Current Research in Microbial Sciences 2 (2021) 100049

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Molecular identification and characterization of *Botrytis cinerea* associated to the endemic flora of semi-desert climate in Chile

Ana-Maria Notte [a], Verónica Plaza [a], Bárbara Marambio-Alvarado [a], Lila Olivares-Urbina [a], Matías Poblete-Morales [b], Evelyn Silva-Moreno [b,c], Luis Castillo [*,a]

[a] *Laboratorio de Biología Molecular y Bioquímica, Departamento de Biología, Universidad de La Serena, Facultad de Ciencias, La Serena, Chile*
[b] *Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Santiago, Chile*
[c] *Instituto de Investigaciones Agropecuarias, INIA-La Platina, Santiago, Chile.*

ARTICLE INFO

Keywords:
Botrytis cinerea
Grey mould disease
Native flora
Genotypes

ABSTRACT

Botrytis cinerea is a phytopathogenic fungus that infects over 200 plant species and can cause significant crop losses in local and worldwide agricultural industries. However, its presence in the endemic flora in the Coquimbo Region and its impact on local flora have not been studied yet. In order to determine whether *Botrytis* spp is present in the native plant in the Coquimbo Region, fifty-two field-samples were analysed. A total of 30 putative *Botrytis* spp were isolated and phenotypic and genetically characterized. The internal transcribed spacer (ITS) analysis of these isolates revealed that it corresponded to genus *Botrytis*. For further confirmation, nuclear protein-coding genes (*G3PDH, HSP60*, and *RPB2*) were sequenced and showed 100% identity against *B. cinerea*. Complementary to this, *Botrytis* can also be clustered in two different groups, group I (*B. pseudocinerea*) and group II (*B. cinerea*), based on DNA polymorphism, the *Botrytis* isolates were identified as member of group II. On the order hand, we investigated the presence and frequency distribution of the transposable elements *boty* and *flipper* in the isolates obtained. The results indicate that 83.3% of the isolates presented both transposable elements, *boty* and *flipper*, indicating that the most prevalent genotype was transpose. In addition, 16.6% of the isolates showed substantially reduced virulence in apple fruit in comparison to B05.10 strain. According to fungicide resistance studies, the results indicate that resistance to Fenhexamid or Boscalid was observed in the 22.6% of isolates. The results show for the first time that *B. cinerea* has not been described before in fourteen new host plants and contributes to our fundamental understanding of the presence of *B. cinerea* in the native plant in the Coquimbo Region and the possible ecological impact of this disease on native and endemic plants.

1. Introduction

Botrytis cinerea is classified as an anamorphic fungus and belongs to the family *Sclerotiniaceae*, it is a phytopathogenic fungus that causes gray mold disease on a wide range of dicotyledonous plants species, including fruits, vegetables and ornamental plants (Jarvis, 1977; Elad *et al.*, 2004; Fillinger and Elad, 2016). In fact, in 2012 *B. cinerea* was supposed by Dean *et al.* (2012), as the second most important plant pathogen worldwide, after *Magnaporthe oryzae*. This due to the fact that *B. cinerea* annually causes enormous economic losses for commercial crops around the world. The identification of *Botrytis* species has traditionally been based on colony morphology and measurements of conidia and conidiophores, however the morphological observations can now be supplemented with molecular characteristics.

The sequence of the internal transcribed spacer (ITS) is used for the genetic identification and fungus phylogenetic relationships (White *et al.*, 1990). For further confirmation, a classification of the genus constructed based on DNA sequence data of three nuclear protein-coding genes (G3PDH, HSP60 and RPB2) is used (Staats *et al.*, 2005). In addition, *B. cinerea* is a phytopathogenic fungus with significant genetic diversity. Diolez *et al.* (1995) identified a retroelement from *B. cinerea* named *boty*, a 6-kb *gypsy*-like retrotransposon, later on, the transposable elements *flipper* (an 1872-bp class II element) was identified in *B. cinerea* (Levis *et al.*, 1997). Giraud *et al.* (1997) defined two sibling sympatric species based on the presence or absence of *boty* and *flipper: transposa* and *vacuma. Transposa* isolates possess both *boty* and *flipper*, whereas these elements are absent in *vacuma* isolates. Different studies have shown a significant differentiation in the frequency

* Corresponding author.
E-mail address: lcastillo@userena.cl (L. Castillo).

https://doi.org/10.1016/j.crmicr.2021.100049
Received 25 April 2021; Received in revised form 19 July 2021; Accepted 20 July 2021
Available online 24 July 2021

distribution of transposable elements among *Botrytis* isolates collected from different host plant and/or geographic regions (Giraud *et al.*, 1997; Giraud *et al.*, 1999; Fournier *et al.*, 2005; Kretschmer and Hahn, 2008; Tanović *et al.*, 2009; Fekete *et al.*, 2012; Samuel *et al.*, 2012; Tanović *et al.*, 2015; Wessels *et al.*, 2016). *Botrytis* can also be clustered in two different groups, group I and group II, based on DNA polymorphism revealed by microsatellite markers (Fournier *et al.*, 2003). Walker *et al.* (2011) carried out extensive research on Group I and named it *B. pseudocinerea* while Group II was named *B. cinerea* sensu stricto. Such information can help to characterize *B. cinerea* and the development of more effective strategies to control and manage the disease.

Fungicides with different modes of action are currently used to control *B. cinerea* such as Phenylpyrroles (Fludioxonil), Hydroxyanilides (Fenhexamid), Dicarboxamides (Iprodione), Succinate Dehydrogenase inhibitors (Boscalid), DeMethylation inhibitors (Tebuconazole) and inhibitors of methionine biosynthesis (Pyrimethanil). However, the intensive use of antifungal led to the rapid selection of resistant strains in various countries, in the field as well greenhouses (Faretra *et al.*, 1989; Yourman and Jeffers, 1999; Moyano *et al.*, 2004; Zhang *et al.*, 2010). In the last decade resistance to Fenhexamid, Pyrimethanil, Fludioxonil, Anilinopyrimidine and Boscalid fungicides have been reported among populations of *B. cinerea* (Latorre et al., 2002; Moyano et al., 2004; Esterio et al., 2007; Fillinger et al., 2008; Fernandez-Ortuno et al., 2012; Fernandez-Ortuno et al., 2013; Fernandez-Ortuno et al., 2014; Esterio et al., 2015). Molecular characterization of *B. cinerea* isolates that were sensitive or resistant to single-site fungicides showed the involvement of major genes of the fungus and a strong association between resistant phenotypes and point mutations (single nucleotide polymorphisms, SNPs) (De Miccolis Angelini *et al.*, 2012). On the other hand, *B. cinerea* possesses multiple ABC (*bcatrA, bcatrB* and *bcatrD*) and MFS (*bcmfs1*) transporter genes that play a role in protection against the plant defense and has also been associated to the transport of several drugs (Movahedi and Heale, 1990a; Movahedi and Heale, 1990b; Walton, 1994; Gomez-Gomez *et al.*, 2002; de Waard *et al.*, 2006).

This pathogen secretes an arsenal of enzymes, toxins, secondary metabolites to help facilitate penetration into the host (Doss, 1999; Choquer *et al.*, 2007). Also, in the *B. cinerea* genome, 118 genes encoding putative Carbohydrate-Active Enzymes (CAZymes) have been identified and can be associated with plant cell-wall degradation to obtain the source of nutrition from dead tissue. This big enzymatic repertoire could explain, in part, the ability of *B. cinerea* to infect over 200 different plant species (Castillo *et al.*, 2017). These CAZymes and other genes are essential for pathogenesis and infection process, some of them play a key role in degrading the cuticle and plant cell wall to enter the plant, others form specialized structures, such as appressoria, to penetrate the epidermis (Mendgen *et al.*, 1996; Plaza *et al.*, 2020). All these mechanisms contribute to *B. cinerea* being a good candidate to survive and spread in extreme climates such as the semi-desert climates in the Coquimbo Region in Chile (Plaza *et al.*, 2018).

B. cinerea has been highly studied and characterized in crop to crops with an economic interest, such as, *Vitis vinifera, Malus sp*., *Fragaria sp*., *Eustoma sp*. Flowers, *Rosa sp*. flowers, *Dianthus sp*., *Solanum sp*., *Vaccinium sp*., *Phaseolus sp., Zea sp*., *Beta sp.*, among others (Haydu and Legard, 2003; Domínguez *et al.*, 2008; Salami *et al.*, 2010; Yin *et al.*, 2011; Leroch *et al.*, 2013; Grant-Downton *et al.*, 2014). Nevertheless, there are few studies focused on analyzing and characterizing the presence and infection of *B. cinerea* and other phytopathogenic fungi associated with native and endemic plants that have little commercial interest.

The Region of Coquimbo in Chile is located in the southern limit of the Atacama Desert and presents a semi-arid climate, it extends from 29 ° 00′S to 32 ° 10′S covering an area of 40,462 km^2 (Novoa and López, 2001). This Region is located in one of the 25 points with the greatest biodiversity worldwide (Myers *et al.*, 2000; Arroyo *et al.*, 2008), it contains a high percentage of native species of plants including 1478 species and 244 naturalized introduced plant species, these species of plants represent a 30% of the total species of the flora of continental Chile (Squeo *et al.*, 2001)., and the most predominant are herbs perennials (44.7%), followed by bushes (27.2%) and annual or bi-annual herbs (23%) (Squeo *et al.*, 2001) and many of them are in the category of Vulnerable and in Danger of extinction (Squeo *et al.*, 2001; Arroyo *et al.*, 2008), due, possibly to water scarcity, human intervention and / or possibly due to viral or fungal infections. In Chile the studies about *B. cinerea* are concentrated mainly in plants of agricultural interest such as *Vitis vinifera* (Latorre *et al.*, 2002; Muñoz *et al.*, 2002; Esterio *et al.*, 2007; Esterio *et al.*, 2011; Esterio *et al.*, 2015; Latorre *et al.*, 2015), however some efforts to identify and characterize of fungi associated with native plants or plants with low commercial interest have been done. Fungi associated with rocks of the Atacama Desert and endophytic fungi associated with the roots of *Chenopodium quinoa* have been identified (Goncalves *et al.*, 2016; Gonzalez-Teuber *et al.*, 2017). In addition, Plaza *et al.* (2018) report the presence of *B. cinerea* causing gray mold disease on the endemic plant *Echinopsis coquimbana* in the Coquimbo Region. Nevertheless, further studies are required to determine the distribution and ecological impact of this pathogen on endemic plants.

