Postharvest incidence 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 primary 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 indicated 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 deliciosa* (A. Chev.) C.F. Liang et A. R. Ferguson) and the second largest exporter of kiwifruit in the Southern Hemisphere, with a production area of more than 8,700 ha. (https://www.odepa.gob.cl/panorama_2019/86/, 2019). The Chilean kiwifruit is produced primarily for export by ship to distant markets in Europe and Asia (https://www.prochile.gob.cl/landing/sectores-productivos/, 2019). 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 (SER) is caused by *Botrytis cinerea* Pers.; Fr. and *Botrytis prunorum* EE Ferrada & Latorre (Elfar et al. 2017). Stem end rot 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, these control strategies were developed based on epidemiological work carried out on *B. cinerea* under field conditions in New Zealand and the USA (California). 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. Therefore, the objectives of this study 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

The study was conducted during the 2016 (S1) and 2017 (S2) growing seasons, in Cachapoal Valley (CV) and 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 and August, 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 and O3) in CV and two (O4 and O5) in 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 instead of full bloom.

***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 an Andersen 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.**

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

166 min and dried in a laminar flow hood. Plates were incubated for 10 days at 20-22°C under
167 12 h light. The results are reported as the percentages of floral parts colonized of the total
168 number of samples.

169 **Incidence of latent infections in fruit**

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

185 **Incidence of stem end rot during storage**

186 Stem end rot incidence in kiwifruit was evaluated after 100 days of storage at 0°C plus
187 two days at 20°C from totals of 320 fruit per orchard. Fruit were hand brushed and their
188 peduncles removed by hand, cured for 24 h at 20°C and packed in 10 kg capacity
189 cardboard boxes with 60-µm thick, low-density polyethylene modified atmosphere bags
190 (MAP, Fresh-Fresh Kiwi, San Jorge Packaging, Santiago, Chile). The fruit were stored in

191 an ethylene-free environment produced by passing the incoming air stream through a
192 potassium permanganate (Bioconservacion, BCN, Spain) ethylene scrubber. The ethylene
193 concentration was monitored every two days, maintaining ethylene concentrations
194 consistently at <0.005 µl liter^{-1} (Zoffoli et al. 2016). Stem end rot incidence was expressed
195 as the percentage of diseased fruit.

196 **Microclimate characterization**

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

207 $$Vpd = Vp\left(1 - \frac{\mathrm{RH}}{100}\right)$$

208 Where water vapor pressure (*Vp*) was:

209 $$Vp = 4.6698\, e^{0.06241\,\mathrm{Tm}}$$

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

214 **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 Anova analysis. Mean differences were separated using the Fisher Least Significant Difference (LSD) test ($P \leq 0.05$). Percentage values of SER were transformed to arcsine square root, 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 recorded 125 mm in CV and 83 mm in SMV. In the last period before harvest, the rainfall recorded 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, 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

Botrytis cinerea was consistently isolated from asymptomatic 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 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 fruit, to predict the later incidence of SER during cold storage. *Botrytis cinerea* colonized all floral parts and the 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. Otherwise, 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 of fruit were related with SER under wet season (S1). When data from wet (S1) and dry (S2)seasons were pulled and analyzed, incidence of latent infection was positively and significantly related to SER incidence from 60 DAFB to harvest.

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

374 Weather conditions and cultural practices (Snelgar et al. 1998) both influence fruit
375 microclimate and so directly affect the colonization of *B*. *cinerea* (Latorre et al. 2015; Elmer
376 and Pyke 1997; English et al. 1989; Valdés-Gómez et al. 2008). Favorable environmental
377 conditions as temperatures between 15°C and 20°C along with relative humidity > 65.5%
378 then lead to sporulation and, in presence of free moisture, the subsequent infection of the
379 fruit (Ciliberti et al. 2016). English et al. (1989) determined that temperature, vapor
380 pressure, wind speed and leaf wetness under the canopy of grapevines are critical
381 variables that affect Botrytis infection in grape. For example, in our study Orchards 4 and
382 5 in SMV had more transparent canopy and reached the lowest cumulated infection
383 periods at harvest and thus, lead the lowest colonization by *B*. *cinerea* in sepals and styles
384 in both years and the lowest SER incidence in S1, reaffirming the prime importance of
385 environmental conditions to *B*. *cinerea* infection. Therefore, strategies with a holistic
386 approach are suggested to reduce the infection during the season in kiwifruit orchards.

