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Management of ripe grape rot on 'Niagara Rosada' grapevine

Manejo da podridão da uva madura em videira 'Niagara Rosada'

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Highlights

The control of *Colletotrichum* spp. is influenced by the vine phenological stage.
The fungus is sensitive to several fungicides with different action modes.
The application manner of fungicides does not influence the pathogen control.
The removal of the mummified bunches reduces the disease incidence.

Abstract

Ripe grape rot disease, caused by *Colletotrichum* spp., threatens grape cultivation under high precipitation and temperatures. Information regarding the control of this disease in American table grapes such as 'Niagara Rosada' is scarce, and this study aims at answering the following questions: a) at which phenological stage does the fungicide application have better control efficiency, b) which fungicides and/or biological products have a better control efficiency, c) do the spray side and target components of the vine influence disease control, and d) does eradication by removing crop residues, applying urea in the soil, or applying lime sulfur influence disease control? To answer these questions, four experiments were conducted in randomized blocks in commercial vineyards located in the municipality of Rosário do Ivaí, PR, Brazil, from 2016 to 2020. In the first experiment, the fungicide application was evaluated in different phenological phases on disease control: a) full flowering, b) end of flowering, c) berries at the pea stage, d) beginning of bunch closure (half berry), e) beginning of bunch ripening, f) half of bunch ripening, and g) beginning and half of bunch ripening. In the second experiment, we evaluated the effects of different fungicides and biological products on disease control. In the third experiment, which was conducted in a factorial arrangement (2×2), the following factors and levels were evaluated for disease control: a) application targets (directed to clusters, and directed to shoots and clusters) and b) application modes

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(on one side of the vine and on both sides of the vine). The fourth experiment was conducted in a factorial arrangement (2×2×2), and the following factors were evaluated for disease control: a) inoculum source – mummified bunches (removed or not), b) application of urea in the soil for the degradation of crop residues (applied or not), and c) application of lime sulfur after winter pruning (applied or not). At the end of each season, the ripe rot incidence in the bunch was evaluated in all experiments. Data were subjected to an analysis of variance, and the means were compared using Fisher's test (LSD) at a significance of 5%. Spraying the fungicide at the final flowering, pea size, and ripening phenological stages (directed to bunches on one side of the vine), reduced the ripe grape rot incidence in the 'Niagara Rosada' grape, and the following fungicides can be used: metiram combined with pyraclostrobin, with or without potassium phosphite or with a biofungicide formulation containing the bacterium *Bacillus amyloliquefaciens* strain d-747, trifloxystrobin combined with tebuconazole, tebuconazole, tetraconazole, captan, and folpet. Removing mummified bunches of previous seasons reduces the disease incidence by eliminating the primary inoculum.
Key words: *Colletotrichum* spp. Fungicides. *Vitis labrusca.*