Our aims were to determine if there was a presence of phytopathogenic fungi *B. cinerea*, a highly pathogenic fungus on native and endemic plants in the Coquimbo Region. This manuscript presents a genetic and phenotypical study, where thirty filamentous fungus *B. cinerea* were characterized, while simultaneously obtaining a panoramic view about this fungal population found on these plants.

2. Materials and methods

2.1. Botrytis isolation from native plant and growth conditions

Native plant leaves were analyzed in 6 points in the semi-deserts climate in the Coquimbo Region, Chile: Caleta Hornos, Quebrada de Talca, Punta Teatinos, El Peñon, Totoralillo and Parque Nacional Fray Jorge 2014 to 2015 (Fig. 1). The samples were collected from tissue of symptomatic and non-symptomatic leaves of endemic plants. The samples were put on petri plates with 2% malt extract agar (MEA) for 4 days in the dark and only fungi similar to *Botrytis cinerea* were selected for analysis. Isolated *Botrytis* selected were grown on Petri plates with 2% MEA for 6 days at 20°C in the dark, then were incubated between 300 to 400 nm light spectrum for 24 hours, to induce sporulation (Tan and Epton, 1973), after which they were incubated in darkness for 24 to 48 hours again. The spores were preserved in glycerol 50% at -80°C.

2.2. DNA extraction, PCR amplification and sequencing

The spores were grown on malt agar for 48 hours at 20°C. Mycelium was harvested, submerged into liquid nitrogen and ground into a powder. Genomic DNA extraction was performed using standard protocols (Sambrook *et al.*, 1989). DNA pellets were dissolved in 50 μL of TE (10mM Tris-HCl [pH 8.0], 1mM EDTA) and quantified using a Nanodrop spectrophotometer. To identify *B. cinerea* species we sequenced the ITS region and *G3PDH, HSP60*, and *RPB2* genes (White *et al.*, 1990; Staats *et al.*, 2005). Primer combinations (Table S1) were designed to amplify the *G3PDH, HSP60*, and *RPB2* regions. For amplifications of ITS region, primers IST1 and ITS4 were used (White *et al.*, 1990). The PCR reaction was conducted on a final volume of 25 μL, containing 1X Expand High Fidelity Buffer with $MgCl_2$, 0.2 μM of each primer, 0.2 mM of each dNTP (GE Healthcare Lifesciences, USA), 1.25 U Expand High Fidelity Taq (Expand™ High Fidelity PCR System kit, Roche) and 10 ng fungal DNA and amplifications were carried out in a Axygen MaxyGene II Thermal Cycler (Axygen a Corning Brand, USA). The following thermocycling pattern was used to amplify *HSP60* and *G3PDH* gene fragments: 95°C for 60 s (1 cycle), 95°C for 30 s, 64°C for 60s, 68°C for 90 s (15 cycles), 95°C for 30 s, 62°C for 60 s, 68°C for 90 s (20 cycles), and then 68°C for 5 min (1 cycle) (Table S2). To amplify RPB2 gene fragment the program was 95°C for 2 min (1 cycle); 95°C for 30 s, 58°C for 60 s, and 68°C for 4 min

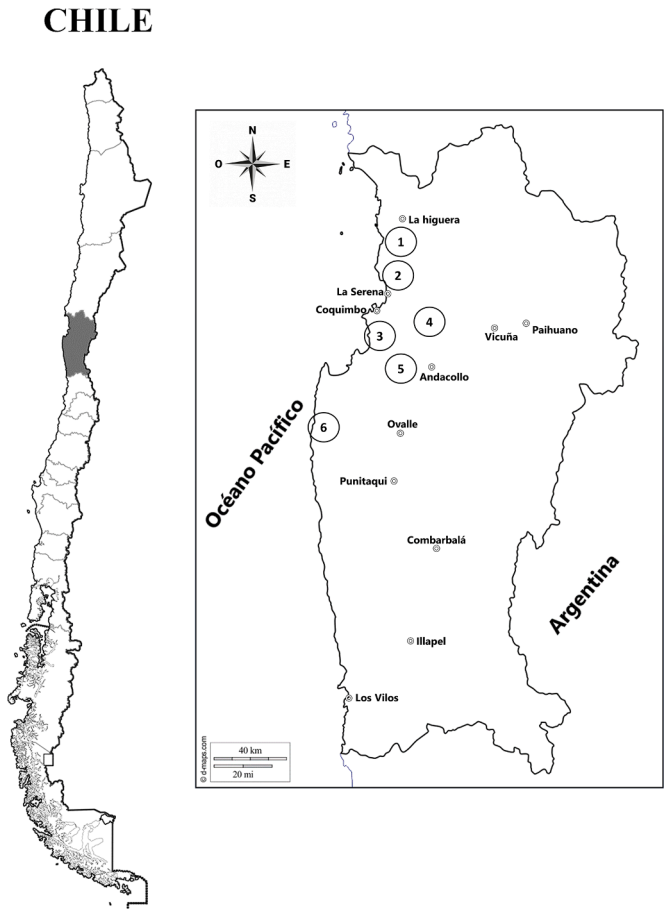

Fig. 1. Map of Coquimbo Region in Chile. Number indicates the geographical locations where native plants were collected. 1. Caleta de Hornos; 2. Punta Teatinos; 3. Totoralillo Sur; 4. Quebrada de Talca; 5. El peñón; 6. Parque Nacional Fray Jorge.

(30 cycles), and then 68°C for 10 min (1 cycle). For amplification of the ITS region, an amplification protocol was used as described by White et al. (1990). The presence of transposons was detected by PCR with the primers *boty*-F and *boty*-R to amplify transposon *boty,* and the primers F300 and F1500 to detect *flipper* (Table S1) (Giraud *et al.*, 1999; Munoz *et al.*, 2010). The amplification protocol consisted of an initial denaturation 95°C for 60 s (1 cycle); 95°C for 30 s, 58°C for 60 s, and 68°C for 90 s (32 cycles), and then 68°C for 10 min (1 cycle) (Table S2).

2.3. Molecular and phylogenetic identification

For the identification of native isolates *Botrytis* sp., analysis of *HSP60, RPB2*, and *G3PDH* gene were sequenced using the same primers by Macrogen (Seoul, Korea). The multiple alignments were performed using CLUSTAL W (Thompson *et al.*, 1994). The phylogenetic trees were constructed based on the neighbor-joining method (Saitou and Nei, 1987) and the topology confirmed with the Maximum Likelihood and Maximum Parsimony methods by using the MEGA X software (Kumar *et al.*, 2018). Distance matrices were calculated by the Kimura 2-parameter method and bootstrap analysis was performed based on 1000 re-samplings (Kumar *et al.*, 2018). Nucleotide sequences of *HSP60, RPB2, G3PDH*, commonly used for differentiation of *Botrytis* species, were obtained from the GenBank website (Table S3). The partial sequences obtained in this study have been deposited in GenBank (Table S4).

2.4. Determination of group I and Group II of B. cinerea

To determine *B. cinerea* (group II) or *B. pseudocinerea* (group I) (Fournier *et al.*, 2003) from the isolates collected from endemic flora we used the *PCR-RFLP* technique. We used the primers Bc-hch262 and Bc-hch520L (Table S1). We amplified it by PCR in a Axygen MaxyGene II Thermal Cycler (Axygen a Corning Brand, USA), each PCR reaction containing a volume of 25 µL, 1X ThermoPol Reaction Buffer with $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP, 1.25 U *taq* DNA Polymesase and 10 ng fungal DNA (*taq* DNA polymerase with ThermoPol Buffer, New England BioLabs), using the program followed by 1 cycles of 4 minutes at 95°C, 30 cycles of 30 seconds at 95°C, 1 minute at 55°C, 60 s at 68°C, finally 1 cycles of 5 minutes at 68°C (Table S2). Restriction digests were done for 1.5 h at 37°C, in a total volume of 20 µL containing 5 µL PCR product, 2 µL reaction buffer, and 1 U restriction enzyme H*ha*I (Fournier *et al.*, 2003).

2.5. Sporulation and infection tests

The fungal isolates were grown on potato dextrose agar (PDA, AppliChem) and synthetic minimal medium (MM) containing 2% sucrose, 0.1% KH_2PO_4, 0.3% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4 \bullet 7H_2O$, pH 5.0 (Plaza et al 2013). The spore production were measured from cultures on PDA, each strain was seeded with 0.5 mm circular section of PDA agar with mycelium of the fungus; the isolate was incubated in PDA for 2 weeks at 20ºC under a 24 h photoperiod (12 h light/ 12 h darkness). The spores were collected and filtered with miracloth (Merck, **USA**) a resuspended in 10 mL water sterile and counted with Neubauer camera. For sclerotia formation, each isolate was seeded with 0.5 mm circular section of PDA agar with mycelium of the fungus in MM for 2 weeks at 17ºC in darkness.

Infection tests of apple fruits were performed as described by Doehlemann *et al.* (2006). Prior to inoculation, the fruit tissues were wounded with a pinprick of a 21G syringe and surface-sterilized by immersion in 75% ethanol for 1 min. Inoculation of the fruits was performed with 5 µL droplets of 2.5×10^5 conidia/mL conidial suspensions for 4 days at 20ºC in Percival incubators (Percival, USA). The B05.10 wild-type strain was used as a control strain due its virulence on apple, tomato or grapevine as well as others fruit or plant (Nafisi *et al.*, 2014; Plaza *et al.*, 2015; Zhang *et al.*, 2016; Plaza *et al.*, 2018; Liu *et al.*, 2019). The pathogenicity of *B. cinerea* isolates in this study was compared to the B05.10 strain.

2.6. Fungicide resistance test

To determine the antifungal susceptibility in samples isolated from field we tested six chemical groups of different antifungals: Fenhexamid, Iprodione, Fludioxonil, Tebuconazole, Pyrimethanil and Boscalid. Agar disk (8 mm diameter) were cut from malt agar 2% and previously inoculated with 10 µL of a conidial suspension 1×10^5 conidia/mL and incubated for 24 hours at 20°C in the dark in Percival incubators (Percival, USA). These disks were then placed upside-down onto plates containing medium with different concentrations of antifungal and all cultures were incubated for 72 hours at 19°C in the dark in Percival incubators (Percival, USA). The Sisler synthetic medium composed of 2% KH_2PO_4, 1.5% K_2HPO_4, 1% $(NH_4)_2SO_4$, 0.5% $MgSO_4 \bullet 7H_2O$, 10% glucose, 2% yeast extract, 12.5% agar (Leroux *et al.*, 1999) was used to Fenhexamid resistant assays. The Fenhexamide concentrations used were: 0, 0.3, 1, 3 and 10 µg/mL and Sisler medium with 0.5% ethanol was used as a control. Meanwhile, the culture medium used with the fungicide Iprodione was 2% Potato Dextrose Agar medium and the concentrations used were: 0.1, 0.3, 0.6, 1.25, 2.5 and 5 µg/mL and PDA medium and 0.5% ethanol was diluted in the medium and used as a control (Dennis and Davis, 1979). To Fludioxonil, Tebuconazole, Pyrimethanil and Boscalid fungicides were tested into nutrient medium composed by 10% glucose, 2% K_2HPO_4, 2% KH_2PO_4 and 10% agar. The

concentrations used were: 0, 0.3, 1, 3, and 10 μg/mL. In a similar way to Fenhexamide and Iprodione, 5% ethanol was used as a control (Latorre *et al.*, 2002; Weber and Hahn, 2011).