The analysis of leaf wetness duration in the CV and SMV orchards, integrated with Tm in an algorithm, allowed to quantify conditions that favoring *B*. *cinerea* infection in both S1 and S2.

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) or under field conditions (Calvo-Garrido et al. 2014; Carisse et al. 2017). Similar to 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 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 levels of infection 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).

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%) and low in S2 (2.7%). These data demonstrate that *B*. *cinerea*

412 infections occur during fruit development. Latent infections of fruit by *Botrytis* spp. have
413 been described in grapes (Holz et al.2003; Sanzani et al. 2012) with full bloom being one
414 of the periods when infection is most likely to occur (Keller et al. 2003; Nair et al. 1995).
415 The results suggest that fruit susceptibility to *B*. *cinerea* was high before 60 DAFB which
416 agrees with the results of Riquelme et al. (2017), who reported that kiwifruit at 20 DAFB
417 was susceptible to controlled inoculation of *Botrytis* spp. They also claim that immature
418 fruit are more susceptible to *Botrytis* infection than mature ones, a result also observed by
419 Wurms et al. (1998). However, this result does not clarify the relationship of the infection in
420 flowering and the early stages of fruit with the appearance of SER during storage as has
421 been explained in some other fruit species, such as table grapes at flowering (Keller et al.
422 2003; Nair and Allen 1993).

423 Since the need to predict SER is important, so that control measures can be
424 implemented under high risk, correlation analysis using different tissues have been
425 proposed. Among these, the percentage of floral parts colonized by *B*. *cinerea* (Michailides
426 and Morgan 1996; Elmer and Pyke 1997) and the inoculum density in leaf tissues
427 (Manning et al. 2010) have proved good SER predictors in kiwifruit. Michailides et al.
428 (2010) described latent infections in fruit as useful 'indicators' of a number of factors that
429 might affect disease development in the orchard. These will include environmental
430 conditions (Mari et al. 2003; Michailides and Morgan 1996) as well as cultural practices.

431 According to the relationship between the floral parts analyzed and SER incidence,
432 style colonization showed the most positive, and significant correlations with SER during
433 the whole of S1, and also at full bloom and at harvest in S2. Nevertheless, this does not
434 necessarily mean that style colonization is the most important inoculum source causing
435 SER. On the contrary, the styles were less frequently colonized compared with the sepals
436 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 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 requires skilled expertise, specific facilities to be applied.

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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677 **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

678

679 [v] Light interception based on the point grid method (Wünsche et al. 1995). Percentage of shadow projected under the canopy on a
680 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
681 area using the IMAGE J 1.49v software (Rasband, National Institute of Health, USA) (Zarate-Valdez et al. 2015). Values shown from
682 S2 at harvest.

683 [w] Values in parenthesis corresponded to standard deviation.

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

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

686 [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
687 before harvest in both growing seasons at rates recommended to control gray mold control.

688 **Table 2**. The microclimate of 'Hayward' kiwifruit orchards in Cachapoal Valley and

689 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**

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

691 [w] Mean daily temperature (Tm).

692 [x] Mean daily relative humidity (HR)

693 [y] Vapor pressure deficit (Vpd)

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

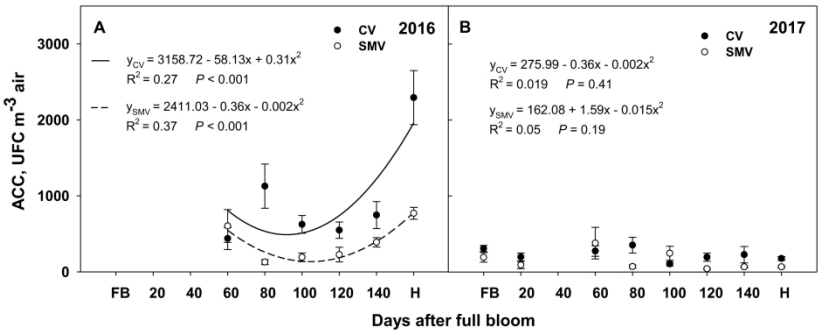

Fig. 1. Mean airborne conidial concentration (ACC) of *Botrytis cinerea* of five 'Hayward' kiwifruit orchards located in Cachapoal Valley (CV: Orchards 1, 2 and 3) and South of Maule Valley (SMV: Orchards 4 and 5) from full bloom (FB) to harvest (H). **A.** Season 2016 (S1). **B.** Season 2017 (S2).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

338x190mm (300 x 300 DPI)

