strain of *Pseudomonas syringae* in a maple nursery. *Plant Pathology* **37**, 573-580.

Manion PD, 1991. Tree Disease Concepts. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Manstretta V, Rossi V, 2015. Effects of temperature and moisture on development of *Fusarium graminearum* Perithecia in maize stalk residues. *Applied Environmental Microbiology* **82**, 184–191.

Marais PG, 1979. Fungi associated with root rot in vineyards in the Western Cape. *Phytophylactica* **11**, 65-68.

Marais PG, 1980. Fungi associated with decline and death of nursery grapevines in the Western Cape. *Phytophylatica* **12**, 9-13.

Maseko BOZ, Coutinho TA, 2002. Pathogenicity of *Phytophthora* and *Pythium* species associated with citrus root rot in South Africa. *South African Journal for Botany* **68**, 327-332.

Mboup M, Bahri B, Leconte M, de Vallavieille-Pope C, Kaltz O, Enjalbert J, 2012. Genetic structure and local adaptation of European wheat yellow rust populations: the role of temperature-specific adaptation. *Evolutionary Applications* **5**, 341-352.

Meehl GA, Stocker TF, Collins WD et al, Z., 2007. Global climate projections. In: Solomon S, Qin D, Manning M, Chen Z et al. (Eds.), Cambridge University Press, Cambridge, U.K. and New York, NY.

Mircetich SM, Matheron ME, 1976. *Phytophthora* root and crown rot of cherry trees. *Phytopathology* **66**, 549-558.

Mittler R, 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science* **11**, 15–19.

Mohammidi M, Ghasemi A., Rahimian H, 2001. Phenotypic characterization of Iranian strains of *Pseudomonas syringae* pv. *syringae* van Hall, the causal agent of bacterial canker disease of stone fruit trees. *Journal of Agricultural Sciences and Technology* **3**, 51-65.

Monaghan J, Zipfel C, 2012. Plant pattern recognition receptor complexes at the plasma membrane. *Current Opinion in Plant Biology* **15**, 349–357.

Moore LW, 1988. *Pseudomonas syringae*: Disease and Ice Nucleation Activity. *Ornamentals Northwest Archives* **12**, 3-16.

Mordecai EA, Gross K, Mitchell CE, 2016. Within-host niche differences and fitness trade-offs promote coexistence of plant viruses. *American Naturalist* **187**, E13–E26.

Morgan W, Kamoun S, 2007. RXLR effectors of plant pathogenic oomycetes. *Current Opinion in Microbiology* **10**, 332-338.

Mostowfizadeh-Ghalamfarsa R, Banihashemi Z, 2005. Identification of soil *Pythium* species in Fars province of Iran. *Iranian Journal of Science and Technology* **29**, 79-87.

Nelson EB, 1994. The disease triangle and the disease cycle. *Turf Grass Trends*, 1-15.

Nogales-Delgado S, Fuentes-Pérez Mdel C, Bohoyo-Gil D, 2013. Cultivar characterization of stone fruits for their minimal processing. *Journal of Food Science and Technology* 52, 2193–2201.

Noormets, A, Sober, A, Pell, EJ, Dickson R, Polida G, Sober J, Isebrands J, Karnosky D, 2000. Stomatal and non-stomatal control of photosynthesis in trembling aspen (Populus tremuloides Michx.) exposed to elevated CO2 and/or O3. *Plant, Cell and Environment* **24**, 327– 336.

Ogundeji AA & Jordaan H, 2017. A stimulation study on the effect of climate change on crop water and chill unit accumulation. *South African Journal of Science* **113**, 1-7.

Oliveira-Garcia E, Valent B, 2015. How eukaryotic filamentous pathogens evade plant recognition. *Current Opinion in Microbiology* **26**, 92–101.

Ostry ME, Venette RC, Juzwik J, 2011.Decline as a disease category: Is it helpful? *Phytopathology* **101**, 404-409.

Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, et al, 2004. Rice yields decline with higher night temperature from global warming. *Proceeding of the National Academy of Sciences of the USA* **101**, 9971–9975.

Pennington JAT, Fisher RA, 2010. Food component profiles for fruit and vegetable subgroups. *Journal of Food Composition and Analysis* **23**, 411–418.

Pfender WF, Vollmer SS, 1999. Freezing temperature effect on survival of *Puccina gramminis sub sp. graminicola* in *Festuca arundinacea* and *Lolium perenne*. *Plant Disease* **83**, 1058-1062.

Pienaar L, Boonzaaier J, 2018. Drought policy brief Western Cape. *Western Cape Department of Agriculture Bureau for Food and Agricultural Policy*, 1-18.

Pritchard SG, Rogers HH, Prior SA, Peterson CM, 1999. Elevated CO_2 and plant structure: a review. *Global Change Biology* **5**, 807–837.

Proffer TJ, Jones AL, Ehret GR, 1987. Biological species of *Armillaria* isolated from sour cherry orchards in Michigan. *Phytopathology* **77**, 941-943.

Rana I, Randhawa S, 2011. Impact of Climatic Variation on Plant Diseases. *H.P State Council for Science Technology and Environment*, 1-20.

Rendina E, Lim YF, Marlow D, Wang Y, Clarke SL, Kuvibidila S, Lucas EA, Smith BJ, 2012. Dietary supplementation with dried plum prevents ovariectomy-induced bone loss while modulating the immune response in C57BL/6J mice. *Journal of Nutritional Biochemistry* **23**, 60–68.

Rollins JA., Habte E, Templer SE, Colby T, Schmidt J, von Korff M, 2013. Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (Hordeum vulgare L.). *Journal of Experimental Botany* **64**, 3201-3212.

Rose JK, Ham KS, Darvill AG, Albersheim P, 2002. Molecular cloning and characterization of glucanase inhibitor proteins: coevolution of a counterdefense mechanism by plant pathogens. *Plant Cell* **14**, 1329-1345.

Sandermann H, 1996. Ozone and plant health. *Annual Review of Phytopathology* **34**, 347–66.

Sarowar MN, Nasrin T, Naznin T, Hossain Z, Rahman MM, 2019. Molecular identification of oomycete species affecting aquaculture in Bangladesh. *Aquaculture and Fisheries* **4**, 105-113.

Scott P, Bader MKF, Burgess T, Hardy G, Williams N, 2019. Global biogeography and invasion risk of the plant pathogen genus *Phytophthora*. *New Environmental Science & Policy Volume* **101**, 175-182.

Shamsbakhsh M, & Rahimian H, 1997. Comparison of pathogenic strains of *Pseudomonas syringae* on various plants in Iran. *Iranian Journal of Plant Pathology* **33**, 133-143.

Shao HB, Chu LY, Jaleel CA, Zhao CX, 2008. Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies* **331**, 215-225.

Shao HB, Liang MA, Shao MA, 2006. Osmotic regulation of 10 wheat (*Triticum aestivum L.*) genotypes at soil water deficits. *Colloids and Surface B: Biointerfaces* **47**, 132-139.

Shao HB, Liang ZS, Shao MA, Sun Q, HU ZM, 2005. Investigation of dynamic changes of photosynthetic characteristics of10 wheat (*Triticum aestivum L*.) genotypes during two vegetative stages at water deficits. *Colloids and Surface B: Biointerfaces* **43**, 221-227.

Smertenko A, Draber P, Viklicky V, Opatrny Z, 1997. Heat stress affects the organization of microtubules and cell division in Nicotiana tabacum cells. *Plant, Cell and Environment* **20**, 1534–1542.

Sohar K, Helama S, Läänelaid A, Raisio J, Tuomenvirta H, 2014. Oak decline in a southern Finnish forest as affected by a drought sequence. Geochronometria 41, 92–103.

Sonoda RM, 2000. Rio Grande gummosis. In: Timmer LW, Garnsey SM, Graham JH, editors. Compendium of Citrus Diseases. St Paul (MN): *APS Press*, 77.

Spaulding P, 1958. Diseases of foreign trees growing in the United States. U. S. *Department of Agriculture Handbook* 139.

Spies CFJ, Mazzola M, McLeod A, 2011. Characterisation and detection of *Pythium* and *Phytophthora* species associated with grapevines in South Africa. *European Journal of Plant Pathology* **131**, 103-119.

Srinivasan C, Dardick C, Callahan A, Scorza R, 2012. Plum (*Prunus domestica*) trees transformed with poplar FT1 result in altered architecture, dormancy requirement, and continuous flowering. *PLoS One* **7**, e40715.

Stevens RB, 1960. Pages 357-429 in: Plant Pathology, an Advanced Treatise, Vol. 3. J.G. Horsfall and A.E. Dimond, eds. Academic Press, NY.

Sutton JC, Sopher CR, Owen-Going TN et al, 2006. Etiology and epidemiology of *Pythium* root rot in hydroponic crops: current knowledge and perspectives. *Summa Phytopathologica* **2**, 307-321.

Tao YH, Zeng F, Ho HH et al, 2011. *Pythium vexans* causing stem rot of Dendrobium in Yunnan Province, China. *Journal of Phytopathology* **159**, 255-259.

Tapsoba H, Wilson JP, 1997. Effects of temperature and light on germination of urediniospores of the pearl millet rust pathogen, *Puccinia substriata* var. *indica*. *Plant Disease* **81**, 1049–1052.

Thirtle C, Townsend RF, Amadi J, Lusigi A, Van Zyl J, 1998. The rate of return onexpendtures of the South African Agricultural Research Council (ARC). *Agrekon* **37**, 612-622.

Thomas FM, Blank R, Hartmann G, 2002. Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *Forest Pathology* **32**, 277-307.

Tian M, Benedetti B, Kamoun S, 2005. A Second Kazal-like protease inhibitor from *Phytophthora infestans* inhibits and interacts with the apoplastic pathogenesis-related protease P69B of tomato. *Plant Physiology* **138**, 1785-1793.

Tian M, Huitema E, da Cunha L, Torto-Alalibo T, Kamoun S, 2004. A Kazal-like extracellular serine protease inhibitor from *Phytophthora infestans* targets the tomato pathogenesis-related protease P69B. *Journal of Biological Chemistry* **279**, 26370-26377.

Tjoelker, MG, Oleksyn, J, Reich, PB, 1998. Seedlings of five boreal tree species differ in acclimation of net photo- synthesis to elevated CO_2 and temperature. *Tree Physiology* **18**, 715–726.

Tollenaere C, Susi H, Laine AL, 2016. Evolutionary and epidemiological implications of multiple infection in plants. *Trends in Plant Science* **21**, 80–90.

Tshabalala PM, 2015. Estimating the economic rate of return to plum research in South Africa. MSc thesis, University of Pretoria, Pretoria.

Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RH et al. 2006. *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* **313**, 1261-1266.

Venette RC, 2009). Implication of global climate change on the distribution and activity of *Phytophthora ramorum*. In: K. McManus, & K. W. Gottschalk (Eds.) *Proceedings 20th U.S. Department of Agriculture interagency research forum on invasive species* (pp. 58–59.)

Verniere C, Cohen S, Raffanel B, Dubois A, Venard P, Panabieres, 2004. Variability in pathogenicity among *Phytophthora* spp. isolated from citrus in Corsica. *Phytopathology* **152**, 476-483.

