ISSN 0100-2945 DOI: http://dx.doi.org /10.1590/0100-29452018443

Revista Brasileira de Fruticultura

Frequency of fungicide application for controlling downy mildew in seedless grape plant 'BRS Vitória'

Reginaldo Teodoro de Souza[1], Rosemeire de Lellis Naves[1],
Marco Antônio Fonseca Conceição[2], Sabrina Marcolino da Costa[3], Taynara Cruz Savini[4]

Abstract - Different application frequencies of metalaxyl + mancozeb were evaluated to control downy mildew in vine plants 'BRS Vitória' in two experiments conducted in Jales, São Paulo, one in the production cycle and another in branches formation cycle. In experimental design of randomized blocks, five treatments were compared (1- two weekly applications; 2- one weekly application; 3- one application every 14 days; 4- one application every 21 days; 5- applications after sporulation) with four replications, each plot with three plants. According to the analysis of the area under the disease progress curve (AUDPC), in both experiments, there were statistically significant differences between the disease control levels provided by the different treatments ($P \leq 0.05$), being the control more efficient when there were two weekly sprayings with metalaxyl + mancozeb, followed by one weekly spraying. Regarding the number of sprayings of the treatment "spraying after sporulation" there was a reduction of over 90% when compared to standard treatment, "two weekly sprayings." This reduction, however, did not result in a significant increase in the percentage of affected leaf area, which was less than 3%, causing no damage to the plant and not interfering with the quality and the physicochemical characteristics of clusters.
Index Terms: *Plasmopara viticola*, chemical control, plant resistance

Frequência de aplicação de fungicida para controle de míldio na cultivar de uva sem semente 'BRS Vitória'

Resumo - Avaliaram-se diferentes frequências de aplicação de metalaxil + mancozebe para o controle de míldio em plantas de videira de 'BRS Vitória', em dois experimentos conduzidos em Jales, São Paulo, sendo um no ciclo de produção e outro no ciclo de formação de ramos. Em delineamento experimental de blocos ao acaso, foram comparados cinco tratamentos (1- duas aplicações semanais; 2- uma aplicação semanal; 3- uma aplicação a cada 14 dias; 4- uma aplicação a cada 21 dias; 5- aplicações após esporulação), com quatro repetições, sendo cada parcela constituída por três plantas. Conforme a análise da área abaixo da curva do progresso da doença (AACPD), nos dois experimentos, houve diferenças estatísticas significativas entre os níveis de controle do míldio proporcionados pelos diferentes tratamentos ($P \leq 0,05$), sendo que o controle foi mais eficiente quando foram realizadas duas pulverizações semanais com metalaxil + mancozebe, seguido por uma pulverização semanal. Em relação ao número de pulverizações, no tratamento "pulverização após a esporulação", houve a redução de mais de 90% quando comparado ao tratamento-padrão, "duas pulverizações semanais". Esta redução, contudo, não implicou um aumento significativo da porcentagem de área foliar afetada, que foi inferior a 3%, não causando prejuízo para a planta e não interferindo na qualidade e nas características físico-químicas dos cachos.
Termos de indexação: *Plasmopara vitícola*, controle químico, resistência de plantas.

Corresponding author:
reginaldo.souza@embrapa.br
Received: May 29, 2017.
Accepted : October 10, 2017.

[1]Agronomist, PhD., Researcher of Embrapa Grape and Wine, Experimental Station of Tropical Viticulture, Jales-SP. Email: reginaldo.souza@embrapa.br; rosemeire.naves@embrapa.br.
[2]Civil Engineer, PhD., Researcher of Embrapa Grape and Wine Experimental Station of Tropical Viticulture E-mail: marco.conceicao@embrapa.br;
[3]Student of Agronomy, Camilo Castelo Branco University, Estrada Projetada s / n, Fazenda Santa Rita, Fernandópolis-SC. E-mail: sahmarcolino39@gmail.com;
[4]Student of Technology in Agribusiness, Faculty of Technology of Jales, Rua Vicente Leporace, 2630, Jardim Trianon.. E-mail: tay.savini@live.com.br.

Introduction

The downy mildew caused by oomycete *Plasmopara viticola* (Berk. & Curt) Berl. De Toni is a disease that occurs in grapevine (*Vitis* sp.) around the world (DE BEM et al., 2016), assuming great importance in humid regions (ATAK et al., 2017), which can cause losses up to 100% in the production (ANGELOTTI et al., 2017) for leading to the total or partial destruction of the inflorescences and fruits and premature drop of leaves, in addition to damage to production of the year, it will also affect the production of the following years (SÔNEGO et al., 2006).

