academicJournals

Vol. 9(40), pp. 3001-3010, 2 October, 2014
DOI: 10.5897/AJAR2014.8609
Article Number: 2DB518047767
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of culture filtrate of *Curvularia inaequalis* on disease control and productivity of grape cv. Isabel

Cristiane Mendes Da Silva[1]*, Katia Regina Freitas Schwan-Estrada[1], Cacilda Marcia Faria Duarte Rios[2], Bruno Neves Batista[1] and Sergio Florentino Pascholati[3]

[1]Universidade Estadual de Maringá – Brazil.
[2]Universidade Estadual do Centro Oeste – Brazil.
[3]Universidade de São Paulo – Brazil.

Received 13 February, 2014; Accepted 9 September, 2014

This study aimed to evaluate the effect of a culture filtrate of the saprophytic fungus *Curvularia inaequalis* on the control of the foliar diseases downy mildew and isariopsis leaf spot of grapevine and on the incidence and severity of grape fruit mildew under field conditions. This study also analyzed the physical and physicochemical characteristics of fruits that were treated preharvest during standard season production and off season production. Concentrations of 0 (absolute control), 1, 5, 10 and 20 ml L^{-1} culture filtrate were tested. The standard treatments that were used as controls were bordeaux mixture, mancozeb, acibenzolar-S-methyl and *Agaricus blazei*. In the case of downy mildew, the concentrations reached a control of approximately 56% compared to that of the absolute control treatment during both seasons. The reduction of the isariopsis leaf spot was approximately 54 and 42% in the standard season and off season, respectively. The severity of fruit mildew was controlled in 62% of the cases, but the incidence was controlled in approximately 11% of the cases. Postharvest, the pH and relationship between solids and acidity (TSS/TTA) of the fruit pulp remained in the ideal range, the fruits showed low juice yield and high total titratable acidity, and the bunch weight, number of bunches and yield per plant were higher in the off season than in standard season and were affected by weather conditions and the pruning season.

Key words: *Vitis labrusca*, biological control, agro-ecology, organic production.

INTRODUCTION

In Southern Brazil, as in other worldwide grape-producing regions, downy mildew caused by *Plasmopara viticola* (Berk and Kurt) Berlese and de Toni is a major disease in grapevines (Dalbó and Schuck, 2003), affecting all of the green plant organs, mainly leaves, inflorescences and young fruits. However, under climatic conditions of high rainfall and temperatures that are favorable to fungal growth, *Isariopsis clavispora* (Berkeley and Curtis) Saccardo, the etiologic agent of isariopsis leaf spot, becomes highly dangerous to vineyards due to the premature defoliation of plants which can cause branch aging and weakening in the following season (Lenz et al., 2009).

Disease management in vineyards still constitutes a major problem for viticulture and when not controlled seriously can be responsible for significant losses in

*Corresponding author. E-mail: crismendes86@hotmail.com

production both in the quantity and quality of berries and their products (Özer et al., 2012; Pinto et al, 2013). According to Chavarria et al. (2007), the grape producer sprays chemicals weekly to guard the harvest, applying the fungicides cymoxanil, azoxystrobin and mancozeb, among other synthetic chemicals that are used in controlling mildew. To control isariopsis leaf spot, the recommended treatments for grape downy mildew and anthracnose are usually enough (Amorim and Kuniyuki, 2005).

When considering the economic and environmental costs of such applications as well as the increasing restrictions that are related to the presence of toxic residues on the fruits, the search for new alternatives is necessary. Among these alternatives, biological control may be an important and justifiable technical alternative, mainly for wine-growers in an agroecological production system, which has few options at a commercial level for controlling these diseases. Therefore, saprobe fungi currently receive special attention as controlling agents as well as potential resistance inducers.

Similarly, the fungus *Curvularia inaequalis* (Shear) belongs to the group of Hyphomycetes dematiaceous fungi and is pathogenic to plants and/or is saprophytic. *C. inaequalis* produces chloroperoxidase, a vanadium enzyme that has similar bromoperoxidase properties and forms hypochlorous acid (HOCl), a strong oxidizing agent and bactericide (Van Schijndel et al., 1993; Hemrika et al., 1999). Hansen et al. (2003) found that the haloperoxidase system of *Curvularia* rapidly acts as an antimicrobial agent against a broad spectrum of bacteria (*Pseudomonas* spp., *Escherichia coli*, *Serratia marcescens*, *Aeromonas salmonicida*, *Shewanella putrefaciens*, *Staphylococcus epidermidis* and *Listeria monocytogenes*), yeasts (*Candida* sp. and *Rhodotorula* sp.) and filamentous fungi (*Aspergillus niger*, *Aspergillus tubigensis*, *Aspergillus versicolor*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Penicillium paxilli*) when cultivated in suspension.

This study aimed to determine the antagonistic potential of the saprophytic fungus *C. inaequalis* which was obtained from the litter of the semi-arid region of the northeastern Brazil in relation to the severity of grape foliar diseases (downy mildew and isariopsis leaf spot) and the incidence and severity of downy mildew in grape berries cv. Isabel under field conditions and to analyze the physical and physicochemical characteristics of fruits that were treated pre-harvest during the standard season and off season using culture filtrates of the saprophytic fungus.

MATERIALS AND METHODS

Obtaining the culture filtrate of the saprophytic fungus *C. inaequalis* from the Brazilian semiarid region

Discs of the saprophytic fungus *C. inaequalis* (CUI) mycelium were transferred to an Erlenmeyer flask containing liquid culture medium potato-dextrose (PD) in the proportion of five mycelial discs for 1000 ml liquid medium. The cultivation was carried out at 25±1°C for seven days in a 12 h photoperiod. After this period, the suspension was filtered through a thin fabric to obtain the culture filtrate.

Field experiment

The experiment was conducted during two consecutive seasons from July to December 2011 (standard season) and from February to June 2012 (late season) in a commercial vineyard with cv. Isabel in Marialva County, Paraná State, Brazil. The county's geographical coordinates are latitude 23°29'8"S, longitude 51°47'34"W and altitude 644 m.a.s.l. (Iapar, 2000). Four-year-old plants that were grafted on Paulsen 1103 rootstock were spaced in 2.5 m x 2.0 m arrays (plant density approximately 5,000 plants ha^{-1}) and trained in a trellis system.

The applied treatments were 0, 1, 5, 10 and 20 ml L^{-1} (0, 1, 5, 10 and 20%, respectively) of the culture filtrate CUI. The zero concentration (0 ml L^{-1} filtrated CUI) consisted of an absolute control without treatment. Furthermore, the standard treatments that were used as controls were bordeaux mixture in a proportion of 1:1:100 (copper sulfate: quicklime: water, v:v:v), mancozeb at 2.5 g p.c. L^{-1} (Manzate® 800, Dow AgroSciences Industrial Ltda.), acibenzolar-S-methyl at 0.05 g p.c. L^{-1} (Bion®, Syngenta Proteção de Cultivos Ltda.) and *Agaricus blazei* (1 g of dry powder per 14 ml water).

