Etiology and epidemiology of gray mold of kiwifruit in Chile.

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Dedicated to Nicolás

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Chapter 1

Introduction

Kiwifruit (*Actinidia* spp.*)* is a recently domesticated fruit crop with a rapid increase in the last decades (O´Rourke 2012). According to the Centro Servizi Ortofrutticoli (CSO) around 3,9 mill tons of kiwifruit were produced in 2016 and one third of this volume was concentrated in the southern hemisphere (Ferguson 2015, 2016). Among the *Actinidia* species, the green-fleshed kiwifruit, *Actinidia chinensis* var. *deliciosa* (A. Chev.) A. Chev.) predominates in the international trade followed by yellow selections of *Actinidia chinensis* var. *chinensis* (A. Chev.) A. Chev (Ferguson 2015).

Chile is the fourth largest producer of 'Hayward' kiwifruit worldwide, only preceded by China, Italy and New Zealand. The total surface (8,720 hectares) is primary established between O´Higgins (34°00'S) and Maule Regions (35°48'S) in the Central Valley of the country (www.odepa.cl) and 182,607 tons of kiwifruit were exported in 2018 (www.odepa.cl). In contrast what happens with the first producer worldwide (China), Chile exports about 85-90% of the total production (Ferguson 2015, 2016). The main markets are Europe (36.1%), Latin America (21.2%), Asia (20.5%) and USA (13.5%), equivalent to 202 million US in returns in the 2017-2018 period (www.odepa.cl)

The kiwifruit

The kiwifruit (*Actinidia* spp.) in the wild is a perennial vine from the rainforest of southeastern of China and comprises at least 55 species (Huang 2016a). Most of the fruit that are being commercialized come from the fruited selection of *A. deliciosa* or *A. chinensis*. 'Hayward' kiwifruit is large, ovoid although slightly flattened laterally with mean weight 80-110 g. The fruit skin is greenish brown, covered with dense, uni or multiseriate ginger-brown

hairs (trichomes), and become easy to remove by the time of harvest. The carpels are persistent and the flesh is light and bright green with a white large and elliptical columella (core) (Huang 2016b). Internally, the kiwifruit comprises an outer and inner pericarp; different distribution of starch accumulation occurs in the outer pericarp depending on cell size, while the small cells accumulate large number the starch grains. The inner pericarp includes locules with two radial rows of seeds within a mucilaginous matrix (Ferguson, 1984). Kiwifruit is classified as high in ascorbate or vitamin C with a high calcium content, in 'Hayward' values between 30 to 400 mg/100 g f.w. has been reported (Ferguson 1991), which are considered high compared with apple. The central core is white and comprises a large, homogeneous parenchyma cells. The softening rate of these cells are slower than the pericarp cells. 'Hayward' is considered the best cultivar for export among the green-fleshed kiwifruit because their size, flavor and their long storage life (Burdon and Lallu 2011; Huang 2016b).

Originated from a humid subtropical climate (Huang 2016a) the kiwifruit requires warm, moist and sheltered environment to growth (Datson and Ferguson 2011) so that several agricultural practices have been introduced in order to obtain a high quality and healthy fruit in the Mediterranean Climate present in Central Valley of Chile.

Postharvest handling

'Hayward' kiwifruit is a climacteric fruit which is commercially harvested when they are un-ripe and firm with soluble solids (SS) between 6.2 and 7.0% (Burdon et al. 2011) and >16.0% dry matter (Crisosto et al. 2011) to assure a long period of storage and achieve a minimum consumer acceptance (12.5% of ripening soluble solids) (Crisosto and Crisosto 2001). In addition, the fruit is highly susceptible to exogenous ethylene (Ilina et al. 2010; Koukounaras and Sfakiotakis 2007), with fruit softening and decay as main deterioration

factors. Several technologies have been introduced to keep the fruit firmness and avoid contamination and action of ethylene. Among those cold chain with ethylene removal, controlled atmosphere storage, modified atmosphere packaging (MAP) and the application of 1-methylcyclopropene (1-MCP) have allowed to increase the storage time and extend the distribution period in the global markets (Lallu and Burdon 2007; Park et al. 2015; Zoffoli et al. 2016). Despite the efforts to produce healthy and high quality fruit, decays during storage still remain as primary risk factor that affects kiwifruit trade.

Gray mold

Several fungal genera have been associated with kiwifruit decays during storage including species of the genus *Diaporthe* (Auger et al. 2013; Díaz et al. 2014; Díaz et al. 2017; Luongo et al. 2011), *Cryptosporiopsis* (Davison et al. 2009; Manning et al. 2003), *Botryosphaeria* (Koh et al. 2003; Pennycook 1985) and *Botrytis* (Burdon and Lallu 2011; Manning et al. 2016; Michailides and Elmer 2000; Pennycook 1985).

Gray mold is the primary postharvest disease in the Chilean kiwifruit industry (Morales and Ulloa 1985; Pinilla et al. 1994; Díaz et al. 2017) and has been widely associated with *Botrytis cinerea*. During kiwifruit development, fruit are infected by the pathogen but remain asymptomatics until symptom expressions during cold storage.

Gray mold symptoms appear during storage infecting different fruit tissues and the name of the disease is called by the position at which it occurs on the fruit, being in increasing order of importance the infections in the stylar-end, body (side) and the stem end of the fruit (Manning et al. 2016).

Stem end rot (SER) is observed as a softening in stem-end area and darkening skin in the affected tissue. As the rot is developed, the darkening on the tissue continued extends down the fruit and the receptacle is detached. Internally, a watery and dark-brown decay is

observed from the receptacle advancing through of the columella (core) (Latorre and Pak 2003) (Fig. 1). Occasionally filamentous, cottony and whitish mycelium with grayish sporulation is observed from the stem end. This mycelium may spread to adjacent fruit, creating “nest” of rotted fruit (Burdon and Lallu 2011). The symptoms express rarely during fruit development; however abundant *B*. *cinerea* sporulation has been frequently observed on senescent petals at the end of bloom and on dead vegetative tissue in the vineyard under New Zealand growing conditions (Elmer and Pyke 1996; Michailides and Elmer 2000).

Causal Agent

Currently, gray mold is produced by *Botrytis cinerea* in ‘Hayward’ kiwifruit worldwide (Brigati et al. 2003; Michailides and Elmer 2000; Opgenorth 1983; Pei et al. 2019; Pennycook 1985). Nevertheless, a recent study reported the presence of *B*. *cinerea* along with *B*. *prunorum* causing gray mold in Chile (Elfar et al. 2017). A similar situation has been described in other crops including grapes (Garfinkel et al. 2017; Harper et al. 2019; Saito et al. 2016; Walker et al. 2011, Zhou et al. 2014), eucalyptus (Liu et al. 2016), blueberries (Saito et al. 2016), and strawberries (Rupp et al. 2017) where more than one species of *Botrytis* have been identified living in sympatry within a host.

Previously, *Botrytis cinerea* and *B*. *prunorum* were identified causing blossom blight in Japanese plum (Ferrada et al. 2016) and calyx-end rot in apple during storage in Chile (Ferrada et al. 2017). However, scarce information about the importance of *B*. *prunorum* in the epidemiology of gray mold in kiwifruit has been studied.

In Chile, gray mold was reported in the 1980s with an estimated prevalence of 35% (Morales and Ulloa 1985). More recently some reports indicate that the prevalence reaches between 7% and 15% under favorable environmental conditions during preharvest (Elfar et

al. 2017; Farías 2009). Similarly, gray mold has been described in the main producer countries of kiwifruit with losses estimated up to 20% in California (Michailides and Morgan 1996), between 32% up to 50% in Italy (Bisiach et al. 1984) and between 2% to 50% in New Zealand (Beever et al. 1984). Recently, the disease has been reported in Greece, Japan, Korea and Turkey.

Moreover, infected kiwifruit induces ethylene production at enough concentration that can triggers premature softening and reduction of shelf life (Niklis et al. 1997; Qadir et al. 1997).

Infection

Successful infection depends on i) the presence/absence and quantity of the pathogen's inoculum (Carisse et al. 2017; Carisse and Van der Heyden 2015; Nair et al. 1995); ii) stage and susceptibility of the crop (Bautista-Baños et al. 1997; McLeod and Poole 1994) and iii) environmental conditions naturally or generated during the growing season by the agricultural practices (Elmer and Pyke 1997; English et al. 1989; Latorre et al. 2015; Valdés-Gómez et al. 2008; Verhoeff 1974).

Botrytis cinerea is a necrotrophic fungus which is disseminated by air (Blanco et al. 2006; Carisse 2016, Carisse et al. 2014) and infects leaves, flowers, sepals, receptacle and stamens of kiwifruit (Michailides and Elmer 2000).

Kiwifruit infection by *B*. *cinerea* occurs in the field, although critical periods depends on weather conditions. Two main routes have been described for *Botrytis* infections. The first route is considered the main way in California (USA). During flowering, petals and anthers become infected, providing the inoculum for frequent and constant sepals and receptacles infections during the fruit development. Consequently, sepals and receptacles provide the inoculum for infections in the stem end which remain latent in the orchard fruit (Michailides

and Morgan 1996; Michailides and Elmer 2000) until subsequent gray mold development during storage.

The second route of infection is considered the main way in New Zealand (Michailides and Elmer 2000). In this case, the infection is concentrated in the picking wound during harvest. Conidia are released from infected leaves and other inoculum sources, adhered to hairs on the kiwifruit surface (Elmer et al. 1997) and transported to small wound in the stem end left by the process of fruit picking where receptacles and vascular tissue are infected (Elmer et al. 1997; Elmer and Michailides 2007; Pennycook 1985; Sharrock and Hallett 1992). Latent infections remain inactive in immature fruit until ripening and senescence (Prusky et al. 2013).

Although the two routes of infection are complementary, it is not clear the importance of each route for kiwifruit under the Chilean conditions.

Predisposing factors

Infection and disease development depends on the susceptibility of the fruit and the predominant environmental conditions. Temperature and relative humidity are the main factors that affect the infection and conidial dispersion. Temperatures between 15°C and 25°C in the presence of free water favor conidial germination (Latorre and Rioja 2002) and infection and development in table grapes (Latorre et al. 2002) and in other crops (Xu et al. 2000).

The pergola training system predominates in ‘Hayward’ kiwifruit (Costa et al. 1999; Costa et al. 2018, Miller et al. 2001), favoring and maintaining the high humidity under the canopy. Nevertheless, the canopy is often dense and compact with low lighting and ventilation, affecting negatively the quality of the fruit (Costa et al. 1999) and increasing the occurrence of postharvest diseases (Manning et al. 2010; Snelgar et al. 1998). Therefore, summer

pruning is suggested to maintain the balance leaf/fruit (Brigati et al. 2003; Snelgar et al. 1998; Manning et al. 2010).

The expression of symptoms associated to SER during storage may be influenced by the maturity of the fruit at harvest and their effect in the pattern of softening (MacRae and Redgwell 1992). Additionally, Poole and McLeod, (1994) indicated that there are differences in the SER prevalence in fruits harvested on the same date, but originated in different shoots, suggesting there are factors fruit dependent in the development of the disease. There is an inverse relationship between fruit maturity, soluble solids content and susceptibility to SER has been determined in 'Hort16A' kiwifruit (*Actinidia chinensis)* after 20 weeks at 0°C (Manning et al. 2016).

Brushing is a common postharvest handling of kiwifruit conducted to remove particles adhered to the trichomes as necrotic floral remains (Mitchel et al. 1994). However, brushing can generate micro-wounds (Massantini et al. 1995) that trigged the anticipated ripening of the fruit and nutrient availability that may influence the success of *Botrytis cinerea* infection (Wurms et al. 1998) and promotes SER development during storage.

Low temperature storage at 0°C reduces fruit softening and maintain kiwifruit quality, decreasing fruit metabolism and delaying development of pathogens. Decreasing temperature by forced air cooling is a common practice in several crops. However, this practice is infrequent in kiwifruit because the incidence of SER increases compared to those were passively cooled (Manning et al. 2016). In addition, the use of bags with low ventilation area saturates the environment and enhances the condensation inside the bag creating an environment conducive to development of decay (Wiley et al. 1999).

Control

Applications of fungicide at flowering and near harvest is a practice suggested in the kiwifruit orchard management in Chile. However, pre-harvest chemical treatments are often unreliable or ineffective because the application not always compromise the site of infection (Michailides and Elmer 2000). Therefore, cultural practices that includes light penetration and removal of necrotic tissues in the canopy decrease the inoculum density in the orchard and become important part of gray mold management.

Complementary to preharvest management, delay storage for 48-72 h between 15 and 20°C with 89% to 95% relative humidity (Bautista-Baños et al. 1997) (curing process) has been introduced as complementary practice to reduce stem end rot in 'Hayward' kiwifruit. Physiological responses, associated to activation of defense mechanisms such as the accumulation of exoquitinases (Wurms et al. 1997a; Wurms et al. 1997b), suberin (Ippolito et al. 1997) and phenolic compounds (Wurms et al. 2005) have been demonstrated in cured fruit. In addition, curing decreases conidial germination, increases cell disruption and dehydration of germ tubes (Sharrock and Hallet 1991).

In Chile, fungicide applications and curing before storage are the main control strategies of SER which were based on epidemiological studies of *B*. *cinerea* under field conditions in New Zealand and California (USA). These conditions may differ from Chilean situation, being little knowledge on the occurrence of latent infections during fruit development and the impact during storage.

Therefore, the hypothesis of this thesis postulates stem end rot of kiwifruit cv. Hayward in Chile is caused by species of the genus *Botrytis* whose infection occurs during fruit development in the field remaining latent until subsequent gray mold development in storage.

The general objective of this thesis is to study the etiology and epidemiology of stem end rot of kiwifruit cv. Hayward in postharvest. The specific objectives were i) to characterize and study the role of *Botrytis* spp. associated with floral tissues and gray mold during kiwifruit development and storage (Chapter 2) ii) to study the temporal dynamic of *B. cinerea* airborne conidia, colonization in floral parts and latent infections in kiwifruit during the fruit development in field (Chapter 3) and iii) to relate the *B*. *cinerea* colonization and stem end rot prevalence in postharvest (Chapter 3).

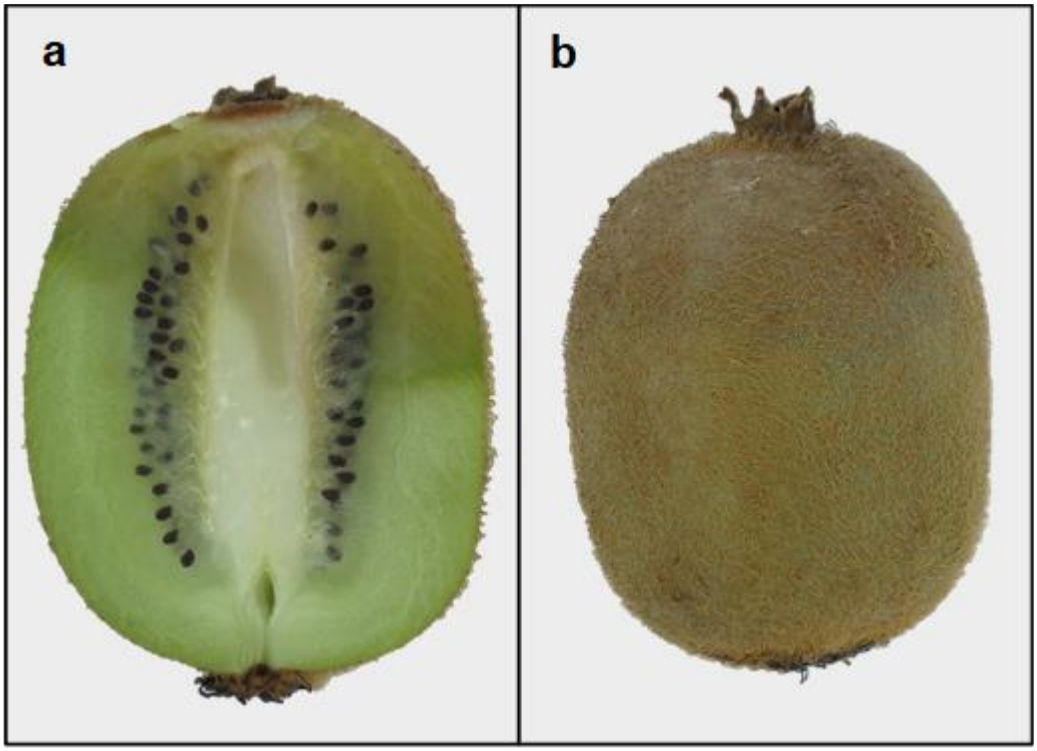

Fig.1. Symptoms of stem end rot in kiwifruit caused by *Botrytis* spp. a) Water soaked tissue and brown decay extended from the stem end to the stylar end. b) Darkening skin in the infected tissue and superficial gray mycelia.

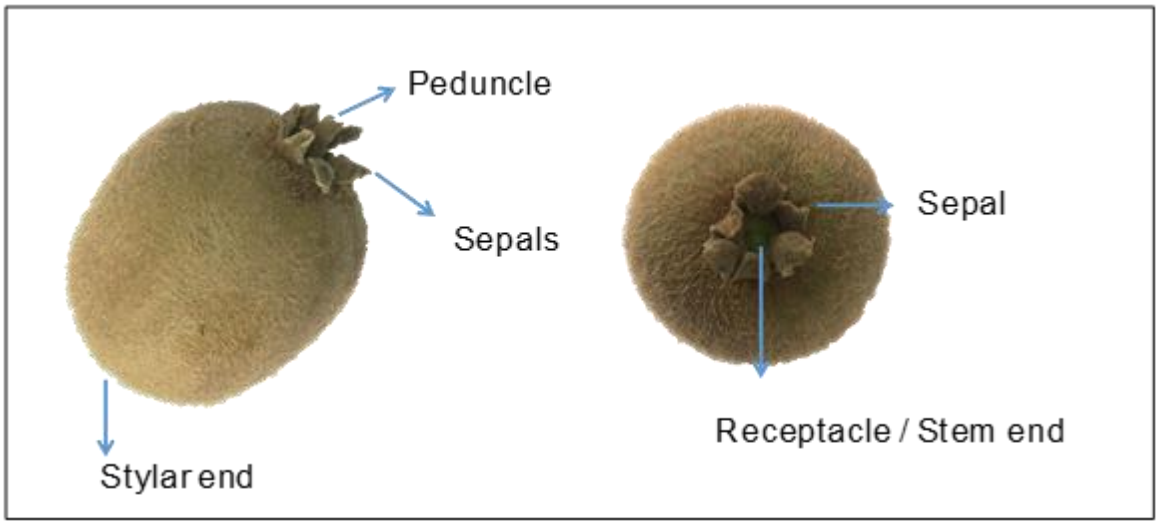

Fig. 2. Arrangement of floral parts in 'Hayward' kiwifruit at harvest. Blue arrows indicate peduncle, sepals, receptacle and stylar end.

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Chapter 2

Characterization of *Botrytis cinerea* and *B. prunorum* obtained from apparently healthy floral structures and postharvest gray mold on 'Hayward' kiwifruit in Chile

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Abstract

Gray mold is the most important postharvest disease of 'Hayward' kiwifruit (*Actinidia chinensis* var. *deliciosa* (A. Chev.). A. Chev) in Chile with a prevalence that varied between 33.1% in 2016 and 7.1% in 2017. Gray mold is characterized by a soft, light to brown watery decay and is caused by *Botrytis cinerea* and *Botrytis prunorum*. However, there is no information related to the importance of *B*. *prunorum* during development and storage of kiwifruit in Chile. For this purpose, flowers and fruit were collected throughout fruit development from five 'Hayward' kiwifruit orchards and two seasons. High (HCP) and low conidial production (LCP) colonies of *Botrytis* sp. were consistently obtained from apparently healthy petals, sepals, receptacles, styles and gray mold from kiwifruits in cold storage. Morphology and phylogenetic analysis using G3PDH, HSP60 and RPB2 partial gene identified *B*. *cinerea* (HCP) and *B*. *prunorum* (LCP). Consistently, *B*. *cinerea* was predominantly isolated from all floral parts in apparently healthy and diseased fruit analyzed. During full bloom, the highest colonization by *B*. *cinerea* and *B*. *prunorum* was obtained in petals followed by sepals. In storage, both *Botrytis* species were isolated from all the diseased fruit (n=644), of which the 6.8% (n=44) corresponded to *B*. *prunorum*. All *Botrytis* isolates grew from 0°C to 30°C in vitro, and were pathogenic on kiwifruit leaves and kiwifruit.

Botrytis cinerea isolates were always more virulent than *B*. *prunorum* isolates. This study confirms the presence of *B*. *cinerea* and *B*. *prunorum* colonizing apparently healthy flowers and floral parts in fruit and causing gray mold in kiwifruit in Chile. These results shown that *B*. *prunorum* played a secondary role in the epidemiology of the gray mold in kiwifruit.

Chile is the second largest exporter of 'Hayward' kiwifruit (*Actinidia chinensis* var. *deliciosa* (A. Chev.). A. Chev) in the southern hemisphere, with over 182,608 ton primary destined to Europe (36.1%) followed by Latin America (21.2%), Asia (20.5%), and the United States of America (13.5%) (www.odepa.cl, 2018). The 88% of the growing area is well established in the Chilean Central Valley, between O´Higgins Region (34° 00' S) and Maule Region (35° 48' S) (www.odepa.cl, 2018) and is characterized by a Mediterranean Climate with warm and dry summer (Sarricolea et al. 2017). In Chile, kiwifruit is harvested with more than 6.2% soluble solids (SS) and 16% dry matter (Zoffoli et al. 1999, 2016) then are cured (24h at 20°C) and stored for 4 to 6 months under controlled atmosphere chambers and modified atmosphere packaging alone or along with 1-methylcyclopropene (1-MCP) at 0°C (Zoffoli et al. 2007, 2016).

Decay and fruit softening negatively affect kiwifruit trade and reduce fruit price in the market (Feng et al. 2006; Zoffoli et al. 1998, 2016). Currently, several pathogens have been identified causing decay in kiwifruit during postharvest, including species of the genera *Sclerotinia* (Lee et al. 2015; Opgenorth 1983; Pennycook 1985), *Diaporthe* (Auger et al. 2013; Díaz et al. 2014, 2017; Li et al. 2016b; Luongo et al. 2011), *Cryptosporiopsis* (Davison et al. 2009; Manning et al. 2003; Manning and Lallu, 1997), *Lasiodiplodia*, *Neofusicoccum* (Pennycook 1985; Zhou et al. 2015), *Fusarium* (Wang et al. 2015; Yang et al. 2017), *Pestalotiopsis* (Li et al. 2016a), *Penicillium* (Prodromou et al. 2018), and *Botrytis* (Elfar et al. 2017; Michailides and Elmer 2000; Pennycook 1985).

Among all of them, gray mold caused by *Botrytis cinerea* is the most important postharvest disease in the Chilean kiwifruit industry (Morales and Ulloa 1985; Latorre 2004, 2018; Latorre and Pak 2003; Pinilla et al. 1994; Díaz et al. 2017) which is present in the main kiwifruit producing countries as well (Brigati et al. 2003; Michailides and Elmer 2000; Opgenorth 1983; Pei et al. 2019; Pennycook 1985). *Botrytis cinerea* is a necrotrophic fungus that attacks leaves, floral structures and senescent tissues of the kiwifruit, using them as inoculum sources for gray mold infections (Elmer and Pyke 1996; Walter et al. 1999; Michailides and Elmer 2000).

Gray mold is often called by the position at which it occurs on the kiwifruit: i) the stylar or blossom end (BER), ii) body, lateral or side rot (BR) and the stem end rot (SER) (Manning et al. 2016). However, the most frequent zone of fruit associated with the gray mold is SER (Michailides and Elmer 2000). The symptoms associated with SER appear after 4 to 8 weeks in cold storage (Pennycook 1985; Brook 1992; Sharrock and Hallett 1992) and they are observed as a softening in stem end area and darkening skin in the affected tissue.

Epidemiologically, the flowering and harvest time are considered as the critical periods for *Botrytis* infections, although constant infections during the season could be favored by the presence of free water (rains or dews) and temperatures (between 15 and 25 ºC) associated with debris on the fruit surface in the vineyard (Michailides and Morgan 1996; Michailides and Elmer 2000).

