

## Article

# Silverleaf (*Chondrostereum purpureum*) effects on Japanese Plum (*Prunus salicina*)

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**Abstract:** Silverleaf is an important trunk disease of fruit crops, like Japanese plum. It is known that *Chondrostereum purpureum* produces wood discoloration, leaves silvering and tree decline, however, the information about the effects on fruit production is scarce. Therefore, the objectives of this study were to determine *C. purpureum* pathogenicity on *Prunus salicina* and effects on physiology, fruit yield and quality, in Chile, in 2019 and 2020. Wood samples from affected plum trees were collected in the Chilean plum productive area. Fungi were isolated plating wood sections from the necrosis margin on culture media. Isolates colonies morphological and molecular characteristics accorded to *C. purpureum* (98%). Representative isolates were inoculated on healthy plum plants and after 65-d incubation, wood necrotic lesions and silver leaves were visible. Fungi were reisolated, fulfilling Koch's postulates. To determine Silverleaf effects, water potential, and fruit yield and quality were measured in healthy and Silverleaf diseased plum plants cv. 'Angeleno'. Water potential was altered on diseased trees, and their yield was reduced in 51% (2019) and 41% (2020), compared to fruit from healthy plants. Moreover, cover-colour, equatorial-diameter and weight were reduced, and fruit were softer, failing to meet the criteria to be properly commercialized and exported to demanding markets.

**Keywords:** Silverleaf disease; *Chondrostereum purpureum*; *Prunus salicina*; Japanese plum

## 1. Introduction

The Japanese plum (*Prunus salicina* Lindl.) is a deciduous stone fruit tree native to China. It is grown globally and Chile cultivates 4,520 ha, mainly 'Angeleno', 'Black Amber' and 'Friar' cultivars. It produces 400,000 t (2019/2020) and is the major fresh plums exporter worldwide (Odepa, 2021).

Fungal trunk diseases have been increasing in recent years, in fruit, ornamental, forest and woody species, worldwide. Some of the reasons of this raise in fruit crops are changes in climate, the incorporation of intensive plant production management measures like high density plantations, the use of dwarfing rootstocks and severe pruning, in order to accelerate plants and fruit production, which stress the plants and alter their behavior (Gramaje et al., 2016; 2020; Grinbergs et al., 2020; France et al., 2017). They are one of the main pathological problems for *Prunus*, which are affected by several fungal taxonomic groups (Gramaje et al., 2012). *Prunus salicina* has been reported as host of different wood fungal pathogens, like *Armillaria* (Cha et al., 2009), *Botryosphaeria* (Ko et al., 2008), *Diplodia* (Mojeremane et al., 2020), *Calosphaeria*, *Jattaea* (Damm et al., 2008a), *Lasioidiplodia* (Damm et al., 2007), *Neofusicoccum* (Li et al., 2019), *Phaeoacremonium*, *Toninia* (Damm et al., 2008b) and *Chondrostereum* (Ramsfield, 2006).

The basidiomycete *Chondrostereum purpureum* (Pers. Ex Fr.) Pouzar is an important wood pathogen which causes Silverleaf disease. While this fungal species has attracted considerable interest in several countries, as a biological agent for woody weeds control in coniferous forest plantations (Becker et al., 1999; 2005; Shamoun, 2000;

Ramsfield, 2006; Hamberg et al., 2021), it can cause severe and destructive disease in some areas of the world, in woody plants, including ornamental, wild, forest and fruit crop species (Bus et al., 1996; Setliff, 2002; Willoughby et al., 2015; Hamberg and Hantula, 2016; France et al., 2017; Merlet et al., 2018; Grinbergs et al., 2019; Hamberg et al., 2021). Over 230 species are recorded as hosts of *C. purpureum* (Spiers and Hopcroft, 1988; Bus et al., 1996; Spiers and Brewster, 1997; Setliff, 2002; Becker et al., 2005). The Rosaceae family has the greatest number of hosts (Spiers et al., 1987; Bus et al., 1996; Simpson et al., 2001; Setliff, 2002; Cloete et al., 2011; France et al., 2017; Grinbergs et al., 2020), and genus *Prunus*, in particular (Bus et al., 1996). Among *Prunus* hosts of *C. purpureum* are: *Prunus armeniaca* (Ozgonen et al., 2006), *P. avium* (Børve et al., 2017), *P. persica* (Spiers et al., 1998), *P. serotina* (Setliff et al., 2002), *P. domestica* (Ogawa and English, 1991) and *P. salicina* (Ramsfield, 2006).

This fungus reproduces through basidiospores, which are airborne disseminated from basidiocarps, developed in dying trunks and branches of infected trees, to fresh wood wounds (Pearce et al., 1994). Mycelia grows through the xylem tissue, producing central wood discoloration (Vartiamäki et al., 2009) and subsequently occluding the vessels. It produces a specific endopolygalacturonase (endoPG) enzyme, which moves to the foliage, inducing silver- greyish colour symptoms on leaves. This silver color is usually visible several seasons after the infection (Senda et al., 2001; Simpson et al., 2001). Subsequently, the fungus kills the plant, and develops purple resupinated carpophores that release spores under high humidity and mild temperature (Pearce et al., 1994).

There is limited information about how wood diseases affect the physiology of fruit crops and fruit production. Moreover, new wood pathogens are being reported every day, and in a wider range of hosts and geographical locations (Damm et al., 2008 a, b; Cloete et al., 2011; Gramaje et al., 2012; Bien and Damm, 2020; Espargham et al., 2020; Hamberg et al., 2021). However, little is known about their impact on trees fruit yield and quality. The most exhaustive research has been performed on grapevines, finding that wood diseases are the main biotic factor limiting vineyards productivity and longevity, causing major economic losses (Bertsch et al., 2013).

As well as for other trunk diseases, the information about Silverleaf effects on fruit crops is scarce. It is an important disease in countries like New Zealand, United States (Washington and Oregon) (Ogawa and English, 1991), Chile (Grinbergs et al., 2020), Australia (Bishop, 1978), France (Bus, 1996), Poland (Głowacka et al., 2017), Latvia (Kaufmane et al., 2019), Norway (Børve et al., 2019), and other European countries (Ogawa and English, 1991).

It can produce severe losses in orchards, and it was the main cause of mortality in peach and nectarine in New Zealand, with losses of 8% per year (Atkinson, 1971). In Chile, it has been found causing disease in different fruit crop species, like in almond, apples, blueberries, cherry, Chilean guava, nectarine, quince, pear-tree and plum (France et al., 2017; Grinbergs et al., 2019; 2020), showing silver leaves and brown central discoloration in branches and main trunks. In blueberry, the effects of Silverleaf on plants and fruit were measured, being water potential and stomatal conductance the most affected physiological parameters, a yield reduction of 40% and problems on fruit quality, like reduction of maturation, colour and weight (France et al., 2017). Nevertheless, there is a lack of information about the effects of Silverleaf in other fruit crops.

There is not information available about the effects of this fungus in Japanese plum. Therefore, the objectives of the present study were to investigate the etiology of the fungal pathogen causing foliar silvering in *Prunus salicina* in Chile, to determine its pathogenicity on plums, and to assess the effects of *C. purpureum* on plums physiology, fruit yield and quality.