Resumo

A podridão da uva madura causada por *Colletotrichum* spp. é uma ameaça ao cultivo de uvas sob condições de alta precipitação e temperaturas elevadas. Devido à carência de informações disponíveis sobre medidas de controle desta doença em uva rústica de mesa 'Niágara Rosada', este trabalho teve como objetivo responder às seguintes questões: a) em quais estádios fenológicos a aplicação de fungicidas tem maior eficiência no seu controle? b) quais fungicidas e produtos biológicos apresentam maior eficiência no seu controle? c) os componentes lado e alvo de pulverização da videira influenciam no seu controle? e d) a erradicação do inóculo por remoção de restos culturais, aplicação de ureia no solo ou aplicação de calda sulfocálcica influenciam no seu controle? Para responder essas questões, foram realizados quatro experimentos em blocos casualizados, em parreirais comerciais localizados em Rosário do Ivaí, PR, Brasil, durante as safras de 2016 a 2020. No primeiro experimento avaliou-se a aplicação de fungicidas nas fases fenológicas: a) pleno florescimento; b) fim do florescimento; c) bagas no estágio de grão de ervilha; d) início do fechamento do cacho (meia baga); e) início do amadurecimento do cacho; f) metade do amadurecimento do cacho; e g) início e metade do amadurecimento do cacho. No segundo experimento avaliou-se a aplicação de diferentes fungicidas e produtos biológicos. No terceiro experimento, realizado em arranjo fatorial 2×2, avaliaram-se os seguintes fatores e níveis: a) alvos de aplicação (direcionada aos cachos, e direcionada aos ramos e aos cachos); e b) modos de aplicação (em um lado da videira somente e nos dois lados da videira). No quarto experimento, realizado em arranjo fatorial 2×2×2, avaliaram-se os seguintes fatores e níveis: a) fonte de inóculo – cachos mumificados da safra anterior (removidos ou não); b) aplicação de ureia no solo para degradação dos restos culturais (aplicada ou não); e c) aplicação de calda sulfocálcica após a poda de inverno (aplicada ou não). No fim do ciclo produtivo, foi avaliada a incidência de podridão da uva madura em todos os experimentos. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Fisher (LSD) a 5% de significância. A pulverização de fungicidas nos estádios fenológicos fim do florescimento, grão ervilha e amadurecimento, direcionada aos cachos em um lado da videira, reduziu a incidência de podridão da uva madura nos cachos de 'Niágara Rosada', podendo ser utilizados os seguintes fungicidas: metiram em

mistura com piraclostrobina, associado ou não ao fosfito de potássio ou ao biofungicida a base de *Bacillus amyloliquefaciens* cepa d-747, trifloxistrobina em mistura com tebuconazol, tebuconazol, tetraconazole, captana, folpete. A remoção de cachos mumificados da safra anterior reduziu a incidência da doença.
Palavras-chave: *Colletotrichum* spp. Fungicidas. *Vitis labrusca.*

Introduction

Fungal diseases are the primary cause of qualitative and quantitative losses in grape production (Shimano & Sentelhas, 2013). The probability of disease occurrence increases when the growing conditions, favorable climate, and susceptibility of the grape cultivar correspond to the conditions for the spread and development of pathogens with inoculum potential in the vine (Garrido, 2017).

Cultivating table grapes with tolerance to some fungal diseases, such as 'Niagara Rosada' reduces production costs and facilitates management, thereby being a favorable option for growers (Ricce et al., 2013). The 'Niagara Rosada' grape can be grown in a two-crop-a-season system in subtropical and tropical regions and is well accepted as a fresh product by the consumer market (Martins et al., 2014). Despite this characteristic in relation to some diseases, this cultivar is susceptible to ripe grape rot, which is accentuated under high precipitation and temperatures during reproductive development (Wang et al., 2015).

Ripe grape rot in Brazil is caused by up to six species of Colletotrichum, with the prevalence of *Colletotrichum viniferum* and *Colletotrichum fructicola* (Echeverrigaray et al., 2020), which are a hemibiotrophic disease whose main inoculum source is the presence of mummified bunches in the vineyard (Silva et al., 2017). For the pathogen's conidia to spread, rain splashes (wind with rain) are necessary (Cannon et al., 2012). Grapes are susceptible to infection by *Colletotrichum* spp. from the flowering to the maturation phase (Greer et al., 2014); however, the disease is only evident when the berries reach maturity, owing to the appearance of small circular brown spots on the berries, which become brown-reddish as the humidity increases with exposure of abundant conidial mass (Weir et al., 2012). Subsequently, with the fungal development to the necrotrophic phase, the fruits mummify and fall (Nogueira et al., 2017).

Few studies have been conducted on ripe grape rot control. Vineyards with a history of *Colletotrichum* spp., and those consisting of old vines, are more susceptible to infection and the development of this disease, making the implementation of preventive management necessary (Garrido & Botton, 2017). Thus, techniques aimed at eliminating the primary inoculum before the growing season begins are essential to reduce disease progression (Carraro et al., 2022). Fungicide sprays typically start at the end of flowering and vary according to seasonal weather conditions to control the disease (Samuelian et al., 2014). In general, protective multisite fungicides have shown moderate efficacy in controlling the disease; however, the number of registered products is limited (Dowling et al., 2020).

