Note

https://doi.org/10.1590/1678-992X-2023-0142

ISSN 1678-992X

SCIENTIA AGRICOLA

Crop Science

# *Botrytis cinerea* infection in *Vitis vinifera* cultivars under cycle inversion

Fernando Albertin[1]*, Jhulia Gelain[2], Juliana Nicolau Maia[1], Louise Larissa May De Mio[1], Luiz Antonio Biasi[1]

[1]Universidade Federal do Paraná – Depto. de Fitotecnia e Fitossanidade, R. dos Funcionários, 1540 – 80035-050 – Curitiba, PR – Brasil.

[2]Clemson University – Dept. of Plant and Environmental Sciences, 105 Collings St – 29634 – Clemson, SC – USA.

*Corresponding author <feralbertin@gmail.com>

Edited by: Sérgio Tonetto de Freitas

Received June 23, 2023

Accepted October 24, 2023

**ABSTRACT**: Grapes for winemaking are harvested in months of high precipitation and temperatures that favor fungal infection. The cycle inversion technique promotes fall-winter harvesting with slower maturation and improved quality. However, the incidence of latent *Botrytis cinerea* Pers. in the berries and the susceptibility of cultivars under this pruning system have yet to be studied. We evaluated the incidence of latent *B. cinerea* in grape berries under cycle inversion at pre-ripening and the susceptibility of different cultivars to *B. cinerea* infection at maturity. The experiment was conducted in a commercial vineyard in São Roque, São Paulo. The red grape cultivars evaluated were Cabernet Franc, Malbec, Syrah, and Marselan, and the white grapes were Viognier and Sauvignon Blanc. The incidence of latent *B. cinerea* in the berries ranged from 1 % to 33 % in the different cultivars and harvest seasons evaluated. Artificially inoculated *B. cinerea* ($10^5$ conidia m $L^{-1}$) did not cause disease in detached, unwounded ripe berries. However, berries from all cultivars showed symptoms of gray mold and signs of the pathogen when artificially inoculated with the same concentration of conidia plus the nutritional contribution of grape juice in a 1:1 ratio.

**Keywords**: latent infection, plant health, viticulture, winter wines

The quality of Brazilian wines has improved through the refinement of oenological and management techniques, such as the cycle inversion technique, used mainly in the states of Minas Gerais and São Paulo (Regina et al., 2011; Brant et al., 2021), resulting in the production of winter wines.

In the wine-producing areas in the southeastern region, as in other parts of Brazil, there is only one harvest season, which coincides with the months of high rainfall and high temperatures, causing an increase in fungal disease incidence and impaired maturation, which affects the quality of the wines produced (Regina et al., 2011). Unlike the regular production cycle, the cycle inversion through two separate prunings transfers the harvest to the autumn-winter season, allowing the bunches to ripen longer in drier weather with a good temperature range, thereby avoiding adverse weather conditions favorable to fungal diseases such as *Botrytis cinerea* Pers. However, there is no information as to whether the change in the flowering period with the cycle inversion technique can interfere with the presence of latent fungi in the berries or even with the susceptibility of these berries during maturation.

*Botrytis cinerea* is one of the most recurrent phytopathogenic fungi that affects grapevines (Magalhães, 2015; González-Fernández et al., 2020). Infection by the fungus occurs before and during flowering, leading to flower drop, damaging fruit formation, and remains latent until the beginning of maturation (Magalhães, 2015). It is known that free water and sugar accumulation in the berries favors the development of the fungus, whose hyphae can directly penetrate the tissues of the berries. However, wounds significantly potentiate this process (Coertze et al., 2001).

There are variations in susceptibility to *B. cinerea* in the different grape cultivars (PlantGrape, 2021). Nevertheless, its incidence in the berries and the susceptibility of the cultivars under this pruning system have yet to be studied. Given this context, this work aimed to evaluate the presence of latent *B. cinerea* in asymptomatic berries of wine grape cultivars managed with cycle inversion. Furthermore, we assessed the susceptibility of grape cultivars to infection by *B. cinerea* in ripe, unwounded berries from vineyards under cycle inversion.