A pathogen's ability to cause major damage in a short time makes the use of fungicides the most important control for susceptible cultivars when grown in areas with high inoculum pressure (SÔNEGO et al., 2003), which traditionally are applied at fixed intervals to keep the surface of the host's organs covered with an effective dosage of the products, being necessary, with this, a large number of sprayings at each crop cycle. The suitability of the moment of fungicide application is determinative of its efficacy, since early or late sprayings have little or no effect in epidemic disease (TESSMANN et al., 2007).

The occurrence of epidemics in crops is dependent on the relationship between hosts and pathogens under the influence of the environment and human interference (KRANZ, 1974). Changes in these factors may promote or inhibit the development of diseases in plants, increasing or reducing the need for pesticide to control them. These changes in cultured species can be made by means of transgenic technique, inducing resistance or tolerance to pathogens, or by means of conventional breeding (BUONASSISI et al., 2017; SÁNCHEZ-MORA et al., 2017).

The Grape Genetic Breeding Program of Embrapa Grape and Wine has sought to introduce resistance to disease in developed cultivars and, in this process, success has been achieved in the development of table grapes 'BRS Nubia', 'BRS Vitória' and 'BRS Isis' that, in field and greenhouse, showed different levels of resistance to downy mildew (RITSCHEL et al., 2015). Studying the impact of resistance of these cultivars on the life cycle of *P. viticola* in favorable environments for their development will assist in establishing parameters for the management of the disease, and consequently, the use of rationally fungicides with a significant reduction in number of applications compared to susceptible cultivars currently grown in Brazil, and the risk of possible emergence of pathogen genotypes resistant to those products.

The objective of this study was to evaluate, in the field, different application frequencies of metalaxyl + mancozeb for the control of downy mildew in grapevine plants 'BRS Vitória'.

Material and methods

The study was conducted at the Tropical Viticulture Experimental Station in Jales, Northwest of São Paulo State (20º 10' S and 50º 36' W; 436 m of altitude), Brazil, in vines of cultivar of seedless grapes BRS Vitória (*Vitis* spp.) grafted on 'IAC-572' and conducted in the Y system.

Two experiments were conducted, occurring the first in the production cycle, from April 8th to July 4th 2014, and the second in branches formation cycle, from October 10th 2014 to May 1st 2015, in which five frequencies of metalaxyl + mancozeb (Ridomil Gold MZ®) were evaluated, using the dosage of 1,7kg.ha^{-1} of active ingredient: 1- two weekly applications; 2 one weekly application; 3- one application every 14 days; 4- one application every 21 days; 5- application after the onset of leaves sporulation.

The experimental design was "randomized blocks" with five treatments and four replications, in which each plot was comprised of three plants. In the central plant of each plot, periodically, in four branches previously marked, the severity of downy mildew was evaluated in ten leaves per branch, determined by the percentage of affected leaf area (ALA) by the disease. Oil spot was considered as symptoms as well as the area necrotized by downy mildew.

With the average values of the percentage of ALA it was determined the progress curve of the disease for each treatment and calculated the area under the disease curve progress (AUDPC) using Fürstenbergere and Canteri program (1999). The data of AUDPC were subjected to analysis of variance by **F** test and the averages were grouped by the Scott-Knott test (1974) at 5% probability. For the statistical analysis it was used the program SISVAR (FERREIRA, 2011).

In the second experiment, at the end of the cycle, during the harvest, physical characteristics and physical and chemical quality of the clusters were evaluated: average mass of the clusters, rachis length, average weight of 20 berries, pH, total soluble solids (TSS), total titratable acidity (TTA), total soluble solids / total titratable acidity (TSS / TTA) and yield per plant. For chemical analysis, it was used a mash of 20 berries each plot. The total soluble solids content (°Brix) was obtained by reading on a bench refractometer, the total acidity (meqL^{-1}) was determined by titration of the must with NaOH 0.1N and bromothymol blue as an indicator and the pH was determined with a digital pH meter.

Results and discussion

Symptoms of downy mildew observed in leaves of ‘BRS Vitória’ differed from symptomatological pattern of the disease in most susceptible cultivars, where the symptom known as oil spot expands fastly, and in high humidity conditions, the underside of the leaf it is possible to observe the sporulation of the pathogen, consisting of a white, dense and cottonous efflorescence aspect, which may compromise the whole leaf lamina (ALMANÇA et al., 2015). In ‘BRS Vitória’, small brown spots were observed on leaves, which expanded slowly, and rapidly became a necrotic leaf tissue (Figure 1). This may be linked to the hypersensitivity reaction, i.e., a plant defense mechanism which has as one of the features the quick and located collapse of the tissue around the infection site, caused by the release of toxic compounds, which also act in some cases, directly on the pathogen, causing its death (AGRIOS, 2004).