To identify the best manners of controlling ripe grape rot on 'Niagara Rosada' grape, this study aims to answer the following questions: (a) at which phenological stage is the application of fungicides more efficient in controlling ripe grape rot, (b) which fungicides are more efficient in terms of control, (c) do the spray side and target of the vine influence its control, and (d) do the practices of crop residue removal, application of urea to the soil, and application of lime sulfur influence its control?

Material and Methods

Experimental area description

Four experiments were conducted to control ripe grape rot in 'Niagara Rosada' (*Vitis labrusca* L.) in commercial vineyards located in the municipality of Rosário do Ivaí, Paraná, Brazil. The municipality is located in a range of 400–800 m a.s.l., with steep slopes of up to 45%, average minimum temperatures between 13 and 15 °C, and maximum temperatures between 26 and 28 °C (Instituto Paranaense de Desenvolvimento Econômico e Social [IPARDES], 2007).

The experiments were conducted during the 2016 season (24°13'20.84"S and 51°17'13.12"W), 2017 season (24°13'26.77"S and 51°17'18.61"W), 2018 season (24°13'28.11"S and 51°17'20.49"W), 2019 season (24°12'30.14"S and 51°17'18.68"W), and 2020 season (24°12'51.7"S and 51º16'11.1"W). In each season, an experimental area was chosen to conduct the experiments to ensure an initial inoculum source by considering the history of the occurrence of diseases in the area and preventing the uneven distribution of inoculum between seasons, provided that treatments in one season would not compromise the results of the following season. In all experimental areas, 'Niagara Rosada' vines were grafted onto 'Riparia do Traviu' rootstock trained in a four-wire vertical positioning system (VSP), with an average spacing of 0.80 to 0.90 m between vines and 1.7 to 1.9 m between rows. Each plot consisted of 5-6 vines, with an average area of 8.5 m2. Pruning, fertilization, pest control, and weed control were conducted according to the standard recommendations for the cultivar for the region (Genta et al., 2010; Kishino et al., 2019b; Kishino, 2019; Kishino et al., 2019a; Calegari et al., 2019).

Experiment description

For fungicide applications in Experiments 1 and 3, a ready mixture of dithiocarbamate and strobilurin (Cabrio® Top) was used at a commercial dose (2 $g.L^{-1}$) with a spray volume of 588.0 $L.ha^{-1}$. In Experiments 1, 2, and 3, a JSF 11001 fan sprayer was used at an angle of 45º to ensure good penetration into the vegetative canopy. In Experiments 2 and 3, an average of six applications were made between vine flowering and ripening, respecting a re-entry interval of 28 days for the application of fungicides to harvest the grapes. Commercial doses of each fungicide were used in all experiments. The experiments were performed as follows:

Fungicide application at different phenological stages to control ripe grape rot

The experiments were conducted during the 2016, 2017, 2018, and 2019 harvest seasons. A randomized block design with eight treatments and four replications was used as the statistical model. The treatments included the application time of the fungicide according to the phenological stage of the vines: a) control (without application), b) full flowering (50% fall of the calyptra), c) end of flowering (complete fall of the calyptra), d) berries at the pea-size stage, e) beginning of the bunch closing (half berry stage), f) beginning of the bunch ripening (berries beginning to soften), g) half of the bunch ripening (berries with an intermediate accumulation of soluble solids), and h) beginning and half of the bunch ripening (berries beginning to soften and with an intermediate accumulation of soluble solids) (Coombe, 1995). Fungicide sprays were directed to the shoots and bunches.

Fungicide applications to control the ripe grape rot

The experiments were conducted during the 2016, 2017, 2018, 2019, and 2020 seasons, and a randomized block design with four replicates was used as the statistical model.

