Factors that Predisposing to *Persea americana* Mill. to Infections of Botryosphaeriaceae Species in Central Zone of Chile

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Chapter 1

General Introduction

The Chilean fruticulture is a mature and dynamic industry, which has established itself in important markets worldwide, thanks to its constant concern to offer fruit of quality demanded by consumers. Chile is also regarded as the leading exporter of fresh fruit from the southern hemisphere, because the main focus of the fruit sector has been to maintain competitiveness and meet the annual demand for fresh fruit and off-season; despite the distance to destination countries, which has meant a challenge for the fruit industry, given that fruit to withstand transport conditions for long periods of time and postharvest long life is required. The effects of adverse weather conditions, such as drought and frost in spring and winter, have caused seasonal fluctuations in fruit available in different seasons. Also, the exchange rate and the international economy have influenced in production costs and the value obtained by product in the different countries. In another hand, the porter problems which have caused the fruit to remain in storage for long periods, which cause effects on quality of life in post-harvest.

In the fruit industry has been essential to preserve an optimal phytosanitary standard to prevent disturbance to the quality of the product exported, and thus achieve the interest of maintaining traditional markets and expanding the offering to new countries, to increase the participation of Chilean fruit internationally.

The cultivation of avocado trees in Chile

Chile is considered one of the largest avocados producing countries in the world, with 29.289 ha planted mainly between Coquimbo Region (29° 20'S to 32° 15'S) and O'Higgins Region (33° 51'S to 35° 01'S) being Valparaiso Region the most productive (Muñoz, 2018).

The avocado is a subtropical fruit, which requires managements to adapt the Mediterranean climate conditions that exist in central Chile. Such managements have allowed adapting to the availability of water, soil salinity, irrigation, climate, among others, but production in Chile is presenting problems in terms of productivity, due to the effects of drought and frost. Also, there is a rise in production costs mainly due to higher cost of labor and energy. Therefore, is required optimize resources to overcome constraints and increase the productivity of orchards considering edaphoclimatic and management aspects, as well as water availability.

Export of avocados

Avocados are produced in México, Chile, Israel, South Africa, Spain, Peru, Brazil and USA, supplying the international market year-round. In Chile, the main exported variety is Hass, which are oval, with rough skin that turns to black in ripening. Pulp is green-yellowish and has small to medium seed (White *et al.,* 2005). These fresh fruits are increasingly fruit valued by consumers (Johnson and Kotzé, 1994). The export of Chilean Hass avocado is for shipping to markets like USA, Europe, Asia and South America, so it is essential to produce fruit with long postharvest life, which

supports long periods of storage and retaining the quality required by countries of destination.

Commercially, the quality of avocado fruit is rated according to size, oil content (dry matter), firmness but mainly absence of defects (Landahl *et al.,* 2009). Therefore, is essential to ensure that harvesting and packaging to be careful to avoid damaging the fruits. Currently, it has grown the supply of other producing countries, which have the ability to achieve higher production volumes, which is necessary to reach markets in periods of peak demand, with quality fruits and stable volumes, to achieve high prices and to maintain profitability, which is achieved only if the fruit keeps well on the tree, there is good availability of water and nutrients, and storage conditions are appropriate.

Storage should be under controlled conditions of temperature, humidity, CO_2 and O_2 to avoid changes that alter the fruit before marketing and consumption, because the Hass avocado is susceptible to chilling injury when stored at temperatures below 4-5°C and due to their high respiratory rate and ethylene release, could have a short-media shelf life (Defilippi *et al.*, 2014).

Effect of pathogens in the deterioration of the fruit

The damage that can cause some abiotic factors and management techniques used preharvest can cause stress on the trees, which affects their vigor and thus productivity. It also makes him more susceptible to pathogens that might be in the orchard, which can cause alterations in the quality of the fruit produced.

The spores of pathogenic fungi must attach to the surface, germinate, produce penetration structures or penetrate directly through wounds, and activate pathogenicity factors in order for the process of decay to develop (Prusky, 1996).

Immature avocado fruits are relative resistant to postharvest rots. However, as fruits start to ripen, they can be severely affected by rots and physiological disorders (White *et al.,* 2005). Some postharvest rots in avocado fruit can be the result of latent infections initiated on the tree during the growing season (Hopkirk *et al.,* 1994). The latent infections are a condition in which the pathogen spends long periods during the host's life in a quiescent stage until, under specific circumstances, it becomes active (Verhoeff, 1974). Therefore, the occurrence and maintenance of pathogen latent on or with the host indicate a dynamic equilibrium among host, pathogen, and environment, without any visible symptoms (Jarvis, 1994).

Some avocado disease with latent infections are anthracnose caused by *Colletotrichum gloeosporioides* in worldwide (Dann *et al*., 2013) and stem end rot caused by a fungal complex of some species of the family Botryosphaeriaceae such as *Lasiodiplodia theobromae* in Italy (Dann *et al.,* 2013; Garibaldi *et al.,* 2012) and *Neofusicoccum luteum* in California (Twizeyimana *et al.,* 2013), along with other identified species, such as *Colletotrichum gloeosporioides* in California (Twizeyimana *et al.*, 2013), *Pestalotiopsis clavispora* in Chile (Valencia *et al.*, 2011), *P. versicolor* in South Africa (Darvas and Kotze, 1987) and *Phomopsis* sp. in California (Twizeyimana *et al.,* 2013), which significantly reduces fruit quality and postharvest life (Menge and Ploetz, 2003; Johnson and Kotze, 1994).

Pathologies associated with Botryosphaeriaceae species.

The Botryosphaeriaceae encompasses a range of morphologically diverse Ascomycota fungi that are pathogens, endophytes or saprobes, mainly on woody hosts. They are found in all geographical and climatic areas of the world, with the exception of the Polar Regions (Phillips *et al.*, 2013).

The anamorphic asexual stage of Botryosphaeriaceae family is commonly observed in nature (Jacobs *et al.*, 1998) and pycnidia can be observed protruding from the bark in or around the canker tissue as well as on surrounding dead bark and twigs. The conidia ooze out of pycnidia in a ribbon-like gelatinous matrix and are usually disseminated by rainwater (Baskarathevan *et al.*, 2013; Eskalen *et al.*, 2013; Ridgway *et al.,* 2011; Úrbez-Torres *et al.,* 2010; Van Niekerk *et al.,* 2010; Michailides and Morgan, 1993).and irrigation splash when the water trajectory of sprinkler system come into contact with the canopy (Eskalen *et al.*, 2013; Úrbez-Torres *et al.,* 2010; Michailides and Morgan, 1993).

The members of Botryosphaeriaceae family can cause cankers and fruit rot on a wide variety of woody hosts and can survive as saprophytes or parasites and some species can survive as endophytes in symptomless tissue, with latent infections (Twizeyimana *et al.*, 2013). Their frequent association with plant diseases has stimulated substantial interest in these fungi, and much of this interest has been focused on the systematics of species and genera.

Using anamorph characteristics combined with DNA sequence data has allowed elucidation of the species involved in branch canker and fruit rot disease complexes,

because the teleomorphs of Botryosphaeriaceae spp. in nature or laboratory cultures are rarely seen (McDonald and Eskalen, 2011). In addition, the use of morphological and genomic information has led clarification of the pathogens Botryosphaeriaceae spp. involved in branch canker in almond (Inderbitzin *et al.,* 2010), avocado (McDonald and Eskalen, 2011), blueberry (Espinoza *et al.,* 2009), grape (Baskarathevan *et al.,* 2013; Díaz *et al.,* 2013; Morales *et al.,* 2012; Van Niekerk *et al.,* 2010; Úrbez-Torres *et al.,* 2006), mango (Slippers *et al.,* 2005) olive (Moral *et al.*, 2010), walnut (Díaz *et al.*, 2018), and fruit rot in avocado (Twizeyimana *et al.,* 2013), olive (Lazzizera *et al.*, 2008), and apple (Cáceres *et al.*, 2016), among others.

Effect of Botryosphaeriaceae species in growing avocado

The species of Botryosphaeriaceae are considered like opportunist pathogens in avocado trees, because did not damage to healthy trees, and post latent phase have ability to rapidly cause disease when their host are under stress (Slippers and Wingfield, 2007).

Death of young trees. The disease can be a serious problem in new plantings that are established with nursery stock that is latently infected at the graft union; these infections can kill the graft union (Dann *et al.*, 2013). The fungus remains in the graft union after it heals and, when a later stress occurs, the tree collapses and dies. The main symptom is graft death with rootstock that has green shoots and leaves. The brownish discoloration in the wood at the graft union is a key symptom (Menge and Ploetz, 2003).

Leaf blight. When leaves are infected but are attached to healthy stems, leaves often are mostly green with necrosis only in brown patches along leaf margins and at tips (Dann *et al.*, 2013).

Dieback. Dieback occurs in small twigs, retaining dead leaves and fruits. The leaves turn brown and the fruit completely black with advanced stages of softening, which may be for several months in these twigs (McDonald and Eskalen, 2011).

Branch Canker. Initial symptoms correspond to rough protuberances on the bark in trunk and twigs, with internal diseased tissue or dead. Subsequently develop cankers on the trunk, branches and twigs that cause necrosis, friable bark, often with whitish hard exudates (perseitol). Under canker wood becomes reddish brown to brown and can damage to the center of the trunk (Dann *et al.*, 2013; Menge and Ploetz, 2003; Johnson and Kotze, 1994).

If the infection reaches vascular tissue, it can stop water and nutrients transport from xylem and translocation of assimilates reserves to sinks. This blockage causes weakening and decay of the wood at the infection site, which eventually can lead to wilting or death of the branch (Eskalen *et al.*, 2013). The avocado trees accumulate reserves in the bark, therefore, the disruption in flow, affects the accumulation and availability of reserves located on the trunk and branches, which are necessary for fruiting the following season (Chanderbali *et al.*, 2013).

For infection can occur is required injuries, so it was considered that the wounds of pruning, girdling, chilling injury, mechanical damage, bark split by the wind, wounds graft, etc., could allow the entry of the pathogen (Dann *et al.*, 2013). Also, the

incidence and severity of these canker is higher in trees subjects some stress, such as: drought, nutritional deficiencies, flooding, extreme temperatures or damage by insects or other pathogens that increase tree susceptibility (Dann *et al.*, 2013; Eskalen *et al.,* 2013; McDonald and Eskalen, 2011; Slippers and Wingfield, 2007; Menge and Ploetz, 2003; Johnson and Kotze, 1994). Another factor is the planting distance, as if planted a few meters repeated pruning is needed, that may increase the incidence of the disease (Dann *et al.,* 2013).

It has been determined that the Hass variety is more susceptible than other commercial varieties and that disease is most often detected in Guatemalan rootstocks than Mexican rootstock (Dann *et al.,* 2013).

Canker is less important in mature trees because they can remain productive, if the spread of these pathogens is limited to perform severe pruning to remove infected tissue. This operation could cause lowering of performance depending on how many twigs are removed (Eskalen and McDonald, 2009), but this action limiting the development of pycnidia and perithecia, and sporulation to healthy tissue. However, in severe cases the main trunk is completely surrounded, thereby the damage is irreversible, and disease kills the tree ends (Dann *et al.,* 2013).

Various species within the Botryosphaeriaceae family have been isolated from cankers on avocado from many different countries, including Mexico, New Zealand, Peru, South Africa, Chile, Central and South America, Spain, and the United States (McDonald and Eskalen, 2011).

In Chile branches canker in avocado was first reported in 1986, showing that it was caused by *Dothiorella* sp. (Pinto de Torres *et al.,* 1986), which currently is attributed to *Fusicoccum aesculi* Corda (synonymous *D. gregaria* Sacc. and *D. dominiacana* Petr. & Cif.) which is the anamorph of *Botryosphaeria berengiana*, *B. ribis* and *B. dothidea* (Acuña, 2010; Latorre, 2004; Besoain *et al.,* 2002).

Advances in molecular analysis and taxonomy of the species of the family Botryosphaeriaceae have allowed other anamorphs are identified, so it is important to define what new species are associated with this disease in Chile, such as *Neofusicoccum australe* (*B. australis*) in the northern center of the country (Auger *et al.,* 2013).

Effect of Botryosphaeriaceae species in quality postharvest

Stem end rot. Conidia of blighted leaves, branches and trunks with canker and / or dieback, could infect the inflorescences from emerging. The infection remains dormant inside the fruit until the environment is favorable for the pathogen (Aly *et al.,* 2011). This damage is characterized by rot at the scar left after removal of the stem during harvest (Dann *et al.,* 2013; Menge and Ploetz, 2003). The symptoms start with vascular browning, which is caused by chilling injury after long periods of storage at low temperatures (White *et al.,* 2005), the damage continues with discoloration and softening of the pulp joint to the fruit peduncle, these lesions grow rapidly compromising the fruit completely with the progress of maturation (White *et al.,* 2005). The initial symptom may be detected by a slight softening near the peduncle union, and the development of mycelium and conidia in the abscission

zone, when the stem is removed (Johnson and Kotze, 1994). Also, under high inoculum load new infections may occur and appear in the peel, irregular surface injuries amber to reddish-brown (Menge and Ploetz, 2003). The damage extending through the fruit and it is covered with mycelium and gray spores (Eskalen, 2009).

It is known that species of Botryosphaeriaceae are generally recovered in greater numbers from stem-end rot of avocado fruit than other fungi. These fungi are often associated with severe rots that vary by geographical region (Twizeyimana *et al.*, 2013).

The symptoms are not apparent until an advanced stage of maturity (Eskalen and McDonald, 2009), which is expressed in destine, causing rejection of the fruit, directly affecting the profitability of exporting companies and those involved in the chain production, so it is necessary to determine the cause of the infection and what conditions pre- and postharvest fruit predispose to the development of this pathology.

In Chilean fruit, stem end rot was detected in importing and consumers countries of Chilean avocado, but to date there is not scientific reports indicating associated Botryosphaeriaceae species.

Hypothesis and objectives

Hypothesis

Branch canker, dieback, and stem end rot detected in the central zone of Chile are caused by Botryosphaeriaceae species, being the edaphoclimatic and management factors that predispose the tree and the fruit to be colonized and infected by these pathogens.

General objective

To identify the causal agents and to determine the main biotic, abiotic and management factors that predispose the avocado orchards of the central zone of Chile to develop branch canker, dieback and stem end rot.

Specific objectives

1st To identify species of Botryosphaeriaceae family associated with branch canker, dieback and stem end rot of fruits in avocado orchards of central Chile.

2nd To determine edaphoclimatic and management factors that influence in the incidence and severity of these problems pathological at orchard level.

3rd To create a predictive model to segregate fruit by risk of developing stem end rot in avocados stored for long periods.

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Figure 1. Symptoms of Dieback. Branches retain dead dry leaves and fruits completely black with advanced level of softening (photographs taken by Ana L. Valencia).

Figure 2. Symptoms of Branch Canker. Trunk with rough protuberances, friable bark and cankers. Wood inner tissue with necrosis reddish brown (photographs taken by Ana L. Valencia).

Figure 3. Symptoms of Stem End Rot. Fruits with softening and necrosis near of peduncle union, and cavities with white-grey mycelium (photographs taken by Ana L. Valencia).

Chapter 2

Characterization and Pathogenicity of Botryosphaeriaceae Species Obtained from Avocado Trees with Branch Canker and Dieback, and from Avocado Fruit with Stem End Rot in Chile

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Characterization and Pathogenicity of Botryosphaeriaceae Species Obtained from Avocado Trees with Branch Canker and Dieback, and from Avocado Fruit with Stem End Rot in Chile

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Abstract

Several species of the Botryosphaeriaceae family have been associated with branch canker, dieback and stem end rot in avocado (*Persea americana* Miller). In Chile, the incidence of diseases affecting the avocado tree increased from 2011 to 2016, which coincided with a severe drought that affected avocado production. Moreover, distant countries importing avocados from Chile also reported an increase of stem end rot of ripe avocados. Therefore, the aims of this study were to identify the pathogen species associated with branch canker, dieback, and stem end rot of avocado in Chile and to study their pathogenicity. This study was conducted between

2015 and 2016 in ‘Hass’ avocado orchards located in the main avocado producing regions in Chile. A diverse collection of fungal species was recovered from both necrotic woody tissue and necrotic tissue on harvested ripe fruit. On the basis of morphology and phylogenetic analyses of the internal transcribed spacer region (ITS1-5.8S-ITS2) and the translation elongation factor 1-α (TEF1-α) gene, eight species in the Botryosphaeriaceae family were identified: *Diplodia mutila*, *D. pseudoseriata*, *D. seriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *Neofusicoccum australe, N. nonquaesitum*, and *N. parvum*. For each of these species, pathogenicity studies were conducted on 1-year-old healthy ‘Hass’ avocado plants. All isolates produced brown gum exudate and caused necrosis in the vascular system three weeks after inoculation. *N. nonquaesitum, N. parvum*, and *D. pseudoseriata* were the most virulent species. Necrotic lesions and cavities with white mycelia near the peduncle union were observed on ‘Hass’ avocado fruit inoculated postharvest. *L. theobromae*, *N. australe*, and *N. parvum* were significantly more virulent than the other tested species in the Botryosphaeriaceae family. This study identified and characterized the pathogenicity of Botryosphaeriaceae species in Chile, which will prove useful future research on these pathogens directed at establishing effective control strategies in avocado.

The family Botryosphaeriaceae encompass a morphologically diverse family of Ascomycota fungi (Phillips et al. 2013). Some species can survive as endophytes in symptomless tissue (Twizeyimana et al. 2013) and become pathogenic when the host is subjected to stress conditions (Dann et al. 2013; Slippers and Wingfield

2007). Species in the Botryosphaeriaceae family are associated with a wide variety of woody hosts (Slippers and Wingfield 2007). On *Persea americana* Mill., the species of Botryosphaeriaceae have been associated with branch canker and dieback in avocado trees in different countries. In this sense, Menge and Ploetz (2003) have indicated that *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not is the most common pathogen reported in Chile, Mexico, New Zealand, Peru, South Africa, and the United States. In addition, *B. obtusa* (Schwein.) Shoemaker and *B. rhodina* (Berk. & M.A. Curtis) Arx have been reported in Mexico and the United States. Other species previously reported are *Diplodia mutila* (Fr.) Mont. in the United States (Chen et al. 2014); *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves in the United States (McDonald and Eskalen 2011); *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl in Tanzania (Alves et al. 2008); *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips in Chile (Auger et al. 2013) and the United States (McDonald et al. 2009); *N. luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips in the United States (McDonald et al. 2009); *N. nonquaesitum* Inderb., Trouillas, Bostock & Michailides in the United States (Carrillo et al. 2016); and *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips in Italy (Guarnaccia et al. 2016), Spain (Zea-Bonilla et al. 2007) and the United States (McDonald et al. 2009).

On avocado, branch cankers are typified by necrotic, friable bark, often with whitish hard exudates of perseitol, a crystalline polyhydric alcohol produced by avocados. Internally, the wood becomes reddish brown (Auger et al. 2013; Dann et al. 2013; McDonald and Eskalen 2011; Menge and Ploetz 2003). Infection under the bark,

make it possible for the fungus to colonize the vascular tissue and affect water and nutrient transport throughout the xylem and the translocation of assimilates through the phloem tissue (Eskalen et al. 2013). This blockage causes weakening and decay of the woody tissue, which can eventually lead to branch wilt and dieback (Auger et al. 2013; McDonald and Eskalen 2011). This condition can also affect the accumulation and availability of the reserves necessary for fruiting during the following season, which are primarily located in the trunk and branches (Chanderbali et al. 2013). Thus, branch canker and dieback can reduce the productivity of the orchard.

Latent fruit infections of Botryosphaeriaceae species can also occur in orchards affected by branch canker and dieback (Hopkirk et al. 1994; Menge and Ploetz 2003). This infection can remain dormant until the environment is favorable for the pathogen (Aly et al. 2011). Rotting is rarely observed on immature avocado fruit before harvesting, because fruit will ripen only when it is removed from the tree causing increases in the rate of respiration and ethylene production (Yahia and Woolf 2011). Moreover, as the fruit ripen, there is a decrease in the concentrations of the antifungal diene, a preformed antifungal component of avocado (Hopkirk et al. 1994; Prusky and Keen 1993) which increases the fruit susceptibility to infection.

Avocado stem end rot starts at the scar left after the removal of the stem during harvest (Dann et al. 2013; Menge and Ploetz 2003). The initial symptom may be a slight softening at the union where the stem attaches to the fruit that continues with the discoloration of the pulp. These symptoms are followed by the development of mycelia and conidia in the abscission zone (Dann et al. 2013; Johnson and Kotzé

1994). These lesions grow rapidly as the fruit matures, completely compromising the fruit (White et al. 2005). The pathogens associated with stem end rot include species of the Botryosphaeriaceae family and other fungal species, such as *Colletotrichum gloesporioides* (Penz.) Penz. & Sacc, *C. acutatum* J.H. Simmonds, *Thyronectria pseudotrichia* (Schwein.) Seeler, *Phomopsis perseae* Zerova, *Pestalotiopsis clavispora* (G.F. Atk.) Steyaert, and *P. versicolor* (Speg.) Steyaert (Dann et al. 2013; Darvas and Kotzé 1987; Hartill and Everett 2002; Menge and Ploetz 2003; Valencia et al. 2011). However, anamorphs of species in the Botryosphaeriaceae have been considered to be the important causal agents of avocado stem end rot throughout the world (Dann et al. 2013; Menge and Ploetz 2003), including *D. mutila* in the United States (Inderbitzin et al. 2010), *L. theobromae* in Italy (Dann et al. 2013; Garibaldi et al. 2012), *N. australe* in Chile (Montealegre et al. 2016) and Turkey (Akgül et al. 2016), *N. luteum* in the United States (Twizeyimana et al. 2013), *N. mangiferae* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips in Taiwan (Ni et al. 2009), *N. mediterraneum* Crous, M.J. Wingf. & A.J.L. Phillips in the United States (Inderbitzin et al. 2010), and *N. parvum* in Italy (Guarnaccia et al. 2016) and Mexico (Molina-Gayosso et al. 2012).

Chile is considered one of the largest avocado producing countries in the world, with 30,078 ha planted between the Atacama Region (25° 17′S to 29° 30’S) and the O’Higgins Region (33° 51′ S to 35° 01′ S), with the Valparaiso region (32° 02’S to 33° 57’S) being the most productive (ODEPA-CIREN 2017). In Chile, the avocados are produced in high-density planting, which requires frequent pruning (Schaffer et

al. 2013) thus increasing the infection risk through pruning wounds (Dann et al. 2013; Eskalen et al. 2013).

The primary avocado fruit cultivar exported from Chile is ‘Hass’, which is sent by overseas transport to distant markets in the United States, Europe, and Asia. Therefore, it is essential to produce fruit with a long postharvest shelf life that can withstand prolonged storage. However, since 2014, the incidence of stem end rot has increased in import markets that consume Chilean fruit. The quality of sanitation is very important to guarantee the distribution and sale of the Chilean avocados in these markets, such as the Asian market, but especially in the European markets, where maturation chambers are used for reducing the ripening times of the fruit, by increasing temperature, ethylene concentration and relative humidity in storage, which allows avocados to be sold ready-to-eat.

In Chile, there is not sufficient information about all the pathogens associated with branch canker, dieback, and stem end rot. This critical information is needed to develop cultural strategies for disease management. Therefore, the aims of this study were to identify the pathogen species associated with branch canker, dieback, and stem end rot of avocado in Chile and to study their pathogenicity.

Materials and Methods

Field sampling. Sampling was conducted in 16 ‘Hass’ avocado orchards located between Illapel (31° 37’S) and Peumo (34° 24’S) in the spring of 2015 and 2016. In each orchard, 15 symptomatic or healthy trees were randomly selected from

an area of approximately 900 m^2. A total of 75 wood samples of branch and or discs cut from trunks were obtained per orchard (five wood samples from each tree) and transported to the laboratory where they were immediately processed. A total of 45 fruits per orchard (three fruits per tree with peduncle attached) were harvested and transported to the laboratory in plastic bags. The fruits were surface disinfected by immersion in 75% ethanol for 30 s and then placed in humidity chambers (100% relative humidity) for 7 to 31 days at 20ºC until they reached the firmness of consumer maturity. Additionally, 15 fruits were harvested by orchard, and sent to the laboratory to determine dry matter content; because avocados are harvested at about 23% dry matter in Chilean production.

Fungal isolation. The wood samples were disinfected by immersion in 96% ethanol for 15 s and immediately flamed for 15 s, and the outer burned tissues were removed aseptically. Small wood pieces and pulp pieces of mature 'Hass' fruit (3 to 5 mm) were removed from the margins between the healthy and symptomatic tissues and placed in Petri dishes containing 2% potato dextrose agar acidified with 0.5 ml $liter^{-1}$ of 92% lactic acid plus 0.05% tetracycline (APDAt) (Díaz et al. 2013). The plates were incubated at 20°C in darkness for 14 to 21 days. The colony features were used to tentatively identify morphologically isolates of Botryosphaeriaceae species, because only some isolates generated conidiophores and conidia at this stage. Therefore, conidial features were later used to identify these isolates to species level. All isolates with these features were transferred to acidified potato dextrose agar (APDA). Pure cultures were obtained from hyphal tip transfers. All the isolates were stored in 1.5 ml of sterile 20% glycerol at 5°C.

Morphological characterization. Colony morphology was characterized on APDA after 5, 12, and 27 days at 25°C in darkness. Conidiophores and conidia were produced by placing small pieces of mycelia on 2% water agar amended with autoclaved pine needles and incubated for 21 days at 15°C in darkness or from 30-day-old colonies cultivated on APDA at 20°C in darkness. The length and width of 50 conidia were measured, and the mean and standard deviation were calculated. The color, shape and the presence or absence of septation of conidia were also determined. The descriptions of Phillips et al. (2013) were used to identify the isolates obtained.

Based on the morphological characteristics, 11 isolates obtained from wood and 17 isolates obtained from fruits were selected for further characterization of their growth rates at various temperatures, by gene sequence analysis, and pathogenicity testing.

Temperature effects on mycelial growth. Mycelial discs (5 mm in diameter) of 28 selected isolates were transferred to APDA in Petri dishes and incubated for 4 days, at 5, 10, 15, 20, 25, 30, 35, and 40°C in darkness. For each isolate, three replicate plates were incubated, and the experiment was conducted twice. The colony diameter was measured daily, and the average growth rate was determined on the second and third days at each temperature, prior to the mycelia reaching the edges of the plates.

DNA extraction, PCR amplification and sequencing. Mycelia plugs from each isolate (*n* = 28) were obtained from 4-day-old colonies cultivated on APDA at 20°C

and placed into 2 ml tubes with glass and metal beads. The tubes were placed into a Mini-Beadbeater-24 (Bio Spec Products Inc., Bartlesville, OK, USA), for 30 s at 3500 strokes per min. The DNA was obtained using a Wizard® Genomic DNA purification kit (Promega Corporation, Madison, USA). The ITS1-5.8S-ITS2 region of the ribosomal DNA was amplified using primers ITS1 and ITS4 (White et al. 1990), and the amplification of the partial sequence of the translation elongation factor 1-alpha (TEF1-α) was conducted using primers TEF1-728F and TEF1-986R (Carbone and Kohn 1999). Polymerase chain reactions (PCR) was performed with a 50 µl reaction mixture containing 5X PCR reaction buffer, 25 mM $MgCl_2$, 10 mM dNTPs, 10 µM of each primer, 1 U GoTaq® G2 Flexi DNA polymerase (Promega Corporation, Madison, USA), and 50 to 100 ng μl^{-1} DNA. Negative controls with sterile water, a positive control of an isolate of *N. parvum* obtained in a previous study, and DNA samples of 28 selected isolates were used in each PCR reaction. The PCR conditions using the primers ITS1 and ITS4 were 5 min of initial denaturation at 95°C, followed by 35 cycles of 30 s of denaturation at 95°C, 30 s alignments at 55°C, 45 s of extension at 72°C, and an extension end of 7 min at 72°C. For the primers TEF1-728 and TEF1-986, the PCR conditions were 5 min of initial denaturation at 95°C, followed by 30 cycles of 20 s of denaturation at 95°C, 20 s alignments at 55°C, 45 s of extension at 72°C, and an extension end of 7 min at 72°C. Next, 5 µl of PCR product of each PCR reaction was examined by electrophoresis at 100 V on a 1.5% agarose gel (w/v) in 1X TAE buffer and visualized with UV light. A 100 bp DNA ladder (Maestrogen, Prion lab) was used as the size marker. The PCR product was purified and sequenced by Macrogen Inc. (Seoul, South Korea), and the sequences obtained were edited using Proseq v. 2.91 (Filatov 2002) and were aligned using Clustal X

v.2.0 (Larkin et al. 2007). The sequences of Botryosphaeriaceae species obtained in this study (Table 1) were compared using BLASTn analysis with sequences existing in the GenBank database (Table 2).

Phylogenetic analyses. The phylogenetic analysis was performed using Mega 6 (Tamura et al. 2013) with the ITS and TEF1-α datasets separate and combined, including sequences of *Melanops tulasnei* as an outgroup (Table 2). The combined sequences of the ITS and TEF1-α regions were aligned using Clustal W (Larkin et al. 2007), and uninformative terminal regions were excluded from the analysis. The partition homogeneity test was conducted using PAUP 4.0 (Swofford 2002) to determine the statistical congruence between the ITS and TEF1-α dataset and to determine if they could be combined in a single dataset (P = 0.05).

The maximum parsimony analysis was performed using a heuristic search option and the tree bisection reconnection (TBR) algorithm with 1000 bootstrap replications and complete deletion of gaps and missing data. In addition, tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RCI) were registered.

Pathogenicity testing. The pathogenicity tests were conducted on 'Hass' avocado plants and fruits.

Pathogenicity testing on young trees. One-year-old 'Hass' avocado grafted onto Mexicola rootstock, were kept in the shade for 60 days with optimal nutrition and irrigation. All plants (*n* = 36) were wounded with a scalpel before inoculation with a 7-day-old mycelial plug (5 mm in diameter) of *D. mutila* (PALUC1M, PALUC18M),

D. pseudoseriata (PALUC406M), *D. seriata* (PALUC14M, PALUC404M), *D. iberica* (PALUC3M, PALUC399M), *N. nonquaesitum* (PALUC4M, PALUC407M), and *N. parvum* (PALUC16M, PALUC411M). Control plants were treated with sterile agar discs (5 mm in diameter). The wounded zone in the stem was sealed with Parafilm to avoid dehydration. The experimental design was completely randomized with three replicates for each treatment. The length of the necrotic inner lesion that developed in the stem was measured. These data were subjected to statistical analysis using a one-way analysis of variance (ANOVA), and the means were separated by Tukey's HSD test (P = 0.05), using SAS v.9 (SAS Institute, Cary, NC). Koch's postulates were confirmed after the reisolation from pieces of damaged stem placed on APDA at 20°C for 7 days. The experiment was repeated once.

Pathogenicity testing on harvested fruit. Pathogenicity testing of Botryosphaeriaceae isolates was conducted on 'Hass' avocado fruit that were harvested at maturity (25.6% mean dry matter). The fruits (*n* = 54) were surface disinfected by immersion for 30 s in 75% ethanol and wounded in the peduncle cavities before inoculation with 7-day-old mycelial discs (5 mm diameter) of *D. mutila* (PALUC45F, PALUC451F), *D. seriata* (PALUC10F, PALUC426F, PALUC455F, PALUC467F), *D. iberica* (PALUC7F), *L. theobromae* (PALUC449F), *N. australe* (PALUC439F, PALUC454F, PALUC474F PALUC490F), and *N. parvum* (PALUC6F, PALUC44F; PALUC415F, PALUC470F, PALUC496F). Control fruit were treated with sterile agar discs of 5 mm diameter. The wounded zone in the fruit was sealed with Parafilm to avoid dehydration. The fruit were placed in humidity chambers (100% RH) at 20 ºC for 10 days. The experimental design was a completely randomized design with three

replicates of each treatment. The length of the necrotic inner lesions that developed in fruit was measured, and these data were subjected to statistical analysis using a one-way analysis of variance (ANOVA), and the means were separated by Tukey's HSD test (P = 0.05) using SAS v.9 (SAS Institute, Cary, NC).

