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EFFECT OF FUNGICIDES AND BIOLOGICAL PRODUCTS IN CONTROLLING RUST AND GRAY MOLD IN GRAPEVINES

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RESUMO

A ferrugem e a podridão cinzenta são doenças frequentes e com alto potencial destrutivo na cultura da videira. Com o objetivo de avaliar a eficácia de diferentes fungicidas e produto biológico no controle dessas doenças, foi realizado um experimento com a cultivar Niágara Rosada, no município de Louveira-SP, no período de fevereiro a abril de 2017. O delineamento experimental adotado foi o de blocos ao acaso com 10 tratamentos e quatro repetições, sendo cada parcela composta por 5 plantas. Foram realizadas 9 aplicações dos produtos a intervalos semanais, com volume variando de 400 L a 1000 L/ha. O critério de avaliação da ferrugem foi a severidade, tendo como base a porcentagem de área foliar afetada pela doença (0 a 100%) e para a podridão cinzenta a incidência de cachos doentes (0 a 100%). Todos os tratamentos promoveram redução significativa das doenças em relação à testemunha. Os melhores níveis de controle da ferrugem foram obtidos com o tratamento boscalida+piraclostrobina (1 L de p.c./ha), sendo esse superior ao fluxapiroxade+piraclostrobina (0,5L) + *Bacillus amyloliquefaciens* (0,5 kg), *B. amyloliquefaciens* (0,5 kg), óleo essencial de *Melaleuca alternifolia* (1,5 L), dimoxistrobina+boscalida (1 L) e semelhante aos demais tratamentos. As parcelas tratadas com fluxapiroxade+piraclostrobina (0,5 L) + *B. amyloliquefaciens* (0,5 kg) apresentaram a menor incidência da podridão cinzenta, sendo superior aos tratamentos dimoxistrobina+boscalida (1 L), boscalida+cresoxim metílico (0,5 L), e pirimetanil (1,5 L) e semelhante aos demais tratamentos. De acordo com os resultados obtidos, a utilização de óleo essencial *M. alternifolia* do produto biológico *B. amyloliquefaciens* pode representar uma importante ferramenta para o manejo da ferrugem e da podridão cinzenta da videira.

Palavras-chaves: *Phakopsora euvitis, Botrytis cinerea, Vitis* spp.

ABSTRACT

Grape rust and gray mold are diseases in grapevines with high destructive potential. In this study, the effectiveness of different fungicides and biological products in controlling these diseases was evaluated using the cultivar “Niágara Rosada” in the municipality of Louveira-SP, from February to April 2017. A randomized block design was used, with 10 treatments and four replicates and five plants per plot. Each treatment was applied nine times at weekly intervals, with application volumes in the range of 400–1000 L/ha. The evaluation criterion for rust was the severity, based on the percentage of leaf area affected by the disease (0 to 100%), whereas gray mold was evaluated using the incidence of diseased bunches (0 to 100%). All treatments were effective in controlling both diseases and significantly reduced the disease incidence in the experimental plot compared with that in the control plots. The best rust control was obtained by treating the plants with boscalide+pyraclostrobin (1 L c.p./ha), which was superior to fluxapyroxade+pyraclostrobin (0.5 L) + *Bacillus amyloliquefaciens* (0.5 kg), *B. amyloliquefaciens* (0.5 kg), *Melaleuca alternifolia* essential oil (1.5 L), and dimoxystrobin+boscalide (1 L). The plants treated with fluxapyroxad+pyraclostrobin (0.5 L) + *B. amyloliquefaciens* (0.5 kg) had a lower incidence of gray mold than did those treated with dimoxystrobin+boscalide (1 L), boscalide+kresoxim methyl (0.5 L), and piremethanil (1.5 L). These results suggest that the use of *M. alternifolia* essential oil and *B. amyloliquefaciens* may represent an important tool for the management of rust and gray mold in grapevines.

Keywords: *Phakopsora euvitis, Botrytis cinerea, Vitis* spp.