Vicente AR, Manganaris GA, Sozzi GO, Crisosto CH, 2009. Chapter 5—Nutritional quality of fruits and vegetables. In: Florkowski WJ, Shewfelt RL, Brueckner B, Prussia SE, eds. Postharvest handling. 2. San Diego: Academic, 57–106.

von Tiedemann A, Firsching KH, 2000. Interactive effects of elevated ozone and carbon dioxide on growth and yield of leaf rust-infected versus non-infected wheat. *Environmental Pollution* **108**, 357–363.

Wahid A, Gelani S, Ashraf M, Foolad MR, 2007. Heat tolerance in plants: an overview. *Environmental and Experimental Botany* **61**, 199–223.

Wang S, Boevink PC, Welsh L, Zhang R et al, 2017. Delivery of cytoplasmic and apoplastic effectors from *Phytophthora infestans* haustoria by distinct secretion pathways. *New Phytologist* **216**, 205–215.

Way H, Chapman S, McIntyre L, Casu R, Xue GP, et al, 2005. Identification of differentially expressed genes in wheat undergoing gradual water deficit stress using a subtractive hybridisation approach. *Plant Science* **168**, 661–670.

Wenneker M, Janse JD, de Bruine JA, 2011. Bacterial canker of plum trees, caused by *Pseudomonas syringae* pathovars, as a serious threat for plum production in the Netherlands. *Communications in Agricultural and Applied Biological Sciences* **76**, 575-8.

Werres S, Wagner S, Luttman R, Diener HA, 2007. Survival of *Phytophthora ramorum* in recirculating irrigation water and subsequent infection of Rhododendron and Viburnum. *Plant Disease* **91**, 1034-1044.

Whisson SC, Boevink PC, Wang S, Birch PRJ, 2016. The cell biology of late blight disease. *Current Opinion in Microbiology* **34**, 127-135.

Whitmore AP, Whalley WR, 2009. Physical effects of soil drying on roots and crop growth. *Journal of Experimental Botany* **60**, 2845–2857.

Whitmore AP, Whalley WR, 2009. Physical effects of soil drying on roots and crop growth, *Journal of Experimental Botany* **60**, 2845-2857.

Wilcox WF, Mircetich SM, 1985. Pathogenicity and relative virulence of seven *Phytophthora* spp. on Mahaleb and Mazzard cherry. *Phytopathology* **75**, 221-226.

Wustman BA, Oksanen E, Kannosky DF, Sober J, Isebrands GR, Hendrey GR, Pregitzer KS, Padila GK, 2001. Effects of elevated CO_2 and O_3 on aspen clones vary in O_3 sensitivity: can CO_2 ameliorate the harmful effects of O_3? *Environmental Pollution* **115**, 473–481.

Yang X, Hong X, 2016. Diversity of population of *Phytophthora*, *Phytopythium* and *Pythium* species recovered from sediments in an agricultural run-off sedimentation reservoir. *Plant Pathology* **65**, 1118-1125.

Yang X, Richardson PA, Olson HA, Hong CX, 2013. Root and stem rot of begonia caused by *Phytopythium helicoides* in Virginia. *Plant Disease* **97**, 1385.

Yordanov I, Velikova V, Tsonev T, 2000. Plant responses to drought, acclimation, and stress tolerance. *Photosynthetica* **38**, 171–186.

Zwart DC, Kim S, 2012. Biochar amendment increases resistance to stem lesions caused by *Phytophthora* spp. in Tree Seedlings. *American Society for Horticultural Science* **47**, 1736–1740.

CHAPTER 2

Identification of oomycetes associated with plum orchards in the Western Cape Province of South Africa

2.1 Abstract

Plums are one of the largest exported fruit crops in South Africa. In the past few years, there has been a slow decline of plum trees in the Western Cape Province of South Africa. It has been suggested that this is due to climate change, in combination with various biotic factors such as bacteria, oomycetes and nematodes. Sampling was conducted during 2017 and 2018 at several plum orchards in the Western Cape Province, to determine if oomycetes were among the causal agents of the plum tree decline. The aim of this study was to isolate and identify oomycetes isolated from plum trees displaying symptoms of trunk cankers, gummosis and brown/red lesions in the inner bark, as well as from the rhizosphere soil of apparently healthy and diseased trees in this region. Baiting technique was used to isolate oomycetes from the rhizospheric soil, followed by the transfer of lesions on the baiting material onto selective media NARPH. Oomycetes on infected plant material were plated directly onto the selective media. The isolates were identified as *Phytophthora*, *Phytopythium* and *Pythium* based on the amplification and sequencing of the ITS region of the ribosomal DNA. Species recovered from the rhizosphere soil were *Phytophthora multivora*, *Phytopythium vexans*, *Pythium coloratum*, *P. diclinum*, *P. irregulare*, and *P. ultimum*. The only species that was recovered from infected plant material was *Phytopythium vexans*. Overall, the results revealed that only *Phytopythium vexans* was associated with symptomatic plum trees. This is the first study to provide evidence of the association of *Phytopythium vexans* with plum trees.

Keywords: oomycetes, Internal transcribed spacer (ITS), soil baiting

2.2 Introduction

Plums are one of the most economically important stone fruit crops in the genus *Prunus* belonging to the family Rosaceae (Okie and Ramming, 1999; Venter et al, 2014). In South Africa, Western Cape Province is the largest producer of stone fruit (Hortgro, 2016). The South African plum industry is well established with 70% of the plum production supplied to the export market in Europe, the United Kingdom and the Middle East (Hortgro, 2017; Nyawo, 2017).

Recently, there has been a slow decline of plum trees in major orchards located in the Western Cape. Symptoms of this slow decline are gradual reduction in growth and vigor, and discoloration and chlorosis of leaves that leads to defoliation (Wen-Hsiung, 2009). Plum trees are long-lived and over time they become susceptible to pathogen attack and extreme environmental conditions. The combination of these stress factors negatively affects the overall function of a plum tree. Extreme environmental conditions such as drought usually weakens the resistance of the trees, thus making the tree susceptible to pathogen attack. Between 2014-2019, the Western Cape Province experienced the worst drought since 1904, which has negatively affected the plum trees (Botai et al, 2017; Pienaar and Boonzaaier, 2018). Due to the drought farmers resort to almost seven hours of irrigation twice or three times a day to compensate for the water loss. This creates a potential for water-logging which is a favorable environment for zoospore release, since the incidence and severity of Phytophthora infections is closely related to soil moisture conditions (Utkhede and Smith, 1996).

It has been suggested that the slow decline of plum trees in the Western Cape is due to the prolonged drought periods, in combination with pathogenic microbes such as *Pseudomonas syringae* pv. *syringae*, oomycetes, infection by ring nematodes, fungal pathogens and phytoplasmas (Wenneker et al, 2011; Gurdeep et al, 2012). This type of complex was reported in peach orchards in the United states, were trees suffered from peach tree short-life syndrome, where trees are often killed by the combination of nematodes, bacterial canker and cold injury (Okie et al, 2009). The primary disease symptoms displayed by the infected trees were cankers on the trunks of the trees. Advanced symptoms on the diseased trees were gummosis from the cankers, brown/red lesions on the inner bark and eventually death of the trees. Trunk cankers are an economically important disease in the plum industry, and the one pathogen most commonly associated with it is *Pseudomonas syringae* (Wenneker et al, 2011). However, other pathogens,

including fungi, are also capable of causing cankers, therefore, diagnosis should be approached with an open mind (Nelson and Hudler, 2007).

Limited information is available on the role played by oomycetes, if any, in the development of trunk canker and gummosis. The main symptoms displayed by the diseased plum trees suggesting oomycete involvement is the 'bleeding' of the canker, as well as the brown to red color of the lesion on the inner bark (Hayden et al, 2013). Infected stone fruit trees usually exudes gum from the bark, and the gum darkens with age. This disease is usually caused by one of the three *Phytophthora* species; *P. cactorum*, *P. cinnamomi* and *P. cambivora*, and *P. cactorum* is the most widely spread (Adem, 2010). Aerial *Phytophthora* infections have been reported in infected almond trees. Almond trees are fasting growing and their rapid growth this usually results in weak tissues that split easily, creating entry for pathogens. During extreme rainfall and irrigation, the wood remains wet for long periods which create favorable conditions for aerial *Phytophthora* infections (Yamashiti, 2018). Some oomycetes capable of causing plant diseases belong to the genera *Pythium* and *Phytopythium*, and they are usually abundant in the rhizosphere soil of infected citrus trees (Maseko and Coutinho, 2002; Benfradj et al, 2017). Species belonging to these genera are capable of causing devastating plant diseases either as a single pathogen or in complexes with other pathogens (Belhaj et al, 2016; Larousse and Galiana, 2017). *Phytophthora cactorum* has been frequently isolated from cankers located below ground, and *P. citricola* is usually limited to above ground portion of almond trees (Browne and Viveros, 1999). *Phytophthora megasperma* has also been isolated from diseased plum in Turkey which displayed symptoms of decline such as leaf discoloration, twig dieback and reddish brown cankers on roots and stems (Kurbetli et al, 2017). There is evidence that proves *Phytophthora* species to be causal agents of trunk cankers of stone fruit in various countries, however, they have not been investigated in plum trees in South Africa.

The role played by oomycetes in plum tree decline in the Western Cape Province of South Africa is unclear and has not yet been investigated. Therefore, the aim of this study was to isolate and identify the oomycetes isolated from trees displaying trunk cankers, gummosis, and brown/red lesions on the inner bark, as well as from rhizosphere soil collected from diseased and healthy trees using molecular techniques.

2.3 Materials and methods

2.3.1 Sampling and isolation of oomycetes

In 2017-2018, six farms located in the Western Cape Province of South Africa were selected for investigation, due to reports of slow decline in plum trees (Figure 3). In order to determine the cause of slow tree decline, sampling was conducted at two different periods, March 2017 and October 2018. The population density often differs throughout the year; therefore, sampling at different times will ensure that the entire population is represented (Shearer and Shea, 1987; Bush et al, 2003).

Trees displaying visible symptoms of stem cankers, gummosis, shoot-dieback and wilt were tested for the presence of oomycetes (Figure 1). Samples compromised of soil and fine roots (50 samples in total) from the rhizosphere soils of healthy and diseased trees, including plant material (20 samples in total). At each sampling site, i.e. orchard, trees were randomly selected, debris was removed and about 4-5 cm of topsoil was collected (Bose et al, 2018). They differed in age and cultivar. The soil samples were combined/per orchard and were stored in brown paper bags in room temperature until baiting. Trees displaying symptoms were randomly selected and infected plant material was collected.

Soils were divided into 100 g and placed into 350 ml plastic containers and were flooded with deionized water overnight to the depth twice as that of the soil. Floating debris was removed with paper towels, and soil samples were baited with citrus leaves, plum leaves, and white rose petals. Samples were incubated at room temperature and baits were monitored regularly for 5-10 days for signs of infections. Infected leaves developed blackened lesions, and infected rose petals developed water-soaked and brown lesions. Lesions from infected baits were removed from the water, blotted dried and plated into Petri dishes containing *Phytophthora*-selective medium NARPH (Masago et al, 1977). Petri dishes were incubated at 25^{o}C in the dark, and pure cultures were established on ½ PDA (Potato Dextrose Agar 20 g (Sigma-Aldrich, USA) and Nutrient Agar 7 g (Merck, USA)) and incubated at the same conditions.