Pathogenicity testing on stored fruit. The 'Hass' avocado fruits were surface disinfected and wounded as described above. Later, the fruits were inoculated with 7-day-old mycelial discs (5 mm diameter) of *D. mutila* (PALUC45F, PALUC451F), *D. seriata* (PALUC426F, PALUC455F), *D. iberica* (PALUC7F), *L. theobromae* (PALUC449F), *N. australe* (PALUC439F, PALUC490F), and *N. parvum* (PALUC6F, PALUC415F, PALUC470F, PALUC496F). Control fruit were treated with sterile agar discs (5 mm diameter). All fruit (*n* = 65) were stored in cardboard boxes, sealed with plastic film to avoid contamination. These fruits were maintained for 15, 30 or 45 days at cold storage of 5ºC plus 7 days at 20ºC in a normal atmosphere (without changes in atmosphere composition of CO_2 and O_2). The treatments were distributed as a completely randomized design with five replicates per treatment. The length of the necrotic inner lesion in fruit was measured, and these data were subjected to statistical analysis using an analysis of variance (ANOVA) with treatments arranged factorially with 13 isolates at 3 storage times. The means were separated by Tukey's HSD test (*P* = 0.05) using SAS v.9 (SAS Institute, Cary, NC). To fulfill Koch's postulates, pieces of fruit obtained from margins of symptomatic tissue of both tests were placed on APDA to 20ºC for 7 days. The isolates were re-identified based on their cultural and morphological features.

Results

Field sampling. Of the 16 orchards, six orchards had symptoms associated with branch canker and dieback on both young plants used for replanting and adult trees. Symptoms included branches with wilting leaves and inflorescences, and small fruits with abnormal development. In addition, harvested fruit from 11 of the 16 orchards showed stem end rot on ripe fruit. On severely affected fruit, white to grey mycelia appeared on the peel near the peduncle, and occasionally black pycnidia with masses of gray conidia completely covered the fruit. The inner tissue also had cavities with white mycelia and necrosis deep in the pulp. In less affected fruit, mycelia and conidia were absent in the peel, but after removal of the peduncle a slight necrosis of the pulp internally extending until the equatorial zone was observed.

Fungal isolation. Thirty-six and 88 isolates obtained from the samples of wood and fruit samples, respectively, showed characteristics of species belonging to Botryosphaeriaceae. These isolates originated from orchards in different geographical locations with different climatic conditions (Table 1).

Morphological characterization. Isolates obtained were grouped into seven groups based on their colony and conidial features.

Group 1 *Diplodia mutila.* The colony grew primarily flattened (nearly absent of dense aerial mycelia) and was initially white, turning olive gray to black. The pycnidia were usually aggregated, black, semi globose, and partially erumpent on pine needles. The conidia were initially hyaline, aseptate, smooth, oblong to ovoid, with both ends

rounded, and with age, pale brown with one septum, reaching sizes of 20.6 to 30.3 x 9.4 to 17.9 µm (Table 3). Ascomata were not observed. These isolates were morphologically similar to the description of *D. mutila* (Alves et al. 2004).

Group 2 *Diplodia seriata*. The colony grew primarily flattened, initially white and later turned gray to black. The pycnidia usually were aggregated, black, erumpent and partially emergent on pine needles. The conidia were initially light brown, aseptate, smooth, oblong, with their base truncate or rounded and dark brown with age, of 17.6 to 32.3 x 7.7 to 14.2 µm (Table 3). Ascomata were not observed. These isolates were morphologically similar to the description of *D. seriata* (Phillips et al. 2013) and *Diplodia pseudoseriata* (Perez et al. 2010).

Group 3 *Dothiorella iberica.* The colony grew primarily flattened, initially white with gray centers and turned from brown to dark gray or black. The pycnidia were usually thick-walled, solitary, black, and globose. The conidia were dark brown with one septum, smooth, and obovoid with a rounded apex and truncate base, and some conidia were slightly constricted at the septum of 22.7 to 28.8 x 8.5 to 13.7 µm (Table 3). Ascomata were not observed. These isolates were morphologically similar to the description of *D. iberica* (Phillips et al. 2005).

Group 4 *Lasiodiplodia theobromae.* The colony developed abundant filamentous mycelia, which initially were white with a gray center and turned gray to dark gray or black. The pycnidia were usually simple or aggregated and black in color. The paraphyses were present as hyaline, cylindrical, and hyphal-like with rounded ends, 51.8 µm long and 3.47 µm wide on average. The conidia were hyaline, with granular

contents, thick-walled, subovoid to ellipsoid-ovoid, dark brown with one septum, developing longitudinal striation with age, of 16.7 to 26.2 x 11.1 to 14.5 µm (Table 3). Ascomata were not observed. These isolates were morphologically similar to the description of *L. theobromae* (Phillips et al. 2013).

Group 5 *Neofusicoccum australe.* The colony developed dense filamentous mycelia with tufts of aerial mycelia around the edges, which initially were white and turned to light gray to dark gray or black. The pycnidia were usually solitary, black and globose. The conidia were hyaline, aseptate, with granular content, fusiform-obovoid, with a truncate to lightly rounded base, of 17.7 to 29.9 x 6.2 to 13.6 µm (Table 3). Ascomata were not observed. These isolates were morphologically similar to the description of *N. australe* (Phillips et al. 2013).

Group 6 *Neofusicoccum nonquaesitum.* The colony developed dense filamentous mycelia, which initially were white and turned to light gray to dark gray to black. The pycnidia were usually simple or aggregate, black, and, in some cases, had a short neck. The conidia were hyaline, aseptate, with granular content, and fusiform, of 18.7 to 31.7 x 4.9 to 9.4 µm (Table 3). Ascomata were not observed. These isolates were morphologically similar to the description of *N. nonquaesitum* (Inderbitzin et al. 2010; Phillips et al. 2013).

Group 7 *Neofusicoccum parvum.* The colony developed dense filamentous mycelia that were primarily aerial, which initially were white and turned to light and dark gray to black. The pycnidia were usually simple or aggregated, of black color, and globose. The conidia were initially hyaline, aseptate, with granular content, fusiform,

and with time, the conidia were biseptate with a light brown middle cell, of 15.7 to 29.1 x 4.9 to 10.9 µm (Table 3). Ascomata were not observed. These isolates were morphologically similar to the description of *N. parvum* (Phillips et al. 2013).

Temperature effects on mycelial growth. The isolates in the groups of *D. mutila*, *N. nonquaesitum, D. seriata*, and *N. parvum* had similar mycelial growth in the range of 5 to 35°C and optimal mycelial growth at 25°C. Isolates in the groups of *D. iberica* and *N. australe* grew at these same temperatures but had less mycelial growth at these temperatures. The isolate PALUC406M of *D. pseudoseriata* grew between 5 and 30°C with optimal mycelial growth at 25°C. Additionally, isolate PALUC449F in the group of *L. theobromae* grew between 5 and 40ºC with optimal mycelial growth between 25 and 35°C (Fig. 1).

DNA extraction, PCR amplification and sequencing. The ITS1-5.8S-ITS2 region of 28 isolates of Botryosphaeriaceae family were amplified, obtaining 521 to 563 bp fragments. All sequences shared a 99 to 100% identity with published sequences of Botryosphaeriaceae species deposited in the GenBank (Table 2). However, the isolates PALUC3M, PALUC399M, and PALUC7F, which were morphologically identified as *D. iberica* showed 99% identity (95% Query cover) with GenBank sequences of *D. iberica* and *D. sarmentorum* (Table 2).

The partial amplification of the TEF1-α sequence generated fragments of 251 to 322 bp, and in BLAST analyses revealed 95 to 100% identity with published sequences of Botryosphaeriaceae species deposited in the GenBank (Table 2). Again, isolates PALUC3M, PALUC399M, and PALUC7F had 88 to 89% identity (99-100% query

cover) with the GenBank sequences of *D. iberica*, and 97% identity (87% query cover) with the GenBank sequences of *D. sarmentorum* (Table 2). All the ITS and TEF1-α sequences obtained in this study were deposited in GenBank (Table 1).

Phylogenetic analysis. The separate phylogenetic analyses of the ITS and TEF1-α sequences clustered Botryosphaeriaceae isolates obtained in this study with clades of *D. mutila*, *D. pseudoseriata*, *D. seriata*, *L. theobromae*, *N. australe*, *N. nonquaesitum*, and *N. parvum* (data not shown). The isolates PALUC3M, PALUC399M, and PALUC7F in their phylogenetic analysis of the ITS sequences clustered with clades of *D. iberica* (95% bootstrap support). Additionally, the phylogenetic analysis of the TEF1-α sequences, showed isolates clustered with the clade of *D. iberica* (91% bootstrap support) (data not shown).

The partition homogeneity test indicated that there was not a significant difference ($P = 0.12$) between the ITS and TEF1-α dataset. Therefore, a concatenated analysis was performed. The combined ITS and TEF1-α dataset included 53 taxa of 607 total characters with 324 parsimonious informative characters. The maximum parsimony analysis returned the seven most parsimonious trees with the following scores: CI = 0.75, RI = 0.96, and RCI = 0.72. The phylogenetic analysis of the combined ITS and TEF1-α datasets from Botryosphaeriaceae species produced a phylogenetic tree with two primary clades. In the first clade (72% bootstrap support), isolates obtained in this study clustered as *N. australe, N. nonquaesitum, N. parvum*, and *D. iberica*. The second clade (100% bootstrap support) of isolates obtained in this study clustered with clades of *L. theobromae*, *D. mutila*, *D. pseudoseriata*, and *D. seriata* (Fig. 2). Isolates of *D. mutila* (PALUC18M, PALUC451F), *D. pseudoseriata*

(PALUC406M), *D. seriata* (PALUC 404M, PALUC10F; PALUC467F), *D. iberica* (PALUC399M, PALUC7F), *L. theobromae* (PALUC449F), *N. australe* (PALUC439F, PALUC454F), *N. nonquaesitum* (PALUC4M), and *N. parvum* (PALUC16M, PALUC6F; PALUC496F) were deposited as living cultures in the Chilean Microbial Genetic Resources Collection (CChRGM; www.cchrgm.cl).

Pathogenicity testing.

Pathogenicity testing on young trees. All the isolates tested were pathogenic on 1-year-old avocado plants. Brown gummy exudate and necrosis developed in the vascular system three weeks after inoculation. In some cases, wilted leaves near the site of inoculation were observed, and fruiting bodies were observed in the stems inoculated with *D. mutila*, *N. nonquaesitum,* and *N. parvum.* The *N. nonquaesitum* isolates were the most virulent isolates ($P = 0.0184$), producing vascular lesions of 117.5 mm in diameter on average. The *N. parvum* and *D. pseudoseriata* isolates were the second most virulent isolates, causing necrotic lesions above 80 mm in diameter. *D. mutila*, *D. iberica*, and *D. seriata* isolates were less virulent with mean lesion sizes less than or close to 60 mm in diameter (Fig. 3). All isolates were successfully reisolated from symptomatic inoculated plants and re-identified based on their cultural and morphological features. Control plants remained symptomless and isolates of Botryosphaeriaceae species were not detected from the pieces of stem on APDA, thus fulfilling Koch's postulates.

Pathogenicity testing on harvested fruit. Isolates of Botryosphaeriaceae species were pathogenic on 'Hass' avocado fruits, producing black lesions near the peduncle

union zone with light brown and softening of the pulp. In certain cases, there were cavities in the pulp with vascular browning. Additionally, abundant white-gray mycelia grew near the site of inoculation. *L. theobromae* isolate PALUC449F was the most virulent ($P < 0.0001$), producing average vascular lesions of 67.5 mm in diameter. *N. parvum* (isolates PALUC6F, PALUC44F, PALUC415F, PALUC470F, PALUC496F), *N. australe* (isolates PALUC439F, PALUC454F, PALUC474F, PALUC490F), *D. mutila* (isolates PALUC45F, PALUC451F), and *D. seriata* (isolates PALUC10F, PALUC426F, PALUC467F) were the second most virulent isolates, causing lesions greater than or close to 40 mm in diameter. In contrast, the isolates of *D. seriata* (PALUC455F) and *D. iberica* (PALUC7F) were significantly less virulent ($P < 0.0001$) (Fig. 4). All isolates were re-isolated in APDA from tissue obtained from the margins of decay in the pulp of inoculated fruits with 100% fungal recovery. The isolates were re-identified based on their cultural and morphological features. Control fruits remained symptomless and isolates of Botryosphaeriaceae species were nor reisolated from pieces of pulp on APDA. Therefore, Koch's postulates were completed.

Pathogenicity testing on stored fruit. Fruit stored for 15 days or more days showed chilling injury, such as vascular and mesocarp browning. On inoculated fruit, a superficial mycelial growth on the peel and internal necrosis of the pulp and vascular tissue that extended from the inoculation site to the rest of the fruit was observed. Analysis of the average lesion diameter indicated significant differences between the isolates of the same species and isolates of distinct species when they were inoculated in fruit that were stored for 15 and 30 days at 5ºC ($P < 0.0001$). However,

there were no differences on fruit maintained for 45 days under such conditions (Table 4). On fruit that were incubated for 15 days at 5ºC, the most severe damage was caused by *N. parvum* (isolates PALUC470F and PALUC415F), *L. theobromae* (isolate PALUC449F), *D. seriata* (isolate PALUC426F), *D. mutila* (isolates PALUC45F and PALUC451F), and *N. australe* (isolates PALUC439F and 490F), with lesions greater than or close to 50 mm in diameter. Additionally, isolates of *D. iberica* (isolate PALUC7F) and *N. parvum* (isolate PALUC496F) had similar average lesion diameters (approximately 20 mm). On fruit stored for 30 days at 5ºC plus 7 days at 20ºC, the most severe damage was caused by isolates of *D. mutila* (isolates PALUC45F and PALUC451F), *N. parvum* (isolate PALUC496F), and *D. seriata* (isolate PALUC455F), with lesions greater than or close to 50 mm in diameter. Conversely, *D. iberica* (isolate PALUC7F) caused lesions of 26 mm in diameter on average. Control fruit developed small necrotic lesions around the inoculation site which were associated with inoculation damage. There were significant differences between isolates and storage times ($P < 0.0001$). Similarly, the interaction between these factors was significant ($P < 0.0001$). The fungal recovery was 100 %, respectively, from symptomatic inoculated fruit for each storage time. The isolates were re-identified based on their cultural and morphological features.

Discussion

In this study, eight species in the family Botryosphaeriaceae were identified: *Diplodia mutila, D. pseudoseriata, D. seriata, Dothiorella iberica, N. nonquaesitum*, and *N.*

parvum, which were demonstrated to be pathogenic to 'Hass' avocado plants. In addition, *Diplodia mutila*, *D. seriata*, *Dothiorella iberica*, *L. theobromae*, *N. australe*, and *N. parvum*, demonstrated to be pathogenic and cause stem end rot on 'Hass' avocado fruit. In Chile, *N. australe* had been previously reported in avocado trees (Auger et al. 2013) and fruits (Montealegre et al. 2016). To our knowledge, this is the first report of *D. mutila, D. seriata*, *D. iberica, N. nonquaesitum*, and *N. parvum* associated with branch canker and dieback in avocado in Chile. Additionally, it has not been previously reported that *D. mutila, D. seriata*, *D. iberica, L. theobromae, and N. parvum* were associated with stem end rot of avocados in Chile. Therefore, this study is also the first to report regarding these pathogens in Chilean avocado trees and fruit.

The identification of the isolates obtained in this study suggest that the identification of Botryosphaeriaceae species is only possible with both morphological characterization of colony growth, and sequence comparison of the ITS and TEF1-α gene regions, which were previously described in several taxonomic reports (Crous et al. 2006; Dissanayake et al. 2016; Phillips et al. 2013; Slippers et al. 2017).

The morphological features allowed the identification of the isolates obtained in this study as *D. mutila*, *D. seriata*, *L. theobromae*, *N. australe*, and *N. parvum*. However, the isolate PALUC406M showed morphological features similar with *D. seriata* but had different conidial dimensions and showed better mycelial growth at different temperatures, features that were similar to data reported by Perez et al. (2010). The BLAST analysis and the separate and combined phylogenetic analyses of the ITS and TEF1-α gene regions allowed to identify this isolate as *D. pseudoseriata*.

The morphological identities were confirmed with the combined phylogenetic analysis of ITS and TEF1-α gene regions, which allowed distinction of species most closely related phylogenetically, such as *N. nonquaesitum* from *N. arbuti* and *D. iberica* from *D. sarmentorum* (Phillips et al. 2013). The isolates of PALUC4M and PALUC407M, when compared with the published data of *N. nonquaesitum* and *N. arbuti* (Inderbitzin et al. 2010; Phillips et al. 2013) were similar to *N. nonquaesitum* in the dimensions of their conidia, which is a characteristic that distinguishes *N. nonquaesitum* from *N. arbuti* (Phillips et al. 2013). Similarly, the phylogenetic analysis clustered these isolates with the clade of *N. nonquaesitum*, which included the specimen type and isolates obtained by Carrillo et al. (2016) in avocado wood with branch canker and dieback. Likewise, the isolates PALUC3M, PALUC399M and PALUC7F when compared with the data published on *D. iberica* and *D. sarmentorum* (Phillips et al. 2005) were more similar to *D. iberica,* because the average size and maximum length of the conidia were longer than the conidial size of *D. sarmentorum*, which are characteristics that distinguish both species (Phillips et al. 2013). This distinction was supported by the combined ITS and TEF1-α phylogenetic analysis, which clustered these isolates with clades of *D. iberica* that included the specimen type (Phillips et al. 2005).

In this study, isolates of *D. seriata, D. iberica, N. nonquaesitum*, and *N. parvum* were detected in samples from avocado orchards located in different climatic zones. In this sense, McDonald and Eskalen (2011) have indicated that climatic factors do not provide an explanation for the distribution of the Botryosphaeriaceae species, because their study detected the same species in northern and southern states of

the United states. Under natural conditions, one would expect that the distribution of these species would be localized, because studies of spore dispersal indicated that their spread is mainly associated with splashing water due to rainfall or irrigation and wind dispersal (Eskalen et al. 2013; Úrbez-Torres et al. 2010; Valencia et al. 2015). Mehl et al. (2017a, b) indicated that human-assisted dispersal has played a significant role in the worldwide distribution of Botryosphaeriaceae species associated with agriculture, probably because these fungi persist endophytically in infected but asymptomatic plant material that are sold and transported across country boundaries (Slippers and Wingfield 2007).

Botryosphaeria branch canker and dieback is an important problem in Chilean viticulture, where *D. mutila, D. seriata, N. australe*, and *N. parvum* were detected (Auger et al. 2004; Besoain et al. 2013; Díaz et al. 2013; Morales et al. 2012; Valencia et al. 2015). Similarly, *D. mutila* was detected in the wood of English walnut (Díaz et al. 2018), *Neofusicoccum arbuti, N. australe,* and *N. parvum* in the wood of blueberry (Espinoza et al. 2009), *N. parvum* in kiwi plants (Díaz et al. 2016), and *B. dothidea* in apple trees (Latorre and Toledo 1984). In addition, *D. seriata* was reported to cause black rot in apple fruits (Cáceres et al. 2016).

In this study, *N. parvum*, *D. mutila*, *D. seriata*, and *D. iberica* were obtained from samples of avocado wood and fruit. These results confirmed reports of Twizeyimana et al. (2013) and Guarnaccia et al. (2016) that these pathogens, localized endophytically in wood, could infect fruit. Their results, confirmed that stem end rot can be initiated by fungi causing branch canker. Additionally, it is important to consider that wounding is a prerequisite for infection of Botryosphaeriaceae species

in wood (pruning, wounds, frost damage, and grafting wounds) and fruit (infections of peduncle can be initiated during harvest) (Dann et al. 2013; Hartill and Everett 2002). Therefore, incorporating pre-harvest and post-harvest management strategies, such as cultural practices that limit the dissemination of these pathogens or that decrease stress in avocado trees and fruit as well as effective fungicide applications, could reduce the development of branch canker, dieback, and the incidence of stem end rot (Dann et al. 2013; Hartill et al. 2002; Hartill and Everett 2002; Menge and Ploetz 2003; Twizeyimana et al. 2013).

The pathogenicity results of this study indicated that the Botryosphaeriaceae species obtained from the Chilean orchards are pathogenic to 'Hass' avocado. Similarly, *N. nonquaesitum and N. parvum* were the most virulent species, causing severe damage in the inner vascular tissue of the plant stem. These results were similar to reports of Carrillo et al. (2016) and McDonald et al. (2009) who also measured the length of vascular lesions. In addition, Slippers and Wingfield (2007) indicated that *N. parvum,* and *N. australe* are considered to be the most damaging species in the Botryosphaeriaceae family. *D. pseudoseriata* also caused severe damage in avocado stems. This species was not previously reported to be associated with avocado branch cankers. Therefore, this is the first report of this pathogen causing branch canker and dieback in avocado in the world. The isolates of *D. mutila*, *D. iberica* and *D. seriata* of this study had low virulence in avocado plants. These species are not considered to be common pathogens in avocado trees.

In pathogenicity tests of avocado fruit, isolates of *L. theobromae*, *N. parvum*, and *N. australe* caused severe damage to the pulp of fruit. Other studies indicated that these

pathogenic species could cause stem end rot (Akgül et al. 2016; Twizeyimana et al. 2013), fruit rot (Garibaldi et al. 2012) and black spot (Molina-Gayosso et al. 2012) in avocado fruit. The isolates of *D. mutila*, *D. iberica*, and *D. seriata* had low virulence on avocado fruit. These species are not considered to be common pathogens in avocado fruit.

Avocados are usually stored at 5ºC under normal atmospheric conditions before they are shipped overseas and distributed to consumers. However, the results of the pathogenicity tests on fruit stored for 15 to 45 days at 5ºC under normal atmospheric conditions indicated that all fruit inoculated with Botryosphaeriaceae species developed symptoms of stem end rot. Additionally, these fruits were damaged by chilling injury, and this damage was highest on inoculated fruit, and most severe after 45 days in storage. These results indicate the importance of storing fruit at optimal conditions to reduce the impact of Botryosphaeriaceae infection in avocado fruit. Defilippi et al. (2014) indicated that avocados should be stored under controlled conditions of temperature (4 to 5°C), humidity (90%), CO_2 (6%) and O_2 (4%) to avoid changes that can alter the fruit.

In conclusion, this study identified and characterized the pathogenicity of species in the Botryosphaeriaceae family associated with branch canker, dieback, and stem end rot in avocado orchards located in the primary production region in Chile. This research provides information that can guide future research to study the epidemiology of these pathogens to establish effective prevention and control strategies, to limit the dissemination of these pathogens to the fruit, and to study the

adequate storage conditions of the fruit to prolong their postharvest life until they reach consumers.

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Zea-Bonilla, T., Gonzalez-Sanchez, M. A., Martin-Sanchez, P. M., and Perez-Jimenez, R. M. 2007. Avocado dieback caused by *Neofusicoccum parvum* in the Andalucía Region, Spain. Plant Dis. 91: 1052-1052.

Table 1. Species, location, and GenBank accession numbers of Botryosphaeriaceae isolates obtained from avocado trees with branch canker and dieback, and from avocado fruits with stem end rot in Chile.

Species	Isolate[a]	Location (Latitude)	GenBank accession number[b]	
			ITS	EF1-α
Diplodia mutila	PALUC1M	Ocoa (32° 50' S)	MF568683	MF581780
D. mutila	PALUC18M	Ocoa (32° 50' S)	MF578221	MF687922
D. mutila	PALUC45F	Ocoa (32º 50' S)	MF578747	MF687932
D. mutila	PALUC451F	Melipilla (33º 46' S)	MF578748	MF687933
D. pseudoseriata	PALUC406M	Quillota (32° 53' S)	MF578222	MF687923
D. seriata	PALUC14M	San Felipe (32° 44' S)	MF578223	MF687924
D. seriata	PALUC404M	Jaururo (32° 28' S)	MF578224	MF687925
D. seriata	PALUC10F	Ocoa (32º 50' S)	MF578749	MF687934

D. seriata	PALUC426F	Jaururo (32º 28' S)	MF578750	MF687935
D. seriata	PALUC455F	Melipilla (33º 46' S)	MF578751	MF687936
D. seriata	PALUC467F	Peumo (34º 23' S)	MF578752	MF687937
Dothiorella iberica	PALUC3M	San Felipe (32° 44' S)	MF578225	MF687926
D. iberica	PALUC399M	Alicahue (32° 24' S)	MF578226	MF687927
D. iberica	PALUC7F	San Felipe (32º 44' S)	MF578753	MF687938
Lasiodiplodia theobromae	PALUC449F	Alicahue (32º 24' S)	MF578754	MF687939
Neofusicoccum australe	PALUC439F	Jaururo (32º 28' S)	MF578755	MF687940
N. australe	PALUC454F	Melipilla (33º 46' S)	MF578756	MF687941
N. australe	PALUC474F	Peumo (34º 23' S)	MF578757	MF687942
N. australe	PALUC490F	Melipilla (33º 45' S)	MF578758	MF687943
N. nonquaesitum	PALUC4M	Ocoa (32° 50' S)	MF578228	MF687929

N. nonquaesitum	PALUC407M	Quillota (32° 53' S)	MF578227	MF687928
N. parvum	PALUC16M	Ocoa (32° 50' S)	MF578229	MF687930
N. parvum	PALUC411M	Peumo (34° 23' S)	MF578230	MF687931
N. parvum	PALUC6F	Ocoa (32º 50' S)	MF578759	MF687944
N. parvum	PALUC44F	Ocoa (32º 50' S)	MF578760	MF687945
N. parvum	PALUC415F	Quillota (32º 53' S)	MF578761	MF687946
N. parvum	PALUC470F	Peumo (34º 23' S)	MF578762	MF687947
N. parvum	PALUC496F	Melipilla (33º 45' S)	MF578763	MF687948

[a] Source M = avocado wood and F = avocado fruit

[b] ITS = internal transcribed spacer and EF1-α = translation elongation factor 1-α

Table 2. Sequences of Botryosphaeriaceae species obtained from GenBank that were included in the phylogenetic analysis.

					GenBank accession number[b]	
Species	**Isolate[a]**	**Host**	**Origin**	**Reference**	**ITS**	**EF1-α**
Botryosphaeria dothidea	CMW8000	*Prunus sp.*	Switzerland	Slippers et al. 2004a	**AY236949**	**AY236898**
Diplodia mutila	CBS 112553	*Vitis vinifera*	Portugal	Alves et al. 2004	**AY259093**	**AY573219**
D. mutila	PD61	*Persea americana*	USA	Inderbitzin et al. 2010	GU251117	GU251249
D. mutila	4D33	*Persea americana*	USA	Chen et al. 2014	KF778789	KF778979
D. pseudoseriata	CBS 124906	*Blepharocalyx salicifolius*	Uruguay	Perez et al 2010	**EU080927**	**EU863181**
D. seriata	CBS 112555	*Vitis vinifera*	Portugal	Alves et al. 2004	**AY259094**	**AY573220**
D. seriata	Mz-F1	*Malus domestica*	Chile	Unpublished	KU942427	KU951888
D. seriata	2K33	*Punica granatum*	USA	Chen et al. 2014	KF778795	KF778985

D. seriata	STE-U 5830	*Prunus sp.*	South Africa	Damm et al. 2007	EF445297	EF445364
Dothiorella iberica	CBS 115041	*Quercus ilex*	Spain	Phillips et al. 2005	**AY573202**	**AY573222**
D. sarmentorum	IMI 63581b	*Ulmus sp.*	England	Phillips et al. 2005	**AY573212**	**AY573235**
D. sarmentorum	CBS 115038	*Malus pumila*	Netherlands	Phillips et al. 2005	AY573206	AY573223
D. sarmentorum	CBS 165.33	*Prunus armeniaca*	Unknown	Phillips et al. 2005	AY573208	AY573225
Lasiodiplodia theobromae	CBS 164.96	Fruit on coral reef	Papua N.G.	Phillips et al. 2005	**AY640255**	**AY640258**
L. theobromae	CMW25212	*Mangifera indica*	South Africa	Mehl et al. 2017b	KU997392	KU997128
L. theobromae	CAA006	*Vitis vinifera*	USA	Alves et al. 2006	DQ458891	DQ458876
Neofusicoccum arbuti	CBS 116131	*Arbutus menziesii*	USA	Phillips et al. 2013	**AY819720**	**KF531792**
N. australe	CMW 6837	*Acacia sp.*	Australia	Slippers et al. 2004b	**AY339262**	**AY339270**
N. mediterraneum	PD312	*Eucalyptus sp.*	Greece	Inderbitzin et al. 2010	**GU251176**	**GU251308**
N. mediterraneum	PD67	*Persea americana*	USA	Inderbitzin et al. 2010	GU251191	GU251323

N. nonquaesitum	PD484	*Laurus nobilis*	USA	Inderbitzin et al. 2010	**GU251163**	**GU251295**
N. nonquaesitum	UCR2733	*Persea americana*	USA	Carrillo et al. 2016	KT965281	KT965283
N. parvum	ATCC 58191	*Populus nigra*	N. Zealand	Slippers et al. 2004a	**AY236943**	**AY236888**
Melanops tulasnei	CBS 116805	*Quercus robur*	Germany	Phillips and Alves 2009	**FJ824769**	**FJ824774**
Melanops tulasnei	CBS 116806	*Quercus robur*	Germany	Phillips and Alves 2009	**FJ824770**	**FJ824775**

[a] Acronyms of culture collection: ATCC = American Type Culture Collection, Virginia, USA; CAA: Personal culture collection A Alves, University of Aveiro, Portugal; CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; IMI = International Mycological Institute, CBI-Bioscience, Egham, Bakeham Lane, UK; PD: Culture collection, University of California, Davis, USA; UCR: Culture collection, University of Riverside, California, USA.

[b] ITS = internal transcribed spacer and EF1-α = translation elongation factor 1-α. In bold ex-type specimens.

Table 3. Conidial measurements of Botryosphaeriaceae species obtained from avocado wood and fruit in Chile and their comparison with the type specimens of previous studies.