The cultivation of grapevines (*Vitis* spp.) is integrated in the habits, cultures, and traditions since time immemorial. They are widely grown in the southern, southeastern, and northeastern regions of Brazil and are mainly consumed as fresh fruit (for domestic and export markets) or used in the production of wine, juice, soft drinks, and preserves. The State of São Paulo has a cultivation area of 8,022 ha and a yield of 147,359 tons, which is surpassed only by Rio Grande do Sul and Pernambuco (IBGE, 2021; IEA, 2019). São Paulo's production of table grapes is particularly notable, and it is considered to be the largest Brazilian producer of the cultivar 'Niagara Rosada' (MELLO; MACHADO, 2020).

However, the occurrence of fungal diseases can cause serious damage to grapevines and directly affect productivity, fruit aesthetics, and the quality of the industrialized product (PEARSON; GOHEEN, 1988; REYNIER, 2005; WILCOX et al., 2015). Among the most frequent diseases in São Paulo, leaf rust and gray mold on fruits are the most noteworthy.

The rust-causing fungus *Phakopsora euvitis* Y. Ono, belongs to the Order Pucciniales (INDEX FUNGORUM, 2021), and the first report in Brazil was from Jandaia do Sul in the State of Paraná in March 2001. In 2003, its presence was verified in the State of São Paulo in the municipalities of Indaiatuba, Itupeva, and Louveira (PAPA et al., 2003; TESSMANN et al., 2004). The disease is characterized by the occurrence of yellow, circular uredinial pustules that sporulate on the underside of the leaves (GARRIDO; GAVA, 2014). In the region corresponding to the pustules on the upper surface, necrotic lesions are formed that coalesce and may cover large extensions of the leaf blade, causing dryness and the affected leaves to fall (LEU, 1988; TESSMAN et al., 2004). According to ANGELOTTI et al. (2008), the degree of infection does not vary significantly between young and mature leaves. Severe attacks cause early defoliation in plants and impair the ripening and quality of fruits, leading to production losses, reduced accumulation of reserves, and compromised plant vigor for the next cycle (SÔNEGO et al., 2005; AMORIM et al., 2016). In the advanced stages of the disease, dark brown telia form close to the uredia (TESSMAN et al., 2004). Infected grape leaves are a source of spore dispersion. This can result in repeated cycles of infection in grapevines. Research has revealed that the optimal temperature for the germination of urediniospores is 24 °C, with minimum and maximum temperatures of 8 °C and 32 °C, respectively, which coupled with high humidity, may give rise to an epidemic. In tropical and subtropical climates, the fungus persists only in the uredinial state without requiring an alternative host and can survive in unfavorable conditions in dormant buds (LEU, 1988; AMORIM et al., 2016). In colder regions, the fungus usually occurs at the end of the crop cycle. In a study conducted by ANGELOTTI et al. (2008) to evaluate the genetic resistance of 15 commercial grapevine genotypes, including rootstocks and canopies, to rust through artificial inoculations, 'Niagara Rosada' was found to be the most susceptible, presenting the highest number of pustules per cm^2, the highest pustule diameter (µm), and the highest number of urediniospores per pustule.

Gray mold is a cosmopolitan disease caused by the fungus *Botrytis cinerea* Pers.: Fr. (*Botryotinia fuckeliana* (de Bary) Whetzel). The disease can affect the production and quality of wines and table grapes (LATORRE et al., 2015). In addition to grapevines, *B. cinerea* can cause damage to several other economically important vegetables, fruits, and ornamental species in open field crops and, especially, in protected cultivation (TÖFOLI et al., 2011; GARRIDO; GAVA, 2014). In early spring, new buds and shoots can become infected, turn brown, and dry out. In late spring and before flowering, necrotic and irregular can areas appear on the margins of the few existing leaves of the grapevine (BULIT; DUBOS, 1988). When the peduncles are affected, sap flow is partially interrupted, thus altering the nutrition of the berries in the clusters. The fruits have circular, clear spots, and the skin can be easily detached and expose the grape pulp. Subsequently, the fruit is covered with gray spore masses and starts to rot (BULIT; DUBOS, 1988; AMORIM et al., 2016). The berries can become infected after exposure to the fungal spores at the optimum temperature (15ºC–20ºC) and high relative humidity for a period of at least 16 hours.