Fungicides were sprayed with hand sprayer weekly at approximately 7:00 a.m. under the proper environmental conditions aiming for a high-quality application (Guarany®, adjustable conic nozzle) using three liters of the solution per treatment. The fungicides were applied until the point of runoff from the onset of sprouting on September 1, 2011 for the first season and on February 14, 2012 for the second season for a total of 10 and 8 applications per crop season, respectively. The surrounding plants were treated weekly with mancozeb.

At the appearance of the first symptoms of downy mildew, the disease severity was evaluated at three leaves in the apex of four branches per plant that was previously identified using the diagrammatic scale that is described by Azevedo (1997). The severity assessment of the isariopsis leaf spot began at the end of each crop season by examining the six leaves in the basal area of the plants that were previously identified through the diagrammatic scale that is described by Lenz et al. (2009).

Data on the severity were determined through the area under the disease progress curve (AUDPC) according to Shaner and Finney (1977). Five evaluations for downy mildew and four evaluations for isariopsis leaf spot were respectively conducted at intervals of seven days during both the first and second seasons.

At the end of the experiment during the first season, the symptoms of downy mildew on grape fruits were identified and the incidence and severity of that disease were evaluated. In the following season, downy mildew was not present on the fruits.

The experimental design was a randomized block pattern of nine treatments and five replicates with one plant per plot during the two evaluation periods. The results were submitted to a variance analysis, to a polynomial regression analysis and to a mean comparison by the Tukey test at 5% probability using the statistical program SISVAR (Sisvar 5.1 Build 72, UFLA) (Ferreira, 2011).

Physical and physicochemical determinations

At the end of the field experiment during each season, the number of grape bunches per plant were counted and then harvested for physical and physicochemical analyses of the grape fruits that were produced after each treatment. For these analyses, each treatment

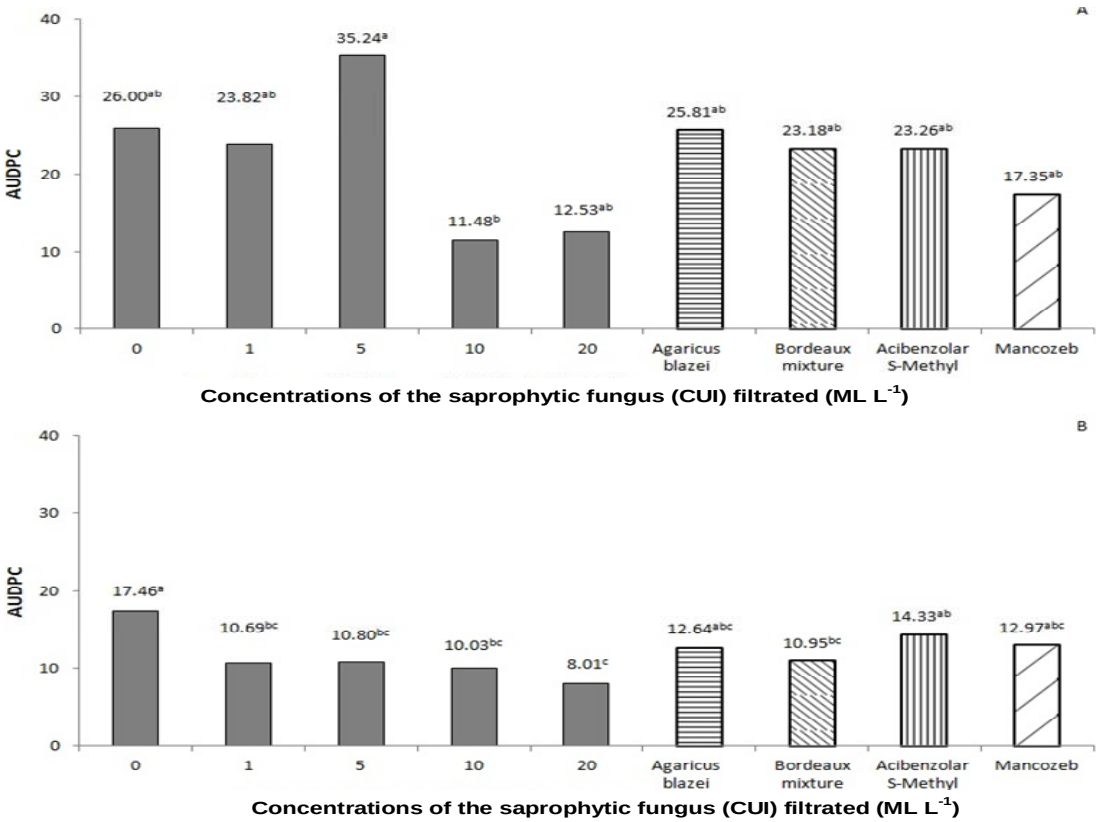

Figure 1. Effect of concentrations of the saprophytic fungus (Curvularia inaequalis) filtrated, in the area under the disease progress curve (AUDPC), on downy mildew in grape cv. 'Isabel', in the first (A) and second year of study (B) under field conditions. [1] Averages followed by different letters differ by the Tukey test (p>0.05). * Significant at 5% probability by test F. The data on severity were transformed through X root for performing the statistical analysis.

consisted of five replicates with three to four bunches of grapes per plot during both of the evaluation periods. The plants were harvested when the grapes presented on average 14 ºBrix.

The first analyses were as follows: a) cluster weight (g) determined using a digital analytical balance; after the extraction of the grape juice, the samples were homogenized and subjected to the following analysis; b) total soluble solids (TSS, °Brix) determined using a portable refractometer (Instituto Adolfo Lutz, 1985); c) total titratable acidity (TTA, %) determined using a titration meter with a 0.1 N NaOH solution; the values are expressed in grams of tartaric acid per 100 ml wort (Instituto Adolfo Lutz, 1985); d) relationship between solids and acidity determined using the ratio TSS/TTA; e) pH determined in using a potentiometer according to the norms that were established by Instituto Adolfo Lutz (1985); f) productivity per plant (g); and g) juice yield (%).

The results were submitted to a variance analysis to a polynomial regression analysis and to a mean comparison by the Tukey test at 5% probability using the statistical program SISVAR (Sisvar 5.1 Build 72, UFLA) (Ferreira, 2011).