## 2. Results

### 2.1. *Chondrostereum purpureum* isolates

Ninety-seven wood samples from Japanese plum trees showing foliar silvering (Figure 1 A) and internal brown circular discolorations in affected branches and main trunks (Figure 1 B) were collected, from 2018 to 2020. Some samples also showed other kind of wood symptoms like wedge shaped and dark brown irregular discolorations, suggesting the presence of a diversity of pathogens. In 98% of the samples, macroscopic and microscopic morphological characteristics of the colonies were consistent to those described for *C. purpureum* (Spiers et al., 2000). Whitish cottony mycelia (Figure 1 C) with clamp connections grew from wood sections from the necrosis progress area, which were surface disinfected and plated on Petri plates containing potato dextrose agar (APDA) (25% PDA, acidified with 0.2% v/v 85% lactic acid) and water agar (WA). After 14 to 21 days of incubation at 25°C, 52% of the isolates developed beige to light pink pseudo-basidiocarps, on the edges of the plate. The fruiting bodies of most of the isolates developed hyaline, apiculate, ovoid basidiospores, 5.3 (4.7–6.8)  $\mu\text{m} \times 3.6$  (3.0–4.5)  $\mu\text{m}$  (n = 50).

Moreover, 57 representative putative *C. purpureum* isolates developed intensely discrete 500 bp bands on agarose gels, (Figure 1 D), after pure cultures DNA amplification, using APN 1 primers, according with *C. purpureum* molecular identification (Becker et al., 1999). The internal transcribed spacer of representative isolates, selected from different host cultivars and collection localities (HMCi7; HMCi121; HMCi147 and HMCi148), was amplified using ITS1 and ITS4 primers (White et al., 1990). Sequences were accessioned to GenBank (MW938164, MW938165; MW938166 and MW938167, respectively).

Nevertheless, in 24% of the samples was possible to isolate other fungal pathogens, most of them associated to wedge shaped and irregular dark-brown discolorations. The species were identified as *Cytospora* (12.6%), *Phomopsis* (9.2%), *Schizophyllum* (17.2%), *Stereum* (7.9%), species in the *Botryosphaeriaceae* family (42.8%), and other fungi (10.3%).

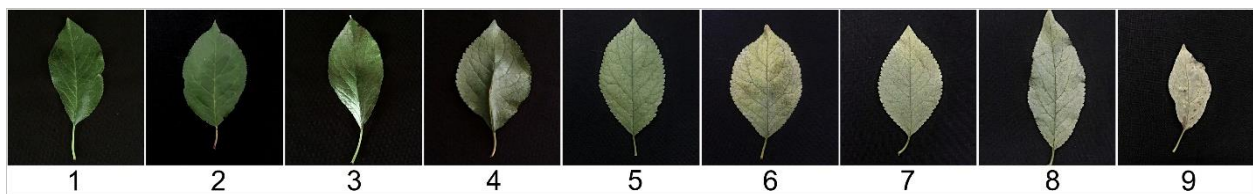




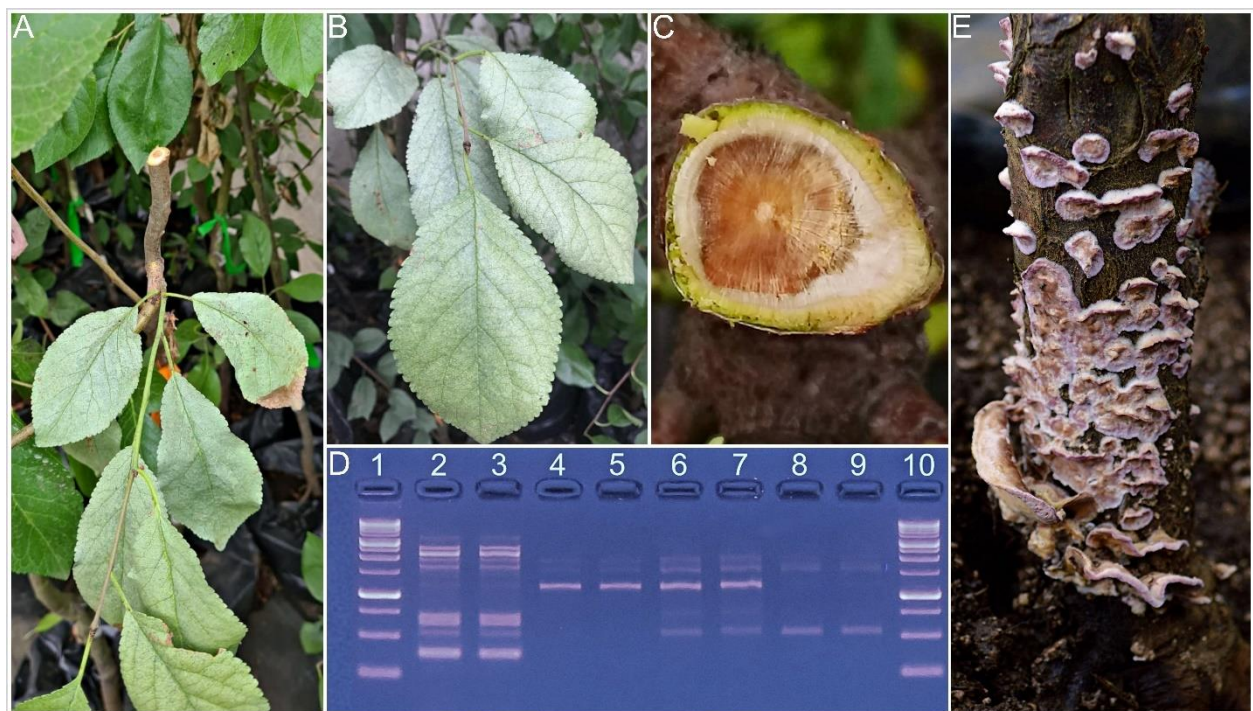
**Figure. 1.** Silverleaf disease caused by *Chondrostereum purpureum* on Japanese plum in Chile; **A)** foliar symptoms on cv. Angeleno, **B)** wood discoloration symptoms on cv. Angeleno, **C)** isolate HMCi7 Genbank: MW938164 and **D)** Gel with DNA from 22 isolates banding patterns after amplification with APN 1 species-specific primers (lanes 2 to 23). Lane 24: positive control isolate RGM 122 Genebank: MK22253.1. Lanes 1 and 25: molecular weight standards.

## 2.2. Pathogenicity tests

Healthy nursery plants were successfully inoculated with *C. purpureum* isolates and reproduced silver foliar symptoms (grade 3 to 8) (Figure 2 and 3 B). After the incubation period, brown central staining was visible when the inoculated branches were transversally cut (Figure 3 A and C). Moreover, one of the inoculated plants, with HMCi121 isolate, developed resupinated purple carpophores in the main trunk, above the soil level, after 20 months of incubation (Figure 3 E).