Although the environmental conditions had been favorable (Figure 2), in both experiments, it was not observed pathogen sporulation on the plants leaves, subjected to different treatments, and no symptoms of downy mildew in clusters. Pereira (2015), evaluating different spraying programs with fungicide for downy mildew control in the ‘BRS Vitória’, in the municipality of Marialva, northwestern region of Paraná State, Brazil, also observed no downy mildew symptom on the clusters, but the leaves showed a small sporulation, formed by a thin fruiting mass of the pathogen (Figure 1).

In both experiments, differences were observed in downy mildew progress curves, determined by the average values of the percentage of affected leaf area by the disease in plants subjected to different treatments (Figure 3) and the mean percentage of leaf area affected were lower in the leaves of vines which received two weekly sprayings of metalaxyl + mancozeb.

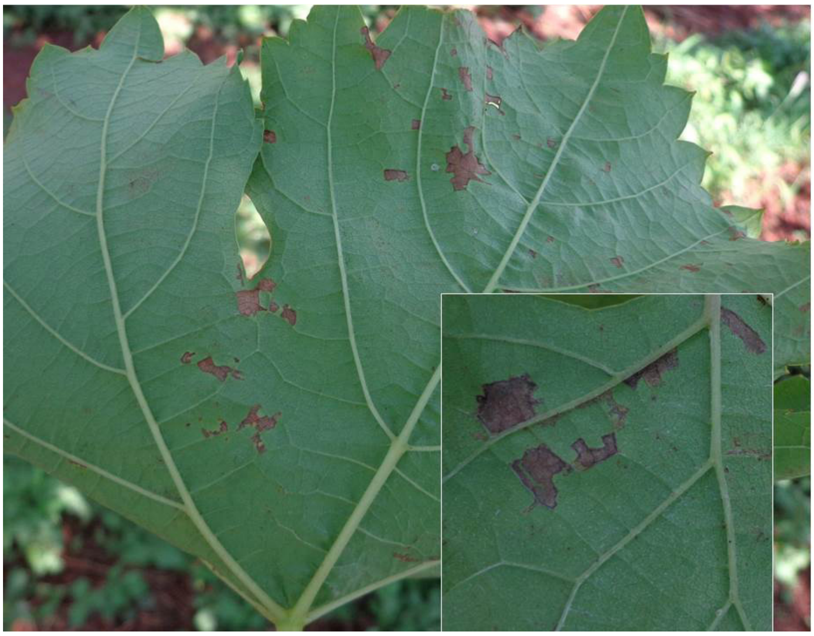

Figure 1. Necrotic spots caused by *Plasmopara viticola* in vine leaves cv. BRS Vitória. (Photo: Carolina Bertuzzi Pereira)

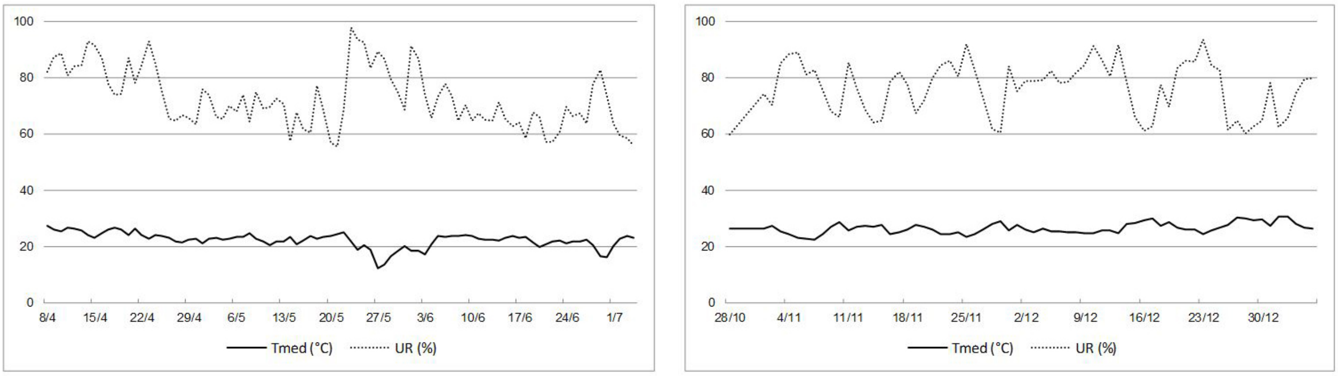

Figure 2. Average temperature and relative humidity recorded by the meteorological station of INMET in the Experimental Station for Tropical Viticulture in Jales, São Paulo, in the period from April 25th to July 4th 2014 (A) and from October 28th 2014 to January 5th 2015 (B).