A recent study indicated that *B*. *cinerea* and *B*. *prunorum* are the causal agents of gray mold in Chile (Elfar et al. 2017). Nevertheless, the importance of *B*. *prunorum* in the gray mold epidemiology in field and postharvest is unknown. The objectives of this study was to characterize and study the role *Botrytis* species associated with floral tissues and decayed fruit during kiwifruit development and storage and to determine the relative importance of *B*. *prunorum*.

Materials and Methods

Sampling and fungal isolation

Vineyards

Sampling was conducted in five commercial vineyards of 'Hayward' kiwifruit (13 to 25 years old) during two growing seasons (2016 and 2017). Kiwifruit vines were planted on their own roots and trained over a pergola system. The vineyards were located between the O´Higgins (34°00'S, 70°42'W) and Maule Regions (35°48'S, 71°49'W) in Central Valley of Chile (Fig. 1).

Samples collected

In the both seasons, kiwifruit with peduncle attached and apparently healthy floral part (sepals, styles and receptacles) were sampled four times during the growing season: i) 60 days after full bloom (DAFB) (4.3% SS), ii) 100 DAFB (4.6% SS), iii) 120 DAFB (5.2% SS) and iv) harvest time (6.5% SS, 160 DAFB). In each sampling day, 240 fruits were collected, obtaining a total of 960 fruits in the season. An additional 240 healthy flowers were sampled at full bloom time in the same five orchards only in 2017 season. Flower and fruit sampled were placed in paper bags and transported in a cool box to the laboratory until assessment.

Floral parts. Fungal isolates were obtained from floral parts of the flowers and fruit collected. In each sampling day, apparently healthy petals (n=1,440), sepals (n=1,440), receptacles (n=240), and styles (n=1,440) were removed from them with a sterile sharp scalpel and then surface-disinfected by dipping (1% sodium hypochlorite plus 0.001% Tween 80) for 2 minutes, rinsed (sterile distilled water) for 1 minute and dried on absorbent paper towels under a laminar flow hood for 5 min. Subsequently, floral parts were separated in groups of six petals, six sepals, three receptacles or six styles and plated on Petri dishes

containing potato dextrose agar (PDA) acidified with 0.5 ml liter^{-1} 92% lactic acid (APDA) plus 0.04% Igepal CO-630 (APDA + I) (Sigma-Aldrich, Atlanta, GA) and were incubated until sporulation, at 20 to 22°C under 12 h light / dark cycle for 7-10 days. Fungal isolates were obtained and colonies were tentatively identified as *Botrytis* sp. according to colony morphology, and conidia and conidiophore characteristics (Ferrada et al. 2016; Mirzae et al. 2008; Pei et al. 2019; Saito et al. 2016; Zhang et al. 2010a, 2010b; Zhou et al. 2014). The total number of *Botrytis* colonies were quantified and visually classified as high conidial producer (HCP) or low conidial producer (LCP). Mycelia from each *Botrytis* HCP (n=231) and LCP (n=32) colony was transferred to a APDA Petri dish, incubated for 2 days at 20°C. Pure cultures were obtained by transferring single hyphal tip to a new APDA Petri dish with the aid of a scalpel and stereoscopic microscope. Similar procedure was conducted for diseased fruit isolates.

Kiwifruits obtained from cold storage. Additionally, in both seasons, 3,200 kiwifruits were collected at commercial harvest maturity (6.5% SS) from the same five orchards indicated above. Then, fruits were cured for 24 h at 20°C, manually cleaning (removing loose hair and debris) and stored in modified atmosphere packaging (5% CO_2 -12% O_2) for 100 days at 0°C plus two days at 20°C in a free ethylene environment (<0.005 µl liter^{-1}). Then, diseased kiwifruits were counted and segregated as SER, BER or BR according to the position of the infection in the fruit: Fungal isolates were obtained from diseased fruit. Three pieces of internal pericarp (3 to 5 mm in length) were excised just behind the advancing margin between decayed and healthy tissue and placed on Petri dishes of 90 mm of diameter with APDA + I at 20-22°C under 12 h light/dark cycle for 10 days. Previously, fruit were surface-disinfected by 2 minutes, rinsed for 1 minute and dried on absorbent paper under a laminar flow hood for 30 min. Fungal isolates were obtained and *Botrytis* isolates were tentatively

identified and classified as HCP (n=231) and LCP (n=32) colonies as described for floral part isolates. Pure cultures were obtained by hyphal tips cultures as described above.

The experimental treatments were distributed at random according to a completely randomized design. Differences between the frequency of type of isolate (HCP and LCP) obtained from floral parts (sepals, receptacles, styles and petals) during flowering was determined by factorial 4 x 2 (F1= floral part; F2=type of isolate) and differences between the frequency of type of isolate (F1) and day of sampling (F2) was determined by bi-factorial 2 x 4. Difference in prevalence of each type of disease (SER, BR, BER) and type of isolate obtained was determined by factorial 3 x 2 (F1= type of disease; F2= type of isolate) each year. Analysis of variances (ANOVA) were separated using Tukey´s test ($P \leq 0.05$). using statistical software SigmaPlot v 12.5 (Systat Software Inc., San Jose, California, USA)

Molecular characterization

A total of 18 isolates, HCP (n=13) and LCP (n=5) colonies obtained from gray mold and apparently healthy floral parts of kiwifruit (petal, sepal, receptacle and style) representing six morphological groups based on colony morphology (sclerotial production and distribution on the Petri dish) were selected for molecular studies (Table 1).

DNA extraction

Botrytis isolates classified as HCP (n=13) and LCP (n=5) were cultivated for 7 days on APDA at 20°C in the dark and then 400 mg mycelium from each isolate were taken using a sterile scalpel and ground with liquid nitrogen in a sterile mortar. DNA extraction was performed according to the manufacturer's instructions (Promega Wizard Genomic Purification Kit, Promega Corp., Madison WI). The DNA was stained with GelRedTM (Biotium Inc., Alameda, CA), and its presence was verified by electrophoresis through a 1% agarose gel using Tris-Acetate-EDTA (TAE) 1.0x at 60 Volts and visualized by UV transillumination at λ = 320 nm.

PCR and phylogenetic analysis

Fragments of three nuclear genes coding to glyceraldehyde-3-phosphate dehydrogenase (G3PDH), heat shock protein 60 (HSP60), and DNA-dependent RNA polymerase subunit II (RPB2) were amplified using the primer pairs G3PDHfor/G3PDHrev, HSP60for/HSP60rev, and RPB2for/RPB2rev, respectively (Ferrada et al. 2016; Staats et al. 2005). The polymerase chain reaction (PCR) was conducted using a 96-well Veriti thermal cycler (Applied Biosystems, Foster City, CA). With a final volume of 25 µl, each PCR mixture contained 5 µl of 10 × PCR buffer, 1.0 µl of 25 mM MgCl2, 0.2 µl of 10 mM dNTPs, 1 µl of a 0.5 mM solution of each primer and 0.13 µl Taq DNA polymerase 5 u/µl (GoTaq® Flexi DNA Polymerase, Promega Wizard Genomic Purification Kit, Promega Corp., Madison WI) and 2 µl of DNA template. In addition, a negative control without genomic DNA was included. The amplification protocol consisted in 1 cycle of preheating at 94°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, 30 s for annealing, an extension at 72°C for 90 s and a final elongation at 72°C for 5 min. The alignment temperature for each gene were 55.0°C for G3PDH, 54.3°C for HSP60 and 49.0°C for RPB2 (Ferrada et al. 2016). The PCR products were stained using GelRedTM (Biotium Inc., Alameda, CA), separated in a 2% agarose gel by electrophoresis (25 min, 85 Volts) and visualized by UV transillumination at λ = 320 nm. The PCR products were purified and sequenced in both directions by Macrogen Inc., South Korea. The sequences obtained were manually edited and aligned using ProSeq v.2.91 (Filatov 2009) and Clustal X 2.0 (Larkin et al. 2007). The consensus sequences were compared with reference sequences of *Botrytis* species, including ex types deposited in the GenBank database (www.ncbi.nlm.nih.gov) using the BLASTn analysis and phylogenetic analysis. The sequences of *Monilia fructigena* (strain 9201) and *Sclerotinia sclerotiorum* (strain 484) were included as outgroup (Table 2).

Partition homogenicity analysis was realized to determined phylogenetic incongruence between sequences using PAUP* (Swofford 2002). Phylogenetic relationships were performed with MEGA 7 using maximum parsimony analysis (Kumar et al. 2016). Tree bisection and reconnection (TBR) algorithm was used as heuristic search with 1,000 random addition sequence replicates. Gaps were treated as missing data. Values of bootstrap support with 1,000 replications were calculated to estimate each branch strength. The tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC) were recorded for each analysis.

Morphological characterization

Conidia and conidiophore morphology

The colonial morphology of the 18 *Botrytis* isolates molecularly identified was characterized. A mycelial plug (5 mm) of 4 day-old APDA culture was plated upside down in the center of a Petri dish (90 mm-diameter) containing APDA for 7 days at 20-22°C under 12 h light/dark cycle. To stimulate conidial production, pea agar medium (PAM) containing per liter 160 g of pea (liquefied for 2 min in 1 liter of distilled water, adjusted to pH 6.0 using HCl), 5 g of sucrose and 25 g of agar) was used (Ferrada et al. 2016). The shape and size of the conidia (n=50) and conidiophores (n=15) were measured and compared with published descriptions (Ferrada et al. 2016; Liu et al. 2016; Mirzae et al. 2008; Pei et al. 2019; Zhang et al. 2010a, b; Zhou et al. 2014).

Five representative isolates of *B*. *cinerea* (F58-16, 106-16, 115-16, 4-17 and 31-17) and five isolates of *B*. *prunorum* (6-16, F102-16, 55-16, F75-16, 112-16) were selected for the conidial production, sclerotial descriptions, effect of temperature on mycelial growth, conidial germination, vegetative incompatibility and pathogenicity tests (Table 1).

Conidial production

For this propose, five isolates of *B*. *cinerea* and five isolates of *B*. *prunorum* were grown on APDA and PAM media. A mycelial plug of a 4 days old APDA culture (5 mm-diameter) was placed in quadruplicate in the middle of a 90 mm-diameter Petri dish and incubated for 7 days at 20°C under a 12 h light/dark cycle. Then, each plate was flooted with 10 ml autoclaved water plus 0.05% Tween 80. The conidial suspensions were filtered through three layers of cheese cloth and the concentration was estimated using a Neubauer chamber under a microscope. The conidial production was expressed as conidia cm^{-2}. The study was conducted twice.

Size, production and distribution of sclerotia

The sclerotial production and distribution the five isolates of *B*. *cinerea* and *B*. *prunorum* was evaluated. A 5 mm-diameter plug of 4 days-old APDA culture was plated in quadruplicate in the center of a Petri dish (90 mm) containing APDA or PAM and incubated for 7 days at 20°C followed by 30 days at 5°C or 20°C, according to Ferrada et al. (2016). The sclerotial production was expressed as numbers of sclerotia cm^{-2} and their distribution depended of the location on the Petri dish: in circle, in the middle, irregular (Martinez et al. 2003). The study was conducted twice.

The conidial and sclerotial production, and sclerotial size were determined using ANOVA and means were separated according to Tukey's test ($P \leq 0.05$) using SigmaPlot v 12.5. Conidial production values were transformed by square root prior the analysis.

Effect of temperature on mycelial growth

The effect of temperature on mycelial growth of five isolates of *B*. *cinerea* and *B*. *prunorum* was determined. With this propose, a 5 mm-diameter mycelial plug taken from a 4 days-old APDA culture was placed upside down in the center of a 90 mm-diameter Petri dish (n=4) containing APDA and incubated in thermal chambers (Velp Scientifica, Milan,

Italy) from 0°C to 40°C (± 1°C) in 5°C intervals, in darkness. Two perpendicular diameters were measured with an digimatic caliper (Mitutoyo Sul Americana Ltda. Suzano, Brazil) after 3 days of incubation and in 24 h interval at 20°C and weekly for 21 days at 0°C. The mycelial growth rate was determined. The temperature inside the chambers and on the surface of the culture media were registered using data loggers (U12 Temp/RH/2 External Channel Logger, HOBO Onset, Computer Corporation, MA, USA). When no growth isolates were observed, they were re-incubated for 5 days at 20°C to determine their viability. The study was conducted twice.

The effect of the temperature in the mycelial growth of both *Botrytis* species was analyzed by a factorial 9 x 2 (F1=temperature; F2=specie). The ANOVA was performed and means were separated according to Tukey's test ($P \leq 0.05$) using SigmaPlot v 12.5. A linear regression model between x = incubation time (°C) and y = mycelial growth (mm) was used to define the growth rate of each *Botrytis* specie.

Conidial germination

Conidial suspensions of isolates of *B*. *cinerea* and *B*. *prunorum* were obtained from 7 days-old colonies on PAM in a cycle of 12 hours of light/12 hours of darkness, removed the mycelia by flooting with sterile water with 0.05% Tween 80, filtered through a three cheese cloth layer and adjusted to 10^6 conidia ml^{-1} with the aid of a Neubauer chamber. Then, 100 µl of a conidial suspension was spread on 90 mm-diameter Petri dishes containing 1.5% water agar and incubated at 20°C and 0°C in a thermal chamber, in darkness. Each isolated was plated in quadruplicated. Germinated conidia were determined from a total of 100 conidia counted per dish at 0-3-6-9-12 and 24 h. A conidium was considered germinated when its germ tube length was equal or longer than the width of the conidium. The experiment was conducted twice.

The effect of the incubation time on the conidial germination at 20°C and 0°C was studied by a factorial 6 x 2 (F1=incubation time; F2=specie) design and analized by ANOVA ($P \leq 0.05$). A sigmoid regression model $y = a/(1 + \varepsilon\ ((x - xo))/b)$ was used to explained the effect of x = the incubation time of conidia (h) and y = the percentage of conidial germination (%).

Vegetative incompatibility test

Vegetative incompatibility between isolates of *B*. *cinerea* and *B*. *prunorum* was determined based on presence or absence of a dark pigmentation along in the interaction or confrontation zone between paired isolates (Beever and Parkes 1993). Two mycelial plugs of 2 cm-diameter were taken from the actively growing margin of 5-day-old APDA colonies of two different isolates and placed 3 cm apart on plates (90 mm-diameter) containing on MEA + 4% NaCl medium and incubated for 2 weeks in the dark. This experiment was conducted twice.

Pathogenicity test

Isolates of *B*. *cinerea* and *B*. *prunorum* were tested for pathogenicity and virulence. For this purpose, mycelium plugs of 3 mm were taken from 4 days-old APDA culture at 20°C and was used to inoculate kiwifruit and kiwifruit leaves. Also, 10 µl of a conidial suspension was used for pathogenicity test in kiwifruit leaves, apples and grapes. Conidial suspension was adjusted to 10^6 conidia ml^{-1} obtained from 7 days-old colonies on PAM in a cycle of 12 hours of light/12 hours of darkness, removed the mycelia by flooting with sterile water with 0.05% Tween 80 and filtered through a three cheese cloth layer.

Kiwifruit. 'Hayward' kiwifruit with peduncle attached were sampling at harvest time (6.8% SS) and at harvest plus 45 days at 0°C (9.4% SS) and were superficially disinfected for 2

minutes, rinsed for 1 minute and dried on absorbent paper under a laminar flow hood for 30 min. Fruit were injured, inoculated with a mycelium plug and wrapped using *Parafilm*. The wound was performed in 12 fruit (n=4) in i) the cheek with a 3 mm-diameter cork borer, ii) in the stem end (fresh picking wound) detaching the peduncle or iii) in the insertion of the sepal to the fruit (receptacle) with a sterile scalpel. An equal number of wounded but non-inoculated fruit was maintained as control. After 5, 7 or 12 days of incubation at 20°C in a humidity chamber (>85%), length of necrotic lesion developed under the inoculated epidermis were measured. Re-isolations were performed from pericarp (3 x 3 mm) located immediately under the inoculation zone, placed on APDA and incubated at 20°C for 7 days under 12 h light/dark cycle to confirm Koch´postulates.

Pathogenicity test was analyzed by bi-factorial design 10 x 2 (F1=isolate; F2=sampling date) ANOVA ($P \leq 0.05$) and mean values were separated according to Tukey's test ($P \leq 0.05$) using SigmaPlot v 12.5

Leaves. Detached leaves of 'Hayward' kiwifruit (n=4) were surface disinfected for 2 min, rinsed for 1 min and dried in a laminar flow chamber for 10 min. Leaves were punctured twice, one on each side of the main vein of the leaf blade with a sterile needle (0.7 mm diameter). Other four leaves were remained non-wounded. Leaves were inoculated with a mycelial plug of each isolate and stored in a humidity chamber (>85%) for 5 days at 20°C. Two perpendicular diameters were measured each necrotic lesion and re-isolation were performed to confirm Koch´postulates.

Apples and grapes. Pathogenicity test was conducted on Fuji apples (n=5, 13.5 % SS) and Red Globe grapes (n = 20, 20.0 % SS). Apple fruits were disinfected in 75% ethanol for 2 min, grapes for 30 s and air-dried. Apples were wounded on the cheek with a sterile borer (3 mm) and grapes with a sterile hypodermic needle, and inoculated with conidial

suspension. The mean of two perpendicular diameters of the lesion was determined and fungal re-isolations were made to confirm Koch´postulates.

Pathogenicity test in leaves, apples and grapes were analyzed by one-way design and ANOVA ($P \leq 0.05$), and mean values separated using Tukey's test ($P \leq 0.05$) in SigmaPlot v 12.5.

Results

Sampling and fungal isolation

Floral parts

Botrytis colonies were quantified during the fruit development from petals (n=1,016 isolates), sepals (n=3,018 isolates), receptacles (n=878 isolates) and styles (n=1,331 isolates.

Two types of *Botrytis* isolates were constantly obtained from all kiwifruit orchard sampled and the two season in study. *Botrytis* colonies with gray mycelia and scarce to high conidial production (HCP) were the most frequently isolated (94.7%) and whitish, fluffy and aerial mycelium with low or null conidial producer (LCP) isolates were less isolated (5.3%).

At full bloom, differences between the frequency of isolation was obtained among the floral parts ($P<0.01$) and type of isolate ($P< 0.001$). The highest values of isolation for both HCP and LCP colonies were obtained from petals averaging 65.5% (±20.65) for HCP and 5.3% (±1.5) for LCP colonies. Sepals, receptacles and styles were also colonized averaging 29.8% (±22.4) and 2.0% (±1.3) in sepals, 23.6% (±23.6) and 1.0% (±0.9) in receptacles and 7.9% (±7.3) and 0.2% (±0.4) in styles, for HCP and LCP colonies, respectively (Fig. 2).

From 60 DAFB to harvest, differences in the frequency of isolation was obtained between HCP and LCP colonies, and the same trend was observed among the day of sampling

(P<0.001) in both years (Table 3). Only the colonization of *B*. *prunorum* in styles at harvest in 2016 season was significantly higher than previous samplings (*P*<0.001). Regardless the season, the frequency of HCP colonies averaged 18.3 % (±4.5) in sepals, 26.8% (±4.7) in receptacles and 12.3% (±11.2) in styles while the frequency of LCP colonies averaged 0.8% (±0.6) in sepals, 1.6% (±1.4) in receptacles and 1.2% (±2.0) in styles.

From kiwifruits obtained from storage

Gray mold prevalence varied between 33.1% (2016) and 7.1% (2017). All diseased fruit (n=644) presented a soft, light to brown watery decay starting from the stem end, cheek or stylar end and HCP and LCP isolates were obtained. A significant difference among type of disease ($P < 0.001$) and type of *Botrytis* isolate ($P < 0.001$) was obtained in both seasons. Stem end rot was the most important disease averaging 30.0% (n=480) of prevalence in 2016 and 6.4% (n=102) in 2017 season. Prevalence of BR varied between 2.4% (n=38) and 0.4% (n=6), and BER varied between 0.8% (n=12) and 0.4% (n=6) of frequency in 2016 and 2017 seasons, respectively (Fig. 3).

Independently of the season, isolates classified as HCP were predominantly obtained from all types of diseases, obtaining from the total of isolates obtained a frequency of 93.1% in SER (n=542), 93.2% in BR (n=41), 94.4% in BER (n=17).

Molecular characterization and phylogenetic analysis

The BLASTn analysis of partial sequences of G3PDH, HSP60 and RPB2 genes showed that HCP isolates (n=13) were 99% to 100% identical to the ex-type isolate of *Botrytis cinerea* and LCP isolates (n=5) were 99% to 100% *Botrytis prunorum*. DNA fragments of genes were amplified, sequenced and 885 (G3PDH), 959 (HSP60) and 1088 (RPB2)-bp DNA fragments were obtained.

Partitioned homogeneity showed a significant level of incongruence (*P* = 0.01) between the G3PDH, HSP60 and RPB2 gene sequences. Therefore, the analysis was performed separately.

Independently of the gene in study, the phylogenetic analyces clearly grouped B. prunorum Chilean sequences along with the ex-type of *B*. *prunorum* isolate in a separated clade. On the contrary, the maximum parsimony analysis of G3PDH, HSP60 and RPB2 partial genes were not informative to delimit *B*. *cinerea* from closest *Botrytis* species.

Sixty-eight sequences of G3PDH of *Botrytis* species obtained from database were separated in clusters along with all Chilean isolates from kiwifruit in this study. Parsimony maximum analysis of G3PDH gene sequences produced 37 parsimonious trees, yielded length tree (TL) =247, consistency index (CI) = 0.581, retention index (RI) = 0.915 and rescaled consistency index (CR) = 0.532. The phylogenetic tree grouped all the *B*. *prunorum* sequences in a clade, with 85% bootstrap support, together the *B*. *prunorum* ex-type. The *B*. *cinerea* sequences were grouped in a different clade with 87% bootstrap support, together with the *B*. *cinerea*, *B*. *fabae*, *B*. *pelargonii* and *B*. *eucalipty* ex-types (Fig. 4).

Phylogenetic analysis of HSP60 sequences produced 72 parsimonious tree, yielded a tree length of 427, consistency index (CI) =0.599, retention index (RI) = 0.919 and rescaled consistency index (CR) = 0.5551. *Botrytis prunorum* sequences were grouped in a clade, with 99% bootstrap support, together with the *B*. *prunorum* ex-type. Sequences of *B*. *cinerea* were grouped in a different clade with 81% bootstrap support, together with the *B*. *cinerea* and *B*. *pelargonii* ex-type (Fig. 5).

Phylogenetic analysis of RPB2 gene sequences produced 100 parsimonious trees, yielded length tree (TL) =463, consistency index (CI) = 0.618, retention index (RI) = 0.932 and rescaled consistency index (CR) = 0.576. Chilean sequences were grouped in three clades. Clade I grouped all sequences of *B*. *prunorum* isolates with 99% bootstrap support, together with the *B*. *prunorum* ex-type. Clade II grouped two Chilean isolates (MK295638;

MK295642), *B*. *cinerea*, and *B*. *fabae* ex-type with 95% bootstrap support. Clade III grouped *B*. *cinerea* isolates together the ex-type, with a 99% bootstrap support (Fig. 6).

Morphological characterization

Botrytis cinerea isolates produced gray to dark-gray wooly mycelia with scarce to abundant sporulation on APDA. Conidiophores on PAM were erect, septate and branched at the top, 936.74 (±482.59) µm length and 10.7 µm (±1.7) width. Conidia were unicellular, hyaline to pale brown, globose to ovoid, 10.99 (±1.26) µm length (L), 6.97 (±0.78) µm width (W), with a 1.60 (±0.20) µm L/W relationship. *B. prunorum* isolates produced white, wooly and aerial mycelia with scarce sporulation on APDA. Conidiophores on PAM were erect, septate, irregularly branched toward the top, 752.9 (±95.2) µm length and 11.3 (±0.5) µm width. Conidia were non-septate, unicellular, hyaline, ovoid to elliptical with a prominent hilum, 11.9 (±0.5) µm length, 6.7 (±0.4) µm width with a 1.8 (±0.1) L/W relationship.

Conidial production

The effect of the composition of the culture media on the conidial production was similar in both species (*P*=0.196). Differences in the conidial production was observed between species of *Botrytis* on APDA and PAM. *Botrytis cinerea* averaged 11.1 (±10.5) x 10^4 conidia cm^{-2} on APDA and 6.6 (±6.0) x 10^4 conidia cm^{-2} on PAM, and *B*. *prunorum* averaged 0.5 (±0.5) x 10^4 conidia cm^{-2} on APDA and 0.3 (±0.7) x 10^4 conidia cm^{-2} on PAM medium.