**Figure. 2.** Silverleaf disease foliar symptoms visual severity scale (1-9). 1= healthy or apparently healthy leaf, 3= epidermis starts detaching the mesophyll and the color is lighter than in healthy leaves, 5= more than 75% of the leaf epidermis is detached from the mesophyll and the leaf is beginning to look gray-silver, 7= 100% of the epidermis is detached from the mesophyll and the leaf is completely white-silver, 9= epidermis peel off from the leaf and mesophyll begins to oxidize. 2, 4, 6 and 8 grades indicate intermediate symptom severity between the previous and the following grade.



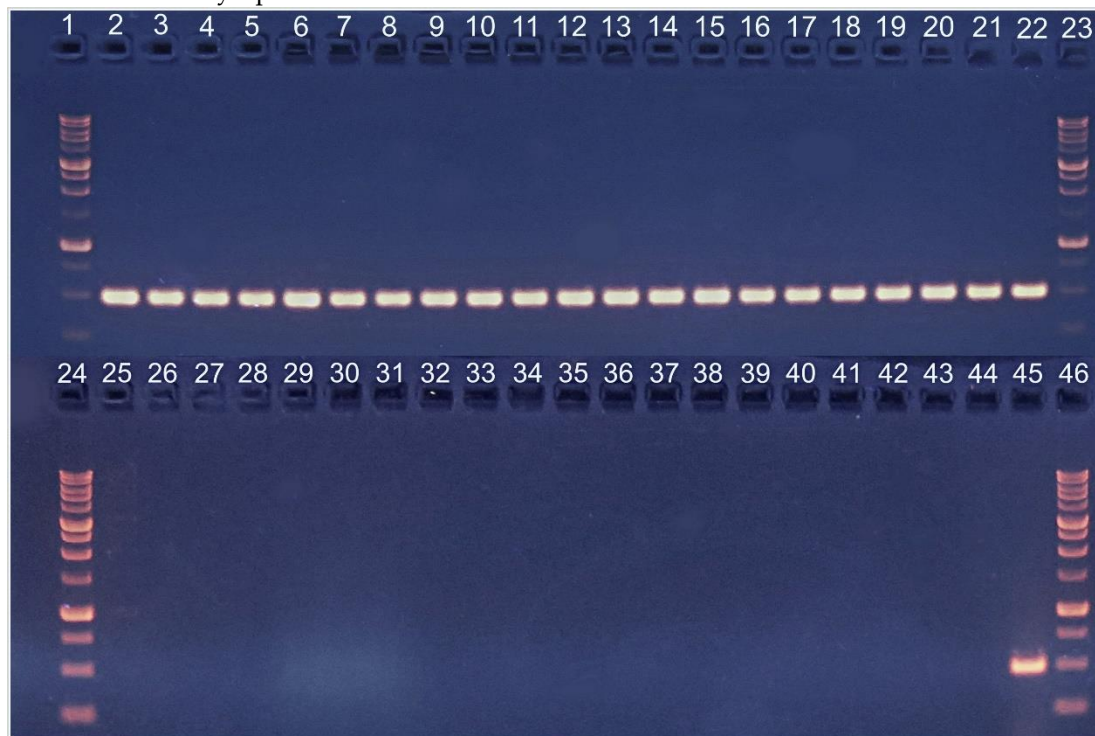
**Figure. 3.** Pathogenicity tests of four *Chondrostereum purpureum* isolates on 2-year plants cv. 'Angeleno'; **A)** transversal cut on inoculated branch at 20 cm from the inoculation point, **B)** foliar symptoms and **C)** necrotic symptoms developed by inoculated plants, after 65-day of incubation. **D)** *C. purpureum* DNA banding patterns after amplification with APM22 primers. Lane 2 and lane 3: inoculated and reisolated *C. purpureum* HMCi7, Lanes 4 and 5: isolated and reisolated

HMCi121, lane 6 and 7: isolated and reisolated HMCi147, and lanes 8 and 9: isolated and reisolated HMCi148. Lanes 1 and 10: molecular weight standards. E) Fruiting bodies of isolate HMCi121, after 20 months of incubation.

It was possible to reisolate *C. purpureum* from inoculated plants, on APDA, while the fungus was not reisolated from controls. The banding patterns in agarose gels, produced by DNA amplification using SCAR primers APM22, were identical between inoculated and reisolated *C. purpureum* isolates (Figure 3 D), fulfilling Koch's postulates.

### 2.3. Silverleaf effects on plum

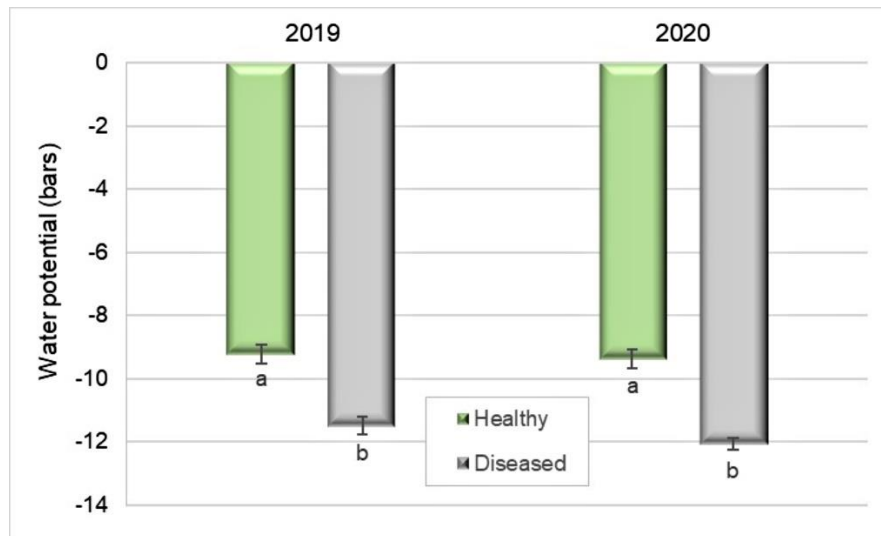
To confirm the presence or absence of *Chondrostereum purpureum* in symptomatic and asymptomatic plants, respectively, the fungus was detected amplifying fungal DNA, directly from the trunk sawdust, using APN1 species-specific primers. A 500 bp band was reproduced in agarose gels, in diseased trees, while DNA from asymptomatic ones did not reproduced the band (Figure 4). In 2020, symptoms were less severe than in 2019 (grade 3, average), and two of the previously selected trees did not showed any foliar symptoms.



**Figure. 4.** *Chondrostereum purpureum* DNA amplification in Silverleaf diseased plum plants, showing foliar symptoms (lanes 2 to 21), and healthy ones (lanes 25 to 44). Lanes 22 and 45: positive controls (isolate HMCi147 GeneBank: MW938166). Lanes 1, 23, 24 and 46: molecular weight standards.