Species	Isolate[a]	Conidial size L x W; Mean ± SD (µm)[b]	L/W[c]	Source
Diplodia mutila	**CBS 112553**	27.4-23.5x14.3-12.4; 25.4±1.0x13.4±0.5	1.9	Alves et al. 2004
D. mutila	PALUC1M	26.9-20.8x14.2-9.4; 24.2±1.3x12.2±1.1	2.0	This study
D. mutila	PALUC18M	29.5-20.6x14.3-10.6; 25.8±1.9x12.8±0.8	2.0	This study
D. mutila	PALUC45F	30.3-20.9 x17.9-10.3; 23.9±2.1 x 13.6±1.5	1.8	This study
D. mutila	PALUC451F	29.8-22.1x14.5-10.2; 25.4±1.6 x 12.5±0.9	2.0	This study
D. pseudoseriata	**CBS 124906**	30.5-23.0x14.0-10.0; N/A	N/A	Perez et al. 2010
D. pseudoseriata	PALUC406M	30.5-24.9x13.3-9.8; 27.3±1.2x11.4±0.7	2.4	This study
D. seriata	**CBS 112555**	28.0-21.5x15.5-11.0; 24.9±1.9x12.9±1.1	1.9	Phillips et al. 2013
D. seriata	PALUC14M	28.6-20.9x13.4-8.8; 24.1±1.8x10.4±1.1	2.3	This study

D. seriata	PALUC404M	32.3-20.2x12.5-9.4; 25.0±2.2x11.2±0.8	2.2	This study
D. seriata	PALUC10F	26.8-18.8x11.9-7.7; 22.2±1.7 x 9.7±0.8	2.3	This study
D. seriata	PALUC426F	25.9-20.9x12.4-10.0; 23.6±1.6 x 10.9±0.8	2.2	This study
D. seriata	PALUC455F	25.2-20.2x14.2-9.1; 22.3±1.3 x 11.2±1.0	2.0	This study
D. seriata	PALUC467F	24.9-17.6x12.0-8.3; 21.8±1.5 x 10.5±0.8	2.1	This study
Dothiorella iberica	**CBS 115041**	28.6-17.2x16.0-8.1; 23.2±1.9x10.9±1.2	2.2	Phillips et al. 2005
D. iberica	PALUC3M	28.8-23.1x13.7-8.5; 26.1±1.5x10.2±0.9	2.6	This study
D. iberica	PALUC399M	27.9-22.7x11.7-8.9; 25.1±1.1x10.5±0.6	2.4	This study
D. iberica	PALUC7F	28.7-22.7x12.8-9.9; 25.7±1.4x11.3±0.6	2.3	This study
Lasiodiplodia theobromae	**CBS 164.96**	32.5-19.0x18.5-12.0; 26.2±2.6x14.2±1.2	1.9	Phillips et al. 2013
L. theobromae	PALUC449F	26.2-16.7x14.5-11.1; 20.9±2.4x12.7±0.8	1.7	This study
Neofusicoccum australe	**CMW 6837**	30.0-18.0x7.5-5.0; 24.7x5.1	4.8	Phillips et al. 2013

N. australe	PALUC439F	24.1-17.7x12.8-9.4; 21.7±1.9x10.5±0.9	2.1	This study
N. australe	PALUC454F	29.9-19.9x13.6-8.5; 24.9±1.9x10.9±0.9	2.3	This study
N. australe	PALUC474F	29.6-22.8x12.3-6.9; 25.2±1.8x8.8±1.6	2.9	This study
N. australe	PALUC490F	23.3-17.9x9.6-6.2; 20.5±1.3x8.1±0.8	2.6	This study
N. nonquaesitum	**PD484**	29.0-17.0x10.5-5.5; 23.2x7.6	3.1	Phillips et al. 2013
N. nonquaesitum	PALUC4M	31.7-23.0x9.4-5.3; 27.0±1.8x7.3±0.7	3.7	This study
N. nonquaesitum	PALUC407M	27.4-18.7x8.5-4.9; 23.9±1.9x6.5±0.8	3.7	This study
N. parvum	**ATCC 58191**	24.0-12.0x10.0-4.0; 17.1±2.1x5.5±0.8	3.2	Phillips et al. 2013
N. parvum	PALUC16M	26.4-16.5x9.7-6.1; 21.2±1.9x8.2±0.9	2.6	This study
N. parvum	PALUC411M	24.6-18.8x8.8-6.5; 21.2±1.3x7.7±0.6	2.8	This study
N. parvum	PALUC6F	23.1-15.7x8.7-4.9; 20.2±1.6x7.2±0.8	2.8	This study
N. parvum	PALUC44F	22.4-17.7x9.6-5.9; 19.9±1.1x7.8±0.7	2.6	This study

N. parvum	PALUC415F	28.6-17.9x10.9-6.4; 21.1±1.9x8.0±0.9	2.6	This study
N. parvum	PALUC470F	23.4-16.1x9.1-5.1; 19.4±1.4x7.3±0.8	2.7	This study
N. parvum	PALUC496F	29.1-19.5x9.8-6.6; 23.2±2.3x8.2±0.8	2.9	This study

[a] measured data of the ex-type is in boldface.

[b] L x W = maximum - minimum length by maximum - minimum width; average ± standard deviation [SD] length by average ± standard deviation [SD] width.

[c] L/W = average length / average width.

N/A = not available.

Table 4. Pathogenicity study of Botryosphaeriaceae species on Hass avocado fruit stored for 15, 30, or 45 days at 5ºC, followed by incubation for 10 d at 20ºC in a normal atmosphere.

Species	**Mean lesion length (mm)[w]**			
isolate	**15d at 5ºC**	**30d at 5ºC**	**45d at 5ºC**	**Mean [w]**
Diplodia mutila				
PALUC45F	46.8 ab	53.5 a	48.1 a	49.4 a
PALUC451F	44.5 ab	43.5 ab	53.2 a	47.0 a
D. seriata				
PALUC426F	50.0 ab	36.5 bcd	59.1 a	48.5 a
PALUC455F	34.9 bc	47.5 ab	58.1 a	46.7 a
Dothiorella iberica				
PALUC7F	17.0 d	26.0 d	46.3 a	29.7 b

Lasiodiplodia theobromae				
PALUC449F	53.0 ab	35.0 bcd	59.3 a	49.1 a
Neofusicoccum australe				
PALUC439F	40.8 b	36.0 bcd	64.8 a	47.2 a
PALUC490F	42.0 ab	35.0 bcd	54.0 a	43.6 a
N. parvum				
PALUC6F	34.5 c	34.0 bcd	55.0 a	41.1 a
PALUC415F	55.5 a	31.0 cd	53.7 a	46.7 a
PALUC470F	50.0 ab	39.0 abc	61.0 a	50.0 a
PALUC496F	21.5 d	44.0ab	66.0 a	43.8 a
Control	7.5 d	19.5 d	24.4 b	17.1 c
Mean	38.3 b	36.9 b	54.0 a	

Analysis of Variance

	df	F	P	df	F	P	df	F	P	df	F	P
Isolates (I)	12	17.8	<0.0001	12	6.5	<0.0001	12	5.8	<0.0001	12	18.3	<0.0001
Time (T)										2	80.4	<0.0001
I x T										24	4.9	<0.0001

[w] Data are the average of five replicates. Means followed by same letters are not significantly different (P =0.05) according to Tukey's test.

[x] Mean lesion length (mm) of fruit maintained during 15 days at 5ºC + 10 days at 20ºC.

[y] Mean lesion length (mm) of fruit maintained during 30 days at 5ºC + 10 days at 20ºC

[z] Mean lesion length (mm) of fruit maintained during 45 days at 5ºC + 10 days at 20ºC

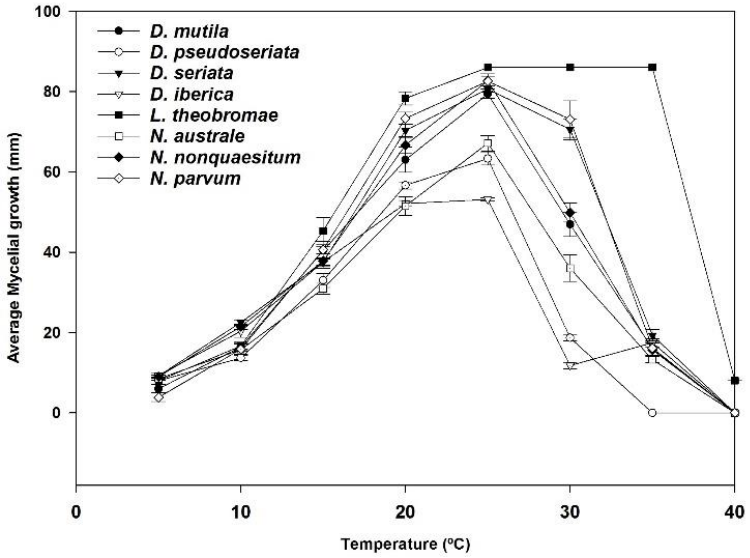

Figure 1. Effects of temperature on the average mycelial growth (mm) of *Diplodia mutila*, *D. pseudoseriata*, *D. seriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *Neofusicoccum australe*, *N. nonquaesitum* and *N. parvum*. The average of the mycelial growth was derived from measurements on the second and third days in acidified potato dextrose agar. Vertical bars represent the standard error of the mean.

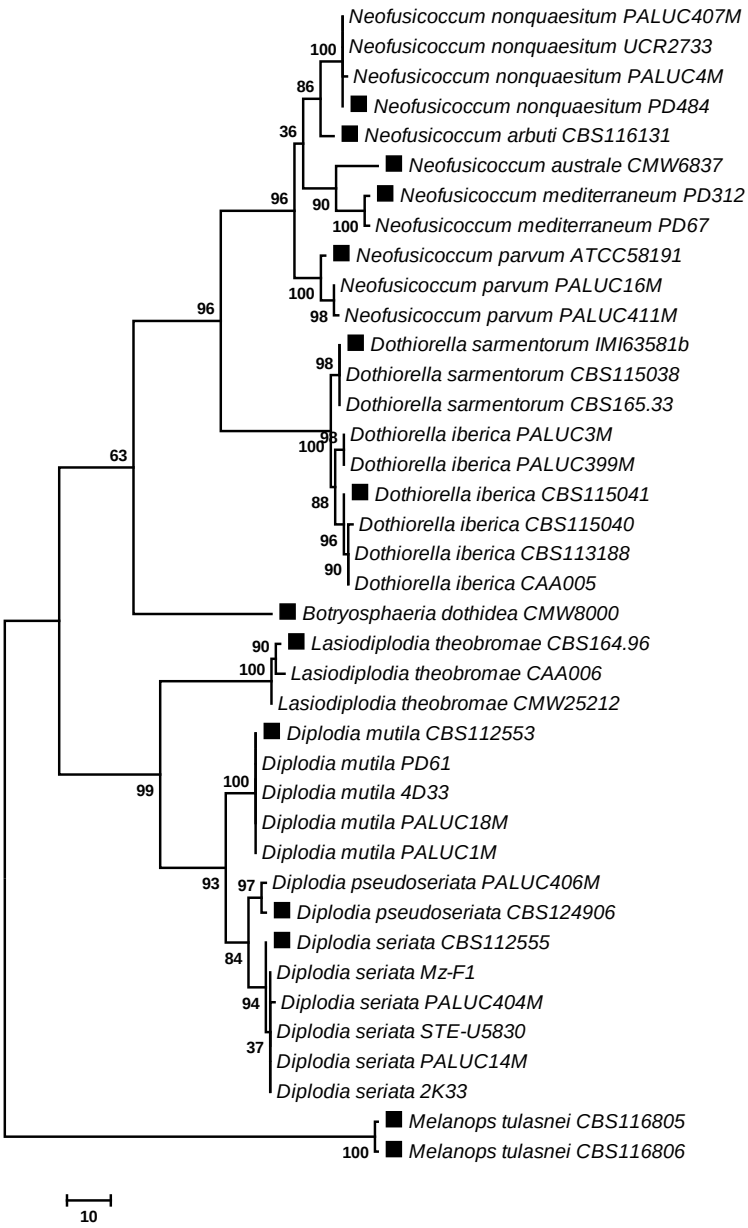

Figure 2. One of the ten most parsimonious phylogenetic trees obtained from the combined ITS and EF1-α datasets. Bootstrap values of 1,000 bootstrap replications are indicated at each node. Black square indicates type specimens, and 'PALUC' denote isolates of avocado wood from this study. CBS116805 and CBS116806 (*Melanops tulasnei*) were added as an outgroup.

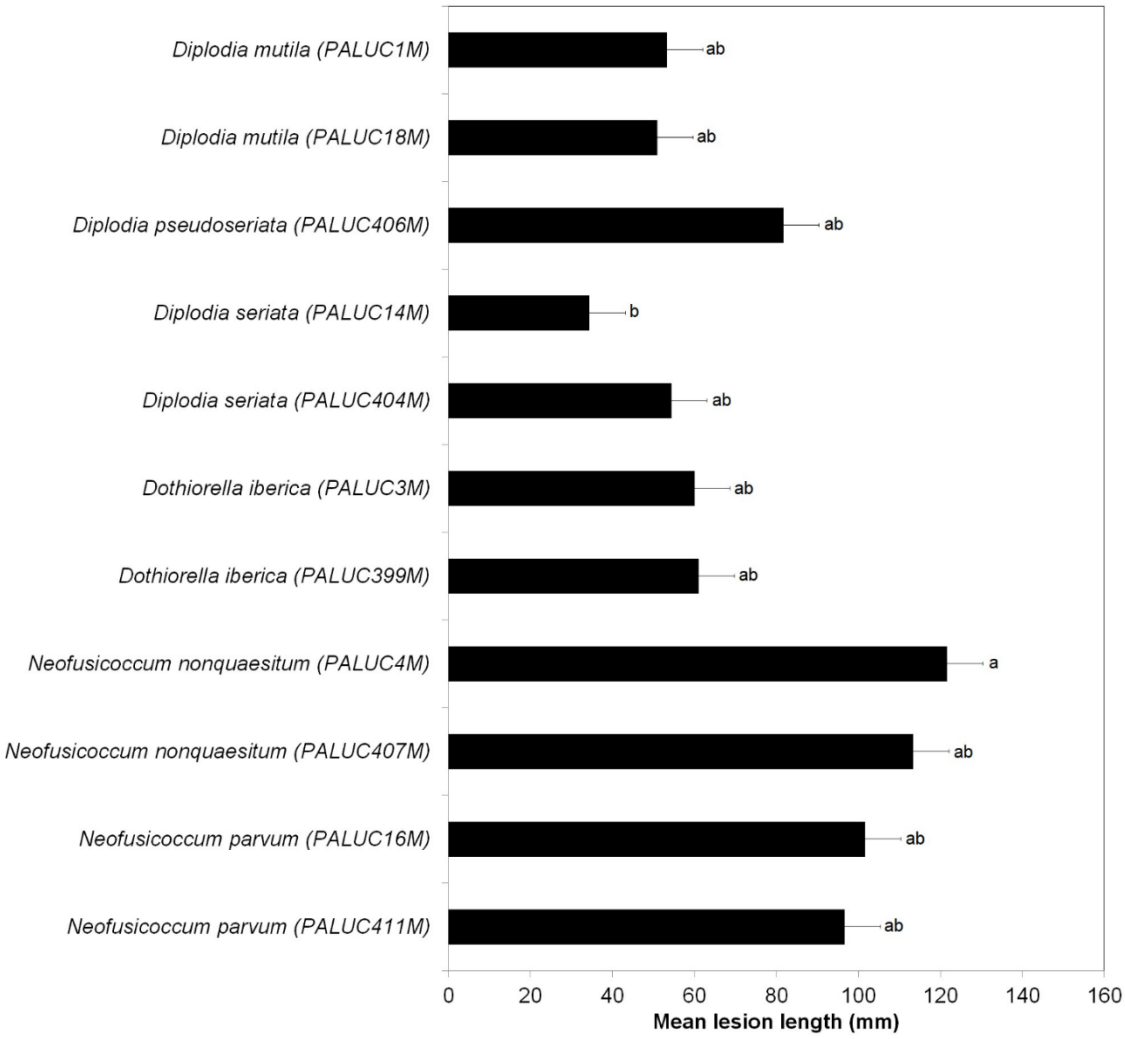

Figure 3. Mean lesion length (mm) caused by Botryosphaeriaceae species in ‘Hass' avocado plants 8 weeks after inoculation. Horizontal bars represent the standard error of the mean, and the mean lesion length followed by same letter are not significantly different according to Tukey's test (P = 0.05).

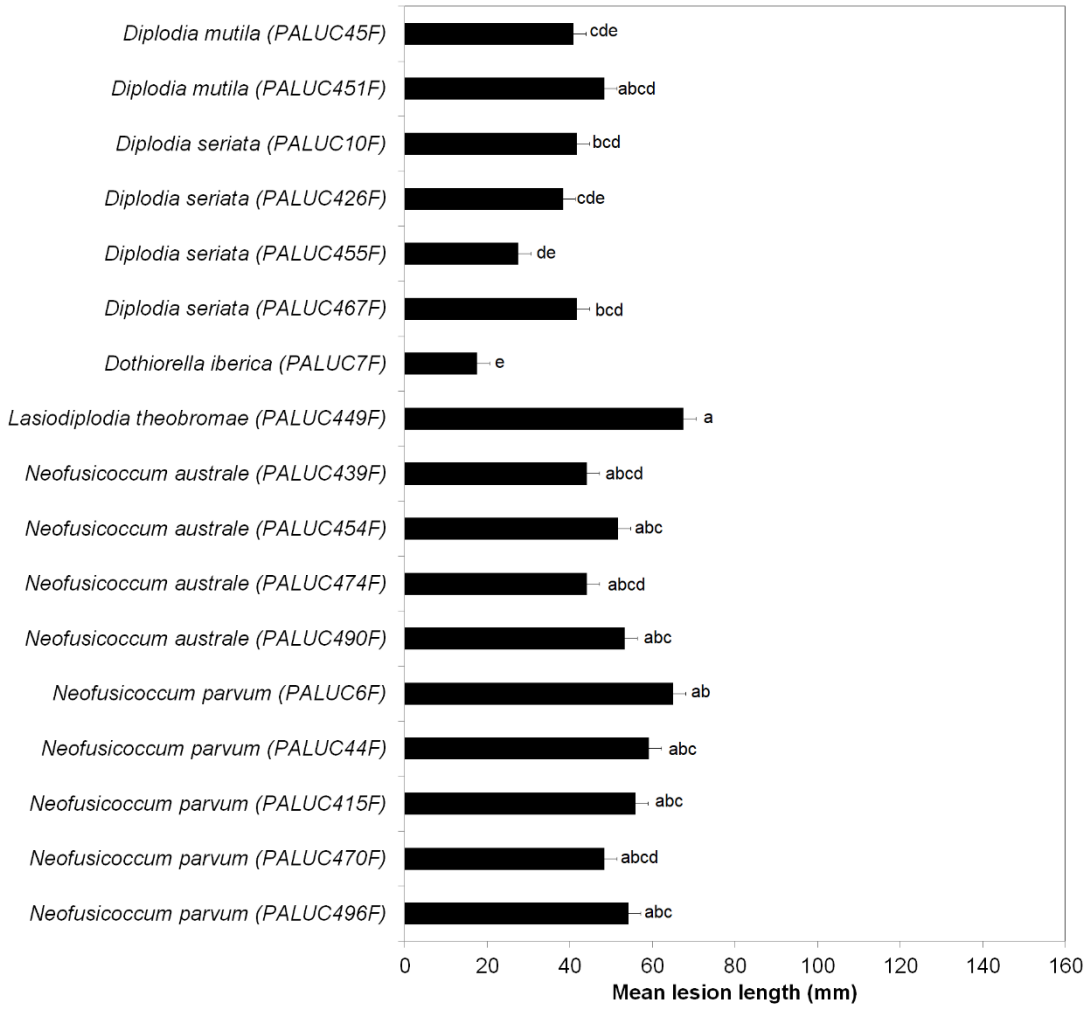

Figure 4. Mean lesion length (mm) caused by Botryosphaeriaceae species in Hass avocado fruit incubated during 10 days at 20ºC after inoculation. Horizontal bars represent the standard error of the mean, and the mean lesion length followed by same letter is not significantly different according to Tukey's test (P = 0.05).

Chapter 3

Effect of Edaphoclimatic Conditions, Planting and Orchard Management in Branch Canker, Dieback and Stem End Rot in Chilean Avocado Orchards.

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Effect of Edaphoclimatic Conditions, Planting and Orchard Management in Branch Canker, Dieback and Stem End Rot in Chilean Avocado Orchards.

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ABSTRACT

In Chile, increasing cases of branch canker and dieback were reported in avocado orchards from 2011 to 2016, which coincided with a severe drought affecting the avocado production area. Additionally, the prevalence of avocado fruit with stem end rot has been increasingly observed in distant consumer countries. The predisposing factors for these diseases in Chilean orchards have not been reported. Therefore, the objective of this study was to identify the main edaphoclimatic condition, planting and management factors associated with branch canker, dieback, and stem end rot in Chile. Analysis of Variance, Principal Component Analysis, Partial Least Squares Analysis, and Partial Least Squares Discriminant Analysis, were performed to

analyze 102 variables associated with prevalence of these diseases in avocado during two consecutives growing season (2014/2015 and 2015/2016). Our study indicated that these diseases are conditioned mainly by planting variables such as: plant age, volume of canopy, diameter of trunk, leaf area index and Planting density. Additionally, the analysis of stem end rot prevalence included postharvest variables, such as: days to maduring and Dry matter of fruits, and climatic conditions as sun exposure in spring, temperature (minimum temperature in spring, average and minimum temperature in winter, and maximum temperature in autumn), relative humidity in several year season and precipitation in spring. On the other hand, with this study was possible to determinate that these diseases can developed in different agroclimatic zone of Chile.

This research allows to guide to agricultures about conditions that pathogens requires for developed of these diseases, which is necessary for stablished appropriate and efficient management practices to avoid dissemination of pathogens and to control the disease.

Avocado (*Persea americana* Miller), is a persistent subtropical tree endemic from Central America and Mexico. Avocados are produced and exported from México, Peru, Chile, Israel, South Africa, Spain, Brazil and USA. Chile is an important avocado exporter, with 29.289 ha planted mainly between Coquimbo Region (29° 20’S to 32° 15’S) and O’Higgins Region (33° 51’S to 35° 01’S) being Valparaiso Region the most productive (Muñoz 2018).

Hass is the main cultivar produced in Chile, which travels toward Europe, North America, Asia and South America. The great problem of Chilean production is the distance with potential and consumers countries. Therefore, is necessary to maintain the fruit quality during postharvest for a long time until reaches to consumers, maintaining the commercial quality, avoiding pest, diseases and wounds that cause damage in the avocado fruits. However, since 2014, the incidence of stem end rot has increased in export markets that consume Chilean fruit, such as the Asian market, but especially in the European markets, where maturation chambers are used for reducing the ripening times of the fruit. These chambers increasing temperature, ethylene concentration and relative humidity in storage, which allows avocados to be sold ready-to-eat.

Stem end rot (SER), is a postharvest rot, which is caused by latent infections initiated on the tree during the growing season (Hopkirk et al. 1994). In latent infections, the pathogen can remain during prolonged periods in a quiescent stage until reaching specific conditions, which can cause that pathogen to become active (Verhoeff 1974). The relationship between host, pathogen and environment in latent infection indicate a dynamic equilibrium, because in such condition there are not symptoms (Jarvis 1994). The infection of fruit can be initiated by fungi causing branch canker and dieback, which are localized endophytically in wood (Guarnaccia et al. 2016; Twizeyimana et al. 2013; Valencia et al. 2019) or by infection of peduncle during harvest (Hartill and Everett 2002).

The initial symptom of stem end rot may be detected by a slight softening in the union area to peduncle, this lesion grows compromising the fruit completely with the

progress of ripening. In severe cases the fruit are covered by mycelium and conidias (Dann et al. 2013; Johnson and Kotzé 1994; Menge and Ploetz 2003; White et al. 2005).

The pathogens associated with stem end rot include fungal species, such as *Colletotrichum gloesporioides* (Penz.) Penz. & Sacc, *C. acutatum* J.H. Simmonds, *Thyronectria pseudotrichia* (Schwein.) Seeler, *Phomopsis perseae* Zerova, *Pestalotiopsis clavispora* (G.F. Atk.) Steyaert, and *P. versicolor* (Speg.) Steyaert (Dann et al. 2013; Darvas and Kotzé 1987; Hartill and Everett 2002; Menge and Ploetz 2003; Valencia et al. 2011). However, anamorphs of Botryosphaeriaceae species have been considered like the most important pathogens of avocado stem end rot throughout the world (Dann et al. 2013; Menge and Ploetz 2003), including *Diplodia mutila* (Fr.) Mont. in Chile (Valencia et al. 2019) and the USA (Inderbitzin et al. 2010), *D. seriata* De Not. in Chile (Valencia et al. 2019), *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves in Chile (Valencia et al. 2019), *L. theobromae* (Pat.) Griffon & Maubl in Chile (Valencia et al. 2019) and Italy (Dann et al. 2013; Garibaldi et al. 2012), *N. australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips in Chile (Montealegre et al. 2016; Valencia et al. 2019) and Turkey (Akgül et al. 2016), *N. luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips in the USA (Twizeyimana et al. 2013), *N. mangiferae* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips in Taiwan (Ni et al. 2009), *N. mediterraneum Crous, M.J. Wingf. & A.J.L. Phillips* in the USA (Inderbitzin et al. 2010), and *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips in Chile (Valencia et al. 2019), Italy (Guarnaccia et al. 2016) and Mexico (Molina-Gayosso et al. 2012).

The branch canker begins with rough protuberances on the bark in trunk and twigs, with internal necrotic tissue. Subsequently, develop cankers on the trunk, branches and twigs that cause, friable bark, often with whitish to brownish exudates of perseitol, a crystalline polyhydric alcohol produced by avocados (Dann et al. 2013; Johnson and Kotze 1994; Menge and Ploetz 2003). Dieback occurs in small twigs, retaining dead leaves and fruits. The leaves turn brown and the fruit completely black with advanced stages of softening, which may be for several months in these twigs (McDonald and Eskalen, 2011).

Branch canker and dieback are two wood diseases associated mainly with a complex of fungal species, of which the most common on avocado is the family Botryosphaeriaceae (Dann et al., 2013; McDonald and Eskalen 2011; Menge and Ploetz 2003). The Botryosphaeriaceae species associated with these wood diseases are *B. obtusa* (Schwein.) Shoemaker and *B. rhodina* (Berk. & M.A. Curtis) Arx have been reported in Mexico and the USA (Menge and Ploetz 2003); *D. mutila* in Chile (Valencia et al. 2019) and the USA (Chen et al. 2014); *D. seriata* and *D. pseudoseriata* C.A. Pérez, Blanchette, Slippers & M.J. Wingfield in Chile *(*Valencia et al. 2019); *Dothiorella iberica* in Chile (Valencia et al. 2019) and the USA (McDonald and Eskalen 2011); *Lasiodiplodia theobromae* in Tanzania (Alves et al. 2008); *Neofusicoccum australe* in Chile (Auger et al. 2013) and the USA (McDonald et al. 2009); *N. luteum* in the USA (McDonald et al. 2009); *N. nonquaesitum* Inderb., Trouillas, Bostock & Michailides in Chile (Valencia et al. 2019) and the USA (Carrillo et al. 2016); *N. parvum* in Chile (Valencia et al. 2019), Italy (Guarnaccia et al. 2016), Spain (Zea-Bonilla et al. 2007), the USA (McDonald et al. 2009).

The infection occurs on injured wood, so it was considered that the wounds of pruning, girdling, chilling injury, mechanical damage, bark split by the wind, wounds graft, etc., could allow the entry of the pathogen (Dann et al. 2013). If the infection reaches vascular tissue, it can stop water and nutrients transport from xylem and translocation of assimilates reserves to sinks. This blockage causes weakening and decay of the wood at the infection site, which eventually can lead wilting or death of the branch (Eskalen et al. 2013). The avocado trees accumulate reserves in the bark, therefore, the disruption in flow, affects the accumulation and availability of reserves located on the trunk and branches, which are necessary for fruiting the following season (Chanderbali et al. 2013).

The Botryosphaeriaceae species are fungus endophytes associated with the plant microbiome of avocado trees (Shetty et al. 2016), which have been considered as opportunist pathogens in avocado trees, because not damage have been observed in healthy trees, but post latent phase has the ability to rapidly cause disease when their host are under stress that increase tree susceptibility (Slippers and Wingfield 2007). The stress associated with these diseases are: drought, nutritional deficiencies, flooding, extreme temperatures or damage by insects or other pathogens (Dann et al, 2013; Eskalen et al, 2013; Johnson and Kotze 1994; McDonald and Eskalen, 2011; Menge and Ploetz, 2003; Slippers and Wingfield, 2007).

The avocados are subtropical species, which requires managements to adapt the Mediterranean climate conditions that exist in central Chile. Such managements have allowed adapting to the availability of water, soil salinity, irrigation, climate,

among others. However, the production in Chile is presenting problems in terms of productivity, due to the effects of drought, which has caused an important decrease of hectares planted with avocado trees in the last 10 years (Muñoz 2018), which coincided with increasing cases of wood diseases in Chilean avocado orchards and reports of fruits with SER in postharvest.

Understanding the relationship between pathogens with avocado host and environment is critical to apply appropriate control measures and thus reduce the economic effect caused by these diseases in orchard and post-harvest. Therefore, the objective of this study is to determine edaphoclimatic, planting and management factors associated with the prevalence of branch canker, dieback and stem end rot in Chile, because these factors predisposing the tree and fruit to be colonized and infected by pathogens of Botryosphaeriaceae species.

MATERIALS AND METHODS

This study was conducted in 16 ‘Hass’ avocado orchards between Illapel (31° 37’S) and Peumo (34° 24’S), during two consecutives growing season (2014/2015 and 2015/2016), from september 2014 to august 2015, and september 2015 to august 2016. This period is associated with the phenological period between flowering and fruit growth. A brief description of the agroclimatic zones, where were localized 16 orchards that were prospected in the two seasons, based on the classification of Pérez and Adonis (2012) is provided in Table 1.

Field sampling. Fifteen symptomatic or healthy trees were selected in each study area, and five wood samples were obtained from each tree and transported to the laboratory where they were processed. A total of 45 fruit (three pedunculated fruits per tree with more than 23% dry matter) were harvested near to symptomatic branches and trunk and transported to the laboratory in plastic bags. The fruit were surface disinfected by immersion in 75% ethanol for 30 s and placing them in humidity chambers for 7 to 31 days at 20ºC in a regular atmosphere until they reached consumer maturity.

Isolation, culture, and identification. Small wood pieces and pulp pieces of mature Hass fruits (3 to 5 mm) were removed from the margins between the healthy and symptomatic tissues and placed in Petri dishes containing 2% potato dextrose agar acidified with 0.5 ml/liter of 92% lactic acid plus 0.05% tetracycline (APDAt) (Díaz et al. 2013). The plates were incubated at 20°C in darkness for 14 to 21 days.

All the isolates were transferred to acidified potato dextrose agar (APDA). Pure cultures were obtained from hyphal tip transfers. All the isolates were stored in 1.5 ml of sterile 20% glycerol at 5°C.

To identify the isolates obtained in this study, the colony morphology was characterized on APDA after 5, 12, and 27 days at 25°C in darkness. The length and width of 50 conidia were measured (mean and standard deviation). The color, shape and the presence or absence of septation in the conidia were also determined. Morphology and measurements of conidia were compared with published

descriptions of mycobank.org, Phillips et al. (2013), Sutton (1980), and Udayanga et al. (2011).

Characterization of orchards. In each consecutive growing season (2014/2015 and 2015/2016) the orchards were characterized with 102 variables registered, associated with:

Climate variables. The climate data were obtained from meteorological stations near to avocado orchards used in this study. The climate variables used were: average temperature (AT, °C), maximum temperature (MAXT, °C), minimum temperature (MINT, °C), solar radiation (RAD, Wm^{-2}), average relative humidity (RH, %), and accumulated precipitation (Pp, mm). These data were separated for each year season: spring (sp, September to December), summer (su, December to March), autumn (au, March to June), and winter (wi, June to september), with measuring frequency of 1 hour.

Planting variables. Latitude (Lat), longitude (Long), altitude (Alt), agroclimatic zone (Zone), plant density (Plants/ha), origin of plants (OPlants, nursery or own plants), age of plant (PlantAge), volume of canopy (VolumeC), diameter of trunk (DiameterT), rootstock variety (Rstock), leaf area index (LAI), soil characteristics (texture, bulk density, pH pHS, electric conductivity ECS, organic matter OMS, Nitrogen NS, Phosphorous PS, Potassium KS, cationic exchange capacity CECS), foliar nutrient content (Nitrogen NF, Phosphorous PF, Potassium KF, Calcium CaF, Magnesium MgF, Copper CuF, Manganese MnF, Zinc ZF), crop evapotranspiration (ETc), and background of biotic (pest and pathogens) or abiotic stress problems

such as drought, salinity, frost, nutrients deficiencies, mechanical damage, and diameter of necrotic inner lesions caused by SER (LFruit). Production parameters, such as: yield, fruit load, weight of fruits, days from harvest to fruit ripening (DaysM), and fruit nutrient content (dry matter DMFruit, Nitrogen NFruit, Phosphorous PFruit, Potassium KFruit, Calcium CaFruit, Magnesium MgFruit, Copper CuFruit, Iron IFruit, Manganese MnFruit, Zinc ZnFruit, Boron BFruit).