The following fungicides were evaluated in the 2016 crop season: a) control, b) tebuconazole, c) metiram and pyraclostrobin, d) thiophanate-methyl, e) procymidone, f) pyrimethanil, and g) iprodione. The following fungicides were evaluated during the 2017 crop season: a) control, b) metiram and pyraclostrobin, c) difenoconazole, d) metconazole, e) tetraconazole, f) azoxystrobin, g) potassium phosphite, and h) boscalide and kresoxim-methyl. The following fungicides were evaluated in the 2018 crop season: a) control; b) metiram and pyraclostrobin; c) methyram, pyraclostrobin, and potassium phosphite; d) mancozeb; e) captan; f) folpet; and g) chlorothalonil. The following fungicides were evaluated in the 2019 crop season: a) control; b) metiram and pyraclostrobin; c) methyram, pyraclostrobin, and potassium phosphite; d) mancozeb; e) Captan; f) folpet; g) trifloxystrobin and tebuconazole; h) biofungicide formulation containing Bacillus amyloliquefaciens strain D-747; and i) metiram and pyraclostrobin, and B. amyloliquefaciens strain D-747. Finally, the following fungicides were evaluated in the 2020 crop season: a) control, b) metiram and pyraclostrobin, c) thiophanate-methyl, d) tetraconazole, e) potassium phosphite, f) mancozeb, g) captan, h) folpet, i) biofungicide containing B. amyloliquefaciens strain D-747, j) metiram and pyraclostrobin, and Bacillus subtilis BV02, and k) metiram and pyraclostrobin, and biofungicide containing B. amyloliquefaciens strain D-747.

Fungicide application manners to control the ripe grape rot

The experiment was conducted during the 2017, 2018, 2019, and 2020 harvest seasons in a randomized block design in a 2x2 factorial arrangement with an additional control and four replications. The following factors and levels were evaluated: a) application targets (directed to bunches and directed to both shoots and bunches) and b) application methods (on one side of the vine only, and on both sides of the vine). The vine side was considered as the accessible side to the right or left of the crop row. While spraying, in treatments with application on only one side of the vine, the sides were alternated each application.

Removal of crop residues to control the ripe grape rot

The experiment was conducted during the 2016 harvest season after winter pruning at the end of dormancy. The experiment design consisted of randomized blocks with three replications, in a factorial arrangement (2x2x2), totaling eight treatments. The following factors and levels were evaluated: a) inoculum source – mummified bunches from the previous crop (removed or not), b) application of urea to the soil to degrade crop residues (applied or not); and c) application of lime sulfur on the vines after winter pruning (applied or not).

For the treatment in which the inoculum source was maintained on the vine, four bunches mummified by ripe grape rot were tied and spaced apart on the second wire of the training system, simulating bunches abandoned by growers during winter pruning. These bunches were selected from the local vineyard after harvesting the previous crop. The experiment components were immediately installed after removing these bunches.

In the treatment in which urea was applied to the soil to degrade crop residues from pruning, this product was solubilized in 20% water at a dose of 1,000 $L.ha^{-1}$. The treatment was applied using a backpack sprayer equipped with an ADGA-02 fan nozzle. Considering the spacing between the lines of 1.5 m, urea was applied in a strip of 0.75 m on each side of the cultivation line.

The lime sulfur (0.11 $mL.L^{-1}$) was applied immediately after the winter pruning and directed to the entire vines, until the runoff, using a 16-L Jacto HD backpack sprayer with a fan nozzle, ADGA-02, with a spray volume of 250 $L.ha^{-1}$.

Disease incidence and control efficiency assessments

The ripe grape rot incidence was assessed at the end of each season. All plots were evaluated by disregarding 50 cm of their borders. The incidence was considered as the percentage of diseased bunches, using the formula ($x \times 100/y$), where x corresponds to the number of diseased bunches, and y is the total number of bunches evaluated.