RESULTS

Field experiment

In this study, concentrations of 10 and 20 ml L^{-1} of the culture filtrate CUI in the first cycle were statistically similar to the standard treatments and reduced the severity of downy mildew by 55.84 and 51.80%, respectively compared to the absolute control treatment. The standard treatments mancozeb, bordeaux mixture and acibenzolar-S-methyl decreased the disease in 33.26, 10.82 and 10.54% of the cases, respectively when compared to the absolute control treatment.

During this season, there was a downtrend AUDPC treatment 1 ml L^{-1} filtrate CUI with an increase of the disease in relation to the 5 ml L^{-1} filtrate treatment followed by stabilization by the 10 and 20 ml L^{-1} filtrate treatment. This pattern led to a low R^2 for the linear regression and non-significant R^2 for the quadratic, requiring further study (Figure 1A). The regression analysis of the second cycle was similar to that of the 2011 harvest, although the results of the AUDPC were more stable (Figure 1B).

In the second year of the study, there were similar reductions in the severity of downy mildew. The highest concentration (20 ml L^{-1}) of the filtrated CUI showed a control of 54.13% when compared to the absolute control treatment. The concentration of 20 ml L^{-1} filtrate was also not significantly different from the other concentrations (1, 5 and 10 ml L^{-1}) or from the bordeaux mixture, *Agaricus*

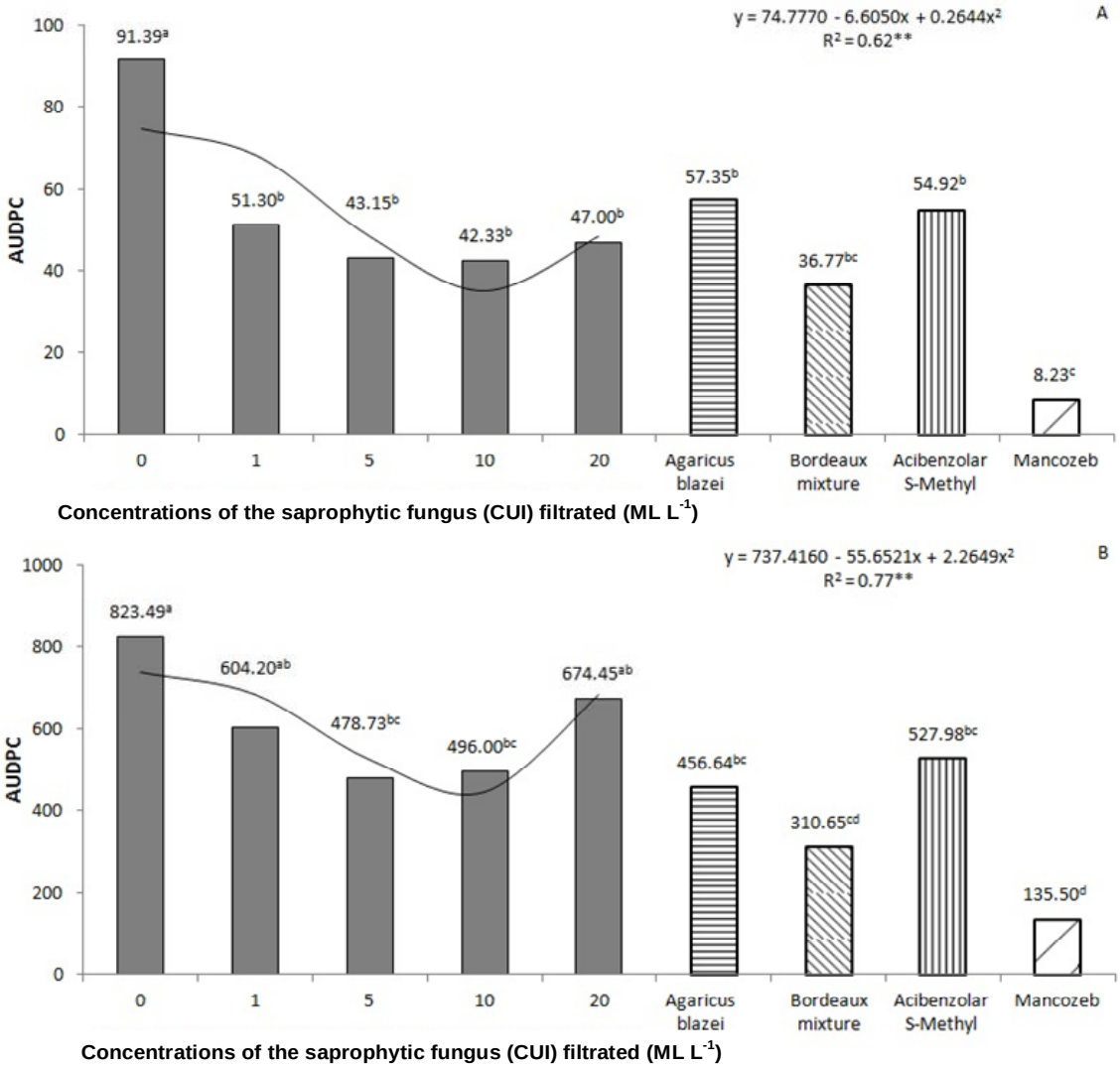

Figure 2. Effect of concentrations of the saprophytic fungus (*Curvularia inaequalis*) strain, in the area under disease progress curve (AUDPC), in isariopsis leaf spot of grapevine cv. 'Isabel', in the first (A) and second years of study (B), under field conditions. [1]Averages followed by different letters differ by the Tukey test ($p>0.05$). ** Significant at 1% probability by test F.

blazei and mancozeb which presented smaller reductions in the disease, decreasing the downy mildew severity by 38.76, 38.17, 42.53, 37.28, 27.61 and 25.72%, respectively, compared to the absolute control treatment (Figure 1B).

Regarding the AUDPC of isariopsis leaf spot in both the first and the second cycles, the concentrations of 1, 5, 10 and 20 ml L^{-1} did not differ significantly from the standard treatments with the bordeaux mixture, *A. blazei* and acibenzolar-S-methyl. In the first cycle, the reductions were 43.87, 52.78, 53.68, 48.57, 59.76, 37.24 and 39.90%, respectively, compared to the absolute control treatment (Figure 2A). Similar to the previous year, the reduction of the severity of isariopsis leaf spot in the second cycle ranged from 18 to 62% (Figure 2B). In both years, studying the AUDPC of this disease, there was a quadratic effect in function to the concentrations of filtrated CUI utilized.

For the incidence of downy mildew on grape bunches, the concentrations of 10 and 20 ml L^{-1} filtrate CUI were statistically similar to the standard treatments of *A. blazei*, mancozeb and bordeaux mixture. The reductions in the incidence rates were 9.89 and 11.14% for 10 and 20 ml L^{-1} filtrate, respectively, and 6.21, 34.75 and 59.07% for the standard treatments, respectively, compared to the absolute control treatment (Table 1).