Management variables. irrigation system (Isystem, drip or micro sprinkler), water applied regarding crop evapotranspiration (H_2OETc), pruning (date DateP, frequency pruning FrP, intensity pruning IP, pruning sealed of wounds: PasteP, FungicideP), girdling, applied nitrogen dosage (UN/ha), applied calcium (Ca+), applied humic acids (HumicA) and growth regulators application date (DateGR), and growth regulators application frequency (FrGR), also were included in this study.

Diseases index. The prevalence of wood disease (branch canker and dieback (CD)) was determined by the percentage of disease trees, according of total trees in each study area (900 m^2). To determine the prevalence of stem end rot (SER) the fruits were surface disinfected by immersion in 75% ethanol for 30 s and then placed in humidity chambers for 7 to 31 days at 20ºC until they reached firmness of consumer maturity, considering diseased fruits of total harvested fruits by study area (N=45).

Statistical analysis. To analyze the climate variables in each growing season were performed one-way analysis of variance (ANOVA). In addition, the analysis of climate variables of consecutives growing seasons was performed with a multifactor ANOVA of two levels, with factors ‘growing season’ and ‘season of the year’. The

results were compared with Tukey's test (P = 0.05), using SAS v.9 (SAS Institute, Cary, NC). The climate variables were contrasted with the Partial Autocorrelation Coefficient (ρk), which tests the autocorrelation between climate variables itself. Values ≤ 0.6 denote no autocorrelation (Montgomery 2001).

Several multivariate analyses were performed to study the scenarios included in this research. These scenarios consider data by season (2014/2015, 2015/2016), and disease data (CD, SER) separately or together: The scenario 1 included data of seasons 2014/2015 and season 2015/2016, with the diseases CD and SER, the scenario 4 included data of season 2014/2015 and the diseases CD and SER, and the scenario 7 included data of season 2015/2016 and the diseases CD and SER.

The dataset included variables associated with edaphoclimatic conditions, planting, crop managements and disease index. The qualitative variables were transformed into categorical scaled (3, 5, 7…n) and by presence (1) or absence (0) (Carot et al. 2003).

The multivariate analyses were performed to analyze the 102 variables, using Chemometrics methods. The methods applied were Principal Component Analysis (PCA), Partial Least Squares Analysis (PLS), and Partial Least Squares Discriminant Analysis (PLS-DA). These analyses were based on the Nonlinear Iterative Partial Least Squares algorithm (NIPALS) (Wold et al. 2001), which allows the analysis of a large number of variables, highly correlated and an ill-conditioned matrix (Ferrer 2007). All variables were centered and standardized to unit variance previous the analysis. All models were validated by a full cross-validation routine, minimizing the

prediction residual sum of squares function (PRESS) to avoid over fitting the models (Cen et al. 2007).

The PCA was performed to determine the relationship among edaphoclimatic, planting and management variables with disease index. The PCA is a method that allows synthesizing the information contained in a large matrix of variables, within a smaller set of factors (principal components), with minimal loss of information (Yañez et al. 2012). Likewise, the PCA allows condensing the information in two ways: identifying relationships between observations that comprise the score matrix, and determining relationships between variables, known as the loadings matrix. Additionally, allows display the relationships between observations and variables in orthogonal planes, that represent the direction of greatest variance contained in the dataset (Cuneo et al. 2013; Saavedra and Cordova 2011).

The PLS method was performed to evaluate the predictor condition over the disease index of the different variables, because the PLS allows analyzing the effects of the predictor variables in the response variables, maximizing the covariance of these matrices, to generate projections in an orthogonal plane and thus be able to develop predictive relationships between them (Kruger and Xie 2012; Wold et al. 2001).

The PLS-DA was performed to determine the relationships between class of observations and orchard and diseases index, because this method allows grouping the observations into classes, according to the contribution of each variable, to determine the causality of possible significant groups, associated to which problem variables are responsible for the behavior of that class and if there are anomalous

or eventually new classes (Barker and Rayens 2003; Brereton and Lloyd 2014; Eriksson et al. 2006).

All chemometrics methods were performed using SIMCA-P v.10 software (Umetrics AB, Sweden).

RESULTS

Field sampling. In 9 orchards were detected symptoms associated with branch cankers and dieback, in young plants of replant and productive adult trees. In some branches and trunk there were friable bark and brown or red-brown inner tissue, and branches with dry leaves, inflorescences, and small fruits with abnormal development.

Evaluated fruits with severe level of damage developed white and grey mycelium on the peel, from the area near to peduncle, and black pycnidia with masses of gray conidia on the peel covering the fruit completely were observed in some cases. The inner tissue was very affected because there were necrosis and cavities in the pulp, reaching great depth, with white mycelium. On the contrary, fruits with low level of damage, did not develop mycelium and conidia in the peel, but when the peduncle was removed, damage in inner tissue advancing internally until equatorial zone was observed.

Fungal isolation. A total of 67 isolates were obtained from 1200 samples of avocados wood. These isolates were identificated as Botryosphaeriaceae species

(Valencia et al. 2019), *Pestalotiopsis* spp., *Colletotrichum* spp., and *Phomopsis* spp. based on their cultural and morphological features. From orchards without symptoms in wood, there were not isolates obtained, because none plate Petri dish developed colony of fungus. Likewise, in 675 samples of avocado fruits 162 isolates were recovered, corresponding to: Botryosphaeriaceae species (Valencia et al. 2019), *Colletotrichum* spp., *Phomopsis* spp., and *Pestalotiopsis* spp. In both growing season the isolates of Botryosphaeriaceae spp obtained from wood and fruits samples were more frequent (Figure 1).

Analysis of variance. The ANOVA of average temperature, maximum temperature, minimum temperature, average radiation, average relative humidity and accumulative precipitation registered between year seasons in each growing season, showed significative difference ($P < 0.0001$). Moreover, the analysis of partial autocorrelation coefficient (ρk), estimated that ρk was near 0.2, therefore, there were not autocorrelation between these variables.

The maximum average temperatures were registered in summer and minimum average temperatures were registered in winter. Moreover, the minimum average temperature showed that season 2015/2016 registered minor average temperature than season 2014/2015. Regarding to maximum temperature in season 2014/2015, there were no difference between spring, summer and autumn, these maximum temperatures registered were highest than maximum temperatures registered in season 2015/2016 in the same year season. Likewise, in both growing season there were difference between minimum temperature of summer and spring but not between autumn and winter (Figure 2).

The average solar radiation registered were most high during summer and spring in season 2014/2015, and during spring in season 2015/2016. In both growing season the less average solar radiation was registered during autumn and winter (Figure 3).

The average relative humidity in season 2014/2015 registered the highest value during winter, and the less value during spring, summer and autumn. Moreover, in season 2015/2016 there were no difference between data registered in spring and summer, and data registered during autumn and winter. However, the highest value was registered during winter and autumn. In another hand, the accumulated precipitation registered were higher in winter for season 2014/2015 and autumn in season 2015/2016 (Figure 3).

Multivariate analysis. The scenarios 1, 4 and 7 were analyzed with PCA, PLS, PLS-DA, because there were more informative and allowed better explain the relationship between orchards conditions in study with different level of prevalence of CD and SER in both growing seasons. The scenario 1 was selected, because included the main variables of planting, associated with these diseases in both growing seasons. The scenario 4 was selected because included the main variables of edaphoclimatic conditions, planting, and crop management associated with these diseases in growing season 2014/2015. Finally, the scenario 7 was selected because included the main edaphoclimatic conditions, planting, and crop management variables associated with these diseases in growing season 2015/2016.

PCA of scenario 1. The PCA performed indicated that the multifactorial model retained two components that explain 75.6% of total variance. The factor 1 (50.4%

explained variance) sorted the orchards according to level of prevalence of CD, in left side the high level and low level in right side. The factor 2 (25.2% explained variance) sorted the orchards in study according to level of prevalence of SER, in above side the high level and low level in below side. In the loadings plot, the factor 1 sorted the planting variables VolumeC, DiameterT, and PlantAge, which are associated with orchards with high level of prevalence of CD. In addition, the factor 2 sorted the planting variable DaysM with high level of prevalence of SER (Figure 4).

PLS-DA analysis of scenario 1. This analysis extracted three components that explain 51.3% of total variance of matrix Y (R^2Y), and the cumulative overall cross-validated Q^2cum (cumulative fraction of the total variation that can be predicted by specific factors, as overall cross-validated) was 41.4% for four classes previously defined. The four classes were then designated: Class 1 (orchards with high level of CD prevalence and high level of SER prevalence); Class 2 (orchards with high level of CD prevalence and low level of SER prevalence); Class 3 (orchards with low level of CD prevalence and high level of SER prevalence); Class 4 (orchards with low level of CD prevalence and low level of SER prevalence). The loadings plot showed that Class 1 is associated with PlantAge, VolumeC, DiameterT, and DaysM; Class 3 is associated with LAI, and Class 4 is associated with Plants/ha. Moreover, Class 2 is associated with DMFruit (Figure 5).

PCA of scenario 4. The PCA performed indicated that the factorial model retained two components that explain 76.1% of total variance. The factor 1 (54.3% explained variance) sorted the orchards in study according to agroclimatic zone in left side the

pre-mountain valley and in right side orchards of inner valley with coastal influence. The factor 2 (21.7% explained variance) sorted the orchards in study according to level of prevalence of CD and SER, in above side the high level of CD prevalence and high level of SER prevalence in below side. The loadings plot, sorted the edaphoclimatic, planting and management variables. In left side, Alt, RADsu, RADsp and MINTsp, which are associated with orchards localized in pre-mountain valley, and in right side Long, RHsp, RHsu, RHau, RHwi and Ppsp, which are associated with orchards localized in inner valley with coastal influence. Moreover, sorted the variables DMFruit and diameterT, with high level of CD prevalence in above site, and the variable FrP with high level of SER prevalence in below site (data not shown).

PLS-DA analysis of scenario 4. This analysis extracted three components that explain 77.2% of total variance of matrix Y (R^2Y), and the cumulative overall cross-validated Q^2cum (cumulative fraction of the total variation that can be predicted by specific factors, as overall cross-validated) was 59.5% for four classes previously defined. Four classes were then designated: Class 1 (orchards with high level of CD prevalence and high level of SER prevalence); Class 2 (orchards with low prevalence of CD and low prevalence of SER); Class 3 (orchards without CD prevalence and high level of prevalence of SER); Class 4 (orchards with low level of prevalence of CD and low level of prevalence of SER). The loadings plot showed that Class 1 is associated with DiameterT and DMFruit, Class 2 is associated with Alt, RADsp and MINTsp. Moreover, Class 3 is associated with FrP and Class 4 with Long, RHsp, RHsu, RHau, RHwi and Ppsp (data not shown).

PCA of scenario 7. The PCA performed indicated that the factorial model retained four components that explain 85.4% of total variance. Factor 1 (45.7% explained variance) sorted the orchards in study according to level of prevalence of CD, in left side the low level of PvCD and high level in right side of PvCD. Factor 2 (24.1% explained variance) sorted the orchards in study according to level of prevalence of SER, in above side the high level of PvSER and low level of PvSER in below side. In the loadings plot, the factor 1 sorted the planting variables VolumeC, DiameterT, PlantAge, LFruit, frost, and Lat, which are associated with orchards with high level of CD and SER prevalence. In addition, the factor 2 sorted the planting variable Alt, RADsu, MINTwi, MAXTau, ATwi, and RADwi, with high level of CD and SER prevalence (data not shown).

PLS-DA analysis of scenario 7. This analysis extracted two components that explain 85.3% of total variance of matrix Y (R^2Y), and the cumulative overall cross-validated Q^2cum (cumulative fraction of the total variation that can be predicted by specific factors, as overall cross-validated) was 77.3% for three classes previously defined. Three class were designated: Class 1 (orchards with high level of CD prevalence and high level of SER prevalence); Class 2 (orchards without prevalence of CD and high level of prevalence of SER); Class 3 (orchards with low level of CD prevalence and high level of prevalence of SER, and orchards with high level of CD prevalence and high level of prevalence of SER). The loadings plot showed that Class 1 is associated with LFruit, frost, PlantAge, VolumeC, and Lat, Class 2 is associated with Plants/ha, and Class 3 is associated with Alt, MINTwi, MAXTau and ATwi (data not shown).

DISCUSSION

This study is the first analysis of edaphoclimatic conditions, planting and crop management variables associated with the prevalence of branch canker, dieback, and stem end rot in avocado orchards of Chile.

The fungal isolation of this study indicated that Botryosphaeriaceae species were more frequently recovered from samples of wood and fruits with symptoms associated with branch canker, dieback and stem end rot. In this sense, Eskalen et al. (2013) indicated that Botryosphaeriaceae family is the most common fungal species belong in avocados with branch canker, and Yahia and Woolf (2011) indicated that the Botryosphaeriaceae species are recovered in greater number from avocado fruit with stem end rot. Likewise, these results were similar with researches developed in Italy (Guarnaccia et al. 2016), the USA (McDonald and Eskalen 2011; Twizeyimana et al. 2013), and Chile (Valencia et al. 2019) for study of these diseases in avocado. On the other hand, some isolates of Botryosphaeriaceae species were recovered from fruits harvested from tree without symptoms, which is explained because these species can survive in fruits as endophyte in symptomless tissue, with latent infections (Twizeyimana et al. 2013).

After identifying the causal agents, the next step was to establish the predisposing factors to obtain an epidemiological analysis and thus establish control strategies. In this sense, the multivariate analysis of scenario 1 showed that orchards with high level of CD prevalence are orchards with old tress, therefore, with more volume and diameter. In this sense, Dann et al. (2013) have indicated that Branch Canker is less

important in mature trees because they can remain productive. However, in severe cases the main trunk is surrounded by these lesions, thereby the damage is irreversible and disease killing the tree. The orchards with low level of CD prevalence are orchards with high planting density and high Leaf Area Index, variables associated mainly with orchards that have young and vigorous trees.

In Chile the high-density planting is a practice common, which requires frequent pruning (Schaffer et al. 2013) thus increasing the infection risk through pruning wounds and could rise the dissemination of pathogens between trees (Dann et al. 2013; Eskalen et al. 2013; McDonald and Eskalen 2011). Therefore, the orchards with low level of CD prevalence with the time could developed these diseases, if pathogens species are present as latent infections. However, if the spread of these pathogens is limited with severe pruning to remove infected tissue, which allow the renewal of the wood for the following growing season, this action could be limiting the development of pycnidia and perithecia, and sporulation towards healthy tissue. (Dann et al. 2013), which could to avoid the dissemination of these pathogens and decrease CD prevalence in the orchards.

Concerning to SER prevalence the PCA and PLS-DA analysis have indicated that orchards with high level of SER prevalence are associated with old trees, and fruits with long time of storage for reaches the maturity index. Moreover, these analyses indicated that low level of SER prevalence are associated with fruits harvested from orchards with young trees, anf fruits harvested with high Dry Matter percentage, which needed few days of storage until reaches the maturity index. These results suggested that old trees must be harvested with high percentage of Dry Matter and

have less storage time, which reduce the likelihood of developing SER. On the other hand, young trees can be harvested with a low percentage of dry matter, because they can remain for longer under storage conditions. Additionally, these results could be associated with the decrease in the concentrations of the antifungal diene, during the ripening of fruits, a preformed antifungal component of avocado (Hopkirk et al. 1994; Prusky and Keen 1993), which increases the fruit susceptibility to infection, and consequently decrease the life postharvest of fruits.

The multivariate analysis of scenario 4 and scenario 7 indicated that the level of CD prevalence and SER prevalence in scenario 4 and scenario 7 were conditioned by planting variables such as PlantAge, VolumeC, DiameterC, and Plants/ha. Hence, the analysis of both scenarios confirmed the results obtained after analysing of scenario 1, where the CD and SER prevalence were highest in orchards with old trees.

The orchards with CD and SER prevalence in scenario 4 and scenario 7 were sorted according to agroclimatic zone, attributed to their latitude, longitude and altitude. In season 2014/2015 all orchards had low or high CD prevalence such in pre-mountain valley as inner valley, but in season 2015/2016 orchards localized in pre-mountain valley, inner valley, and inner valley with coastal influence also had low CD prevalence or high CD prevalence. Hence, the results obtained since scenario 4 and scenario 7 indicated that CD prevalence could be low or high independently of agroclimatic zone. Therefore, the CD prevalence are not conditioned by agroclimatic zone, in orchard with trees infected by pathogens such as Botryosphaeriaceae species, which is coincided with results of McDonald and Eskalen (2011), whom

recovery Botryosphaeriaceae species from avocado with branch canker from orchards localized in north and south counties in California.

Regarding to SER prevalence, in season 2014/2015 the orchards localized in pre-mountain valley had low SER prevalence, but the orchards localized in inner valley and in inner valley with coastal influence had high SER prevalence. However, in season 2015/2016 all orchards had high level of SER prevalence. These differences between scenario 4 and scenario 7 was attributed to climatic conditions in each growing season.

The analysis of interactions between climatic conditions during season 2014/2015 indicated that SER prevalence had a direct proportional and significant relationship with RADsp and MINTsp, whereas that the Relative Humidity, and Ppsp had an inverse proportional and significant relationship. On the contrary, in season 2015/2016 this analysis indicated that SER prevalence had an inverse proportional and significant relationship with MINTwi, MAXTau, and ATwi. Considering these results is possible to indicate that the sun exposure, temperature, relative humidity and precipitation during growing season could explain the differences in level of SER prevalence between consecutives growing season. In this sense, Rivera et al. (2017) indicated that the variability in fruit quality and ripening is due for environmental factors such as temperature, and sun exposure, but also the irrigation management and nutritional content. In regarding to precipitation and relative humidity, Twizeyimana et al. (2013) indicated that in California SER is a minor problem because there are low relative humidity and rainfall during growing season and harvest time, which avoid production and dissemination of inoculum. However, our

results indicated that there are highest levels of SER prevalence when the relative humidity is low in the growing season and low rainfall in spring, which could be associated with water stress that affected Chilean avocado orchards in the last ten years (Muñoz 2018), which causes more susceptibility to trees, and allow that endophytes species to be pathogenic, from inner tissue or new colonization (Dann et al. 2013).

In conclusion, the multivariate analysis of consecutives growing season about wood diseases and stem end rot, allowed identified the main factors associated with edaphoclimatic conditions, planting and crop management. Our study indicated that these diseases are conditioned mainly by planting variables such as: PlantAge, VolumeC, DiameterT, LAI and Plants/ha. Additionally, the analysis of SER prevalence included postharvest variables, such as: DaysM and DMFruits, and climatic conditions of the sun exposure (RADsp), temperature (MINTsp, ATwi, MINTwi, and MAXTau), relative humidity (RHsp, RHsu, RHau, RHwi) and precipitation (Ppsp). On the other hand, with this study was possible to determinate that these diseases can developed in different agroclimatic zone of Chile.

This research allows to guide to agricultures about conditions that pathogens requires for developed of these diseases, which is necessary for stablished appropriate and efficient management practices to avoid dissemination of pathogens and to control the disease.

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Table1. A summary description of the agroclimatic zones, where were localized 16 orchards in study. based on the classification of Pérez and Adonis (2012).

No.	Orchards	Agroclimatic zone	Latitude	Longitude	Altitude (m)
1	San Felipe 1	Pre-mountain Valley	32,7336 S	70,833 O	706
2	Nogales 1	Inner valley	32,7678 S	71,157 O	265
3	Nogales 2	Inner valley	32,7708 S	71,155 O	264
4	Quillota 1	Inner valley with coastal influence	32,8921 S	71,1871 O	211
5	San Felipe 2	Pre-mountain Valley	32,7361 S	70,8446 O	741
6	Ocoa	Inner valley	32,8362 S	71,0442 O	342
7	Jaururo	Inner valley with coastal influence	32,4680 S	71,3126 O	131
8	María Pinto	Inner valley	33,4545 S	71,2013 O	358
9	Alicahue 1	Pre-mountain Valley	32,3967 S	70,8640 O	501
10	Alicahue 2	Pre-mountain Valley	32,4022 S	70,8592 O	590
11	Melipilla 1	Inner valley with coastal influence	33,7694 S	71,1928 O	185
12	Illapel	Pre-mountain Valley	31,596 S	71,0793 O	524
13	La Ligua	Inner valley with coastal influence	32,4557 S	71,2124 O	71
14	Quillota 2	Inner valley with coastal influence	32,8856 S	71,2034 O	148
15	Peumo	Inner valley with coastal influence	34,3853 S	71,2111 O	161
16	Melipilla 2	Inner valley with coastal influence	33,7652 S	71,1228 O	226

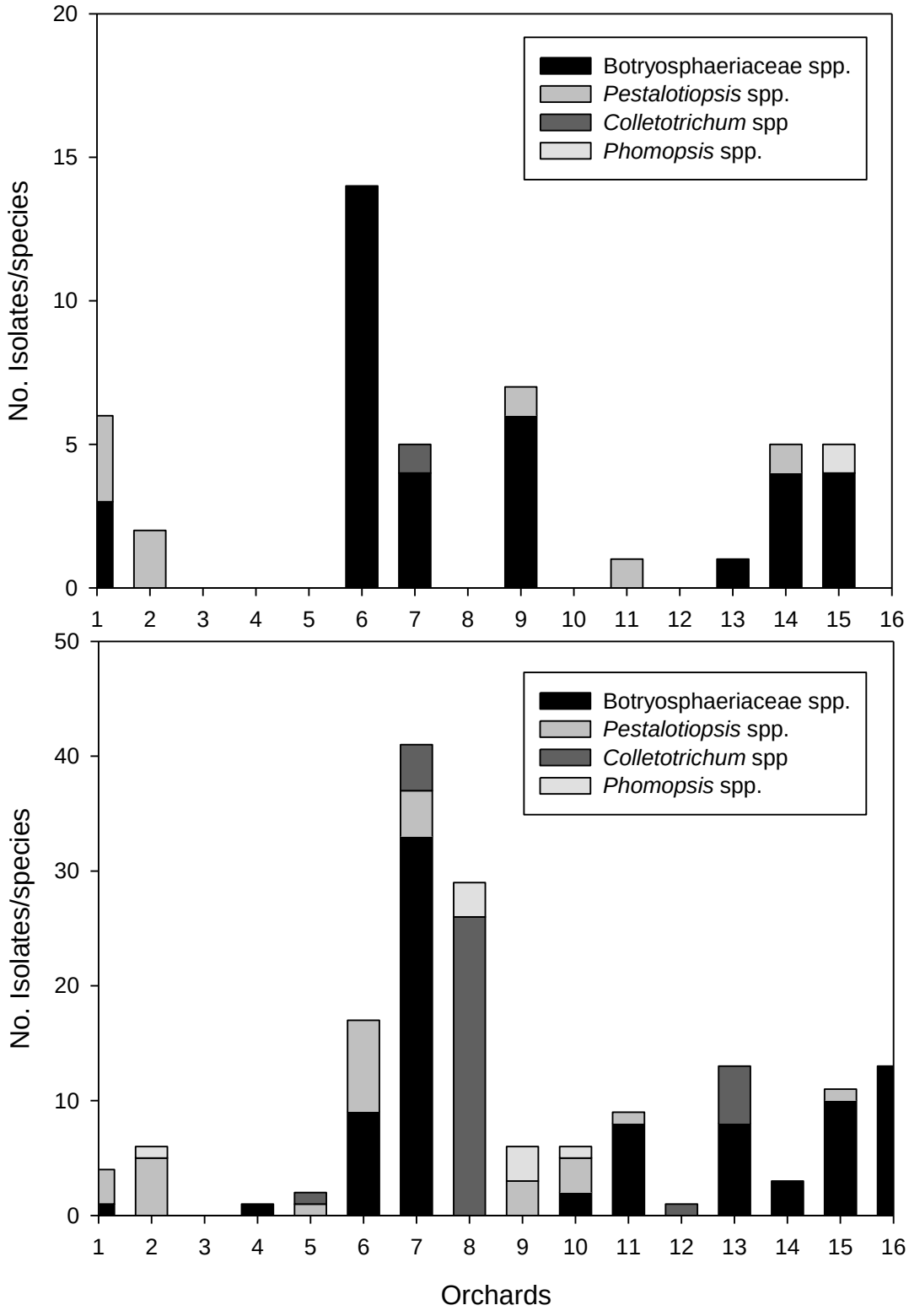

Figure 1. Frequency of genera obtained from samples of avocado wood (above) and fruits (below), localized in orchards of main productive zone of Chile.

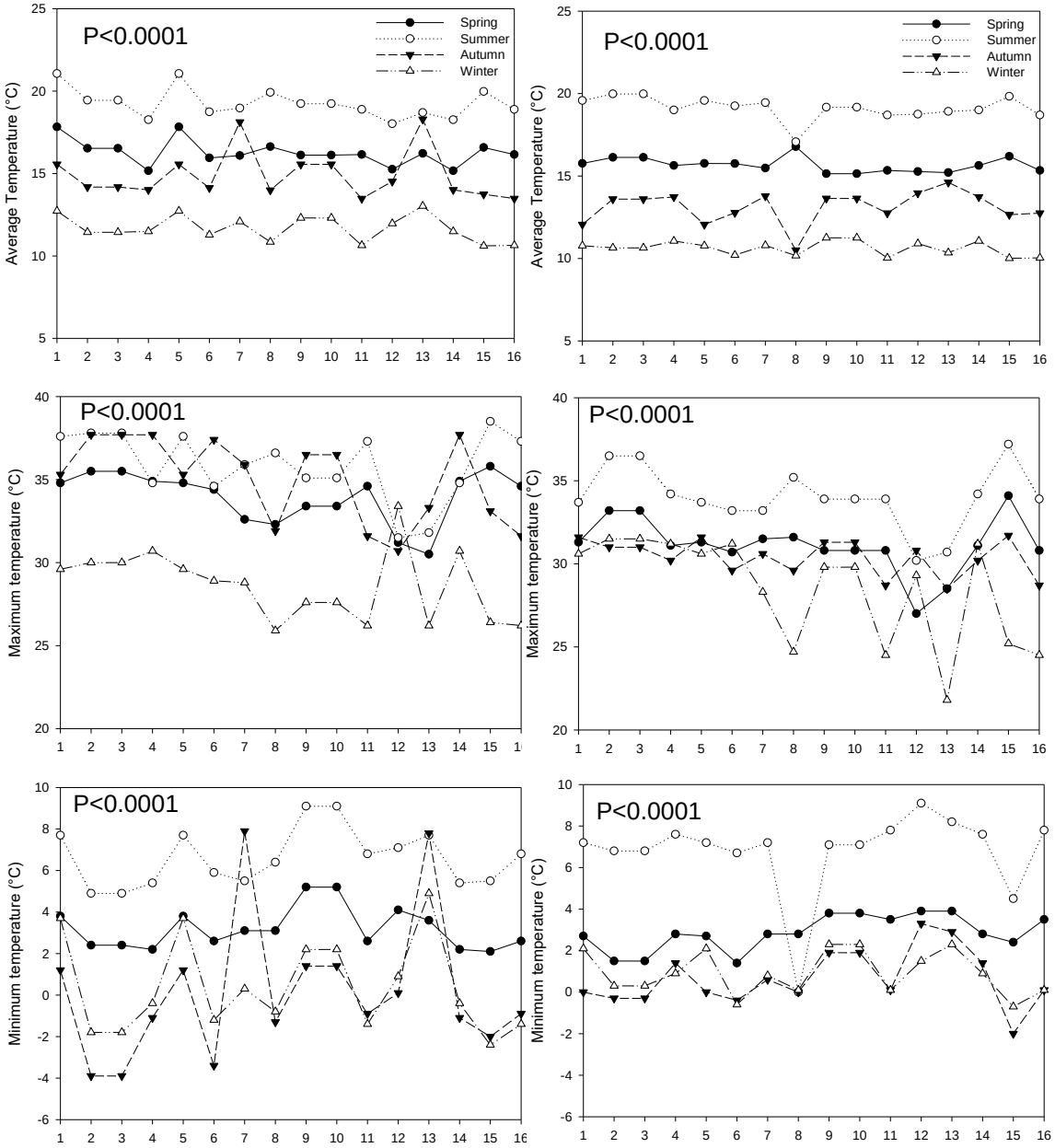

Figure 2. Average temperature, maximum temperature, and minimum temperature, for each year growing season (2014/2015, left graphics; 2015/2016 right graphics), in spring, summer, autumn and winter, corresponding to 16 orchards in study. P value in each graphic was obtained by Tukey's test (P = 0.05).

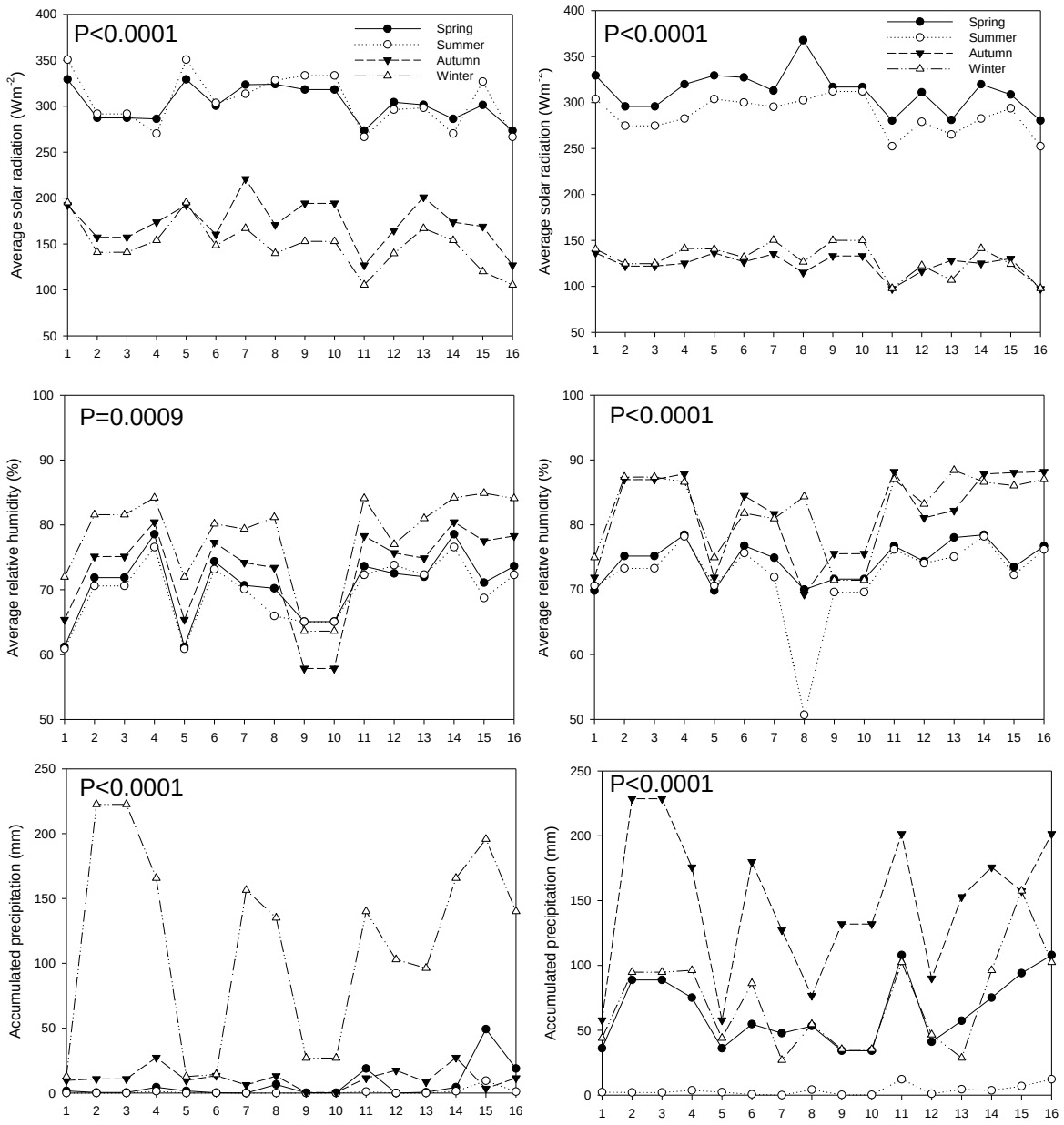

Figure 3. Solar radiation, average relative humidity, and accumulated precipitation, for each year growing season (2014/2015, left graphics; 2015/2016 right graphics), in spring, summer, autumn and winter, corresponding to 16 orchards in study. P value in each graphic was obtained by Tukey's test (P = 0.05).