The control percentage was defined as the difference between the incidence percentage of the evaluated treatment in relation to the control, using the formula [$(x - y) \times 100/x$], where x corresponds to the incidence of the control treatment, and y is the incidence of the evaluated treatment.

Statistical analysis

The original dataset was subjected to a box and Cox transformation. The data were subjected to an analysis of variance with the F test. When significant, the means were compared using Fisher's test (LSD) at a significance of 5% using R Studio.

Results and Discussion

Fungicide application at different phenological stages to control ripe grape rot

The application of fungicides at different phenological stages responded to treatments only under conditions of a moderate incidence of up to 43%, with no response at high or low incidences (Table 1). The weather conditions of the season, and possibly the amount of inoculum, influenced the disease incidence and treatment effectiveness.

Table 1

Incidence of ripe grape rot caused by *Colletotrichum* spp. in 'Niagara Rosada' table grape subjected to fungicide applications in different phenological stages

Phenological stages	Incidence of ripe grape rot (%)				
	2016	2017	2018	2019	Maximum control (%)
Control	23.7 a	43,.3 a	86.6	1.4	0.0
Full flowering	14.4 ab	41.6 ab	82.7	1.5	39.5
End of flowering	NT	14.1 cd	85.6	1.2	67.4
Pea size	8.1 bc	7.4 d	86.0	1.1	83.0
Half berry	3.1 c	11.9 d	87.4	0.0	72.5
Early ripening	4.4 c	12.1 d	85.1	2.9	72.2
Half of the ripening	8.7 bc	40.3 abc	87.4	NT	63.2
Beginning and half of the ripening	1.9 c	15.5 bcd	93.1	NT	92.1
CV %	47.5	58.7	8.81	9,97	
F	8.0 **	3.1 *	0,6 ^{ns}	0,5 ^{ns}	

Means followed by same letters within columns do not differ according to Fisher's test (p<0.05). NT: not tested. ns: non-significant. *: significant (p<0.05). **: significant (p<0.01).

The frequency of rainy periods is the most determining factor for the occurrence of this disease (Carraro et al., 2022), and the average amounts of rainfall in the experiment region were 611, 885, 622, and 518 mm in the 2016, 2017, 2018, and 2019 seasons, respectively. Despite the rainfall amount in the 2017 season, which was higher than that in 2018, the rainfall in 2017 intensified only in October of this year, and in 2018 in September (Instituto das Águas do Paraná [AGUASPARANA], 2022), and as in this year the rainfall occurred after flowering increased the incidence of bunch rot. Thus, the effect of chemical treatment on different phenological stages was significant only in the 2016 and 2017 harvest seasons (Table 1). In 2016, the disease incidence was significantly lower in treatments from the pea-size phase, whereas in 2017, when the treatment at the end of flowering was included, the rot incidence was determined to be significantly lower in treatments from the end of the flowering stage, except for treatments applied in the middle of the ripening phase. The maximum control rates in the years in which a response (2016 and 2017) to the treatments was evident ranged from 39.5% to 92.1%.

The results of this study corroborate that a time window after flowering exists in which disease control can be performed, and that, under high disease pressure, the response period to treatments is more restricted, comprising the end of the flowering and beginning of the maturation stages. The sequential application of strobilurins, trifloxystrobin, and pyraclostrobin and azoxystrobin at the phenological stages of late flowering, pea size, and early ripening, respectively, reduces the ripe grape rot incidence (Steel et al., 2016). In addition, the application of pyraclostrobin during flowering and ripening reduces infection to a level lower than that of the control vines (Samuelian et al., 2014). This is because the sensitivity of the grape bunches to infection via *Colletotrichum* spp. is higher while flowering and in the pea size and ripening stages, as well as lower at the beginning of bunch closure (Oliver, 2016).