#### 2.3.1. Water potential

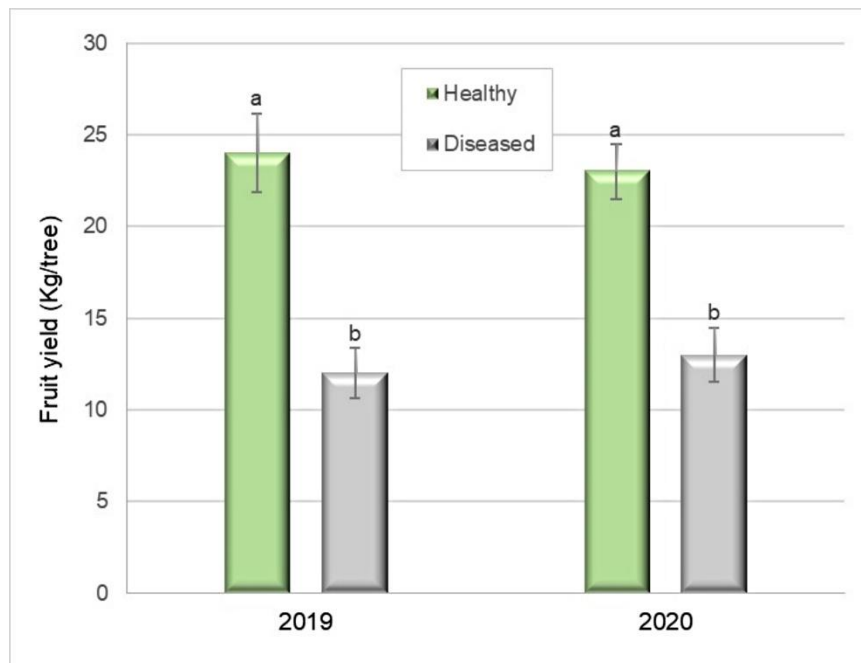
Xylem potential was 19.4 % lower in diseased plants (-11.48 bar) than in healthy ones (-9.22 bar), in 2019 (t-Student  $P < 0.0001$ ). Similarly, in 2020, water potential in diseased plants (-12 bar) was 22.5 % lower than in healthy ones (-9.4 bar) (t-Student  $P < 0.0001$ ) (Figure 5). It was measured a few days after harvest in both years.



**Figure. 5.** Water potential of Japanese plum cv. 'Angeleno' leaves of healthy and Silverleaf diseased trees. Mean of 10 x two trees x 5-leaf samples, in 2019 and 9 x 2 x 5, in 2020. Bars represent standard error of the means and columns with different letters are statistically different.

### 2.3.2. Yield assessment

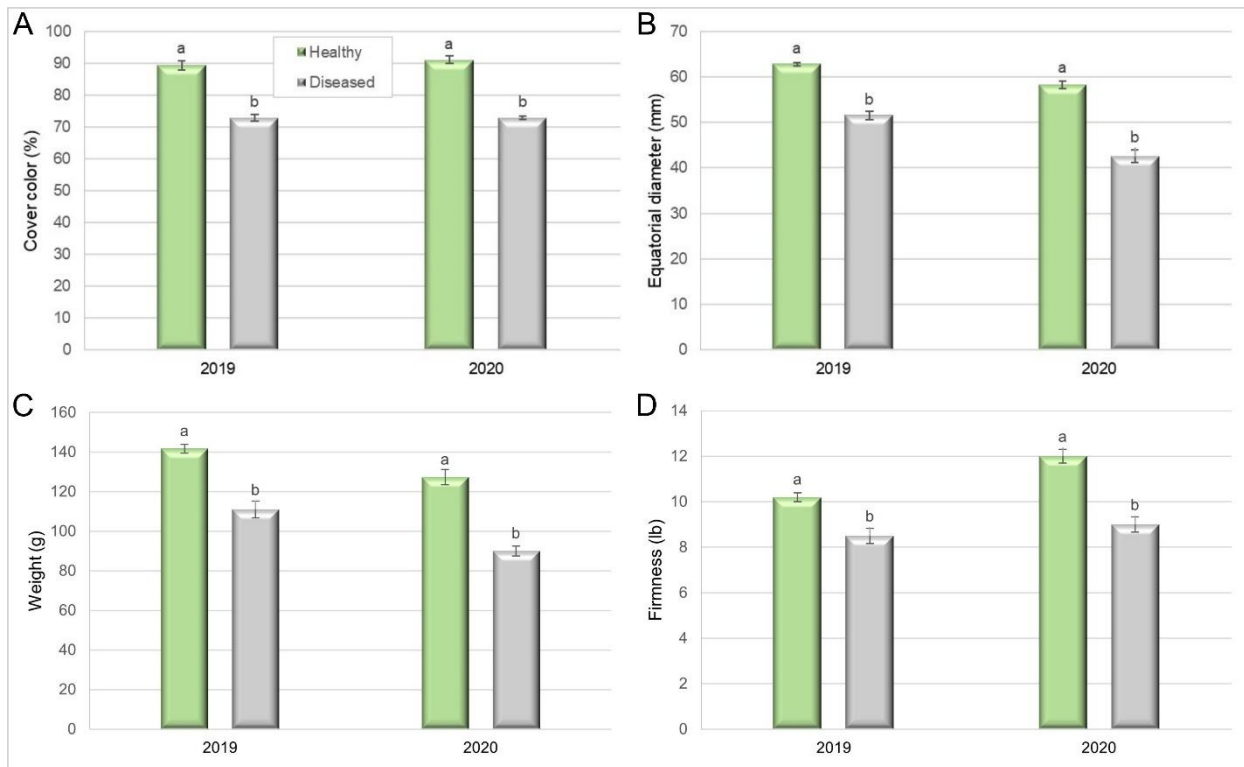
Total yield from Silverleaf diseased trees was 51% lower (12.1 kg per tree) than weight from healthy ones (24.8 kg per tree) ( $t$ -Student  $P = 0.0001$ ) in 2019 (Figure 5). In 2020 total yield was 41% lower (13.7 kg per tree) than fruit from healthy plants (23.5 kg per tree) ( $t$ -Student  $P = 0.0003$ ) (Figure 6).



**Figure. 6.** Fruit yield (kg) on healthy and Silverleaf diseased Japanese plum trees cv. 'Angeleno'. Mean of 10 x 2 trees in 2019 and 9 x 2, in 2020. Bars represent standard error of the means and columns with different letters are statistically different.

### 2.3.3. Fruit quality

In 2019 period, fruit harvested from healthy trees showed 89.3% of cover colour, compared to 72.9% in diseased ones (Pearson Chi square = 16.37,  $P = 0.0001$ ). In the next season, the cover colour in fruit from healthy trees was higher than in the previous year (91.1%), while colour in fruit from diseased trees remained similar than in the previous year (Pearson Chi square = 18.22,  $P < 0.0001$ ) (Figure 7 A).



**Figure. 7.** Quality of fruit from healthy and Silverleaf diseased trees in a Japanese plum orchard cv. 'Angeleno': **A)** cover color, **B)** equatorial diameter, **C)** individual weight and **D)** firmness, in two seasons. Mean of 20 × 10-fruit samples in 2019 and 18 × 10 in 2020., Bars represent standard error of the means and columns with different letters are statistically different.

The equatorial diameter was an affected caliber parameter. In 2019, it was 62.8 mm in fruit from healthy trees, compared to 51.5 mm in fruit from diseased ones (Kruskal - Wallis,  $P = 0.0002$ ). In 2020, equatorial diameter was lower than in the previous season, both in fruit from healthy trees (58.48 mm) and from diseased ones (42.35 mm) (Kruskal - Wallis,  $P < 0.0001$ ) (Figure 7 B).