However, regarding the mildew severity on grape bunches, the reductions ranged from 8 to 86%. The concentrations of 1 and 10 ml L^{-1} filtrate CUI did not differ statistically from the standard treatments. Furthermore, the 5 and 20 ml L^{-1} filtrate concentrations were statistically equivalent to the 10 ml L^{-1} concentration

Table 1. Incidence and severity index of downy mildew on grape bunches treated with saprophytic fungus strain Curvularia inaequalis (CUI). Marialva County, Paraná State, Brazil, 2011 harvest.

Treatments (ml L^{-1})	Incidence of Disease (%)	Severity Index
0	24.01^{bc}	2.52^{a}
1	27.91^{ab}	0.96^{cd}
5	43.36^{a}	2.30^{ab}
10	21.63^{bc}	1.10^{bcd}
20	21.33^{bc}	1.75^{abc}
Agaricus blazei	22.52^{bc}	0.98^{cd}
Bordeaux mixture	9.83^{c}	0.35^{d}
Acibenzolar S-Methyl	24.91^{bc}	1.31^{abcd}
Mancozeb	15.67^{bc}	0.70^{cd}
CV (%)	33.92	46.75

*Averages followed by the same letter in columns do not differ significantly by Tukey test at 5% probability. The severity index data were processed by the root of x to perform statistical analysis.

and to the standard treatment acibenzolar-S-methyl, despite the 5 ml L^{-1} concentration exhibiting the lowest culture filtrated performance. In relation to the absolute control treatment, the concentrations of 1, 5, 10 and 20 ml L^{-1} filtrate decreased the severity of downy mildew on grape bunches by 61.85, 8.63, 56.32 and 30.23%, respectively. The standard treatments acibenzolar-S-methyl, *A. blazei*, bordeaux mixture and mancozeb decreased the severity by 47.93, 61.14, 72.16 and 86%, respectively, compared to the absolute control treatment (Table 1).

Physical and physicochemical determinations

When analyzing the effect of the concentrations of the CUI culture filtrate on the physical and physicochemical characteristics of grape fruits, in general, the juice pH did not vary greatly between the two years ranging from 2.92 to 3.23. In the first cycle, the pH ranged from 3.07 to 3.23 (Table 2) and in the second cycle from 3.02 and 3.18 (Table 3).

In the first cycle, the values for total soluble solids (TSS) reached the minimum value established for a grape juice with identity and quality which is 14.00 °Brix except for the standard treatments acibenzolar-S-methyl (12.50 °Brix), *A. blazei* (12.66 °Brix) and bordeaux mixture (13.55 °Brix) (Table 2). However in the second cycle, the TSS values for all of the treatments were inferior to the established minimum, except for the treatment of mancozeb which showed 15.52 °Brix (Table 3). In relation to the total titratable acidity (TTA) in the first cycle, there was no significant difference between the treatments (Table 2) ranging from 0.90 to 1.37% tartaric acid. In the following year, there was also no significant difference between the treatments (Table 3).

The TSS/TTA ratio is indicative of the grape juice quality, that is, the degree of sweetness, and the limiting values that were established by legislation are between 15 and 45 (Miguel et al., 2009; Pinheiro et al., 2009). However, in the first cycle, the average values for this ratio ranged from 10.06 to 20.25, which were transformed by log (x) to perform statistical analyses. There was no significant difference between the treatments in the first cycle (Table 2). In the next cycle, the TSS/TTA ratio ranged from 16.66 to 27.05, which is consistent with the values that are required for quality standards. The concentration of 5 ml L^{-1} CUI filtrate did not differ statistically from the standard treatments of mancozeb, bordeaux mixture and *A. blazei*, which showed the highest TSS/TTA values (Table 3).

Regarding the juice yield in the first cycle, there was a significant difference between the treatments and the bordeaux mixture had the highest yield (44.39%), followed by acibenzolar-S-methyl, mancozeb, and 0, 1 and 10 ml L^{-1} filtrated (Table 2). In the following year, the juice yield ranged from 8.59 to 19.22, and there was no significant difference between the treatments in this cycle (Table 3).

In the two cycles, there was no significant difference between the treatments for the variable bunch weight. In the first cycle, the mean values varied from 46.75 (*Agaricus blazei*) to 89.74 g (mancozeb) (Table 2), and in the next cycle from 175.92 (acibenzolar-S-methyl) to 268.27 g (20 ml L^{-1} culture filtrate) (Table 3).

For the variable number of bunches per plant, it was noted that concentrations of 0, 5 and 20 ml L^{-1} filtrate CUI in addition to the standard treatment mancozeb produced the highest results ranging from 26.20 to 41.80 bunches per plant. The other treatment ranged from 12 to 18 bunches per plant (Table 2). In the second cycle, there was no significant difference between the treatment

Table 2. Average values of pH, total soluble solids (TSS), total titratable acidity (TTA), ratio (TSS/TTA), juice yield, bunch weight, bunches per plant and plant yield for grapevine cv. 'Isabel' treated with the strain from saprophytic fungus Curvularia inaequalis (CUI), in Marialva County, Paraná State, Brazil, 2011 harvest.

Treatment (ml L^{-1})	pH	TSS (°Brix)	TTA (%)	TSS/TTA	Juice yield (%)	Bunch weight (g)	Bunches per plant	Yield per grapevine (kg)
0	3.09^{ab}	15.74^{abc}	1.29^{a}	1.10^{a}	35.66^{ab}	73.96^{a}	28.80^{abc}	2.14^{ab}
1	3.23^{a}	17.24^{a}	1.02^{a}	1.23^{a}	36.52^{ab}	48.80^{a}	18.40^{bcd}	0.90^{b}
5	3.07^{ab}	14.58^{abc}	1.03^{a}	1.20^{a}	26.09^{b}	79.77^{a}	31.80^{ab}	2.61^{ab}
10	3.10^{ab}	15.53^{abc}	1.08^{a}	1.17^{a}	34.67^{ab}	60.11^{a}	12.40^{d}	0.99^{b}
20	3.09^{ab}	14.90^{abc}	1.27^{a}	1.08^{a}	27.84^{b}	49.80^{a}	26.20^{abcd}	1.40^{b}
Agaricus blazei	2.73^{c}	12.66^{bc}	1.08^{a}	1.13^{a}	29.84^{ab}	46.75^{a}	13.00^{cd}	0.70^{b}
Bordeaux mixture	2.98^{abc}	13.55^{abc}	1.13^{a}	1.07^{a}	44.39^{a}	47.80^{a}	16.60^{bcd}	1.04^{b}
Acibenzolar S-Methyl	2.95^{bc}	12.50^{c}	1.37^{a}	0.97^{a}	39.99^{ab}	49.32^{a}	17.00^{bcd}	1.04^{b}
Mancozeb	3.21^{ab}	16.58^{ab}	0.90^{a}	1.27^{a}	38.11^{ab}	89.74^{a}	41.80^{a}	3.83^{a}
CV (%)	4.42	12.85	31.36	18.29	21.42	36.82	33.60	59.82

Averages followed by the same letter in columns do not differ significantly by the Tukey test at 5% probability. TSS/TA ration data were transformed to log (x) to perform statistical analysis.