Cankered bark tissue, including the inner bark, was rinsed with deionized water to remove dirt, and the outer bark was carefully removed. A sterile scalpel was then used to cut into the inner bark and about 2-3 mm pieces of plant tissue, which included diseased and healthy tissue, was

plated directly into Petri-dishes containing *Phytophthora*-selective medium NARPH. Petri dishes were incubated under the same condition as described above.

2.3.2 Identification of oomycetes isolates

Mycelia from isolates grown on ½ PDA were harvested by scraping them from the agar surface using a sterile spatula. Thereafter, genomic DNA was extracted using the Prepman kit (Applied Biosystems, USA). Molecular identification was performed by amplifying and sequencing the Internal Transcriber Spacer (ITS) regions using primers ITS4 and ITS6 (White et al, 1990; Cooke et al, 2000). Polymerase chain reactions consisted of 25µl reaction mixture containing: 1 µl of DNA template, 5 µl MyTaq reaction buffer (Bioline, UK), 0.5 µl MyTaq DNA polymerase (Bioline, UK), 0.5 µl of each primer and 17.5 µl PCR grade water. The PCR reactions were carried out in the following conditions: denaturation at 94 ^{o}C for 3 min, followed by 35 cycles of 94 ^{o}C for 1 min, 57 ^{o}C for 1 min, 72 ^{o}C for 1 min and 72 ^{o}C for 10 min. The resulting PCR products were separated by 1 % agarose gel electrophoresis. The DNA sequencing facility of the University of Pretoria sequenced the amplicons. Preliminary identification of the amplicons was done using BLAST (Altschul et al, 1990) algorithm available through NCBI GenBank.

2.3.3 Phylogenetic analyses of the sequence data

Sequence data that used in this phylogenetic analysis was retrieved from NCBI GenBank. The selected sequences had a BLAST hit of above 98% similarity. The representative sequences were sequences within each genus. The genera included in the phylogenetic analysis were *Phytophthora*, *Phytopythium*, *Pythium* and the outgroup *Albugo*. The alignments were edited and refined with MEGA.5 software (Tamura et al, 2011), and jModelTest (Darriba et al, 2012) was used to statistically select the best-fit model nucleotide substitution (Posada, 2008). The best-fit model was identified as General Time Reversible (GTR+G). A Maximum Likelihood phylogenetic tree was constructed using GTR+ of G substitution model using PhyML. The branch support was based on 1000 bootstrap replications. The phylogenetic tree was visualized and rooted using MEGA.5 software.

2.4 Results

2.4.1 Sampling and isolation of oomycetes

A total of 44 isolates were recovered from fifty soil samples and 15 infected plant material in the Western Cape Province (Figure 3). No isolates were obtained from 5 plant samples. Oomycetes species were readily isolated from soil samples taken from rhizosphere as compared to samples taken from infected plant tissue. The occurrence and distribution of the species varied from farm to farm. Species dominance differed at each farm throughout the duration of the study. The pathogen diversity also fluctuated among the different baiting material. The highest population diversity was observed during spring (October). The recovered species are shown in Table 1.

2.4.2 Identification of oomycetes isolates

The PCR of the amplified ITS regions of the isolates resulted in a single band of 900 base pairs. Based on the molecular identification using BLAST the species belonged to the genera *Phytophthora, Phytopythium* and *Pythium*. The isolates represented six taxa: *Phytophthora multivora*, *Phytopythium vexans*, *Pythium coloratum*, *P. diclinum*, *P. irregulare* and *P. ultimum* (Figure 2). The only taxon recovered from the infected plant material was *P. vexans*. The most efficient baits used were the rose petals followed by citrus land *Prunus* leaves (Table 1). The isolation success varied considerably during the different sampling times, as well as between different farms. During the first sampling, *P. multivora* and *P. irregulare* were the only taxa recovered. After the second sampling *P. multivora* was not isolated, however, there was a variety of *Pythium* species and *P. vexans* was among the taxa isolated. The species diversity varied among the orchards. *P. multivora, P. coloratum* and *P. vexans* were only recovered at the Wellington area. *P. diclinum* and *P. irregulare* were only recovered in Franschoek. *Pythium ultimum* was the only species recovered in Simondium and *P. vexans* was the only species recovered in Franschoek from infected plant material.

2.4.3 Phylogenetic analysis of sequence data

The taxa in the oomycetes phylogenetic tree clustered into three genera: *Phytophthora, Phytopythium,* and *Pythium* (Figure 2). The outgroup *Albugo candida* is distantly related to all the taxa. *Phytophthora multivora* has a 97% bootstrap statistical value, *P. vexans* 100%, *P. irregulare* 100%, *P. ultimum* 100% and *P. diclinum* and *P. coloratum* 96%.

2.5 Discussion

The aim of this study was to isolate and identify the oomycetes isolated from trees displaying trunk cankers, gummosis, and brown/red lesions on the inner bark, as well as from the rhizosphere soil collected from diseased and healthy plum trees in the Western Cape Province of South Africa. Oomycetes that were detected from the soil and trunks of symptomatic plum trees in this study belonged to three genera: *Phytophthora*, *Phytopythium* and *Pythium*. In total 44 isolates representing six oomycetes species were detected, one *Phytophthora* sp, one *Phytopythium* sp and four *Pythium* spp. All the taxa are known species, viz. *Phytophthora multivora*, *Phytopythium vexans*, *Pythium coloratum*, *Pythium diclinum*, *Pythium irregulare*, and *Pythium ultimum*.

Despite the abundance of oomycetes belonging to *Phytophthora*, *Phytopythium* and *Pythium* being reported at citrus farms, very little is known about the role of oomycetes in infected plum trees in South Africa (Graham and Menge, 2000; Maseko and Coutinho, 2002; Cacciola and Di san Lio, 2008; Benfradj et al, 2017).

In this study, the first sampling was conducted in March 2017 and this was when the Western Cape province was experiencing extreme drought. Symptoms displayed by diseased trees were stem cankers, gummosis, discoloration of the foliage, diebacks, and a few dead trees. The only species that were detected from the soil samples during that time was *Phytophthora multivora* and *Pythium irregulare*. *Phytophthora multivora* is a recently described species that was associated with the decline of natural ecosystems in Western Australia (Scott et al, 2009; Puno et al, 2015). Similar results were observed by Aldaoud et al (2016) who detected *P. multivora* from several soil samples collect from plants displaying dieback symptoms in Australia at the Royal Botanic Gardens Victoria and the Melbourne Museum. *Phytophthora multivora* was also detected from the rhizosphere soil of declining or dead Eucalyptus trees by Scott et al (2009), although in our study we did not detect *P. multivora* on infected plant tissue, they managed to get isolated it from the finer root of infected trees.

During the second sampling the Western Cape Province was experiencing heavy rainfall, and a variety of species were recovered. Three *Pythium* spp. were isolated (*P. coloratum*, *P. diclinum* and *P. ultimum*), and *Phytopythium vexans. Phytopythium vexans* was the most frequently isolated species from soil samples of symptomatic infected plum trees and also the only one

isolated from infected plant tissue. The rest of the species were only isolated from the soil samples. The first report of *Pythium* and *Phytopythium* species being causal agents of citrus gummosis in Tunisia was reported by Benfradj et al (2017). Similarly, they recovered *Pythium dissotocum*, *P. ultimum* and *Phytopythium vexans* from symptomatic infected citrus trees.

Different species were detected during the different sampling times, and the baiting material may have influenced the results. For instance, during the first sampling the only baiting material used was citrus leaves, and for the second samples rose petals and plum leaves were used. These different baiting materials yielded different results. The rose petals recovered a greater species diversity compared to the other baits. Some species might have gone undetected and there might be more diversity of oomycetes than what was recovered from the baits due to several reasons. Certain species may be less competitive under baiting conditions or less responsive to particular baiting material (Arcate et al, 2006). This suggests that in order to get a complete picture of diversity, different baiting material should be used. This will reduce or eliminate any possible bias of baiting material excluding certain taxa (Dick, 1996; Sanchez et al, 2006; Nechwatal et al, 2008).

Environmental conditions are another factor that affects the detection of species. Climate change influences the occurrence of droughts by affecting evapotranspiration (Pereira et al, 2018). Extreme environmental conditions like drought and waterlogging influences the species compositions; therefore, species that thrive under wet and dry conditions will differ (Meisner and de Boer, 2018). This explains why species recovered in this study varied between the two sampling periods. Soil moisture affects the life cycle of oomycetes such as hyphal growth, sporangia formation, and zoospore motility. Wet conditions favor the development and establishment of oomycetes.

During drought conditions the species community consists of species that able to thrive under these harsh conditions. This indicates that *P. multivora* and *P. irregulare* were able to tolerate and survive the drought conditions, compared to other species and there is less competition for food. However, once it rains the water will increase the availability of soluble substrates which will act as food source. This will temporarily relieve the competitive pressure for energy sources, but thereafter the species will resume competition (William and Xia, 2008). Keeping in mind that this will be the recovery phase, and the species that will be abundant will be the ones that

resuscitate faster (Placella et al, 2002). Therefore, recovery phase will influence species composition. This indicates that the *Pythium* and *Phytopythium* species recovered during the second sampling were able to recover quickly and outcompete *P. irregulare* and *P. multivora*.

Species richness is sometimes liked areas, which could be due to different silviculture practices conducted by farmers (Bose et al, 2018). Farmers at plum orchards may be using different fungicides which eliminates some species and not others. The cropping history also plays a role in influencing the species richness (Arcate et al, 2006). Another factor could be season sampling, the samples in this study were conducted in autumn and spring. Meteorological conditions also shape the oomycetes community structure (Lang-Yona et al, 2018). Therefore, in order to increase the accuracy of the detection samples should be taken every season. To our knowledge this is the first report to associate *P. vexans* with diseased plum trees in the plum orchards in the Western Cape Province. This study revealed the diversity of oomycetes present in the rhizosphere soil of plum trees. Having the knowledge of which species are present in these orchards will help in the development of effective disease management strategies.

2.6 Conclusion

Oomycetes from three genera were recovered from the soil and diseased plant material of plum trees in the Western Cape Province. Species that were recovered from the soil samples were *Phytophthora multivora*, *Phytopythium vexans*, *Pythium coloratum*, *P. diclinum*, *P. irregulare* and *P. ultimum*. The only species retrieved from diseased plant material was *P. vexans*, which indicates that this oomycete is associated with diseased plum trees. The occurrence of this pathogen could be related to favorable environmental conditions. Climate change results in the rise of relative humidity, which is ideal for the production of *P. vexans* infective propagules (sporangia and zoospores). There was a change in the variability of species between the two years. In 2017, the only species recovered were *P. multivora* and *P. irregulare* and this was during the drought period. They may have been able to tolerate the extreme drought conditions that other microorganisms found unfavorable. In addition, the choice of baiting material may also have played a role. In 2018, the recovered species were *P. vexans*, *P. coloratum*, *P. diclinum* and *P. ultimum*, and this was during heavy rainfall. During the recovery phase these species have had a better resuscitation strategy than *P. multivora* and *P. irregulare*; thus,

outcompeting them and becoming abundant. In some cases, baiting material may be bias and exclude certain taxa; therefore, different baiting materials should be considered for all experiments. Different species also thrive under different weather condition; therefore, seasonal sampling should be conducted. The internal transcribed spacer (ITS) was used as marker; however, the cytochrome oxidase (cox) mitochondrial DNA should be used since it produces much stronger evidence. Sequence diversity has been reported to be greater in soil communities than in bait communities, so direct DNA extraction from rhizospheric soils should be considered as well. The above recommendations will help provide a more complete picture of the diversity of oomycetes in plum orchards. To our knowledge, this is the first study to report of the occurrence of *P. vexans* in diseased stone fruit trees. This pathogen has been reported in citrus, apple, grapevine farms and woody plantations in South Africa, which indicates that it is important in plant production and should be further investigated. The results of this study have contributed to the knowledge of oomycetes species associated with plum trees in the Western Cape Province of South Africa. Knowing which species are present will help with the development of effective disease management strategies.