The combination of cultivation practices and chemical control has been fundamental for the effective management of rust and gray mold in grapevines. The most notable recommended cultivation practices include, not planting in areas subjected high moisture content, adopting proper rootstocks, careful nitrogen fertilization to prevent excessive plant growth, adequate pruning to promote aeration, penetration of sunlight into the foliage, and proper use of registered fungicides (NOGUEIRA et al., 2017).

Susceptibility of most planted cultivars, such as Italia, Isabel, and Niagara to fungal diseases, along with the occurrence of unfavorable climatic conditions and inappropriate crop management often allow grapevines to grow only through the application of fungicides, which increases the risk of fungicide resistance, food contamination, and environmental damage (NAVES et al., 2006). Currently, there are several active ingredients registered in the country for the control of gray mold, including thiophanate-methyl, captan, iprodione, procymidone, pyrimethanil, cyprodinil, mancozeb, chlorothalonil, and isofetamid. For the treatment of rust, only tebuconazole and metiram mixed with pyraclostrobin is available (AGROFIT, 2021).

In light of the negative impact of rust and gray mold on grapevines, this study aimed to evaluate the effectiveness of new fungicides and biological products in controlling both diseases under commercial crop conditions.

The experiment was conducted between February and April 2017 in a commercial vineyard belonging to Mr. Lucas Strabello in the municipality of Louveira, São Paulo (23°05’17.0” S, 46°56’09.6” W). The cultivar used was ‘Niagara Rosada’ because it is highly susceptible to rust and gray mold and trained it in the espalier system.

We evaluated the following treatments: (% a.i.; c.p./ha) dimoxystrobin+boscalid (20+20, 1 L), boscalid+pyraclostrobin (25.2+12.8; 1 L), boscalid+kresoxim-methyl (20+10; 0.5 L), pyrimethanil (75.7, 1.5 L), fluxapyroxad+pyraclostrobin (16.7+33.3; 0.5 L and 1.0 L, respectively), fluxapyroxad+pyraclostrobin (16.7+33.3; 0.5 L)+*Bacillus amyloliquefaciens* strain MBI600 (5.5 x 10^{10} viable spores/g, 0.5 kg), *Bacillus amyloliquefaciens* strain MBI600 (5.5 x 10^{10} viable spores/g, 0.5 kg), and *Melaleuca alternifolia* (22.25; 1.5 L). We applied each treatment nine times at weekly intervals. We used a pressurized backpack sprayer (CO_2) equipped with a 30 cm bar and three 110/02 nozzles spaced 10 cm apart to spray the plants at a distance of 50 cm with a pressure of 3 bar. Thus, we obtained a volume of 400 to 1000 liters of spray per hectare.

The experiment was conducted using a randomized block design, with ten treatments and four replicates and each plot comprising five plants.

The evaluation criterion for rust was severity based on the percentage of the affected leaf area (0% to 100%). These results were utilized to calculate the area under the disease progression curve (AUDPC) according to CAMPBELL & MADDEN (1990). We performed three evaluations at seven-day intervals. For gray mold, we noted the incidence of the disease seven days after the last spraying. We counted the infected clusters and then calculated the percentage of clusters affected by the disease. For statistical analysis, we used Duncan’s test at a 5% significance level.

There were considerable levels of rust and gray mold in the control plots, which allowed for a clear demonstration of the effects of the treatments on the experimental plots. All treatments significantly reduced the severity of rust and the incidence of gray mold in comparison to the control plots (Table 1; Figs. 1 and 2). We observed the best levels of control for rust using boscalid+pyraclostrobin (1 L of c.p./ha) treatment, whose performance was superior to that of fluxapyroxad+pyraclostrobin (0.5 L) + *B. amyloliquefaciens* (0.5 kg), *B. amyloliquefaciens* (0.5 kg), *M. alternifolia* essential oil (1.5 L), and dimoxystrobin+boscalid (1 L) but not significantly different from the other treatments (Table 1).