Size, production and distribution of sclerotia

In general, *B*. *prunorum* isolates produced sclerotia on PAM at 5°C and 20°C but not on APDA, except for one isolate (112-16) that produced sclerotia on APDA and PAM at 5°C and 20°C. *Botrytis cinerea* produced sclerotia on APDA and PAM at 5°C and 20°C. Differences in the production and size of sclerotia were significant on APDA (Table 4).

Botrytis cinerea sclerotia were black, elongate to spherical, 0.7 to 5.3 µm length and 0.6 to 3.4 µm width on APDA and their distribution was irregular or in circle in the Petri dish on APDA and PAM. *Botrytis prunorum* sclerotia were black, oblong to spherical with 2.0 to 3.0 µm length and 2.0 to 2.5 µm width on PAM and their distribution in circle on APDA and PAM.

Effect of temperature on mycelial growth

Mycelial growth of *Botrytis* species was significantly ($P < 0.001$) affected by the temperature with a significantly interaction (specie x temperature, $P < 0.01$). All isolates of *B. cinerea* and *B. prunorum* grew on a temperature range between 0°C and 30°C with maximum growth observed at 20°C (Fig. 7). None isolate grew at 35°C and 40°C for 3 days. Only *B. prunorum* isolates remained viable at 35°C. The highest mycelial growth rate at 20°C was observed in *B. cinerea* isolates with 26.5 ± 2.0 mm day^{-1} followed by *B. prunorum* isolates with 20.6 ± 4.7 mm day^{-1}. At 0°C, the growth rate was similar between species with 2.7 (±2.3) mm $week^{-1}$ (0.4 mm day^{-1}) and 2.8 ±0.3 mm $week^{-1}$ (0.4 mm day^{-1}) ($P = 0.659$) (Fig. 8).

Conidial germination

Independent of the incubation temperature, conidial germination of *Botrytis* isolates was similar between species and depended on incubation time ($P < 0.001$). The germination ranged between 95.0% to 98.5% in *B*. *cinerea* isolates and 92.8% to 96.5% in *B. prunorum* isolates after 24 h at 20°C. A sigmoid model explained the rate of germination ($R^2 = 0.96$, $P = <0.0001$) for both species and the exponential stage was showed between 3 h and 9 h (Fig. 9). Less than 2% of the conidia of the *B*. *cinerea* (1.3%) and *B*. *prunorum* (1.9%) germinated after 24 h at 0°C.

Vegetative incompatibility test

An interaction zone along the line of confrontation between paired *Botrytis* isolates was consistently observed on MEA + 4% NaCl medium. A black to dark-brown pigmentation was observed on 17 *Botrytis* paired isolates and 26 paired isolates was not shown the dark line (Table 5). No black line was formed when the isolates were paired themselves and between *B*. *prunorum* isolates.

Pathogenicity test

Kiwifruit

All *Botrytis* isolates were pathogen on wounded kiwifruit when were inoculated with mycelial plug on the cheek, stem end and receptacle (Table 6). Regardless the point of inoculation, all isolates caused a light-brown, soft, watery decay from the inoculation point and their virulence depended of the isolate ($P < 0.001$). *Botrytis* isolates were more virulent in kiwifruit sampled at harvest plus 45 days at 0°C (9.4% SS) than those recently harvested (6.8% SS) ($P < 0.001$). Significantly interaction between isolates and maturity stage was showed in all analysis ($P < 0.001$). Reisolations of *B*. *cinerea* and *B*. *prunorum* isolates were 100% obtained from inoculations made on cheek. Besides, *B*. *cinerea* was 100% reisolated from fruit inoculated in all point of inoculation in kiwifruit sampled at harvest plus 45 days at 0°C and 77.8% and 66.7% from stem end and receptacle inoculation in kiwifruit sampled at harvest. *Botrytis prunorum* isolates were less reisolated, averaging 72.2% and 75.0% of successful from stem end and receptacle inoculation in fruit sampled at harvest.

Leaves and other hosts

All *Botrytis* isolates produced a watery soaked lesion followed by a necrotic decay on wounded and non-wounded leaves of kiwifruits when were inoculated with mycelial disc and some *B*. *cinerea* isolates (31-17, 4-17, F58-16) sporulated on the necrotic lesion on the fifth day of incubation (Table 7). Differences in diameter of the necrotic lesion produced were obtained, being *B*. *cinerea* isolates those more virulent with a mean of 24.6 (±1.5) mm, in contrast with *B*. *prunorum* that produce a mean lesion of 5.0 (±5.4) mm. All *B*. *cinerea* isolates and two of *B*. *prunorum* (6-16 and 55-16) were able to produce lesions in non-wounded leaves and the other three *B*. *prunorum* isolates shown a superficial mycelial growth on the leaf. (Table 7). On apples, *Botrytis* species produced dark brown lesions and soft decay and cracking and skin split soft rot on grape cv. Red Globe. Regardless the host, necrotic lesions caused by *B*. *cinerea* isolates were significantly (P=0.00) larger than those caused by *B*. *prunorum.* Reisolation was successful on all inoculated and symptomatic fruit and control fruit remained healthy and no re-isolates were obtained.

Discussion

On the basis of the isolation, morphological and molecular studies performed in this study, *Botrytis cinerea* and *B. prunorum* were identified and consistently isolated from apparently healthy flower structures and from postharvest diseased kiwifruit in Chile. This research determined the presence of HCP (91.84%) and HCP (8.16%) colonies of *Botrytis* genera which were identified as *B*. *cinerea* and *B*. *prunorum*, respectively, according to morphological and molecular analysis (Ferrada et al. 2016; Mirzaei et al. 2008; Pei et al. 2019; Staats et al. 2005). In Chile, gray mold is the prime postharvest disease in 'Hayward' kiwifruit and has been globally associated with *Botrytis cinerea* (Brigati et al. 2003; Díaz et al. 2017; Michailides and Elmer 2000; Opgenorth 1983; Pei et al. 2019; Pennycook 1985).

The present study determined the presence of *B*. *prunorum* and *B*. *cinerea* causing gray mold in 'Hayward' kiwifruit stored at 0°C corroborating the reports by Elfar et al. (2017) and García et al. (2018) who identified *B*. *prunorum* and *B*. *cinerea* causing gray mold in kiwifruit in Chile, respectively. Previously, *B. prunorum* was described by Ferrada et al. (2016) causing blossom blight in Japanese plum and identified in apples and pears causing calyx-end rot (Ferrada et al. 2017). Outside Chile, *B*. *prunorum* was identified colonizing apparently healthy alfalfa (Reich et al. 2017) and grapes cv. Chardonnay in the USA (Wang et al. 2018).

Botrytis species were part of the mycobiota of kiwifruit orchard. Floral parts of the kiwifruit were predominantly colonized by *B*. *cinerea* followed by *B*. *prunorum* with the highest values in flowers, particularly petals. Flowers and floral parts colonized by *Botrytis* species have been considered as important inoculum sources for kiwifruit infections (Brook 1992; Michailides and Morgan 1996; Michailides and Elmer 2000) and other crops including pistachio (Michailides 1991), grapes (Esterio et al. 2011; Keller et al. 2003; Nair et al. 1995; Viret et al. 2004), plums, nectarines (Ferrada et al. 2016; Fourie et al. 2002) and blueberries (Rivera et al. 2013). In addition, flowering has been considered as a critical period of infection (Michailides and Morgan 1996; Michailides and Elmer 2000). Considering the highest values of colonization that were obtained at flowering, these results also suggest that strategies against stem end rot should aim to decrease the inoculum density at flowering.

Furthermore, SER was the most frequent postharvest disease developed in cold stored kiwifruit in Chile, confirming previous reports (Latorre 2004, 2018; Latorre and Pak 2003; Pinilla et al. 1994; Díaz et al. 2017). *Botrytis* species were the causal agents of the 100% of the diseased fruit and *B*. *cinerea* was always the predominant specie with more than 90% of the isolates. In addition, *B*. *prunorum* colonies were isolated alone or accomplish of *B*. *cinerea* from the same tissue and vegetative compatibility was observed among isolates

which suggests that both species coexist and share the same ecological niche. A similar situation has been described in other crops including grapes (Garfinkel et al. 2017; Harper et al. 2019; Saito et al. 2016; Walker et al. 2011, Zhou et al. 2014), eucalyptus (Liu et al. 2016), blueberries (Saito et al. 2016), and strawberries (Rupp et al. 2017) where more than one species of *Botrytis* have been identified living in sympatry within a host.

Botrytis cinerea and *B*. *prunorum* were identified through phylogenetic analysis of 18 *Botrytis* isolates obtained from apparently healthy petals, sepals, receptacles, styles and kiwifruit with gray mold by G3PDH, HSP60 and RPB2 partial genes. The identification through phylogenetic analysis have helped to delimit cryptic *Botrytis* species that morphologically are very similar or equals (Saito et al. 2016; Staats et al. 2005; Staats et al. 2007; Walker et al. 2011). In this study, both species were grouped in two separated clades. However, the information provided by G3PDH gene was not sufficient to distinguish *B*. *cinerea* and *B*. *eucalipty* as different species. These species were delimited with sequences of RPB2 partial gene although two isolates in study and *B*. *cinerea* ex-type (AJ745674 accession number) were grouped together with *B*. *fabae*. Therefore, a concatenate analysis is suggested to clearly delimit *B*. *cinerea* and closest species (Ferrada et al. 2016; Khan et al. 2013; Saito et al. 2016; Staats et al. 2005; Walker et al. 2011; Zhang et al. 2010a, b).

All isolates were consistent with previous morphological descriptions of *B*. *cinerea* and *B*. *prunorum* (Ferrada et al. 2016; Mirzae et al. 2008; Pei et al. 2019; Saito et al. 2016; Zhou et al. 2014) although, phenotype variability was observed among *Botrytis* isolates in this research. In general, white and fluffy mycelia with scarce conidial and sclerotial production was characteristic in *B*. *prunorum* on APDA while gray mycelia, abundant sporulation and high sclerotial production was characteristic in *B*. *cinerea* isolates. These differences tentatively allowed to distingue both species through colonial and morphological characteristics. Nevertheless, one isolate of *B*. *prunorum* was a high sclerotial producer on APDA and PAM at 5°C and 20°C which differed with descriptions realized by Ferrada et al.

(2016). These differences are consistent with previous studies which indicated that *Botrytis* species are able to produce different morphotypes depending of the ecosystem and the season of the year (Coley-Smith 1980). The low capacity of the *B*. *prunorum* to produce sclerotia could negatively affect overwintering and the subsequent conidial dispersion and multiplication in the host when the condition are favorable (Coley-Smith 1980; Nair et al. 1995; Latorre et al. 2015).

The *Botrytis* species grow between 0°C to 30°C, with an optimum at 20°C although *B*. *prunorum* isolates grew at a low rate at 20°C. All isolates grew at 0°C and at a similar growth rate. These results demonstrated the capability of both *Botrytis* species to grow under low temperatures and therefore to be important pathogens in the postharvest of the kiwifruit. On the other hand, independent of the origin location, *Botrytis prunorum* isolates grow more than *B*. *cinerea* at 30°C and was able to remain viable at 35°C, suggesting that *B*. *prunorum* could be adapted to warmer climates.

Botrytis species have been widely considered to be aggressive plant pathogen although an initial or permanent saprophytic phase in some *Botrytis* species has been described, causing non visible symptom in their hosts (Grant-Downton et al. 2014; Shaw et al. 2016; van Kan et al. 2014; Zhang et al. 2016). In this study, all *Botrytis* isolates tested including those isolated from apparently healthy floral parts were pathogenic, producing necrotic lesion on leaves and symptoms of rot on kiwifruit. *Botrytis cinerea* was always more virulent than *B*. *prunorum* varying among the isolates, supporting the results obtained by Ferrada et al. (2016) in plum flowers. However, kiwifruit sampled at harvest time and inoculated in stem end with isolates of *B*. *prunorum* showed no visible symptoms but with successful reisolation, suggesting that infections could be latent. This work also suggests that *B. cinerea* and *B. prunorum* have the capacity to infect other fruit host as apples and grapes. Therefore, both species of Botrytis associated with kiwifruit can be potential inoculum source for calyx-end rot in apple and gray mold in grapes in Chile (Ferrada et al., 2017; Latorre et al. 2015). It is

very common to find in this central area of Chile, apple orchards very close to kiwifruit vineyards and grape of wines vineyards located along with kiwifruit vineyards in Chile.

In the vineyards, kiwifruit leaves turn from soft to hard, breakable and easier to be injured by abiotic factors, favoring the infection by *Botrytis* conidia in the field. Moreover, during wet weather, leaf lesions may develop from secondary spread via adhering debris from infected blossoms (Elmer et al. 1997; Manning et al. 2010; Michailides and Elmer 2000; Pennycook 1985). These results suggest that injured leaves of kiwifruits are potential inoculum sources of *B. prunorum* in the field, and confirmed that *B. prunorum* is a non- strict organ or tissue specialist fungi.

In conclusion, based on the results of this study, the gray mold is the most important postharvest disease in 'Hayward' kiwifruit and is caused for a complex of two species of *Botrytis* in Chile. Both *Botrytis* species are present in the vineyard and postharvest, colonizing asymptomatic flowers and floral parts in fruit during the whole growing season. *B*. *cinerea* is clearly the most prevalent and virulent pathogen and both *B*. *cinerea* and *B*. *prunorum* are able to infect and produce gray mold in postharvest. This results contributed to the knowledge of the etiology and the role of *Botrytis* species in the epidemiology of the gray mold of kiwifruit in Chile.

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Table 1. *Botrytis cinerea* and *B. prunorum* isolates obtained from kiwifruit tissues and decay fruits in storage at 0°C used for morphological and phylogenetic studies.

Specie/Isolate	Host tissue	Sampling day (DAFB)[x]	Origin	Coodinates	Sclerotial distribution	Sclerotial production[y]	GenBank accession numbers[z]		
							G3PDH	*HSP60*	*RPB2*
Botrytis cinerea									
100-17	Sepal	160	San Francisco de Mostazal	34°00'S, 70°42'W	Irregular	moderate	MK256906	MK256888	MK295634
106-16	Receptacle	160	Quinta de Tilcoco	34°22´S, 70°54´W	In circle	abundant	MK256907	MK256889	MK295635
115-16	Receptacle	160	Requinoa	34°20´S, 70°43´W	In circle	moderate	MK256909	MK256891	MK295637
132-16	Sepal	160	Quinta de Tilcoco	34°22´S, 70°54´W	Irregular	low	MK256910	MK256892	MK295638
172-16	Sepal	160	Longaví	35°48'S, 71°49'W	In circle	abundant	MK256911	MK256893	MK295639
31-17	Petal	Bloom	San Francisco de Mostazal	34°00'S, 70°42'W	Irregular	low	MK256912	MK256894	MK295640
33-16	Sepal	120	Longaví	35°48'S, 71°49'W	Irregular	abundant	MK256913	MK256895	MK295641
338-17	Sepal	Bloom	San Francisco de Mostazal	34°00'S, 70°42'W	Irregular	low	MK256914	MK256896	MK295642
41-16	Receptacle	120	Longaví	35°48'S, 71°49'W	-	null	MK256915	MK256897	MK295643
4-17	Petal	Bloom	Quinta de Tilcoco	34°22´S, 70°54´W	Irregular	low	MK256916	MK256898	MK295644
54-17	Petal	Bloom	Longaví	35°48'S, 71°48'W	Irregular	low	MK256917	MK256899	MK295645
F2-17	Decay fruit	Storage	Longaví	35°48'S, 71°48'W	In circle	abundant	MK256921	MK256903	MK295649
F58-16	Decay fruit	Storage	Longaví	35°48'S, 71°49'W	Irregular	low	MK256922	MK256904	MK295650
Botrytis prunorum									
112-16	Style	160	Requinoa	34°20´S, 70°43´W	In circle	abundant	MK256908	MK256890	MK295636
55-16	Receptacle	120	Requinoa	34°20´S, 70°43´W	-	null	MK256918	MK256900	MK295646
6-16	Sepal	120	Longaví	35°48'S, 71°48'W	-	null	MK256919	MK256901	MK295647
F102-16	Decay fruit	Storage	Longaví	35°48'S, 71°49'W	-	null	MK256920	MK256902	MK295648
F75-16	Decay fruit	Storage	Longaví	35°48'S, 71°48'W	-	null	MK256923	MK256905	MK295651

[x] Days after full bloom (DAFB). Bloom: Full bloom. Decay fruit: Fruit sampled from storage after 100 days at 0°C plus 2 days at 20°C.

[y] Sclerotial production: abundant: ≥30 sclerotia dish^{-1}; moderate: ≥15 and < 30 esclerotia dish $^{-1}$; low: ≤ 15 esclerotia dish $^{-1}$.

[z] G3PDH: glyceraldehyde 3-phosphate dehydrogenase; HSP60: Heat shock protein 60; RPB2: DNA-dependent RNA polymerase subunit II.

Table 2. Isolates of species of *Botrytis* and their GenBank accession numbers used in the phylogenetic analysis.

Species	Strain[a]	Origin	GenBank accession numbers		
			G3PDH	HSP60	RPB2
B. aclada	MUCL8415	Germany	AJ704992	AJ716050	AJ745664
	PRI006	N/A	KJ796652	AJ716051	AJ745665
B. allí	MUCL403	The Netherlands	AJ704996	AJ716055	AJ745666
B. byssoidea	MUCL94[d]	USA	AJ704998	AJ716059	AJ745670
	OnionBC-76	China	FJ169652	FJ169661	FJ169681
B. califórnica	X503	USA	KJ937068	KJ937058	KJ937048
	X1348	USA	KJ937071	KJ937061	KJ937051
B. calthae	MUCL1089	Belgium	AJ705000	AJ716061	AJ745672
	MUCL2830	USA	AJ705001	AJ716062	AJ745673
B. caroliniana	CB15[d]	USA	JF811584	JF811587	JF811590
	CA3	USA	JF811586	JF811589	JF811592
B. cinerea	MUCL87[d]	The Netherlands	AJ705004	AJ716065	AJ745676
	Bci-18	Chile	KP120870	KP120877	KP136782
	B05.10	Germany	AJ705002	AJ716063	AJ745674
B. convoluta	MUCL11595	USA–The Netherlands	AJ705008	AJ716069	AJ745680
	9801	The Netherlands	AJ705007	AJ716068	AJ745679
B. croci	MUCL436	The Netherlands	AJ705009	AJ716070	AJ745681
B. deweyae	CBS 134649[d]	UK	HG799521	HG799519	HG799518
B. elliptica	BE0022	The Netherlands	AJ705010	AJ716071	AJ745682
	BE9610	The Netherlands	AJ705011	AJ716072	AJ745683
	BE9714	The Netherlands	AJ705012	AJ716073	AJ745684
B. eucalypti	CERC7170[d]	China	KX301020	KX301024	KX301028
	CERC7160	China	KX301018	KX301022	KX301026
	CERC7208	China	KX301021	KX301025	KX301029
B. euroamericana	B83[d]	Italy	KC191677	KC191678	KC191679
	AK10	USA	KX266727	KX266733	KX266739
B. fabae	MUCL98[d]	Spain	AJ705014	AJ716075	AJ745686
	CBS 109.57	The Netherlands	AJ705013	AJ716074	AJ745685
B. fabiopsis	BC-2	China	EU519211	EU514482	EU514473
	BC-13	China	EU563109	EU563100	EU563115
B. ficariarum	CBS 176.63[d]	Belgium	AJ705015	AJ716076	AJ745687
B. fragariae	D11_H_R4[d]	Germany	KX429702	KX429695	KX429709
	U14_H3	USA	KX429701	KX429694	KX429708
B. galantina	MUCL435	The Netherlands	AJ705018	AJ716079	AJ745689

	MUCL3204	The Netherlands	AJ705017	AJ716078	AJ745690
B. gladiolorum	MUCL3865	The Netherlands	AJ705020	AJ716081	AJ745692
	9701	N/A	AJ705019	AJ716080	AJ745691
B. globosa	MUCL444	Belgium	AJ705022	AJ716083	AJ745693
	MUCL21514	UK	AJ705021	AJ716082	AJ745694
B. hyacinthi	0001	The Netherlands	AJ705023	AJ716084	AJ745695
	MUCL442	The Netherlands	AJ705024	AJ716085	AJ745696
B. mali	BPI412756[d]	USA	EF367129	N/A	N/A
	BPI411770	USA	EF367121	N/A	N/A
B. narcissicola	MUCL2120	Canada	AJ705026	AJ716087	AJ745697
B. paeoniae	HA11	USA	KX266729	KX266735	KX266741
	GBG22	USA	KX266730	KX266736	KX266742
B. pelargonii	CBS 497.50[d]	Norway	AJ704990	AJ716046	AJ745662
	MUCL1152	Norway	AJ705029	AJ716090	AJ745701
B. porri	MUCL3234[d]	-	AJ705032	AJ716093	AJ745704
B. prunorum	Bpru-1.9[d]	Chile	KP339985	KP339999	KP339992
	Kw 2.2.2	Chile	KX196312	KX196316	KX196314
	Kw 4.1.2	Chile	KX196311	KX196315	KX196313
	Bpru-1.5	Chile	KP339984	KP339998	KP339991
	Bpru-1.4	Chile	KP339983	KP339997	KP339990
B. pseudocinerea	X004	USA	KJ796651	KJ796655	KJ796647
	X010	USA	KJ796652	KJ796656	KJ796648
	10091	France	JN692414	JN692400	JN692428
B. pyriformis	SedsarBC-1[d]	China	KJ543484	KJ543488	KJ543492
	SedsarBC-3	China	KJ543486	KJ543490	KJ543494
B. ranunculi	CBS178.63[d]	USA	AJ705034	AJ716095	AJ745706
B. sinoallii	OnionBC-23[d]	China	EU519217	EU514488	EU514479
	OnionBC-59	China	FJ169646	FJ169658	FJ169678
B. sinoviticola	GBC-5	China	JN692413	JN692399	JN692427
B. sphaerosperma	MUCL21481	UK	AJ705035	AJ716096	AJ745708
	MUCL21482	UK	AJ705036	AJ716097	AJ745709
B. squamosa	MUCL1107[d]	USA	AJ705037	AJ716098	AJ745710
B. tulipae	BT9001	The Netherlands	AJ705040	AJ716101	AJ745712
Sclerotium sclerotiorum	484	The Netherlands	AJ705044	AJ716048	AJ745716
Monilinia fructigena	9201	The Netherlands	AJ705043	AJ716047	AJ745715

[a] CBS, CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; CERC, Culture Collection of China Eucalypt Research Centre, Chinese Academy of Forestry, Zhan Jiang,

Guang Dong, China; MUCL, BCCM Belgium Coordinated Collection of Microorganisms; X, Saito et al. 2016.

[b] G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HSP60, heat-shock protein 60; RPB2, DNA-dependent RNA polymerase subunit II;

[d] Isolates are ex-type.

Table 3. *Botrytis cinerea* and *B*. *prunorum* colonization in apparently healthy sepals, receptacles and styles sampled at 60, 100 120 and 160 (harvest) days after full bloom (DAFB) during 2016 and 2017 seasons.

DAFB[w,x]	*Botrytis* colonization, %							
	2016 season				2017 season			
	B. cinerea	*B. prunorum*		Mean	*B. cinerea*	*B. prunorum*		Mean
Sepals								
60	18.0	1.7		9.8	25.7	0.8		13.3
100	14.1	1.8		7.9	22.9	0.3		11.6
120	14.3	0.9		7.6	18.1	0.3		9.2
160	20.3	0.5		10.4	13.0	0.1		6.6
Mean	16.7 B[z]	1.2	A	9.0	19.9 B	0.4	A	10.2
Receptacles								
60	21.3	3.8		12.5	31.3	0.3		15.8
100	22.9	3.3		13.1	26.9	0.3		13.6
120	22.5	2.5		12.5	28.3	1.0		14.6
160	35.0	1.7		18.3	25.8	0.3		13.1
Mean	25.4 B	2.8	A	14.1	28.1 B	0.5	A	14.3
Styles								
60	13.2	0.6	a[y]	6.9	9.9	0.0		5.0
100	11.6	1.7	a	6.7	5.6	0.6		3.1
120	6.6	0.3	a	3.5	6.4	0.6		3.5
160	39.2	5.9	b	22.6	5.8	0.3		3.1
Mean	17.6 B	2.1	A	9.9	6.9 B	0.3	A	3.6

[w] Days after full bloom (DAFB).