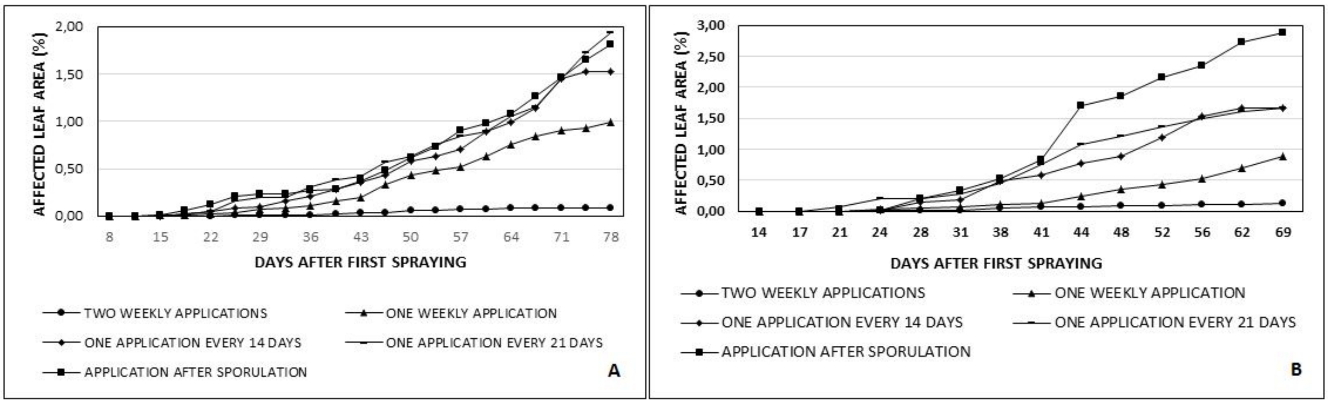

Figure 3. Downy mildew on vines progress curves 'BRS Vitória' subjected to different spraying frequencies of metalaxyl + mancozeb, from April 25th to July 4th 2014 (A) and from October 28th 2014 to January 5th 2015 (B), in Jales, SP.

The first symptoms of downy mildew were observed approximately 20 days after the first spraying on the leaves of the plants submitted for a "one spraying every 21 days" and "spraying after sporulation" treatments, but the average values for the severity of the disease, expressed by the affected leaf area, were lower than 2% and 3% in the first and second experiments, respectively. Naves et al. (2008) studying, in a greenhouse, the development of downy mildew in genotypes with different levels of susceptibility to the disease, observed the onset of symptoms in cv. Thompson Seedless, highly susceptible, four days after the suspension of spraying with fungicides. In a greenhouse, the evaluation of cultivars and advanced selections of the Vine breeding program of Embrapa Grape and Wine for susceptibility to downy mildew, the 'BRS Vitória' behaved similarly to the cultivar Seyve Villard 12375, which was used as a highly resistant standard to disease, being noticed the first symptoms of downy mildew about 30 days after being observed in 'Thompson Seedless' (NAVES et al., 2014).

According to the analysis of the area under the disease progress curve, in both experiments, there were significant and statistical differences between the downy mildew control levels provided by the different treatments ($p \leq 0.05$), once the control was more efficient when twice weekly sprayings metalaxyl + mancozeb, followed by a weekly spraying (Table 1).

In the first experiment, there was no significant difference among the downy mildew control levels, provided by the treatments "a biweekly spraying", "one spraying every 21 days" and "spraying after sporulation" although there had been a reduction of 80% in the number of sprayings received by the plants subjected to the last treatment in comparison with the former (Table 2).

Regarding the number of sprayings of the treatment "spraying after sporulation" there was a reduction of 94.45% and 93.75% when compared to the standard treatment "two weekly sprayings" in the first and second experiments, respectively. That reduction, however, did not result in a significant increase in the percentage of the affected leaf area, which has not reached 2% in the first experiment and even in environmental conditions extremely favorable to the occurrence of disease in the crop production cycle, when the second experiment was performed, it did not reach 3%. These severity levels were not enough to cause any damage to the plant and did not affect the final production, the quality and the physicochemical characteristics of the clusters (Table 3).