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2.8 References

Adem, H, 2010. *Phytophthora* root and trunk rot stone fruit. *Department of Environment and Primary*. Viewed 22 January 2020. http://agriculture.vic.gov.au/agriculture/pests-diseases-and-weeds/plant-diseases/fruit-and-nuts/pome-fruit-diseases/phytophthora-root-and-trunk-rot-stone-fruit.

Aldaoud R, Salib S, Dinh SQ, de Alwis S, Holmes RJ, Edwards J, 2016. Host records for *Phytophthora multivora* associated with dieback in Victoria, Australia. *Australasian Plant Disease Notes* **11**, 1-4.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403 - 410.

Arcate JM, Karp MA, Nelson EB, 2006. Diversity of Peronosporomycete (oomycetes) communities associated with rhizosphere of different plant species. *Microbial Ecology* **51**, 36-50.

Belhaj K, Cano LM, Prince DC, Kemen A, Yoshida K, Dagdas YF, Etherington GJ, Schoonbeek HJ, van Esse HP, Jones JD, Kamoun S, Schornack S, 2016. Arabidopsis late blight: Infection of a nonhost plant by *Albugo laibachii* enables full colonization by *Phytophthora infestans*. *Cell Microbiology* **19**, 1-15.

Benfradja N, Migliorinib D, Luchib N, Santinib A, Boughalleb-M'Hamdi N, 2017. Occurrence of *Pythium* and *Phytopythium* species isolated from citrus trees infected with gummosis disease in Tunisia. *Archives of Phytopathology and Plant Protection* **50**, 286-302.

Bose T, Wingfield MJ, Roux J, Vivas M, Burgess TI, 2018. Community composition and distribution of *Phytophthora* species across adjacent native and non-native forests of South Africa. *Fungal Ecology* **36**, 17-25.

Botai CM, Botai JO, De Wit JP, Ncongwane KP, Adeola AM, 2017. Drought characteristics over the Western Cape Province, South Africa. *Water* 9, 1-16.

Bush EA, Hong CX, Stromberg EL, 2003. Fluctuations of *Phytophthora* and *Pythium* spp. in components of a recycling irrigation system. *Plant Disease* **87**, 1500-1506.

Cacciola SO, Lio G, 2008. Management of citrus diseases caused by *Phytophthora* Spp. In: Ciancio A, Mukerji K. (eds) Integrated management of diseases caused by fungi, phytoplasma and bacteria. *Integrated Management of Plant Pests and Diseases* **3**. Springer, Dordrecht.

Cooke DEL, Drenth JM, Wagels G, Brasier CM, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30**, 17-32.

Darriba D, Taboada GL, Doallo R, Posada D, 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772-772.

Dick MW, 1996. The Saprolegniaceae of environs of Blelham Tarn: Sampling techniques and the estimation of propagule numbers. *Journal of General Microbiology* **42**, 257-282.

Graham JH, Menge JA. 2000. *Phytophthora*-induced diseases. In: Timmer LW, Garnsey SM, Graham JH, editors. Compendium of citrus diseases. 2nd ed. St. Paul (MN): *APS Press* p. 12–15.

Gurdeep S, Virk S, Nagal A, 2012. Citrus diseases caused by *Phytophthora* species. *GERF Bulletin of Biosciences* **3**, 18-27.

HORTGRO, 2016. Key Deciduous Fruit Statistics. *Hortgro* South Africa.

HORTGRO, 2017. Key Deciduous Fruit Statistics. *Hortgro South Africa*.

Hudspeth DSS, Nadler SA, Hudspeth MES, 2000. A cox2 molecular phylogeny of the Peronosporomycetes. *Mycologia* **92**, 674-684.

Kurbetli I, Sulu G, Aydogdu M, Polat I, 2017. First report of crown and root rot of plum caused by *Phytophthora megasperma* in Turkey. *Plant Disease* **101**, 260.

Lang-Yona N, Pickersgill DA, Maurus I, Teschner D, Wehking J, Thines E, Poschl U, Despres VR, Frohlich-Nowisky J, 2018. Species richness, rRNA gene abundance, and seasonal dynamics of airborne plant-pathogenic oomycetes. *Frontiers in Microbiology* **9**, 1-11.

Larousse M, Galiana E, 2017. Microbial partnerships of pathogenic oomycetes. *PLOS Pathogens* **13**, 1-7.

Maseko BOZ, Coutinho TA, 2002. Pathogenicity of *Phytophthora* and *Pythium* species associated with citrus root rot in South Africa. *South African Journal of Botany* **68**, 327-332.

Meisner A, de Boer W, 2018. Strategies to maintain natural biocontrol of soil-borne crop diseases during severe drought and rainfall events. *Frontiers in Microbiology* **9**, 1-8.

Nechwatal J, Wielgoss A, Mendgen K, 2008. Diversity, host, and habitat specificity of oomycete communities in declining reed stands (*Phragmites australis*) of a large freshwater lake. *Mycological Research* **112**, 689-696.

Nelson AH, Huler GW, 2007. A summary of North American hardwood tree diseases with bleeding canker symptoms. *Arboriculture & Urban Forestry* **33**, 122-131.

Nyawo TA, 2017. Fingerprinting and molecular characterization of ARC's apricot and plum collection. Stellenbosch University, Stellenbosch, South Africa.

Okie WR, Ramming DW, 1999. Plum breeding worldwide. *American Society for Horticultural Sciences* **9**, 162-176.

Okie WR, Reighard GL, Nyczepir AP, 2009. Importance of scion cultivar in peach tree short life. *Journal of the American Pomological Society* **63**, 58-63.

Pereira VR, Blain GC, de Avila AMH, Pires RCM, Pinto HS, 2017. Impacts of climate change on drought: changes to drier conditions at the beginning of the crop growing season in southern Brazil. *Bragantia* **77**, 201-211.

Pienaar L, Boonzaaier J, 2018. Drought policy brief Western Cape. *Western Cape Department of Agriculture Bureau for Food and Agricultural Policy*, 1-18.

Placella SA, Brodie EL, Firestone MK, 2012. Rainfall-induced carbon dioxide pulses result from sequential resuscitation of phylogenetically clustered microbial groups. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 10931-10936.

Posada D, 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253-1256.

Puno VI, Laurence MH, Guest DI, Liew ECY, 2015. Detection of *Phytophthora multivora* in the Wollemi Pine site and pathogenicity to *Wollemia nobilis*. *Australasian Plant Pathology* **44**, 205-215.

Sanchez J, Sanchez-Cara J, Gallego E, 2000. Suitability of ten plant baits for the rapid detection of pathogenic *Pythium* species in hydroponic crops. *European Journal of Plant Pathology* **106**, 209-214.

Scott PM, Burgess TI, Barber PA, Shearer BL, Stukely MJ, Hardy GE, Jung T, 2009. *Phytophthora multivora* sp. nov., a new species recovered from declining Eucalyptus, Banksia, Agonis and other plant species in Western Australia. *Persoonia* **22**, 1-13.

Shearer BL, Shea SR, 1987. Variation in seasonal population fluctuations of *Phytophthora cinnamomi* within and between infected *Eucalyptus marginata* sites of southwestern Australia. *Forest Ecology and Management* **21**, 209-230.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S, 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution* **28**, 2731 - 2739.

Utkhede RS, Smith EM, 1996. The effect of three irrigation practices on phytophthora crown and root rot of apple trees under field conditions. *European Journal of Plant Pathology* **102**, 507-510.

Venter A, Joubert E, de Beer D, 2014. Nutraceutical value of yellow- and red-fleshed South African plums (*Prunus salicina Lindl.*): Evaluation of total antioxidant capacity and phenolic composition. *Molecules* **19**, 3084-3109.

Wen-Hsiung KO, 2009. Nature of slow and quick decline of macadamia trees. *Botanical Studies* **50**, 1-10.

Wenneker M, Janse JD, De Bruine JA, 2011. Bacterial canker of plum trees, caused by *Pseudomonas syringae* pathovars, as a serious threat for plum production in the Netherlands. *Communications in Agricultural and Applied Biological Sciences* **76**, 575-578.

White TJ, Bruns T, Lee SJ, Taylor JL, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, SninskyJJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York (NY): Academic Press; p. 315-322.

Williams MA, Xia K, 2009. Characterization of the water-soluble soil organic pool following the rewetting of dry soil in a drought-prone tallgrass prairie. *Soil Biology and Biochemistry* **41**, 21-28.

Yamashita, TT, 2018. Bark necrosis and gummosis-Diagnosis and treatment. *The Future of Agriculture*. Viewed 22 January 2020. https://www.fusion360inc.com/bark-necrosis-and-gummosis-diagnosis-and-treatment/.

2.9 Figures

Figure 1. Symptoms of cankers on the trunk of the tree (A). Symptoms of gummosis affecting one of the main branches of the tree (B). Brown to red lesion on the wood following the removal of the bark (C). Death of a tree (D).