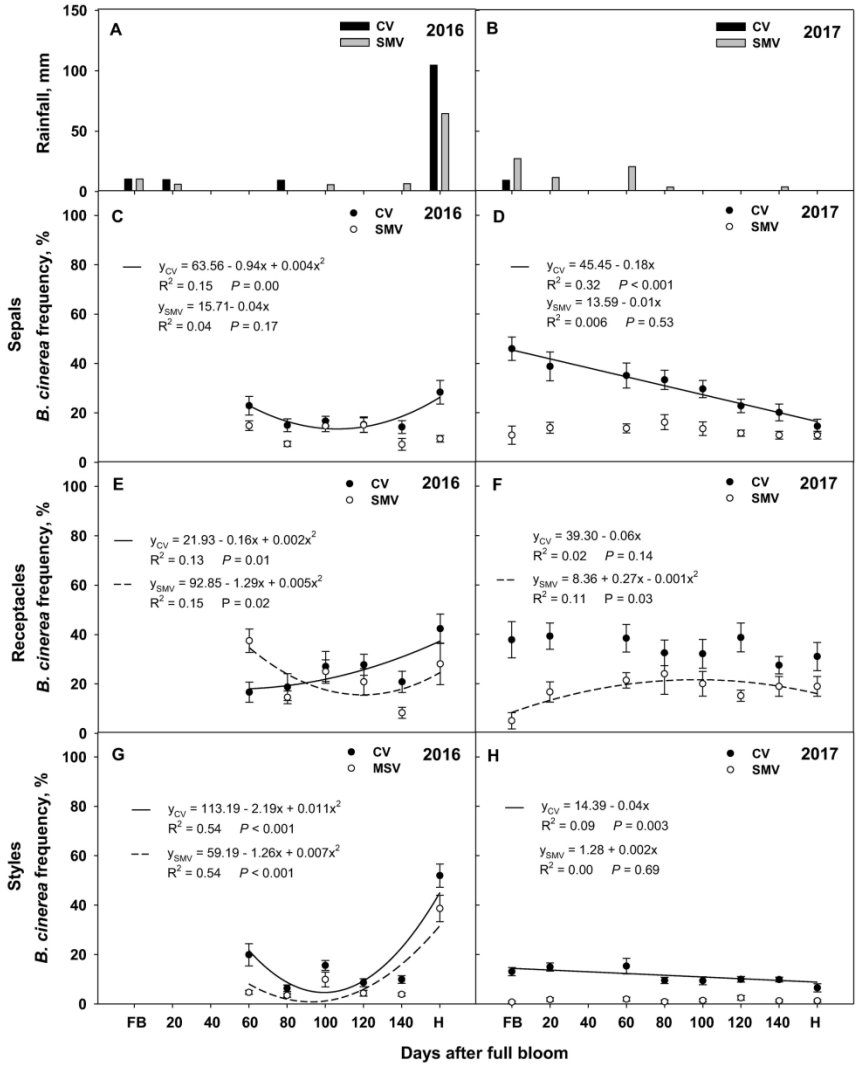

Fig. 2. Cumulated rainfall (A, B) and temporal dynamic of *Botrytis cinerea* affecting sepals (C, D), receptacles (E, F), and styles (G, H) of 'Hayward' kiwifruit from full bloom (FB) to harvest (H) in five orchards located in Cachapoal Valley (CV) and South of Maule Valley (SMV) in 2016 season (A, C,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

190x254mm (300 x 300 DPI)

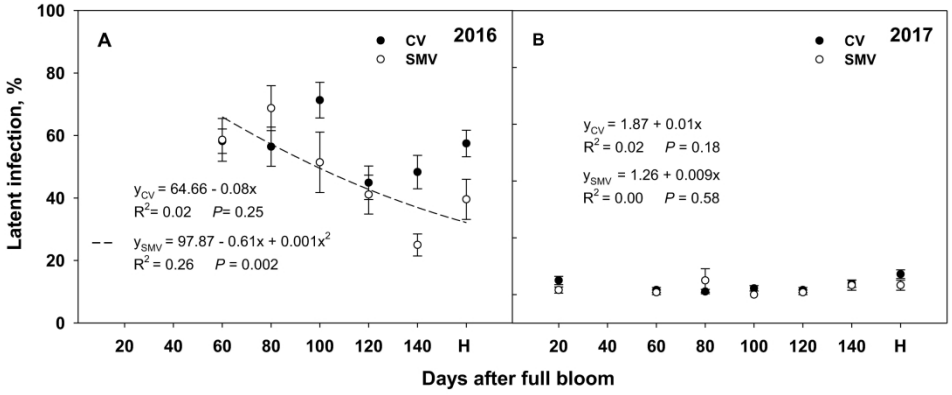

Fig. 3. Temporal dynamic of *Botrytis cinerea* latent infection in 'Hayward' kiwifruit from 20 DAFB to harvest (H) of three orchards in Cachapoal Valley (CV) and two in South of Maule Valley (SMV). **A.** 2016 season. **B.** 2017 season. Latent infection was determined after 6-10 days of incubation at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