Primary infections by *Colletotrichum* spp. in grape bunches can occur from flowering; however, they remain quiescent until ripening, when they progressively result in berry rot. After infection, berries begin to rot and produce conidia even with low rainfall, which can favor the rapid spread of spores to uninfected berries in the same or neighboring clusters, causing cycles of secondary infections (Ji et al., 2021). Therefore, applications in the early stages of berry development are important for the control of primary infections; however, they must be associated with the application at ripening, with the aim of controlling possible secondary inocula.

Fungicide applications to control the ripe grape rot

The fungicide methyram mixed with pyraclostrobin reduced the incidence of rot compared with the control treatment in the 2016, 2017 and 2020 seasons, as well the tebuconazole in 2016, the tetraconazole in 2017, the mixtures of methyram and pyraclostrobin with potassium phosphite or with *B. amyloliquefaciens* strain d-747, captan and trifloxystrobin mixed with tebuconazole in 2019, and the folpet in 2020 season. The control rates of treatments that significantly reduced the disease incidence ranged from 71.5 to 100% (Table 2).

Colletotrichum species are sensitive to fungicides with different action modes (He et al., 2019), and this facilitates the management of ripe grape rot, as control typically depends on the sequential use of fungicides throughout the growing season (Ji et al., 2021).

Strobilurin group fungicides are efficient in ripe grape rot management programs (Samuelian et al., 2014; Steel et al., 2016). When testing this chemical group alone with the application of azoxystrobin, the ripe grape rot incidence was insignificantly reduced; however, commercial mixtures with dithiocarbamate and triazole, pyraclostrobin, and trifloxystrobin with tebuconazole resulted in a reduction. Commercial mixtures showed a similar control efficiency. However, the re-entry interval period for metiram plus pyraclostrobin was 30 days (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2020a), whereas the interval was 10 days for trifloxystrobin with tebuconazole (MAPA, 2020b), and this mixture can be used closer to the harvest, if necessary.

Azoxystrobin is reported to be the most effective fungicide for controlling ripe grape rot (Horikawa et al., 2018). However, resistance from *Colletotrichum* spp. has been reported in associated with ripe grape rot (Yokosawa et al., 2020). Because it is a fungicide with a single action site, azoxystrobin tends to select resistant populations more easily. Resistance to fungicide sensitivity may be inherent or may have arisen because of selection pressure via application frequency and/or inoculum density (Dowling et al., 2020).

Table 2

Incidence of ripe grape rot caused by *Colletotrichum* spp. in 'Niagara Rosada' table grape subjected to the application of different fungicides and bioproducts between the flowering and ripening phases

Treatments	IIncidence of ripe grape rot (%)					
	2016	2017	2018	2019	2020	Maximum control (%)
Control	10.6 ab	42.8 a	84.0	6.8 a	32.6 abcd	0.0
Tebuconazole	1.9 cd	NT	NT	NT	NT	82.1
Metiram and pyraclostrobin	0.6 d	5.1 c	85.0	4.3 ab	11.6 ef	94.3
Thiophanate-methyl	10.0 abc	NT	NT	NT	33.3 abcd	5.6
Procymidone	14.4 a	NT	NT	NT	NT	0,0
Pyrimethanil	11.2 ab	NT	NT	NT	NT	0.0
Iprodione	13.7 a	NT	NT	NT	NT	0.0
Difenoconazole	NT	27.3 ab	NT	NT	NT	36.2
Metconazole	NT	17.8 ab	NT	NT	NT	58.4
Tetraconazole	NT	12.2 b	NT	NT	33.3 abcde	71.5
Azoxystrobin	NT	18.2 ab	NT	NT	NT	57.5
Potassium phosphite	NT	18.3 ab	NT	NT	31.9 abcde	57.2
Boscalide and kresoxim-methyl	NT	24.8 ab	NT	NT	NT	42.1
Metiram and pyraclostrobin, and potassium phosphite	NT	NT	88.5	0.7 bc	NT	89.7
Mancozeb	NT	NT	83.6	3.1 abc	15.3 cdef	54.4
Captan	NT	NT	85.7	0.9 bc	20.4 bcdef	86.7
Folpet	NT	NT	81.9	3.4 abc	8.1 f	75.15
Chlorothalonil	NT	NT	88.8	NT	NT	0.0
Trifloxystrobin and tebuconazole	NT	NT	NT	0.5 bc	NT	92.6
Bacillus amyloliquefaciens strain D-747	NT	NT	NT	3.3 ab	68.9 a	51.5
Metiram and pyraclostrobin, and e *Bacillus subtilis* BV02	NT	NT	NT	NT	36.2 bcdef	0.0
Metiram and pyraclostrobin, and *B. amyloliquefaciens* strain D-747)	NT	NT	NT	0.0 c	34.9 abcd	100.0
CV %	46.7	6.7	7.9	2.1	0.7	
F	3.2 *	3.9 **	0.9 ns	2.5 *	2.3 *	