[x] Each sampling day, apparently healthy sepals (n=1,440), receptacles (n=240) and styles (n=1,440) were cultivated on APDA +I for 7-10 days at 20-22°C under 12 h light cycle.

[y] Values followed by the same lowercase letter in the same column did not differ significantly according to Tukey's test ($P < 0.05$).

[z] Means followed by the same uppercase letter in each column did not differ significantly according to Tukey's test ($P < 0.05$).

Table 4. Analysis of morphological characteristics of *Botrytis cinerea* and *Botrytis prunorum* isolates from apparently healthy floral tissues and postharvest decayed kiwifruit.

Parameters	*B. cinerea*	*B. prunorum*	*P* value
Conidia production at 20°C (conidia cm^{-2}, 10^4)			
APDA	11.1 (±10.5)	0.5 (±0.5)	< 0.001
PAM	6.6 (±5.9)	0.3 (±0.7)	< 0.001
Sclerotia production at 5°C (n° cm^{-2})			
APDA	2.1 (±3.1)	1.5 (±3.3)	<0.001
Range	0.5-7.6	0.0-7.5	
PAM	2.1 (±3.8)	2.1 (±4.4)	0.08
Range	0.1-8.8	0.0-10.0	
Sclerotia production at 20°C, (n° cm^{-2})			
APDA	1.5 (±1.9)	0.9 (±2.1)	<0.001
Range	0.4-4.8	0.0-4.6	
PAM	0.5 (±0.4)	1.7 (±3.3)	0.38
Range	0.0-0.9	0.0-7.6	
Sclerotia length at 5°C (mm)			
APDA	3.6 (±1.7)	0.6 (±1.3)	0.02
Range	0.7-5.3	0.0-3.0	
PAM	2.9 (±1.0)	2.1 (±2.0)	0.44
Range	1.3-3.7	0.0-4.2	
Sclerotia length at 20°C (mm)			
APDA	5.3 (±2.6)	0.5 (±1.2)	0.01
Range	0.8-7.6	0.0-2.6	
PAM	3.3 (±1.9)	1.3 (±1.9)	0.14
Range	0.0-4.8	0.0-4.2	
Sclerotia width at 5°C (mm)			
APDA	2.8 (±1.19)	0.5 (±1.1)	0.02
Range	0.6-3.43	0.0-2.5	
PAM	2.4 (±0.82)	1.4 (±1.3)	0.18
Range	1.0-3.1	0.0-2.9	
Sclerotia width at 20°C (mm)			
APDA	3.7 (±1.7)	0.5 (±1.0)	0.01
Range	0.7-5.0	0.0-2.3	
PAM	3.0 (±1.8)	1.0 (±1.5)	0.09
Range	0.0-4.3	0.0-3.4	

[y] APDA: Acidified potato dextrose agar, PAM: Pea agar medium.

[z] Mean values of 5 isolates each specie followed by standard deviation in parenthesis.

Table 5. Incompatibility tests of *Botrytis cinerea* and *B. prunorum* isolates obtained from apparently healthy floral tissues and postharvest decayed kiwifruit.

F58-16	106-16	115-16	4-17	31-17	6-16	F102-17	55-16	F75-16	112-16	Isolate	Specie
										F58-16	***Botrytis cinerea***
										106-16	
										115-16	
										4-17	
										31-17	
										6-16	***Botrytis prunorum***
										F102-17	
										55-16	
										F75-16	
										112-16	

Box in black: incompatible, gray: compatible; white: uncertain.

Table 6. Water soaked decay (mm) caused by *Botrytis cinerea* and *B. prunorum* isolates on kiwifruit sampling at harvest and after 45 days of cold storage at 0°C.

Specie/Isolate	Inoculation site														
	Cheek					Stem end					Receptacle				
	Harvest		Storage		Mean	Harvest		Storage		Mean	Harvest		Storage		Mean
Botrytis cinerea															
F58-16	21.8	a	28.9	ab	25.3	8.2	a	9.4	ab	8.8	7.6	ab	7.7	ab	7.7
106-16	21.8	a	32.9	a	27.3	1.6	cd	8.3	abc	4.9	4.2	abcd	8.7	a	6.4
115-16	22.8	a	28.2	ab	25.5	4.8	abc	9.7	a	7.3	2.1	abcd	9.6	a	5.9
4-17	24.4	a	32.7	a	28.5	6.9	ab	9.5	ab	8.2	6.8	abc	9.2	a	8.0
31-17	24.4	a	33.8	a	29.1	7.6	ab	9.8	a	8.7	8.0	a	9.6	a	8.8
Mean	23.0		31.3			5.8		9.3			5.7		9.0		
Botrytis prunorum															
6-16	18.6	a	23.3	bc	21.0	3.0	cd	6.8	bcd	4.9	1.1	cd	4.4	c	2.8
F102-16	18.4	a	21.3	c	19.9	0.0	d	5.6	cd	2.8	2.1	bcd	4.4	c	3.2
55-16	21.2	a	21.8	c	21.5	0.0	d	6.2	cd	3.1	0.0	d	2.7	c	1.4
F75-16	17.1	a	22.3	c	19.7	0.0	d	0.6	e	0.3	0.8	d	3.5	c	2.2
112-16	20.4	a	23.0	bc	21.7	0.0	d	5.1	d	2.6	0.8	d	5.1	bc	3.0
Mean	19.1		22.3			0.6		4.9			1.0		4.0		
ANOVA	Df		F		*P*	df		F		*P*	df		F		*P*
Isolates (I)	9		11.84		<0.001	9		27.60		<0.001	9		15.98		<0.001
Maturity stage (MS)	1		74.34		<0.001	1		117.30		<0.001	1		53.64		<0.001
Interaction (IxMS)	9		74.34		0.02	9		3.55		0.001	9		2.08		0.046

[y] Twelve kiwifruit per isolate were inoculated each inoculation site. Storage: kiwifruit stored at 0°C for 45 days. All kiwifruits were inoculated with a mycelial plug of 4 days-old on the cheek, stem end or receptacle and incubated in a humidity chamber for 5, 7 or 12 days at 20°C, respectively. [z] Values followed by a different letter in the same column means that there are significant differences according Tukey´s test (P ≤ 0.05). the same column means that there are significant differences according Tukey´s test (P ≤ 0.05).

Table 7. Pathogenicity of *Botrytis cinerea* and *Botrytis prunorum* isolates on leaves of 'Hayward' kiwifruit, apples cv. Fuji and grapes cv. Red Globe.

Isolate	Diameter of necrotic lesion (mm)[y,z]			
	Kiwifruit leaves		Apple	Grape
	Wounded	Non-wounded		
B. cinerea				
F58-16	22.8 a	25.9 ab	28.2 bcde	14.7 bc
106-16	26.5 a	19.7 abc	35.4 abcd	12.5 c
31-17	25.3 a	26.8 a	47.1 a	19.5 a
115-16	25.3 a	27.8 a	40.0 abc	13.2 c
4-17	23.3 a	24.7 ab	43.5 ab	18.2 ab
Mean	24.6	25.0	38.8	15.6
B. prunorum				
6-16	13.4 ab	6.7 abc	25.7 cde	7.8 d
112-16	1.8 b	0.0 c	22.9 def	6.0 d
55-17	7.6 b	0.0 c	26.8 cde	6.1 d
F102-16	1.8 b	3.2 bc	8.7 f	10.1 cd
F75-16	0.6 b	0.0 c	20.0 ef	9.7 c
Mean	5.0	2.0	20.8	7.9

[y] Leaves (n=4) were inoculated with a 3 mm diameter of mycelium disc, apples (n=4) and grapes (n=20) were inoculated with 10 µl of a conidial suspension of 10^6 conidia ml^{-1}. Leaves were incubated for 5 days and apples and grapes for 7 days at 20°C.

[z] Values followed by a different lowercase letter in the same column means there are significant difference according to Tukey´s multiple comparison test (≤ 0.05).

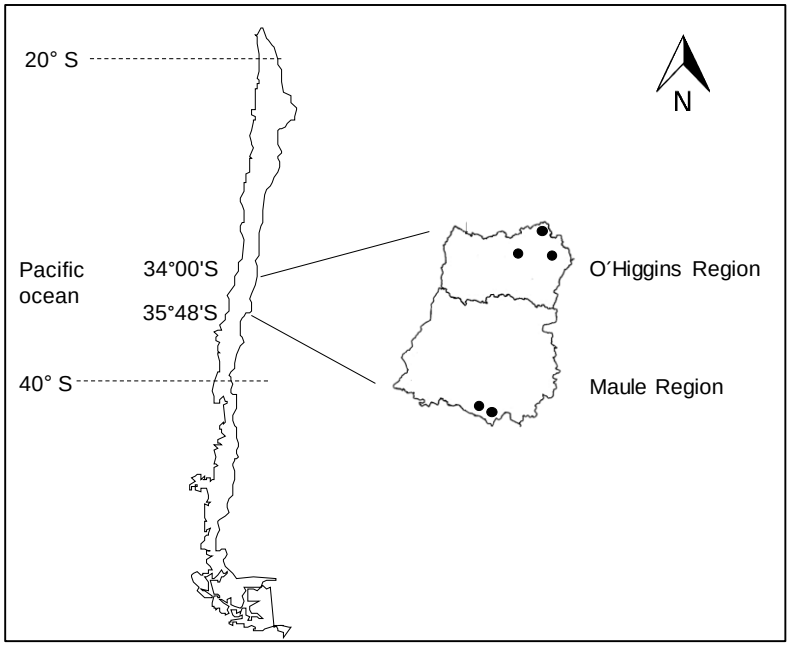

Fig. 1. Map of continental Chile showing the zone central where fruits and flowers with apparently healthy floral parts were sampled from five ‘Hayward’ kiwifruit orchards (three located in O’Higgins Region and two located in Maule Region).

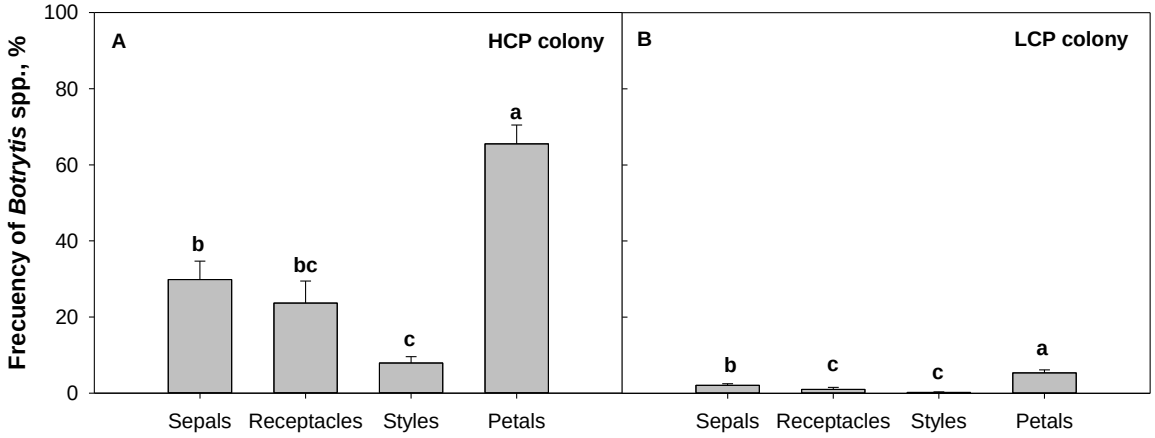

Fig. 2. Frequency of isolation of *Botrytis* colonies from flowers in 2017 season classified as **A**. high conidial producer (HCP, *B*. *cinerea*) and **B.** low conidial producer (LCP, *B*. *prunorum*). Mean values of colonization of sepals (n=1,440) receptacles (n=240), styles (n=1,400) and petals (n=1,440) are presented. Values followed by the same letter did not differ significantly according to Tukey's test ($P < 0.05$). Vertical bar=standard error.

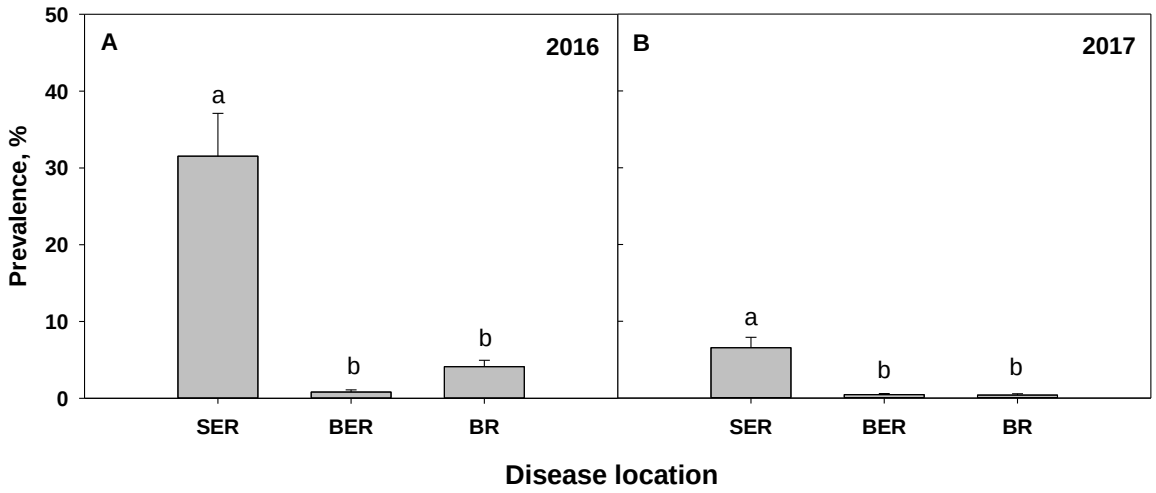

Fig. 3. Prevalence of gray mold analyzed according to the position of the infection: stem end rot (SER), blossom end rot (BER) and body rot (BR). **A.** Prevalence obtained in 2016 season. **B.** Prevalence obtained in 2017 season. A total of 1,600 fruit were evaluated each season. Values followed by the same letter did not differ significantly according to Tukey's test ($P < 0.05$). Vertical bar=standard error.

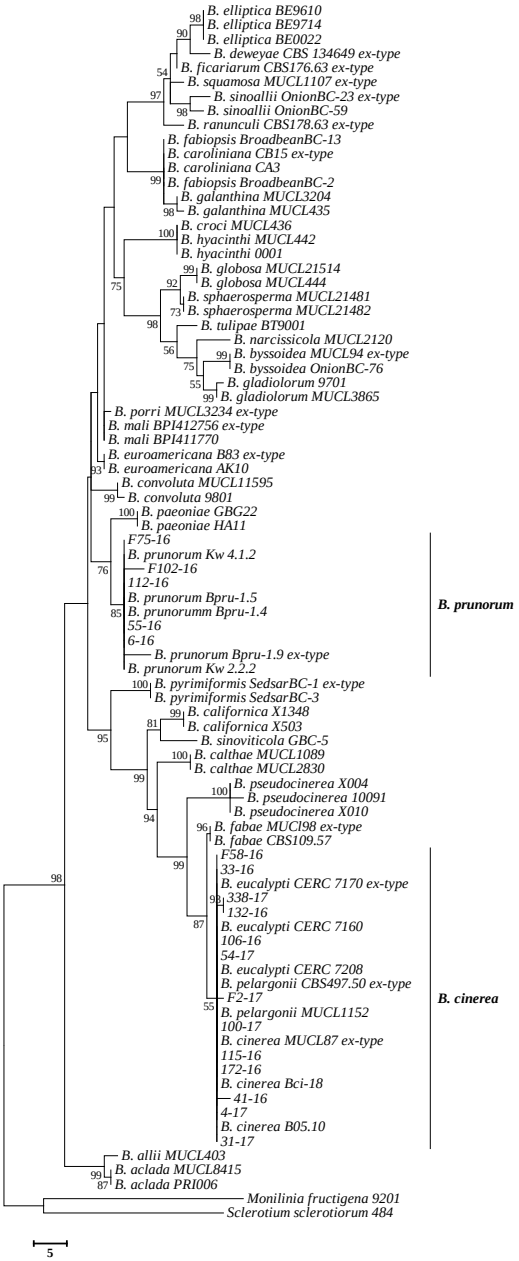

Fig. 4. Phylogenetic analysis of 85 taxa of *Botrytis* was inferred by maximum parsimony method of G3PDH partial gene sequences. Bootstrap support with 1000 replicates, values >50% were shown at the nodes. Isolates highlighted in bold were analyzed in this study. *S*. *sclerotiorum* and *M*. *fructigena* were used as outgroups.

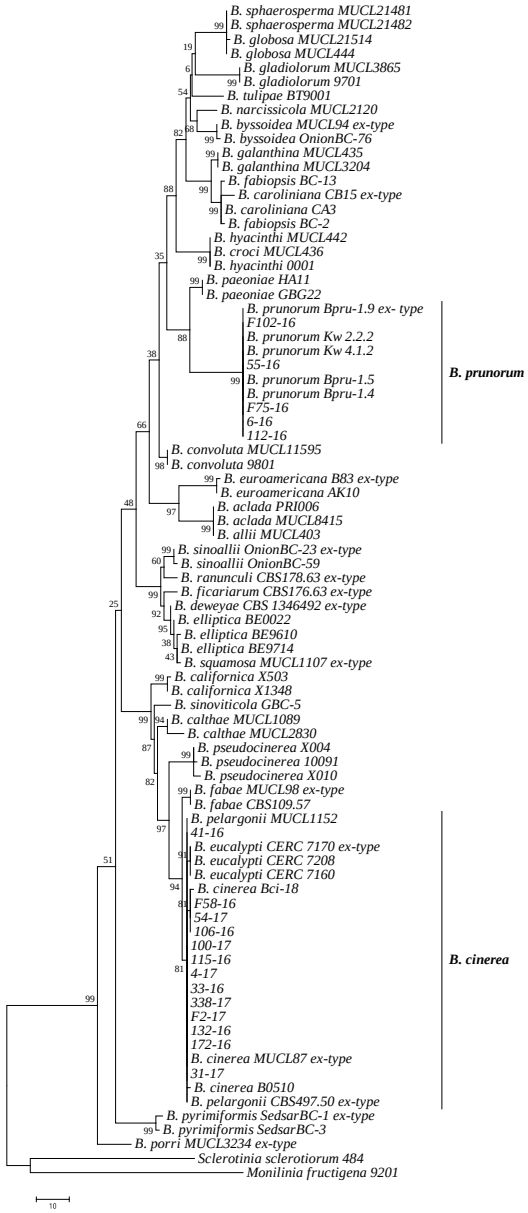

Fig. 5. Phylogenetic analysis of 87 taxa of *Botrytis* was inferred by maximum parsimony method of HSP60 partial gene sequences. Bootstrap support with 1000 replicates, values >50% were shown at the nodes. Isolates highlighted in bold were analyzed in this study. *S. sclerotiorum* and *M. fructigena* were used as outgroups.

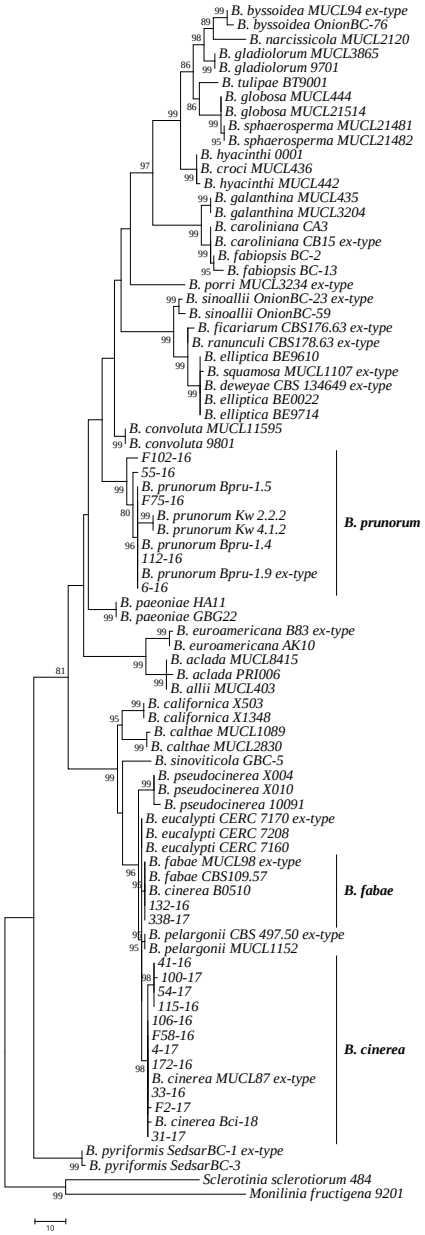

Fig. 6. Phylogenetic analysis of 87 taxa of *Botrytis* was inferred by maximum parsimony method of RPB2 partial gene sequences. Bootstrap support with 1000 replicates, values >50% were shown at the nodes. Isolates highlighted in bold were analyzed in this study. *S*. *sclerotiorum* and *M*. f*ructigena* were used as outgroups.

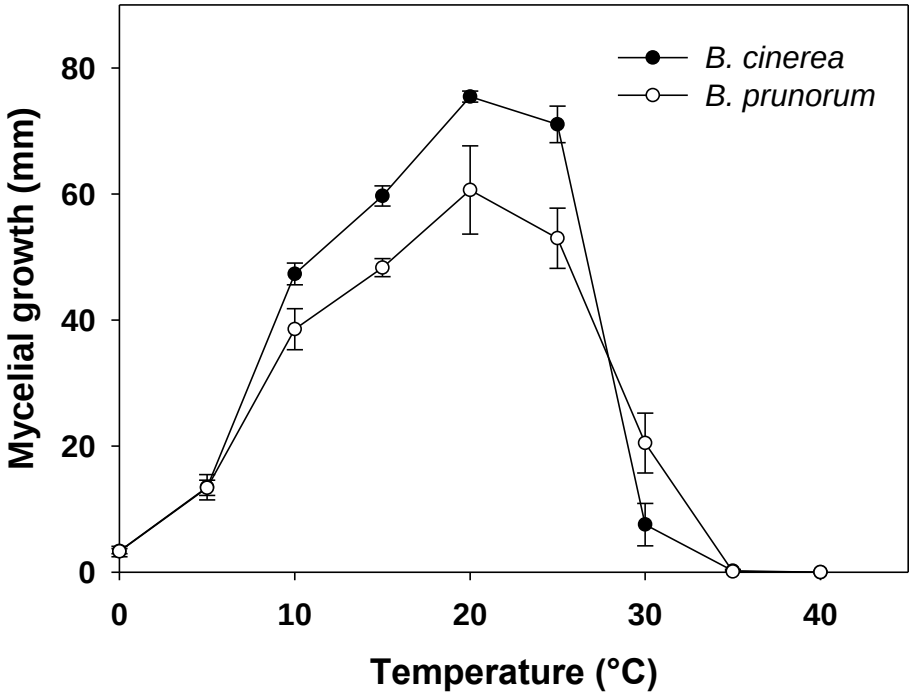

Fig. 7. Effect of the temperature (0°C to 40°C) on the mean diameter of mycelial growth of five isolates of *B. cinerea* and five isolates of *B. prunorum* for 3 days on acidified potato dextrose agar (APDA). Vertical bar = standard error.