Boscalid+pyraclostrobin (1 L c.p./ha) and boscalid+kresoxim-methyl (1.5 L c.p./ha) provided the best residual effects by maintaining rust severity levels below 10%. By contrast, the severity was 70% in the control plots. The treatments based on fluxapyroxad+pyraclostrobin (1.0 L and 1.5 L c.p./ha, respectively) and *M. alternifolia* essential oil (1.5

L c.p./ha) exhibited an intermediate effect, while *B. amyloliquefaciens* (0.5 kg c.p./ha), fluxapyroxad+pyraclostrobin + *B. amyloliquefaciens* (0.5 L c.p./ ha + 0.5 kg c.p./ha, respectively), and dimoxystrobin+boscalid (1L c.p./ha) were the least effective (Fig. 1).

Fluxapyroxad+pyraclostrobin (0.5 L) + *B. amyloliquefaciens* (0.5 kg) was the most effective in controlling gray mold. Its performance was superior to that of dimoxystrobin+boscalid (1 L), boscalid+kresoxim-methyl (0.5 L), and pyremethanil (1.5 L) and similar to that of other treatments (Table 1).

Mixtures of carboxamide (boscalid and fluxapyroxad) and strobilurin (dimoxystrobin, pyraclostrobin, and kresoxim-methyl) were most effective in controlling rust and gray mold, possibly due to the joint action of these fungicides on two specific sites of the fungal respiratory system, which blocks energy production in the fungal cells. Carboxamides act on Complex II by inhibiting the enzyme succinate dehydrogenase, while strobilurins act on Complex III by inhibiting electron transport in mitochondria and preventing the formation of ATP. Moreover, the simultaneous effect of the two compounds on the same physiological process reduces the probability resistance against the treatment, and therefore, it is highly recommended for rotational use. The collapse in energy production promoted by the products is directly reflected in spore germination, germ tube elongation, appressorium formation, mycelial growth, and sporulation. Therefore, the products directly regulate the infection process and affect the reproduction of phytopathogenic fungi (TÖFOLI et al., 2016).

Additionally, a mixture of strobilurins and carboxamides result in products having considerable mobility in the plant (mesostemic and translaminar action), thus enabling their rapid absorption and movement in the treated tissue and resulting in high levels of protective, curative, antisporulant, and residual action as well as resistance to rain (TÖFOLI et al., 2012, TÖFOLI et al., 2014). These characteristics are very important given the epidemiological aspects of rust and gray mold. In tropical and subtropical conditions, the fundamental characteristic of *P. euvitis* is the ability to survive on the vine as urediniospores (NARUZAWA et al., 2006) and penetrate the leaves through the stomata after germination (LEU, 1988). According to NARUZAWA et al. (2006), a greater number of stomata on the bottom surface of the grape leaf could induce greater germination of urediniospores. In the case of *B. cinerea*, the spores produced in spring can infect leaves and new clusters of flowers and may remain dormant until the beginning of fruit maturation (SÔNEGO et al., 2005). The products obtained by mixing strobilurins and carboxamides are able to protect plants from these infections, even if the particular areas have not been sprayed with adequate amounts of the fungicides, because of their aforementioned characteristics.

Treatment using pyrimethanil (1.5 L) reduced the severity of rust and the incidence of gray mold in grapevines, and its performance was superior to that of the control treatment. Pyrimethanil belongs to the anilinopyrimidine group, and it has a different mode of action compared to carboxymides and strobilurins. It acts by inhibiting the secretion of proteins and enzymes related to pathogenesis (GISI; MÜLLER, 2007). The product is used worldwide to control gray mold in grapes; however, there have been cases of reduced sensitivity to pyremethanil (ROSSLENBROICH; STUEBLER, 2000; LATORRE et al., 2002; LATORRE; TORRES, 2012).

The results obtained for *M. alternifolia* essential oil treatment corroborate the results of FIALHO et al. (2015), who detected a significant reduction of rust in grapevine plants in the field. with scores below 1.0 in the treated plots and an average severity of 2.3 in the control plots. In *in vitro* experiments, treatment with 1% *M. alternifolia* essential oil resulted in the inhibition of urediniospore germination by approximately 80% (HOMEYER et al., 2015). Even though it is made up of more than 100 different chemical substances, the antimicrobial activity of *M. alternifolia* essential oil is attributed to monoterpene terpinen-4-ol, which accounts for approximately 40% of the total chemical composition (BUSTOS-SEGURA et al., 2015).