338x190mm (300 x 300 DPI)

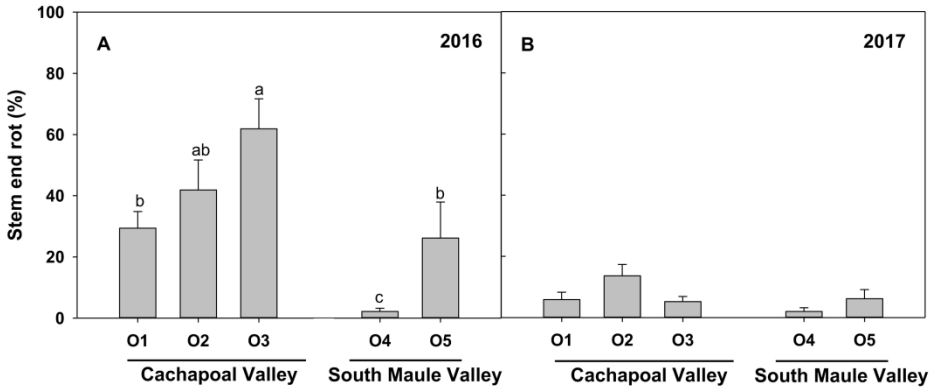

Fig. 4. Stem end rot incidence (%) after 100 days at 0°C plus 2 days at 20°C in five 'Hayward' kiwifruit orchards located in Cachapoal Valley (Orchards 1, 2 and 3) and South of Maule Valley (Orchards 4 and 5). **A.** 2016 growing season. **B.** 2017 growing season. Vertical bars = the standard error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

338x190mm (300 x 300 DPI)