Table 3. Average values of pH, total soluble solids (TSS), total titratable acidity (TTA), TSS/TA ratio, juice yield, bunch weight, bunches per plant and plant yield for grapevine cv. 'Isabel' treated strain from saprophytic fungus Curvularia inaequalis (CUI), in Marialva County, Paraná State, Brazil, 2012 harvest.

Treatment (ml L^{-1})	pH	TSS (°Brix)	TTA (%)	TSS/TTA	Juice yield (%)	Bunch weight (g)	Bunches per plant	Yield per grapevine (kg)
0	3.08^{ab}	12.74^{b}	1.30^{a}	17.62^{b}	0.31^{a}	208.84^{a}	25.80^{a}	1.46^{a}
1	3.04^{abc}	10.80^{b}	1.24^{a}	16.66^{b}	0.27^{a}	208.84^{a}	20.60^{a}	1.34^{a}
5	3.02^{bc}	11.96^{b}	1.34^{a}	21.45^{ab}	0.32^{a}	221.85^{a}	21.40^{a}	1.24^{a}
10	3.06^{abc}	12.00^{b}	1.26^{a}	19.25^{b}	0.31^{a}	223.18^{a}	35.20^{a}	2.37^{a}
20	3.18^{a}	12.66^{b}	1.24^{a}	19.55^{b}	0.35^{a}	268.27^{a}	23.40^{a}	1.61^{a}
Agaricus blazei	3.07^{abc}	11.96^{b}	1.34^{a}	21.54^{ab}	0.30^{a}	179.14^{a}	22.20^{a}	1.01^{a}
Bordeaux mixture	3.12^{ab}	13.30^{ab}	1.28^{a}	21.90^{ab}	0.33^{a}	240.29^{a}	26.50^{a}	1.56^{a}
Acibenzolar S-Methyl	3.06^{abc}	10.96^{b}	1.30^{a}	18.36^{b}	0.27^{a}	175.92^{a}	27.80^{a}	1.28^{a}
Mancozeb	2.93^{c}	15.52^{a}	1.32^{a}	27.05^{a}	0.31^{a}	229.83^{a}	21.40^{a}	1.14^{a}
CV (%)	2.38	10.63	7.08	15.64	15.72	38.59	57.29	68.52

Averages followed by the same letter in columns do not differ significantly by the Tukey test at 5% probability. The TTA data were transformed to $(1/\sqrt{x})$ to perform statistical analysis.

ranging from 20.6 to 35.20 bunches per plant (Table 3).

Tables 2 and 3 present the plant production for the concentrations of the filtrate CUI in the first and second crop cycles, respectively. In the first cycle, there were higher productivities for the treatments with mancozeb (3.83 kg), 0 ml L^{-1} culture filtrate (2.14 kg) and 5 ml L^{-1} of culture filtrate (2.61 kg) in which the last two treatments

did not differ from the other treatments. In the following season, there was no difference between the treatments, and the plant yield ranged from 1.01 (*A. blazei*) to 2.37 kg per plant (10 ml L^{-1} filtrate).

DISCUSSION

Since 1979, there have been reports on grapevine disease control using strains of antagonistic fungi that are supported by the results presented in this work. For example, Bogdanova et al. (1979) reported that *Fusarium gibbosum* strains that were isolated from grapevine leaves and fruits restrained the disease development when sprayed onto detached leaves before inoculation with *P. viticola* zoospores.

Falk et al. (1996) analyzed from 1992 to 1995, the downy mildew incidence and severity on bunches and leaves of grapevine cv. Chancellor and cv. Lakemont that were treated with a microconidial suspension of *Fusarium proliferatum* G6. The authors observed that for cv. Chancellor in 1992, the grapevines that were treated with the microconidial suspension of *F. proliferatum* G6 showed a 71% reduction in the downy mildew severity. This treatment was not significantly different from the treatment with mancozeb which is similar to the results presented in this work and the various concentrations of the filtrated CUI in both of the evaluated years did not differ from the standard treatments that exhibit control over this disease. However, in relation to the cv. Lakemont, the authors found that the reduction of the incidence of downy mildew in grapevine leaves that were treated with the antagonist suspension decreased from 68.7 to 37% (control 46%) in 1992 and from 40.8 to 20.8% (control 49%) in 1994. The disease severity was reduced by 79 and 67% in 1992 and 1994, respectively.

Previously, Musetti et al. (2006) isolated 126 endophytic fungi from grapevine leaves and tested their activities on *P. viticola* on leaf discs. Of these isolates, only five fungi that were identified as *Alternaria alternata* induced ultra-structural changes in the mycelium of *P. viticola* and restrained sporulation. The most likely hypothesis is that *A. alternata* produces toxic compounds similar to other antagonistic microorganisms.

The results from this study in the control of both downy mildew and isariopsis leaf spot can most likely be explained by the possible occurrence of chlorination as a result of antagonistic interactions between microorganisms through the chloroperoxidase system presented by antagonist microbial interactions in terrestrial ecosystems (Bengtson et al., 2009). In addition, the haloperoxidase system may be involved, such as that of *Curvularia* which has a broad spectrum of action and exerts its lethal effect against bacteria, yeasts and filamentous fungi. It is likely but has not been experimentally verified that the haloperoxidase of *Curvularia* oxidizes halogenates, such as bromide, chloride and iodide, which in the presence of hydrogen peroxide produce reactive oxygen species with antimicrobial properties (Wolfson and Sumner, 1993).

However, one cannot categorically support these explanations, although the *C. inaequalis* filtrate produced some substance(s) that operate in the partial control of the studied diseases. However, it is unknown whether this production is due to a direct action and/or induction of resistance in grapevines. In addition to most of the studies on this subject which are old, few studies were found in the available literature on these antagonistic microorganisms.

Nevertheless, before biological control by any other biocontrol agents can be practically implemented, it is essential to determine how this biological control may be affected by changing environmental conditions. Overall, it is important to learn as much as possible regarding the ecology of these biocontrol organisms and their interactions with the pathogen, host plant and soil and rhizosphere microbial communities and their surrounding environments. Many attempts at biological control have resulted in inconsistent or unsatisfactory disease control under varying environmental conditions and locations (Larkin and Fravel, 2002), as in the case of this study in which the results obtained in the control of isariopsis leaf spot were similar during both years of study; but the AUDPC values were approximately 10 times higher in the first crop season when compared to the second crop season.