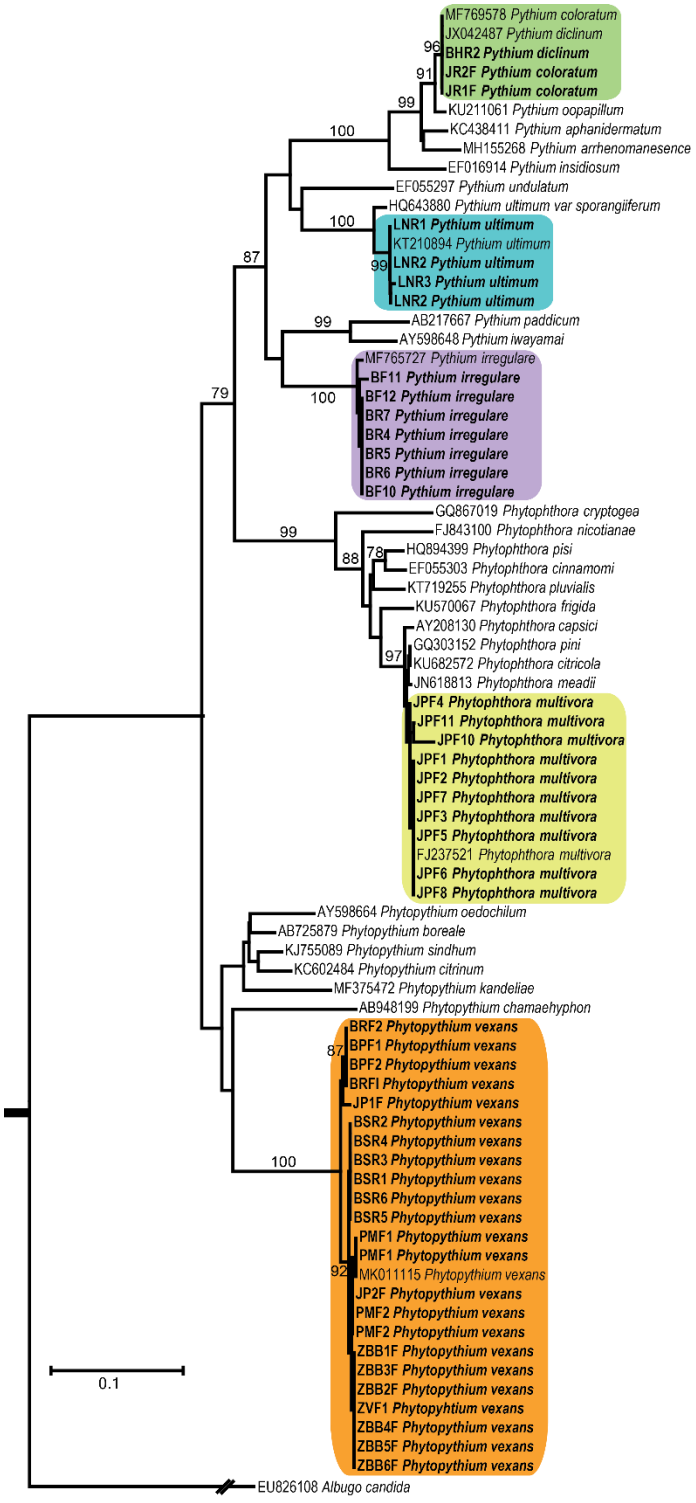

Figure 2. Maximum Likelihood (ML) tree for oomycetes species recovered in this study. The tree was constructed using complete Internal Transcribed Spacer (ITS) region. The taxon names in bold font within highlighted clades indicate isolates recovered from this study. Numbers on the branch shows bootstraps values ≥ 70%.

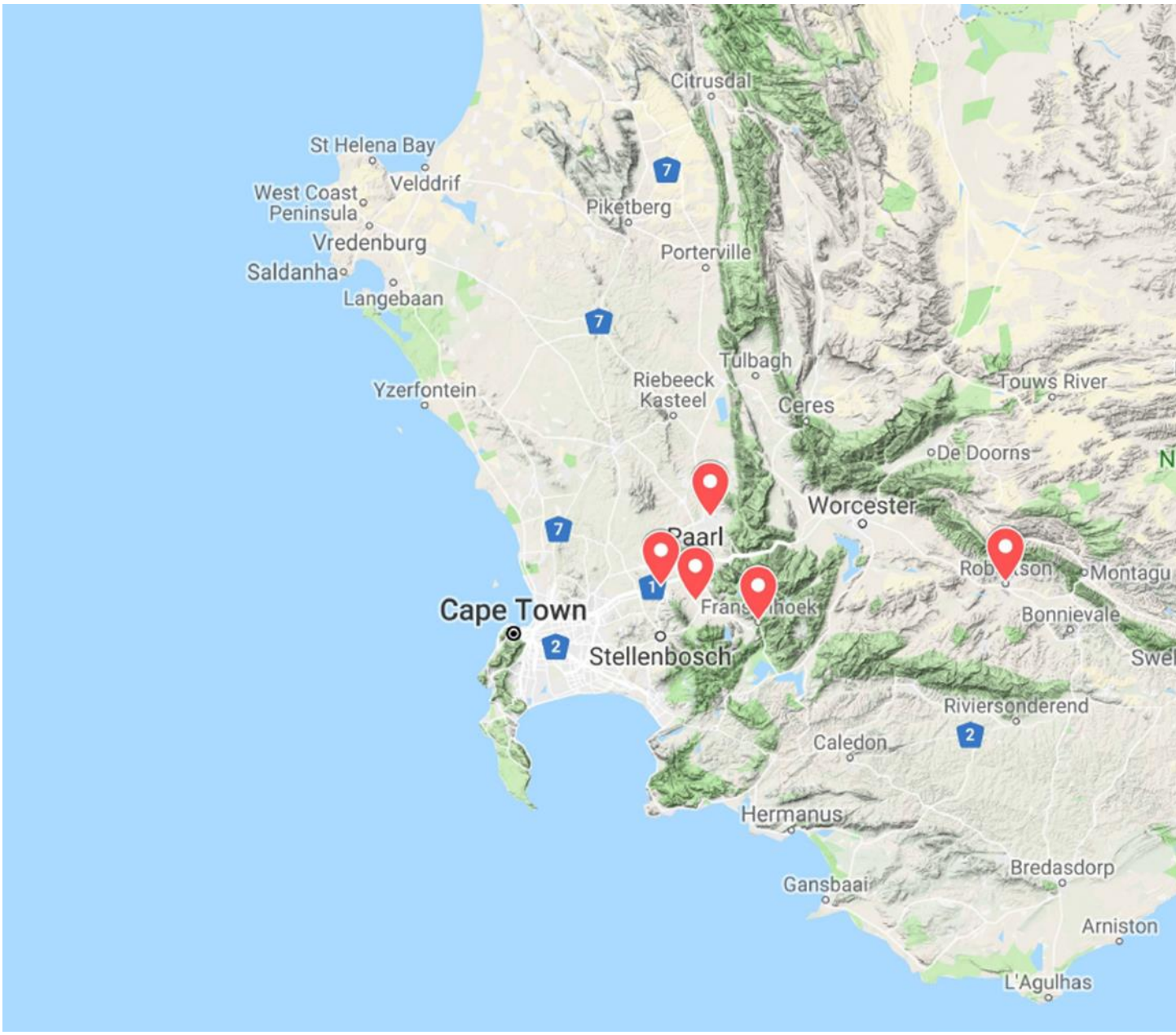

Figure 3. Map of plum production areas in the Western Cape Province of South Africa, red pins representing sampling sites.

Table 1. List of oomycete species isolated from rhizosphere soil and infected plant tissues from symptomatic plum trees.

Species identity	Isolate number	Material	Location*
P. multivora	JPF1	Soil	Wellington
P. multivora	JPF2	Soil	Wellington
P. multivora	JPF3	Soil	Wellington
P. multivora	JPF4	Soil	Wellington
P. multivora	JPF5	Soil	Wellington
P. multivora	JPF6	Soil	Wellington
P. multivora	JPF7	Soil	Wellington
P. multivora	JPF8	Soil	Wellington
P. multivora	JPF10	Soil	Wellington
P. multivora	JPF11	Soil	Wellington
P. irregulare	BR4	Soil	Franschoek
P. irregulare	BR5	Soil	Franschoek
P. irregulare	BR6	Soil	Franschoek
P. irregulare	BR7	Soil	Franschoek
P. irregulare	BF10	Soil	Franschoek
P. irregulare	BF11	Soil	Franschoek
P. irregulare	BF12	Soil	Franschoek
P. diclinum	BHR2	Soil	Franschoek
P. coloratum	JR1J	Soil	Wellington
P. coloratum	JR2F	Soil	Wellington
P. ultimum	LNR1	Soil	Simondium
P. ultimum	LNR2	Soil	Simondium
P. ultimum	LNR3	Soil	Simondium
P. vexans	JP1F	Soil	Wellington
P. vexans	JP2F	Soil	Wellington
P. vexans	BRF1	Soil	Wellington
P. vexans	BRF2	Soil	Wellington
P. vexans	BPFI	Soil	Wellington
P. vexans	BPF2	Soil	Wellington
P. vexans	BSR1	Soil	Wellington
P. vexans	BSR2	Soil	Wellington
P. vexans	BSR3	Soil	Wellington
P. vexans	BSR4	Soil	Wellington
P. vexans	BSR5	Soil	Wellington
P. vexans	BSR6	Soil	Wellington
P. vexans	PMF1	Soil	Wellington
P. vexans	PMF2	Soil	Wellington
P. vexans	ZBB1F	Plant tissue	Franschoek
P. vexans	ZBB2F	Plant tissue	Franschoek
P. vexans	ZBB3F	Plant tissue	Franschoek
P. vexans	ZBB4F	Plant tissue	Franschoek
P. vexans	ZBB5F	Plant tissue	Franschoek
P. vexans	ZBB6F	Plant tissue	Franschoek
P. vexans	ZVF1	Soil	Franschoek

* Samples from some orchards were combined.

CHAPTER 3

Pathogenicity of oomycetes and *Pseudomonas syringae* pv. *syringae* on plum seedlings in greenhouse trials

3.1 Abstract

Recently, field observations reported the appearance of trunk cankers and gummosis in plum orchards in the Western Cape Province of South Africa. This disease is economically important and the pathogen that has been the most frequently recovered is *Pseudomonas syringae* pv. *syringae*. However, cankers observed on some trees resembled those caused by oomycetes, i.e. black discolored outer bark with gummosis and discolored internal tissue present at the tree base. A study was conducted during 2017 and 2018 to identify if oomycetes were responsible for these symptoms. *Phytopythium vexans* was isolated most frequently from the cankers on the aboveground parts of the trees. *Phytophthora multivora was* only isolated from the soil. In this study, pathogenicity trial was conducted to investigate the role (if any) of *P. vexans, P. multivora* and *P. syringae* pv. *syringae* on overall tree health of two plum cultivars under greenhouse environment. Stem inoculations and sand-infestation pot trial were conducted on one-year-old plum seedlings. Roots were inoculated with either *P. vexans or P. multivora*, and *P. syringae* pv. *syringae* was inoculated into the stem in one treatment. The disease severity was compared between seedlings that were infected with a single pathogen, and with those that were co-infected. Seedlings that were stem-inoculated developed lesions after six weeks, and the respective pathogen was re-isolated. The lesions on seedlings inoculated with *Phytophthora* and *P. syringae* pv. *syringae* were larger compared to the ones inoculated only with *P. syringae* pv. *syringae*, and the ones inoculated with *Phytopythium and P. syringae pv. syringae*. For root inoculations there were no disease symptoms, i.e. neither root rot or canker development was observed. Results confirmed that *P. syringae* pv. *syringae* was the cause of the cankers and gummosis. Both *P. vexans* and *P. multivora* were not pathogenic to the plum seedlings under greenhouse conditions.

Keywords: oomycetes, *Pseudomonas syringae*, plum, pathogenicity

3.2 Introduction

Globally, stone fruits such as apricots, cherries and plums are often infected by *Pseudomonas syringae*, causing dieback, bud necrosis and blast, wilting, cankers and gummosis (Scortichini, 2010). This bacterial canker of plum is often referred to as plum decline and it is one of the most economically important diseases of stone fruits (Wenneker et al, 2011). It has been suggested that several abiotic and biotic factors could be the cause of this decline. Trunk cankers and gummosis were observed during field surveys conducted in plum orchards located in the Western Cape Province of South Africa. Cankers were observed on trunks, branches and twigs of infected trees. The Western Cape Province experienced extreme drought conditions between 2014-2018 (Botai et al, 2017). Drought was reported as the major contributing factor for reduction in plum production in 2016 (Pienaar and Boonzaaier, 2018). As drought positively influence disease development (Velasquez et al, 2018; Sinha et al, 2019), hence, those plum tree in the West Cape Province become more susceptible to plant pathogens (Wegulo et al, 2013). For instance, although *Phytophthora* diseases are frequent in poorly drained soils, they are also reported in well drained or dry soil (Desprez-Loustau et al, 2006).