The experiment was conducted in a non-irrigated commercial vineyard in the municipality of São Roque, São Paulo, Brazil, during the 2019 and 2021 seasons. The experimental area was located at 23°35′37.5″ S, 47°9′40″ W, altitude 890 m. The climate is Cfb according to the Köppen classification - humid subtropical climate without a dry season with a temperate summer (Alvares et al., 2013). The temperatures ranged from 23.1 °C in the hottest month, Feb, to 15.5 °C in the coldest month, July. The average rainfall was circa 270 mm in Jan and 27 mm in June, according to the Integrated Agrometeorological Information Center (Abramides et al., 2019).

All cultivars were trained to a three-wire vertical trellis with the low wire 0.9 m above ground and two spur cordons per vine and submitted to the cycle inversion technique. The red *Vitis vinifera* L. cultivars used were: Cabernet Franc (clone 214) and Malbec (clone 596), planted in 2011; Syrah (clone 174) and Marselan (clone 980), planted in 2013, all on the rootstock Paulsen 1103, with a spacing of 1.5 m between plants and 2.5 m between rows. The white *V. vinifera* cultivars were Viognier (clone 1042) and Sauvignon Blanc (clone 242), planted in 2016, also grafted onto the rootstock Paulsen 1103, with a spacing of 1.2 m between plants and 2.5 m between rows. In the 2021 season, the Syrah and Marselan cultivars had already been removed for not responding to the canopy management used.

Two distinct prunings were necessary to carry out the cycle inversion. The first was formative, and the second was required for production. Formative pruning was carried out in Sept, when the one-year-old canes were cut back to two buds, with the inflorescence coming from the sprouting removed after its appearance. Production pruning was carried out based on the maturation of branches with incomplete lignification, consisting of pruning of canes with an average of five to seven buds. Hydrogen cyanamide was applied to the two apical buds. All cultivars had their second pruning in Dec, except for Syrah, which was pruned one month after the others to reproduce the management approach adopted south of Minas Gerais, where the pruning technique was perfected and developed.

As regards management, the rows were kept clean, and the spaces between the rows were maintained with mowed spontaneous cover. In the canopy, the leaves around the bunches were removed when the berries were groat-size, at stage 73, according to the Biologische Bundesanstalt für Land und Forstwirtschaft, Bundessortenamt und Chemische Industrie (BBCH) scale (Lorenz et al., 1995). At veraison, the plants were wrapped with a white anti-hail screen to protect against pests and wild animals. Furthermore, spraying of insecticides and fungicides for *B. cinerea* with a rotation of the active ingredients chlorothalonil, captan, procymidone, and thiophanate-methyl was carried out, as well as fertilization, according to technical recommendations.

A total of 20 berries were collected randomly to evaluate latent *B. cinerea*, with a pedicel of approximately 2 to 3 mm, 20 days after stage 81 (BBCH) from each plant selected. They were submitted to the overnight freezing incubation technique (ONFIT) (Luo and Michailides, 2001). First, the surface was disinfected by immersing the fruits in a 70 % alcohol solution for 1 min, followed by immersion in 0.5 % sodium hypochlorite for a further 1 min, and rinsed three times in sterilized distilled water, followed by drying on sterilized paper towels. Afterwards, the berries were placed in a freezer for 15 h (–16 °C) and then placed on a suspended grid, without touching each other, inside transparent plastic boxes (Gerbox®) containing 20 mL of sterilized distilled water in the bottom. The boxes were randomly placed on shelves and incubated in a room between 21 and 26 °C. The experimental design used was completely randomized (CRD) with six replications per red grape cultivar and 12 replications per white grape cultivar, containing ten berries per replicate. The experiment had two replications each season, with the same number of berries sampled.

The evaluation was carried out on the tenth day after incubation, observing the occurrence of necrosis and signs of pathogens with a stereoscopic microscope (20×) and confirmation of fungal structures in an optical light microscope (400×). Another evaluation was performed four days after the tenth day evaluation to ensure that fungi that are slower to sporulate were not overlooked.

The primary fungi identified were isolated from the berries. For this, the structures were visualized under the stereoscopic microscope and placed on Petri dishes with Potato Dextrose Agar (PDA) culture medium, incubated in a growth chamber at 23 °C in the dark for ten days.