This result is mainly due to the favorable conditions for disease development in the second year, making it an unusual year for controlling this disease with a large inoculum pressure of the etiologic agent and conditions of warm and humid weather (high temperatures with an average minimum temperature of 17°C and average maximum temperature of 28°C and a relative humidity of approximately 80.5% mainly in the last month of the experiment during which the disease was evaluated) (Figure 3). Furthermore, the experiment was conducted in the off season during the second year with rainy periods occurring between December and February. This pruning requires more frequent spraying of the vineyard to ensure good control of diseases during the cycle. Therefore, depending on the climate, farmers usually spray every two or three days, but in both experimental years, weekly sprays were performed which may have influenced the increased severity of the disease during the second year compared to the first.

According to Sharma et al. (2009), since 1984 several reports in the related literature have demonstrated the potential of microbial antagonists in the biological control of postharvest diseases of fruits and vegetables. In this sense, some microbial antagonists that have been used for the successful control of postharvest diseases of grapevine include *Aureobasidium pullulans* on grapevine gray mold (*Botrytis cinerea*) (Schena et al., 2003) and soft rot (*Monilinia laxa*) (Barkai-Golan, 2001), as well as

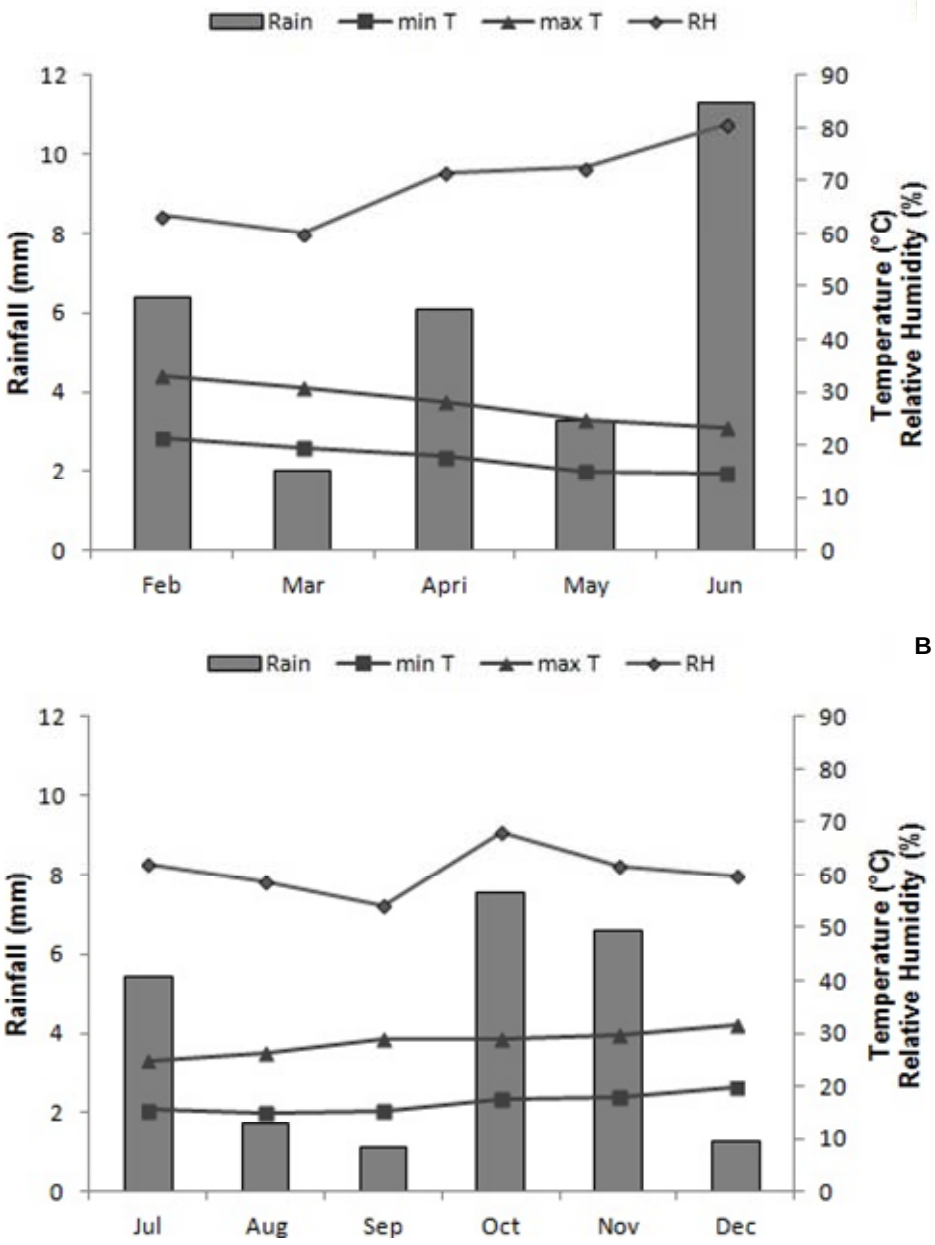

Figure 3. Monthly rainfall (mm), minimum and maximum monthly average temperature (°C) and monthly relative humidity prevailing in the vineyard studied, the periods from July to December 2011 harvest (A) and February to June 2012 harvest (B), in Marialva County, Paraná State, Brazil.

Metschnikowia fructicola (Karabulut and Baykal, 2003) and *Trichoderma harzianum* (Batta, 2007) both on *B. cinerea*.

Cañamás et al. (2011) studied the efficiency of different formulations of *Candida sake* CPA-1 in vineyards of cv. Cabernet Sauvignon on the incidence and severity of *Botrytis cinerea* (gray mold) and observed that in the first year of study, the treatments reduced the incidence of disease between 36 and 40% and that the severity in all of the treatments with *C. sake* was equivalent to the severity with conventional fungicide treatment. The following year, the treatments with *C. sake* cells without heat treatment and that were formulated in liquid solution combined with Fungicover® 5% and the conventional fungicide reduced the incidence of gray mold by up to 90% and the severity from 2.7% to 0.12 and 0.07%, respectively.

Regarding the physicochemical analyses according to Rizzon et al. (2004), the ideal pH range for a good quality grape juice is between 3.1 and 3.3 similar to the values

that were found in this study, although during the second year of study, the pH was more acidic than in the first year. In addition, the use of grapes with a TTA from 0.4 to 0.6%, lower than those values that were obtained in this study is recommended.

Pereira et al. (2008), in analyzing the grape juices of different cultivars, found a total titratable acidity of 0.8% tartaric acid for the cv. Isabel. The acidity in grapes comes from tartaric, malic and citric acids, and this characteristic is related to the edaphic and environmental conditions, especially near the ripening phase in addition to the cultivar and cultivation methods that are used during grape development (Santana et al., 2008) which can explain the high tartaric acid content in the grape juices of this experiment.