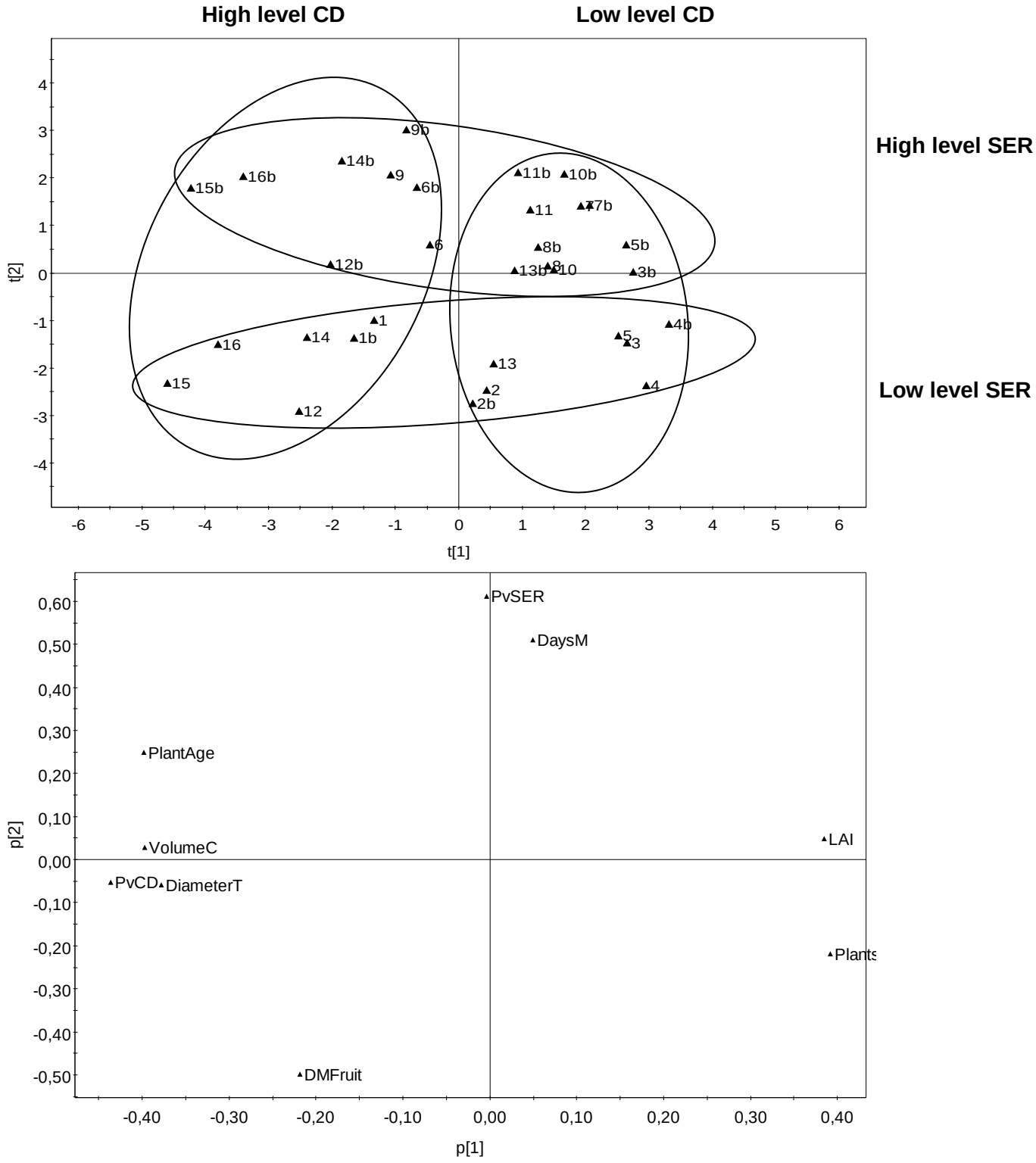

Figure 4. Principal Component Analysis of scenario 1. Scores plot for level of diseases in orchards. Loadings plots for planting variables (32 observations).

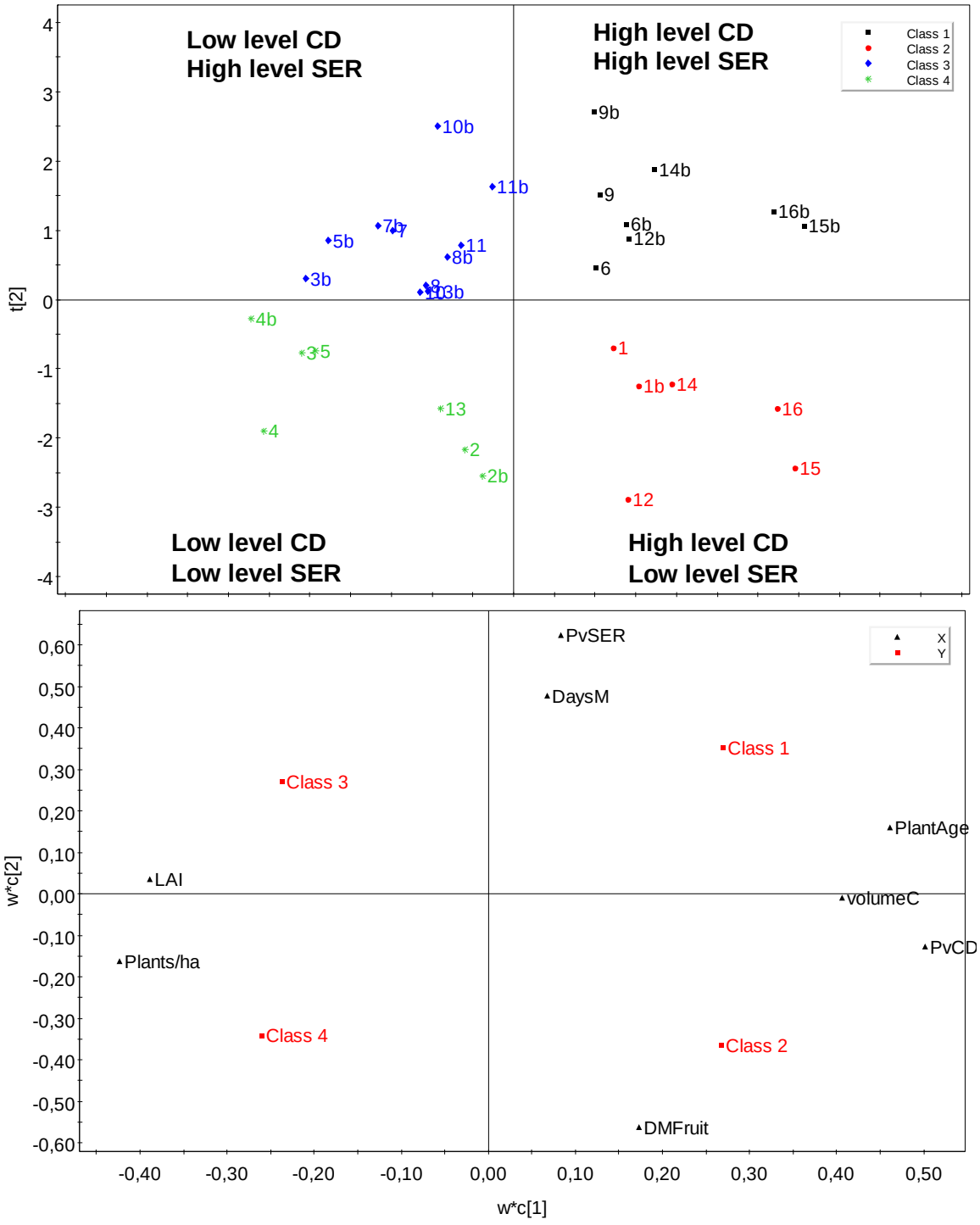

Figure 5. Partial Least Squares Discriminant Analysis of scenario 1. Scores plot for level of diseases in orchards. Loadings plots for planting variables (32 observations).

Chapter 4

Predictive model to segregate avocados by risk of developing stem end rot in fruits stored for long periods

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Predictive model to segregate fruit by risk of developing stem end rot in avocados stored for long periods

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Keywords: Prevalence, severity, stem end rot, avocado.

ABSTRACT

Stem end rot is a severe postharvest disease that since 2014 is more frequent in import markets that consume Chilean fruits. Several fungal species have been associated with this disease in worldwide. Being the most common the species of Botrysphaeriaceae family. In Chile there are not studies about predisposing factors to development of this disease. Therefore, the objetive of this study was to create a predictive model to segregate fruit by risk of developing stem end rot in avocados stored for long periods. This study was conducted in ‘Hass’ avocado orchards

between 2015 and 2016. The orchards selected were localized in the primary productive avocado regions, where diverse fungal species were recovered from ripening fruit. Analysis of Variance, Principal Component Analysis, Partial Least Squares Analysis, Partial Least Squares Discriminant Analysis, and Ridge Regression were performed to analyze 102 variables associated with prevalence and severity of this disease in avocado.

This study showed that Stem End Rot is conditioned mainly by agroclimatic conditions of growing season and data of nutritional content of fruits harvested. Moreover, indicated that orchards localized in different altitude and longitude also can develop Stem End Rot. Therefore, in Chile Stem End Rot could be present in several agroclimatic conditions, and the management in growing season is determinant for the develop of this disease.

The predictive model developed in this study is a method that allow to segregate healthy fruits of fruits with high risk of developing this postharvest disease. Therefore, is a guide about management required in each growing season, to avoid increase of prevalence and severity level in fruits postharvest.

1. Introduction

Avocado (*Persea americana* Miller), is a persistent subtropical tree endemic from Central America and Mexico. Avocados are produced and exported from México, Peru, Chile, Israel, South Africa, Spain, Brazil and USA. Chile is an important avocado producer, with 29.289 ha planted mainly between Coquimbo

Region (29° 20’S to 32° 15’S) and O’Higgins Region (33° 51’S to 35° 01’S) being Valparaiso Region the most productive (Muñoz, 2018). In Chile, the main exported avocado cultivar is ‘Hass’, which travels toward Europe, North America, Asia and other countries of South America.

The main problem of Chilean production is the distance with potential and consumers countries. Therefore, is necessary to produce fruits with long postharvest life to maintain the fruit quality for a long time until reaches to consumers. However, since 2014, the incidence of stem end rot has increased in export markets that consume Chilean fruit, such as the Asian market, but especially in the European markets, where maturation chambers are used for reducing the ripening times of the fruit. These chambers increasing temperature, ethylene concentration and relative humidity in storage, which allows avocados to be sold ready-to-eat.

Stem end rot is a postharvest disease, which is caused by latent infections initiated on the tree during the growing season (Hopkirk et al., 1994). In latent infections, the pathogen can remain during prolonged periods in a quiescent stage until reaching specific conditions, which can cause that pathogen to become active (Verhoeff, 1974). The relationship between host, pathogen and environment in latent infection indicate a dynamic equilibrium, because in such condition there are not symptoms (Jarvis, 1994). The latent infection remain latent until avocados are removed from the tree (Prusky et al., 1983), because this action causes a reduction in the concentration of antifungal diene, a preformed antifungal component of avocado (Hopkirk et al., 1994; Prusky and Keen, 1993), which increases the fruit susceptibility to infection.

The infection of fruit can be initiated by fungi causing branch canker and dieback, which are localized endophytically in wood (Valencia et al., 2019; Guarnaccia et al., 2016; Twizeyimana et al., 2013) or by infection of peduncle during harvest (Hartill and Everett, 2002).

The initial symptom of stem end rot may be detected by a slight softening in the union area to peduncle, this lesion grows compromising the fruit completely with the progress of ripening. In severe cases the fruit are covered by mycelium and conidias (Dann et al., 2013; White et al., 2005; Menge and Ploetz, 2003; Johnson and Kotzé, 1994).

The pathogens associated with stem end rot include fungal species, such as *Colletotrichum gloesporioides* (Penz.) Penz. & Sacc, C. acutatum J.H. Simmonds, *Thyronectria pseudotrichia* (Schwein.) Seeler, *Phomopsis perseae* Zerova, *Pestalotiopsis clavispora* (G.F. Atk.) Steyaert, and *P. versicolor* (Speg.) Steyaert (Dann et al., 2013; Valencia et al., 2011; Menge and Ploetz, 2003; Hartill and Everett, 2002; Darvas and Kotzé, 1987). However, anamorphs of Botryosphaeriaceae species have been considered like the most important pathogens of avocado stem end rot throughout the world (Dann et al., 2013; Menge and Ploetz, 2003), including *Diplodia mutila* (Fr.) Mont. in Chile (Valencia et al., 2019) and the USA (Inderbitzin et al., 2010), *D. seriata* De Not. in Chile (Valencia et al., 2019), *Dothiorella iberica* A.J.L. Phillips, J. Luque & A. Alves in Chile (Valencia et al., 2019), *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl in Chile (Valencia et al., 2019) and Italy (Dann et al., 2013; Garibaldi et al., 2012), *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips in Chile (Valencia et al., 2019; Montealegre

et al., 2016) and Turkey (Akgül et al., 2016), *N. luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips in the USA (Twizeyimana et al., 2013), *N. mangiferae* (Syd. & P. Syd.) Crous, Slippers & A.J.L. Phillips in Taiwan (Ni et al., 2009), *N. mediterraneum* Crous, M.J. Wingf. & A.J.L. Phillips in the USA (Inderbitzin et al., 2010), and *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips in Chile (Valencia et al., 2019), Italy (Guarnaccia et al., 2016) and Mexico (Molina-Gayosso et al., 2012).

Understanding the relationship between pathogens with avocado host and environment is critical to avoid development of stem end rot in avocados. In this sense, is necessary to apply appropriate pre-harvest strategies to limit the dissemination of these pathogens (Dann et al., 2013; Twizeyimana et al., 2013). To avoid changes of quality postharvest Defilippi et al. (2014) have indicated that is necessary to maintain avocados stored under controlled conditions of temperature, humidity, CO_2 and O_2. These strategies allow to reduce the economic effect caused by this disease in post-harvest.

The predictive models have arisen by the need to develop disease management programs (Jeger, 2004), with the objective of reducing costs related to the disease, such by the cost of pesticide applications as by yield losses (Pfender et al., 2011), considering the spatial and temporal characteristics of a pathogenic population and its interaction with a susceptible host of economic importance. In avocados, there are not a predictive model associated to stem end rot, which is necessary to know the potential life postharvest of fruits produced in specific planting, edaphoclimatic and orchard management conditions. Therefore, the objective of this study to create

a predictive model to segregate fruit by risk of developing stem end rot in avocados stored for long periods.

2. Materials and methods

This study was conducted in 16 ‘Hass’ avocado orchards between Illapel (31° 37’S) and Peumo (34° 24’S) (Table 1), during two consecutives growing season (2014/2015 and 2015/2016), from september 2014 to august 2015, and september 2015 to august 2016. This period is associated with the phenological period between flowering and fruit growth.

2.1. Field sampling.

Fifteen trees were selected in each study area, and a total of 45 fruit (three pedunculated fruits per tree with more than 23% dry matter) were harvested near to symptomatic branches and trunk and transported to the laboratory in plastic bags. The fruit were surface disinfected by immersion in 75% ethanol for 30 s and placing them in humidity chambers for 7 to 31 days at 20ºC in a regular atmosphere until they reached consumer maturity.

2.2. Isolation, culture, and identification.

Small pulp pieces of mature Hass fruits (3 to 5 mm) were removed from the margins between the healthy and symptomatic tissues and placed in Petri dishes containing 2% potato dextrose agar acidified with 0.5 ml/liter of 92% lactic acid plus

0.05% tetracycline (APDAt) (Díaz et al., 2013). The plates were incubated at 20°C in darkness for 14 to 21 days.

All the isolates were transferred to acidified potato dextrose agar (APDA). Pure cultures were obtained from hyphal tip transfers. All the isolates were stored in 1.5 ml of sterile 20% glycerol at 5°C.

To identify the isolates obtained in study, the colony morphology was characterized on APDA after 5, 12, and 27 days at 25°C in darkness. The length and width of 50 conidia were measured, and the mean and standard deviation were calculated. The color, shape and the presence or absence of septation in the conidia were also determined. Morphology and measurements of conidia were compared with published descriptions of mycobank.org, Phillips et al. (2013), Sutton (1980), and Udayanga et al. (2011).

2.3. Characterization of orchards.

In each consecutive growing season (2014/2015 and 2015/2016) the orchards were characterized with 102 variables registered, associated with:

2.3.1. Climate variables.

The climate data were obtained from meteorological stations near to avocado orchards used in this study. The climate variables used were: average temperature (AT, °C), maximum temperature (MAXT, °C), minimum temperature (MINT, °C), solar radiation (RAD, Wm^{-2}), average relative humidity (RH, %), and accumulated precipitation (Pp, mm). These data were separated for each year season: spring (sp,

September to December), summer (su, December to March), autumn (au, March to June), and winter (wi, June to september), with measuring frequency of 1 hour.

2.3.2. Planting variables.

Latitude (Lat), longitude (Long), altitude (Alt), agroclimatic zone (Zone), plant density (Plants/ha), origin of plants (OPlants, nursery or own plants), age of plant (PlantAge), volume of canopy (VolumeC), diameter of trunk (DiameterT), rootstock variety (Rstock), leaf area index (LAI), soil characteristics (texture, bulk density, pH pHS, electric conductivity ECS, organic matter OMS, Nitrogen NS, Phosphorous PS, Potassium KS, cationic exchange capacity CECS), foliar nutrient content (Nitrogen NF, Phosphorous PF, Potassium KF, Calcium CaF, Magnesium MgF, Copper CuF, Manganese MnF, Zinc ZF), crop evapotranspiration (ETc), and background of biotic (pest and pathogens) or abiotic stress problems such as drought, salinity, frost, nutrients deficiencies, mechanical damage, and diameter of necrotic inner lesions caused by SER (LFruit). Production parameters, such as: yield, fruit load, weight of fruits, days from harvest to fruit ripening (DaysM), and fruit nutrient content (dry matter DMFruit, Nitrogen NFruit, Phosphorous PFruit, Potassium KFruit, Calcium CaFruit, Magnesium MgFruit, Copper CuFruit, Iron IFruit, Manganese MnFruit, Zinc ZnFruit, Boron BFruit).

2.3.3. Management variables.

The irrigation system (Isystem, drip or micro sprinkler), water applied regarding crop evapotranspiration (H_2OETc), pruning (date DateP, frequency pruning FrP, intensity pruning IP, pruning sealed of wounds: PasteP, FungicideP), girdling,

applied nitrogen dosage (UN/ha), applied calcium (Ca+), applied humic acids (HumicA) and growth regulators application date (DateGR), and growth regulators application frequency (FrGR), also were included in this study.

2.3.4. Disease index.

The prevalence of stem end rot (SERpv) was determined when the fruits reaches the consumer maturity, considering diseased fruits of total harvested fruits by study area (N=45). The SER severity (SERs) was calculated using the ordinal scale of White et al. (2005), this scale has four degrees: 0 = health fruits; 1 = low severity (10% infected tissue); 2 = moderate severity (25% infected tissue); and 3 = severe damage (50% infected tissue).

2.4. Statistical analysis.

To analyze the dataset of 102 variables associated with geographic location, planting, productive, climate, management, and disease index. The qualitative variables were transformed in scaled (3, 5, 7…n) and by presence (1) /absent (0) (Carot, 2003).

The 102 variables were analyzed from nine scenarios generated, which consider data by growing season and disease. Three scenarios were selected that proved to be more descriptive with respect to the phenomenon under study (3, 6, 9). These scenarios consider data by season (2014/2015, 2015/2016), and disease data (SER): Scenario 3. Seasons:2014/2015, 2015/2016, Disease: SER; Scenario 6. Season:2014/2015, Disease: SER; Scenario 9. Season: 2015/2016, Disease: SER.

Several multivariate analyses were performed, using chemometrics methods. The methods applied were Principal Component Analysis (PCA), Partial Least Squares Analysis (PLS), and Partial Least Squares Discriminant Analysis (PLS-DA). These analyses were based on the nonlinear iterative partial least squares algorithm (NIPALS) (Wold et al., 2001), which allows the analysis of a large number of variables, highly correlated and an ill-conditioned matrix (Ferrer, 2007). All variables were centered and standardized to unit variance previous the analysis. All models were validated by a full cross-validation routine, minimizing the prediction residual sum of squares function (PRESS) to avoid over fitting the models (Cen et al., 2007).

The PCA was performed to determine the relationship among orchard variables and disease index, in each selected scenario (3, 6 and 9), because PCA is a method that allows synthesizing the information contained in a large matrix of variables, within a smaller set of factors (principal components), with minimal loss of information (Yañez et al., 2012). Likewise, the PCA allows condensing the information in two ways: identifying relationships between observations that comprise the score matrix, and determining relationships between variables, known as the loadings matrix. Additionally, allows display the relationships between observations and variables in orthogonal planes, that represent the direction of greatest variance contained in the dataset (Cuneo et al., 2013; Saavedra and Cordova, 2011).

The PLS method was performed to evaluate the predictor condition of orchard variables of selected scenarios (3, 6 and 9) over the disease index, because the PLS allows analyzing the effects of the predictor variables in the response variables,

maximizing the covariance of these matrices, to generate projections in an orthogonal plane and thus be able to develop predictive relationships between them (Kruger and Xie, 2012; Wold et al., 2001).

The PLS-DA was performed to determine the relationships between class of observations and orchard and diseases variables in selected scenarios (6 and 9), because this method allows grouping the observations into classes, according to the contribution of each variable, to determine the causality of possible significant groups, associated to which problem variables are responsible for the behavior of that class and if there are anomalous or eventually new classes (Barker and Rayens, 2003; Brereton and Lloyd, 2014; Eriksson et al., 2006).

Finally, the predictors variables obtained in PLS analysis of scenario 3, were subjected to Ridge Regression, which is an alternative regression method to ordinary least square that allows treating regressors with multicollinearity (Ryan, 2009). This method was used to study the prevalence of stem end rot (SERpv).

All chemometrics methods were performed using SIMCA-P v. 10 software (Umetrics AB, Sweden), and the Ridge Regression was performed using Statgraphics 18 software (StatPoint, USA).

3. Results

3.1. Field sampling.

All orchards used in this study had fruits with stem end rot. The fruits with severe level of damage developed white and grey mycelium on the peel, from the area near to peduncle, and there are black pycnidia with masses of gray conidia on the peel covering the fruit completely in some cases. The inner tissue is very affected because there are necrosis and cavities in the pulp, reaching great depth, with white mycelium. On the contrary, Fruits with low level of damage, have not developed mycelium and conidia in peel, but when is removed the peduncle there are damage in inner tissue, which advances internally until equatorial zone.

3.2. Fungal isolation.

From a total of 675 samples of avocado fruits were recovered 162 isolates, which corresponding to: Botryosphaeriaceae spp., *Colletotrichum* spp., *Phomopsis* spp., and *Pestalotiopsis* spp. In both growing season the isolates of Botryosphaeriaceae spp obtained from fruits samples were more frequent (Figure 1).

3.3. Statistical analysis

3.3.1. PCA of scenario 3.

The PCA performed indicated that the multifactorial model retained two components that explain 81.7% of total variance. The factor 1 (64.0% explained variance) sorted the orchards according to climatic conditions of temperature, radiation and precipitation, in left side the high level of Ppau and high level of ATau,

MINTau, ATwi, MAXTwi, RADsu, RADau, RADwi in right side. The factor 2 (17.7% explained variance) sorted the orchards in study according to level of prevalence and severity of SER, in above side the low level and high level in below side. In the loadings plot, the factor 2 sorted the variables CaFruit and DMfruit in above side, and DaysM in below side (Figure 2).

3.3.2. PLS-DA analysis of scenario 3.

This analysis extracted two components that explain 59.5% of total variance of matrix Y (R^2Y), and the cumulative overall cross-validated Q^2cum (cumulative fraction of the total variation that can be predicted by specific factors, as overall cross-validated) was 54.7% for four classes previously defined. The four classes were then designated: Class 1 (orchards with low level of SER prevalence and severity, low level of DaysM, and high level of DMFruit, ATau, MINTau, ATwi, MAXTwi, RADsu, RADau, RADwi); Class 2 (orchards with low level of SER prevalence and severity, high level of CaFruit, DMFruit, and Ppau); Class 3 (orchards with high level of SER prevalence and severity, high level of DaysM, and Ppau); Class 4 (orchards with high level of SER prevalence and severity, and high level of ATau, MINTau, ATwi, MAXTwi, RADsu, RADau, RADwi, low level of CaFruit, DMFruit and high level of DaysM). (Figure 3).

3.3.3. PCA of scenario 6.

The PCA performed indicated that the multifactorial model retained two components that explain 80.0% of total variance. The factor 1 (40.6% explained variance) sorted the orchards according to altitude, longitude, and climatic conditions

of temperature, radiation, relative humidity and precipitation, in left side the high level of altitude, ATsp, ATsu, RADsu, MINTsp, MINTsu and high level of Longitude, Ppsp, RHsp, RHsu, RHau, RHwi in right side. The factor 2 (28.7% explained variance) sorted the orchards in study according to level of prevalence and severity of SER, in above side the low level and high level in below side. In the loadings plot, the factor 2 sorted the variables MAXTsp, Ca+, KF, FeFruit and BFruit in above side, and MINTwi, MgF, MnFruit and ZnFruit in below side (data not shown).

3.3.4. PLS-DA analysis of scenario 6.

This analysis extracted two components that explain 59.6% of total variance of matrix Y (R^2Y), and the cumulative overall cross-validated Q^2cum (cumulative fraction of the total variation that can be predicted by specific factors, as overall cross-validated) was 39.3% for four classes previously defined. The four classes were then designated: Class 1 (orchards with low level of SER prevalence and severity, and high level of MAXTsp, Ca+, KF, FeFruit and BFruit, Ppsp, RHsp, RHsu, RHau and RHwi); Class 2 (orchards with low level of SER prevalence and severity, high level of altitude, ATsp, ATsu, RADsp, MINTsp, MINTsp, Ca+, FeFruit and BFruit); Class 3 (orchards with high level of SER prevalence and severity, high level of altitude, RADsp, RADsu, MINTsu, MINTsp, MnFruit and ZnFruit); Class 4 (orchards with high level of SER prevalence and severity, and high level of MgF, MnFruit, ZnFruit and BFruit) (data not shown).

3.3.5. PCA of scenario 9

The PCA performed indicated that the multifactorial model retained three components that explain 85.1% of total variance. The factor 1 (52.8% explained variance) sorted the orchards according to altitude, longitude and climatic conditions of temperature, relative humidity and precipitation, in left side the high level of altitude, ATwi, MINTsp, and MINTwi, and high level of longitude, RHsp, RHwi and Ppsp in right side. The factor 2 (22.0% explained variance) sorted the orchards in study according to level of prevalence and severity of SER, in above side the high level and low level in below side. In the loadings plot, the factor 2 sorted the variables VolumeC and LFruit in above side, and low level of these variables in below side (data not shown).

3.3.6. PLS-DA analysis of scenario 9.

This analysis extracted two components that explain 82.4% of total variance of matrix Y (R^2Y), and the cumulative overall cross-validated Q^2cum (cumulative fraction of the total variation that can be predicted by specific factors, as overall cross-validated) was 53.4% for three classes previously defined. The three classes were then designated: Class 1 (orchards with high level of SER prevalence and severity, and high level of longitude, volumeC, LFruit, Ppsp and RHsp, and low level of altitude, ATwi, MINTsp, and MINTwi); Class 2 (orchards with low level of SER prevalence and severity, low level of volumeC, LFruit); Class 3 (orchards with low level of SER prevalence and severity, high level of altitude, MINTsp, MINTwi and ATwi, and low level of longitude, Ppsp, RHsp, RHwi) (data not shown).

3.3.7. Ridge regression of scenario 3.

The regressors for Ridge Regression were obtained from a PLS analysis that explained 53.4% total variance (R^2Y), and the Q^2cum was 46.0%, considered prevalence of SER as respond variable (Y). The Ridge regression indicated a level of explained variance of 81.2% for SER prevalence (Figure 4). The model obtained was:

SERpv = 1.13329 + 0.0587917*ATau - 0.0182358*MINTau - 0.152862*ATwi + 0.0226904*MAXTwi + 0.000884054*Ppau - 0.000142616*RADsu + 0.000426552*RADau + 0.00216797*RADwi - 0.0238042*DMFruit - 0.01213*CaFruit + 0.0137662*DaysM.

This predictive model incorporated climatic variables, such as: ATau, MINTau, ATwi, MAXTwi, Ppau, RADsu, RADau, RADwi, and variables associated with conditions of fruits, such as: DMFruit, CaFruit, DaysM.

4. Discussion and conclusion

The multivariate analysis and the predictive model, performed in this study were consistent in to indicate that Chilean avocado orchards with different edaphoclimatic, planting and management conditions, during two consecutive growing seasons, can be affected for SER, if climatic conditions and nutritional content of fruits allow the increasing of susceptibility of fruits and the development of pathogens associated with this disease, especially of Botryosphaeriaceae family.

The fungal isolations performed in ripe avocado fruits indicated that species of Botryosphaeriaceae family were more frequently recovered. In this sense, Guarnaccia et al. (2016), Twizeyimana et al. (2013), McDonald and Eskalen (2011), and Yahia and Woolf (2011) have indicated that is frequent that the Botryosphaeriaceae species recovered in greater number from avocado with rots. These pathogens species also can cause Branch Canker and Dieback in avocado tree (Valencia et al., 2019; Guarnaccia et al., 2016; Dann et al., 2013; Twizeyimana et al., 2013; McDonald and Eskalen, 2011; Menge and Ploetz, 2003). The infection of avocado tree by these pathogens species is associated with wounds occurred during the growing season, caused by pruning, girdling, chilling injury, mechanical damage, bark split by the wind, wounds graft, etc. Moreover, the trees are more susceptible when are under stress, such as drought, nutritional deficiencies, flooding, extreme temperatures or damage by insects o pathogens (Dann et al., 2013; Eskalen et al., 2013; McDonald and Eskalen, 2011; Slippers and Wingfield, 2007; Menge and Ploetz, 2003; Johnson and Kotze, 1994). Therefore, preharvest conditions are limitations for the development of these wood disease, which could be directly associated with increasing of SER prevalence, if the fruit are infected and postharvest conditions are not adequate. In this sense, since 2011 in Chile, the effect of drought has caused decrease of productivity and hectares planted (Muñoz, 2018), which coincided with increasing cases of wood diseases in Chilean avocado orchards and SER in ripe fruits imported from Chile (Valencia et al., 2019).

The harvested fruits have high variability of origin, quality and ripening, these differences are due to the broad range of climatic, planting and management

conditions during the growth of fruits (Rivera et al., 2017; Hofman et al., 2013). Therefore, the postharvest life and time for ripening is due for preharvest and postharvest conditions, to maintain the quality of fruits. In addition, Hofman et al. (2013) indicated that factors influencing the ripening rate can affect ripe fruit quality. In this sense, the results of this study indicated that the prevalence of SER also is conditioned by preharvest conditions of the climate and nutritional content of fruits. This research has not included in the analysis postharvest management and conditions for stored the fruits during long periods, because all fruits were stored in same conditions of temperature, relative humidity and regular atmosphere. Therefore, is necessary include these variables in new multivariate analysis, for to known if postharvest conditions could be also associated with level of SER prevalence, and to determine if there are some management during the stored that can to reduce the level of damage of this pathology. Because the risk of quality loss is higher the longer the time from harvest to consumption, thus, fruit that ripen more quickly had less rotting, than those taking longer to ripen (Hopkirk et al., 1994). In this study, the results of scenario 3 indicated that the orchards with fruits that have high level of DaysM have high level of SER prevalence, therefore, these fruits require optimal postharvest conditions because need more time of storing until reaches of ripeness.