This study used the DNA extraction protocol using CTAB (cetyltrimethylammonium bromide) with certain modifications (Doyle and Doyle, 1987; Pereira et al., 2019). The species were identified by PCR (Polymerase chain reaction) using the methodology proposed by Li et al., 2012, through forward primers G3PDH-F1 and G3PDH-F2 and reverse primer G3PD-R for identification of *B. cinerea* and *Botrytis caroliniana* X.P. Li & G. Schnabel, respectively. A reaction without DNA was used for the negative control, and a sample previously identified as *B. cinerea* was used as a positive control.

For evaluating the susceptibility of the berries to *B. cinerea*, two sampled clusters were randomly selected from each plant, marked 20 days before collection, washed with water to remove possible traces of fungicides, dried, and then bagged with tissue paper to avoid direct contact with the sprays until harvest time. Bunches were collected between stages 83 and 85 according to the BBCH phenological scale. The bagged bunches were unwrapped, and nine uninjured berries of each cultivar were selected with a 3 mm pedicel.

A conidial suspension was prepared from *B. cinerea* colonies after 15 days of growth in PDA medium. Two mL of sterilized distilled water was added, and the colonies were scraped off with a sterilized Drigalsky loop. The suspension was filtered through sterilized gauze, stirred, and the concentration of conidia measured in a Neubauer chamber under an optical light microscope and adjusted to a concentration of $10^5$ conidia m $L^{-1}$.

The fruit was packed in plastic cups with sterilized and moistened cotton to maintain humidity. The containers were then placed in Gerbox® boxes in a room at a temperature between 21 and 26 °C, and the fruit was evaluated daily for seven days by observing the onset of symptoms and signs of fungus. The area under the disease progress curve (AUDPC) was calculated by adding the area of the trapezoids using the formula: $AUDPC = (y_1 + y_2) / 2 \times (t_2 - t_1)$ (Shaner and Finney, 1977), where y is equal to the number of infected berries in percentage and t the time in days of each evaluation.

The experimental design used was the CRD, consisting of six grape cultivars (four red and two white) with nine replications each, each berry being considered a replication, and four treatments (water, juice, suspension, and suspension plus juice from the cultivars in a 1:1 ratio). The experiment was initially carried out using only conidial suspension without juice. Next, conidial suspension and conidial suspension plus berry juice were tested. Both experiments were repeated twice. Aliquots of 10 µL of sterile distilled water and sterile distilled water plus juice were deposited on unwounded berries from different cultivars as controls.

Aliquots of 30 µL of the suspension were deposited on Petri dishes containing 10 mL of Water-Agar (WA) medium to evaluate the germination capacity of the isolate under the same temperature conditions of incubation as the berries. Germination was stopped 24 h after deposition by adding approximately 1 mL of Lactophenol plus blue dye for better visualization under an optical light microscope (200×).

The data collected in the experiments were analyzed using the R® Software version 3.6.1. In the ONFIT experiment, the data transformed with the constant +0.05 were submitted to the Shapiro-Wilk normality test and the Bartlett test of homogeneity, followed by an analysis of variance and comparison of means by the Tukey test ($p < 0.05$). In the *B. cinerea* susceptibility experiment, the data were submitted to the Kruskal-Wallis test, followed by the Dunn test with *p*-value adjustment by Bonferroni.

The berries of all cultivars collected during stage 81 (BBCH) in the 2019 and 2021 seasons showed a high incidence of endophytic fungi, such as *Clasdosporium* sp., *Fusarium* sp., *Pestalotiopsis* sp., and *Colletotrichum* sp. (data not shown). The genus *Botrytis* had incidences ≤ 33 % but with variations between cultivars (Table 1). The Sauvignon Blanc and Syrah cultivars were the most susceptible in the 2019 season, followed by Marselan, Viognier, Malbec, and Cabernet Franc. In the 2021 season, the incidence of *Botrytis* sp. was lower in white cultivars, but 'Cabernet Franc' and 'Malbec' showed the same trend in both seasons.

A fragment of 238 base pairs (bp) was amplified by PCR using a species-specific primer (G3PDH-F1), confirming the *B. cinerea* species.

In tests with the inoculation of a *B. cinerea* conidia suspension without adding juice on unwounded berries, no symptoms or signs of the pathogen were observed in any of the cultivars.