Gomes et al. (2011) found that grapevines cv. Isabel that were treated with fungicides showed the lowest proportions of pulp yield differing from the other alternative treatments. The maximum pulp yields that were obtained for each treatment were 72.31% EcoLife® 95 days after pruning (DAP); 69.39% EcoLife® + 71 potassium phosphite 102 DAP; 63.43%, potassium phosphite 102 DAP; 66.67% fungicides 95 DAP; 70.84% Rocksil® 95 DAP; and 81.31%, Agro-Mos® 95 DAP. The results of Gomes et al. (2011) were higher than those that were obtained in this work; however, Di Piero et al. (2005) explain that in some cases, the use of elicitors for the variable weight, bunch weight and berry diameter may prejudice the yield most likely due to the dose and the number of applications which require additional study. In addition, the allocation of plant resources for defense can generate greater energy expenditure.

In the studies of Sato et al. (2008), the average grape bunch weight (cv. Isabel) in northern Paraná State was 125.1 g, and Pereira et al. (2008) averaged 130.55 g, but Kishino et al. (2007) state that the grapevine Isabel produces an average bunch weight of approximately 200 g. According to Grangeiro et al. (2002), the climatic conditions, mainly temperature and luminosity at the time of blooming differentiation may be mainly responsible for the increase in the bunch mass. Furthermore, Neis et al. (2010) concluded that the average productivity of grapevines is higher when pruning is conducted in March and April; these factors could explain the increase in the bunch mass and subsequent yield per plant during the second cycle of this work.

Assis et al. (2011) studied the productive behavior of grapevine cv. Isabel and obtained similar results to those of this study observing a value of 47.4 bunches per plant. Previously, Sato et al. (2008) observed in different rootstocks that the cultivar Isabel had higher number of bunches than those that were obtained by Assis et al. (2011) and in this study in which an average of 76.1 bunches per plant was observed.

The productivity values of this study were lower than those obtained by Sato et al. (2008) which showed a productivity of 9.60 kg per plant (cv. Isabel). Gomes et al. (2011) analyze the effect of resistance inducers on the productivity of grapevine cv. Isabel observed reductions at different levels when the plants were treated with Agro-Mos®, Rocksil®, EcoLife® and EcoLife® + potassium phosphite compared to plants that were treated with fungicides and potassium phosphite, indicating that plants possibly utilize their resources to defend themselves, creating a state of preconditioning that results in an associated adaptive cost, that is, low productivity which may also explain the values that were obtained in this study.

Based on the results of this study, the culture filtrate from the saprobe fungus *C. inaequalis* was efficient in controlling the foliar diseases downy mildew and isariopsis leaf spot, as well as restraining the severity of downy mildew on grape bunches. However, with the weather conditions being extremely favorable for disease development and high inoculum pressure, the efficiency was lower. Postharvest of the grape fruits showed no physicochemical changes regarding the use of this product, but when analyzing the physical features, poor performance was observed which may have been generated by both the experiment development time and climatic conditions as well as by environmental penalties caused by the dose applied and/or the amount of the elicitor applications. Thus, further studies are required in order to clarify issues related to the physical and physicochemical characteristics.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

The authors acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship granted to the first author, CNPq for the Research Productivity Grant to the second and fifth authors, Project SISBIOTA, CNPq and FAPESP who funded this research and Mr. Laércio (producer) who allowed for the use of an area of his property for the trial.

REFERENCES

Amorim L, Kuniyuki H (2005). Doenças da videira. In: Amorim L, Bergamin A, Camargo LEA (eds) Manual de Fitopatologia: Doenças de plantas cultivadas. 4 ed. São Paulo: Agronômica Ceres, 2:736-757.

Assis AM, Yamamoto LY, Souza FS, Borges RS, Roberto SR (2011). Evolução da maturação e características físico-químicas e produtivas das videiras 'BRS Carmem' e 'Isabel. Rev. Bras. Frutic. 33(1):493-498. http://dx.doi.org/10.1590/S0100-29452011000500066

Azevedo LAS (1997). Manual de quantificação de doenças de plantas. São Paulo: Novartis Biociências- Setor Agro, P. 114.

Barkai-Golan R (2001). Postharvest Diseases of Fruit and Vegetables: Development and Control. Amasterdam: Elsevier Sci. P. 432.

Batta YA (2007). Control of postharvest diseases of fruit with an invert

emulsion formulation of Trichoderma harzianum Rifai. Postharv. Biol. Technol. 43(1):143–150. http://dx.doi.org/10.1016/j.postharvbio.2006.07.010

Bengtson P, Bastviken D, Boer W, Öberg G (2009). Possible role of reactive chlorine in microbial antagonism and organic matter chlorination in terrestrial environments. Environ. Microbiol. 11(6):1330–1339. http://dx.doi.org/10.1111/j.1462-2920.2009.01915.xPMid:19453612

Bogdanova VN, Marzhina LA, Dima SG (1979). Izucheniye antibioticheskoy activnosti gribov protiv mild'yu vinograda (analysis of antibiotic activity of fungi against grape mildew). Mikroorg. Virusy. pp. 43-50.

Cañamás TP, Vi-as I, Torres R, Usall J, Solsona C, Teixidó N (2011). Field applications of improved formulations of Candida sake CPA-1 for control of Botrytis cinerea in grapes. Biol. Control. 56(2):150–158. http://dx.doi.org/10.1016/j.biocontrol.2010.11.007

Chavarria G, Santos HP, Sônego OR, Marodin GAB, Bergamaschi H, Cardoso LS (2007). Incidência de doenças e necessidade de controle em cultivo protegido de videira. Rev. Bras. Frutic. 29(3):477-482. http://dx.doi.org/10.1590/S0100-29452007000300014

Dalbó MA, Schuck E (2003). Avaliação do uso de fosfitos para o controle do míldio da videira. Agrop. Catarinense. 16(3):33-35.

Di Piero RM, Kuhn OJ, Pascholati SF (2005). Indução de resistência e a produtividade das culturas. In: Cavalcanti LS, Di Piero RM, Cia P, Pascholati SF, Resende MLV, Romeiro RS. Indução de resistência em plantas a patógenos. Piracicaba: FEALQ, pp. 239-255.

Falk SP, Pearson RC, Gadoury DM, Seem RC, Sztejnberg A (1996). Fusarium proliferatum as a biocontrol agent against grape downy mildew. Phytopath. 86(10):1010-1017. http://dx.doi.org/10.1094/Phyto-86-1010

Ferreira DF (2011). Sisvar: a computer statistical analysis system. Ciênc. Agrotec.35(6):1039-1042.