Means followed by same letters within columns do not differ according to Fisher's test (p<0.05). NT: not tested. ns: non-significant. *: significant (p<0.05). **: significant (p<0.01).

Fungicides of the triazole chemical group, such as tebuconazole (Rizzotto, 2015) and tetraconazole (Oliver, 2016) have been shown to inhibit the mycelial growth of *Colletotrichum gloeosporioides* in vitro (Rizzotto, 2015). In general, these products have shown good efficacy in controlling Colletotrichum spp., but lower than that of strobilurins (Defrancesco et al., 2018). Therefore, they are typically used in mixtures with strobilurins, which lead to greater efficiency in controlling the disease (Dowling et al., 2020).

Captan (Oliver, 2016) and folpet (Moral et al., 2018) have been shown to be active in inhibiting the conidial germination and mycelial elongation of Colletotrichum isolates (Oliver, 2016). These compounds are considered more active because they are multisite and have moderate efficacy against diseases caused by Colletotrichum spp. (Dowling et al., 2020). In theory, fungicides with an action mode aimed at inhibiting germination and conidia growth are more efficient for controlling ripe grape rot than those that inhibit mycelial elongation, such as captan (Oliver, 2016) and folpet (Moral et al., 2018).

Targeting a management approach with rotations of action mechanisms, fungicides with strobilurin alone or in a mixture can be recommended during the flowering stage (Steel et al., 2012), and broad-spectrum protective fungicides such as captan and folpet during fruit development and ripening (Daykin & Milholland, 1984; Moral et al., 2018).

Fungicides used to control Botrytis cinerea, such as chlorothalonil, iprodione, and pyrimethanil, were ineffective in controlling ripe grape rot (Steel et al., 2013).

Fungicide application manners to control the ripe grape rot

The application factors on different sides of the vine (one side only or both sides), in a VSP training system, and spraying target (directed to shoots and bunches or only to bunches) did not show significant interactions with each other and were evaluated independently. Neither interfered with the control efficiency (Table 3).

Table 3
Incidence of ripe grape rot caused by *Colletotrichum* spp. in 'Niagara Rosada' table grape subjected to the different manners of fungicide application

Contrasts		Incidence of ripe grape rot (%)			
		2017	2018	2019	2020
Control		42.8 a	75.3	0.9 a	23.4
Factorial		2.7 b	84.0	0.9 b	8.5
Spray target (ST)					
Bunches		3.4	84.1	1.0	11.9
Shoots and bunches		2.1	83.9	1.0	5.1
Vine side (VS)					
One side only		3.6	91.0	0.1	10.4
Both sides		1.8	76.9	1.0	6,6
Causes of variation	GL	F			
ST	1	0.8 ns	0.2 ns	0.0 ns	1.8 ns
VS	1	1.0 ns	7.0 ns	1.7 ns	0.3 ns
ST × VS	1	0.0 ns	0.0 ns	0.0 ns	2.0 ns
Control × factorial	1	93.4 **	2.5 ns	14.3 **	2.6 ns
Residue	12				

Means followed by same letters within columns do not differ according to Fisher's test (p<0.05). NT: not tested. ns: non-significant. **: significant (p<0.01).