Pseudomonas syringae pv. *syringae* has frequently been identified as the causal agent of bacterial canker of stone fruit trees (Kennelly, 2007; Kim et al, 2017). Although, the majority of the diseased trees in the field displayed bacterial cankers, some trees displayed cankers that resembled those caused by oomycetes, notably *Phytophthora* spp. Several *Phytophthora* species are capable of causing trunk cankers on various tree hosts (Kenaley et al, 2014). These cankers are called 'bleeding cankers', they are dark-sunken cankers with sap oozing from them. Members of the oomycetes are well known, economically important pathogens that cause the destructive diseases of both forest and agricultural trees both in the field and in nurseries (Kamoun, 2003; Kamoun and Smart, 2005; Derevnina et al, 2016; Benfradj et al, 2017).

In Europe, *Phytophthora ramorum* and *P. cinnamomi* have been isolated from trees displaying symptoms of bleeding cankers (Brown and Brasier, 2007). *Phytophthora* spp. have also been associated with the decline of *Quercus* spp. in Eastern and North-central USA (Balci et al, 2007). In South Africa, bleeding cankers caused by *Phytophthora* spp. has been reported on *Quercus cerris* in the Western Cape Province (Oh et al, 2011). Citrus is another host that has been targeted by oomycetes. Canker lesions and gummosis caused by *Phytophthora* spp. have been

observed on Clementine mandarin and Troyer citrange rootstocks since 2002 in the Western Cape Province (Schutte and Botha, 2010). Other oomycetes that have been linked to gummosis of citrus are *Pythium* and *Phytopythium*. They were reported in all the major citrus growing regions in Tunisia experiencing gum disease (Benfradj et al, 2017).

Currently, limited information is available on the role played by oomycetes in the development of trunk cankers and gummosis of plum trees, since most research has focused on bacterial cankers. In addition, research has mainly focused on single host-single disease interactions; however, in nature plants interacts with multiple pathogens which sometimes results in co-infection (Tollenaere et al, 2016; Kozanitas et al, 2017). During an infection, the more virulent pathogen may takeover, or both pathogens may coexist in the same host. This multiple-pathogen complex may influence the severity of the disease expression (Abdullah et al, 2017).

Although, *Pseudomonas syringae* pv. *syringae* has been identified as the causal agent of cankers on stone fruit trees, the possibility that oomycetes may also play a role in this disease has not been investigated. Therefore, the aim of this study is to investigate the potential pathogenicity of *P. multivora* and *P. vexans* on plum seedlings in a greenhouse trial, and further investigate if there is a synergistic effect when seedlings are infected with both these pathogens and *Pseudomonas syringae* pv. *syringae*.

3.3 Materials and method

3.3.1 Bacterial strains

Strains of *Pseudomonas syringae* pv. *syringae* used in this study were obtained from the Plant pathogenic bacteria research group culture collection (BCC 1068) which was isolated from diseased plum trees in the Western Cape. Cultures were grown on King's B agar (200 g Difco proteose peptone, 15 ml glycerol, 1.5 g K_2HPO_4, 1.5 g $MgSO_4$, 15 g agar, 1.5 g boric acid, 8 ml cephalexin, 2 ml cyclohexamide, 1000 ml distilled water) at 25 ^oC before use.

3.3.2 Oomycetes isolates

Phytophthora multivora and *Phytopythium vexans* used in this study were obtained from soil/plant tissue from diseased plum trees in the Western Cape. Cultures were grown on V8 agar (200 ml V8 juice, 3 g $CaCO_3$, 15 agar, 800 ml distilled water) before use.

3.3.3 Plant material

Sun kiss plum cultivars grafted on to a Mariana rootstock were used as host material in these assays. The seedlings were planted a year before the inoculation trial was conducted. For sand-infestation system, one-year old trees were planted in pots (30 cm diameter x 30 cm deep) containing potting soil, and two plastic pipes (2 cm diameter x 30 cm length) were placed on each side of the stem. The trees were placed on tables in a greenhouse at 20 to 25^{o}C temperatures, watered often and pots were arranged in a randomized block design. The seedlings were divided into seven treatments with seven seedlings in each treatment, and the trial was repeated. The treatments were: control, *P. syringae* only, *P. syringae* with *P. multivora, P. syringae* with *P. vexans*, i.e. 4 treatments. A number of trees in the greenhouse had developed bacterial canker naturally due to their exposure to the disease from infected plum trees in the greenhouse. Forty-two of these trees were included in this trial and the treatments were as follows: control, infected with *P. multivora* only, and *P. vexans* only.

3.3.4 Seedling root inoculations

Inocula of *P. vexans* and *P. multivora* were prepared as follows: 1 liter of vermiculite and 10 g millet seeds were mixed with 600 ml V8 broth (120 ml juice, 480 ml water and 2 g calcium carbonate) and autoclaved twice on two consecutive days. Thereafter the mixture was inoculated with 10 (1 cm) squares of 7-day-old cultures (from active growing margins) and the flasks were incubated at 20 ^{o}C in the dark. The inoculum slurries were then rinsed thrice to remove excess nutrients, and a small amount of each slurry was plated onto selective media to confirm inoculum viability. The plastic pipes were removed, and 5 g of inoculum was added into each hole (5 g x 2 pipes), thereafter the holes were covered with soil. For the control trees, slurry that did not contain any mycelial mats was used. Each seedling was placed in an individual plastic container to prevent water and pathogen exchange between seedlings. The seedlings were then flooded with water for 24 hours following inoculation. After 24 hours, the containers were emptied to allow the soil to drain. The seedlings were well watered, monitored regularly and the greenhouse

was maintained between 21-24^{o}C. After six weeks the seedlings were removed from the pots, the soil was removed from the root systems and roots were examined and decay was scored. Root decay was scored on a 0–6 scale, where 0= no root decay, 1 = less than 5% root tip decay, 2 = 5–25%, 3 = 25–50%, 4 = 50–75%, 5 = 75–99% root tip decay and 6 = dead.

Individual feeder roots were selected at random from each seedling, the roots were then surface sterilized with 50% ethanol and plated onto NARPH medium to confirm infection of root tissue. They were incubated at 25^{o}C in the dark, and pure cultures were established on ½ PDA (Potato Dextrose Agar 20 g and Nutrient Agar 7 g) and incubated at the same conditions for 10 days.

3.3.5 Molecular identification of the reisolated oomycetes

For DNA extraction, mycelia were harvested by scraping them from the agar surface. Thereafter, genomic DNA was extracted using the Prepman kit (Applied Biosystems, USA) following the manufacturer's protocol. Molecular identification was performed by amplifying and sequencing the Internal Transcriber Spacer (ITS) regions using primers ITS4 and ITS6 (White et al, 1990; Cooke et al, 2000). Each 25 µl PCR mixture consisted of: 1 µl of DNA template, 5 µl MyTaq reaction buffer (Bioline, UK), 0.5 µl MyTaq DNA polymerase (Bioline, UK), 0.5 µl of each primer and 17.5 µl PCR grade water. The PCR reactions were carried out in the following conditions: denaturation at 94 ^{o}C for 3 min, followed by 35 cycles of 94 ^{o}C for 1 min, 57 ^{o}C for 1 min, 72 ^{o}C for 1 min and 72 ^{o}C for 10 min. The DNA sequencing facility of the University of Pretoria sequenced the amplicons. Preliminary identification of oomycetes was done using the BLAST algorithm (Altschul et al, 1990) available through the NCBI GenBank.

3.3.6 Seedling stem inoculations

The same plum seedlings as above were used for the stem inoculation trial, the stems were inoculated with *Pseudomonas syringae* pv. *syringae*. Stem inoculations were performed a week after the root inoculations. Single colonies were suspended in phosphate buffer, spun down at 3500 rmp for 10 minutes and re-suspended in phosphate buffer. The concentration of the inoculum was measured using a spectrophotometer, with an optical density of 0.2 (OD_{600}) being ~2x10^{8} CFU/ml (Debener et al, 1991). The bark of the stem was removed with a sterile scalpel. The bacteria were inoculated into the plant by pipetting 200 µl of bacterial suspension into the wound. The inoculation areas were covered with parafilm and plastic tape. Controls were inoculated with sterile deionized water. The seedlings were well watered and kept at the same

conditions as described above. The health of the seedling was monitored regularly. After six weeks, disease symptoms were recorded and scored. Disease symptoms were scored on a 0-4 scale, where 1 = no symptoms, 2 = canker, 3 = canker and gummosis and 4 = dead. Parafilm and duct tape were also removed, and disease severity was evaluated by measuring the lesion that developed around the inoculation areas. Reisolation was done by removing pieces of infected bark and placing them onto King's B agar and incubated at 25 ^{o}C for three days. Thereafter, bacterial colonies were streaked onto King's B agar for purification and incubated at the same conditions as described above.

3.3.7 Molecular identification of the reisolated bacteria

DNA was extracted using the ZymoBiomics DNA miniprep kit (Zymo Research) following the manufacturer's protocol. Each 25 µl PCR reaction consisted of: 1 µl of DNA template, 1 µl reaction buffer (Supertherm), 0.25 dNTPs (Fermentas), 1µl MyTaq DNA polymerase (Supertherm), 1.25 µl Mg_2Cl (Supertherm), 0.2 µl of each primer and 6 µl PCR grade water (WhiteSci). The PCR reactions were carried out in the following conditions: denaturation at 94 ^{o}C for 2 min, followed by 30 cycles of 95 ^{o}C for 45 s, 57 ^{o}C for 45 s, 72 ^{o}C for 1 min and 72 ^{o}C for 5 min. The PCR products were separated by 1 % agarose gel electrophoresis in 1X TAE buffer and then visualized. The DNA sequencing facility of the University of Pretoria sequenced the amplicons. Preliminary identification of bacterial isolates was done using the BLAST algorithm available through the NCBI GenBank.

3.3.8 Statistical analyses

The stem lesion lengths of the treatments were analyzed using ANOVA using the SigmaXL software (Salim et al, 2011). All statistics were considered significant at $P \leq 0.05$. Controls were excluded from the statistical analysis as lesions did not form and disease systems did not develop on these seedlings.

3.4 Results

3.4.1 Seedling root inoculations

After six weeks, seedlings were examined for any root decay or the development of lesions on the roots or at the interface between the above and belowground parts of the trees. All the

uninfected control seedlings did not develop any symptoms, and the seedlings soil-inoculated with *P. vexans* and *P. multivora* developed cankers and gummosis on the stems (Figure 1). Furthermore, no feeder and main root decay was observed on seedlings. Discoloration or diseased tissue was also not observed when the outer layer of the roots was scraped off. Neither of the oomycete species was isolated from plated root tissue material of these seedlings. In addition, seedlings that were soil-inoculated with *P. vexans* and *P. multivora* after they were naturally infected with bacterial canker did not develop any new disease symptoms or any root decay they maintained their initial symptoms which were cankers and gummosis.