Gomes ECS, Leite RP, Silva FJA, Cavalcanti LS, Nascimento LC, Silva SM (2011). Manejo do míldio e ferrugem em videira com indutores de resistência: produtividade e qualidade pós-colheita. Trop. Plant Pathol. 36(5):332-335.

Grangeiro LC, Leão PC, Soares JM (2002). Caracterização fenológica e produtiva da varie¬dade de uva Superior Seedless cultivada no vale do São Francisco. Rev. Bras. Frutic. 24(2):552-554. http://dx.doi.org/10.1590/S0100-29452002000200054

Hansen EH, Albertsen L, Schafer T, Johansen C, Frisvad JC, Molin S, Gram L (2003). Curvularia haloperoxidase: antimicrobial activity and potential application as a surface disinfectant. J. App. Environ. Microbiol. 69(8):4611–4617.

Hemrika W, Renirie R, Macedo-Ribeiro S, Messerschmidt A, Wever R (1999). Mutagenesis of the Active Site Residues His Saccharomyces cerevisiae and Site-directed from Curvularia inaequalis in Vanadium-containing Chloroperoxidase Heterologous Expression of the 496, Lys353, Arg360, and Arg490. J. Biol. Chem. 274(34):23820–23827. http://dx.doi.org/10.1074/jbc.274.34.23820 PMid:10446144

Iapar (2000). Instituto Agronomico do Parana. Cartas Climaticas do Parana. Versao 1.0. 2000. (formato digital) 1 CD.

Instituto Adolfo Lutz (1985). Normas analíticas: métodos químicos e físicos para análises de alimentos. 3.ed. São Paulo: Instituto Adolfo Lutz.

Karabulut OA, Baykal N (2003). Biological control of postharvest diseases of peaches and nectarines by yeasts. J. Phytopath. 151(3):130–134. http://dx.doi.org/10.1046/j.1439-0434.2003.00690.x

Kishino AY, Carvalho SLC, Roberto SR (2007). Viticultura tropical: o sistema de produção do Paraná. Londrina: IAPAR, P. 366.

Larkin RP, Fravel DR (2002). Effects of varying environmental conditions on biological control of Fusarium wilt of tomato by nonpathogenic Fusarium spp. Phytopath. 92(11):1160-1166. http://dx.doi.org/10.1094/PHYTO.2002.92.11.1160 PMid:18944240

Lenz G, Costa ID, Balardin RS, Marques LN, Arrué A, Stefanelo MS, Zemolim CR (2009). Elaboração e validação de escala diagramática para quantificação da mancha de isariopsis da videira. Ciênc. Rural. 39(8):2301-2308. http://dx.doi.org/10.1590/S0103-84782009000800005

Miguel ACA, Dias JRPS, Albertini S, Spoto MHF (2009). Pós-colheita de uva 'Itália' revestida com filmes à base de alginato de sódio e armazenada sob refrigeração. Ciênc. e Tecnol. de Alim. 29(2):277-282. http://dx.doi.org/10.1590/S0101-20612009000200006

Musetti R, Vecchione A, Stringher L, Borselli S, Zulini L, Marzani C, D'Ambrosio ML, Toppi LS, Pertoti I (2006). Inhibition of Sporulation and Ultrastructural Alterations of Grapevine Downy Mildew by the Endophytic Fungus Alternaria alternata. Phytopath. 96(7):689-698. http://dx.doi.org/10.1094/PHYTO-96-0689 PMid:18943142

Neis S, Reis EF, Santos SC (2010). Produção e qualidade da videira cv. Niágara rosada em diferentes épocas de poda no sudoeste goiano. Rev. Bras. Frutic. 32(4):1146-1153. http://dx.doi.org/10.1590/S0100-29452010000400024

Özer C, Solak E, Öztürk1 L., Özer N (2012). The development of powdery mildew-tolerant grape cultivars with standard quality characteristics by crossbreeding. Afri. J. Agric. Resea. 7(9):1374-1380.

Pereira GE, Lima LCO, Regina MA, Rosier JP, Ferraz V, Junior MM (2008). Avaliação do potencial de cinco cultivares de videiras americanas para sucos de uva no sul de minas. Ciênc. Agrotec. 32(5):1531-1537. http://dx.doi.org/10.1590/S1413-70542008000500026

Pinheiro ES, Costa JMC, Clemente E, Machado PHS, Maia GA (2009). Estabilidade Físico-Química e Mineral do Suco de Uva obtido por Extração a Vapor. Rev. Ciênc. Agron. 40(3):373-380.

Pinto, KMS, Nascimento LC, Oliveira AK, Leite RP, Silva JP (2013). Resistência induzida em frutos de videira 'Isabel' (*Vitis labrusca*) e seus efeitos sobre a qualidade pós-colheita. Rev. Bras. Fruti. 35(1):210-217. http://dx.doi.org/10.1590/S0100-29452013000100024

Rizzon LA, Meneguzzo J, Manfroi L (2004). Processamento de uva, vinho tinto, graspa e vinagre. Brasília: Embrapa Informação Tecnológica.

Santana MTA, Siqueira HH, Reis KC, Lima LCO, Silva RJL (2008). Caracterização de diferentes marcas de sucos de uva comercializados em duas regiões do Brasil. Ciênc. e Agrotecn. 32(3):882-886.http://dx.doi.org/10.1590/S1413-70542008000300027

Sato AJ, Silva BJ, Santos CE, Bertolucci R, Santos R, Carielo M, Guiraud MC, Fonseca ICB, Roberto SR (2008). Caracteristicas físico -químicas e produtivas das uvas 'Isabel' e 'Brs-Rúbea' sobre diferentes porta-enxertos na região do norte do Paraná. Rev. Bras. Frutic. 30(2): 553-556. http://dx.doi.org/10.1590/S0100-29452008000200050

Schena L, Nigro F, Pentimone IA, Ippolito A (2003). Control of postharvest rots of sweet cherries and table grapes with endophytic isolates of Aureobasidium pullulans. Postharv. Biol. Technol. 30(3):209–220.http://dx.doi.org/10.1016/S0925-5214(03)00111-X

Shaner G, Finney RE (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopath. 67(8):1051-1056.http://dx.doi.org/10.1094/Phyto-67-1051

Sharma RR, Singh D, Singh R (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biol. Control. 50(3):205-221. http://dx.doi.org/10.1016/j.biocontrol.2009.05.001

Van Schijndel JWPM, Vollenbroek EGM, Wever R (1993). The chloroperoxidase from the fungus Curvularia inaequalis; a novel vanadium enzyme. Biochim Biophys Acta. 1161(2-3):249-256. http://dx.doi.org/10.1016/0167-4838(93)90221-C

Wolfson LM, Sumner SS (1993). Antibacterial activity of the lactoperoxidase system - A review. J. Food Protect. 56(10):887-892.