Fruit individual weight was also a parameter affected by Silverleaf. In 2019, fruit harvested from healthy trees weighed 141.73 g and 111.34 g in diseased trees (t-Student test,  $P < 0.0001$ ). In the next season, fruit from healthy trees weighed 127.48 g and 90 g in diseased trees (Figure 7 C) (t-Student test,  $P < 0.0001$ ). Finally, fruit from diseased trees (10 lb) was softer than fruit from healthy ones (8.26 lb), in 2019 (Kruskal - Wallis,  $P = 0.0005$ ), as well as in 2020 (12 lb in fruit from diseased and 8.56 in fruit from healthy trees) (Kruskal - Wallis,  $P < 0.0001$ ) (Figure 7 D), indicating a shorter postharvest life for fruit from diseased trees.

The other measured parameters: pH, titratable acidity, soluble solids, skin color and background color, were not different between fruit harvested from diseased and healthy trees (data not shown).

### 3. Discussion

In the present study, *Chondrostereum purpureum* was successfully isolated from the necrotic margin in stained wood of Japanese plums showing Silverleaf foliar symptoms.



The latter were similar to those described by France et al., (2009) and Grinbergs et al., (2019; 2020), for other fruit hosts. *C. purpureum* is a primary invader of woody angiosperms which enters its host through a fresh wounds, and is followed by the infection of aggressive saprobic fungi such as *Trametes versicolor* and *Schizophyllum commune* (Shaumon et al., 2000; Becker et al., 2005;). However, *C. purpureum* it is still present in these old trees infected by several pathogens, and it is also able to produce foliar symptoms, as it was demonstrated in this study, with the symptoms observation and *C. purpureum* molecular detection and isolation.

The isolated fungi developed whitish cottony mycelia, and it was also possible to detect it directly from wood, through clear staining bands on agarose gels, when fungal DNA was amplified with APN1 species-specific primers (Becker et al., 1999). Moreover, the Japanese plum plants inoculated with *C. purpureum* isolates, clearly showed foliar symptoms, developing silver-greyish leaves in the inoculated branches, and also internal wood necrosis and fruiting bodies. Besides, reisolated fungi showed the same cultural features as the inoculated ones, and identical banding patterns on agarose gels, when SCAR fingerprinting markers (Becker et al., 1999) were used to amplify their DNA, thus, fulfilling Koch's postulates.

It was demonstrated that wood diseases alter physiological parameters in different hosts (Petit et al., 2006; Fontaine et al., 2015). Likewise, in this study the water potential was lesser in diseased plants, compared to healthy ones. Similarly, in 2020, diseased plants water potential was lesser than in healthy ones. These differences can be explained by *C. purpureum* growth through the xylem tissues of the host (Pearce et al., 1994), besides from the systematic silvering of leaves (Spiers et al., 1987). During the infection process, *C. purpureum* penetrates through starch granules and cell walls, producing the occlusion of tree vessels (Spiers et al., 1998). Moreover, transpiration, stomatal conductance and leaf area decrease significantly while leaf-silvering intensity increases (Spiers et al., 1987). The resulting physiology disruption and dehydration, combined with fungal toxins, finally cause the death of the host (Pearce, 1996; Hamberg et al., 2015; Hamberg and Hantula, 2016).

On the other hand, intensity of symptoms may change between seasons, like in some trees of the present study. Intensity was lower in some plants, despite of the presence and viability of the fungus, which was confirmed by PCR and microbiological isolation, as occurred in apple (Grinbergs et al., 2021), where the reversion of foliar symptoms was first described. Damaged wood on the branches and trunk are the battlegrounds of microorganisms; pathogens and endophytes. While vascular tissue is being destroyed by pathogens (Xing et al., 2020), endophytic microorganisms are behaving as their antagonists and/or inducing the plant resistance (Grinbergs et al., 2021), and thus, modulating disease expression (Bailey et al., 2008).

Foliar symptoms remained similar in their intensity (grade 3 to 6 in the severity scale) (Figure 2) in most of the diseased analyzed trees (95%) during 2019 and 2020. In 2019, a reduction of 51% of total fruit weight was recorded in Silverleaf diseased plants, compared to healthy ones. Moreover, in 2020, the reduction was 41%. The fungal pathogen inhabiting the vessels and necrosing tissue decreased the nutrient translocation, affecting the number and weight of fruits per tree. Similarly, it has been recorded the decrease in fruit yield due to several wood pathogens (Bien and Damm, 2020). The results were consistent with yield assessments performed in other fruit crops in Chile, such as apples and blueberries (France et al., 2017; Grinbergs et al., unpublished work). Although there are authors who point out yield losses due to the damaging effects of *C. purpureum* infections in stone and pome fruits (Atkinson, 1971; Bishop, 1978; Spiers, et al., 1987; Bus et al., 1996; Spiers et al., 2000), and blueberries (France et al., 2017), as far as we concern, this is the first record about yield losses in Japanese plum related to the detrimental effects caused by *C. purpureum*.

Regarding fruit quality, our results showed that important quality components like fruit weight, cover color, equatorial diameter and firmness were influenced by Silverleaf disease. Similar results were reported by Spiers et al. (1987; 1997) in apple orchards, with



smaller and fewer fruit, reduced color, an increased incidence of physiological damages like water core, short post-harvest storage and softening of fruit. Furthermore, Simpson et al. (2001) stated that the spread of *C. purpureum* in woody tissues and the loss of photosynthetic capability eventually leads to tree death.

In conclusion, our work has demonstrated that *C. purpureum* alters physiological parameters like water potential in Japanese plum trees, and negatively impacts in fruit yield and quality. The latter can downfall the orchards productivity and, moreover, the fruit harvested from diseased trees do not meet the requirements to be properly commercialized and even less to be exported to demanding markets.

#### 4. Materials and Methods

##### 4.1. Collection of samples

Collections of wood samples from silverleaf affected plum trees showing foliar silvering and internal wood discoloration symptoms were conducted in the Chilean Japanese plum productive area, from Metropolitana Region (33° 42' 16.11" S, 70° 59' 11.82" W) to Ñuble Region (36° 37' 24.98" S, 72° 0' 23.39" W). Two nurseries and 27 orchards of different plum cultivars, mainly Angeleno, Black Amber, Friar, Fortune and Larry Anne, were prospected. Symptoms were recorded and photographed.

##### 4.2. Isolation and purification

In the laboratory, bark was removed from the samples and 0.5-cm wooden pieces were cut from the margin of the discoloration area. Pieces were superficially disinfected using 10% v/v sodium hypochlorite bleach (4.9% chlorine) for 4 min and aseptically plated on Petri plates containing APDA and WA. Plates were incubated at 25°C and darkness, until mycelial development. Pure cultures were obtained by transferring hyphal tips to fresh PDA plates and incubating them at 25°C.