**Table 1** – Average incidence (%) of *Botrytis cinerea* from two assays, in berries of red and white grape cultivars under cycle inversion system, collected from stage 81 (Biologische Bundesanstalt für Land und Forstwirtschaft, Bundessortenamt und Chemische Industrie - BBCH), in the 2019 and 2021 seasons, with the Overnight Freezing incubation technique (ONFIT) (15 h at –16 °C).

| Cultivar | *Botrytis* sp. (%) | |
|---|---|---|
| | 2019 | 2021 |
| Cabernet Franc | 2 b | 1 a |
| Malbec | 3 b | 1 a |
| Syrah | 33 a | - |
| Marselan | 9 b | - |
| CV (%) | 54.74 | 26.65 |
| Cultivar | *Botrytis* sp. (%) | |
| | 2019 | 2021 |
| Viognier | 8 b | 0 a |
| Sauvignon Blanc | 20 a | 1 a |
| CV (%) | 48.88 | 39.07 |

Means followed by the same lowercase letter in the column do not differ significantly by Tukey's test ($p < 0.05$).

In the second test model, with pathogen suspension plus grape juice in a 1:1 ratio, the incidence of the disease was observed, though it varied between cultivars (Table 2). In treatments with water only, juice only, or conidia suspension in water, the berries did not show fungal development even after seven days of inoculation in the cultivars and seasons evaluated. On the other hand, treatment with conidial suspension plus juice, regardless of the total soluble solids (SS) content, resulted in gray mold symptoms. In the 2019 season, with SS ranging from 14.6 to 21.6 °Brix, incidences ≥ 89 % were observed in most of the cultivars, except for Sauvignon Blanc, with 56 % of incidence. In the 2021 season, all tested cultivars showed an incidence of 100 % at the end of the evaluations, and SS ranging from 19.4 to 23.8 °Brix.

The time for the pathogen to infect and show symptoms varied from one cultivar to another (Figure 1). The Syrah cultivar showed the longest time from the inoculation needed for the appearance of symptoms and signs, namely, a period of four days, in the 2019 season. For the other cultivars, two days were enough for the onset of symptoms and signs, reaching more than 89 % of berries infected seven days after inoculation, except for 'Sauvignon Blanc' with 56 % infected. In 2021, symptoms and signs were observed starting on the second day of inoculation and reaching an incidence of 100 % berries infected seven days after inoculation. In the 2019 season, the AUDPC indicated that the cultivars Cabernet Franc, Malbec, Marselan, and Viognier did not differ, presenting the highest values with 539, 490, 490, and 550, respectively, and 'Syrah' had the lowest AUDPC value, with 279. 'Sauvignon Blanc' did not differ statistically from 'Syrah' or other cultivars. In the 2021 season, the AUDPC did not differ between the cultivars evaluated.

This study identified that latent *B. cinerea* is present even with the cycle inversion in grape cultivars Cabernet Franc, Malbec, Syrah, Marselan, Viognier and Sauvignon Blanc. The overnight freezing incubation technique (ONFIT) is used on several fruit trees to observe latent pathogens. This technique eliminates superficial microorganisms and degrades the cells of the berry epidermis, leading to the emergence of latent fungi. The presence of latent fungi in berries can result in symptom onset during harvesting and processing, which can interfere with the quality of the wine through metabolites that disturb the yeasts in alcoholic fermentation (Fugelsang and Edwards, 2010).

The period from inflorescence to full bloom is highly vulnerable to pathogen infection due to the natural opening of the flower (Giovannini and Manfroi, 2013; Magalhães, 2015). In this work, latent *Clasdosporium* sp., *Fusarium* sp., *Pestalotiopsis* sp., and *Colletotrichum* sp. were found in berries, which serves as a warning for future studies that aim to identify the species and confirm pathogenicity to better understand the relevance of these fungi to the grape healthcare and interference in the winemaking process.

**Table 2** – Average incidence (%) of *Botrytis cinerea* from two assays, seven days after inoculation, in berries of red and white grape cultivars under cycle inversion, collected between stages 83 and 85 (Biologische Bundesanstalt für Land und Forstwirtschaft, Bundessortenamt und Chemische Industrie -BBCH), in the 2019 and 2021 seasons, kept under favorable conditions of humidity and temperature. Average soluble solids content (SS) of berry juice, water deposition, juice deposition, inoculation with conidia suspension and inoculation with conidia suspension plus berry juice (1:1).