The EC_{50} value is the effective fungicide concentrations causing 50% inhibition of the germ-tube growth according to described by Weber and Hahn (2011). Fungicide sensitivity categories (sensitive and resistant) were defined according to the discriminatory doses that differentiate resistant from sensitive isolates as follows: Fenhexamid resistant ($EC_{50} \geq$ 7.68 μg/mL); Iprodione resistant ($EC_{50} \geq 2$ μg/mL); Fludioxonil ($EC_{50} \geq$ 3.2 μg/mL); Tebuconazole ($EC_{50} \geq 1.65$ μg/mL); Pyrimethanil ($EC_{50} \geq 9$ μg/mL) and Boscalid ($EC_{50} \geq 6.4$ μg/mL). The mean colony diameter minus the diameter of the inoculation disk was measured and expressed as the percentage of the mean colony diameter of the untreated control. Each test was performed three times.

2.7. Similarity analysis of B. cinerea isolates

To know the similarity in *Botrytis* isolates we performed an analysis of *Multidimensional Scaling non metric, NMDS* using the correlation matrix by index of similarity Bray-Curtis using PRIMER v6 program (Clarke and Gorley, 2006). This analysis was based on results of ITS sequencing, detection transposon *boty* and *flipper*, infection test and resistance boscalid antifungal.

3. Results

3.1. Isolations and fungal identification

Our studies were conducted in 2014/ 2015 and a total of Fifty-two field-sampled plants were processed (Fig. 1 and S1). From these, a total of 85 fungal strains were obtained. Thirty fungal colonies obtained were initially white, then became gray to brown after 6 days. Analysis of light micrographs displayed elliptical conidia on MEA. The overall morphology suggests the isolates are members of the genus *Botrytis* and a total of thirty putative *Botrytis* spp were isolated in 17 plant species (Table 1).

3.2. Sequence analyses

To determine their identity, all isolates were subjected to DNA sequence analysis. ITS analysis of this isolate revealed that all isolates correspond to genus *Botrytis*. For further confirmation, nuclear protein-coding genes (*G3PDH, HSP60*, and *RPB2*) were sequenced (Table S1 and S4). The partial sequences of the *HSP60* (900 bp), *RPB2* (926 bp) and *G3PDH* (888 bp) genes analyzed by Blast NCBI report a high percentage of identity of all isolates obtained with strains and isolates of *Botrytis cinerea* (Table S4). Therefore, based on these results, the phylogenetic analysis of genes was performed with strains belonging to species of the genus *Botrytis*, the phylogenetic position observed by the individual analysis of the genes (Fig. S2-S4) and concatenated genes (*HSP60, RPB2* and *G3PDH*; 2714 bp) groups all isolates obtained in a single clade together with *B. cinerea* B05.10 (Fig. 2 and S5). Based on DNA polymorphism evidenced by the molecular marker Bc-*hch* and the restriction enzyme H*haI,* a through the Restriction *Fragment Length Polymorphism* (PCR-RFLP) technique, two cryptic species can be differentiated, *B. cinerea* and *B. pseudocinerea.* The analysis showed that the 100% of isolates belong to the species of *B. cinerea* (group II) (data not shown).

To investigate the presence and frequency of the transposable elements in populations of the *Botrytis* isolates in the endemic plant, the retrotransposon (*boty*), and the transposon (*flipper*) was detected. PCR-based detection of the two transposable elements showed that, in all the sampled hosts, only three possible *Botrytis* types existed. The results showed that 25 of the 30 wild *Botrytis* isolates presented both transposes in their genome, indicating that they present the genotype *transposa* (83.3%). On the other hand, three wild *Botrytis* isolates had only the *boty* transposon (10%) in their genome, indicating that they present the *boty* genotype, and two isolates belong exclusively to the *vacuma* genotype (6.7%), whereas the *flipper* genotype was absent (Fig. 3 and Fig S6). The results of these analyses indicate that the most predominant genotype in the isolates was *transposa*, where only Totoralillo and PNBFJ localities

Table 1
B. cinerea isolated in this study.

Strain	Host Plant	Conservation	Geographic Origin	Year	Coordenates
Bc.ad01	*Adesmia bedwelli*	OD	PNBFJ Quebrada	2015	30°70′43′′S 71°67′41′′O
Bc.ad02	*Adesmia bedwelli*	OD	PNBFJ Quebrada	2015	30°70′43′′S 71°67′41′′O
Bc.ar01	*Aristolochia chilensis*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.ar02	*Aristolochia chilensis*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.ar03	*Aristolochia chilensis*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.cu01	*Cumulopuntia sphaerica*	NE	Caleta de Hornos	2015	29°37′54′′S 71°17′03′′O
Bc.eq01	*Equinopsis coquimbana*	OD	Caleta de Hornos	2014	29°37′54′′S 71°17′03′′O
Bc.eua01	*Eulychnia acida*	OD	Peñon	2015	30°8′56′′S 71°12′40′′O
Bc.eub01	*Eulychnia breviflora*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.fu.01	*Fuchsia lycioides*	OD	PNBFJ Bosque	2015	30°65′19′′S 71°68′81′′O
Bc.he01	*Heliotropium stenophyllum*	OD	Caleta de Hornos	2014	29°37′54′′S 71°17′03′′O
Bc.he02	*Heliotropium stenophyllum*	OD	Peñon	2015	30°8′56′′S 71°12′40′′O
Bc.he03	*Heliotropium stenophyllum*	OD	Peñon	2015	30°8′56′′S 71°12′40′′O
Bc.he04	*Heliotropium stenophyllum*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.li01	*Lithraea caustica*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.ma01	*Malva nicaeensis*	IK	Quebrada de Talca	2014	30°0′21′′S 71°1′20′′O
Bc.my01	*Myrcianthes coquimbensis*	D	Punta Teatinos	2014	29°50′24′′S 71°6′56′′O
Bc.ox01	*Oxalis gigantea*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.ox02	*Oxalis gigantea*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.pl01	*Pleocarphus revolutus*	OD	Peñon	2015	30°8′56′′S 71°12′40′′O
Bc.po01	*Porlieria chilensis*	VU	PNBFJ Quebrada	2015	30°70′43′′S 71°67′41′′O
Bc.po02	*Porlieria chilensis*	VU	PNBFJ Quebrada	2015	30°70′43′′S 71°67′41′′O
Bc.sc01	*Schinus molle*	OD	Peñon	2015	30°8′56′′S 71°12′40′′O
Bc.sc02	*Schinus molle*	OD	Peñon	2015	30°8′56′′S 71°12′40′′O
Bc.se01	*Senna cumingii*	OD	PNBFJ Quebrada	2015	30°70′43′′S 71°67′41′′O
Bc.se02	*Senna cumingii*	OD	PNBFJ Quebrada	2015	30°70′43′′S 71°67′41′′O
Bc.se03	*Senna cumingii*	OD	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.tr01	*Trichocereus deserticola*	NE	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.tr02	*Trichocereus deserticola*	NE	Totoralillo	2015	30°4′6′′S 71°22′27′′O
Bc.tr03	*Trichocereus deserticola*	NE	Totoralillo	2015	30°4′6′′S 71°22′27′′O

D= Danger, V= Vulnerable, OD= Out of danger, IK= Insufficiently known, NE= Not evaluated, PNBFJ= Parque Nacional Bosque de Fray Jorge.

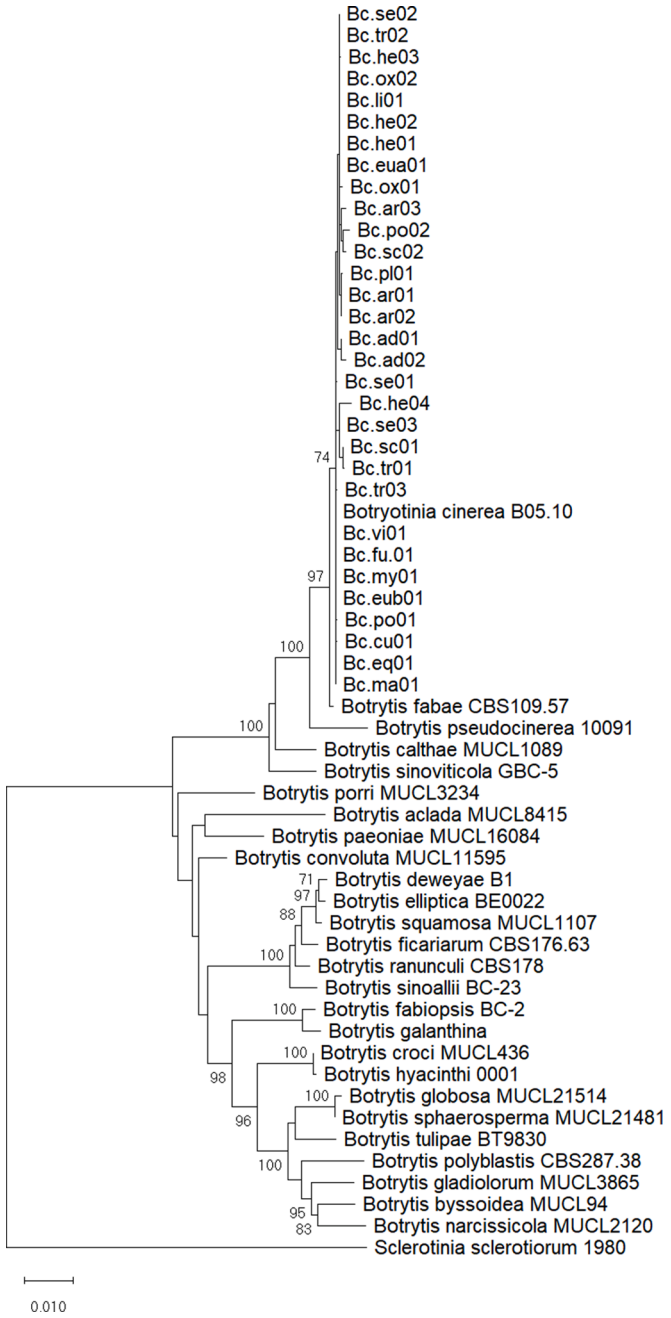

Fig. 2. Maximum Likelihood phylogenetic tree based on *HSP60, RPB2 AND G3PDH*. Sequences showing the phylogenetic position of native isolated of *B. cinerea*. Bootstrap values (>70%) based on 1000 replications are shown at the nodes of the tree. Bar, 1 substitutions per 1000 nt. *Sclerotinia sclerotiorum* was used as the outgroup.

presented *vacuma* and *boty* genotypes, meantime *vacuma* genotype was found only in the PNBFJ (Fig. 3).