##### 4.3. Identification and characterization

The identification was focused on isolates resembling *Chondrostereum purpureum*. Fungal colonies showing whitish cottony mycelia were preliminary selected. Subsequently, the isolates were identified by their cultural characteristics after 7 and 14-days of incubation on PDA, at 25°C, the morphology of the mycelia, presence of clamp connections, and morphometry of spores from pseudo-basidiocarps.

Furthermore, the isolates were identified by molecular means. Pure cultures were incubated on PDA at 25°C for 7 days. Total nucleic acids were isolated from fresh mycelium using the CTAB method, and genomic DNA was amplified using APN 1 *C. purpureum* specific primers described by Becker et al. (1999), following the protocols described by Grinbergs et al. (2020). The PCR products (20 µl) were analyzed on 1.5% agarose gels, using a 1 kb DNA ladder (Maestrogen Inc., Xiangshan Dist., Hsinchu, Taiwan) as molecular size standard. Electrophoresis was performed at 7.5 V/cm for 2 h, and gels were stained with ethidium bromide (1 µg/mL). Gels were visualized under UV-light ( $\lambda=365$  nm) transillumination (Clear View standard UV transilluminator) and digitally recorded (G16, Cannon).

The internal transcribed spacer of representative isolates selected from different host cultivars and collection localities, was amplified using ITS1 and ITS4 primers (White et al., 1990), GoTaq® Green Master Mix 2X (Promega) and 30 denaturation cycles at 94°C for 1 min, annealing at 52°C for 1.5 min and extension at 72°C for 2 min. Fungal DNA was quantified using a Qubit fluorometer (2.0, Invitrogen). Electrophoresis, staining and visualization were conducted as described for APN1 gels. The PCR products were purified and sequenced by MacroGen (Macro-gen Inc.) and the nucleotide sequences were assembled and edited using the Sequencher software version 5.4.6 (Gene Codes Corporation, MI, USA). Subsequently, sequences were compared with the GenBank database using the BLAST software (Basic Local Alignment Search Tool program).

Furthermore, colonies showing different characteristics like dark colour, were preliminary identified by the morphometry of their reproductive structures. In the case of basidiomycete fungi, which colonies are similar to *C. purpureum*, they were discriminated by the colony shape and density, and also by their DNA amplification using APN1 species-specific primers (Grinbergs et al., 2019).

#### 4.4. Pathogenicity

Four representative isolates were inoculated on healthy 2-year nursery plants cv. Angeleno. Fresh cuts were aseptically performed on the main 1-year old lateral branches (1 to 1.5 cm diameter). Cuts were inoculated with 0.7 diameter mycelial plugs collected from an actively growing colony of *C. purpureum* representative isolates HMCi7, HMCi121, HMCi147 and HMCi148. Sterile agar was used for controls. Inoculum was covered with petroleum jelly and plastic film, to avoid dehydration. Five plants were inoculated with each treatment in the beginning of spring 2019 and incubated at screenhouse for 65 days, at environmental temperature. Plants were periodically monitored to observe the occurrence of foliar symptoms which severity was recorded using a visual scale (Figure 2). After the incubation period, branches were cut and analyzed at the laboratory. Branches were longitudinally cut and necrotic symptoms were recorded. Small wood pieces 0.5 cm were cut from the necrosis margin and aseptically plated on APDA. The reisolated fungi were purified, following the protocol described above.

Genomic DNA was extracted from the reisolated fungi and amplified using *C. purpureum* SCAR species-specific primers APM22D13F (58- GGGGTGACGAGGACGACGG TG-38), Tm 563.2° C and APM22D13R (58-GGGGTGACGACATTATACTGCAGGTAGT AG-38), previously described by Becker et al. (1999). PCR conditions were those indicated by the authors. Electrophoresis, staining and visualization were conducted as described above. Subsequently, banding patterns from the inoculated isolates were compared with the reisolated ones, to confirm Koch's postulates.

#### 4.5. Silverleaf effects on plum

To determine silverleaf effects on plum trees water potential, and fruit yield and quality were measured in a 22-year orchard cv. 'Angeleno' with planting distances of 4.5 x 3 m (5.6 hectares), located in Maule Region (34° 58' 58.21"S O, 71°16' 37.01"W), in 2019 and 2020 harvest periods. Twenty healthy and 20 diseased trees were selected, homogeneous in their height and architecture, and located on two adjacent rows, to avoid topography and other differences among them. Two and two trees were analyzed together.

Trees were selected based on the absence of foliar silver symptoms for healthy ones, and on the presence of them in diseased ones (grade 3 or more in the symptoms severity scale) and widespread throughout the canopy for diseased plants, meaning more than the 80% of the leaves showing silverleaf symptoms, from healthy to severely affected.

The Silverleaf foliar symptoms visual severity scale (1-9) was built with leaves from plum trees cv. Angeleno naturally infected with *C. purpureum*, showing different levels of silverleaf symptoms. Grades were assigned to the different foliar symptoms visual intensities: 1= healthy or apparently healthy leaf, 3= epidermis starts detaching the mesophyll and the color is lighter than in healthy leaves, 5= more than 75% of the leaf adaxial epidermis is detached from the mesophyll and the leaf is beginning to look gray-silver, 7= 100% of the epidermis is detached from the mesophyll and the leaf is completely white-silver, 9= epidermis peel off from the leaf and mesophyll begins to oxidize. 2, 4, 6 and 8 grades indicate intermediate symptom severity between the previous and the following grade. Epidermis detachment on leaves adaxial surface was confirmed using a Stereo Microscope at 15 x (Olympus SZ61).

Additionally, the presence or absence of *C. purpureum* was confirmed through the amplification of DNA isolated from sawdust collected from the main trunk (Grinbergs et al., 2020), using APN1 specific primers (Becker et al., 1999), following the previously described protocol.

#### 4.5.1. Water potential

Xylem potential was measured on diseased and healthy trees, in March 1st 2019 and March 10th 2020 (about 3-6 days after harvest). For both periods, it was measured on light exposed leaves (five leaves per plant), which were previously covered for 2 h to avoid sunlight. It was measured using a Scholander-type pressure chamber (Model 615, PMS Instruments, USA).

#### 4.5.2. Yield assessment

Fruit was harvested on February 27th 2019 and March 4th 2020, in the same dates that producers harvested the orchard. Before harvesting the fruit from each selected healthy and diseased tree, 10 random fruits from one lateral representative branch of each tree, were weighed and set aside, for posterior quality parameters analysis, at the laboratory. Subsequently, the remaining fruit of each tree were individually collected and weighed.

#### 4.5.3. Fruit quality

Quality parameters were measured on the 10 previously reserved fruit. Measured parameters were: individual weight (g), cover colour (%) and background colour (%), based on a plum colour chart (Rivero et al., 2013), caliber (polar and ecuatorial diameter measured through a digital caliper), titrasuppl acidity measured by potentiometric titration with NaOH 0,1N (Hanna, pH 211, Romania) expressed as citric acid proportion (%) (AOAC, 2000), pH, and soluble solids, measured with a digital refractometer (Brix degrees) (Atago, Pocket PAL- refractometer, Japan). For firmness measurement, two cheeks per fruit were cut and the firmness was directly measured in both sides, using a manual firmness penetrometer (Dimeri, Chile). Media from both cheeks was subsequently analyzed. The average of measurements (n=10) was calculated for each parameter, for further analysis.