Several studies have demonstrated the inhibitory capacity of *B. amyloliquefaciens* on *B. cinerea*. Under field conditions, ALMANÇA et al. (2013) conducted two experiments on the cultivars Chardonnay and Tempranillo trained in the espalier system and obtained positive results. In the experiment on the cultivar Chardonnay, the results after treatment using *B. amyloliquefaciens* did not differ significantly from the control treatment, which included fungicides thiophanate-methyl, mancozeb, and pyrimethanil. In the area with the cultivar Tempranillo, there was greater incidence of gray mold due to the occurrence of hail. However, lower values of both incidence and severity were verified in plants treated using *B. amyloliquefaciens* than in plants treated with the fungicides. In addition to demonstrating the direct action *B. amyloliquefaciens* on *B. cinerea,* studies conducted by ZHOU et al. (2020) showed that *B. amyloliquefaciens* also has the ability to activate the plant's immune system, which could further reduce the severity of gray mold.

The high level of control against gray mold observed after fluxapyroxad+pyraclostrobin (0.5 L) + *B. amyloliquefaciens* (0.5 kg) treatment offers a new perspective for integrated and sustainable management of the disease.

The present results suggest that *M. alternifolia* essential oil and the biological product *B. amyloliquefaciens* could be a vital tool for managing rust and gray mold in grapevines. Moreover, the integration of different mechanisms of action presented by the products can serve as an important approach to prevent the occurrence of resistant races of the pathogens evaluated herein.

Table 1 – Effects of fungicides and biological products on the severity and area under the disease progression curve (AACPD) for leaf rust and the severity of gray mold on fruits of grapevines (Vitis spp. 'Niagara Rosada'), trained in espalier systems, Louveira, SP, 2017.

Treatments and doses (kg or L c.p./ha)	Rust		Gray Mold
	Severity (%)*	AACPD	Severity (%)*
Control	70.00 a	779.00 a	29.25 a
Dimoxystrobin+boscalid (1 L)	18.00 bc	211.25 bc	7.25 c
Boscalid+pyraclostrobin (1 L)	8.00 d	74.00 c	4.25 cde
Boscalid+kresoxim-methyl (0.5 L)	8.25 cd	86.75 bc	10.75 b
Pyrimethanil (1.5 L)	14.00 bcd	175.50 bc	4.75 cde
Fluxapyroxad+pyraclostrobin (0.5 L)	16.50 bcd	166.25 bc	4.50 cde
Fluxapyroxad+pyraclostrobin (1.0 L)	13.25 bcd	123.25 bc	2.25 de
Fluxapyroxad+pyraclostrobin (0.5 L)+*B. amyloliquefaciens* (0.5 kg)	18.75 bc	224.75 bc	1.75 e
B. amyloliquefaciens (0.5 kg)	24.25 b	268.75 b	4.75 cde
M. alternifolia essential oil (1.5 L)	19.75 b	164.00 bc	3.00 de
CV	20.92	18.84	20.56

*Original averages. For statistical analysis, the data were previously converted to arcsin sqrt (x/100). Averages followed by the same letter do not diverge from Duncan's test at a 5% significance level.

Figure 1 - Rust progression curves for grape leaves (Vitis spp. 'Niagara Rosada') treated with different fungicides and biological products. Louveira, SP, 2017. Severity: 0% to 100%.