| Cultivar | Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SS | | Water | | Juice | | Conidia suspension | | Conidia suspension + Juice (1:1) | |
| | 2019 | 2021 | 2019 | 2021 | 2019 | 2021 | 2019 | 2021 | 2019 | 2021 |
| C. Franc | 17.6 | 20.4 | 0 | 0 | 0 | 0 | 0 | 0 | 100 a | 100 a |
| Malbec | 20.0 | 19.4 | 0 | 0 | 0 | 0 | 0 | 0 | 89 b | 100 a |
| Syrah | 14.6 | - | 0 | - | 0 | - | 0 | - | 89 b | - |
| Marselan | 16.4 | - | 0 | - | 0 | - | 0 | - | 89 b | - |
| Viognier | 21.6 | 23.8 | 0 | 0 | 0 | 0 | 0 | 0 | 100 a | 100 a |
| S. Blanc | 16.8 | 21.0 | 0 | 0 | 0 | 0 | 0 | 0 | 56 b | 100 a |

Means followed by the same lowercase letter in the column do not differ significantly by Dunn's test ($p < 0.05$).

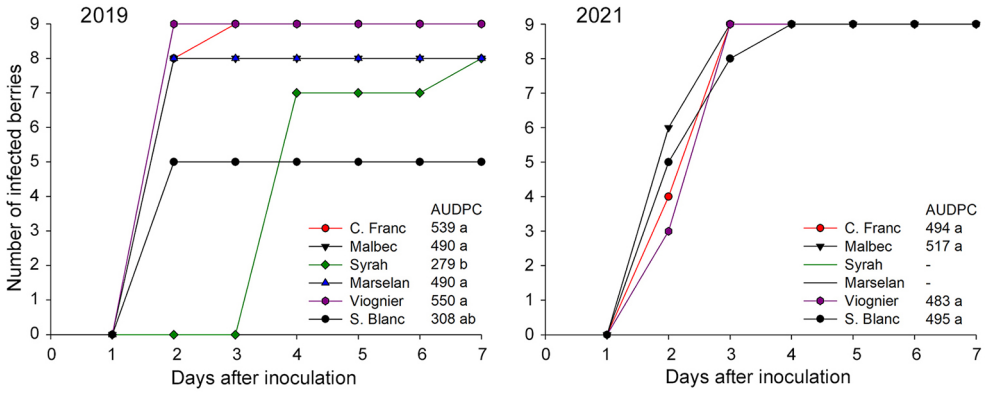


**Figure 1** – Cumulative curve of mean incidence of *Botrytis cinerea* from two assays and area under the disease progress curve (AUDPC), in number of berries (n = 9) and seven days after inoculation, of red and white grape cultivars under cycle inversion system, inoculated with conidial suspension plus juice (1:1), collected between stages 83 and 85 (Biologische Bundesanstalt für Land und Forstwirtschaft, Bundessortenamt und Chemische Industrie - BBCH), in the 2019 and 2021 seasons. Means followed by the same lowercase letter in the column do not differ significantly by Dunn's test ($p < 0.05$).

In this study, even managing fungal diseases with multisite and site-specific fungicides, the average incidence of *Botrytis* sp. was 12 % for red cultivars and 14 % for white cultivars. This fact may be due to the 40 mm of rain recorded during the flowering period in the 2019 season. On the other hand, in the 2021 season, the average incidence of *Botrytis* sp. for the remaining white and red cultivars was approximately 1 %.

Flowering is one of the developmental phases most susceptible to the primary infection by *B. cinerea* present from the previous season. Natural openings, such as flowers, act as a gateway for the pathogen, which causes the flower buds to dry (at the ends or completely) or remain latent until the beginning of maturation (Magalhães, 2015; Carisse et al., 2018; Haile et al., 2017). The most significant losses occur during maturation due to fungus penetration, mainly through openings caused by hail, cracks caused by water imbalance or excess vigor, and attacks by bees, powdery mildew, and moth caterpillars (Magalhães, 2015). Although the broadly optimal temperature for pathogen germination is between 15 °C and 25 °C with humidity ≥ 90 %, germination occurs at lower temperatures in the presence of free water, even if at a slower rate (Pearson and Goheen, 1990; Wilcox et al., 2015). This demonstrates that the pathogen infection may occur in the berries, even with the cycle inversion, if conditions are favorable. Green berries have a high concentration of organic acids, which, together with phytoalexins, will prevent the germination of *B. cinerea* spores (De Kock and Holz, 1991). However, when berries fall, for abiotic reasons, physiological disorders, or other phytopathogens, the pathogen can lodge in the scar site and remain there until the opportune moment for the disease to develop (Giovannini and Manfroi, 2013).