3.4.2 Seedling stem inoculations

The comparisons between the isolates were made based on the size of discolored lesions produced underneath the bark at the site of inoculation. Discolored lesions, stem cankers and gummosis indicated the pathogenicity of the isolates.

Six weeks after inoculation, the lesion length of the of the treatments in the uninfected trees were as follows: *Pseudomonas syringae* pv. *syringae* was 9±10 cm, *P. syringae* pv. *syringae and P. multivora* was 13±14 cm, *P. syringae* pv. *syringae* and *P. vexans* 2±3 cm and the controls did not develop any discoloration (Figure 2). *Pseudomonas syringae* pv. *syringae* was reisolated from treatment seedlings, but not from the controls. Significant differences ($P < 0.05$) were observed for the lesion lengths among the treatments with length ranging from 2-14 cm (Tables 1 and 2). The lesion lengths were longer in the *P. syringae* pv. *syringae and P. multivora* treatment, compared to the other treatments (Figure 3). The overall disease symptoms of the treatments were scored as follows: *Pseudomonas syringae* pv. *syringae* was 3 = canker and gummosis, *P. multivora* was 2 = canker, *P. vexans* 2 = canker, *P. syringae* pv. *syringae and P. multivora* was 2 = canker, *P. syringae* pv. *syringae* and *P. vexans* was 3 = canker and gummosis, and the controls did not develop any disease symptoms (Figure 4).

3.5 Discussion

Plum trees in the Western Cape Province displayed symptoms of foliage discoloration, dieback, cankers and gummosis. During sampling, several oomycetes were isolated from the rhizosphere soil of symptomatic plum trees, which included *Phytophthora multivora. Phytopythium vexans* was the only oomycetes species isolated from cankers on symptomatic trees. However, neither

oomycetes species was shown to be pathogenic to any of the two plum cultivars used as a host in this study.

In this study, the pathogenicity tests were conducted in July. *i.e.* in winter, and during this season plum trees are usually dormant. Robin et al (1994) demonstrated seasonal differences in stem lesion development, where trees were most susceptible to *P. cinnamomi* during their active stage compared to the dormancy stage. Navarros et al (2015) also demonstrated this difference by conducting stem inoculation trials using various *Phytophthora* species in two different seasons, summer and winter. Their results showed that during winter, the dormant trees developed much smaller lesions than those observed on actively growing trees. *Phytophthora cactorum* and *P. citricola* have also been reported to be more successful in infection and colonization during warmer periods, than cooler months (Browne and Viveros, 1999). This may be a plausible reason for oomycetes isolates in our study could not cause visible disease symptoms.

Second factor that may have influenced the results of the pathogenicity tests could be the type of rootstock used in the trials. The plum seedlings used in this study were grafted with Marianna rootstocks, which are to some extent resistant to oomycetes infection, particularly to *Phytophthora* species (Browne, 2017). Browne (2017) conducted a series of greenhouse trials to examine the resistance of certain plum rootstocks (Hanse, Marianna, Lovell etc.) to *Phytophthora* species, and the Marianna rootstock was shown to be highly resistant to crown and root rot compared to other tested rootstocks.

Furthermore, seedlings in this study were well watered unlike the plum trees in the Western Cape Province, which were experiencing extreme drought conditions during 2014-2018 that could have participated in the disease development. Water stress negatively impacts a host's resistance, predisposing them to microbial infection (Sturrock et al, 2011; Klutsch et al, 2017; Devkota et al, 2018). Since the drought period was prolonged the infection by *P. vexans* may have been repeated from year to year, which reduced the host resistance, greater inoculum build-up, and rapid spread (Brasier, 1995). Seedlings in our trial were not subjected to prolonged stress; allowing the host to defend to still have resistance.

Plum trees observed in the field also displayed symptoms of bacterial canker which is caused by *Pseudomonas syringae* pv. *syringae.* This is one of the most virulent bacteria responsible for

bacterial canker of stone fruit trees (cherries, apricots, plums etc.). This bacterium is considered an opportunistic pathogen that infects a host plant with compromised resistance, which may be due to abiotic stresses. Factors that increase the incidence of bacterial canker are wounding, and dormant trees have been reported to be more susceptible to this disease compared to those in actively growing stage, and dual infection also increases disease severity (Moore, 1988). Gasic et al (2012) conducted a study to test the pathogenicity of *P. syringae* pv. *Syringae* on several stone fruit species, and the pathogen was successful in causing disease symptoms. Similarly, in our study under the greenhouse environment, *P. syringae* pv. *syringae* successfully infected our host tree species leading to disease development. The symptoms included moderate to severe stem canker and gummosis. In this study it was also observed that seedlings that were co-infected with *P. syringae* pv. *syringae and P. multivora* developed longer lesions, followed by *P. syringae* pv. *syringae* only treatment, and smallest lesions in the *P. syringae* pv. *syringae* and *P. vexans* treatment. In cases were a single host tree is co-infected with more than one pathogen, the disease symptoms may be more severe than when it is a single pathogen involved (Lamicchane and Venturi, 2015).

In South Africa, research on oomycetes especially *Phytophthora* spp. being the causal agent of root rot of stone fruit hosts has never been conducted. Therefore, insufficient information is available regarding the association of oomycetes with plum trees. Even though bleeding cankers were observed in our set of co-infected seedlings yet the pathogenicity yet the pathogenicity of oomycetes could not be confirmed. Therefore, further greenhouse trials should be conducted to confirm the role of *P. vexans* on stem canker development in plum trees, this is essential because *P. vexans* was the only oomycetes species that was recovered from infected plant tissue of symptomatic plum trees collected from the Western Cape Province.

3.6 Conclusion

This study aimed to determine if *P. vexans* and *P. multivora* were pathogens of plum seedlings, and to further investigate if dual infection with *Pseudomonas syringae* pv. *syringae* had a synergistic effect. Based on the greenhouse trials, it can be concluded that the oomycetes species used in this study were not pathogenic to the one-year-old seedlings; however, *P. syringae* pv. *syringae* showed moderate to high level of pathogenicity. Pseudomonas syringae pv. syringae

was able to infect the seedlings and was also re-isolated from the inoculated seedlings. Plum seedlings infected with either of the oomycetes species did not manifest any symptoms neither they could be re-isolated from inoculated plum seedlings. This could be due to Marianna rootstock used in this study was resistant to oomycetes. It has to be noted that the plum trees in the natural field were exposed to drought condition for approximately four years, which possibly made them more susceptible to microbial infection than those in the greenhouse trials. For future studies, other rootstocks should be considered, trials should be conducted during different seasons, and environmental stress factors should also be introduced.

3.7 Acknowledgements

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3.8 References

Abdullah AS, Moffat CS, Lopez-Ruiz FJ, Gibberd MR, Hamblin J, Zerihun A, 2017. Host–multi-pathogen warfare: Pathogen interactions in co-infected plants. Front. *Plant Science* **8**, 1-12.

Agriculture, Forestry and Fisheries, 2017. A profile of the South African plum market value. *Department of Agriculture, Forestry and Fisheries*. South Africa.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403 - 410.

Balci Y, Balci S, Eggers J, MacDonald WL, 2007. *Phytophthora* spp. associated with forest soils in Eastern and North-Central U.S. oak ecosystems. *Plant Disease* **91**, 705-710.

Benfradja N, Migliorinib D, Luchib N, Santinib A, Boughalleb-M'Hamdi N, 2017. Occurrence of *Pythium* and *Phytopythium* species isolated from citrus trees infected with gummosis disease in Tunisia. *Archives of Phytopathology and Plant Protection* **50**, 286-302.

Botai CM, Botai JO, De Wit JP, Ncongwane KP, Adeola AM, 2017. Drought characteristics over the Western Cape Province, South Africa. *Water* **9**, 1-16.

Brasier CM, 1995. *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. *Annuals Sciences Forest* **53**,47 – 58.

Brown, Brasier, 2007. Colonization of tree xylem by *Phytophthora ramorum*, *P. kernoviae* and other *Phytophthora* species. *Plant Pathology* **56**, 227-241.

Browne GT, 2017. Resistance to *Phytophthora* Species among Rootstocks for Cultivated *Prunus* Species. *HORTSCIENCE* **52**, 1471-1476.

Browne GT, Viveros MA, 1999. Lethal cankers caused by *Phytophthora* spp. in almond scions: Specific etiology and potential inoculum sources. *Plant Disease* **83**, 739-745.

Cooke WE, Drenth A, Duncan JM, Wagels G, Brasier CM, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* **30**, 17-32.

Debener T, Lehnackers H, Arnold J, Dangl JL, 1991. Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to phytopathogenic *Pseudomonas syringae* isolate. *The Plant Journal* **3**, 289-302.

Derevnina L, Petre B, Kellner R, Dagdas YF, Sarowar MN, Giannakopoulou A, De la Concepcion JC, Chaparro-Garcia A, Pennington HG, van West P, Kamoun S, 2016. Emerging oomycete threats to plants and animals. *Philosophical Transactions of the Royal Society B* **371**, 1-9.

Desprez-Loustau M, Marcais B, Nageleisen L, Piou D, Vannini A, 2006. Interactive effects of drought and pathogens in forest trees. Annals of Forest Science, *Springer Verlag/EDP Sciences* **63**, 597-612.

Devkota P, Enebak SA, Eckhardt LG, 2018. The Impact of drought and vascular-inhabiting pathogen invasion in *Pinus taeda* Health. *International Journal of Forestry Research*, 1-9.

Gasic K, Prokic A, Ivanovic M, Kuzmanovic N, Obradovic N, 2012. Differentiation of *Pseudomonas syringae* Pathovars Originating from Stone Fruits. Journal *Pesticides. Phytomedicine (Belgrade)* **27**, 2012, 219-229.

HORTGRO, 2017. Key deciduous fruit statistics. *Hortgro* South Africa.

Kamoun S, 2003. Molecular genetics of pathogenic oomycetes. *Eukaryotic Cell* **2**, 191-199.

Kamoun S, Smart CD, 2005. Late blight of potato and tomato in the genomics era. *Plant Disease* **89**, 692-699.

Kenaley SC, Rose C, Sullivan PJ, Hudler GW, 2014. Bleeding canker of European beech in Southeastern New York State: *Phytophthora* species, spatial analysis of disease, and periodic growth affected trees. *HORTSCIENCE* **32**, 113-125.

Kennelly MM, 2007. *Pseudomonas syringae* of disease of fruit trees. *The American Phytopathological Society* **91**, 1-14.

Kim GH, Jang JS, Koh YJ, 2017. Occurrence and epidemics of bacterial canker of kiwifruit in Korea. *Plant Pathology Journal* **33**, 351-361.

Klutsch JG, Shamoun SF, Erbilgin N, 2017. Drought stress leads to systemic induced susceptibility to a nectrotrophic fungus associated with mountain pine beetle in *Pinus banksiana* seedlings. *PLoS ONE* **12**, 1-18.

Kozanitas M, Osmundson TW, Linzer R, Garbelotto M, 2017. Interspecific interactions between the Sudden Oak Death pathogen *Phytophthora ramorum* and two sympatric *Phytophthora* species in varying ecological conditions. *Fungal Ecology* **28**, 86-96.

Lamichhane JR, Venturi V, 2015. Synergisms between microbial pathogens in plant disease complexes: a growing trend. *Frontiers in Plant Science* **6**,385.