3.3. Phenotypic characterization of Botrytis isolates

Botrytis isolates sporulation was compared by counting the number of conidia formed by each one of them. The results showed significant variation in conidial production on MEA when compared with the reference strain B05.10. Twenty four isolates strains showed significant reduction in the conidial production in comparison to B05.10 from no conidial production such as *Bc.ox01* or *Bc.sc02* that showed the highest conidial production in this group that was 4×10^7 conidia/mL (± 8.3×10^6), the average of conidial production for the twenty four isolate was 1×10^7 conidia/mL (± 3.4×10^6) (Fig. 4a, b) significantly lower in comparison to B05.10 that was 6.3×10^7 conidia/mL (± 1.3×10^7). The remaining strains showed similar results that reference strain B05.10.

Sclerotia (size and number) were compared with the reference strain B05.10, isolates sclerotia formation was variable among and between them. Thirty six percentage of isolates presented (*Bc.he01, Bc.my01, Bc. se02, Bc.he03, Bc.pl01, Bc.tr01, Bc.ar01, Bc.eub01, Bc.ox01, Bc.ox02 y Bc. tr02*) significantly more sclerotia when compared to B05.10 (Fig. 5a, b) and thirteen percentage of the isolates did not produce sclerotia (*Bc. ad02, Bc.ma01, Bc.po02)*. On the other hand, twenty-eight isolate strains that were able to produce sclerotia, seventy-five percentage produced smaller sclerotia in comparison to B05.10 (Fig. 5a, b).

3.4. Pathogenicity assays

To evaluate the role of *Botrytis* isolates in fruit infection, a pathogenicity test was carried out on apple by inoculating 5 µL of a solution containing 2.5×10^5 conidia/mL of isolate strain. Four dpi, disease development was measured as the diameter of the expanding lesions. All the *Botrytis* isolates used in the study were pathogenic to apple fruit even though variability existed among isolates, in terms of disease severity (decay depth) on fruit (Fig. 6a, b). Furthermore, in five native isolates the disease incidence were significantly lower in comparison to reference strain B05.10, where the strain *Bc.oxo01* was the least pathogenic when compared to the others isolates and control strain. This data supports the idea that *B. cinerea* isolates have not lost the ability to infect another host.

3.5. Resistance profiles of Botrytis isolates

Susceptibility to six active ingredients representing six chemical classes of fungicides was determined, including Phenylpyrroles (Fludioxonil), Hydroxyanilides (Fenhexamid), Dicarboxamides (Iprodione), Succinate Dehydrogenase inhibitors (Boscalid), DeMethylation inhibitors (Tebuconazole) and inhibitors of methionine biosynthesis (Pyrimethanil). In total, four different resistance profiles were detected for the 30 *Botrytis* isolates used in this study (Table 2). Resistance to Fenhexamid or Boscalid was observed in the 22.6% of isolates, whereas 77.4% of isolates were susceptible to the six fungicides and none of isolates were multidrug-resistant. The *Bc.se01* isolate was moderately resistant to Fenhexamid, meantime *Bc.ar03, Bc.he03, Bc.he04* and *Bc. vi01* were slightly resistant, *Bc.ar01* was moderately resistant and *Bc. he02* highly resistant.

4. Discussion

Botrytis cinerea is a phytopathogenic fungus that can cause significant crop losses in the worldwide agricultural industries. However, its presence in the endemic flora in the Coquimbo Region has not yet been studied. In this present study, we conducted molecular and phenotypic analyses of thirty *B. cinerea* isolates from the native plants in the Coquimbo Region. Our analysis shows that ITS analysis of these isolates revealed that it corresponded to the genus *Botrytis*. For further confirmation, nuclear protein-coding genes (*G3PDH, HSP60*, and *RPB2*) were sequenced and the phylogenetic analysis of DNA sequence data showed 100% identity against *B. cinerea*. Classically, *B. pseudocinerea* and *B. cinerea*, two morphologically cryptic species, have been treated as *B. pseudocinerea* Group I, and *B. cinerea* sensu stricto Group II (Walker *et al.*, 2011). Erroneously *B. pseudocinerea* has been referred to previously as *B. cinerea* Group I (Fournier *et al.*, 2002; Martinez *et al.*, 2005). Consistent with the results of the sequencing of the *G3PDH, HSP60*, and RPB2 genes, the PCR-RFLP characterization of the native isolates obtained in this study indicated all the isolates within group II or what is classically known as *B. cinerea* sensu strict, revealed by the Bc-hch locus. Coincidentally with the findings of Fournier *et al.* (2003) the isolates

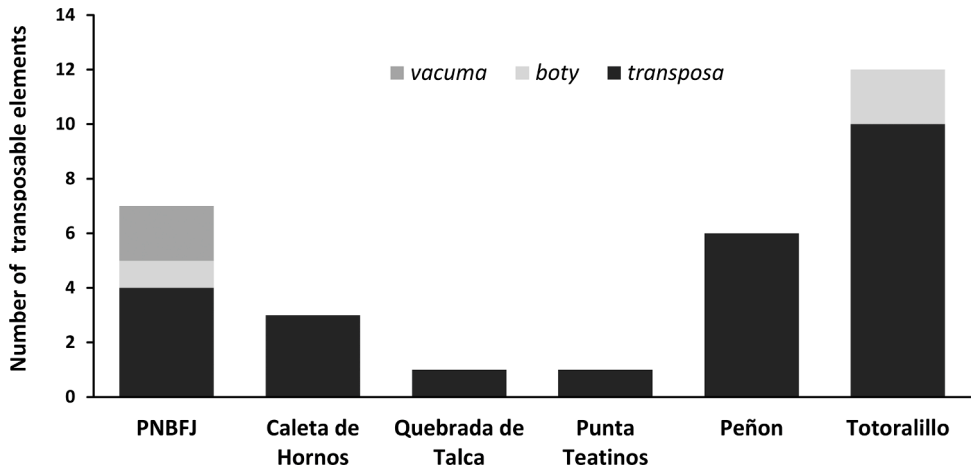

Fig. 3. Frequency distributions of transposable element types (*boty, flipper, transposa*, and *vacuma* type) in *Botrytis cinerea* populations collected from several hosts in Chile. 83% of the isolates presented both transposable elements (*boty* and *flipper*), indicating that the most prevalent genotype was transpose.

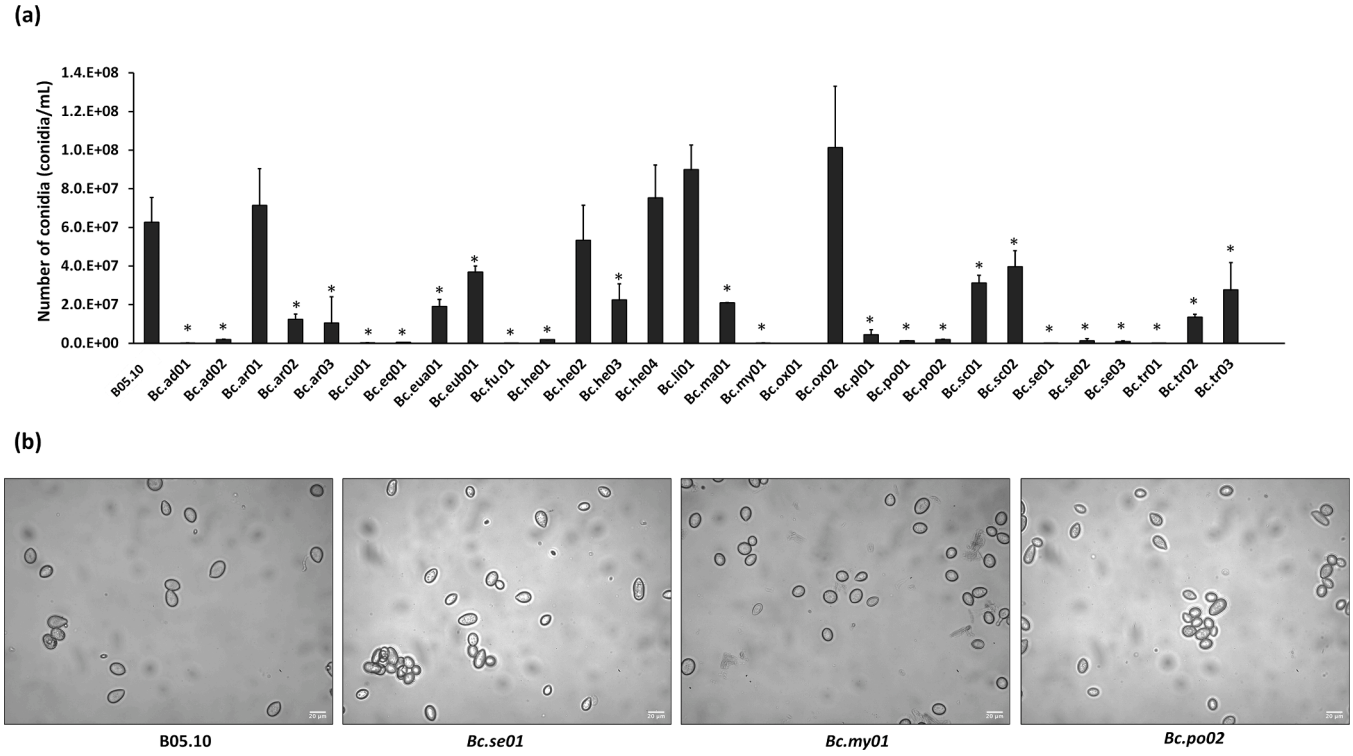

Fig. 4. Conidia production in *Botrytis cinerea* isolates in Coquimbo region. (a). Quantification of conidia production in the isolates grown for two weeks on MEA at 20 °C under a 24 h photoperiod (12 h light/12 h darkness). (b). Representative photographs of conidia suspensions in isolates strains. The experiments were performed in triplicates and the data was represented by means ± standard deviations. Asterisks indicate significant differences ($p<0.05$, Mann-Whitney U test) between the B05.10 WT strain and the isolates.

classified as Group II present a variable susceptibility to Fenhexamid. This analysis shows that *B. cinerea* is present in seventeen species of native/endemic plants where only three genera have been described in the literature as hosts of this fungus, the genus *Fuchsia, Heliotropium* and *Echinopsis* (Fillinger & Elad, 2016; Plaza *et al.*, 2018). Therefore, it is the first time that *B. cinerea* has been described in fourteen new host plant interactions. That could be explained because *B. cinerea* secretes numerous cell wall–degrading enzymes (CWDEs) and metabolites to breach the plant cell wall, obtaining the source of nutrition from dead tissue and also the ability to avoid plant resistance mechanisms (Staats *et al.*, 2005; Choquer *et al.*, 2007; van Baarlen *et al.*, 2007; Hahn *et al.*, 2014; Nakajima & Akutsu, 2014; Castillo *et al.*, 2017; Plaza *et al.*, 2020). This strategy could explain, in part, why *B. cinerea* can attack a wide range of host plants, including Chilean native plants.

