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 (28º 27 to 35º 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.

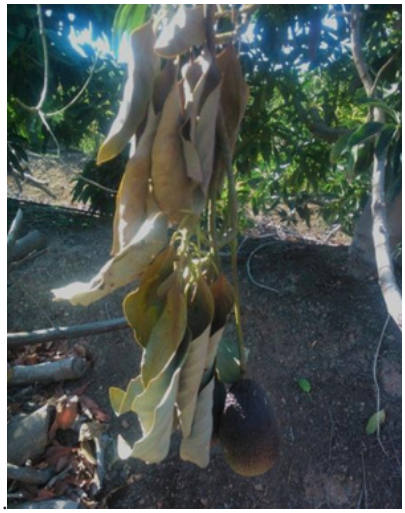

Figure 1. Branches dieback with leaves and fruits death (white arrow) attached to branches

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.

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