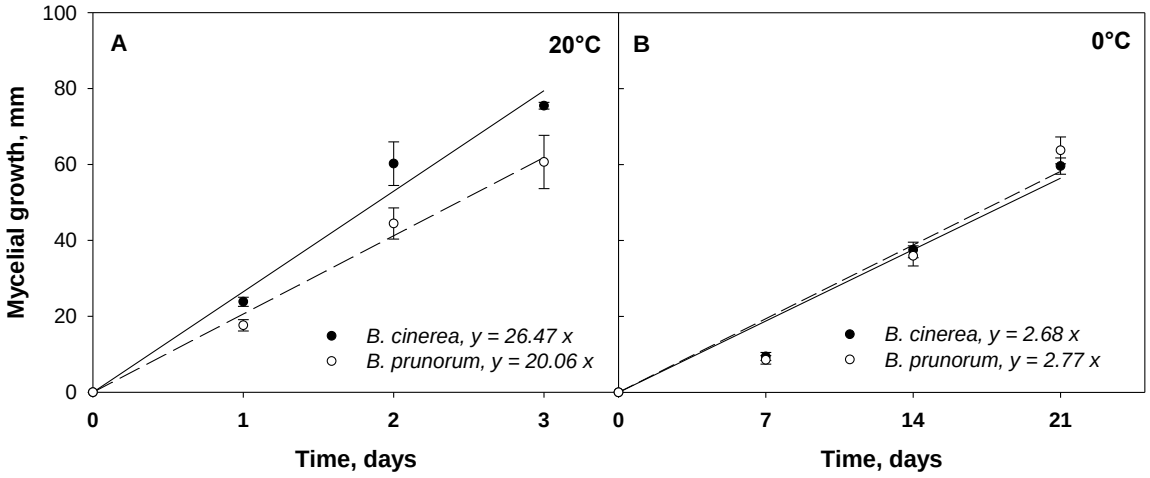

Fig. 8. Effect of the temperature on the mycelial growth of five isolates of *B*. *cinerea* (continuous line) and *B*. *prunorum* (dashed line). A. Mycelial growth daily measured on APDA at 20°C. B. and weekly measured at 0°C. Vertical bar=standard error.

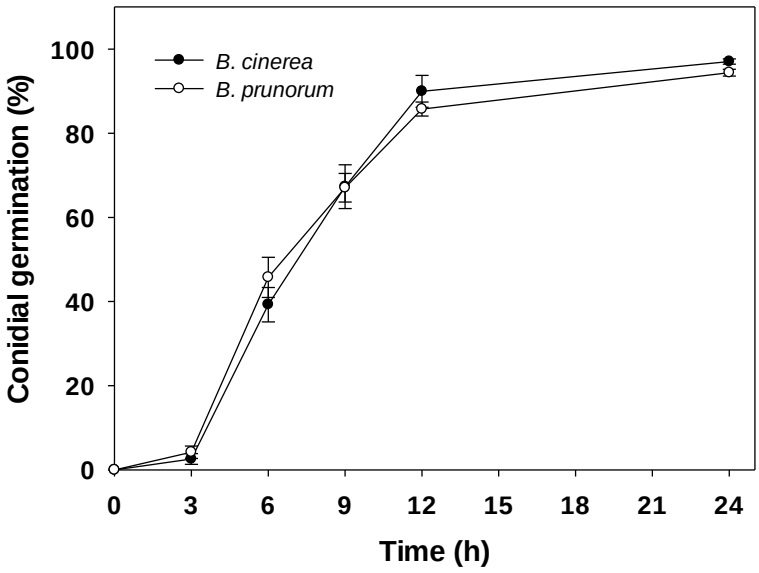

Fig. 9. Conidial germination of *Botrytis cinerea* and *B. prunorum* isolates at 20°C on water agar 1.5%. Values showed corresponded to the average of five isolates each specie. Vertical bar = standard error.

Chapter 3

Postharvest prevalence of stem end rot in 'Hayward' kiwifruit is related to preharvest *Botrytis cinerea* colonization of floral parts and latent infection

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Abstract

Stem end rot (SER) caused by *Botrytis cinerea* is the most important postharvest disease in the Chilean kiwifruit industry. Relationships between the postharvest occurrence of SER in 'Hayward' kiwifruit and the temporal dynamics of earlier *B. cinerea* colonization of the floral parts (petals, sepals, receptacles, styles) was studied in five orchards over two consecutive seasons in Chile. Weather conditions in the first season favored *B*. *cinerea* infection with roughly constant colonization of floral parts up to about 120 days after full bloom but colonization then increased up until harvest. In the second season colonization was roughly constant throughout. Latent infections of the fruit occurred in both seasons but were high in the first season and low in the second. Incidence of latent infections at harvest were the best predictors ($r>0.8$) of postharvest SER. The number of preharvest infection periods calculated using temperature, leaf wetness and relative humidity satisfactorily predicted SER incidence by an exponential model, $R^2 = 0.90$, $P<0.001$. Results indicate environmental variables play key roles in the temporal dynamics of *B*. *cinerea* colonization. Quantification

of latent *B*. *cinerea* infections in asymptomatic fruit close to harvest, is a practicable way to predict later incidence of SER during storage.

Chile is one of the world's largest producers of 'Hayward' kiwifruit (*Actinidia chinensis var. deliciosa* (A. Chev.). A. Chev) and the second largest exporter of kiwifruit in the Southern Hemisphere, with a production area of more than 8,700 ha (www.odepa.cl, 2018). The Chilean kiwifruit is produced primarily for export by ship to distant markets in Europe and Asia (www.prochile.cl, 2018). Hence, it is crucial the storage life of the fruit is as long as possible. Postharvest technologies to complement cold storage, such as curing (holding fruit at ambient temperatures prior to cold storage to promote wound healing), controlled atmosphere storage, modified atmosphere packaging (MAP) and 1-methylcyclopropene (1-MCP) application, are widely used to reduce kiwifruit softening and extend storage life (Lallu and Burdon 2007; Park et al. 2015; Pennycook and Manning 1992; Poole and McLeod 1994). Despite these efforts to maximize fruit quality to market, postharvest decay remains the primary risk factor affecting Chile's kiwifruit trade.

Stem end rot is caused by *Botrytis cinerea* Pers.; Fr. and *Botrytis prunorum* EE Ferrada & Latorre (Elfar et al. 2017). Stem end rot (SER) is the postharvest disease of greatest importance to the kiwifruit industry, and particularly for 'Hayward' (Manning et al. 2010; Park et al. 2015). Losses due to SER are highly variable between seasons and between orchards indicating both weather effects and also microclimate/management effects (Pennycook 1985; Michailides and Morgan 1996). Incidence of SER is around 2% to 3% in New Zealand (Michailides and Morgan 1996; Beever et al. 1984), up to 15% in Chile (Farías 2009) and up to 20% in Italy (Bisiach et al. 1984).

Botrytis cinerea is a polyphagous fungus that has the ability to colonize different tissues within a host (Latorre et al. 2015; Giraud et al. 1999; Martinez et al. 2005). In kiwifruit, *B. cinerea* can colonize flowers, leaves and fruit tissues. These become potential inoculum

sources for SER when environmental conditions are favorable and the fruit is susceptible (Elmer and Pyke 1996; Jarvis 1994). The symptoms of SER occur primarily in stored kiwifruit, while preharvest expression of the disease is uncommon (Pennycook 1985; Sharrock and Hallett 1992).

Similar to other postharvest diseases (Mari et al. 2003; Fourie et al. 2002; Rivera et al. 2013), *B*. *cinerea* infection in kiwifruit occurs in the field, through the colonization of sepals and receptacles relatively early in fruit development and well before harvest (Michailides and Elmer 2000; Michailides and Morgan 1996). It can also occur in the small wound in the stem end caused by picking (Elmer and Michailides 2007; Sharrock and Hallett 1992).

Thus *B. cinerea* produces latent infections that are later activated when the physiological and biochemical conditions of the host and the environmental conditions are optimal for the development of the disease (Michailides et al. 2010; Prusky et al. 2013). Paraquat and/or freezing temperature treatments have been used to demonstrate the presence of non-visible pathogens in apple (Biggs 1995), grape (Holz et al. 2003; Sanzani et al. 2012), peach (Emery et al. 2007), sweet cherry (Adaskaveg et al. 2000), prune (Luo et al. 2001, Luo and Michailides 2001) and other fruit hosts (Fourie et al. 2002; Northover and Cerauskas 1994, Prusky et al. 1981). However, few studies have described latent infections in immature kiwifruit during fruit development or their potential value as predictors of postharvest SER.

Airborne conidial concentrations (ACC) and environmental conditions are two key factors that influence the success of the establishment of a latent infection (Elad and Evensen 1995; Prusky et al. 2013, Verhoeff 1974). Temperature, relative humidity and wetness duration have been described as the main environmental factors that affect the infection and dispersion of *B*. *cinerea* in table grapes (Broome et al. 1995; English et al. 1989), blueberry (Rivera et al. 2013) and strawberry (Bulger et al. 1987; Xu et al., 2000). However, the effects of such factors on SER in kiwifruit have not yet been identified.

In Chile, the major strategies to control SER during cold storage involve applications of chemical products at flowering and/or at harvest (Latorre and Pak, 2003). In addition, postharvest 'curing' is used to induce defense mechanisms within the fruit (Pennycook and Manning 1992; Poole and McLeod 1994; Wurms et al. 1997). However, this control strategy was developed based on epidemiological work carried out on *B. cinerea* under field conditions in New Zealand and the USA (California). However, the climatic conditions in Chile are distinct, so the conclusions from these studies may not apply directly here. Thus, knowledge is needed of the temporal dynamics of *B*. *cinerea* colonization and infection during fruit development in Chile. This new information should permit more appropriate recommendations for SER control and more accurate risk assessments to be made under Chilean growing conditions. The objectives of this study, therefore, were to determine the temporal dynamics of *B. cinerea* colonization in the floral parts and in fruit of 'Hayward' kiwifruit during development and their relationships with SER occurrence during the fruit's subsequent period of cold storage. This study included the somewhat contrasting weather conditions of two sequential growing seasons.

Materials and Methods

Experimental site characteristics

The study was conducted during the 2016 (S1) and 2017 (S2) growing seasons, in the Cachapoal Valley (CV) and the south of the Maule Valley (SMV). Both are major production areas for 'Hayward' kiwifruit in the Central Valley in Chile. These areas have a Mediterranean climate (Csb1) according to the Köpper climate classification (Sarricolea et al. 2017). The historical mean annual temperature range is 14-16°C; the coldest month is July (mid-winter) 8-9°C and the hottest month is January (mid-summer) 20-22°C. The main

rainfall period is concentrated between May to August (august-winter), with the mean annual rainfalls varying between 446 mm in CV and 773 mm in SMV (www.ine.cl).

Five commercial 'Hayward' kiwifruit orchards were selected: three (O1, O2, O3) in the CV and two (O4, O5) in the SMV (Table 1). The vines in all orchards were trained to a pergola system and irrigated and fertilized following the usual agronomic practices for commercial kiwifruit production in Chile (www.comitedelkiwi.cl).

Sampling

Fruit (or flower) sampling and capture of airborne conidia was done every 20 days. In S1 sampling started 60 days after full bloom (DAFB), when fruit diameter was 50 mm and the soluble solid content (SSC) was 4.3 %, and continued until harvest. In S2, sampling was from full bloom, and by 20 DAFB fruits were 20 mm in diameter and SSC was 4.0%, and continued through to harvest when the SSC was 5.5-6.2%. At full bloom, a total of 12 flowers per replicate were collected randomly. Later, fruit sampling used 27 fruit per replicate. Flower and fruit samples were transported to the laboratory in an ice chest and were stored at 10°C (flowers) or 0°C (fruit) until assessment. At harvest, 80 additional fruits per replicate were collected and stored at 0°C. Four replicates were used per sampling day. Sampling was carried out in four rows from 12 to 16 vines. To represent as much of the plot as possible, sampled vines-rows were spaced between 5 and 10 m apart. The same vine-rows were used to determine ACC. At full bloom, in S2, the sampling was carried out before a fungicide application. Due to technical problems, the S1 sampling was conducted from 60 DAFB.

***Botrytis cinerea* identification**

Botrytis cinerea and *B. prunorum* were identified from a total of 10 *Botrytis* colonies obtained from culture of sepals, receptacles, styles, fruit and airborne conidial capture. Identification was based on the colonial characteristics, colony's conidial production and the morphology of the conidia and conidiophores (Ferrada et al. 2016; Mirzae et al. 2008; Pei et al. 2019). The culture medium was potato dextrose agar acidified with 0.5 ml liter^{-1} of 92% v/v lactic acid (APDA) with incubation at 20°C under 12 h light/dark cycle for 10 days. In addition, the morphological identification was corroborated with a molecular study using BLASTn analysis of the HSP60 gene fragment sequences.

Because *B*. *cinerea* was predominantly isolated from ACC, floral part colonization, latent infection and SER (> 90% of the total of *Botrytis* isolates in average), the results obtained were grouped with *B*. *prunorum* and then expressed as *B*. *cinerea* frequency.

Airborne conidial concentration (ACC)

To determine the ACC for *B*. *cinerea*, airborne conidia were captured using a volumetric spore sampler for agar plates (Burkard Manufacturing. Co. Ltd., Ricksmanworth, Hertfordshire, UK). This used a 100-hole sieve plate and three agar Petri dishes (90 mm diameter) per replicate. The spore sampler was operated once per plate for 30 s (20 liter min^{-1}) around midday and was located in two vine-row spaces, 1.8 m above the ground near the fruit and away from senescent leaves (Michailides and Elmer 2000; Mundy et al. 2012). The Petri dishes contained APDA plus 0.1% v/v Igepal CO-630 (Sigma-Aldrich, Atlanta, GA) and were incubated for 7 to 10 days at 20-22°C, with a 12 h day/night light cycle. Colony counts of *B*. *cinerea* were expressed as colony forming units per cubic meter of air (CFU m^{-3}) (Fernández et al. 2011).

***Botrytis cinerea* colonization in floral parts and latent infection in fruit.**

Floral parts

To determine the presence of *B. cinerea* in floral parts of kiwifruit, petals (n=72), sepals (n=72), styles (n=72) and receptacles (n=12) were removed from 12 flowers or fruits per replicate and placed in labeled Petri dishes containing APDA plus 0.1 % v/v Igepal CO-630. Before plating, the various floral parts were surface-disinfected (1% w/v sodium hypochlorite, plus 0.001% v/v Tween 80 for 2 min), washed in sterile distilled water for 1 min and dried in a laminar flow hood. Plates were incubated for 10 days at 20-22°C under 12 h light. The results are reported as the percentages of floral parts colonized of the total number of samples.

Incidence of latent infections in fruit

Latent infections in fruit were determined using the overnight freezing incubation technique (ONFIT). Apparently healthy fruit (n=15 per replicate) were randomly collected with peduncle attached on each sampling date and gently hand brushed to remove all senescent floral debris (necrotic petals, stamens and styles). Fruit were then surface-disinfected as was described for floral parts, washed in sterile water for 1 min, dried for 15 min in a laminar flow and frozen for 24 h at -20°C (Holz et al. 2003; Sanzani et al. 2012). Subsequently, fruit were incubated in a moist chamber at 20°C. Infection by *B*. *cinerea* was confirmed by noting the presence of soft decay with light brown pulp and the abundant gray aerial mycelia at the stem end. The kiwifruit infection was assessed after 6 days, and symptomatic fruit were retained for an additional 4 days, awaiting corroboration by sporulation. Pieces of tissues were immediately taken from under the receptacle from asymptomatic and symptomatic fruit, plated on APDA for 7 days at 20°C to determine the presence of *B*. *cinerea*. The development of symptoms of SER and the signs of *B*. *cinerea* in apparently healthy fruit after the freezing treatment were considered to be latent infections.

Incidence of stem end rot during storage

Stem end rot incidence in kiwifruit was evaluated after 100 days of storage at 0°C plus two days at 20°C from totals of 320 fruit per orchard. Fruit were hand brushed and their peduncles removed by hand, cured for 24 h at 20°C and packed in 10 kg capacity cardboard boxes with 60-µm thick, low-density polyethylene modified atmosphere bags (MAP, Fresh-Fresh Kiwi, San Jorge Packaging, Santiago, Chile). The fruit were stored in an ethylene-free environment produced by passing the incoming air stream through a potassium permanganate (Bioconservacion, BCN, Spain) ethylene scrubber. The ethylene concentration was monitored every two days, maintaining ethylene concentrations consistently at <0.005 µl liter^{-1} (Zoffoli et al. 2016). Stem end rot incidence was expressed as the percentage of diseased fruit.

Microclimate characterization

Considering that environmental conditions are relevant variables affecting pathogen infection; temperature, relative humidity, leaf wetness and rainfall were monitored in each orchard using sensors located in horizontal position, 1.8 m above the ground and below the kiwifruit canopy. Temperature and relative humidity were recorded using a data logger (U12 Temp / RH / 2 External Channel Logger, HOBO Onset, Computer Corporation, MA, USA). Leaf wetness duration (WET) was recorded using a dielectric leaf wetness sensor (Echo Decagon Devices, WA, USA) and the times were expressed in hours. All devices recorded at 15 min intervals from 60 DAFB in S1 and from full bloom to harvest in S2. Mean daily temperature (Tm, ±0.35°C) and mean relative humidity (RH, ±2.5%) were calculated. Vapor pressure deficit (*Vpd*, kPa) was calculated as (Kaye and Laby 1957):

$$Vpd = Vp\left(1 - \frac{\mathrm{RH}}{100}\right)$$

Where water vapor pressure (*Vp*) was:

$$Vp = 4.6698\, e^{0.06241\,\mathrm{Tm}}$$

Growing degree days (GDD) were calculated from full bloom to harvest using Tm with a threshold value of 10°C (Salinger and Kenny 1995). Rainfall data (mm), Tm and HR from full bloom to 60 DAFB in S1 were obtained from weather data recorded by meteorological stations located in each of the orchards.

Infection periods

The weather data collected were used to calculate the number of infection periods for *B. cinerea* that occurred within each 20-days period from full bloom. The infection periods were determined using the model proposed for *B*. *cinerea* infection on table grapes (Broome et al. 1995). One infection period was recorded if the temperature fluctuated between 14 and 25°C and if leaf wetness occurred continuously for 6 h or more. An infection period was *not* counted if during such a period, the conditions favoring infection (described above) were interrupted continuously for >4 h.

Statistical analyses

The dynamic of ACC and colonization of sepals, receptacles, styles and latent infection in fruit during the growing season (DAFB) were explained with best-fit models using $P \leq 0.05$.

Stem end rot differences between orchards were determined by a one-way analysis. Mean differences were separated using the Fisher Least Significant Difference (LSD) test ($P \leq 0.05$). Percentage values were transformed an arcsine square root transformation of the proportion, prior to analysis

Pearson´s correlation was used to determine associations between ACC, *B*. *cinerea* colonization in sepals, receptacles, styles, latent infection in fruit and SER incidence with ($P \leq 0.05$).

The relationship between the accumulated number of infection-risk periods and SER at harvest was determined using the best statistical model.

The analyses were carried out using the statistical software SigmaPlot v 12.5 (Systat Software Inc., San Jose, California, USA).

Results

Botrytis cinerea identification

Botrytis cinerea (n=5) and *B*. *prunorum* (n=5) were identified from isolates cultured in APDA obtained from airborne conidial capture plates, petals, sepals, receptacles, styles and fruit. *B*. *cinerea* colonies were white to gray and cottony with abundant sporulation on erect and free conidiophores, branched at the top with ellipsoidal, ovoid to globose non-septated and hyaline conidia of size 9.2 ± 0.8 x 7.1 ± 0.9 µm. *Botrytis prunorum* colonies were white, fluffy and cottony with nil to low sporulation in erect conidiophores with ellipsoidal to ovoid conidia with a slightly protuberant hilum of size 11.8 ± 1.6 x 6.9 ± 0.9 µm. The *B. prunorum* counts were combined with the *B. cinerea* ones because of the low colonization values obtained (< 3%). A BLASTn search analysis using the HSP60 fragment gene sequence of all isolates in the study corroborated the identity of between 99 to 100% of *B*. *cinerea* and *B*. *prunorum*.

Microclimate conditions

In both seasons, CV and SMV experienced a typical Mediterranean summer (Table 2). The GDD from full bloom to harvest varied from 1,324 to 1,379 GDD in S1 and from 1,189

to 1,373 GDD in S2. The average daily maximum temperature was recorded between December and February (60-100 DAFB) averaging 19.8±0.9°C in S1 and 20.1±1.3°C in S2.

Rainfall was greater in S1 than in S2. The total accumulated rainfall in S1 was 125 mm in CV and 83 mm in SMV. In the last period before harvest rainfall was 104.7 mm in CV and 60.2 mm in SMV. In contrast, no important rainfall events occurred in CV (2.9 mm) or SMV (41.0 mm) during S2.

The average of accumulated WET duration in CV was 865 h (S1) and 662 h (S2), while the equivalent values for SMV were 788 h (S1) and 371 h (S2) (Table 2).

Airborne conidial concentration

The concentrations of airborne *B*. *cinerea* conidia averaged 674 CFU m^{-3} (from 60 DAFB to harvest) in S1 and 177 CFU m^{-3} (from 0 DAFB to harvest) in S2 (Fig. 1).

In S1, the ACC temporal dynamics were similar between CV and SMV, these are both satisfactorily fitted by quadratic regressions ($P < 0.001$). The concentrations were always higher in CV than in SMV, the averages were 685 CFU m^{-3} in CV and 288 CFU m^{-3} in MSV. The highest concentrations were obtained at the end of the season with maxima of 2,293 CFU m^{-3} in CV and 770 CFU m^{-3} in MSV (Fig. 1A). In S2, mean ACC values were 3.8-fold lower than those in S1 and were similar between CV and MSV throughout the season. The highest values of ACC in S2 occurred on 80 DAFB in CV (352 CFU m^{-3}) and on 60 DAFB in MSV (379 CFU m^{-3}) while the lowest values were at harvest in CV (27 CFU m^{-3}) and at 120 DAFB in MSV (41 CFU m^{-3}) (Fig. 1B).

***Botrytis cinerea* in floral parts and latent infections in fruit**

Floral parts

Botrytis cinerea was consistently isolated from apparently healthy petals, sepals, receptacles and styles (Fig. 2). The temporal dynamics differed significantly between seasons (S1 and S2) and locations (CV and SMV).

Across all floral parts, the levels of *B*. *cinerea* found in the CV orchards were similar or higher than those in the SMV orchards. This was true across both seasons. Levels were either constant or increased towards harvest in S1. However, no clear temporal pattern emerged during S2. In S1 and S2, respectively, the mean frequencies of isolation were: 15.1 and 21.4% in the sepals, 24.0 and 26.1% in the receptacles and 14.8 and 6.4% in the styles. In S2, the average colonization of petals by *B*. *cinerea* was 70.9%.

In S1, the temporal dynamics of *B*. *cinerea* in sepals, receptacles and styles were significantly explained by quadratic regressions, with the exception of the sepals in SMV, where little variation occurred with time. In the sepals, the frequency of isolation averaged 18.8% with a slight increase at harvest (Fig. 2C). In the receptacles, the frequency of *B*. *cinerea* averaged 25.6% in CV and 22.4% in SMV (Fig. 2E). The highest levels of *B*. *cinerea* in the styles was at harvest when the levels averaged 17.0% in CV and 13.2% in MSV (Fig. 2G).

In S2, the temporal dynamics of *B*. *cinerea* either decreased or was constant with time. The isolation frequency in the sepals decreased linearly from bloom (45.9%) to harvest (14.6%) in CV ($R^2 = 0.32$, $P < 0.001$) (Fig. 2D). The isolation frequency through the season remained constant in SMV, averaging 12.7%. In the receptacles, no significant relationship was found between *B*. *cinerea* isolation frequency and time in CV (Fig. 2F). Meanwhile, in MSV, the isolation frequency increased between 80 and 120 DAFB and then decreased to harvest. In the styles, the *B*. *cinerea* isolation frequency remained low and constant in both CV and MSV (Fig. 2.H).

Incidence of latent infection in fruit

During S1, fruit with latent *B*. *cinerea* infections developed abundant aerial mycelia and light brown pulp in the stem end at each sampling day. The averages across sampling days were 49.8% in S1 and 2.7% in S2.

In general, similar latent infections occurred with time in CV in S1, values ranging from 40 to 60% until harvest. Meanwhile, in SMV, the latent infection rate decreased from 65% and to 40% (Fig. 3A). In S2, the latent infection rates were low (<10%), remained constant with time and were not significantly explained by a linear regression (Fig. 3B).

Stem end rot incidence in storage

Stem end rot developed in fruit stored at 0°C for 100 days with significantly different incidences between seasons and orchards. Mean incidence varied between seasons, being 31.5% in S1 and 6.6% in S2 ($P < 0.001$). In S1, the SER incidence varied between a low of 1.9% in O4 and a high of 60.3% in O3. In S2, the mean incidence was 2.8-fold lower than in S1 and did not differ significantly between orchards (P = 0.14) (Fig. 4).