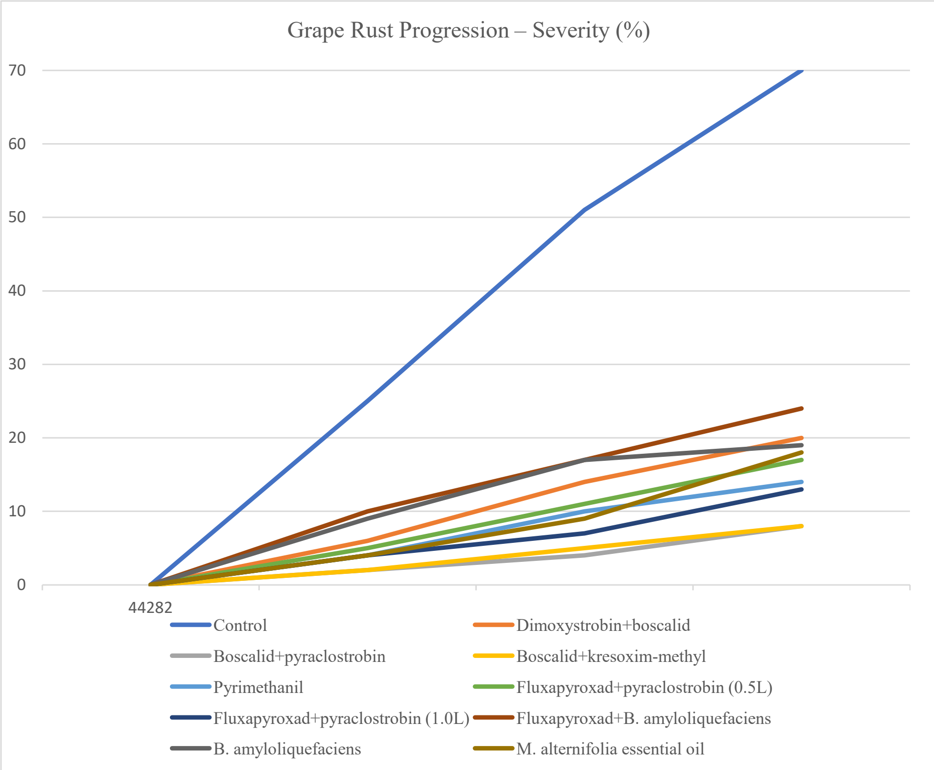
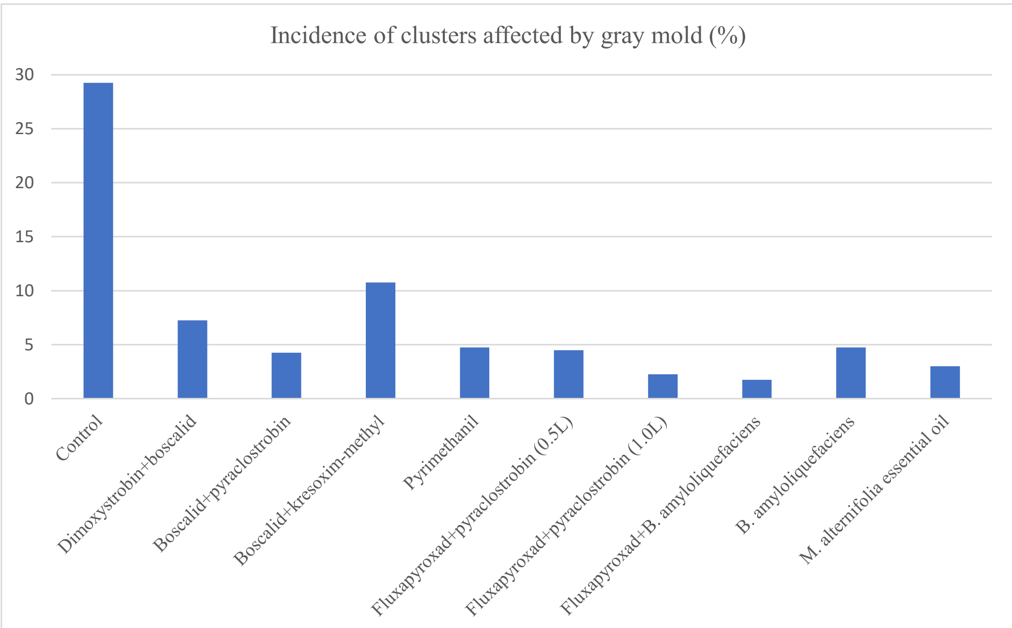


Figure 2 - Effect of fungicides and biological products on the percentage of clusters on grapevines (Vitis spp. 'Niagara Rosada'), affected by gray mold. Louveira, 2017.



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