Some *Colletotrichum* species, such as *C. nymphaeae* and *fructicola,* have been isolated from lesions on vine leaves and shoots (Guginski-Piva et al., 2018). However, most grapevine green tissues do not develop *Colletotrichum* (Samuelian et al., 2012), which explains why target spraying on shoots and bunches did not increase the effectiveness of controlling ripe grape rot.

Note that the significant economic damage caused by this disease is because of the bunches rotting. Therefore, by reducing the amount of fungicide used, the application directed only to the bunches on one side of the vine, provided that each application alternates sides, becomes the most viable alternative.

Removal of crop residues to control the ripe grape rot

The source factors of the initial inoculum, application of urea in the soil, and application of lime sulfur did not present significant interactions among themselves, which were evaluated separately. Only the presence of the inoculum source influenced the disease incidence, and the mummified bunches from the previous harvest significantly increased this variable (Table 4).

Table 4
Incidence of ripe grape rot caused by *Colletotrichum* spp. in ‘Niagara Rosada’ table grape subjected to removal of crop residues

Contrasts		Incidence of ripe grape rot (%)
		2016
Not removed		71.2 a
Removed		18.7 b
Urea applied in the soil (UAS)		
Applied		45.1
Not applied		44.8
Surfur lime (SLA)		
Applied		45.4
Not applied		44.5
Causes of variation	GL	F
SOI	1	136.9 **
UAS	1	0.0 ns
SLA	1	0.0 ns
SOI × UAS	1	3.0 ns
SOI × SLA	1	0.2 ns
UAS × SLA	1	0.5 ns
Residue	14	

Means followed by same letters within columns do not differ according to Fisher’s test ($p<0.05$). NT: not tested. ns: non-significant. **: significant ($p<0.01$).

Agricultural practices are typically employed in the management of *Colletotrichum* spp. in several fruit species in temperate climates. In general, they aim to adjust conditions to reduce the production and dissemination of the inoculum. For most fruit trees, removing diseased and mummified fruits and symptomatic tissues is recommended to reduce the inoculum in the area (Dowling et al., 2020).

Mummified bunches remaining from a previous season can generate potential amounts of conidia that disperse and lead to primary infections that settle on flowers and grape berries. In the grape cycle, the primary inoculum is responsible for the long-term progression of this disease, causing infections that occur between the flowering and beginning of maturation (Ji et al., 2021). Thus, reducing inoculum sources, such as mummified bunches left on trellises and in the soil from previous crops, is important for disease control.

During the winter, viable spores are found in mummified berries, peduncles, canes, and spurs, demonstrating that the pathogen can overwinter in different plant tissues and vine parts, and that the conidia produced in the agricultural remains of the vine in the soil have the potential to be dispersed by wind and rain (Samuelian et al., 2012). However, eliminating the inoculum source by applying urea to the soil and the application of lime sulfur as a winter treatment after pruning did not significantly interfere with the disease amount.

Considering the results of this study and the corrosive potential of lime sulfur in vineyard wires and application equipment (Garrido & Botton, 2015), its application for inoculum eradication is not recommended.

Conclusions

Spraying fungicides at the final flowering, pea, and ripening phenological stages and spraying bunches on one side of the vine, was demonstrated to reduce the ripe grape rot incidence in 'Niagara Rosada' grape. The following fungicides can be used: metiram combined with pyraclostrobin, with or without potassium phosphite or with a biofungicide formulation containing *Bacillus amyloliquefaciens* strain d-747, trifloxystrobin combined with tebuconazole, tebuconazole, tetraconazole, captan, and folpet. In addition, removing mummified bunches left from previous seasons reduces the disease incidence by eliminating the primary inoculum.

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