Infection periods

Environmental conditions favoring *B*. *cinerea* infection were recorded from full bloom to harvest in S1. The number of infection periods ranged from a maximum of 18 in O3 to a minimum of 11 in O4 which were concentrated at 80 DAFB and at harvest (Figure 5A). At least one period of infection occurred during each time interval except for from 0 to 40 DAFB in which none were recorded. The second season was drier and less conducive to infection. There were four infection periods in O1, O4 and O5 and none in O2 and O3 (Figure 5B). A positive relationship between the cumulated number of infection periods from full bloom to harvest and of SER in cold storage was observed (r = 0.95). This relationship is best represented by the exponential model (y=exp (0.23 x), $R^2 = 0.90$, $P < 0.001$) where x = the

cumulative number of infection periods and y = the incidence of SER (%) (Fig. 6). To illustrate this positive relationship by two examples, when the number of infection periods exceeded 15, the percentage of infected fruit was approximately 30%, while with when there were 17 infection periods, the percentage of infected fruit exceeded 50%.

Relationships between colonization in floral parts and latent infections in fruit with stem end rot incidence during storage

Pearson´s coefficients (r) were used to describe the relationships between *B*. *cinerea* colonization in floral parts or as latent infections in fruits observed from full bloom or from 60 DAFB to harvest and the subsequent SER incidence during storage in both seasons. In S1, the relationships were positive and significant with the highest values between 140 DAFB and harvest (Fig. 7A). In S2, significant relationships were found between colonization and SER in the sepals (r = 0.47) and the styles (r = 0.47) at full bloom and the styles at harvest (r = 0.56) (Fig. 7B). To obtain a more robust result, given the two contrasting seasons, the S1 and S2 data were combined and used to determine the relationship between SER and the presence of *B*. *cinerea* in each tissue and at each sampling date. The significance of Pearson´s coefficients for all variables increased from full bloom to the end of the season, with the highest values at harvest (Fig. 7C). All variables at harvest were significantly related to the postharvest incidence of SER. The style colonization was positively and significantly related to SER from 120 DAFB until harvest (r = 0.75) (Fig. 7C). Receptacle colonization was significantly related to SER only at harvest while sepal colonization was significantly relater to SER only at full bloom (only measured in S2) and at harvest (both seasons). The most significant (r = 0.8) and consistent relation was between latent *B*. *cinerea* infections of the fruit through the season and SER.

Discussion

The temporal dynamics of *B*. *cinerea* colonization associated with floral parts and fruit was studied during fruit development, in five 'Hayward' kiwifruit orchards in two regions and over two growing seasons (2016, 2017). Using this information, we were able to identify the best 'indicator' tissue, e.i. latent infections of fruits, to predict the later incidence of SER during cold storage. *Botrytis cinerea* colonized all floral parts and key sources of inoculum associated with SER were the style from 120 DAFB until harvest, the receptacle at harvest and the sepals at full bloom and harvest. However, environmental conditions affected the temporal dynamics, inoculum density of *B*. *cinerea* in the orchard and the most important factors appear to be episodes of rainfall and surface wetness. Our study indicates that the presence of *B*. *cinerea* in all floral parts and latent infections were related with SER in S1. However, in S2 barely significant relationships were obtained because of the low incidence of SER. Also, latent infection incidence was positively and significantly related to SER incidence from 60 DAFB to harvest when data from both seasons were pooled and analyzed.

Notwithstanding the application of a botryticide at full bloom in both seasons, the colonization of *B*. *cinerea* was demonstrated in all floral parts evaluated. This result indicates *B*. *cinerea* is a component of the mycoflora of flowers and fruit and that it is able to survive through the season within these inoculum sources. In this study, the temporal dynamics of *B*. *cinerea* in S1 was similar to the results obtained by Michailides and Morgan (1996) in California, with the highest values at the end of the season. The high wetness duration due recurrent rainfall events along with warm temperature (14°C to 25°C) before harvest would seem to explain the high values of colonization at the end of S1 and the beginning of the following season. In grapes, Hill et al. (2019) showed that RH and surface wetness duration were key environmental determinants of botrytis epidemics and similar results were obtained by Rivera et al. (2013) in blueberry.

Weather conditions and cultural practices (Snelgar et al. 1998) both influence fruit microclimate and so directly affect the colonization of *B*. *cinerea* (Latorre et al. 2015; Elmer and Pyke 1997; English et al. 1989; Valdés-Gómez et al. 2008). Favorable environmental conditions as temperatures between 15°C and 20°C along with relative humidity > 65.5% then lead to sporulation and, in presence of free moisture, the subsequent infection of the fruit (Ciliberti et al. 2016). For example, our orchards in SMV (O4, O5) had less dense canopies and the lowest cumulated infection periods at harvest and thus, had the lowest colonization by *B*. *cinerea* in sepals, styles and ACC in both years and the lowest SER incidence in S1, reaffirming the prime importance of environmental conditions to *B*. *cinerea* infection. Therefore, strategies with a holistic approach in order to reduce the infection rate during the season in kiwifruit orchards are suggested.

The analysis of leaf wetness duration in the CV and SMV orchards integrated with Tm in an algorithm allowed quantified conditions favoring *B*. *cinerea* infection in both S1 and S2. Similar results were found by English et al. (1989) who distinguished two microclimates under grapevine canopies when temperature, vapor pressure, wind speed and leaf wetness are included together in the analysis.

After ONFIT, previously asymptomatic kiwifruit developed abundant aerial mycelia and light brown pulp in the stem end, demonstrating that *B*. *cinerea* infections occur during fruit development. These latent infections were shown to be strongly associated with the development of SER during storage (r=0.8). Overall the incidence of latent infections was high in S1 (49.8%)with a high value (55.0%) at the beginning of the sampling date (60 DAFB) in S1, with multiple rain events and free moisture before full bloom and 20 DAFB but these resulted in low cumulative infection periods (0-2). Latent infections of fruit by *Botrytis* spp. have been described in grapes (Holz et al.2003; Sanzani et al. 2012) with full bloom being one of the periods when infection is most likely to occur (Keller et al. 2003; Nair et al. 1995). The results suggest that fruit susceptibility to *B*. *cinerea* was high before 60 DAFB which

agrees with the results of Riquelme et al. (2017), who reported that kiwifruit at 20 DAFB was susceptible to controlled inoculation of *Botrytis* spp. They also claim that immature fruit are more susceptible to *Botrytis* infection than mature ones, a result also observed by Wurms et al. (1998). However, this result does not clarify the relationship of the infection in flowering and the early stages of fruit with the appearance of SER during storage as has been explained in some other fruit species, such as table at flowering (Keller et al. 2003; Nair and Allen 1993).

Models using microclimatic factors to predict *Botrytis* infections in a range of hosts have been developed both under controlled conditions (Broome et al. 1995; Bulger et al. 1987) and under field conditions (Calvo-Garrido et al. 2014; Carisse et al. 2017). Similar to Broome et al. (1995) with table grapes and Rivera et al. (2013) with blueberries, we found an exponential model best explained the relation between the number of infection periods (14°C<T<25°C, >90% RH along with at least 6 h of wetness duration) and the incidence of postharvest decay. Our results show that with zero infection periods in S2 a few infected kiwifruits were nevertheless found during storage. This suggests environmental factors other than the ones we considered could be involved (Blanco et al. 2006; English et al. 1989). The inclusion of ACC has been found to improve models based on microclimatic climatic factors that predict *Botrytis*-caused diseases (Blanco et al.2006; Carisse et al. 2017; Xu et al. 2000). Also, Carisse and McNealis (2018), reported a significant correlation between ACC and losses produced by *Botrytis* fruit rot in strawberry. In our results, the ACC differed between seasons, with higher mean values in S1 and lower ones in S2 which also fits with the SER incidences in the two seasons. The ACC changes daily depending on host tissue (Jaspers et al. 2016; Ciliberti et al. 2016) and environmental conditions favorable to sporulation and conidial release (Blanco et al. 2006; Sosa-Alvarez et al. 1995). A more dynamic model is required to explain the interaction factors associated with latent infections.

Since the need to predict SER is important, so that control measures can be implemented under high risk, correlation analysis using different tissues have been proposed. Among these, the percentage of floral parts colonized by *B*. *cinerea* (Michailides and Morgan 1996; Elmer and Pyke 1997) and the inoculum density in leaf tissues (Manning et al. 2010) have proved good SER predictors in kiwifruit. Michailides et al. (2010) described latent infections in fruit as useful 'indicators' of a number of factors that might affect disease development in the orchard. These will include environmental conditions (Mari et al. 2003; Michailides and Morgan 1996) as well as cultural practices.

According to the relationship between the floral parts analyzed and SER incidence, style colonization showed the most positive, and significant correlations with SER during the whole of S1, and also at full bloom and at harvest in S2. Nevertheless, this does not necessarily mean that style colonization is the most important inoculum source causing SER. On the contrary, the styles were less frequently colonized compared with the sepals and receptacles. This finding is supported by the previous work which indicates stylar rot in kiwifruit is unusual (Bisiach et al. 1984) and is not associated with postharvest SER (Fermaud and Gaunt 1995). Therefore, these results reaffirm that colonization of floral parts may more closely reflect the environmental conditions of the season (Michailides et al. 2010).

It is important to realize that a kiwifruit is the results of the pollination of numerous styles (about 35) and reflects the fecundity of perhaps 1000 ovules. Lastly, it is worth noting that petals fall just a few days after flowering, whereas the sepals and styles are retained by the fruit through to harvest offering a substrate for *B. cinerea* throughout this roughly five-month period from bloom to harvest. Hence these sources, rather than the very temporary petals, are more likely to infect the fruit. Symptoms of *Botrytis* infection during storage appear at the stem end, suggesting that it is the sepal and receptacle tissues that are contact with the fruit. Of course, due to variable environmental conditions and the fruit's own disease defense

mechanisms, not all these infected tissues will result in SER (Sharrock and Hallett 1992). Sepals and receptacle were significantly correlated with SER in this study during S1 similar to the results of a Californian study (Michailides and Morgan, 1996). Also SER infections rarely occur in some Chinese *Actinidia* cultivars (yellow-fleshed) such as 'Hort16A' or 'Jintao' where sepals hardly remain on the fruit (Manning et al. 2003).

In Chile, recording of incidence of latent infections in kiwifruit can be indicative of years in which high incidence of SER (S1) will occur, or of low ones (S2). This advance warning of likely high or low SER is at least two months after full bloom and some three months before harvest. This is similar to the timing of analysis of methods recommended using level of colonization of *B*. *cinerea* in sepals and receptacles as predictors of SER (Michailides and Morgan 1996). Nevertheless, it is likely that not all infections on floral parts will result in latent infections of the fruit and then in a subsequent rot (Sanzani et al. 2012). In addition, the presence of latent infection by B. cinerea in grape berries using a quantitative real-time PCR (qPCR) detection method was demonstrated and with an efficiency higher than obtained from freezing technique (Sanzani et al. 2012) even at low inoculum pressure. However, values obtained from qPCR technique was highly correlated with freezing technique (Sanzani et al. 2012), indicating that both methods were similarly reliable. However, qPCR is an expensive method which require skilled expertise, specific facilities to be apply.

In summary, *B*. *cinerea* colonization of the floral parts remaining on the fruit was present during the whole season and was able to harbor latent infections in fruit early in the season. Microclimatic conditions played a significant role in the SER epidemiology, affecting the temporal dynamics of colonization and inoculum density by *B*. *cinerea* and determined the occurrence of conducive season to infection or unfavorable season as S1 instead of S2. Furthermore, latent infections detected by ONFIT from 60 DAFB in kiwifruit might be a valuable, practical and straightforward tool for the early and accurate prediction of postharvest SER and might be useful to producers, to identify plots or orchards with a

potential high SER incidence and, timely to apply control managements and take properly decisions about the storage duration or proximity of destination markets. Nevertheless, we suggest further evaluation of this method is undertaken using fruit from multiple orchards, to more precisely determine the critical values needed to estimate the risk of significant losses due to SER during subsequent storage.

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Table 1. Characteristics of the 'Hayward' kiwifruit orchards used in the study

Characteristics	Cachapoal Valley			South of Maule Valley	
	Orchard 1	Orchard 2	Orchard 3	Orchard 4	Orchard 5
Planting year	1994	2006	2006	2004	2004
Location	34°00'S, 70°42'W	34°20´S, 70°42´W	34°22´S, 70°54´W	35°48'S, 71°48'W	35°48'S, 71°49'W
Plot (hectare)	6.4	1.8	4.7	3.5	6.0
Row length (m)[w]	193.4 (0.6)	100 (3.4)	123.8 (44.6)	112.2 (140.6)	328.5 (11.6)
Vine Spacing (m x m)	5.0 x 3.5	3.5 x 3.0	4.7 x 2.5	3.5 x 3.0	3.5 x 3.0
Light interception (%) [v,w]	94.7 (2.7)	88.6 (8.1)	91.5 (1.8)	77.0 (12.7)	88.6 (2.3)
Irrigation system	micro-sprinkler[y]	drip irrigation	drip irrigation	micro-sprinkler[x]	micro-sprinkler[x]
Fungicide program[z]	Iprodione	Iprodione	Fenhexamid	Iprodione	Iprodione

[v] Light interception based on the point grid method (Wünsche et al. 1995). Percentage of shadow projected under the canopy on a grid of 19 to 35 frame of 1 x 0.5 m. Each frame was photographed and the percentage of shaded area was calculated over the total area using the IMAGE J 1.49v software (Rasband, National Institute of Health, USA) (Zarate-Valdez et al. 2015). Values shown from S2 at harvest.

[w] Values in parenthesis corresponded to standard desviation.

[x] The micro-sprinklers were 30 cm above the ground

[y] The micro-sprinklers were 100 cm above the ground.

[z] Iprodione (Rovral 4Flow, Bayer; 2.5 liter ha^{-1}) or fenhexamid (Teldor, Bayer; 1.2 liter ha^{-1}) were applied at full bloom and one week before harvest in both growing seasons at rates recommended to control gray mold control.

Table 2. The microclimate of 'Hayward' kiwifruit orchards in Cachapoal Valley and South of Maule Valley from full bloom to harvest in S1 (2016), and S2 (2017)

Valley	GDD (°C) [v]		Tm (°C)[w]		RH (%)[x]		Vpd (kPa)[y]		WET (h)[z]	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Cachapoal Valley (CV)										
O1	1,324	1,373	18.2	18.3	74.7	75.3	3.9	3.7	1,130.9	499.6
O2	1,354	1,189	18.3	18.7	71.1	73.4	4.5	4.0	680.8	1190.0
O3	1,379	1,360	18.2	18.9	71.6	70.4	4.4	4.6	784.6	177.7
Mean	**1,353**	**1,307**	**18.2**	**18.6**	**72.5**	**73.0**	**4.3**	**4.1**	**865.42**	**622.44**
South of Maule Valley (SMV)										
O4	1,359	1,373	17.7	20.1	73.6	67.0	3.9	5.4	790.5	245.8
O5	1,311	1,359	17.8	19.6	73.5	68.1	3.9	5.1	785.5	496.3
Mean	**1,335**	**1,366**	**17.7**	**19.8**	**73.6**	**67.6**	**3.9**	**5.2**	**788.0**	**371.1**

[v] Growing degree day accumulation base 10°C from full bloom to harvest.

[w] Mean daily temperature (Tm).

[x] Mean daily relative humidity (HR)

[y] Vapor pressure deficit (Vpd)

[z] Accumulated leaf wetness duration (WET) from full bloom to harvest.

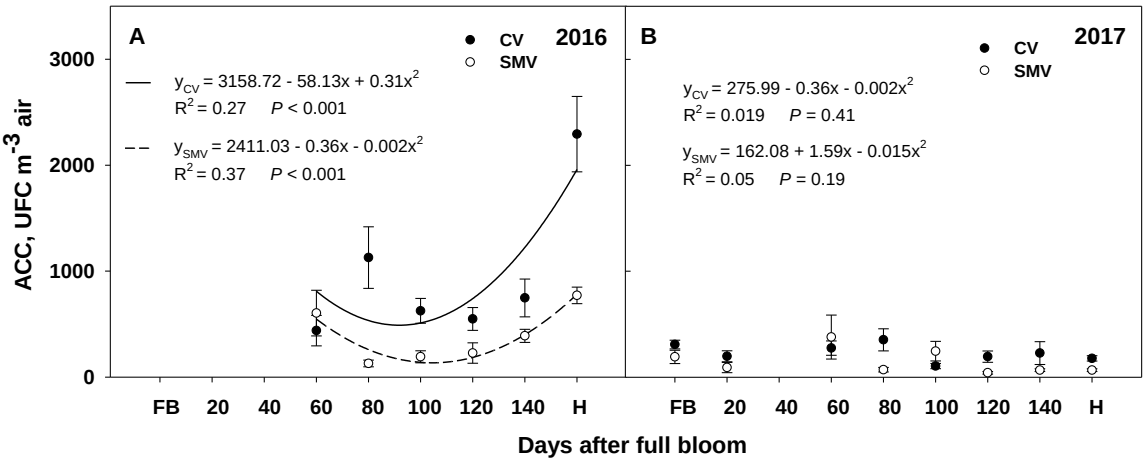

Fig. 1. Mean airborne conidial concentration (ACC) of *Botrytis cinerea* of five 'Hayward' kiwifruit orchards located in Cachapoal Valley (CV: Orchard 1, 2 and 3) and South of Maule Valley (SMV: Orchard 4 and 5) from full bloom (FB) to harvest (H). **A**. Season 2016 (S1). **B**. Season 2017 (S2). Mean values were obtained from the average of 4 replicates of 3 measures each. Vertical bar = standard error. Regressions were significant when $P \leq 0.05$.

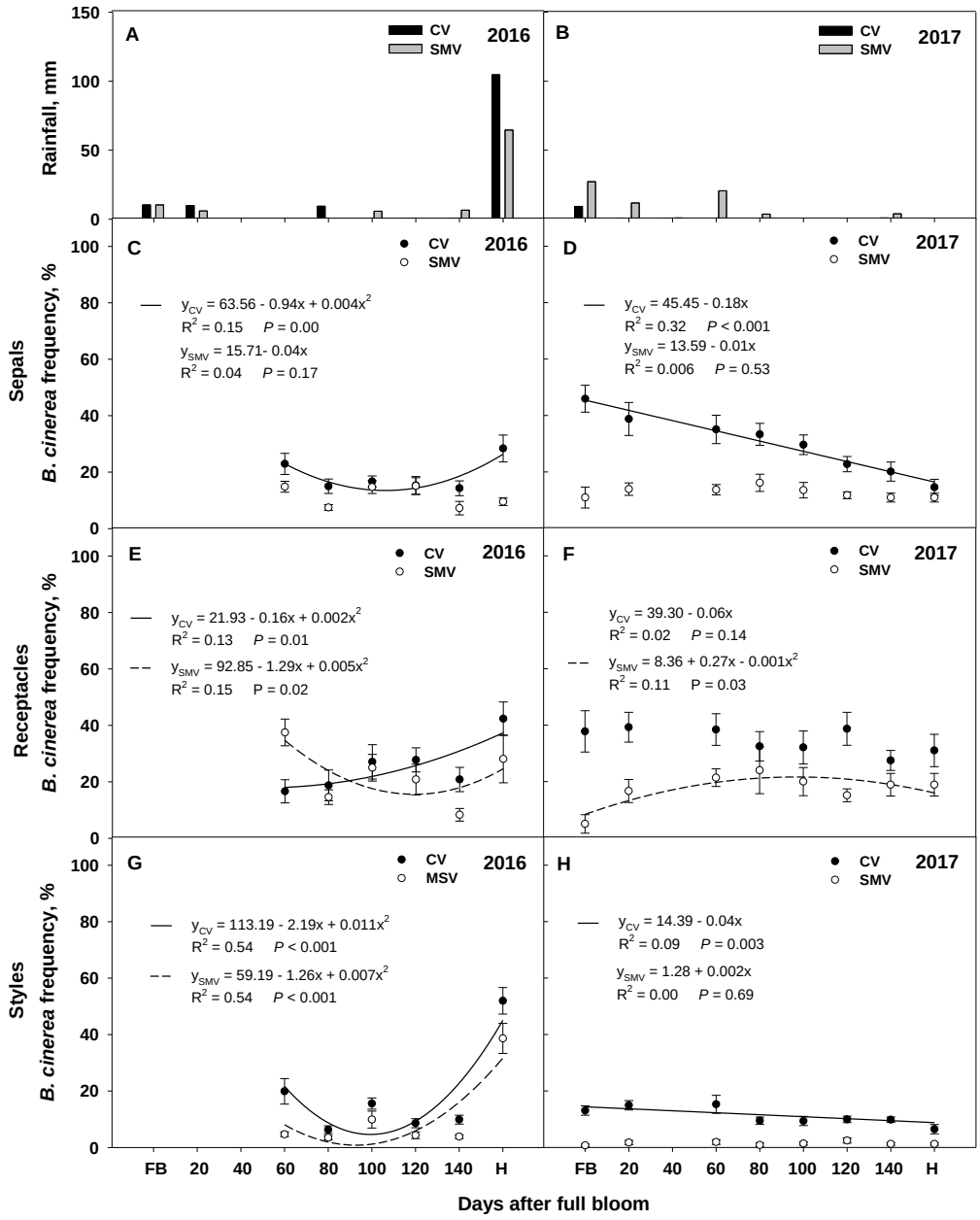

Fig. 2. Cumulated rainfall (A, B) and temporal dynamic of *Botrytis cinerea* affecting sepals (C, D), receptacles (E, F), and styles (G, H) of 'Hayward' kiwifruit from full bloom (FB) to harvest (H) in five orchards located in Cachapoal Valley (CV) and South of Maule Valley (SMV) in 2016 season (A, C, E, G) and 2017 season (B, D, F, H). Vertical bar = standard error. Regressions were significant when $P \leq 0.05$.

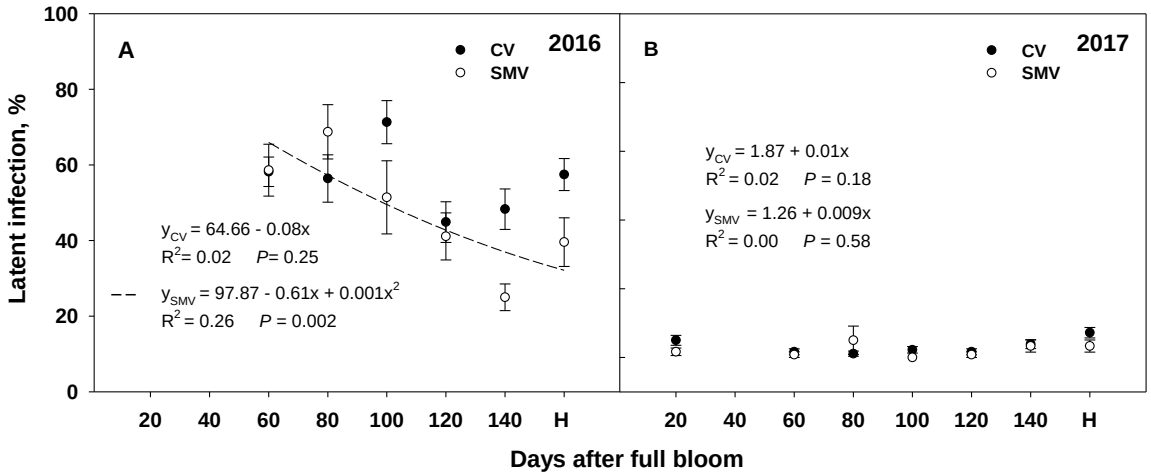

Fig. 3. Temporal dynamic of *Botrytis cinerea* latent infection in 'Hayward' kiwifruit from 20 DAFB to harvest (H) of three orchards in Cachapoal Valley (CV) and two in South of Maule Valley (SMV). **A.** 2016 season. **B.** 2017 season. Latent infection was determined after 6-10 days of incubation at 20°C in a wet chamber prior overnight freezing incubation (n = 15, 4 replicates). Vertical bar = standard error. Regressions were significant when $P \leq 0.05$.