The presence and frequency distribution of the transposable elements *boty* and *flipper* in the isolates showed that the genotype *transposa* has more frequency with 83.3%, after transposon *boty* (10%) and *vacuma* (6.7%). This result is according to Muñoz *et al.* (2002) where reported similar frequencies of *transposa, boty* and *vacuma* in grapes, tomato and blueberry in Chile, in addition they did not find any isolate with only transposable element *flipper*, identical results have been found in this study or in others, this supports the idea that the predominant genotypes in Chile are *transposa* (Esterio *et al.*, 2011). However, this frequency could be associated to the host, differences in genotype

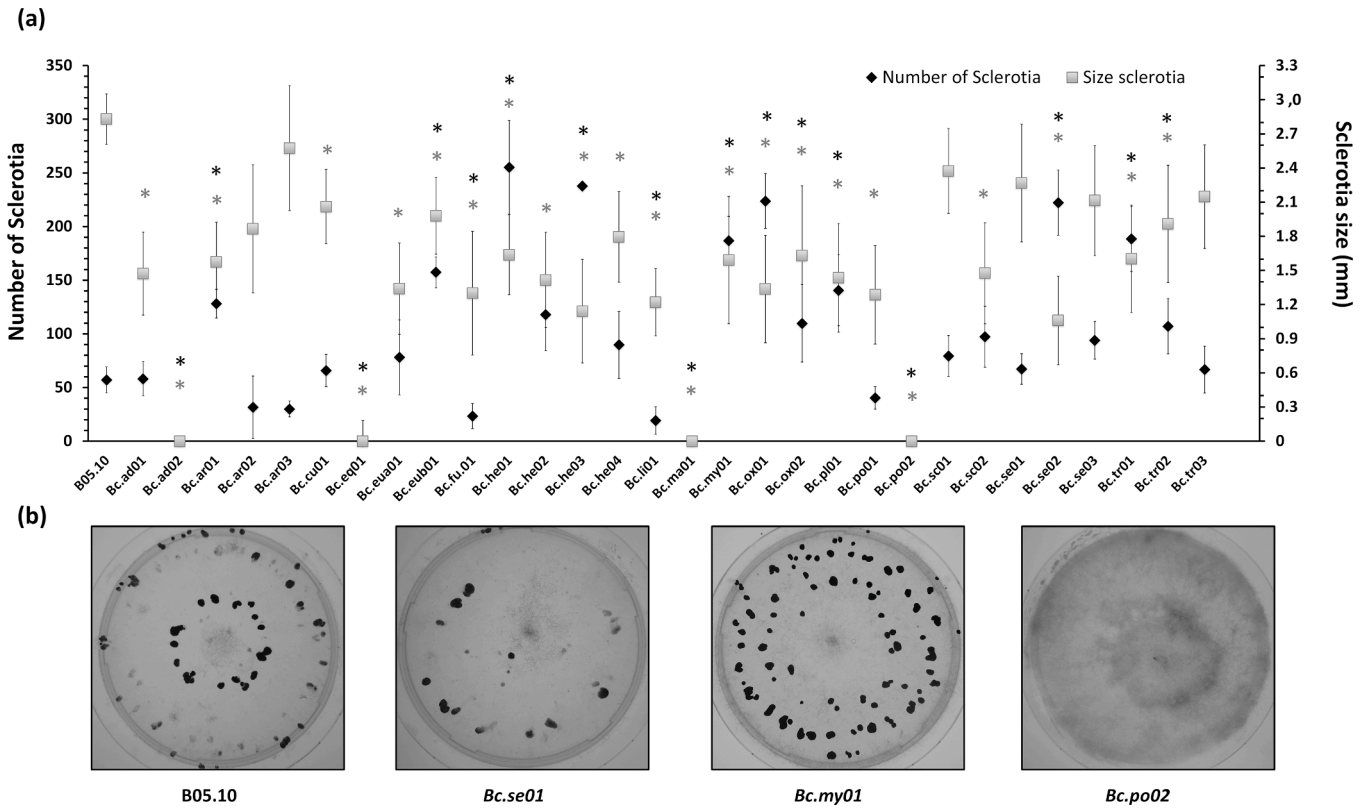

Fig. 5. Sclerotia production in *Botrytis cinerea* isolates in Coquimbo region. (a) (above) Sclerotia formation was induced by inoculating the strains on MEA plates in the darkness for two weeks. The form represents number of sclerotia and size sclerotia. (b) (down) Representative pictures are shown for some strains. The experiments were performed in triplicates and the data was represented by means ± standard deviations. Asterisks indicate significant differences ($p<0.05$, Mann-Whitney U test) between the B05.10 WT strain and the isolates. Black asterisk represents significance in number of Sclerotia and gray asterisk represents significance in size sclerotia.

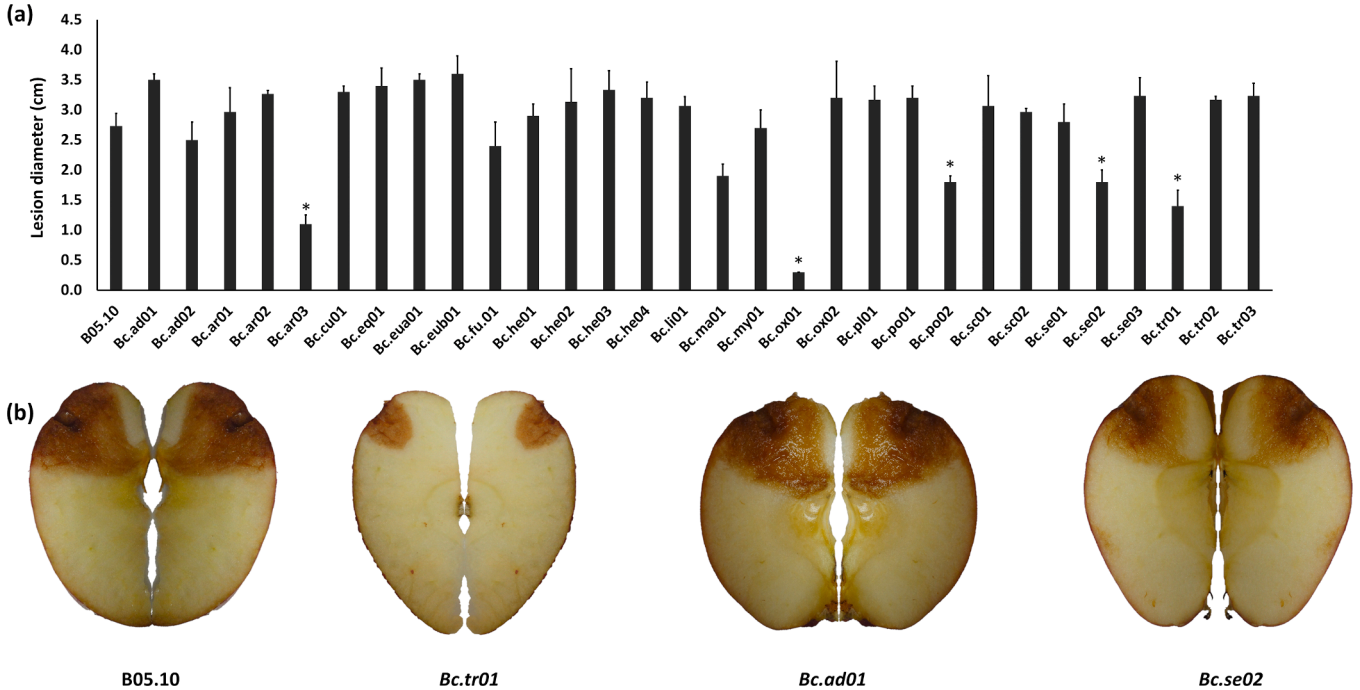

Fig. 6. Virulence assay of the *Botrytis* isolates. (a) Apple fruit tissues were wounded with a pinprick after which 5 mL droplets (2.5×10^5 conidia/mL) were inoculated. After 4 dpi at 20 °C, sizes of the lesions were measured. (b) Representative photographs of apple fruits infected with isolates strains at 7 dpi. The experiments were performed in triplicates and the data was represented by means ± standard deviations. The experiments were performed in triplicates and the data was represented by means ± standard deviations. Asterisks indicate significant differences ($p<0.05$, Mann-Whitney U test) between the B05.10 WT strain and the isolates.

Table 2
Sensitivity of *B. cinerea* isolates to the antifungal drug.