Statistical analysis. All data were analyzed using InfoStat 2020 statistical software. Before testing for statistical significance, normality and homoscedasticity were evaluated by Shapiro - Wilk test, residues independence analysis and graphic methods. Normal distributed data were analyzed by T-Student test and nonnormal distributed data were tested using the Kruskal-Wallis test. Proportions were compared using Pearson's Chi square independence test..

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## References

1. Atkinson, J. D. 1971: Diseases of tree fruits in New Zealand. Bulletin 81. Department of Scientific and Industrial Research, Auckland. 406 p.
2. Bailey, B., Bae, H., Strem, M., Crozier, J., Thomas, S., Samuels, G., Vinyard, B. y Holmes, K. 2008. Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. *Biol. Control*, 46: 24–35.
3. Becker, E., Ball, L. and Hintz, W. 1999. PCR Based genetic markers for detection and infection frequency analysis of the biocontrol fungus *Chondrostereum purpureum* on Stika Alder and Trembling Aspen. *Biol. Control*, 15: 71 - 80.
4. Becker, E., Shamoun, S. and Hinz, W. 2005. Efficacy and environmental fate of *Chondrostereum purpureum* used as a biological control for red alder (*Alnus rubra*). *Biol. Control*, 33 (2005) 269–277.



5. Bertsch, C., Ramirez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C., and Fontaine, F. 2013. Grapevine trunk diseases: complex and still poorly understood. *Plant Pathol.*, 62: 243–65.
6. Bien, S. and Damm, U. 2020. Prunus trees in Germany—a hideout of unknown fungi? *Mycol. Prog.*, 19:667–690. <https://doi.org/10.1007/s11557-020-01586-4>.
7. Bishop, G. C. 1978. Studies on silver leaf disease of stone and pome fruit trees. Ph.D. Thesis, University of Adelaide, 128 p.
8. Børve, J., Ippolito, A., Tanovic, B., Michalecka, M., Sanzani, S.M., Ponitowska, A., Mari, M., and Hrustic, J. 2017. Fungal diseases. In Quero Garcia, Pulawska, Lang, Iezzoni (eds). *Cherries. Botany, production and uses*. CABI pp. 338-364.
9. Børve, J., Talgø, V., Stensvand, A., 2019. Silver leaf in Norwegian *Prunus domestica* plum orchards. *IOBC Abs* p.219.
10. Bus, V., Spiers, A., Brewster, D. and Hofstee, M. 1996. Preliminary screening of apple germplasm for resistance to Silverleaf infection. *N. Z. J. Crop Hort. Sci.*, 24:1-6.
11. Cha, J., Lee, S., Chun, K., Lee, S. and Ohga, S. 2009. Armillaria root rot caused by *Armillaria Tabescens* on *Prunus Salicina* in a Korean garden. *J. Fac. Agric. Kyushu Univ.*, 54. 10.5109/16103.
12. Cloete, M., Fourie, P., Damm, U., Crous, P. and Mostert, L. 2011. Fungi associated with die-back symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease pathogens. *Phytopathol. Mediterr.*, 50. 176-190. 10.14601/Phytopathol\_Mediterr-9004.
13. Damm, U., Mostert, L., Crous, P. and Fourie, P. 2008 b. Novel *Phaeoacremonium* species associated with necrotic wood of *Prunus* trees. *Persoonia*, 20:87–102.
14. Damm, U., Crous, P., Fourie, P. 2007. *Botryosphaeriaceae* as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. *Mycologia*, 99:664–680.
15. Damm, U., Crous, P. and Fourie, P. 2008 a. Afissitunicate ascus mechanism in the *Calosphaeriaceae*, and novel species of *Jattaea* and *Calosphaeria* on *Prunus* wood. *Persoonia*, 20:39–52.
16. Espargham, N., Mohammadi, H. and Gramaje, D. 2020. A survey of trunk disease pathogens within *Citrus* trees in Iran. *Plants*, 9, 754; doi:10.3390/plants9060754.
17. France, A., Grinbergs, D. and Carrasco, J. 2017. First detection of Silverleaf (*Chondrostereum purpureum*) on rabbiteye blueberry (*Vaccinium ashei*) and disease damages. *Acta Hort.*, 1180:277. doi.org/10.17660/ActaHortic.2017.1180.37.
18. Glowacka, A. and Rozpara, E. 2017. Evaluation of several dessert cultivars of plum, new under climatic conditions of Poland. *Hort. Sci. (Prague)*, 44: 126–132.
19. Gramaje, D., Agustí-Brisach, C., Pérez-Sierra, A., Moralejo, E., Olmo, D., Mostert, L., Damm, U. and Armengol, J. 2012. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain) *Persoonia*, 28; 1–13
20. Gramaje, D., Baumgartner, K., Halleen, F., Mostert, L., Sosnowski, M.R., Úrbez-Torres, J.R. and Armengol, J. 2016. Fungal trunk diseases: A problem beyond grapevines? *Plant Pathol.*, 65: 355–356. doi: 10.1111/ppa.12486.
21. Grinbergs, D., Chilian, J., Carrasco-Fernandez, J., France, A., Moya-Elizondo, E. and Gerding, M. 2020. A PCR-based method for the rapid detection of *Chondrostereum purpureum* in apple. *Plant Dis.*, 104 (3): 702 - 707. doi.org/10.1094/PDIS-10-19-2086-RE.
22. Grinbergs, D., Chilian, J., Lisboa, K. and France, A. 2019. First report of Silverleaf disease caused by *Chondrostereum purpureum* on murta (*Ugni molinae* Turcz.) in Chile. *Plant Dis.* doi.org/10.1094/PDIS-12-18-2285-PDN.
23. Grinbergs, D., Chilian, J., Padilla, N., Reyes, M. France, A. Gerding, M. and Ernesto A. Moya-Elizondo. 2021. Endophytic microorganisms associated with reversion of silverleaf disease symptoms in apple. *Phytopathology*, <https://doi.org/10.1094/PHYTO-12-20-0548-R> (AP6)
24. Hamberg, L., Vartiamaä, H. and Hantula, J. 2015. Breeding Increases the Efficacy of *Chondrostereum purpureum* in the Sprout Control of Birch. *PLoS ONE*, 10(2): e0117381. doi:10.1371/journal.pone.0117381
25. Hamberg, L. and Hantula, J. 2016. The efficacy of six elite isolates of the fungus *Chondrostereum purpureum* against the sprouting of European aspen. *J. Environ. Manage.*, 171: 217 - 224.
26. Hamberg, L., Saksa, T. and Hantula, J. 2021. Role of *Chondrostereum purpureum* in biocontrol of trees. *Appl. Microbiol. Biotechnol.*, 105: 431-440.
27. Kaufmane, E., Grāvē, I. and Ikase, L. 2019. Plum research and growing in Latvia. *Proc. Latv. Acad. Sci., Section B*, Vol. 73 (2019), No. 3 (720), pp. 195–206.
28. Ko, Y., Yao, K., Chen, C., Liu, C., Maruthasalam, S. and Lin, C. 2008. First report of gummosis disease of plum (*Prunus salicina*) caused by a *Botryosphaeria* sp. in Taiwan. *Plant Dis.*, 92(3):483. doi.org/10.1094/pdis-92-3-0483a
29. Li, Q., Tang, L., Sun, W., Huang, S., Guo, T., Mo, J., Fan, M., Zhang, Z. and Hsiang, T. 2019. First report of stem canker and dieback caused by *Neofusicoccum parvum* on Plum in Guangxi, Southern China. *Plant Dis.*, 103 (11): 2952-2952. doi.org/10.1094/PDIS-03-19-0434-PDN.
30. Merlet, L., Wiseman, M., Serdani, M. and Putnam, M. L. 2018. First report of Silver Leaf caused by *Chondrostereum purpureum* on *Vaccinium corymbosum* in Oregon. *Plant Dis.*, 102(10): 2041. <https://doi.org/10.1094/PDIS-02-18-0312-PDN>.
31. Mojeremane, K., Lebenya, P., du Plessis, I., van der Rijst, M., Mostert, L., Armengol, J. and Halleen, F. 2020. Cross pathogenicity of *Neofusicoccum australe* and *Neofusicoccum stellenboschiana* on grapevine and selected fruit and ornamental trees. *Phytopathol. Mediterr.*, 59 (3): 581-593. doi: 10.14601/Phyto-11609.
32. Odepa, Estudios y Políticas Agrarias. 2021. Ministerio de Agricultura. Evolución de la Fruticultura Chilena en los Últimos 20 Años. Oficina de estudios y políticas Agrarias. <https://www.odepa.gob.cl/rubros/frutas-frescas>.
33. Ogawa, J. and English, H. 1991. Diseases of temperate zone tree fruit and nut crops. University of California, Oakland, CA, USA. Publication 3345. 461 p.