The multivariate analysis of scenario 3 indicated that there was high level of SER prevalence in orchards with high level of Ppau, ATau, MINTau, ATwi, MAXTwi, RADsu, RADau and RADwi, which coincided with period between summer and winter, when occurs the growth of fruits (Salazar-García et al., 2013). However, SER

prevalence is high only when the harvested fruits during spring have low content of CaFruit and DMFruit. These results were consistent with Rivera et al. (2017), which indicated that matter content, calcium content and minimum temperature are some variables that significantly influencing the overall ripening behavior of ‘Hass’ avocado fruits. Therefore, is necessary take account that these variables allowing to know the risk level of develop SER in ripe fruits.

The multivariate analysis of scenario 6 and scenario 9 showed that the level of SER prevalence and severity also were conditioned by climatic conditions of each growing season and nutritional content of the fruits. However, the analysis of scenario 6 indicated that orchards with high level of SER prevalence is associated with high level of RAD in summer, and fruits with high level of MnFruit and ZnFruit, by the contrary orchards with low level of SER prevalence and severity, have high level of Pp, RH, AT and MAXT during spring. Likewise, were orchard that have fruits with high level of FeFruit.

In scenario 9 high level of SER prevalence have relationship with orchards that have high level of longitude, therefore, are orchards localized near the Chilean coast, with high level of precipitation and relative humidity in spring, and trees with large volume of canopy (older trees). Whereas, orchards with low level of SER prevalence and severity are localized near of premountain, with high level of MINTsp, MINTwi and ATwi. In this sense, Hofman et al. (2013) indicated that the susceptibility of fruits diseases and disorders can increase with increased maturity, warmer orchards temperatures, higher rainfall and older trees.

Rotting is rarely observed in immature fruits, but as the fruits ripen, they can be severely damaged by rot (White et al., 2005; Hopkirk et al., 1994). Therefore, the predictive model developed in this study is a method that allow to segregate healthy fruits of fruits with high risk of developing postharvest diseases. Including in the analysis agroclimatic conditions of growing season and data of nutritional content of fruits harvested. Moreover, indicated that orchards localized in different altitude and longitude also can develop SER. Therefore, in Chile SER could be present in several agroclimatic conditions, and the management in growing season is determinant for the develop of SER.

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Table1

A summary description of the agroclimatic zones, where were localized 16 orchards in study. based on the classification of Pérez and Adonis (2012).

No.	Orchards	Agroclimatic zone	Latitude	Longitude	Altitude (m)
1	San Felipe 1	Pre-mountain Valley	32,7336 S	70,833 O	706
2	Nogales 1	Inner valley	32,7678 S	71,157 O	265
3	Nogales 2	Inner valley	32,7708 S	71,155 O	264
4	Quillota 1	Inner valley with coastal influence	32,8921 S	71,1871 O	211
5	San Felipe 2	Pre-mountain Valley	32,7361 S	70,8446 O	741
6	Ocoa	Inner valley	32,8362 S	71,0442 O	342
7	Jaururo	Inner valley with coastal influence	32,4680 S	71,3126 O	131
8	María Pinto	Inner valley	33,4545 S	71,2013 O	358
9	Alicahue 1	Pre-mountain Valley	32,3967 S	70,8640 O	501
10	Alicahue 2	Pre-mountain Valley	32,4022 S	70,8592 O	590
11	Melipilla 1	Inner valley with coastal influence	33,7694 S	71,1928 O	185
12	Illapel	Pre-mountain Valley	31,596 S	71,0793 O	524
13	La Ligua	Inner valley with coastal influence	32,4557 S	71,2124 O	71
14	Quillota 2	Inner valley with coastal influence	32,8856 S	71,2034 O	148
15	Peumo	Inner valley with coastal influence	34,3853 S	71,2111 O	161
16	Melipilla 2	Inner valley with coastal influence	33,7652 S	71,1228 O	226

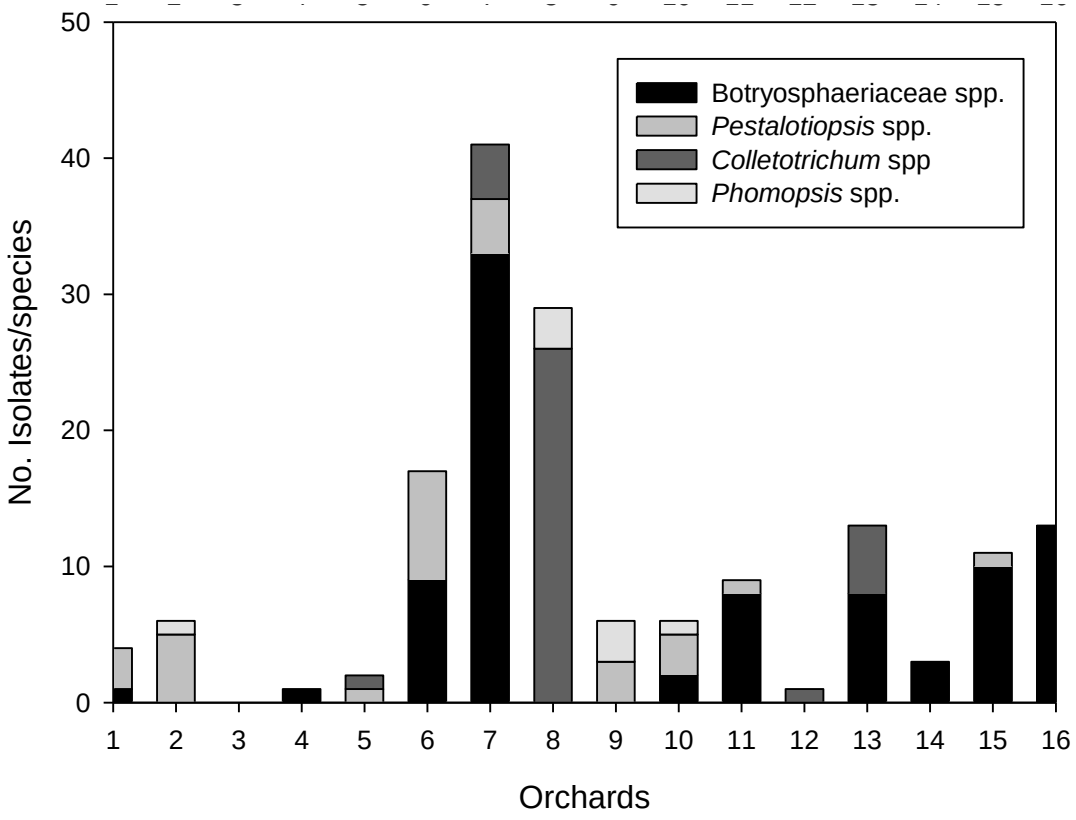

Fig. 1. Frequency of genera obtained from samples of avocados, localized in orchards of main productive zone of Chile.

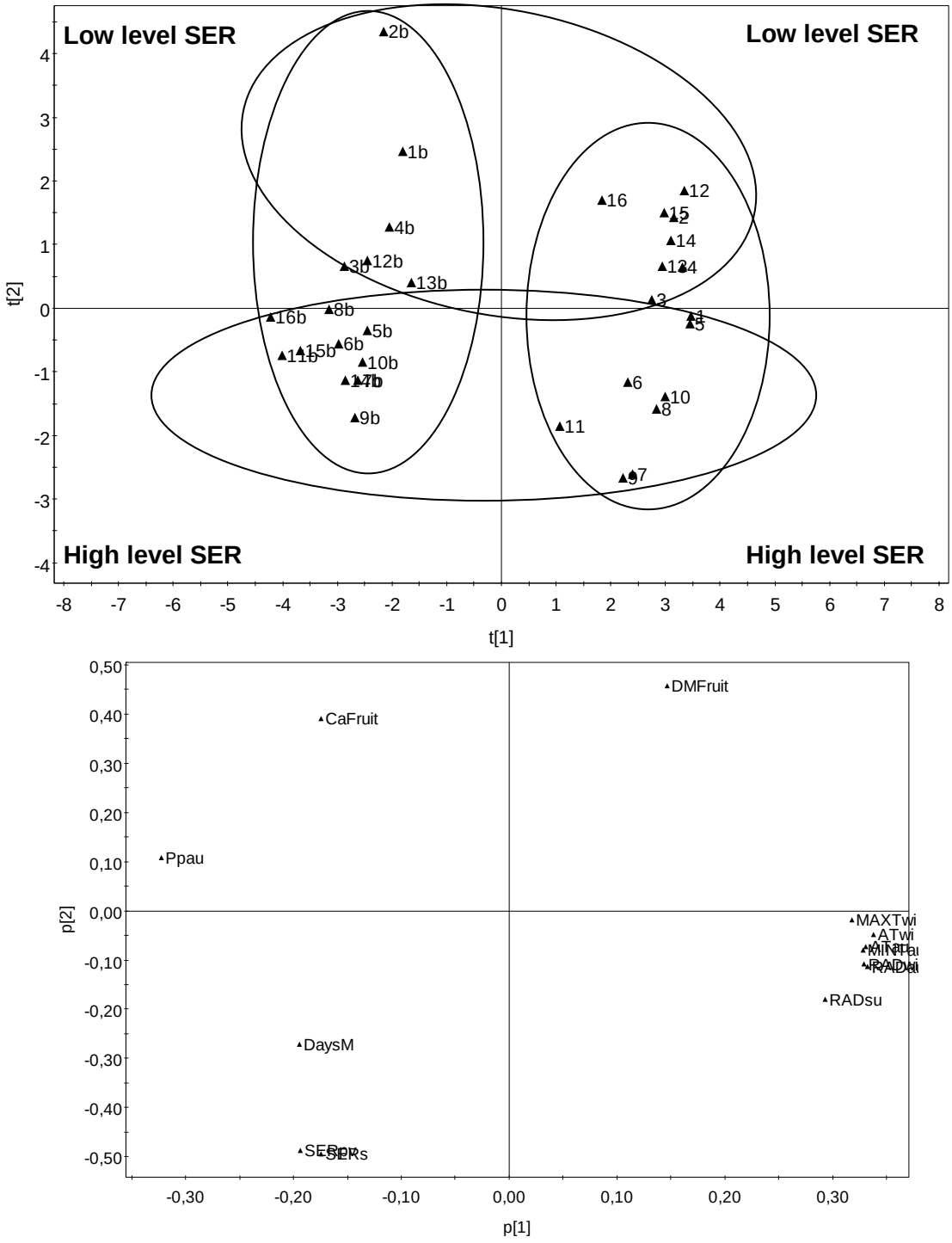

Fig. 2. Principal Component Analysis of scenario 3. Scores plot for level of diseases in orchards. Loadings plots for climate and nutritional content of fruits variables (32 observations).

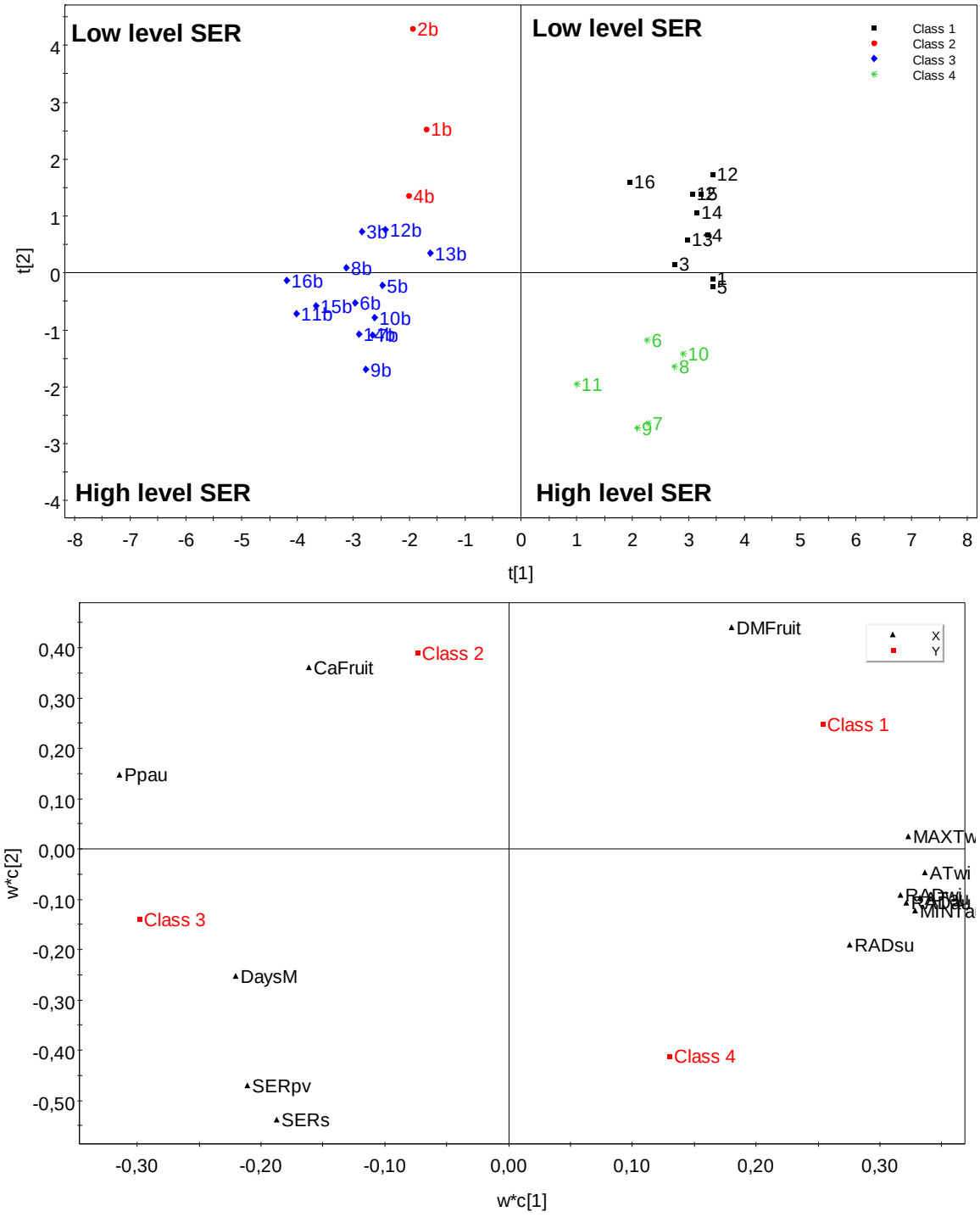

Fig. 3. Partial Least Squares Discriminant Analysis of scenario 3. Scores plot for level of diseases in orchards. Loadings plots for climate and nutritional content of fruits variables (32 observations).

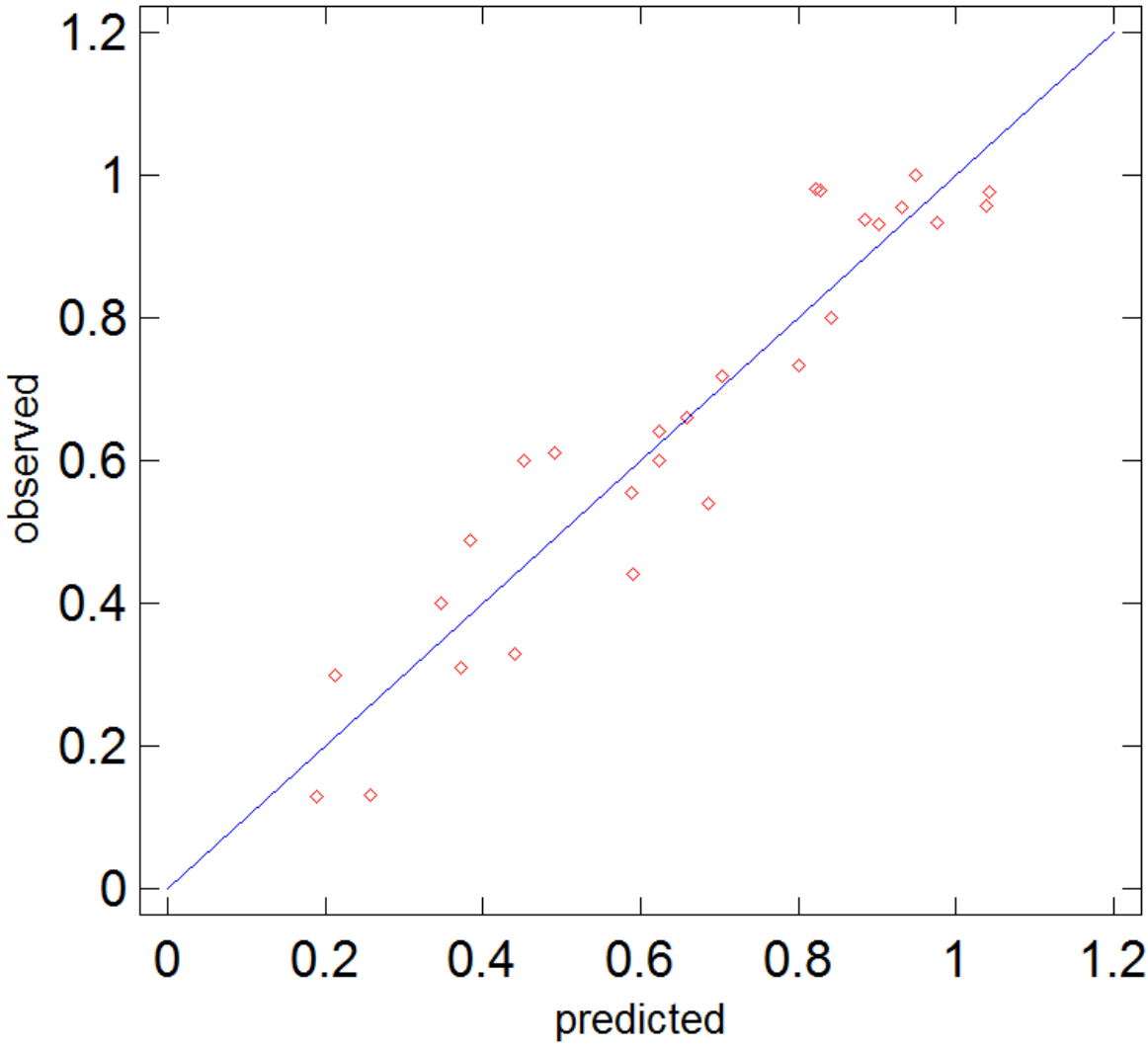

Fig. 4. Models obtained with Ridge Regression from scenario 3. Relationship between SER prevalence associated with climatic and nutritional content of fruits variables (32 observations).

Chapter 5

General Discussion

The increase of cases of orchards with symptoms of diseases in the wood and the arrival of Chilean fruit with peduncular rot in more distant countries, than inspired this doctoral thesis allowed to know in greater depth which species are associated with these pathologies, the predisposing conditions for the development of these diseases and to generate predictive model for SER prevalence, which allow to segregate the fruit by planting and climatic conditions in each orchard, because the symptoms are not observed at the orchard level or immediately after harvest, but manifests during postharvest , and in fruit that has several days of travel.

In this study, the isolates obtained in avocado wood and fruits were identified as species of Botryosphaeriaceae and Diaporthaceae family, and genus *Phomopsis*, *Colletotrichum*, *Pestalotiopsis* and *Alternaria.* However, was coincident that in both growing season the major number of isolates were identified as species of Botryosphaeriaceae family. In *Persea americana* Mill., the species of Botryosphaeriaceae family have been associated with wood damage and postharvest fruit rot since several decades ago. In México, New Zealand, Perú, South Africa and the USA the main reports have been of genus *Dothiorella*, *Lasiodiplodia* and *Neofusicoccum* (Dann *et al.*, 2013; McDonald *et al.*, 2009; Menge and Ploetz, 2003). In Chile, there are only reports of *Botryosphaeria dothidea* and

Neofusicoccum australe (Montealegre *et al*., 2016; Auger *et al*., 2013; Latorre, 2004).

The isolates identified in wood were *Diplodia mutila*, *D. pseudoseriata*, *D. seriata*, *Dothiorella iberica*, *Neofusicoccum nonquaesitum and N. parvum*. In fruits the isolates identified were *D. mutila*, *D. seriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *N. australe and N. parvum*. In addition, our results indicated that isolates identified were pathogenic to Hass avocado plants and fruits

To our knowledge, this is the first report of *D. mutila*, *D. seriata*, *D. iberica*, *N. nonquaesitum*, and *N. parvum* associated with branch canker and dieback in avocado in Chile. Additionally, it has not been previously reported that *D. mutila*, *D. seriata*, *D. iberica*, *L. theobromae*, and *N. parvum* were associated with stem end rot of avocados in Chile.

The isolates of *N. nonquaesitum*, *N. parvum* and *D. pseudoseriata* were most virulent pathogens in one-year-old Hass avocado trees grafted onto Mexicola rootstock, and the isolates of *L. theobromae* and *N. parvum* were the most pathogenic species in ripe avocado fruits, which is an important contribution about knowledge of these pathologies in Chile.

The main results of multivariate analysis (PCA, PLS, PLS-DA), which included the nine scenarios of growing seasons and diseases, allowed to determine that the factors most related with CD prevalence are diameter of trunk, volume of canopy, Leaf area index, age of plants and plant density. Moreover, these analyses indicated that the prevalence of SER included the same variables for CD prevalence in

addition with DaysM and DMFruit. Additionally, the analysis of SER prevalence as variable dependent indicated that the high level is associated with variables of climatic conditions and nutritional content of fruits. In this sense, there were high level of SER prevalence in orchards with high level of Ppau, ATau, MINTau, ATwi, MAXTwi, RADsu, RADau and RADwi with fruits with low content of CaFruit and DMFruit. These results were consistent with Rivera *et al.* (2017), which indicated that matter content, calcium content and minimum temperature are some variables that significantly influencing the overall ripening behavior of 'Hass' avocado fruits. Therefore, is necessary take account that these variables allowing to know the risk level of develop SER in ripe fruits.

Internationally, Branch Canker and Dieback have been attributed to different stress such as nutritional deficiencies, salinity, extreme temperatures, excessive radiation and injuries, caused by mechanical damage caused mainly by over loading, excessive pruning and ringing (Dann *et al*., 2013). Therefore, the results obtained in this study allow to indicate that is very important to avoid aging of plants and stressful conditions (for example, ringed, deficit irrigation, excessive use of growth regulators). Likewise, is necessary the renew foliage by performing invigorating pruning early in the season (closer to winter outings) and promote root growth.

In relation to Stem End Rot, the development of this disease has been attributed to the contamination of the fruit during the harvest either by conidia of the environment that infect the peduncle, or by conidia that are dragged in the harvest scissors from a fruit infected to a healthy fruit, because the pathogens related to Stem End Rot require a way to enter the fruit (wounds), which can be a key element when defining

harvesting procedures (Dann *et al*., 2013; Hartill and Everett, 2002). In this sense, with this study is possible to indicate that is necessary to reduce the pressure of these pathogens maintaining an optimum quality and storage condition in the fruit, from the orchard to the final consumer, because optimal storage conditions during DaysM allow have fruits with low level of SER prevalence, which were harvested from orchards with young trees, that were harvested with high Dry Matter percentage and that needed few days of storage until reaches the maturity index. These results suggested that old trees must be harvested with high percentage of Dry Matter and have less storage time, which reduce the likelihood of developing SER. On the other hand, young trees can be harvested with a low percentage of dry matter, because they can remain for longer under storage conditions. Additionally, these results could be associated with the decrease in the concentrations of the antifungal diene, during the ripening of fruits, a preformed antifungal component of avocado (Hopkirk *et al*., 1994; Prusky and Keen, 1993), which increases the fruit susceptibility to infection, and consequently decrease the life postharvest of fruits.

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Chapter 6

Conclusions

This research identified and characterized the pathogenicity of species in the Botryosphaeriaceae family associated with branch canker, dieback, and stem end rot in avocado orchards located in the primary production region in Chile. Therefore, this research provides information that can guide future research to study the epidemiology of these pathogens to establish effective prevention and control strategies, to limit the dissemination of these pathogens to the fruit, and to study adequate fruit storage conditions to prolong their postharvest life until they reach consumers.

The multivariate analysis of edaphoclimatic, planting and management conditions indicated than the incidence and severity of these disease depend heavily of climatic conditions during growing seasons and planting variables. Therefore, to avoid a significant impact on productivity and profitability in the production of avocados, caused by branch canker and dieback in orchards, and stem end rot in exporting companies, is necessary consider some managements that allows to tree to maintain vigor, to avoid the aging of plants and stressful conditions. Likewise, is necessary the renew foliage by performing invigorating pruning early in the season and promote root growth.

Chapter 7

Annexes

Annex 7.1.1. Abstract. Valencia A. L, and Gil P.M. 2015. Conditions detected in avocado orchards to develop canker dieback caused by Botryosphaeriaceae species in Chile. VIII World Avocado Congress. Lima, Perú.

Abstract

Species of Botriosphaeriaceae family in Chilean production of Hass avocado can cause canker dieback in young and old avocado trees, which affect trunk and branch, producing damage and tissue death. The infection begins at the vascular tissue and is spread systemically to others healthy parts of the plant, altering the water and nutrients distribution. This condition can affect the accumulation and availability of reserves, which are mainly located on the trunk and branches, necessary for fruiting the following season; thus, canker dieback can reduce productivity of the orchard. Also, this disease produced in preharvest can generate latent infections inside the fruit, causing rot that affects the normal development of fruit during postharvest.

A prospective research is being developed in Chilean Hass avocado orchards, from Illapel (31° 37'S) until Melipilla (33°33'S). Some of the data being registered to study its relationship with the disease are orchard age, climatic variables, soil chemical and physical features, irrigation management, and abiotic stress problems, such as

drought, salinity, extreme temperatures, wind and mechanical damage. Other variables that are being take into account are pruning and girdling managements, pest and other pathogens that could raise host susceptibility.

So far, we have found that there are abiotic factors that predispose to damage avocados trees and fruits by this complex of fungi. Symptoms have been detected in most of the visited orchards, but the incidence and severity depend heavily of pre and postharvest handling.

Annex 7.1.2. Poster. Valencia A. L, and Gil P.M. 2015. Conditions detected in avocado orchards to develop canker dieback caused by Botryosphaeriaceae species in Chile. VIII World Avocado Congress. Lima, Perú.

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CONDITIONS DETECTED IN AVOCADO ORCHARDS TO DEVELOP CANKER DIEBACK CAUSED BY BOTRYOSPHAERIACEAE SPECIES IN CHILE

CONDICIONES DETECTADAS EN HUERTOS DE PALTOS PARA DESARROLLAR CANCROSIS Y MUERTE REGRESIVA CAUSADAS POR ESPECIES BOTRYOSPHAERIACEAE EN CHILE

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INTRODUCCIÓN

The symptoms associated with Botryosphaeriaceae species in the world are death of graft union; leaf blight, dieback, cankers, stem end rot and fruit rot (Dann et al., 2013; Eskalen et al., 2013; McDonald & Eskalen, 2011; Menge & Ploetz, 2003; Slippers & Wingfield, 2007; White et al., 2005).
Stress in avocado tree, such as drought, wet, extreme temperature, nutrients deficiencies, and wounds from mechanical damage by wind, grafting, girdling, pest and other pathogens, raise susceptibility host to Botryosphaeria species (Dann et al., 2013; Eskalen et al., 2013; Johnson & Kotzé, 1994; McDonald & Eskalen, 2011; Menge & Ploetz, 2003; Slippers & Wingfield, 2007). The predisposing factors for development of species detected in Chilean orchards are not reported. Therefore, the objective of this study is to known factors that are predisposing to the development of cankers and branches dieback caused by Botryosphaeriaceae species in Chilean orchards.

MATERIALS AND METHODS

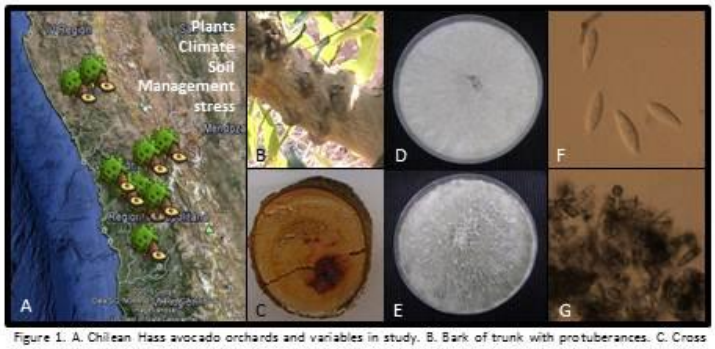

Figure 1. A. Chilean Hass avocado orchards and variables in study. B. Bark of trunk with protuberances. C. Cross section of canker in branches. D-E. White and grey-black colony of *Neofusicoccum* sp. in APDA. F-G. Conidia of *Neofusicoccum* sp. in early and later development.

RESULTS AND DISCUSSION

The pathogens of Botryosphaeriaceae species have ability to rapidly cause disease when their hosts are under stress (Slippers & Wingfield, 2007), particularly drought stress (Dann et al., 2013). This factor is very important because was the most common abiotic stress detected in this study (71.4%) and in Chile the drought extends from the north to the center-south of the country, directly affecting the productive zone of avocados.
The pruning is a common management strategy (71.4%) that is mainly used annually to improve lighting, reduce overgrowth in high density planting and avoid increasing the severity of the disease in some orchard. The pruning wounds increase risk for branch canker development (Eskalen et al., 2013). Therefore, is necessary to know the risk period and sealed the wound branch with latex amended with a cupric commercial fungicide (Dann et al., 2013; Menge & Ploetz. 2003).

CONCLUSION

In Chilean avocado production zone, there are abiotic factors that are predisposing factors to the development of disease caused by Botryosphaeriaceae species, and that is necessary have into take some managements to avoid a significant impact, because the incidence and severity depend heavily of preharvest handling, mainly of pruning intensity.

ACKNOWLEDGEMENTS

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Annex 7.1.3. Proceeding. Valencia A. L, and Gil P.M. 2015. Conditions detected in avocado orchards to develop canker dieback caused by Botryosphaeriaceae species in Chile. VIII World Avocado Congress. Lima, Perú.

Conditions detected in avocado orchards to develop canker dieback caused by Botryosphaeriaceae species in Chile

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ABSTRACT

Species of Botriosphaeriaceae family in Chilean production of Hass avocado can cause canker dieback in young and old avocado trees, which affect trunk and branch, producing damage and tissue death. The infection begin at the vascular tissue and is spread systemically to others healthy parts of the plant, altering the water and nutrients distribution. This condition can affect the accumulation and availability of reserves, which are mainly located on the trunk and branches, necessary for fruiting the following season; thus, canker dieback can reduce productivity of the orchard. Also, this disease produced in preharvest can generate latent infections inside the fruit, causing rot that affects the normal development of fruit during postharvest.

A prospective research is been developed in Chilean Hass avocado orchards, from Illapel (31° 37'S) until Melipilla (33°33'S). Some of the data being registered to study its relationship with the disease are orchard age, climatic variables, soil chemical and physical features, irrigation management, and abiotic stress problems, such as drought, salinity, extreme temperatures, wind and mechanical damage. Other variables that are being take into account are pruning and girdling managements, pest and other pathogens that could raise host susceptibility.

So far, we have found that there are abiotic factors that predispose to damage avocados trees and fruits by this complex of fungi. Symptoms have been detected in most of the visited orchards, but the incidence and severity depend heavily of pre and postharvest handling.
Keywords: Predisposing factors, fungal trunk pathogens, complex of fungi, avocado tree.