Strain	Host plant	Fludioxonil EC_{50} 3.2 μg/mL	Iprodione EC_{50} 2 μg/mL	Fenhexamid EC_{50} 7.68 μg/mL	Tebuconazole EC_{50} 1.65 μg/mL	Pyrimethanil EC_{50} 9 μg/mL	Boscalid EC_{50} 6.4 μg/mL
Bc.ad01	*Adesmia bedwelli*	S	S	S	S	S	S
Bc.ad02	*Adesmia bedwelli*	S	S	S	S	S	S
Bc.ar01	*Aristolochia chilensis*	S	S	S	S	S	MR
Bc.ar02	*Aristolochia chilensis*	S	S	S	S	S	S
Bc.ar03	*Aristolochia chilensis*	S	S	S	S	S	SR
Bc.cu01	*Cumulopuntia sphaerica*	S	S	S	S	S	S
Bc.eq01	*Equinopsis coquimbana*	S	S	S	S	S	S
Bc.eua01	*Eulychnia acida*	S	S	S	S	S	S
Bc.eub01	*Eulychnia breviflora*	S	S	S	S	S	S
Bc.fu.01	*Fuchsia lycioides*	S	S	S	S	S	S
Bc.he01	*Heliotropium stenophyllum*	S	S	S	S	S	S
Bc.he02	*Heliotropium stenophyllum*	S	S	S	S	S	HR
Bc.he03	*Heliotropium stenophyllum*	S	S	S	S	S	SR
Bc.he04	*Heliotropium stenophyllum*	S	S	S	S	S	SR
Bc.li01	*Lithraea caustica*	S	S	S	S	S	S
Bc.ma01	*Malva nicaeensis*	S	S	S	S	S	S
Bc.my01	*Myrcianthes coquimbensis*	S	S	S	S	S	S
Bc.ox01	*Oxalis gigantea*	S	S	S	S	S	S
Bc.ox02	*Oxalis gigantea*	S	S	S	S	S	S
Bc.pl01	*Pleocarphus revolutus*	S	S	S	S	S	S
Bc.po01	*Porlieria chilensis*	S	S	S	S	S	S
Bc.po02	*Porlieria chilensis*	S	S	S	S	S	S
Bc.sc01	*Schinus molle*	S	S	S	S	S	S
Bc.sc02	*Schinus molle*	S	S	S	S	S	S
Bc.se01	*Senna cumingii*	S	S	MR	S	S	ND
Bc.se02	*Senna cumingii*	S	S	S	S	S	S
Bc.se03	*Senna cumingii*	S	S	S	S	S	S
Bc.tr01	*Trichocereus deserticola*	S	S	S	S	S	S
Bc.tr02	*Trichocereus deserticola*	S	S	S	S	S	S
Bc.tr03	*Trichocereus deserticola*	S	S	S	S	S	S

S = Sensitive, LR = Slightly Resistant, MR = Moderately Resistant, HR = Highly Resistant, ND = Not Determined.

frequencies have been reported among populations in tomato, cucumber, grape, and strawberry, where *transposa* genotypes were predominant in the populations, while in kiwifruit and apple fruit the genotypes *vacuma* were prevailing (Samuel *et al.*, 2012). Similar results were found in the frequency of the transposable elements in Raspberry fruit, or Pear blossoms (Fournier *et al.*, 2005; Tanović *et al.*, 2009; Wessels *et al.*, 2016). Also, the frequency and distribution of transposon types varied markedly between geographic regions where India/Nepal and Australia were predominant by *boty* or *transposa* respectively, similar results have been reported in Europe (Kretschmer and Hahn, 2008; Fekete *et al.*, 2012; Tanović *et al.*, 2015), however in Bangladesh the predominant genotype was *flipper* (Isenegger *et al.*, 2008). Until now there is no plausible explanation about these genetic differentiations between regions, however it has been hypothesized that the climate, the geographic region or the isolation of some areas could have played a key role in the predominance of some genotypes on a worldwide scale (Isenegger *et al.*, 2008). Also, it has been proposed that change in the *vacuma* and *transposa* frequencies was most likely caused by differences in saprotrophic and pathogenic fitness (Martinez *et al.*, 2005). This differential distribution also could be explained because *transposa* has showed themselves to be more virulent than the other genotypes, that could explain why in our study the frequency of *transposa* has been more successful in native plant (Martinez *et al.*, 2003; Martinez *et al.*, 2005).

Differences in phenotypes from *B. cinerea* isolates also were found in this study, that some isolates of this fungus lose the ability to produce conidia or sclerotia formation and revealed a great diversity among the isolates concerning both features. A decrease in conidial production was also found in the isolates and significantly more sclerotia but smaller in comparison to B05.10, suggesting different abilities of the isolates to survive in these hosts. The growth rate, virulence and fungicide sensitivity have been studied in *B. cinerea*, where diversity in conidia and sclerotia formation were also found (Nicot *et al.*, 1996; Rebordinos *et al.*, 2000; Tanović *et al.*, 2009; Tanović *et al.*, 2015; Isaza *et al.*, 2019; Fedele *et al.*, 2020). The results of the pathogenicity test showed that disease incidence cause by isolates with *vacuma, transposa* or *boty* genotypes after wound inoculation of the apple fruit were similar. Although it has been suggested in *B. cinerea* that a relationship between genotypes and their pathogenicity. Martinez *et al.* (2005) demonstrated that *B. cinerea* with *transposa* genotypes were significantly more virulent than *vacuma* isolates, however other studies did not find differences among the isolates in each genetic group or correlation in isolates to different hosts, thereby making it the species where it is difficult to establish relationships between pathogenicity and genotype or phenotype characteristics (Rebordinos *et al.*, 2000; Samuel *et al.*, 2012; Tanović *et al.*, 2015; Isaza *et al.*, 2019).

To control *B. cinerea* several synthetic fungicides are widely used in the agriculture like Iprodione, Fludioxonil, Fenhexamid, Tebuconazole, Pyrimethanil and Boscalid. Our results show different grades of resistance to Fenhexamid or Boscalid, but no antifungal resistance was found in the other fungicides used in this study. *B. cinerea* represent a classical high-risk pathogen in fungicide resistance development, due to its high genetic variability (Leroux *et al.*, 2002; Rajaguru and Shaw, 2010). Resistance in *B. cinerea* has been associated to target site modifications, target site overexpression, efflux pump activation and detoxification. Fenhexamid acts by inhibiting the 3-ketoreductase involved in demethylation during the ergosterol biosynthesis and several mutations in this gene has been involved to different levels of Fenhexamid resistance. In addition, reports of Boscalid resistance in *B. cinerea* isolates from several host have been documented in the last ten years and associated with mutations in the *sdhB gene* (succinate dehydrogenase inhibitors) (Debieu *et al.*, 2001; Albertini *et al.*, 2002; Leroux *et al.*, 2002; Albertini and Leroux, 2004; Leroux *et al.*, 2010). This could explain partially why *B. cinerea* has been found with different levels of sensitivity to Fludioxonil, Fenhexamida, Boscalid, isolates from berries from the grapevine, Strawberry, Blackberry, and Champagne vineyards (Fillinger *et al.*, 2008; Esterio *et al.*, 2011; Fernandez-Ortuno *et al.*, 2012; Fernandez-Ortuno *et al.*, 2013; Fernandez-Ortuno *et al.*, 2014). Our results are in concordance with others studies, where have been detected Fenhexamid or Boscalid resistance in *B. cinerea* isolates from Full bloom, Ripe berries, Grapes, Pistachio orchards, Strawberry, Blueberry or herbaceous perennial Heuchera (Esterio *et al.*, 2011; Fernandez-Ortuno *et al.*, 2012; Moorman *et al.*, 2012; Grabke *et al.*, 2013; Saito *et al.*, 2016; Esteriol *et al.*, 2017; Avenot *et al.*, 2020). However, this is the first report where *B. cinerea* isolates from native plants in Chile were resistant to Fenhexamid and Boscalid. Further studies will be necessary to determine whether *B. cinerea* has far migrating spores, but one could imagine a human, insect or bird mediated transmission of the fungus that would favor the dispersion of infection between plant species.

5. Conclusions

In summary, this is the first study to provide evidence that *B. cinerea* can cause gray mold disease on a wide range of native and endemic plants in Chile. We provide evidence for phenotypes and genotypes diversity in these *B. cinerea* isolates. These findings are of great epidemiological importance for regions and the distribution and the possible ecological impact of this disease on native and endemic plants. Our results will help in the future for further studies toward improved target control strategies.

Ana Notte: Collection samples from native/endemic plant, Formal analysis, Investigation, review draft manuscript. Veronica Plaza: Formal analysis, Investigation, Methodology, Writing - original draft. Bárbara Marambio-Alvarado: Formal analysis and virulence data. Lila Olivares-Urbina: Formal and Investigation analysis and Genetic analysis. Matías Poblete-Morales: Informatic analysis in molecular and phylogenetic identification and assistance in the creation of Fig. 2 and Fig S2-S4. Evelyn Silva-Moreno: helped during the research work and helped for drafting the manuscript. Luis Castillo: The design concept, the content, original draft, as well as the visualization have been prepared and the manuscript revised.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by grants FIC-R BIP 3048588-0 GORE Coquimbo and Research Department of Universidad de La Serena [DIDULS] PT17122 and PT13121, La Serena, Chile.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2021.100049.

References

Albertini, C, Leroux, P., 2004. A Botrytis cinerea Putative 3-keto Reductase Gene (ERG27) that is Homologous to the Mammalian 17β-Hydroxysteroid Dehydrogenase type 7 gene (17β-HSD7). Eur. J. Plant Pathol. 110 (7), 723–733.

Albertini, C, Thebaud, G, Fournier, E, Leroux, P., 2002. Eburicol 14α-demethylase gene (CYP51) polymorphism and speciation in Botrytis cinerea. Mycol. Res. 106 (10), 1171–1178.

Arroyo, M, Marquet, P, Marticorena, C, Simonetti, J, Cavieres, L, Squeo, F., 2008. El hotspot chileno, prioridad mundial para la conservación. *Diversidad de Ecosistemas, Ecosistemas Terrestres*. Diversidad de Chile: Patrimonios y Desafíos, pp. 94–97.