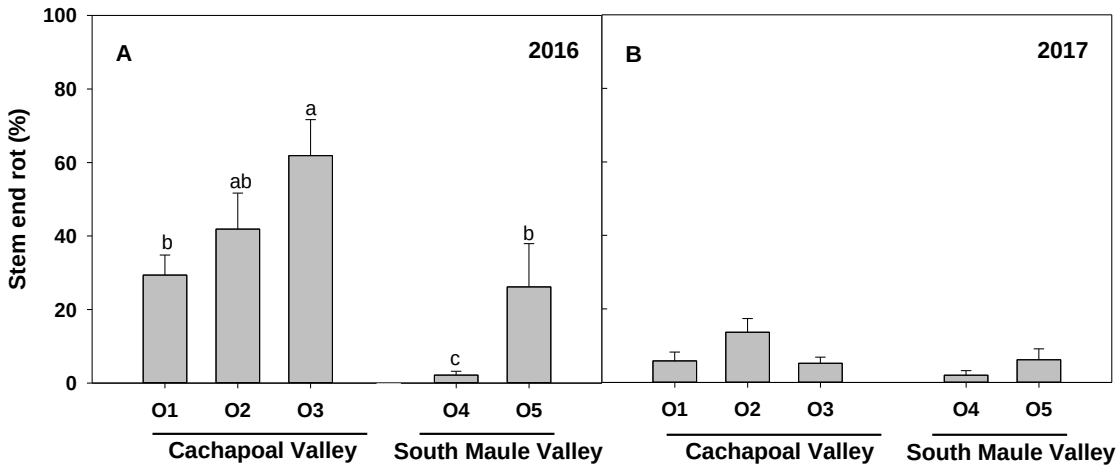

Fig. 4. Stem end rot prevalence (%) after 100 days at 0°C plus 2 days at 20°C in five kiwifruit orchards located in Cachapoal Valley (Orchard 1, 2 and 3) and South of Maule Valley (Orchard 4 and 5). **A.** 2016 growing season. **B**. 2017 growing season. Vertical bars = the standard error of four replicates of 80 fruit each. Means followed by the same letter indicate significant differences according to Fisher LSD test ($P \leq 0.05$). n. s= non significant.

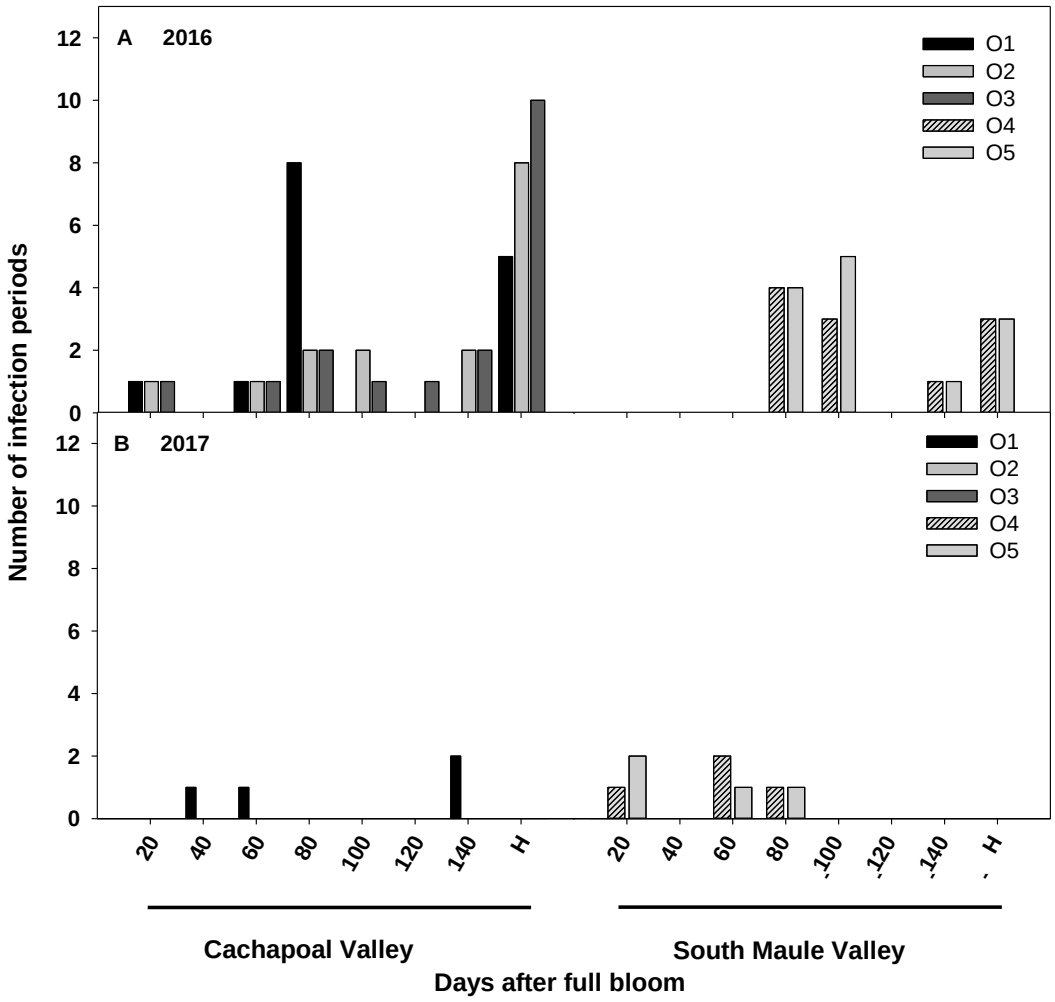

Fig. 5. Infection periods counted between full bloom to harvest in five orchards located in Cachapoal Valley (Orchard 1, 2 and 3) and South of Maule Valley (Orchard 4 and 5). **A.** 2016 season (S1). **B.** 2017 season (S2). One infection period was estimated if 14°C < T < 25°C, RH > 90% or leaf wetness for at least 6 hours (Broome et al. 1995). T: mean temperature; RH: Relative humidity.

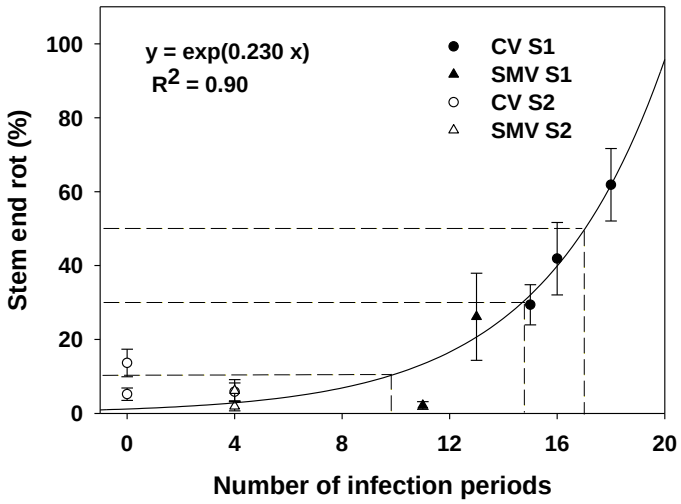

Fig. 6. Exponential relationship between the cumulated number of infection periods based on the algorithm of *Botrytis cinerea* model (Broome et al. 1995) from full bloom until harvest and stem end rot prevalence after 100 days at 0°C plus 2 days at 20°C. Stem end rot prevalence was estimated from five kiwifruit orchards in 4 replicates of 80 fruit each season. Season 2016 (S1) in Cachapoal Valley (CV, solid circle) and South of Maule Valley (SMV, solid triangle) and from season 2017 (S2) in Cachapoal Valley (CV, empty circle) and South of Maule Valley (SMV, empty triangle).

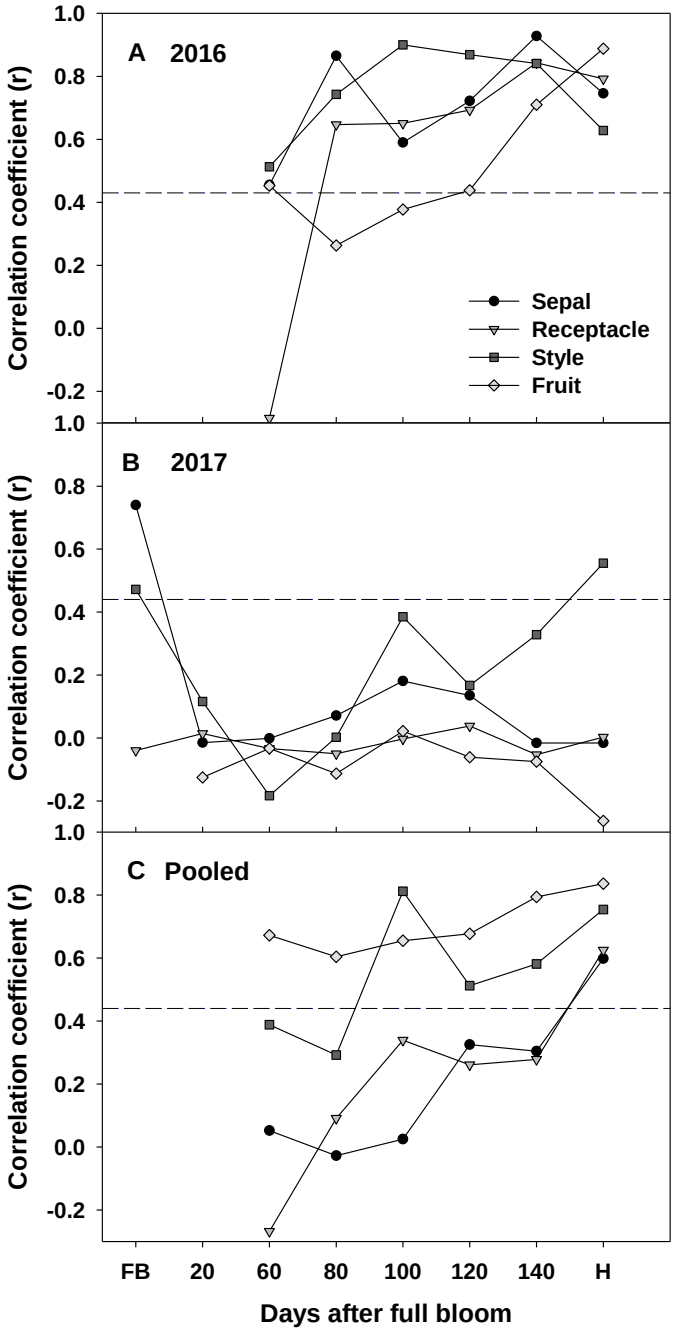

Fig. 7. Pearson's coefficients obtained from the correlations between postharvest stem end rot prevalence and the presence of *Botrytis cinerea* in sepals, styles, receptacles and latent infection in fruit, from full bloom to harvest. **A**. Season 2016 (S1). **B**. Season 2017 (S2). **C**. Pearson's coefficients were calculated using data from S1 and S2 at each sampling day. Values above dashed line indicate significant correlations ($P \leq 0.05$).

Chapter 4

General Discussion and Conclusions

In ‘Hayward’ kiwifruit, the stem end rot (SER) is a disease where the symptoms are visualized during stored kiwifruits at low temperatures and not during the fruit development in the orchard so it is not possible to segregate infected from healthy fruits at harvest in Chile. *Botrytis cinerea* has been widely associated with SER; however, the recently identification of *B*. *prunorum* causing gray mold in cold stored kiwifruit showed the scarce information about causal agents and their role in the SER epidemiology on fruits in Chile. Therefore, this doctoral thesis determined the relative importance of isolates of *B*. *cinerea* and *B*. *prunorum* associated with gray mold and apparently healthy floral parts attached to the fruit during the fruit development. In addition, the understanding of SER in Chile has been based on epidemiological studies that were conducted under different climatic conditions, which considered two different critical infection periods: flowering and harvest time. However, it is not clear the most relevant critical period for infection under Chilean conditions. Therefore, this doctoral thesis research was focused on studying some epidemiological aspects in Chile.

Based on the results obtained, SER was caused by *B*. *cinerea* and *B*. *prunorum* and was the main postharvest disease in kiwifruit in Chile confirming previous reports (Díaz et al. 2017; Morales and Ulloa 1985; Pinilla et al. 1995). In addition, both *Botrytis* species were well established in the orchards, colonizing petals, sepals, receptacles and styles where *B*. *cinerea* was the predominant specie. *Botrytis cinerea* has been associated with SER in the main kiwifruit producing countries (Brigati et al. 2003; Michailides and Elmer 2000; Opgenorth 1983; Pei et al. 2019; Pennycook 1985) and in Chile was identified in the 1980s (Morales and Ulloa 1985), less a decade after the first commercial kiwifruit plantation. In this research *B*. *cinerea* was identified along with *B*. *prunorum* suggesting both species are part

of the mycobiota of kiwifruit orchard and may use floral parts as potential inoculum sources for stem end rot infections.

Some isolates of *B*. *cinerea* and *B*. *prunorum* used in this study were obtained from apparently healthy floral structures; however all of these isolates were pathogenic in kiwifruit, leaves and apples and grapes where *B*. *cinerea* isolates were the most virulent. This information contributes to a better knowledge of SER produced for these *Botrytis* species.

In addition, this research corroborated that the latent infections in the pericarp stem end area occurs during the fruit development and not only at harvest, supporting the study performed by Michailides and Morgan (1996). On the other hand, the prevalence of latent infections discriminated seasons with high and low SER prevalence, proving to be a good predictor of SER in kiwifruit before the harvest time.

Correlation and regression analysis determined that the colonization dynamic and inoculum density were affected by the environmental conditions of the season. Similar with the results obtained by Rivera et al. (2013) in blueberries, the prevalence of SER was better explained by an exponential model which integrated microclimatic conditions integrated in a mathematical algorithm which estimates the infection risk as a function of the temperature and duration of wetness. Fungicide application, senescent leaf and floral debris removal and canopy management during the fruit development have been studied in order to minimize decay postharvest in kiwifruit (Brigati et al. 2003; Latorre and Pak 2003; Snelgar et al. 1998; Manning et al. 2010) and other crops (English et al. 1989; Hildebrand et al. 2001; Rivera et al. 2013; Valdés-Gómez et al. 2008). Therefore, strategies with a holistic approach in order to reduce the infection rate during the season in kiwifruit orchards are suggested.

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Annexes

Annex 1

Effect of the Jintao kiwifruit floral remains removal on the incidence of stem end rot in different types of packaging during storage

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Effect of the Jintao kiwifruit floral remains removal on the incidence of stem end rot in different types of packaging during storage

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Abstract

Packaging in modified atmosphere or perforated bags and storage at low temperature are critical practices to extend "Jintao" kiwifruit storage time, decreasing the metabolic rate, reducing water loss and affecting the development of pathogens. In the industry, the fruit are brushed and then packed to be exported to destination. The objective of the study was to evaluate the effect of the presence of floral remains on the stem end rot caused by *Botrytis* spp in kiwifruit cv. Jintao from two orchards under different relative humidity conditions produced by the bag types (perforated o modified atmosphere packaging). The inoculum level maintained on sepals and styles was quantified at harvest, cultivating these asymptomatic tissues on acidified potato dextrose agar plus Igepal for 7 days at 20°C The fruit were cured by 48 h, packed, with or without the presence of floral remains (brushing treatment), and stored without bag, perforated (0.9%) or modified atmosphere packaging at 0°C. After 90 days, it was observed that the fruit brushed and packed without floral remains had a high prevalence of stem end rot, in a perforated bag (94% relative humidity) or under modified atmosphere packaging (98% relative humidity) than without bag. This study

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suggests that high relative humidity inside the packaging and the damage induced by removal of floral remains favors the expression of symptoms caused by *Botrytis* spp.

Keywords: *Actinidia chinensis*, *Botrytis* spp., postharvest, brushing.

Introduction

Postharvest managements have been developed to maintain the quality of the fruit, delaying the senescence and ripening of kiwifruit. The modified atmosphere packaging (MAP) has allowed extending the commercial life of the fruit, reducing the softening and retarding ripening. However, fluctuations in temperature can cause condensation inside the packaging favoring the development of pathogens (Wiley et al., 2009). Brushing is a common practice in kiwifruit conducted to remove particles adhered to the trichomes as necrotic floral remains (Mitchel et al., 1994). However, brushing can generate micro-wounds (Massantini et al., 1995) that trigged the anticipated ripening of the fruit and nutrient availability that may influence the success of *Botrytis cinerea* infection (Wurms et al., 1998) and development of stem end rot during storage.

Studies determined an inverse relationship between fruit maturity, soluble solids content and susceptibility to stem end rot (Bautista-Baños et al., 1997; Brook, 1990). Epidemiological studies indicated external fruit contamination by *B. cinerea* conidia is a major source of inoculum for infections of sepals and receptacles (Michailides and Elmer, 2000). There is a direct relationship between the number of viable conidia at harvest and the incidence of stem end rot (Elmer et al., 1997). We studied the effect of removal of floral remains on the stem end rot caused by *Botrytis* spp. in kiwifruit cv. Jintao in different conditions of humidity produced by the types of packaging.

Materials and Methods

Kiwifruit "Jintao" with similar maturity (Table 1) and size at harvest, were collected with the peduncle attached from 2 orchards of central valley (O'Higgins region) in Chile. Once the fruit arrived at the laboratory, the peduncle was removed and the fruit kept at 20°C, 50% relative humidity for 48 hours (curing). Twenty fruit were selected per replication; they were hand brushed, removing trichomes, sepals and styles; and other fruit remained without brushing as control.

Then, the fruit in each group were placed in 10-kg carton boxes using two types of packaging: i) a high density 10-µ, 0.9%-perforated polyethylene bag (PB) and ii) a 60-µ, low-density polyethylene modified atmosphere bag (MAP, FF Kiwi, San Jorge Packaging, Chile). The storage was carried for 90 days at 0°C in an ethylene-free environment produced by passing air through a potassium permanganate ethylene scrubber.

The ethylene concentration was monitored every two days and was consistently < 0.005 µl L^{-1}. Concentrations of CO_2 and O_2 inside the modified atmosphere bags were determined extracting 20 mL of air from the bag and injecting into a O_2/CO_2 gas analyzer (CheckPoint, Dansensor A/S, Ringsted, Denmark) at 7, 20, 30, 60 and 90 days of storage (Figure 1). Maturity and quality parameters were determined before and after storage (after 90 days at 0 ° C and 2 days at 20 ° C) considering fruit mass, firmness (penetrometer FT 327, Milan, Italy) and soluble solid concentration with refractometer (Atago, Master Alpha, Tokyo, Japan)

***Botrytis* spp., and other fungus genera infecting apparently healthy sepals and styles at harvest and stem end rot prevalence after storage.**

Sepals and Jintao kiwifruit styles were surface disinfected with sodium hypochlorite 1% plus 0.05% Tween 80, rinsed on sterile distilled water and cultivated on acidified potato dextrose agar plus Igepal for 7 days at 20 ° C.

The fungus genera present in each tissue were identified by microscopic morphology, determining the proportion of sepals and styles infected by each pathogen, more than one pathogen per tissue were reported (4 replicates of 15 petri dishes of 6 sepals and 6 styles each). After storage, stem end rot prevalence was calculated as a percentage of the diseased fruit by *Botrytis* spp at the stem end respect to total fruit evaluated (20 fruit per replicate) after 90 days at 0°C and 2 days at 20°C.

Statistical Analysis.

The effect of the type of packaging and the presence of floral remains on stem end rot at postharvest was evaluated under a bifactorial model (4x2) with 4 replicates of 20 fruit each. Tukey's test was used to evaluate differences between means using P value ≤0.05.The mean value of the *Botrytis* spp. inoculum level between orchards obtained from sepals and styles were compared using Tukey's test (P value <0.05) under a complete random design. Percentage values were transformed to arcsine √% for statistical analysis, but the original data is presented. Fruit quality was assessed on four replications of 12 fruit each. Tukey's test was used to evaluate differences between means using P value ≤0.05.

Results

Removal of floral tissue remains by brushing and the use of plastic bag (perforated or MAP) affected the prevalence of stem end rot caused by Botrytis spp in cold storage. In both orchards, the prevalence of stem end rot was higher in brushed fruit (Orchard 1 P = 0.01 and Orchard 2 P <0.01), varying on the type of packaging used. Modified atmosphere bags

(98.5% relative humidity, figure 1) obtained a higher or equal prevalence of stem end rot than the perforated bags (95% relative humidity), depending on the orchard. No interaction was detected between factors (P value> 0.05). The same trend was observed after 2 days at 20 ° C (Table 2 and Table 3).

The proportion of infected tissue by Botrytis spp was higher in sepals than styles, but no significant differences between orchards was observed, however orchard 2 had a higher percentage of stem end rot than Orchard 1 after 90 days at 0°C (Table 4). Different fungus genera were detected on asymptomatic sepals and styles at harvest. (Table 5).

After cold storage, the content of soluble solids in brushed fruits varied according the type of packaging. Fruits without and perforated bag obtained higher soluble solid content than the fruits in modified atmosphere (15.1%, 14.6% and 13.3% respectively) data not shown.

Discussion

Stem end rot is the main disease affecting the long term storage of kiwifruit. The prevalence of the disease is more critical in *Actinidia deliciosa* than *Actinidia chinensis* suggesting that genetic characteristics are important factors associated with the susceptibility.

Postharvest practices oriented to fruit brushing, including cleaning the fruit surface and removing the sepals and styles, are widely recommended in the kiwifruit industry. Additionally, the dehydration is controlled by a saturated packaging such as the incorporation of a perforated bag or the modified atmosphere packaging.

This study demonstrated that under controlled brushing conditions, packaging of Jintao kiwifruit under perforated or modified atmosphere bags the prevalence of decayed fruit

increases significantly when brushed kiwifruit was incorporated in the packaging. Gray mold symptoms were primarily located at the stem end, starting from the infection at the scar of the sepal wounded from the receptacle. The disease was not present in the control fruit, without polybags.

Different fungus genera were detected at harvest on non-symptomatic sepals and styles but not all expressed during storage. *Botrytis* spp appeared mainly on sepals, and caused gray mold symptoms at the stem end during storage; otherwise in the case of the styles, the genus *Cladosporium* and the members of Pleosporaceae family (*Alternaria* and *Stemphylium* spp) were the most frequents but did not appear the symptoms at the stylar end during storage under humidity condition (data not shown).

Conclusions

The type of packaging and the presence of floral remains affect the expression of stem end rot on 'Jintao' kiwifruit. High humidity induced by perforated or non perforated modified atmosphere bag increased significantly the incidence of decayed fruit. Brushed fruit were more susceptible to the infection of *Botrytis* and stem rot symptoms appears after 90 days at 0°C, suggesting that postharvest practices can affect the infection during storage under saturated conditions.

Acknowledgments

The authors are grateful to Kiwigold Chile Spa, for providing the fruit that were used in this work.

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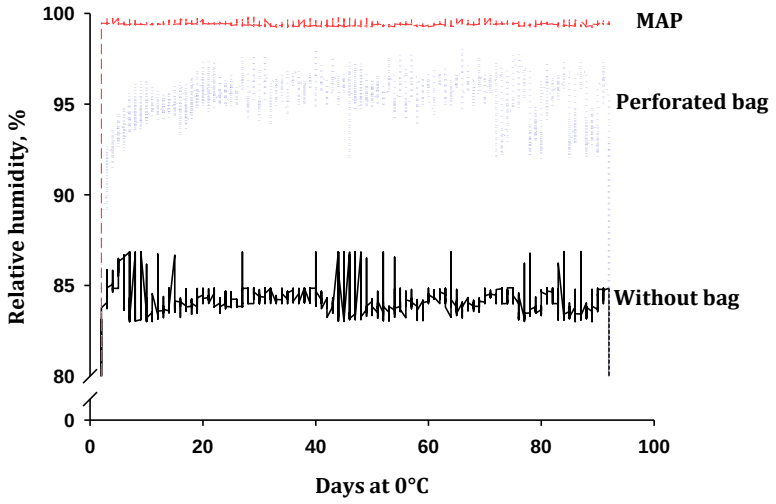

Fig. 1. Relative humidity maintained inside boxes with kiwifruit packed in perforated bag, modified atmosphere(MAP) and control without bag for 90 days at 0 ° C.