Moore LW, 1988. *Pseudomonas syringae*: Disease and ice nucleation activity. *Ornamental NorthWest Archives* **12**, 3-16.

Navarro S, Sims L, Hansen E, 2015. Pathogenicity to alder of *Phytophthora* species from riparian ecosystems in western Oregon. *Forest Pathology* **45**, 356-366.

Oh E, Wingfield BD, Wingfield MJ, Roux J, 2011. First report of *Phytophthora cinnamomi* associated with stem cankers of *Quercus cerris* in South Africa. New Disease Report 24, 11.

Pienaar L, Boonzaaier J, 2018. Drought policy brief Western Cape. *Western Cape Department of Agriculture Bureau for Food and Agricultural Policy*, 1-18.

Robin C, Dupuis F, Desprez-Loustau ML, 1994. Seasonal changes in northern red oak susceptibility to *Phytophthora cinnamomi*. *Plant Disease* **78**, 369-374.

Salim B, Bakheit MA, Kamau J, Nakamura I, Sugimoto C, 2011. Molecular epidemiology of camel trypanosomiasis based on ITS1 rDNA and RoTat 1.2 VSG gene in the Sudan. *Parasites Vectors* **4,** 1-5.

Schuttle GC, Botha WJ, 2010. *Phytophthora citrophthro* a trunk and branch canker on Clementine manderins in the Western Cape Province of Pretoria. *South African Journal of Plant and Soil* **27**, 215-220.

Scortichini M, 2010. Epidemiology and predisposing factors of major bacterial diseases of stone and nut fruit tree species. *Journal of Plant Pathology* **92**, S73–S78.

Sinha R, Irulappan V, Mohan-Raju B, Suganthi A, Senthil-Kumar M, 2019. Impact of drought stress on simultaneously occurring pathogen infection in field-grown chickpea. *Science Reports* **9**, 1-15.

Sturrock RN, Frankel SJ, Brown AV, Hennon PE, Kliejunas JT, Lewis KJ, Worrall JJ, Woods AJ, 2011. Climate change and forest diseases. *Plant Pathology* **60**, 133-149.

Tollenaere C, Susi H, Laine AL, 2016. Evolutionary and epidemiological implications of multiple infection in plants. *Trends in Plant Sciences* **21**, 80-90.

Velásquez AC, Castroverde CDM, He SY, 2018. Plant and pathogen warfare under changing climate conditions. *Current Biology* **28**, 619-634.

Virk SGS, Nagal A, 2012. Citrus diseases caused by *Phytophthora* species. *GERF Bulletin of Biosciences* **3**, 18-27.

Wegulo S, Giesler L, Harveson R, Jackson-Ziems TA, Liu B, Korus K, 2013. Impacts of Drought on Disease Development and Management. *Plant Pathology* **537**, 125-127.

Wenneker M, Janse JD, De Bruine JA, 2011. Bacterial canker of plum trees, caused by *Pseudomonas syringae* pathovars, as a serious threat for plum production in Netherlands. *Communications in Agricultural Applied Biological Science* **76**, 575-578.

3.9 Figures

Figure 1. Symptoms displayed by seedlings after being inoculated with *Phytophthora multivora* (A and B), *Phytopythium vexans* (C), *Pseudomonas syringae* pv. *syringae* and *P. vexans* (D), and *P. syringae* pv. *syringae* (F).

Figure 2. Exposed inner barks showing canker lesions after being root inoculated with oomycetes species and stem inoculated with *Pseudomonas syringae* pv. *syringae*. Control seedlings (A) did not develop any lesions, seedling infected with only *P. syringae* pv. *syringae* (B) developed lesions with size 9±10 cm. *P. syringae* pv. *syringae* with *P. multivora* (C) lesion size of 13±14 cm, and *P. syringae* pv. *syringae* with *P. vexans* (D) 2±3 cm.

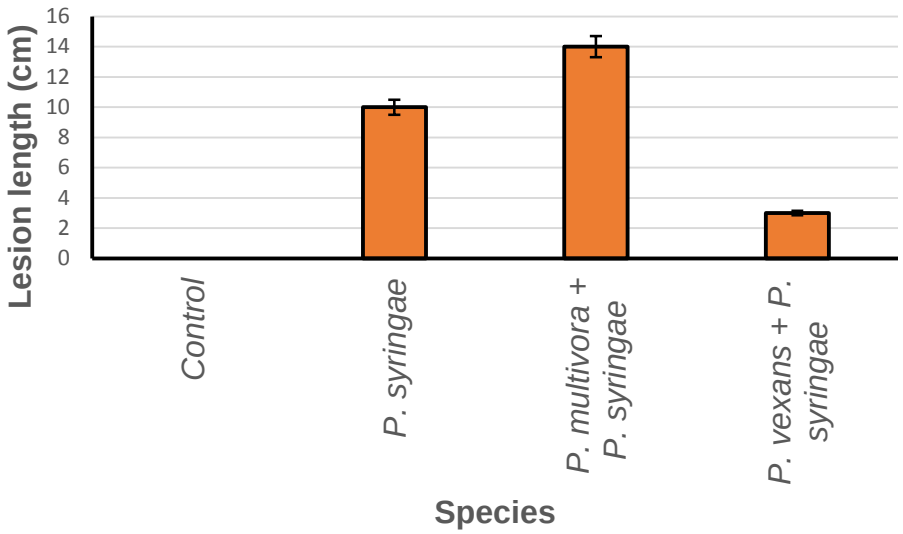

Figure 3. Lesion lengths in the stems of one-year-old plum seedlings after six weeks of root inoculation with oomycetes species and stem inoculations with *Pseudomonas syringae* pv. *syringae*.

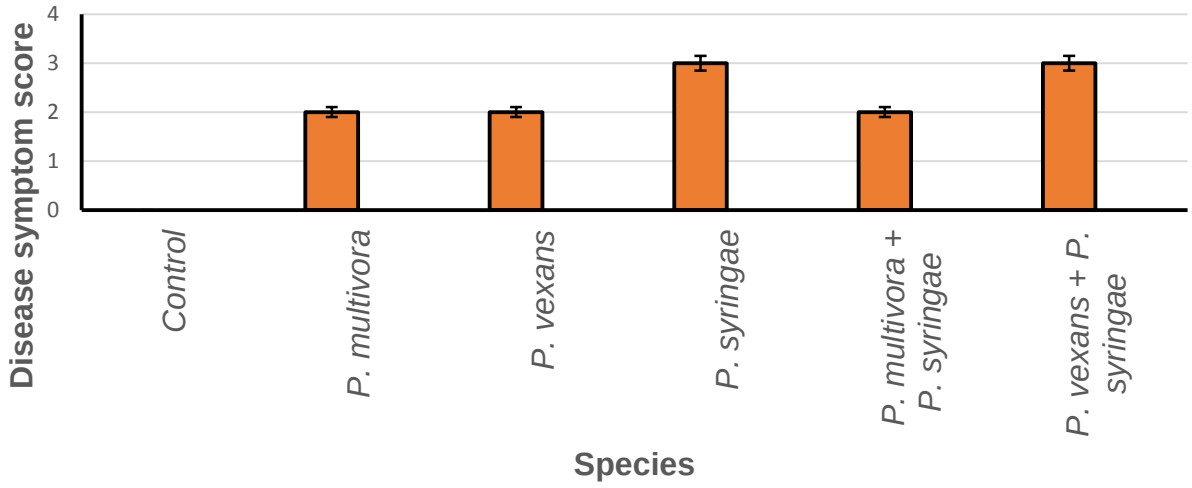

Figure 4. Mean plum seedling overall disease score, measured six weeks after root inoculations with oomycetes species and stem inoculations with *Pseudomonas syringae* pv. *syringae*. Disease symptoms were scored on a 0-4 scale, where 1 = no symptoms, 2 = canker, 3 = canker and gummosis and 4 = dead.

Table 1. Mean values and standard error results for the lesions caused by *Pseudomonas syringae* pv. *syringae*, *Phytophthora multivora* and *Phytopythium vexans* in the stem inoculation trial.

Data Summary				
Treatments	**N**	**Mean**	**Std. Dev**	**Std. Error**
Ps. syringae	14	10.2143	1.3688	0.3658
Ps. syringae* and *P. multivora	14	14.0714	0.9169	0.245
Ps. syringae* and *P. vexans	14	1.8571	0.8644	0.231

ANOVA Summary					
Source	**Degrees of Freedom** **DF**	**Sum of Squares** **SS**	**Mean Square** **MS**	**F-Stat**	**P-value**
Between treatments	2	1091.576	545.788	473.0206	1.1102e-16
Within treatments	39	44.9996	1.1538		
Total:	41	1136.5756			

Table 2. The results for Turkey's post hoc test for the lesions caused by *Pseudomonas syringae* pv. *syringae*, *Phytophthora multivora* and *Phytopythium vexans* in the stem inoculation trial.

Treatment pair	**Turkey HSD Statistic**	**Turkey HSD p-value**	**Turkey HSD Inference**
Ps. syringae **vs** ***Ps. syringae* and *P. multivora***	13.4356	0.0010053	** p<0.01
Ps. syringae **vs** ***Ps. syringae* and *P. vexans***	29.1104	0.0010053	** p<0.01
P. multivora **vs** ***P. vexans***	42.5459	0.0010053	** p<0.01

*Length differs significantly at P<0.01

SUMMARY

The aim of this thesis was to determine the role played by oomycetes in plum tree decline observed in the Western Cape Province of South Africa from 2016-2018. At this time, extreme drought conditions were experienced in the province. Thus, the focus of this study was to identify and characterize oomycetes isolated from both diseased plum tree tissue and rhizosphere soil, and to test their pathogenicity on two plum cultivars.

Chapter 1 reviewed previous literature on plant diseases caused by oomycetes and *P. syringae* pv. *syringae*, and how the disease triangle and climate change influenced disease development. Temperature and moisture were reported as factors that influence disease development by weakening plant hosts when conditions are unfavorable, and also they influence pathogen occurrence and establishment. The literature also highlighted the importance of the interrelations of stress factors involved in decline, including the effect of co-infection in a single host plant.

Chapter 2 of this thesis focused on conducting field surveys and sampling five plum orchards in the Western Cape Province. During the survey, a few trees displayed symptoms of bleeding cankers, which suggested that an oomycete might be a possible causal agent. Isolations from the diseased plant material and soil samples were conducted followed by molecular identifications. Six oomycetes species were identified, which are: *Phytophthora multivora, Phytopythium vexans, Pythium coloratum, P. diclinum, P. irregulare and P. ultimum. Phytopythium vexans* was the only oomycetes that was isolated from infected plant material.

The pathogenicity of *P. multivora* and *P. vexans* (isolated in this study) was determined in Chapter 3 on Sun kiss plum cultivars in a greenhouse environment. *Pseudomonas syringae* pv. *syringae* was included because bacterial canker symptoms were concurrently observed in the field. The pathogenicity trials showed that neither *P. vexans* nor *P. multivora* were able to cause symptoms and were not re-isolated from the inoculated seedlings. Seedlings infected with *P. syringae* showed symptoms typical of bacterial canker and the pathogen was re-isolated from the infected seedlings. Co-infection trials revealed that seedlings inoculated with *P. multivora* and *P. syringae* had larger lesion size compared to other combinations.