34. Ozgonen, H., Erkilic, A. and Baloglu, S. 2006. First report of *Chondrostereum purpureum* causing silverleaf disease of apricot (*Prunus armeniaca*) in Malatya, Turkey. *N. Z. J. Crop Hortic. Sci.*, 34:4, 357-357, DOI: 10.1080/01140671.2006.9514426
35. Pearce, R.B., Sumer, S., Doran, S.J., Carpenter, T.A. and Hali, L.D. 1994. Non-invasive imaging of fungal colonization and host response in the living sapwood of sycamore (*Acer pseudoplatanus* L.) using nuclear magnetic resonance. *Physiol. Mol. Plant Pathol.*, 45: 359-384.
36. Li, Q., Tang, L., Sun, W., Huang, S., Guo, T., Mo, J., Fan, M., Zhang, A. and Hsiang, T. 2019. First report of stem canker and dieback caused by *Neofusicoccum parvum* on plum in Guangxi, Southern China. *Plant Dis.*, doi.org/10.1094/PDIS-03-19-0434-PDN
37. Ramsfield, R.D. 2006. Risk assessment of inundative biological control with *Chondrostereum purpureum* in N. Z. *J. For. Sci.*, 36 (1): 11-20.
38. Rivero, M., Quiroga, M., Gonzalez, O. and Moraga, L. 2013. Plum postharvest manual. INTA, Instituto de Investigaciones Agropecuarias. [https://inta.gob.ar/sites/default/files/script-tmp-ficha\\_n\\_1\\_-\\_cosecha.pdf](https://inta.gob.ar/sites/default/files/script-tmp-ficha_n_1_-_cosecha.pdf)
39. Senda, M., Narita, T., Akada, S., Okuno, T. and Miyairi, K. 2001. Characterization of an endopolygalacturonase gene *cppgl* from phytopathogenic fungus *Chondrostereum purpureum*. *J. Gen. Plant Pathol.* 67: 41 - 44.
40. Setliff, E. C. 2002. The wound pathogen *Chondrostereum purpureum*, its history and incidence on trees in North America., *Austral. J. Bot.* 50: 645-651.
41. Shamoun, S. 2000. Application of biological control to vegetation management in forestry. Proceedings of the X International Symposium on Biological Control of Weeds. 4-14 July 1999, 87-96 p.
42. Simpson, R. M., Van Hekezen, R., Van Lune, F., Brewster, D. and Spiers A. G. 2001. Extracellular enzymes of *Chondrostereum purpureum*, causal fungus of silverleaf disease. Pp. 202-208. In: *N. Z. Plant Prot.*, Vol. 54. Proceedings of a conference, Quality Hotel, Palmerston North, New-Zealand.
43. Spiers A, Edwards W, Hopcroft D. 1987. Effects of Silverleaf infection on ultrastructure of foliage of *Prunus*, *Rosa*, and *Populus*. *N. Z. J. Bot.*, 25 (3): 411 - 423.
44. Spiers, A. Brewster, D. Bus, V. and Hopcroft, H. 1998. Seasonal variation in susceptibility of xylem tissue of *Malus*, *Pyrus*, *Prunus*, and *Salix* species to *Chondrostereum purpureum* in New Zealand. *Mycol. Res.*, 102 (7): 881 - 890.
45. Spiers, A. G. and Brewster, D. 1997. Evaluation of chemical and biological treatments for control of *Chondrostereum purpureum* infection of pruning wounds in willows, apples, and peaches. *New Zeal. J. Crop Hort.*, 25 (1): 19-31.
46. Spiers, A., Brewster, D., Slade, A. and Gardiner, S. 2000. Characterization of New Zealand isolates of *Chondrostereum purpureum* with regard to morphology, growth, pathogenicity and RAPD bandings patterns. *Mycol. Res.* 104(4): 395-402.
47. Spiers, A.G. and Hopcroft, D.H. 1988. Factors affecting *Chondrostereum purpureum* infection of *Salix*. *Eur J. For. Pathol.*, 18: 257-278.
48. Valiuškaitė, A. 2002. Micromycetes infecting stone fruit trees. *Biologija*, 1:18-21.
49. Vartiamaäki H, Hantula J, Uotila A. 2009. Susceptibility of silver birch pruning wounds to infection by white-rot fungus (*Chondrostereum purpureum*), a potential bioherbicide. *Silva Fenn.*, 43(4): 537 - 547.
50. White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungi ribosomal RNA genes for phylogenetics. In: *PCR protocols. A guide to methods and applications*: 315-322. Academic Press, San Diego, CA.
51. Willoughby, I. H, Seier, M. K., Stokes, V. J., Thomas, S. E. and Varia, S. 2015. Synthetic herbicides were more effective than a bioherbicide based on *Chondrostereum purpureum* in reducing resprouting of *Rhododendron ponticum*, a host of *Phytophthora ramorum* in the UK. *Forestry*, 88: 336 - 344.
52. Xing, J., Li, P., Zhang, Y., Li, J., Liu, Y., Lachenbruch, B., Su, X. and Zhao, J. 2020. Fungal pathogens of canker disease trigger canopy dieback in poplar saplings by inducing functional failure of the phloem and cambium and carbon starvation in the xylem. *Physiol. Mol. Plant Pathol.*, 112: 101523.