No vineyard is free of *B. cinerea* due to this pathogen's various alternative hosts (Choquer et al., 2007; Wilcox et al., 2015). The pathogen is one of the most damaging in Brazilian vineyards during the flowering period until harvest. An interesting fact is that grapes partially affected by gray mold can be used in winemaking; however, if their quantities are large, the enzyme laccase produced by the fungus, which causes the oxidation of certain phenols, will modify the aroma and stabilization of the color (Giovannini and Manfroi, 2013).

Studies on the time of *B. cinerea* infection in the field in vineyards using cycle inversion have yet to be found. They should be explored to expand knowledge and, consequently, improve management.

As regards the susceptibility of different grape cultivars to *B. cinerea*, the fungus could infect healthy, unwounded berries only after the nutritional contribution of grape juice with the spore suspension. Regardless of the number of infected berries, all cultivars were susceptible to infection. Spores suspended in water alone could not infect any berry of the different cultivars tested. The germ tube length of spores amended with juice was greater than without the nutrient, which may have favored the infection process (data not shown). There are reports in the literature of direct infection by hyphae in adjacent infected berries (González-Domínguez et al., 2015), and studies to confirm this type of infection in healthy berries for the cultivars evaluated are needed.

*Botrytis cinerea* is a necrotrophic fungus that kills host plant cells to feed and develop reproductive structures. However, its efficacy relates to the energy input from water and nutrients. The penetration of *B. cinerea* into the vine, especially into the berry, occurs typically with the germination of a single germ tube with direct penetration, with or without the formation of an appressorium (Coertze et al., 2001). In field conditions associated with factors such as bee damage, berry cracking, and others, the pathogen is favored by the leakage of pulp juice, which enhances penetration and subsequent infection.

The work with inoculation of *B. cinerea* demonstrated that its spores could only penetrate ripe berries with nutritional input from the cultivars' juice under the conditions of the experiment. For other inoculum concentrations, temperatures, maturation stages, and places of cultivation of the vines, direct infection without nutrient input can occur, as has been reported by Ciliberti et al. (2015) in a study on 'Sauvignon Blanc' in the region in Villenave d'Ornon – France and Pukekohe – New Zealand (Tyson et al., 2022). According to the experience of our work group, in other cultivars of grapes, such as table grapes produced in the São Francisco Valley – Brazil, infection by *B. cinerea* occurs in unwounded berries even without nutrient input. Several studies have proven that nutrients improve the infectious and colonization process. It was observed that the cavity of harvested raspberries resulted in a moist and nutritious environment, which favors the attack by *Rhizopus* sp. (Davis, 1991). The same condition occurs when berry peduncles are broken and generate exudation of sugars, which favors infection by this fungus (Lisker et al., 1996) and leads to infection by *B. cinerea* in the field. Furthermore, Baggio et al. (2016) observed that the ability to penetrate directly into peaches and nectarines that were not injured and with the external nutritional supply, showed a more effective production of enzymes by *Rhizopus stolonifer* (Ehrenb.) Vuill.

All cultivars were susceptible to infection in unwounded berries. After the second day of inoculation, berries from all cultivars showed a high incidence of fungus, regardless of the season, except for 'Syrah' in the 2019 season, which took four days. A rapid infection of the fungus was observed in Thompson Seedless and Flame Seedless berries, which recorded an incidence of around 40 % in 24 h at a temperature of 20 °C (Latorre et al., 2002). The results in the present study can be correlated with the AUDPC, which demonstrated that the Syrah cultivar had the lowest value compared to the others and may be linked to the lower SS of the berries, resulting in a longer time for the infection.

Detecting pathogens surviving on the berries indicates the need for monitoring and attention to the potential for damage. The fact that *B. cinerea* directly penetrated the surface of mature berries of wine grapes without wounds, only when the juice of the respective cultivars was added to the suspension of conidia, alerts producers in São Paulo who use cycle inversion to avoid damage to berries that can cause juice to leak and encourage infection.