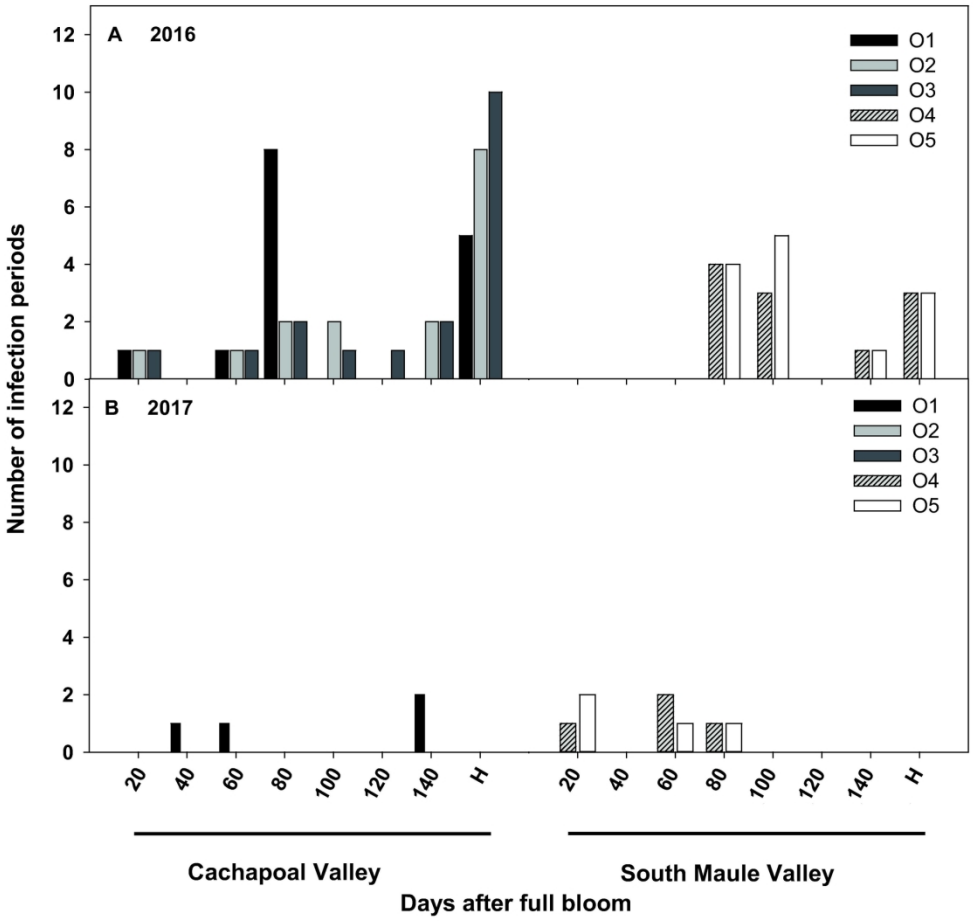

Fig. 5. Infection periods counted between full bloom to harvest in five orchards located in Cachapoal Valley (Orchards 1, 2 and 3) and South of Maule Valley (Orchards 4 and 5). **A.** 2016 season (S1). **B.** 2017 season (S2). One infection period was estimated if 14°C < T < 25°C, RH > 90% or leaf wetness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

175x174mm (300 x 300 DPI)

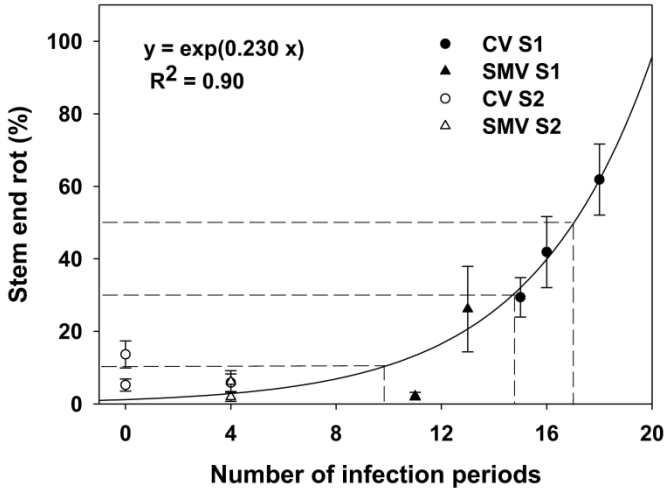

Fig. 6. Exponential relationship between the cumulated number of infection periods based on the algorithm of *Botrytis cinerea* model (Broome et al. 1995) from full bloom until harvest and stem end rot incidence after 100 days at 0°C plus 2 days at 20°C. Stem end rot incidence was estimated from five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

338x190mm (300 x 300 DPI)

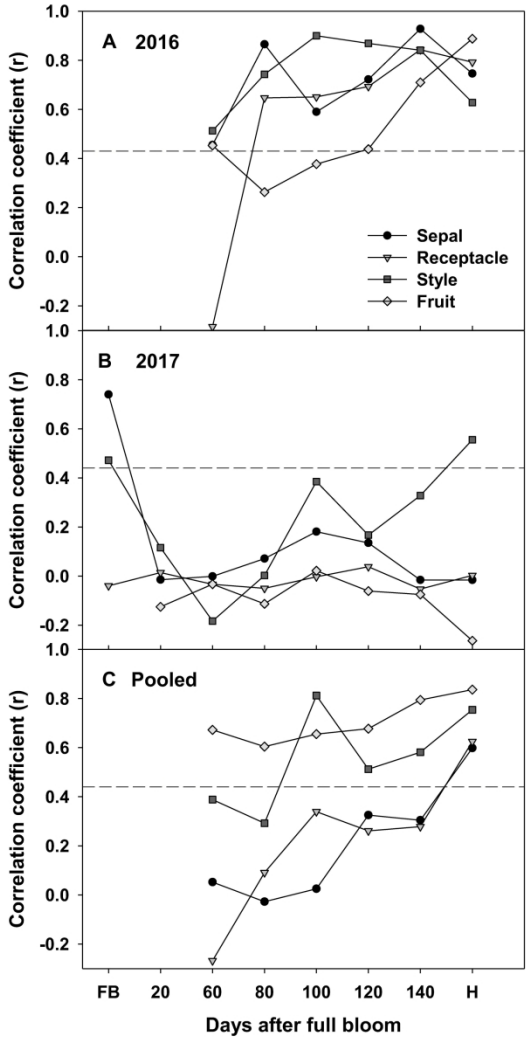

Fig. 7. Pearson's coefficients obtained from the correlations between postharvest stem end rot incidence and the presence of *Botrytis cinerea* in sepals, styles, receptacles and latent infection in fruit, from full bloom (FB) to harvest (H). **A.** Season 2016 (S1). **B.** Season 2017 (S2). **C.** Pearson's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

190x338mm (300 x 300 DPI)