RESUMEN

Especies de la familia Botryosphaeriaceae en la producción chilena de Paltos Hass, pueden causar cancrosis y muerte regresiva en árboles jóvenes y adultos, lo cual afecta al tronco y ramas, produciendo daño y muerte de tejido. La infección comienza en el tejido vascular y es diseminada sistémicamente a otras partes sanas, alterando la distribución de agua y nutrientes. Esta condición puede afectar la acumulación y disponibilidad de reservas, las cuales están principalmente localizadas en los troncos y ramas, necesarias para fructificar en la siguiente temporada; en sí, la cancrosis y muerte regresiva pueden reducir la productividad del huerto. También, esta enfermedad producida en precosecha puede generar infecciones latentes al interior del fruto, causando pudrición que afecta el normal desarrollo del fruto durante postcosecha.

Una investigación prospectiva está siendo desarrollada en huertos chilenos de palto Hass, desde Illapel (31° 37'S) hasta Melipilla (33°33'S). Algunos de los datos que están siendo registrados para estudiar la relación con la enfermedad son: edad del huerto, variables climáticas, características físicas y químicas del suelo, manejo de riego, y problemas por estrés abiótico, tales como sequía, salinidad, temperaturas extremas, viento y daño mecánico. Otras variables que están siendo tomadas en cuenta son poda y anillamiento, plagas y otros patógenos que podrían aumentar la susceptibilidad del hospedante.

Hasta el momento, hemos encontrado que hay factores abióticos que predisponen al daño en árboles y frutos de palto por este complejo de hongos. Los síntomas han sido detectados en muchos de los huertos visitados, pero la incidencia y severidad dependen fuertemente del manejo en pre y postcosecha.

Palabras clave: Factores predisponente, Hongos patógenos del tronco, complejo fúngico, palto.

INTRODUCTION

Avocados are produced commercially in México, Chile, Israel, South Africa, Spain, Peru, Brazil and USA, supplying the international market year round. Chile is an important avocado exporter and producer, with 31.727 ha planted along 3rd to 6th Region (28° 27⍁ to 35° 01⍁ S) (ODEPA-CIREN, 2014), being the 5th region the most productive.

Hass is the main cultivar produced in Chile, which travels toward North America, Europe, Asia and South America. The great challenge of Chilean production is the distance with potential and consumers countries. Therefore, is necessary to maintain quality postharvest for a long time until reaches to consumers, maintaining the commercial quality, avoiding pest, diseases and wounds that can cause damage in the avocado fruits.

The Botryosphaeriaceae family encompasses a range of morphologically diverse Ascomycota fungi that are pathogens, endophytes or saprobes, mainly on woody hosts. They are found in all geographical and climatic areas of the world, with the exception of the Polar Regions (Phillips *et al.,* 2013).

Members of the Botryosphaeriaceae family causing cankers and fruit rot on a wide variety of woody hosts, and can survive as saprophytes or parasites and some species can survive as endophytes in symptomless tissue, with latent infections (Twizeyimana *et al.,* 2013). The *Botryosphaeria* spp. are considered like opportunist pathogens in avocado trees, because did not damage to healthy trees, and post latent phase have ability to rapidly cause disease when their host are under stress (Slippers & Wingfiels, 2007). The symptoms detected in the world are death of graft union; leaf blight, dieback, cankers, stem end rot and fruit rot (Dann *et al.,* 2013; Eskalen *et al.,* 2013; Johnson & Kotzé, 1994; McDonald & Eskalen, 2011; Menge & Ploetz, 2003; Slippers & Wingfield, 2007; White *et al.,* 2005).

Various species within the Botryosphaeriaceae family have been isolated from cankers on avocado from many different countries, including Mexico, New Zealand, Peru, South Africa, Chile, Spain, and the United States (McDonald & Eskalen, 2011).

The first specie Botryosphaeriaceae family detected in Chilean Hass avocado tree is *Dothiorella* sp. in 1986 (Pinto de Torres *et al.,* 1986), currently known as *Fusicoccum aesculi* Corda (Synonymous *Dothiorella* gregaria Sacc. and D. *dominicana* Petr. y Cif.) (Acuña, 2010; Latorre, 2004; Besoaín *et al.,* 2002), anamorph of *Botryosphaeria berengiana* (Acuña, 2010; Besoaín *et al.,* 2002; Latorre, 2004), B. *ribis* and B. *dothidea* (Acuña, 2010; Latorre, 2004). The second and last report in Chile of *Botryosphaeria* spp. was *Neofusicoccum australe*, anamorph of B. *australis* in 2013 (Auger *et al.,* 2013).

Understanding the interaction of plant pathogen with their avocado host is critical to apply appropriate control measures and to reduce the lost yield. In this sense, there are researches which have indicates that stress in avocado tree, such as drought, wet, extreme temperature, nutrients deficiencies, and wounds from mechanical damage by wind, grafting, girdling, pest and other pathogens, raise susceptibility host to *Botryosphaeria* species (Dann *et al.,* 2013; Eskalen *et al.,* 2013; Johnson & Kotzé, 1994; McDonald & Eskalen, 2011; Menge & Ploetz, 2003; Slippers & Wingfield, 2007).

The predisposing factors for development of species detected in Chilean orchards are not reported. Therefore, the objective of this study is to known factors that are predisposing to the development of cankers and branches dieback caused by Botryosphaeriaceae species in Chilean orchards.

MATERIALS AND METHODS

The prospective research has been developed in Chilean Hass avocado orchards, from Illapel (31° 37'S) until Melipilla (33° 33'S). The orchards are located in the provinces of Illapel (4th region), San Felipe (5th region), Petorca (5th region), Quillota (5th region) and Melipilla (Metropolitan region).

Characterization of orchards

In this prospection, were used nine orchards, and two sectors by each orchards, like sample area, which were characterized with several data registered, such as: origin of plants (Nursery or own plants), plant age, rootstock, distance between plants, climatic variables, soil chemical and physical features, irrigation management, and abiotic stress problems, such as drought, salinity, extreme temperatures, wind and mechanical damage. Other variables that were taken into account are pruning and girdling managements, pest and other pathogens that could raise host susceptibility.

Study of diseased orchards

The study of disease in each orchard has considered trees with symptoms associated with the disease, distribution of tree with the disease and severity. Since trees with cankers and dieback, were obtained five samples by each symptom, from damaged zone on tress. Each pruned branches of samples were refrigerated until analysis. The tools used in each tree were disinfected with sodium hypochlorite to prevent spread of the disease and the wounds of trees were sealed with latex amended with a commercial fungicide.

The bark of each sample was removed prior to surface disinfection with 96% ethanol for 15s and immediately flamed for 15s. The outer tissues were removed aseptically in a laminar flow chamber. Small wood pieces (3-5 mm) were taken from the margins between healthy and discolored tissues and placed in Petri dishes containing 2% potato dextrose agar acidified with 0.5 ml/liter of 92% lactic acid (APDA) plus 0.005% tetracycline and 0.01% streptomycin. The plates were incubated for 14 to 21 days at 20°C until fungal colonies were observed.

The colony characteristics in APDA was considered because allow distinguishing anamorph of the Botryosphaeriaceae family with other pathogenic or saprophytes fungi, because in early development have white colony and grey-black colony in the later development (Phillips *et al.,* 2013).To produce asexual structures, including conidia and conidiophores, small pieces of mycelium were placed in APDA, and maintained during 30d at 20°C in darkness. The width and length of 30 conidia were measured and the mean and standard deviation were calculated. Also, shape, color, and the presence or absence of septation in the conidia was considered.

RESULTS

Characterization of orchards

The features detected in orchards visited have indicated that plants were obtained in nursery (100%), located near of each region. The rootstock is Mexicola (100%), which was not affected by the disease.

The age of plant was more or equal than three years (14.3%), ten years (35.7%) and 15 years (50%).

The planting density is more than 250 plants/ha (42.9%), 500 plants/ha (21.4%) and 1000 plants/ha (35.7%). The soils are clay (28.5%) or loam clay (71.4%).

The irrigation systems are drip (21.4%) with 8 to 12h by week and micro sprinkler (71.4%) with 6 to 10h by week, depending on the availability of water. On the other hand, the fertilizers are used to provide N, P, K, S, B, Ca, and Zn, in all the orchards for to maintain nutritional plants state.

The pruning is a common management strategy that is mainly used annually to improve lighting, reduce overgrowth and avoid increasing the severity of the disease in some orchard (71.4%). The girdling is used to avoid the translocation of assimilates and reserves to roots, and to improve the development of fruits but in orchards visited this strategy was less common (35.7%).

The drought was the most common abiotic stress detected (71.4%), being the orchards located in 4th region and 5th region most affected.

In some orchard there are effects of frost (28.5%), sunburn (28.5%), salinity (21.4%), mechanical damage and wounds by wind (7.1%), and biotic stress by *Saissetia oleae* (28.5%), thrips (42.9%) and red mites (42.9%).

Study of diseased orchards

In the orchards were found symptoms associated with the disease caused by Botryosphaeriaceae species (Figure 1). The detection of protuberances in trunk and brunches, branches dieback, cankers, friable bark and inner tissue brown or red-brown only in graft, it allows distinguish this disease from others diseases that occasionally affect avocados. Only one orchard visited does not have the disease.

The branches dieback was detected in young plants in replant and productive adult trees, being the trees of more than 15 years which have the most severity (71.4%). In some branches there are dry leaves and dry inflorescences. Also, there are branches with small fruits and abnormal development.

The identification of Botryosphaeriaceae species obtained from brown and dark red-brown inner tissue of branches was on the basis of colony and morphology of conidia (Phillips *et al.,* 2013). The isolates were identified as PALUCM3, PALUCM7, PALUCM10 and PALUCM13. On APDA, PALUCM3 produced white cottony and clear grey colonies and conidia were hyaline, smooth, obovoid or fusiform with base sub truncate, with granular contents (23.3) 20.1 to 26.0 × (10.9) 8.6 to 12.7 µm with a length/width ratio of 2.2 ± 0.2, with absence of septation. On APDA, PALUCM7 produced white cottony and clear grey colonies and conidia were hyaline, fusiform (23.3) 19.1 to 31.9 × (8.2) 5.0 to 14.4 µm with a length/width ratio of 3.2 ± 0.5, with presence of one or two septum and darker brown middle cell in mature state. PALUCM10 on APDA produced white cottony and dark grey colonies, the conidia were hyaline, obovoid or ellipsoid (22.8) 13.6 to 29.1 × (8.2) 6.9 to 10.8 µm with a length/width ratio of 2.5 ± 0.5, with absence of septation. On APDA, PALUCM13 produced white cottony and clear grey colonies, the conidia were hyaline, ovoid-obovoid, or fusiform, (22.8) 17.0 to 29.3 × (10.3) 6.5 to 12.5 µm with a length/width ratio of 2.4 ± 0.2, with absence of septation.

Figure 1. Branches dieback with leaves and fruits death (white arrow) attached to branches

Figure 2. Bark of trunk with protuberances (white arrow) and its damaged or dead inner tissue.

Figure 3. Cross section of canker in branches, with infection on vascular tissue (white arrow)

DISCUSSION

The regular distribution of this disease, with high severity on trees of more than 15 years, could be caused by the predisposing factors detected in this research, because the disease caused by Botryosphaeriaceae species is considered of minor importance on mature trees if these are in optimal conditions (Dann *et al.,* 2013).

The symptoms just were detected in the graft of Hass variety, which have mainly features of Guatemalan race, and the Mexicola rootstock was not affected by disease symptoms, which coincides with Dann *et al.* (2013) that have indicated that Mexican race rootstock is less affected than Guatemalan material.

In Chilean orchards, the high density planting requires more intensive canopy management, such as more frequent pruning, which may lead to an increased risk for branch canker development (Eskalen *et al.,* 2013). Therefore, is necessary to know the risk period and sealed the wound branch with latex amended with a cupric commercial fungicide (Dann *et al.,* 2013; Menge & Ploetz. 2003). By another hand, in some orchards with pruning for control the disease, is necessary consider that severe pruning also cause possible decrease in yield if branches with cankers are removed (Eskalen & McDonald, 2009), but this action allow to obtain healthy branches and limit the inoculum on pycnidia and perithecia, only if there are disinfection of tools, and pruned branches with disease are located away of healthy trees, to avoid spread of the disease.

The management of each orchard is to respond to crop needs throughout of the season, balancing tree nutrition and irrigation to maintain health of tree and minimize stress. Because the pathogens of Botryosphaeriaceae species have ability to rapidly cause disease when their hosts are under stress (Slippers & Wingfield, 2007), particularly drought stress (Dann *et al.,* 2013) that is an important stress because causes physiological damage and the water status is essential for plant development and growth. This factor is very important because in Chile the drought extends from the north to the center-south of the country, directly affecting the productive zone of avocados.

The symptoms detected allow indicating that initially the bark of branches and trunk has rough protuberances and death of inner tissue. In more severe infections have caused cankers with dark and friable bark, often with the dried brown-white exudate of perseitol. Under the canker, the bark and wood turns red-brown or brown and can penetrate into the heartwood, which is consistent with national and international reports of this disease (Acuña, 2010; Auger *et al.,* 2013; Besoaín *et al.,* 2002; Dann *et al.,* 2013; Eskalen *et al.,* 2013; Johnson & Kotzé, 1994; Latorre, 2004; McDonald & Eskalen, 2011; Menge & Ploetz, 2003; Pinto de Torres *et al.,* 1986; Slippers & Wingfield, 2007; White *et al.,* 2005). The main problem with this disease is if the infection reaches vascular tissue, because it can stop water and nutrients transport from xylem and translocation of assimilates reserves to sinks, this blockage causes weakening and decays of the wood at the infection site, which eventually can lead to wilting or death of the branch (Eskalen *et al.,* 2013). The avocado trees accumulate reserves in the bark, therefore, the disruption in flow, affects the accumulation and availability of reserves located on the trunk and branches, which are necessary for fruiting the following season (Chanderbali *et al.,* 2013). This effect would be the cause of low productivity in orchards with high incidence and severity of the disease.

The identification of these pathogens is necessary to improve the knowledge of this disease, to address this pathologic problem and generate accurate solutions to agroindustry, because there are complex of fungi associated with the disease in some cases.

CONCLUSIONS

This research has allows conclude that in Chilean avocado production zone, there are abiotic factors that are predisposing factors to the development of disease caused by Botryosphaeriaceae species, and that is necessary have into take some managements to avoid a significant impact on productivity and profitability in the production of avocados, because the incidence and severity depend heavily of preharvest handling, mainly of pruning intensity.

ACKNOWLEDGMENTS
PhD scholarship CONICYT-PCHA 21140282 and Project CONICYT-PAI 21140282

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Annex 7.2.1. Abstract. Valencia A.L., Gil P.M., Rosales M., Saavedra-Torrico J., Martiz J., and Link A. 2016. Estudio de condiciones predisponentes en Persea americana variedad Hass, para el desarrollo de cancrosis, muerte regresiva y pudrición peduncular causadas por Botryosphaeriaceae detectadas en Chile. 67° Congreso Agronómico y 14° Sochifrut. Santiago, Chile.

Resumen

Desde enero de 2015 hasta septiembre de 2016, se ha realizado un estudio para determinar las principales causas de cancrosis, muerte regresiva y pudrición peduncular en palto cv Hass. Para ello se han muestreado huertos desde Illapel (31° 37' S) hasta Peumo (34º 23'S), donde se han obtenido trozos de ramas y frutos, para determinar la presencia de hongos de la familia Botryosphaeriaceae. Además, se han registrado datos de manejos agronómicos y condiciones edafoclimáticas que pudieran influir en la incidencia y severidad de estos problemas fitopatológicos a nivel de huerto y en la calidad y condición de los frutos. Los datos han sido analizados por análisis multivariado (PCA y PLS), para definir los factores más influyentes tanto en incidencia como en severidad de tales patologías.

Preliminarmente, se ha observado que la incidencia y severidad de estas enfermedades dependen fuertemente del nivel de estrés hídrico, presión de plagas y época e intensidad de poda, además del manejo de los restos de madera con síntomas, lo que se espera corroborar con la temporada que está terminando.

Agradecimientos Beca Doctorado Nacional CONICYT-PCHA 21140282, Proyecto CONICYT- PAI Tesis Doctoral con empresa 781413002 y Exportadora SUBSOLE S.A.

Annex 7.3.1. Invitation. Valencia A.L., Gil P.M., Rosales M., Saavedra-Torrico J., Link A., and Gonzalez F. 2016. Seminario Proyecto CONICYT+PAI 781413002: Estudio de Factores asociados y especies causantes de enfermedad en la madera y pudrición peduncular en huertos de paltos Hass de Chile. Santiago, Chile.

Invitación

Marlene Rosales, Directora de Investigación y Postgrado, de la Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, les invita a participar de un Seminario por término del Proyecto CONICYT de Tesis de Doctorado en la empresa **"Factores que predisponen a *Persea americana* Mill. a la infección de especies de la familia Botryosphaeriaceae en la zona central de Chile"**, Folio N° 781413002.

El objetivo de esta convocatoria es difundir los resultados obtenidos en el marco del Proyecto, realizado en la temporada 2014-2015 y 2015-2016, en huertos distribuidos desde Illapel a Peumo, con síntomas asociados a muerte de ramas, cancrosis y pudrición peduncular.

Esta actividad se realizará el martes 20 de junio a las 10:00 horas, en la Sala de Postgrados (Segundo piso), de la Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile. Ubicada en Vicuña Mackenna 4860, Macul, Región Metropolitana.

Los cupos son limitados, por lo que solicitamos confirmar asistencia al correo alvalenc@uc.cl, previo al miércoles 14 de junio de 2017.

Enfermedad en la madera

Pudrición peduncular

Agradecimientos

CONICYT+PAI Concurso Nacional Tesis de Doctorado en la empresa, 781413002.
CONICYT+PCHA Beca Doctorado Nacional, convocatoria 2014. 21140282.
SUBSOLE S.A.
Dirección de Investigación y Postgrados
Facultad de Agronomía e Ingeniería Forestal
Fundo Los Agustinos, San Felipe; FullPal S.A. Nogales; Cuatro Palmas S.A., Quillota; Parcela El Río, Ocoa; Agrícola El Roble, Jaururo; Fundo Quilhuica, María Pinto; Parcela La Muralla, Alicahue; Fundo El Cardal, Melipilla; Parcela 147, Illapel; Parcela 5, La Ligua; La Rosa SOFRUCO, Peumo; Agrícola Doña Adriana, El Carmen de Las Rosas, Francisco González (Asesor), Gonzalo Vargas (Asesor).

Annex 7.3.2. Program. Valencia A.L., Gil P.M., Rosales M., Saavedra-Torrico J., Link A., and Gonzalez F. 2016. Seminario Proyecto CONICYT+PAI 781413002: Estudio de Factores asociados y especies causantes de enfermedad en la madera y pudrición peduncular en huertos de paltos Hass de Chile. Santiago, Chile.

10:00-10:15 Registro de los participantes

10:15-10:30 Palabras de bienvenida

10:30-10:45 Marco teórico del Proyecto. Pilar Gil (Profesora guía de Tesis y encargada del Proyecto ante CONICYT y PUC).

10:45-11:20 Importancia de la enfermedad en la madera a nivel de huerto. Francisco González (asesor Paltos zona central).

11:20-11:55 Importancia de la enfermedad en postcosecha. Andrés Link (Contraparte empresa Subsole S.A. Proyecto).

11:55-12:20 Coffe break (Financiado por la Dirección de Investigación y Postgrados)

12:20-12:55 Presentación de Resultados obtenidos a nivel de huerto. Ana L. Valencia (Tesista Proyecto).

12:55-13:30 Presentación de Resultados obtenidos a nivel de postcosecha. Ana L. Valencia (Tesista Proyecto).

13:30-14:00 Ronda Final de Preguntas

Annex 7.4.1. Abstract. Valencia A.L., Gil P.M., and Rosales M. 2017. Caracterización y patogenicidad de especies de Botryosphaeriaceae obtenidas desde madera y frutos de Persea americana en huertos chilenos. XXV Congreso SOCHIFIT, XIX Congreso ALF y LVII APS Caribbean división meeting. Chillán, Chile.

Resumen

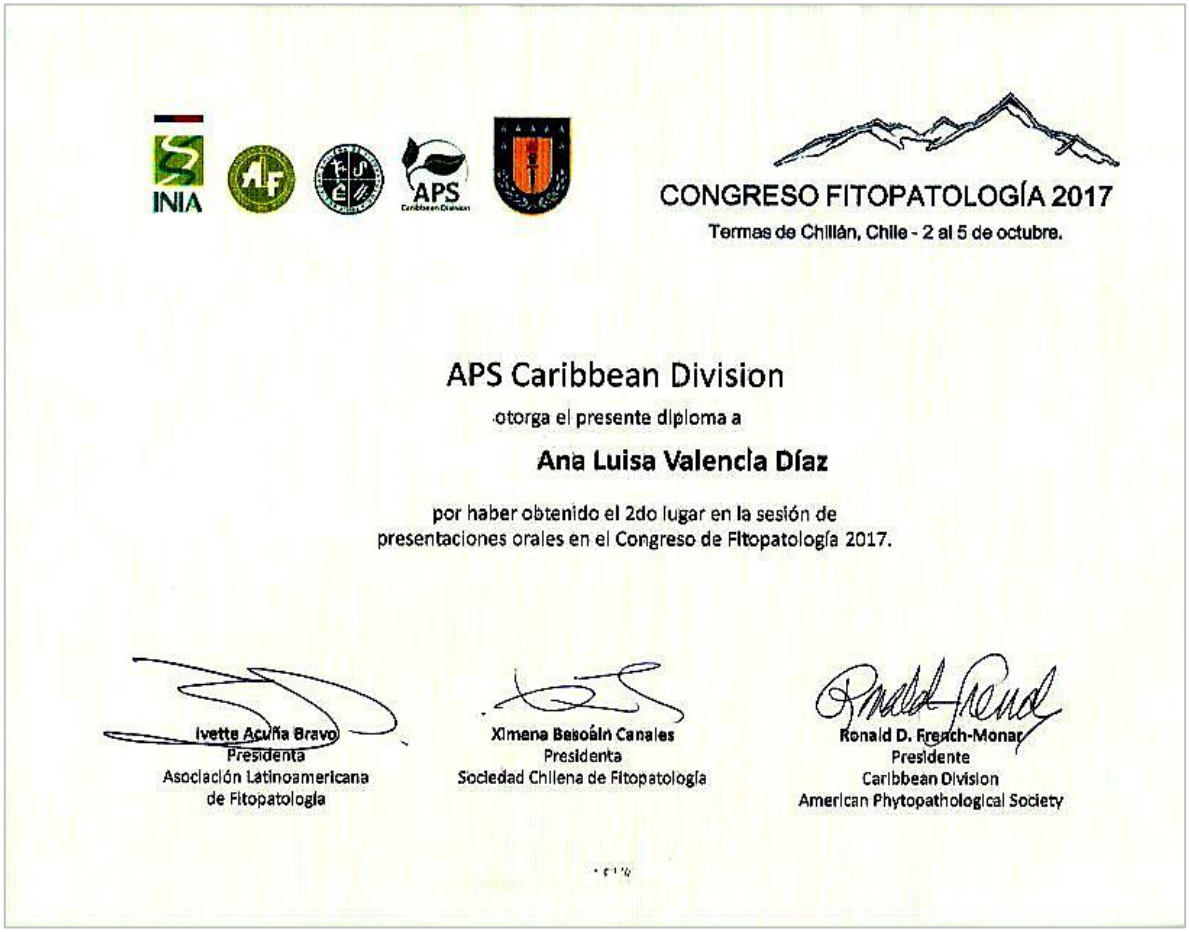
En Chile, se han registrado síntomas de cancrosis y muerte regresiva en paltos (*Persea americana*) y pudrición peduncular en paltas Hass de exportación. El objetivo de esta investigación ha sido caracterizar e identificar los patógenos asociados a estas enfermedades. Para esto, se obtuvo muestras de madera y frutos en huertos desde Illapel (IV región) hasta Peumo (VI región). Se sembró trozos de madera y frutos desde la zona de avance del daño se sembró en APD (20ºC por 7 días). De un total de 42 aislados de madera y 88 aislados de frutos, tentativamente identificados como *Diplodia, Dothiorella, Lasiodiplodia y Neofusicoccum*, se seleccionaron 12 aislamientos de madera y 17 aislamientos de paltas, para su caracterización morfológica, térmica, molecular (genes ITS1/ITS4 y EF728/EF986) y estudios de patogenicidad. La patogenicidad se determinó en plantas de palto Hass sobre portainjerto Mexicola, durante 2 meses y en paltas Hass (25,57% materia seca), incubadas en cámaras húmedas a 20ºC/10 días. Los resultados demostraron la presencia de colonias fungosas blancas y picnidios negros con conidias ovoides hialinas/marrón, septadas/aceptadas; fusiformes hialinas y septadas, dependiendo del aislamiento). En función de las características morfológicas de las conidias y de los análisis moleculares, estos aislamientos se identificaron como *Diplodia mutila, Diplodia seriata, Diplodia pseudoseriata,*

Dothiorella iberica, Dothiorella rosulata, Lasiodiplodia theobromae, *Neofusicoccum australe, N. nonquaesitum y N, parvum.* En conclusión, los resultados de este trabajo demostraron la presencia de *especies de la familia Botryosphaeriaceae en Persea americana*. Sin embargo, estos resultados no descartan que estas especies coexistan con otros hongos patógenos.

Agradecimientos

CONICYT-PAI Proyecto Tesis de Doctorado en la empresa 781413002 y CONICYT-PCHA Beca Doctorado Nacional 21140282.

Annex 7.4.2. Prize. Valencia A.L., Gil P.M., and Rosales M. 2017. Caracterización y patogenicidad de especies de Botryosphaeriaceae obtenidas desde madera y frutos de Persea americana en huertos chilenos. XXV Congreso SOCHIFIT, XIX Congreso ALF y LVII APS Caribbean división meeting. Chillán, Chile.



CONGRESO FITOPATOLOGÍA 2017

Termas de Chillán, Chile - 2 al 5 de octubre.

APS Caribbean Division

otorga el presente diploma a

Ana Luisa Valencia Díaz

por haber obtenido el 2do lugar en la sesión de presentaciones orales en el Congreso de Fitopatología 2017.

Ivette Acuña Bravo
Presidenta
Asociación Latinoamericana de Fitopatología

Ximena Besoáin Canales
Presidenta
Sociedad Chilena de Fitopatología

Ronald D. French-Monar
Presidente
Caribbean Division
American Phytopathological Society

Annex 7.5.1. Poster. Valencia A.L., Gil P.M., and Rosales M. 2018. Characterization and Pathogenicity of Botryosphaeriaceae Species, obtained from Wood and Fruits of Persea americana in Chilean Orchards. Avocado Brainstorming. South Africa.

Avocado Brainstorming 2018

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

CONICYT Gobierno de Chile

Characterization and pathogenicity of Botryosphaeriaceae species, obtained from wood and fruits of *Persea americana* in Chilean orchards.

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INTRODUCTION

In Chile, since 2014, there are increasing reports of wood diseases in avocado orchards and stem end rot in avocado fruit exported to distant consumer countries. Those symptoms have been associated to Botryosphaeriaceae species and other pathogens in avocado plants and fruits, however little information is available about the causal pathogens. Botryosphaeriaceae infection symptoms are commonly death of graft union, leaf blight, dieback, branch cankers and fruit stem end rot (Dann et al., 2013; Eskalen et al., 2013; McDonald & Eskalen, 2011; Menge & Ploetz, 2003; Slippers & Wingfield, 2007; White et al., 2005). Therefore, the aim of this study was to identify species associated with these diseases and to study their pathogenicity..

METHODOLOGY

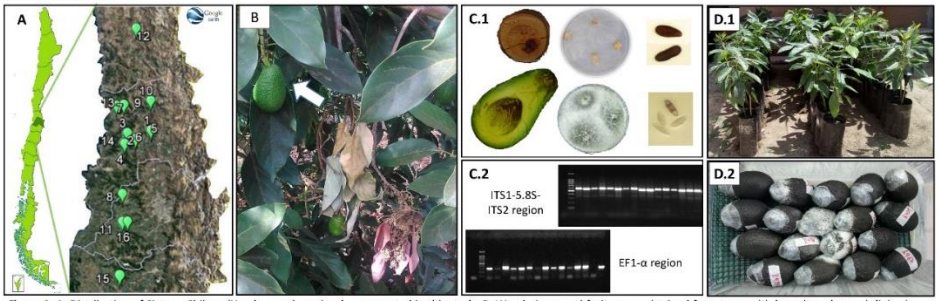

Figure 1. A. Distribution of Sixteen Chilean 'Hass' avocado orchards, prospected in this study. B. Wood pieces and fruits were obtained from trees with branch canker and dieback. C.1. Cross section of symptomatic branches and fruits; white to grey-black colony in APDA, and conidias. C.2. Electrophoresis gel of Internal Transcribed Spacer region (ITS1-5.8S-ITS2), and Translation Elongation Factor 1- α (EF1-α) gene, used in Blast and Phylogenetic Analysis. D.1. Pathogenicity tests on Hass avocado plants. D.2. Pathogenicity tests on Hass avocado fruits.

RESULTS

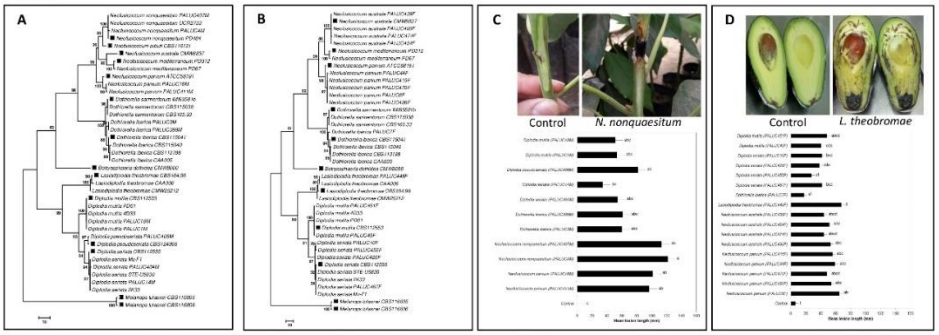

Figure 2. Phylogenetic analysis of isolates obtained in this study (PALUC), and sequences of Botryosphaeriaceae species available in GenBank databases. A. Isolates obtained from trees with Branch Canker and Dieback. B. Isolates obtained from fruits with Stem end rot. C. Results of Pathogenicity test of Botryosphaeriaceae species obtained in Hass avocado plants and fruits.

CONCLUSION

In this study diverse species of Botryosphaeriaceae family were obtained: *Diplodia mutila, D. pseudoseriata, D. seriata, Dothiorella iberica, Lasiodiplodia theobromae, Neofusicoccum australe, N. nonquaesitum*, and *N. parvum*. This research is the first study that describes identification and pathogenicity of Botryosphaeriaceae spp. in avocado orchards of Chile, which provides information for future research about impacts and epidemiology of these pathogens, which is critical to develop cultural strategies for disease management.

ACKNOWLEDGEMENTS

Doctoral scholarship CONICYT-PCHA 21140282, Project CONICYT-PAI 781413002, and Subsole S.A.

Annex 7.5.2. Poster. Valencia A.L., Gil P.M., Rosales M., and Saavedra-Torrico J. 2018. Factors detected in avocado orchards to develop branch canker, dieback and stem end rot caused by Botryosphaeriaceae species in Chile. Avocado Brainstorming South Africa.