The pathogen *B. cinerea* was detected as latent in grape cultivars Cabernet Franc, Malbec, Syrah, Marselan, Viognier, and Sauvignon Blanc under a cycle inversion system in the region of São Roque – São Paulo. The *B. cinerea* fungus directly penetrated the surface of mature berries of fine, unwounded grapes and presented symptoms under laboratory conditions when the conidia suspension used for inoculation was amended with juice from the respective cultivars.

## Acknowledgments

This study was financially supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação da Universidade Federal do Paraná (FUNPAR). We wish to thank the Vitivinícola Góes Ltda. for allowing this study to be carried out in part of their vineyards.

## Authors' Contributions

**Conceptualization:** May De Mio LL, Biasi LA. **Methodology:** Albertin F, Gelain J, May De Mio LL. **Investigation:** Albertin F, Gelain J, Maia JN. **Supervision:** May De Mio LL, Biasi LA. **Writing – Original Draft:** Albertin F, Gelain J. **Writing – Review & Editing:** Albertin F, Gelain J, May De Mio LL, Biasi LA.

## References

Abramides PLG, Brunini O, Pedro Júnior MJ, Camargo MBP, Ernandes ES, Blani GC, Brunini APC, Brigante RS, Santos RL. 2019 CIIAGRO. Available at: https://www.ciiagro.sp.gov.br/ciiagroonline/ [Accessed Dec 25, 2019].

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G. 2013. Köppen's climate identification map for Brazil. Meteorologische Zeitschrift 22: 711-728. https://doi.org/10.1127/0941-2948/2013/0507

Baggio JS, Gonçalves FP, Lourenço SA, Tanaka FAO, Pascholati SF, Amorim L. 2016. Direct penetration of *Rhizopus stolonifer* into stone fruits causing rhizopus rot. Plant Pathology 65: 633-642. https://doi.org/10.1111/ppa.12434

Brant LAC, Souza CR, Mota RV, Fernandes FP, Gonçalves MGM, Menezes MD, et al. 2021. Macro scale analysis of Syrah vineyards under winter growing cycles: Agronomical and ecophysiological responses. Scientia Agricola 78: 1-9. https://doi.org/10.1590/1678-992X-2020-0055

Carisse O, McNealis V, Kriss A. 2018. Association between weather variables, airborne inoculum concentration, and raspberry fruit rot caused by *Botrytis cinerea*. Phytopathology 108: 70-82. https://doi.org/10.1094/PHYTO-09-16-0350-R

Choquer M, Fournier E, Kunz C, Levis C, Pradier J, Simon A, et al. 2007. *Botrytis cinerea* virulence factors: new insights into a necrotrophic and polyphageous pathogen. FEMS Microbiology Letters 277: 1-10. https://doi.org/10.1111/j.1574-6968.2007.00930.x

Ciliberti N, Fermaud M, Roudet J, Rossi V. 2015. Environmental conditions affect *Botrytis cinerea* infection of mature grape berries more than the strain or transposon genotype. Phytopathology 105: 1090-1096. https://doi.org/10.1094/PHYTO-10-14-0264-R

Coertze S, Holz G, Sadie A. 2001. Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea*. Plant Disease 85: 668-677. https://doi.org/10.1094/PDIS.2001.85.6.668

Davis RP. 1991. Postharvest soft rot (leak disease). In: Ellis MA; Converse RH, Willians RN, Williamson B. Compendium of raspberry and blackberry diseases and insects. APS Press, Saint Paul, MN, USA.

De Kock PJ, Holz G. 1991. Colonization of table grapes by *Botrytis cinerea* in the western Cape Province. Phytophylactica 23: 73-80.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

Fugelsang KC, Edwards CG. 2010. Wine Microbiology: Practical Applications and Procedure. 2ed. Springer, New York, NY, USA.

Giovannini E, Manfroi V. 2013. Viticulture and Enology: Making Great Wines in Brazilian *Terroirs* = Viticultura e Enologia: Elaboração dos grandes vinhos nos terroirs brasileiros. 2ed. IFRS, Bento Gonçalves, RS, Brazil (in Portuguese).