Avenot, HF, Morgan, DP, Quattrini, J, Michailides, TJ., 2020. Phenotypic and molecular characterization of fenhexamid resistance in Botrytis cinerea isolates collected from pistachio orchards and grape vineyards in California. Crop. Prot. 133, 105133.
Castillo, L, Plaza, V, Larrondo, LF, Canessa, P., 2017. Recent Advances in the Study of the Plant Pathogenic Fungus Botrytis cinerea and its Interaction with the Environment. Curr. Protein Pept. Sci. 18 (10), 976–989.
Choquer, M, Fournier, E, Kunz, C, Levis, C, Pradier, JM, Simon, A, Viaud, M., 2007. Botrytis cinerea virulence factors: new insights into a necrotrophic and polyphageous pathogen. FEMS Microbiol. Lett. 277 (1), 1–10.
Clarke, K, Gorley, RN., 2006. PRIMER v6: user manual/tutorial. PRIMER-E, Plymouth. 29, 1060–1065.
De Miccolis Angelini, RM, Rotolo, C, Masiello, M, Pollastro, S, Ishii, H, Faretra, F., 2012. Genetic analysis and molecular characterisation of laboratory and field mutants of Botryotinia fuckeliana (Botrytis cinerea) resistant to QoI fungicides. Pest Manage. Sci. 68 (9), 1231–1240.
de Waard, MA, Andrade, AC, Hayashi, K, Schoonbeek, HJ, Stergiopoulos, I, Zwiers, LH., 2006. Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. Pest Manage. Sci. 62 (3), 195–207.
Dean, R, Van Kan, JA, Pretorius, ZA, Hammond-Kosack, KE, Di Pietro, A, Spanu, PD, Rudd, JJ, Dickman, M, Kahmann, R, Ellis, J, et al., 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13 (4), 414–430.
Debieu, D, Bach, J, Hugon, M, Malosse, C, Leroux, P., 2001. The hydroxyanilide fenhexamid, a new sterol biosynthesis inhibitor fungicide efficient against the plant pathogenic fungus Botryotinia fuckeliana (Botrytis cinerea). Pest Manage. Sci. 57 (11), 1060–1067.
Dennis, C, Davis, RP., 1979. Tolerance of Botrytis cinerea to iprodione and vinclozolin. Plant Pathol. 28 (3), 131–133.
Diolez, A, Marches, F, Fortini, D, Brygoo, Y., 1995. Boty, a long-terminal-repeat retroelement in the phytopathogenic fungus Botrytis cinerea. Appl. Environ. Microbiol. 61 (1), 103–108.
Doehlemann, G, Berndt, P, Hahn, M., 2006. Different signalling pathways involving a G alpha protein, cAMP and a MAP kinase control germination of Botrytis cinerea conidia. Mol. Microbiol. 59 (3), 821–835.
Domínguez I, Cedeno L, Briceño A, Pino H, Quintero K, Rodriguez L. 2008. Primer reporte en Venezuela de Botrytis cinerea causando quema foliar en Lisianto (Eustoma grandiflorum) First report of Botrytis cinerea in Venezuela causing leaf blight on Lisianto (Eustoma grandiflorum).
Doss, RP., 1999. Composition and enzymatic activity of the extracellular matrix secreted by germlings of botrytis cinerea. Appl. Environ. Microbiol. 65 (2), 404–408.
Elad, Y, Williamson, B, Tudzynski, P, Delen, N, 2004. Botrytis spp. and diseases they cause in agricultural systems – an introduction. Botrytis: Biology, Pathology and Control. Kluwer Academic Publishers, Dordrecht.
Esterio, M, Araneda, MJ, Román, A, Pizarro, L, Copier, C, Auger, J., 2015. First report of Boscalid resistant botrytis cinerea isolates carrying the mutations H272R, H272Y, P225L, and P225H from table grape in Chile. Plant Disease 99 (6), 891-891.
Esterio, M, Auger, J, Ramos, C, Garcia, H., 2007. First report of fenhexamid resistant isolates of botrytis cinerea on grapevine in Chile. Plant Disease 91 (6), 768.
Esterio, M, Muñoz Vera, G, Ramos, C, Cofré, G, Estévez, R, Salinas Rojas, A, Auger, J, 2011. Characterization of botrytis cinerea isolates present in thompson seedless table grapes in the central valley of Chile. Plant Disease 95, 683–690.
Esteriol, M, Copier, C, Román, A, Araneda, MJ, Rubilar, M, Pérez, I, Auger, J., 2017. Frequency of fungicide-resistant Botrytis cinerea populations isolated from Thompson Seedless table grapes in the Central Valley of Chile. Ciencia e investigación agraria 44, 295–306.
Faretra, F, Pollastro, S, Di Tonno, AP., 1989. New natural variants of Botryotinia fuckeliana (Botrytis cinerea) coupling benzimidazole-resistance to insensitivity toward the N-phenylcarbamate diethofencarb. Phytopathologia Mediterranea 28 (2), 98–104.
Fedele, G, Gonzalez-Dominguez, E, Si Ammour, M, Languasco, L, Rossi, V, 2020. Reduction of Botrytis cinerea Colonization of and Sporulation on Bunch Trash. Plant Disease 104 (3), 808–816.
Fekete, E, Fekete, E, Irinyi, L, Karaffa, L, Arnyasi, M, Asadollahi, M, Sandor, E, 2012. Genetic diversity of a Botrytis cinerea cryptic species complex in Hungary. Microbiol Res 167 (5), 283–291.
Fernandez-Ortuno, D, Bryson, PK, Grabke, A, Schnabel, G., 2013. First report of fludioxonil resistance in botrytis cinerea from a strawberry field in Virginia. Plant Disease 97 (6), 848.
Fernandez-Ortuno, D, Chen, F, Schnabel, G, 2012. Resistance to Pyraclostrobin and Boscalid in Botrytis cinerea Isolates from Strawberry Fields in the Carolinas. Plant Disease 96 (8), 1198–1203.
Fernandez-Ortuno, D, Grabke, A, Bryson, PK, Beasley, ED, Fall, LA, Brannen, PM, Schnabel, G., 2014. First report of Fludioxonil resistance in botrytis cinerea from a blackberry field in Georgia. Plant Disease 98 (6), 848.
Fillinger, S, Elad, Y., 2016. Botrytis - The Fungus, the Pathogen and its Management in Agricultural Systems. Springer International Publishing, New York, NY.
Fillinger, S, Leroux, P, Auclair, C, Barreau, C, Al Hajj, C, Debieu, D, 2008. Genetic analysis of fenhexamid-resistant field isolates of the phytopathogenic fungus Botrytis cinerea. Antimicrob Agents Chemother 52 (11), 3933–3940.
Fournier, E, Giraud, T, Albertini, C, Brygoo, Y., 2005. Partition of the Botrytis cinerea complex in France using multiple gene genealogies. Mycologia 97, 1251–1267.
Fournier, E, Giraud, T, Loiseau, A, Vautrin, D, Estoup, A, Solignac, M, Cornuet, JM, Brygoo, Y., 2002. Characterization of nine polymorphic microsatellite loci in the fungus Botrytis cinerea (Ascomycota). Mol. Ecol. Notes 2 (3), 253–255.
Fournier, E, Levis, C, Fortini, D, Leroux, P, Giraud, T, Brygoo, Y, 2003. Characterization of Bc-hch, the Botrytis cinerea homolog of the Neurospora crassahet-c vegetative incompatibility locus, and its use as a population marker. Mycologia 95 (2), 251–261.
Giraud, T, Fortini, D, Levis, C, Lamarque, C, Leroux, P, Lobuglio, K, Brygoo, Y., 1999. Two sibling species of the botrytis cinerea complex, transposa and vacuma, are found in sympatry on numerous host plants. Phytopathology 89 (10), 967–973.
Giraud, T, Fortini, D, Levis, C, Leroux, P, Brygoo, Y., 1997. RFLP markers show genetic recombination in Botryotinia fuckeliana (Botrytis cinerea) and transposable elements reveal two sympatric species. Mol. Biol. Evol. 14 (11), 1177–1185.
Gomez-Gomez, E, Ruiz-Roldan, MC, Di Pietro, A, Roncero, MIG, Hera, C., 2002. Role in pathogenesis of two endo-beta-1,4-xylanase genes from the vascular wilt fungus Fusarium oxysporum. Fungal Genet. Biol. 35 (3), 213–222.
Goncalves, VN, Cantrell, CL, Wedge, DE, Ferreira, MC, Soares, MA, Jacob, MR, Oliveira, FS, Galante, D, Rodrigues, F, Alves, TM, et al., 2016. Fungi associated with rocks of the Atacama Desert: taxonomy, distribution, diversity, ecology and bioprospection for bioactive compounds. Environ. Microbiol. 18 (1), 232–245.
Gonzalez-Teuber, M, Vilo, C, Bascunan-Godoy, L., 2017. Molecular characterization of endophytic fungi associated with the roots of Chenopodium quinoa inhabiting the Atacama Desert, Chile. Genom. Data 11, 109–112.
Grabke, A, Fernández-Ortuño, D, Schnabel, G., 2013. Fenhexamid Resistance in Botrytis cinerea from Strawberry Fields in the Carolinas Is Associated with Four Target Gene Mutations. Plant Disease 97 (2), 271–276.
Grant-Downton, RT, Terhem, RB, Kapralov, MV, Mehdi, S, Rodriguez-Enriquez, MJ, Gurr, SJ, van Kan, JA, Dewey, FM., 2014. A novel Botrytis species is associated with a newly emergent foliar disease in cultivated Hemerocallis. PLoS One 9 (6), e89272.
Hahn, M, Viaud, M, van Kan, J, 2014. The genome of botrytiscinerea, a ubiquitous broad host range necrotroph. In: Dean, RA, Lichens-Park, A, Kole, C (Eds.), Genomics of Plant-Associated Fungi and Oomycetes: Dicot Pathogens. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 19–44.
Haydu, JJ, Legard, DE., 2003. An economic analysis of preharvest fungicide applications to control botrytis fruit rot in annual strawberries in Florida. HortScience HortSci 38 (1), 124.
Isaza, L, Zuluaga, Y, Marulanda, M., 2019. Morphological, pathogenic and genetic diversity of Botrytis cinerea Pers. in blackberry cultivations in Colombia. Revista Brasileira de Fruticultura 41.
Isenegger, D, Ades, P, Ford, R, Taylor, P., 2008. Status of the Botrytis cinerea species complex and microsatellite analysis of transposon types in South Asia and Australia. Fungal Diversity 29.
Jarvis W 1977. Botryotinia and Botrytis species: taxonomy, physiology and pathogenicity - a guide to the literature.
Kretschmer, M, Hahn, M., 2008. Fungicide resistance and genetic diversity of Botrytis cinerea isolates from a vineyard in Germany /Fungizidresistenz und genetische Diversität von Botrytis-cinerea-Isolaten eines deutschen Weinbergs. J. Plant Dis. Protect. 115 (5), 214–219.
Kumar, S, Stecher, G, Li, M, Knyaz, C, Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35 (6), 1547–1549.
Latorre, B, Elfar, K, Ferrada, E., 2015. Gray mold caused by Botrytis cinerea limits grape production in Chile. Ciencia e investigación agraria 42, 1-1.
Latorre, BA, Spadaro, I, Rioja, ME., 2002. Occurrence of resistant strains of Botrytis cinerea to anilinopyrimidine fungicides in table grapes in Chile. Crop. Prot. 21 (10), 957–961.
Leroch, M, Plesken, C, Weber, RW, Kauff, F, Scalliet, G, Hahn, M., 2013. Gray mold populations in German strawberry fields are resistant to multiple fungicides and dominated by a novel clade closely related to Botrytis cinerea. Appl. Environ. Microbiol. 79 (1), 159–167.
Leroux, P, Chapeland, F, Desbrosses, D, Gredt, M., 1999. Patterns of cross-resistance to fungicides in Botryotinia fuckeliana (Botrytis cinerea) isolates from French vineyards. Crop. Prot. 18 (10), 687–697.
Leroux, P, Fritz, R, Debieu, D, Albertini, C, Lanen, C, Bach, J, Gredt, M, Chapeland, F., 2002. Mechanisms of resistance to fungicides in field strains of Botrytis cinerea. Pest Manage. Sci. 58 (9), 876–888.
Leroux, P, Gredt, M, Leroch, M, Walker, AS., 2010. Exploring mechanisms of resistance to respiratory inhibitors in field strains of Botrytis cinerea, the causal agent of gray mold. Appl. Environ. Microbiol. 76 (19), 6615–6630.
Levis, C, Fortini, D, Brygoo, Y., 1997. Flipper, a mobile Fot1-like transposable element in Botrytis cinerea. Mol. Gen. Genet. 254 (6), 674–680.
Liu, Y, Liu, JK, Li, GH, Zhang, MZ, Zhang, YY, Wang, YY, Hou, J, Yang, S, Sun, J, Qin, QM., 2019. A novel Botrytis cinerea-specific gene BcHBF1 enhances virulence of the grey mould fungus via promoting host penetration and invasive hyphal development. Mol. Plant Pathol. 20 (5), 731–747.
Martinez, F, Blancard, D, Lecomte, P, Levis, C, Dubos, B, Fermaud, M., 2003. Phenotypic differences between vacuma and transposa subpopulations of botrytis cinerea. Eur. J. Plant Pathol. 109 (5), 479–488.
Martinez, F, Dubos, B, Fermaud, M., 2005. The role of saprotrophy and virulence in the population dynamics of botrytis cinerea in vineyards. Phytopathology 95 (6), 692–700.
Mendgen, K, Hahn, M, Deising, H., 1996. Morphogenesis and mechanisms of penetration by plant pathogenic fungi. Annu. Rev. Phytopathol. 34, 367–386.
Moorman, GW, Walker, AS, May, S, 2012. First report of fenhexamid-resistant botrytis cinerea causing gray mold on Heurchera in a North American greenhouse. Plant Disease 96 (1), 147.
Movahedi, S, Heale, JB., 1990a. Purification and characterization of an aspartic proteinase secreted by botrytis-cinerea Pers Ex Pers in culture and in infected carrots. Physiol. Mol. Pl. Path. 36 (4), 289–302.
Movahedi, S, Heale, JB., 1990b. The roles of aspartic proteinase and endo-pectin lyase enzymes in the primary stages of infection and pathogenesis of various host tissues