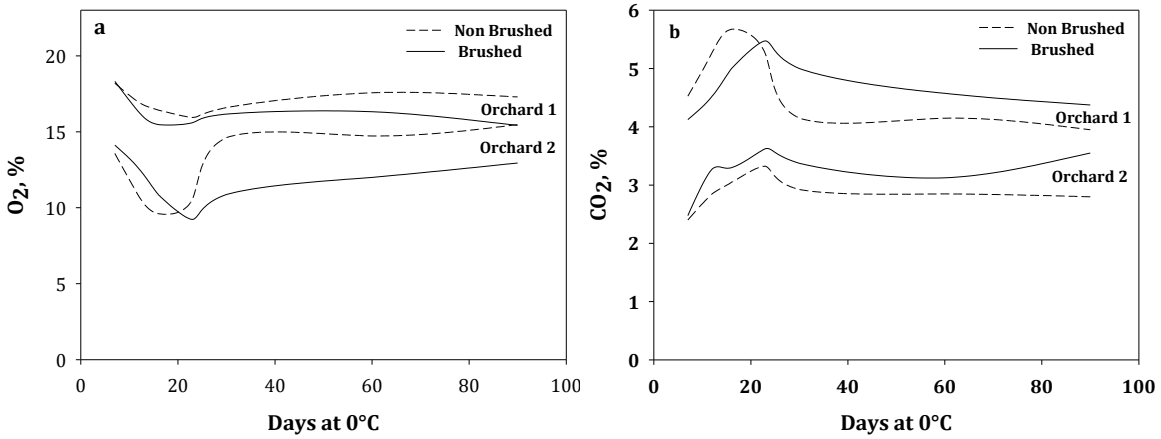

Fig. 2. CO_2 and O_2 concentration in modified atmosphere packaging during storage of brushed and non-brushed 'Jintao' kiwifruit from Orchard 1 and Orchard 2.

Table 1. Average of fruit mass (g). firmness (N) and soluble solids (%) at harvest from orchard 1 and 2.

Orchard	Fruit mass (g)	Firmness (N)	Soluble solids (%)
Orchard 1	86.85 a	47.93 n.s.	12.24 b
Orchard 2	103.03 b	46.91 n.s.	10.33 a

Different letters indicate significantly differences according to Student´t test (P value < 0.05).

Average values represent 4 replicates with 12 fruits each.

Table 2. Effect of the different packages on the percentage of stem end rot on brushed or non- brushed ‘Jintao’ kiwifruit maintained at 0° C for 90 days.

Treatment	Orchard 1			Orchard 2		
	Non brushed	Brushed	Mean	Non brushed	Brushed	**Mean**
Without bag	0.00	1.25	0.63 a	1.25	12.50	**6.88 a**
Perforated bag	0.00	3.75	1.88 a	10.00	21.25	**15.63 b**
Modified atmosphere	5.00	12.50	8.75 b	21.25	30.00	**25.63 b**
Mean	**1.67 A**	**5.83 B**		**10.83 A**	**21.25 B**	
ANOVA	Df	F	P	Df	F	P
Packaging (P)	2	9.20	0.00	2	13.62	<0.001
Brushing (BRS)	1	8.04	0.01	1	16.27	<0.001
Interaction P x BR	2	0.84	0.45	2	1.17	0.33

Means followed by the same letter in each column did not differ significantly according to Tukey's pairwise multiple comparison test ($P < 0.05$). Data were arcsine $\sqrt{(x/100)}$ transformed before the analysis but the nontransformed data are presented.

Table 3. Effect of the different packages on the percentage of stem end rot on brushed or non-brushed 'Jintao' kiwifruit maintained at 0° C for 90 days + 2 days at 20°C.

Treatment	Orchard 1			Orchard 2		
	Non brushed	Brushed	Mean	Non brushed	Brushed	Mean
Without bag	0.00	1.25	**0.63 a**	1.25	12.50	**6.88 a**
Perforated bag	0.00	3.75	**1.88 a**	10.00	22.50	**16.25 b**
Modified atmosphere	10.00	13.75	**11.88 b**	25.00	33.75	**29.38 c**
Mean	**3.33 A**	**6.25 B**		**12.08 A**	**22.92 B**	
ANOVA	Df	F	P	Df	F	P
Packaging (P)	2	28.28	<0.001	2	18.17	<0.001
Floral remains (FR)	1	5.36	0.03	1	16.99	<0.001
Interaction P x FR	2	0.45	0.64	2	1.34	0.29

Means followed by the same letter in each column did not differ significantly according to Tukey's pairwise multiple comparison test ($P < 0.05$). Data were arcsine $\sqrt{(x/100)}$ transformed before the analysis but the nontransformed data are presented.

Table 4. Proportion of *Botrytis* spp. (%) on asymptomatic sepals and styles of 'Jintao' Kiwifruit from orchard 1 and 2 at harvest.

	Botrytis spp. prevalence (%)	
	Orchard 1	Orchard 2
Sepals	10.97 b	10.64 b
Styles	0.50 a	1.79 a
Mean	5.74 n.s.	6.22 n.s

Different letters in columns indicate significantly differences according to T student test (P value < 0.05). Values represent the average of 4 replicates with 90 sepals or styles each. n.s.: non-significant differences between mean values.

Table 5. Proportion of different fungus genera different than *Botrytis* sp. on asymptomatic sepals and styles from orchard 1 and 2 at harvest.

	Sepals		Styles	
	Orchard 1	Orchard 2	Orchard 1	Orchard 2
Pleosporaceae	19.17	36.67	37.17	39.00
Cladosporium sp.	5..83	10.83	31.17	47.00
Epicoccum sp.	7.78	30.83	5.58	7.77
Penicillium sp.	4.17	0.28	0.68	0.86
Rhizoctonia sp.	1.11	6.74	0.00	0.17
Macrophomina sp.	0.56	2.50	1.33	0.17
Fusarium sp.	0.83	0.00	0.52	4.85
Trichoderma sp	0.56	0.28	0.18	0.17
Sclerotinia sp.	0.83	0.00	0.00	0.00
Others	14.72	1.39	2.43	1.49

Annex 2

IV International Symposium on Postharvest Pathology. Skukuza, Sudáfrica. 2017.

Effect of the Jintao kiwifruit floral remains on the incidence of stem end rot in different types of packaging during storage.

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Packaging and storage at low temperature are critical practices to extend "Jintao" kiwifruit storage time while decreasing the metabolic rate and delaying the development of pathogens. In the industry, the fruits are brushed and then packed to be exported to destination. The objective of the study was to evaluate the effect of the presence of floral remains on the stem end rot caused by *Botrytis* spp in kiwi cv. Jintao from two orchards under different conditions of relative humidity. The inoculum level maintained on sepals and styles was quantified by cultivating these tissues on acidified potato dextrose agar plus Igepal for 7 days at 20 °C The fruits were cured for 48 h, packed with or without the presence of floral remains, and stored without bag, in perforated (0.9%) or modified atmosphere bag (4,1 and 4,7%°CO2) at 0°C. After 90 days, it was observed that the fruit brushed and packed without floral remains had a high prevalence of stem end rot, and this was even higher in fruits stored in a perforated bag (92% relative humidity) and modified atmosphere (98%

relative humidity) than without bag. This study suggests that high relative humidity inside the packaging and the damage induced by removal of floral remains favours the expression of symptoms caused by *Botrytis* spp.

Effect of the Jintao kiwifruit floral remains removal on the incidence of stem end rot in different types of packaging during storage

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INTRODUCTION

Commercial brushing of Jiantao kiwifruit is done to remove floral remains during the process of packaging. Different bags can be used during the storage and export to avoid dehydration. However, physical damage caused by brushing on the insertion of sepals can promote the infection of *Botrytis* spp. depending on relative humidity produced by the bag. The objective of the study was to evaluate the effect of the removal of floral remains on the stem end rot caused by *Botrytis* spp. in kiwifruit cv. Jintao under varied conditions of humidity determined by different types of packaging.

METHODOLOGY

Harvest Jintao kiwifruits from two orchards located in Maule Region, Chile (34°56'S, 71°12'W and 34°51'S, 71°15'W).

Determining proportion of *Botrytis* spp. and other genera latent infections a)sepals (n=6) and b) styles (n=10, 4 replicate).

Removing floral remains fruits were:
a) hand brushed or b) non brushed. (n=20, 4 replicates).

Fig. 1. Jintao kiwifruit

Cold storage The fruits were packed and stored at 0°C for 90 days in a) perforated bag (0,9% ventilated area) b)Modified atmosphere packaging (MAP) c) without bag.

Stem end rot prevalence was determined

RESULTS

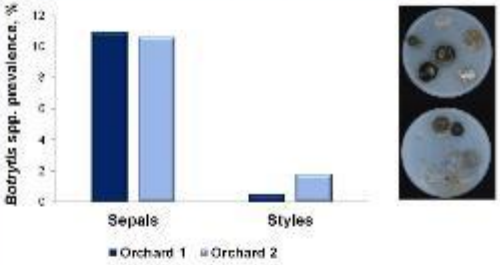

Fig 2. Prevalence of *Botrytis* spp. [%] on asymptomatic sepals and styles of 'Jintao' kiwifruit from orchard 1 and 2 at harvest.

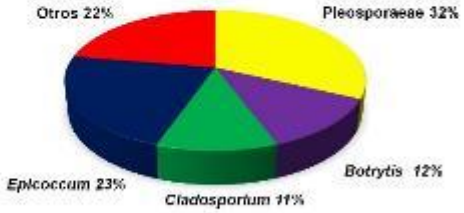

Fig 3. Average percentage of isolates from different fungal genera on asymptomatic Jintao sepals from fruits of both orchards.

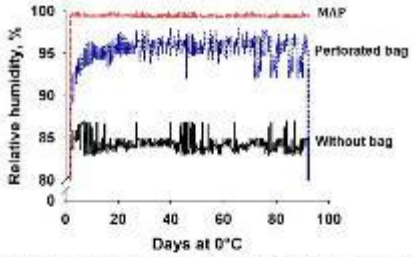

Fig 4. Relative humidity maintained inside boxes with kiwifruit packed in perforated bags, modified atmosphere packaging (13,8%O_2 and 4,3%CO_2) and control without bags for 90 days at 0 ° C.

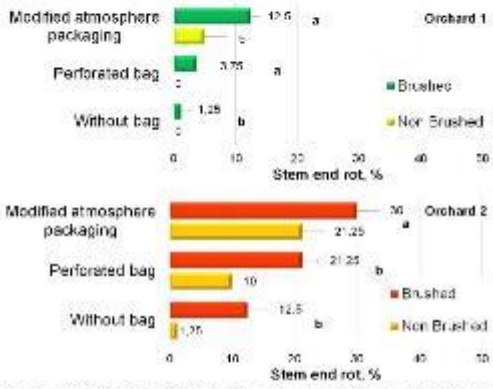

Fig 5. Effect of the different packages on the percentage of stem end rot on brushed and unbrushed 'Jintao' kiwifruit maintained at 0° C for 90 days. Significant differences between brushing in both orchards ($p<0,05$).

CONCLUSIONS

Perforated or modified atmosphere bags increased significantly the prevalence of decayed fruit. Brushed fruits were more susceptible to the infection of *Botrytis* spp. than unbrushed fruits.

ACKNOWLEDGEMENTS

The authors are grateful to Kiwigold Chile Spa, for providing the fruit used in this work.

Annex 3

XVII International Botrytis symposium Santa Cruz, Chile. 2016

Infection risk and *Botrytis* spp. latent infections on kiwifruit cv. Hayward during the season and coldstorage

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Stem end rot caused by *Botrytis* spp is the main postharvest disease of kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A. R. Ferguson cv. 'Hayward'). The symptoms appear in coldstorage but the infection is produced in the field. The occurrence of latent infections on fruits in the orchard was studied in Chile. The aims of this study were to evaluate the prevalence of Botrytis spp. infection on fruits and floral remains and determine the infection risk periods on basis of weather conditions on field. Kiwifruits cv. Hayward from five different Chilean locations were collected through the growing season. Latent infections on fruits, sepals and receptacles were evaluated. In all orchards, tissues and during all phenological stages in study, were observed *Botrytis* spp. latent infections. The prevalence on all kiwifruit tissues was constant until 20 days before harvest (140 days after flowering-DAF), being different between orchards. The mean prevalence varied on fruits (39-59%), sepals (10-24%) and receptacles (14-29%) and it increased at harvest (160 DAF) between 30-58%, 7-

41% and 22-47%, respectively. It was proposed an algorithm to estimate the periods of *Botrytis* spp. infection risks. The algorithm was based on >6 hr of free moisture or > 90% relative humidity between 14°C and 25°C. A significant correlation between the infection risk and the incidence on sepals was obtained. A similar analysis was realized between the infection risk and the stem end rot prevalence in coldstore and immature fruit. The results of this study indicated that *Botrytis* spp. could produce asymptomatic infection on fruit and floral remains during the whole season, which prevalence at coldstorage is affect by weather conditions in the field.

INFECTION RISK AND *Botrytis* spp. LATENT INFECTIONS ON KIWIFRUIT CV. HAYWARD DURING THE SEASON AND COLDSTORAGE

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INTRODUCTION

Stem end rot caused by *Botrytis* spp. is the main postharvest disease of kiwifruit (*Actinidia deliciosa*) which infection ocurrs in the orchard. In Chile, there is little knowledge about the occurrence of latent during fruit development in field infections. The aims of this study were a) to determine the relationship on sepals, receptacles, styles and fruits and the stem end rot incidence in posharvest and b) to determine latent infection risk periods on basis of weather conditions during the 2015-2016 season.

METHODOLOGY

2015-2016 Sampling: Every 20 days since 60 days after flowering (daf) to harvest (160 daf) from 5 Hayward kiwifruit orchards localized in the Región de O´Higgins (34°0´S, 70°42´W) and Región del Maule (35°48´S, 70°42´W), Chile.

Latent infections a) sepals, b) receptacles c) styles y d) frozen kiwifruits incubated in humid chamber (Fig. 2).

Fig. 1. Water soaked appearance of stem end rot caused by *Botrytis* spp. on kiwifruit cv. Hayward.

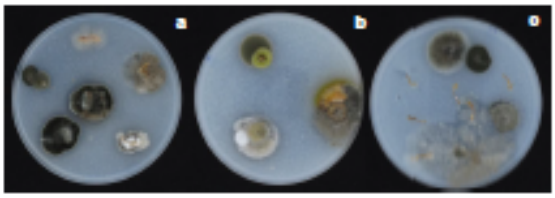

Fig. 2. Latent infections on a) sepals, b) receptacles and c) styles.

Infection risk period: It was based on Broome *et al.* 1995 model. a) Temperature > 15°C and <25°C y b) at least 6 hr of wetness or 95% relative humidity.

RESULTS

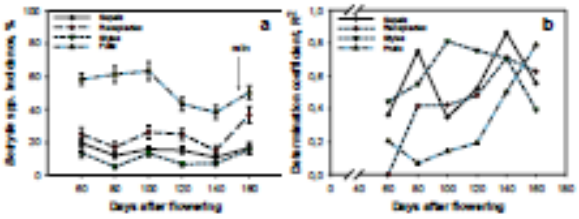

Fig. 3. a) *Botrytis* spp. incidence on sepals, receptacles, styles and fruits through the season. The incidence was evaluated after 7-10 days at 20°C. b) Determination coefficient of lineal regressions between *Botrytis* spp. prevalence on different tissues and the incidence of stem end rot at 90 days (0°C coldstorage).

Relationship between latent infections and stem end rot at coldstorage

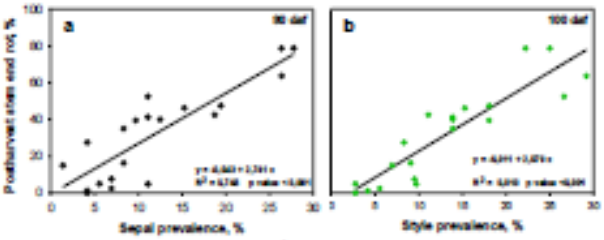

Fig. 4. Linear regressions between *Botrytis* spp incidence on a) sepals and b) styles at 80 daf and 100 daf, respectively, and stem end rot at coldstorage

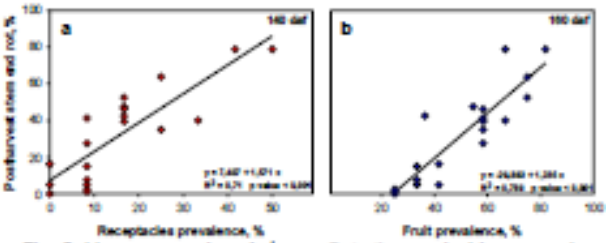

Fig. 6. Linear regressions between *Botrytis* spp. incidence on a) receptacles and b) fruits at 140 daf and 160 daf, respectively, and stem end rot at coldstorage

Infection risk periods

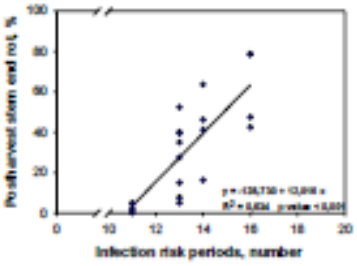

Fig. 8. Linear regression between number of infection risk periods at 160 daf and stem end rot incidence at 90 days (0°C). It was based on Broome *et al.* 1995

CONCLUSION

Asymptomatic infection caused by *Botrytis* spp. on fruit and floral remains were associated to stem end rot prevalence on postharvest, which was affected by infection risk periods on field.

Annex 4

2017 American Phytopathological Society Annual Meeting, San Antonio, Texas.

Kiwifruit susceptibility to *Botrytis cinerea* and *Botrytis prunorum* during fruit development in Chile

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Gray mold, the main postharvest disease of kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A. R. Ferguson 'Hayward') is caused by *Botrytis cinerea* and *Botrytis prunorum*. Symptoms appears in colstorage but infections produced in the field. Fruit tissue susceptibility to *B*. *cinerea* (isolate Kw_F58) and *B*. *prunorum* (isolate Kw_6) was studied since 20 d after flowering (fruit immature) until near harvest of 'Hayward' kiwifruit. Fruits were injured and inoculated on the cheek, in the picking wound, and at the base of the berry, in the insertion of the sepal with the receptacle with a mycelial plug (3 mm) or a conidial suspension (10^6 conidia mL^{-1}) and evaluated after 5, 7 or 12 d at 20°C and 100% humidity. Results showed that the fruits were susceptible to *B*. *cinerea* and *B*. *prunorum* during all the season. Fruits collected early in the season were more susceptible than those collected at harvest, varying according to the wounded tissue and pathogen. Kiwifruits inoculated on cheeks with conidial suspension were susceptible during all the season. Inoculation on sepal insertion was more susceptible early, while picking wound was susceptible only 20 d after

flowering. The isolate of *B*. *cinerea* was significantly (*P*<0.001) more virulent than *B*. *prunorum* in all fruit inoculated tissues. Differences in fruit susceptibility throughout the season could be attributed to host defence mechanisms that vary within the season. These results suggest that early fruit protection could reduce stem end rot in kiwifruit in postharvest period.

287-P

Kiwifruit susceptibility to *Botrytis cinerea* and *Botrytis prunorum* during fruit development in Chile.

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INTRODUCTION

Gray mold the main postharvest disease of kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A. R. Ferguson 'Hayward') is caused by *Botrytis cinerea* and *Botrytis prunorum* (1). Symptoms develop during storage but the infection is produced in the field (2). The aim of study was to determine the kiwifruit susceptibility to *Botrytis cinerea* and *Botrytis prunorum* during fruit development.

METHODOLOGY

Sampling Hayward kiwifruits were collected 20 days after full bloom (DAFB) to harvest (160 DAFB) from a orchard located in O'Higgins Region, Chile (34°00'S 70°42'W).

Isolates *Botrytis cinerea* and *Botrytis prunorum* were isolated from natural infected kiwifruit (Figure 1)

Fig. 1. Colony morphology of the *Botrytis* isolates

Inoculation: Were performed with a 5 days old mycelium plug or 10 µl of 10^6 conidia/mL solution on a) the cheek, b) the picking wound, c) the insertion of the sepal to the fruit (Figure 2).

Fig. 2. a) wound on the cheek, b) picking wound, c) wound in the insertion of the sepal to the fruit.

RESULTS

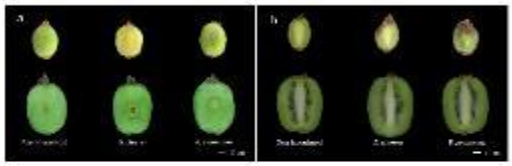

Fig 3. Kiwifruit susceptibility (First line: 20 dafb, second line: 160 dafb) inoculated by *Botrytis* species. Inoculation were performed at the a) cheek after 5 days or b) picking wound after 7 days at 20°C.

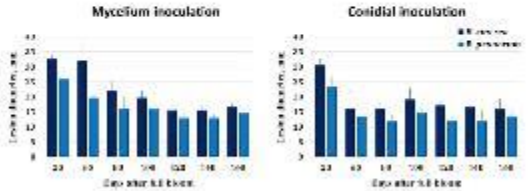

Fig 4. Mean diameter of the lesion produced on the cheek caused by inoculation of two *Botrytis* isolates. Fruits were maintained for 5 days at 20° C. Bars indicate standard deviation

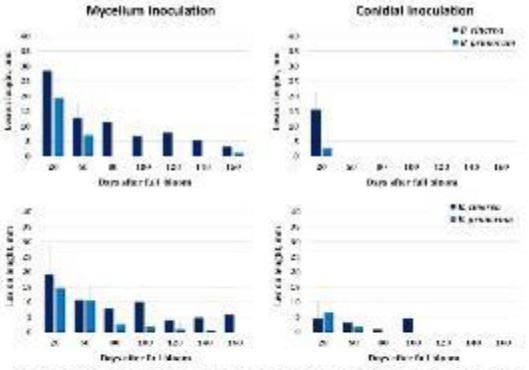

Fig 5. Lesion height produced by *Botrytis* species on the picking wound (above) or in the insertion of sepals in the kiwifruit (below). Fruits were maintained for 7 days at 20°C. Bars indicate standard deviation.

CONCLUSIONS

Kiwifruit harvested early in the season were signicantly more susceptible than 160 dafb to *Botrytis* infection. The isolate of *Botrytis cinerea* was more virulent than *B. prunorum* in all the tissues (p value < 0.05)

ACKNOWLEDGEMENTS

The authors are grateful to Conicyt Conicyt Scholarship 21170512 and Dirección de Investigación y Postgrado de la Facultad de Agronomía e Ingeniería Forestal de la Pontificia Universidad Católica de Chile.

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Annex 5

XXV Congreso de Sociedad Chilena de Fitopatología, XIX Congreso Latinoamericano de Fitopatología, LVII APS Caribbean Division Meeting, Chillán, Chile. 2017

Importancia de las infecciones quiescentes en restos florales y frutos de kiwi cv. Hayward y su relación con la prevalencia de la pudrición peduncular en poscosecha en Chile.

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La pudrición peduncular causada por especies del género *Botrytis* es la principal enfermedad durante el almacenaje en frío del kiwi, siendo altamente variable entre temporadas. Esta variabilidad podría explicarse por el momento de la infección, describiéndose dos momentos críticos: floración y cosecha. Sin embargo, existe escasa información que relacione la infección latente en tejidos florales y del fruto, las condiciones climáticas del huerto y el desarrollo de la pudrición peduncular durante el almacenamiento en frío. Para determinar esta relación, frutos de kiwi cv. Hayward fueron colectados cada 20 días desde floración a cosecha desde 5 huertos comerciales ubicados entre la región de

O´Higgins y del Maule durante las temporadas 2015-16 y 2016-17. Se determinó la incidencia de infecciones latentes (IL) en sépalos, receptáculos, estilos y frutos inmaduros; además se cuantificó la prevalencia de la pudrición peduncular en poscosecha (PP) luego de 100 días a 0°C. Los resultados indicaron que durante la temporada 2015-16, con condiciones climáticas favorables para el desarrollo del patógeno, las infecciones latentes en todos los tejidos fueron correlacionadas positivamente a la PP, observándose en general las más altas correlaciones con frutos a cosecha. Durante la temporada 2016-17 se observaron condiciones climáticas menos favorables y sólo la IL en sépalos durante floración se correlacionó positivamente con la incidencia de PP. En conclusión, un alto inóculo a la floración junto con condiciones climáticas favorables durante la temporada que permitan mantener el nivel de infección latente en los frutos en el campo, determinarían la presencia de pudrición peduncular en poscosecha.