Avocado Brainstorming 2018

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE

Factors detected in avocado orchards to develop branch canker, dieback and stem end rot caused by Botryosphaeriaceae species in Chile

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INTRODUCTION

In Chile, increasing cases of branch canker and dieback were reported in avocado orchards from 2011 to 2016, which coincided with a severe drought affecting the avocado production area. Additionally, the prevalence of avocado fruit stem end rot has been increasingly observed in distant consumer countries. The predisposing factors for these diseases in Chilean orchards have not been reported. Therefore, the objective of this study was to identify the main factors that are predisposing to the development of branch canker and dieback (CD), and stem end rot (SER) caused by Botryosphaeriaceae species in Chilean orchards.

METHODOLOGY

This study was conducted during the seasons 2014/15 and 2015/16 in 16 'Hass' avocado orchards, between Illapel (31° 37'S) and Peumo (34° 24'S), localized in the main avocado productive regions (Figure 1). The dataset considered 102 variables associated with geographic location, weather, plantation, management, yield, abiotic and biotic stress. To analyze those variables a Multivariate analysis was performed using Chemometrics Methods (NIPALS algorithm). This algorithm allow the analysis of measurements of a large number of variables, highly correlated and non well-conditioned matrix (Ferrer, 2007). Principal Component Analysis and Partial Least Squares (PLS) were performed to select representative variables. Additionally, a PLS discriminant analysis (PLS-DA) was performed to determine classes since the analysis of relations between variables and observations (avocado orchards), and the interactions among variables to study the relation of variables and classes.

All computes were performed using SIMCA-P v.10 software (Umetrics AB, Sweden). From variables obtained in PLS analysis was performed and a Ridge Regression analysis was performed for prevalence of each disease with Statgraphics 18 (StatPoint, USA).

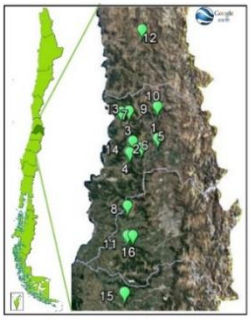

Figure 1. Distribution of avocado orchards, prospected in this study.

RESULTS

The PLS and Ridge Regression analysis of each disease have indicated a level of prediction of 78.8 % for CD prevalence, and 71.6 % for SER Prevalence. These models, using variables associated with age of plants, diameter of trunk, Leaf area index, volume of canopy and plants/ha for CD Prevalence. Furthermore, using Days from bloom to fruit ripening and Dry matter of fruits, with variables indicated previously for SER Prevalence (see Figure 2A y 2B). The PLS-DA with predictive capacity 72.5% (R^2Y), has indicated that Class 1 grouped orchards with low level of CD and severe level of SER, Class 2 grouped orchards with low level of CD and low level of SER, Class 3 grouped orchards without CD and severe level of SER, and Class 4 grouped orchards without CD and SER. The analysis of interactions between variables have indicated that CD with SER, Volume canopy with CD, and Dry matter of fruits with SER are highly correlated.

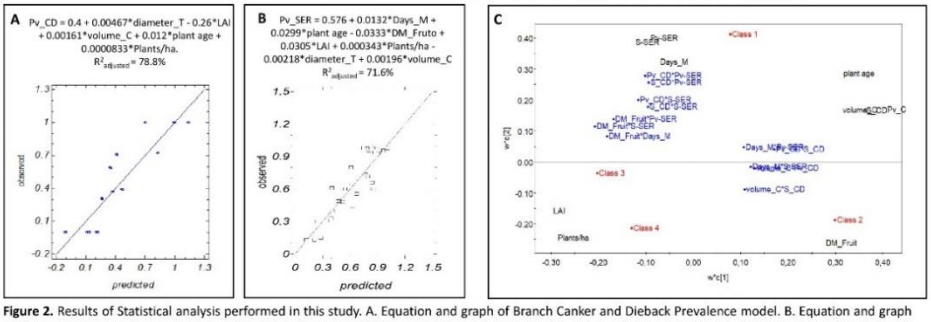

Figure 2. Results of Statistical analysis performed in this study. A. Equation and graph of Branch Canker and Dieback Prevalence model. B. Equation and graph of Stem End Rot Prevalence model. C. Scatter Plot of PLS-DA with classes (red letters), variables (black letters) and interactions between variables (blue letters).

CONCLUSION

The prevalence of CD and SER are directly related and correlated with age of plants, volume of canopy, diameter of trunk, and inversely correlated with LAI and plants/ha. This research is the first study that describes that age and plant size are the main factors associated with prevalence of CD and SER in Chile.

ACKNOWLEDGEMENTS

Doctoral scholarship CONICYT-PCHA 21140282, Project CONICYT-PAI 781413002, and Subsole S.A.

Annex 7.6. Report. Valencia A.L., Gil P.M., Rosales M., Saavedra-Torrico J., Martiz J., and Link A. 2018. Botryosphaeriaceae en palto: agentes causales de enfermedades de la madera y pudrición peduncular del fruto. Redagrícola Chile. Especial paltos y cítricos/Fitosanidad. ISSN 0718- 0802.

Figura 1a. La cancrosis consiste en heridas de gran magnitud a nivel de corteza, que pueden estar presentes en ramas y tronco, las cuales se originan a partir de protuberancias cuya corteza es friable y se desprende fácilmente.

Figura 1b. Al interior de ramas con cancrosis se puede observar tejido interno necrótico.

BOTRYOSPHAERIACEAE EN PALTO: AGENTES CAUSALES DE ENFERMEDADES DE LA MADERA Y PUDRICIÓN PEDUNCULAR DEL FRUTO

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En Chile desde hace un tiempo muchos productores de paltas han observado sus huertos con árboles decaídos, con ramas secas o con protuberancias a nivel de corteza, todos síntomas de enfermedades en la madera. Lo anterior ha sido más frecuentemente observado en huertos de edad avanzada y que han sido severamente afectados por algún tipo de estrés, como por ejemplo la sequía ocurrida en la zona central de Chile en los últimos años. Por otra parte, muchas empresas exportadoras de paltas chilenas han visto que sus envíos llegan con problemas de calidad, por la presencia de pudriciones, entre ellas la pudrición peduncular (conocida también como stem end rot), lo cual ha provocado una gran preocupación y diversas acciones para su mitigación. En el caso de los huertos con enfermedades asociadas a la madera, el escenario es preocupante, pues se ha observado que los productores han manejado esta enfermedad con podas sucesivas para remover la totalidad de los síntomas en madera, lo cual no ha sido una medida efectiva, pues han provocado que aumente la severidad en los sectores enfermos y se afectan sectores de plantas jóvenes que inicialmente estaban sanos.

SÍNTOMAS Y EPIDEMIOLOGÍA DE LA ENFERMEDAD

Especies de la familia Botryosphaeriaceae han sido reportadas en otros países que producen y consumen palta, tanto en daño en madera como en pudriciones de postcosecha, tal es el caso de México, Nueva Zelanda, Perú, Sudáfrica y Estados Unidos, habiéndose identificado previamente los patógenos del género *Dothiorella*, *Lasiodiplodia* y *Neofusicoccum*, como causantes de estas enfermedades (Dann et al. 2013; McDonald et al. 2009; Menge and Ploetz 2003). En Chile si bien existen algunos reportes previos, referidos a *Dotryosphaeria dothidea* y *Neofusicoccum australe* (Montealegre et al. 2016; Auger et al. 2013; Latorre 2003), el aumento de los casos de huertos con síntomas y llegadas de fruta con pudrición peduncular ha hecho necesario conocer con mayor profundidad cuáles son las especies asociadas con estas patologías, las condiciones que propician la infección y las condiciones predisponentes, para así poder contar con medidas de manejo para prevenir el desarrollo de estas enfermedades y estudiar posibles alternativas de control efectivas tanto para aplicar en madera como en frutos.

Los síntomas asociados a las enfermedades causadas por Botryosphaeriaceae son cancrosis, muerte regresiva y pudrición peduncular. La cancrosis consiste en heridas de gran magnitud a nivel de corteza, que pueden estar presentes en ramas y tronco, las cuales se originan a partir de protuberancias cuya corteza es friable y se desprende fácilmente, en dónde se puede observar tejido interno necrótico (Figura 1). La muerte regresiva, se caracteriza por la presencia de una pudrición café presente desde la corteza hasta el tejido vascular, y que causa muerte de ramas desde el ápice, las cuales se secan y retienen hojas, inflorescencias y frutos (Figura 2). Este síntoma es posible de ser observado tanto en árboles adultos como en árboles jóvenes. La pudrición peduncular, por su parte, es el problema de mayor impacto y preocupación en la industria, ya que se caracteriza porque en fruta madura se observa pudrición en pulpa desde la zona de unión al pedúnculo, la que avanza internamente hacia la zona ecuatorial, que en casos severos puede incrementar el síntoma de pardeamiento de pulpa y haces vas-

culares (Figura 3). Lamentablemente este síntoma no es observado a nivel de huerto ni inmediatamente después de la cosecha, sino que se manifiesta durante la postcosecha, y en fruta que tiene varios días de viaje.

Las enfermedades se generan cuando existe un hospedero susceptible, un patógeno y las condiciones predisponentes. Si la infección se genera en la madera, ésta puede alcanzar tejido vascular, deteniendo el flujo de agua y nutrientes vía xilema y la translocación de fotoasimilados, lo que finalmente termina causando marchitez y decaimiento en las ramas (Eskalen et al., 2013), con lo cual se obtiene árboles decaídos, de baja producción y corta longevidad. Asimismo, dado que el palto acumula reservas en la madera, la detención del flujo puede afectar la acumulación y disponibilidad de reservas localizadas en troncos y ramas, las cuales son necesarias para el desarrollo reproductivo de la siguiente temporada (Chanderbali et al., 2013), produciendo problemas de rendimiento y/o fruta de corta vida de poscosecha en huertos con alta prevalencia y severidad de estas enfermedades. Por otra parte, el inóculo presente en el campo genera infecciones latentes en la fruta, que desarrollan pudrición peduncular, lo cual se manifiesta en destino, generando rechazo en los recibidores y con ello pérdidas económicas significativas.

La **cancrosis** consiste en **heridas** de gran magnitud a **nivel de corteza**, que pueden estar presentes en **ramas** y **tronco**. Se originan a partir de **protuberancias** cuya corteza es **friable** y se desprende fácilmente, en dónde se puede observar **tejido interno necrótico**.

Las **enfermedades** se generan cuando **existe un hospedero susceptible**, un **patógeno** y las condiciones predisponentes.

Figura 2a. Rama con muerte regresiva, cuya muerte se inicia desde el ápice y retienen hojas, inflorescencias y frutos.

Figura 2b. Al interior de ramas con muerte regresiva se puede observar tejido interno necrótico.

GENERACIÓN DE CONOCIMIENTO RESPECTO A LAS ENFERMEDADES CAUSADAS POR BOTRYOSPHAERIACEAE EN CHILE

Para poder determinar las especies patógenas asociadas y los factores predisponentes para el desarrollo de los síntomas descritos tanto en huertos como en frutas de exportación, la Bióloga y Fitopatóloga Ana Luisa Valencia ha llevado a cabo una tesis doctoral en la Facultad de Agronomía e Ingeniería Forestal de la Pontificia Universidad Católica de Chile, dirigida por la Dra. Pilar Gil y la Dra. Marlene Rosales, con el financiamiento de CONICYT (Beca Doctorado Nacional CONICYT-PCHA 21140282 y Proyecto Tesis con la Empresa CONICYT-PAI 781413002), en conjunto con la empresa Subsole S.A (patrocinante de Proyecto Tesis con la Empresa). A continuación, se señalan los principales resultados y conclusiones de dicho trabajo.

Figura 3a. Paltas 'Hass' inoculadas con *Neofusicoccum parvum*, mantenidas durante 15 días en 5°C y adicionalmente 10 días en 20°C. Nótese el incremento del síntoma de pardeamiento de pulpa y haces vasculares.

Figura 3b. Paltas 'Hass' sin inocular, mantenidas durante 15 días en 5°C y adicionalmente 10 días en 20°C. Nótese que no hay síntoma de pardeamiento de pulpa y haces vasculares.

Figura 4. Pruebas de patogenicidad en plantas 'Hass' sobre portainjerto Mexícola de un año. Plantas inoculadas con *Neofusicoccum parvum* (Figuras a la izquierda) y planta sin inocular (Figuras de la derecha). Nótese el color café del exudado de Perseitol, que sin patógenos es de color blanco.

Durante las **temporadas 2014-2015 y 2015-2016** se realizó una prospección en **16 huertos de paltos** Hass localizados desde **Illapel a Peumo.**

Se debe tener en cuenta que los patógenos relacionados a **pudrición peduncular** requieren una **vía de ingreso al fruto (heridas)**, lo cual puede ser un elemento **clave al definir procedimientos de cosecha**.

PROSPECCIÓN EN HUERTOS CON SÍNTOMAS Y ESPECIES DETECTADAS

Durante las temporadas 2014-2015 y 2015-2016 se realizó una prospección en 16 huertos de paltos Hass localizados desde Illapel a Peumo. En ambos períodos se identificaron árboles enfermos y se cortaron trozos de madera con cancrosis y muerte regresiva; además se cosecharon 45 frutos en cada sitio de estudio, desde árboles sanos y enfermos. En los árboles enfermos la cosecha se realizó desde ramas cercanas a ramas con muerte regresiva. Luego, los frutos fueron desinfectados superficialmente por inmersión y colocados en cámaras húmedas a 20°C para inducir la maduración y el desarrollo de pudriciones.

Los aislados obtenidos tanto en madera como en fruto corresponden a especies de la familia Botryosphaeriaceae, especies de la familia Diaporthaceae, y a los géneros *Colletotrichum*, *Pestalotiopsis* y *Alternaria*. Sin embargo, fue coincidente en ambas temporadas que se obtuvo un mayor número de aislados pertenecientes a especies de la familia Botryosphaeriaceae, por lo que el análisis posterior contempla principalmente la identificación de estas especies.

Se seleccionaron 11 aislados obtenidos desde madera y 17 aislados obtenidos desde frutos. Se realizó la caracterización morfológica, térmica y molecular con lo cual se identificaron en madera 7 especies de la familia Botryosphaeriaceae: *Diplodia mutila*, *D. pseudoseriata*, *D. seriata*, *Dothiorella iberica*, *Neofusicoccum nonquaesitum* y *N. parvum*; y 6 especies de la familia Botryosphaeriaceae en fruto: *D. mutila*, *D. seriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *N. australe* y *N. parvum*.

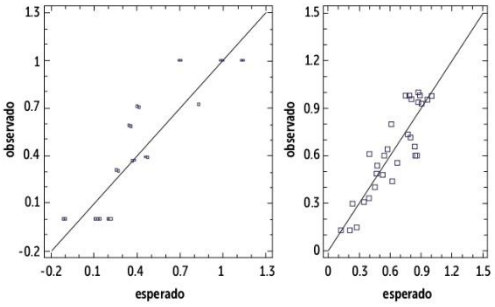
Se realizaron pruebas de patogenicidad para corroborar la capacidad de generar la sintomatología atribuida a daño en la madera (Figura 4) y en paltas, con lo cual se determinó que todas las especies detectadas son patógenas. Sin embargo, *N. nonquaesitum*, *N. parvum* y *D. pseudoseriata* en plantas de variedad Hass sobre portainjerto Mexícola resultaron ser las que desarrollan mayor severidad de daño en la madera, mientras que, en fruto, las especies más severas corresponden a *Lasiodiplodia theobromae* y *N. parvum*.

FACTORES PREDISPONENTES

A nivel internacional, la cancrosis y muerte regresiva han sido atribuidas a distintos tipos de estrés tales como deficiencias nutricionales, salinidad, temperaturas extremas, exceso de radiación y heridas, causadas por daño mecánico causado principalmente por exceso de carga, podas excesivas y anillado (Dann et al. 2013). En relación con la pudrición peduncular, se ha atribuido el desarrollo de estas enfermedades a la contaminación de la fruta durante la cosecha ya sea por conidias del ambiente que infectan el pedúnculo, o bien por conidias que son arrastradas en las tijeras de cosecha desde una fruta infectada a una fruta sana (Everett 2014).

Para determinar los factores asociados al desarrollo de estas enfermedades en huerto chilenos, se caracterizaron 16 huertos de paltos Hass para lo cual se registraron 102 variables en ambas temporadas (2014-2015 y 2015-2016). Tales variables contemplan información referente a ubicación geográfi-

Figura 5. Ecuaciones de la regresión obtenida a partir del modelo PLS, en el cual se analizaron 102 variables en las temporadas 2014-2015 y 2015-2016, para 16 huertos ubicados desde Illapel a Peumo, para predecir la prevalencia de las enfermedades en la madera (Pv_CD) y prevalencia de la pudrición peduncular de frutos (Pv_PP).



Pv_CD = 0.4 + 0.00467* diámetro de tronco - 0.26* índice área foliar + 0.00161* volumen de canopia + 0.012* edad de la planta + 0.0000833* plantas/ha.
R^2ajustado = 78.8%.

Pv_PP = 0.576 + 0.0132* días a madurez + 0.0299* edad de la planta - 0.0333* materia seca fruto + 0.0305* índice área foliar + 0.000345* plantas/ha - 0.00218* diámetro de tronco + 0.00196* volumen de canopia.
R^2ajustado = 71.6%.

ca del huerto, particularidades de la plantación, características edafoclimáticas, sistema de riego, manejos (poda, anillado, inyecciones, fertilización y uso de reguladores de crecimiento), estrés abiótico, patógenos y plagas presentes, rendimiento, calibre, prevalencia y severidad de cancrosis, muerte regresiva y pudrición peduncular. Con lo anterior, se realizó un análisis multivariado (Análisis de Componentes Principales, PCA y Regresión de Mínimos Cuadrados Parciales, PLS), analizando nueve escenarios que contemplan el análisis de cada temporada y de ambas temporadas con cada enfermedad o las enfermedades en conjunto. Este análisis se llevó a cabo con la colaboración del Dr. Jorge Saavedra de la Pontificia Universidad Católica de Valparaíso, quien tiene vasta experiencia en este tipo de análisis multivariantes. Los resultados obtenidos mediante PCA y PLS, fueron sometidos a Regresión Múltiple de forma de obtener modelos predictivos. En estos modelos se consideraron las principales variables asociadas con la prevalencia de las enfermedades en madera y la pudrición peduncular de frutos (Figura 5). Como principales resultados del estudio, se estableció que los factores más fuertemente relacionados con la prevalencia y severidad de enfermedades en la madera son diámetro de tronco, volumen de canopia, índice de área foliar, edad y densidad de plantación, mientras que en frutos, las variables corresponden a las indicadas para enfermedades en la madera, más el tiempo (días) que existe desde cosecha a madurez de consumo y también el contenido de materia seca del fruto.

Considerando los antecedentes expuestos, se puede señalar que, junto con evitar la presencia del inóculo en campo (eliminando restos de poda fuera del huerto) es muy importante evitar condiciones estresantes para las plantas que lleven a su envejecimiento (por ejemplo, anillado, riego deficitario, uso excesivo de antigiberélicos), renovar follaje realizando podas vigorizantes ojalá temprano en la temporada (lo más cerca de salidas de invierno que sea posible) y potenciar el crecimiento de raíces (propiciando una buena aireación del suelo, buen contenido de humedad, evitando condiciones de anegamiento, etc). Respecto a la pudrición peduncular, se debe tener en cuenta que los patógenos relacionados a pudrición peduncular requieren una vía de ingreso al fruto (heridas), lo cual puede ser un elemento clave al definir procedimientos de cosecha. Asimismo, es necesario reducir la presión de estos patógenos manteniendo una calidad y condición de almacenamiento óptima en la fruta, desde el huerto hasta el consumidor final.

En Chile no existen productos registrados para palto que controlen efectivamente los patógenos asociados con esta enfermedad en madera y en fruta. Sin embargo, existen reportes internacionales (Everett y Timudo-Torrevilla, 2007) que mencionan que aplicaciones a nivel de huerto de cobre puede bajar el nivel de presión de estos patógenos. Ra

Annex 7.7.1. 3MT. Jornada de Investigación de Postgrados. Enero 2018. Dirección de Investigación y Postgrados. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile.

Annex 7.7.2. Poster. Jornada de Investigación de Postgrados. Enero 2018. Dirección de Investigación y Postgrados. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile.

Characterization and Pathogenicity of Botryosphaeriaceae Species, obtained from Wood and Fruits of *Persea americana* in Chilean Orchards.

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INTRODUCTION

The symptoms associated with Botryosphaeriaceae species in avocado plants and fruits are death of graft union; leaf blight, dieback, branch cankers, stem end rot and fruit rot (Dann et al., 2013; Eskalen et al., 2013; McDonald & Eskalen, 2011; Menge & Ploetz, 2003; Slippers & Wingfield, 2007; White et al., 2005). In Chile, since 2014, increased the reports of wood diseases of Chilean avocado orchards and stem end rot in distant consumer countries of Chilean fruits. Therefore, the aim of this study was to identify species associated with these diseases and to determine its pathogenicity.

MATERIALS AND METHODS

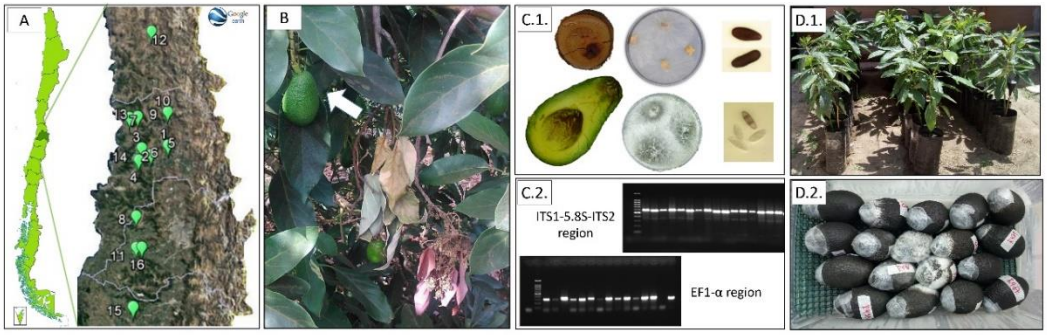

Figure 1. A. Distribution of Sixteen Chilean 'Hass' avocado orchards, prospected in this study. B. Wood pieces and fruits were obtained from trees with Branch Canker and Dieback. C.1. Cross section of symptomatic branches and fruits; white to grey-black colony in APDA, and conidias. C.2. Electrophoresis gel of Internal Transcribed Spacer region (ITS1-5.8S-ITS2), and Translation Elongation Factor 1- α (EF1-α) gene, used in Blast and Phylogenetic Analysis. D.1. Pathogenicity tests of Hass avocado plants. D.2. Pathogenicity tests of Hass avocado fruits.

RESULTS

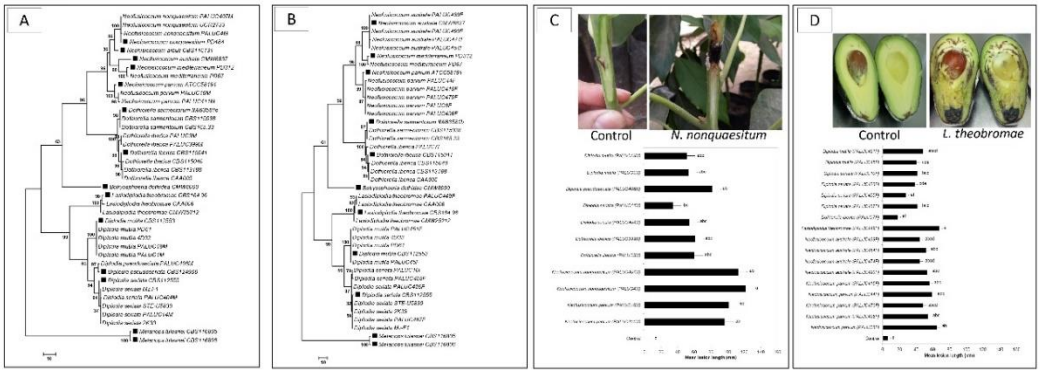

Figure 2. Phylogenetic analysis of isolates obtained in this study (PALUC), and Botryosphaeriaceae species available in GenBank databases. A. Isolates obtained from trees with Branch Canker and Dieback. B. Isolates obtained from fruits with Stem end rot. C. Results of Pathogenicity test of Botryosphaeriaceae species obtained in Hass avocado plants and fruits.

CONCLUSION

Eight Botryosphaeriaceae spp. were found in this study: *Diplodia mutila, D. pseudoseriata, D. seriata, Dothiorella iberica, Lasiodiplodia theobromae, Neofusicoccum australe, N. nonquaesitum*, and *N. parvum*. This research is the first study that describes identification and pathogenicity of Botryosphaeriaceae spp. in orchards of main productive region of Chile, which provides information for future research about impacts and epidemiology of these pathogens to establish effective prevention and control strategies.

ACKNOWLEDGEMENTS

Doctoral scholarship CONICYT-PCHA 21140282, Project CONICYT-PAI 781413002, and Subsole S.A.

Annex 7.8.1. 3MT. I Simposio: "Innovación para una agricultura más sostenible". Diciembre 2018. Dirección de Investigación y Postgrados. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile.

Annex 7.8.2. Poster. I Simposio: "Innovación para una agricultura más sostenible". Diciembre 2018. Dirección de Investigación y Postgrados. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile.

FACULTAD DE AGRONOMÍA E INGENIERÍA FORESTAL
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I SIMPOSIO DE INNOVACIÓN
PARA UNA AGRICULTURA MÁS SOSTENIBLE
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DICIEMBRE - 2018

doctorados

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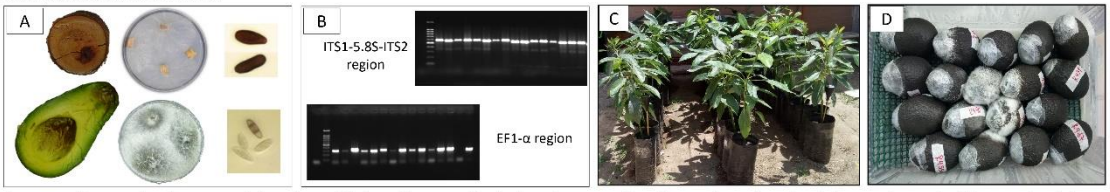

Figure 1. A. Cross section of symptomatic branches and fruits; white to grey-black colony in APDA, and conidias. B. Electrophoresis gel of Internal Transcribed Spacer region (ITS1-5.8S-ITS2), and Translation Elongation Factor 1- α (EF1-α) gene, used in Blast and Phylogenetic Analysis. C. Pathogenicity tests of Hass avocado plants. D. Pathogenicity tests of Hass avocado fruits.

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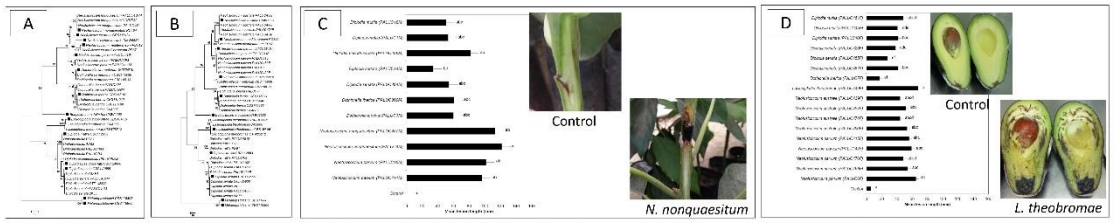

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Annex 7.8.3. Prize. I Simposio: "Innovación para una agricultura más sostenible". Diciembre 2018. Dirección de Investigación y Postgrados. Facultad de Agronomía e Ingeniería Forestal. Pontificia Universidad Católica de Chile.

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Annex 7.9.1. Abstract. Valencia A.L., Gil P.M., and Rosales M. 2019. Caracterización y patogenicidad de especies de Botryosphaeriaceae obtenidas desde madera y frutos de P*ersea americana* en huertos chilenos. IX World Avocado Congress. Colombia.

Abstract

En Chile, han aumentado los hallazgos de paltos con cancrosis, muerte regresiva, y frutos exportados con pudrición peduncular. Para identificar los agentes causales de estas patologías, se realizó una prospección en 16 huertos chilenos de palto Hass, desde Illapel (31° 37' S) hasta Peumo (34º 23'S), en la cual se obtuvieron muestras de ramas y frutos Para inducir la expresión de los síntomas de pudrición peduncular, se colocaron los frutos cosechados en cámaras húmedas a 20°C, hasta alcanzar la madurez de consumo,

Se sembró trozos de madera y frutos desde la zona de avance del daño en APD (20ºC por 7 días). De un total de 42 aislados de madera y 88 aislados de frutos, tentativamente identificados como *Diplodia*, *Dothiorella*, *Lasiodiplodia* y *Neofusicoccum*, se seleccionaron 12 aislamientos de madera y 17 aislamientos de paltas, para su caracterización morfológica, térmica, molecular (genes ITS1/ITS4 y EF728/EF986) y estudios de patogenicidad. La patogenicidad se determinó en plantas de palto Hass sobre portainjerto Mexicola, durante 2 meses y en paltas Hass (25,57% materia seca), incubadas en cámaras húmedas a 20ºC/10 días. Los resultados demostraron la presencia de colonias fungosas blancas y picnidios negros con conidias ovoides hialinas/marrón, septadas/aseptadas; fusiformes hialinas y septadas, dependiendo del aislamiento). En función de las características

morfológicas de las conidias y de los análisis moleculares, estos aislamientos se identificaron como *Diplodia mutila*, *Diplodia seriata*, *Diplodia pseudoseriata*, *Dothiorella iberica*, *Lasiodiplodia theobromae*, *Neofusicoccum australe*, *N. nonquaesitum* y *N, parvum*. En las pruebas de patogenicidad todos los aislados identificados como especies de la familia Botryosphaeriaceae demostraron ser patogénicos.

En conclusión, los resultados de este estudio demostraron la presencia de especies de la familia Botryosphaeriaceae en *Persea americana* causando cancrosis, muerte regresiva y pudrición peduncular. Sin embargo, estos resultados no descartan que estas especies coexistan con otros hongos patógenos.

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Annex 7.9.2. Abstract. Valencia A.L., Gil P.M., and Rosales M. 2019. Estudio de factores predisponentes en *Persea americana* variedad hass, para el desarrollo de cancrosis, muerte regresiva y pudrición peduncular en huertos chilenos. IX World Avocado Congress. Colombia.

Abstract

En Chile, han aumentado los hallazgos de paltos con cancrosis, muerte regresiva, y frutos exportados con pudrición peduncular, lo cual coincide con la severa sequía que ha afectado la zona productiva en los últimos 10 años. Para identificar los agentes causales de estas patologías, se realizó una prospección en 16 huertos chilenos de palto Hass, desde Illapel (31° 37’ S) hasta Peumo (34º 23’S), en la cual se han obtenido muestras de ramas y frutos Para inducir la expresión de los síntomas de pudrición peduncular, se colocaron los frutos cosechados en cámaras húmedas a 20°C, hasta alcanzar la madurez de consumo,

En cada huerto se registraron datos edafoclimáticos, de plantación y de manejo con el objetivo de determinar los factores que influyen en la prevalencia de estos problemas fitopatológicos a nivel de huerto, por análisis multivariado, usando PCA y PLS-DA.

El análisis de las 102 variables registradas en cada huerto indicó que el factor predisponente principal para el desarrollo de estas tres enfermedades es la edad del árbol, dado que los mayores niveles de prevalencia de estas enfermedades se registraron en huertos con árboles de mayor edad, con baja densidad de plantación y altos niveles de IAF. Asimismo, se observó variaciones en temporadas

consecutivas del cultivo, principalmente por los cambios en las condiciones climáticas por año, siendo la temperatura, radiación, humedad relativa y precipitaciones las variables que están estrechamente relacionadas con huertos con alta prevalencia de estas enfermedades.

Este estudio es el primer estudio realizado en Chile para conocer los agentes causales y los factores predisponentes para el desarrollo de cancrosis, muerte regresiva y pudrición peduncular en paltos Hass. A partir de esta investigación, los productores podrán tener certeza de las condiciones requeridas por estos patógenos para desarrollar estas patologías.

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