by different isolates of botrytis-cinerea Pers Ex pers. Physiol. Mol. Pl. Path. 36 (4), 303–324.
Moyano, C, Gómez, V, Melgarejo, P., 2004. Resistance to pyrimethanil and other fungicides in botrytis cinerea populations collected on vegetable crops in Spain. J. Phytopathol. 152 (8-9), 484–490.
Munoz, C, Gomez Talquenca, S, Oriolani, E, Combina, M., 2010. Genetic characterization of grapevine-infecting Botrytis cinerea isolates from Argentina. Rev Iberoam Micol 27 (2), 66–70.
Muñoz, G, Hinrichsen, P, Brygoo, Y, Giraud, T, 2002. Genetic characterisation of Botrytis cinerea populations in Chile. Mycol. Res. 106 (5), 594–601.
Myers, N, Mittermeier, RA, Mittermeier, CG, da Fonseca, GA, Kent, J, 2000. Biodiversity hotspots for conservation priorities. Nature 403 (6772), 853–858.
Nafisi, M, Stranne, M, Zhang, L, van Kan, JA, Sakuragi, Y., 2014. The endo-arabinanase BcAra1 is a novel host-specific virulence factor of the necrotic fungal phytopathogen Botrytis cinerea. Mol. Plant Microbe Interact 27 (8), 781–792.
Nakajima, M, Akutsu, K., 2014. Virulence factors of Botrytis cinerea. J. General Plant Pathol. 80 (1), 15–23.
Nicot, P, Mermier, M, Vaissière, B, Lagier, J., 1996. Differential spore production by Botrytis cinerea on agar medium and plant tissue under near-ultraviolet light-absorbing polyethylene film. Plant Disease 80, 555–558.
Novoa, JE, López, D, 2001. IV Región: el escenario geográfico físico. In: Squeo, F, Arancio, G, Gutierrez, J (Eds.), Libro Rojo de la Flora Nativa y de los Sitios Prioritarios para su Conservación: Región de Coquimbo.
Plaza, V, Bustamante, C, Silva-Moreno, E, Castillo, L., 2018. First report of botrytis cinerea causing gray mold disease on the endemic plant echinopsis coquimbana in the coquimbo region, Chile. Plant Disease 102 (7), 1448–1449.
Plaza, V, Lagues, Y, Carvajal, M, Perez-Garcia, LA, Mora-Montes, HM, Canessa, P, Larrondo, LF, Castillo, L., 2015. bcpmr1 encodes a P-type Ca(2+)/Mn(2+)-ATPase mediating cell-wall integrity and virulence in the phytopathogen Botrytis cinerea. Fungal Genet. Biol. 76, 36–46.
Plaza, V, Silva-Moreno, E, Castillo, L., 2020. Breakpoint: cell wall and glycoproteins and their crucial role in the phytopathogenic fungi infection. Curr. Protein Pept. Sci. 21 (3), 227–244.
P Rajaguru, BA, Shaw, MW., 2010. Genetic differentiation between hosts and locations in populations of latent Botrytis cinerea in southern England. Plant Pathol. J. 59 (6), 1081–1090.
Rebordinos, L, Vallejo, I, Santos, M, Collado, I, Carbú, M, Cantoral, J., 2000. Análisis genético y relación con patogenicidad en Botrytis cinerea. Revista Iberoamericana de Micología 17, S37–S42.
Saito, S, Michailides, TJ, Xiao, CL., 2016. Fungicide resistance profiling in botrytis cinerea populations from blueberry in California and Washington and their impact on control of gray mold. Plant Disease 100 (10), 2087–2093.
Saitou, N, Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4 (4), 406–425.
Salami, P, Ahmadi, H, Keyhani, A, Akram, A., 2010. The effect of Botrytis Cinerea and Rhizopus Stolonifer on pre-harvest energy losses of strawberry production in Iran. J. Am. Sci. 6.
Sambrook, J, Fritsch, EF, Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press.
Samuel, S, Veloukas, T, Papavasileiou, A, Karaoglanidis, GS., 2012. Differences in frequency of transposable elements presence in botrytis cinerea populations from several hosts in Greece. Plant Disease 96 (9), 1286–1290.
Squeo, FA, Arancio, GM, Muñoz, C., M., Gutiérrez JR, 2001. Diversidad vegetal de la IV Región de Coquimbo, Chile. In: Squeo, F, Arancio, G, Gutierrez, J (Eds.), Libro Rojo de la Flora Nativa y de los Sitios Prioritarios para su Conservación: Región de Coquimbo.
Staats, M, van Baarlen, P, van Kan, JA., 2005. Molecular phylogeny of the plant pathogenic genus Botrytis and the evolution of host specificity. Mol. Biol. Evol. 22 (2), 333–346.
Tan, KK, Epton, HAS., 1973. Effect of light on the growth and sporulation of Botrytis cinerea. Trans. Brit. Mycol. Soc. 61 (1), 145–157.
Tanović, B, Delibašić, G, Milivojević, J, Nikolić, M., 2009. Characterization of Botrytis cinerea isolates from small fruits and grapevine in Serbia. Arch. Biol. Sci. 61.
Tanović, B, Hrustic, J, Mihajlovic, M, Grahovac, M, Delibasic, G., 2015. Botrytis cinerea in raspberry in Serbia II: Growth rate and virulence of isolates. Pesticidi i fitomedicina 30, 9–16.
Thompson, JD, Higgins, DG, Gibson, TJ., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acid. Res. 22 (22), 4673–4680.
van Baarlen, P, Woltering, EJ, Staats, M, van Kan, JAL., 2007. Histochemical and genetic analysis of host and non-host interactions of Arabidopsis with three Botrytis species: an important role for cell death control. Mol. Plant Pathol. 8 (1), 41–54.
Walker, AS, Gautier, AL, Confais, J, Martinho, D, Viaud, M, Le, PCP, Dupont, J, Fournier, E., 2011. Botrytis pseudocinerea, a new cryptic species causing gray mold in French vineyards in sympatry with Botrytis cinerea. Phytopathology 101 (12), 1433–1445.
Walton, JD., 1994. Deconstructing the Cell-Wall. Plant Physiol. 104 (4), 1113–1118.
Weber, RWS, Hahn, M., 2011. A rapid and simple method for determining fungicide resistance in Botrytis. J. Plant Diseases Protect. 118 (1), 17–25.
Wessels, B, Linde, C, Fourie, P, Mostert, L., 2016. Genetic population structure and fungicide resistance of Botrytis cinerea in pear orchards in the Western Cape of South Africa. Plant Pathol. 65.
White T, Bruns T, Lee S, Taylor J, Innis M, Gelfand D, Sninsky J 1990. Amplification and direct sequencing of fungal Ribosomal RNA Genes for phylogenetics. 315-322.
Yin, YN, Kim, YK, Xiao, CL., 2011. Molecular characterization of boscalid resistance in field isolates of Botrytis cinerea from apple. Phytopathology 101 (8), 986–995.
Yourman, LF, Jeffers, SN., 1999. Resistance to benzimidazole and Dicarboximide fungicides in greenhouse isolates of botrytis cinerea. Plant Disease 83 (6), 569–575.
Zhang, CQ, Liu, YH, Zhu, GN., 2010. Detection and characterization of benzimidazole resistance of Botrytis cinerea in greenhouse vegetables. Eur. J. Plant Pathol. 126 (4), 509–515.
Zhang, Z, Li, H, Qin, G, He, C, Li, B, Tian, S., 2016. The MADS-Box transcription factor Bcmads1 is required for growth, sclerotia production and pathogenicity of Botrytis cinerea. Sci. Rep. 6, 33901.