González-Domínguez E, Caffi T, Ciliberti N, Rossi V. 2015. A mechanist model of *Botrytis cinerea* that includes weather, vine growth stage, and the main infection pathways. Plos One 10: e0140444. https://doi.org/10.1371/journal.pone.0140444

González-Fernández E, Piña-Rey A, Fernández-González M, Aira MJ, Rodríguez-Rajo FJ. 2020. Identification and evaluation of the main risk periods of *Botrytis cinerea* infection on grapevine based on phenology, weather conditions and airborne conidia. The Journal of Agricultural of Science 158: 88-98. https://doi.org/10.1017/S0021859620000362

Haile ZM, Pilati S, Sonego P, Malacarne G, Vrhovsek U, Engelen K, et al. 2017. Molecular analysis of the early interaction between the grapevine flower and *Botrytis cinerea* reveals that prompt activation of specific host pathways leads to fungus quiescence. Plant, Cell and Environment 40: 1409-1428. https://doi.org/10.1111/pce.12937

Latorre BA, Rioja ME, Lillo C. 2002. Effect of temperature on flower and berry infections caused by *Botrytis cinerea* on table grapes = Efecto de la temperatura en el desarrollo de la infeccion producida por *Botrytis cinerea* en flores y bayas de uva de mesa. Ciencia e Investigacíon Agraria 29: 145-151 (in Spanish).

Li X, Kerrigan J, Chai W, Schnabel G. 2012. *Botrytis caroliniana* a new species isolated from blackberry in South Carolina. Mycologia 104: 650-658. https://doi.org/10.3852/11-218

Lisker N, Keren-Shacham Z, Sarig P, Zhtkhi Y, Ben-Arie R. 1996. The biology and pathology of the fungus *Rhizopus stolonifer*, cause of black mould disease of table grapes in Israel. Plant Pathology 45: 1099-1109. https://doi.org/10.1046/j.1365-3059.1996.d01-10.x

Lorenz DH, Eichhorn KW, Bleiholder H, Klose R, Meier U, Weber E. 1995. Phenological growth stages of grapevine (*Vitis vinifera* L. spp. *vinifera*): codes and descriptions according to the extended BBCH scale. Australia Journal of Grape and Wine Research 1: 100-110. https://doi.org/10.1111/j.1755-0238.1995.tb00085.x

Luo Y, Michailides TJ. 2001. Factors affecting latent infection of prune fruit by *Monilinia fructicola*. Phytopathology 91: 864-872. https://doi.org/10.1094/PHYTO.2001.91.9.864

Magalhães N. 2015. Treatise on Viticulture: the Vine, the Vineyard and the *Terroir* = Tratado de Viticultura: A Videira, a Vinha e o *Terroir*. 2ed. Esfera Poetica, Lisboa, Portugal (in Portuguese).

Pearson RC, Goheen AC. 1990. Compendium of Grape Diseases. American Phytopathological Society Press, Saint Paul, MN, USA.

Pereira WV, Padilha CAN, Kaiser JAO, Nesi CN, Fischer JMM, May De Mio LL. 2019. *Monilinia* spp. from imported stone fruits may represent a risk to Brazilian fruit production. Tropical Plant Pathology 44: 120-131. https://doi.org/10.1007/s40858-018-0243-z

PlantGrape. 2021. Catalogue of vines cultivated in France. IFV-INRAE 2009-2020. Institut Agro, Montpellier, France. Available at: https://plantgrape.plantnet-project.org/en/ [Accessed Apr 28, 2022]

Regina MA, Mota RV, Favero AC, Shiga TM, Silva LHJ, Souza WC, et al. 2011. Physicochemical characterization of wine grape cultivars under double pruning management in the northeast region of São Paulo = Caracterização físico-química de uvas viníferas cultivadas em regime de dupla-poda no nordeste do estado de São Paulo. Revista Brasileira de Viticultura e Enologia 3: 84-92 (in Portuguese).

Shaner G, Finney RE. 1977. The effect of nitrogen fertilization on the expression of slow mildewing resistance in Knox wheat. Phytopathology 67: 1051-1056.

Tyson JL, Middleditch CL, Fullerton RA. 2022. The effect of grape berry growth stage on germination of *Botrytis cinerea* in New Zealand. Australasian Plant Pathology 51: 79-90. https://doi.org/10.1007/s13313-021-00839-4

Wilcox WF, Gubler WD, Uyemoto JK. 2015. Compendium of Grape Diseases, Disorders, and Pests. 2ed. American Phytopathological Society Press, Saint Paul, MN, USA.