The significant reduction in the number of applications in relation to susceptible cultivars currently grown in Brazil represents a major advance in order to establish economically viable programs and environmentally sustainable for the control of fungal diseases of the vine, whereas in the tropics, about 70 annual sprayings in crops of fine grapes (*Vitis vinifera* L.), and 35 sprayings in rough grape cultivars, like 'Niágara Rosada' (*Vitis labrusca* L.), are held, which represents around 20% of the total operational costs of the culture (MELLO, MAIA, 2003). A significant reduction in the number of sprayings in susceptible cultivars grown in these regions has been achieved only with the use of a plastic cover, that, in the North region of Paraná State, Brazil, reduced up to 75% the fungicide applications for downy mildew control in 'BRS Clara' in comparison with the anti-hail net (GENTA et al., 2010). In the Northwest region of São Paulo State, Brazil, the management of sprayings based on phytosanitary warning systems, coupled with the use of plastic sheeting, reduced in 70% the number of sprayings for downy mildew control on plants 'BRS Morena' (HOLCMAN, 2014). However, although it is a promising technology, the protected cultivation involves higher costs due to the implementation of the covering system and of the less durability of the plastic canvas, which is three years, on average, in relation to anti-hail net.

As the pathogen sporulation on the leaves was not observed, the only spraying carried out in plants subjected to treatment "spraying after sporulation" was not due to the observation of those signals, but to an initial spraying carried out in all plants before the start of the sprayings differentiated by the treatments. Therefore, probably it is possible to produce table grapes of 'BRS Vitória' in the Northwest region of São Paulo State, without spraying for downy mildew control. However, the cultivar has proven susceptible to powdery mildew and rip rot *Glomerella* or rot ripe grape (MAIA et al., 2016), which requires researches to seek alternatives to conventional fungicides to control those diseases. Thus, it is believed that an organic crop cultivar can be established in this region, contributing for the economical viability of the activity and ecological sustainability so that it can offer a better quality product for the consumer.

Table 1.Area under the downy mildew progress curve on grape 'BRS Vitória' under different spraying frequencies metalaxyl + mancozeb.

Treatment	AUDPC*	
	1º Exp.	2º Exp.
2 Weekly sprayings	2.72 A	3.66 A
1 Weekly spraying	24.31 B	16.08 B
1 Biweekly spraying	35.14 C	41.08 C
1 Spraying 21 days	40.71 C	45.41 C
Spraying after sporulation	41.24 C	69.03 D

[1]Average followed by the same letter in the columns do not differ by the Scott-Knott test ($p \leq 0.05$).
*AUDPC: Area under the disease progress curve

Table 2. Number of sprayings to control downy mildew on plants 'BRS Vitória' under different spraying frequencies of metalaxyl + mancozeb in Jales, SP.

Treatment	Number of spraygns	
	1º Exp.	2º Exp.
2 Weekly sprayings	18	16
1 Weekly spraying	09	08
1 Biweekly spraying	05	05
1 Spraying 21 days	03	03
Spraying after sporulation	01	01

Table 3. Average mass of clusters, rachis length, twenty berries mass, pH, total soluble solids (TSS), total titratable acidity (TTA) and production of vines 'BRS Vitória' under different application frequencies of metalaxyl + mancozeb .

TREATMENT	Mass Cluster (g)	Rachis length (cm)	Mass 20 Berries (g)	pH	TSS (ºBrix)	TTA (meqL^{-1})	TSS/TTA	Production Plant (kg)
2 Weekly sprayings	155.9 ^{ns}	10.73 ^{ns}	62.50 ^{ns}	3.97 ^{ns}	19.3 ^{ns}	1.45 ^{ns}	10.68 ^{ns}	3.12 ^{ns}
1 Weekly spraying	155.9	10.59	58.75	4.04	19.0	1.58	11.08	3.12
1 Biweekly spraying	186.6	11.01	60.75	4.13	18.4	1.62	11.39	3.73
1 Sprayng 21 days	176.1	11.35	58.25	4.18	17.5	1.65	12.06	3.52
Spraying after sporulation	164.9	10.50	56.75	4.22	18.1	1.65	13.35	3.23

ns: not significant F test at 5% of probability; TSS: Total Soluble Solids; TTA: Total Titratable Acidity

References

AGRIOS, G. N. **Plant pathology**. 5th ed. San Diego: Elsevier Academic Press, 2004. 922p.

ALMANÇA, M.A.K.; LERIN, S.; CAVALCANTI, F.R. Doenças da videira. **Informe Agropecuário**, Belo Horizonte, v.36, n.289, p.7-12, 2015.

ANGELOTTI, F.; HAMADA, E.; MAGALHÃES, E.E.; GHINI,R.; GARRIDO, L. da R.; PEDRO JÚNIOR, M.J. Climate change and the occurrence of downy mildew in Brazilian grapevines. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.52, n.6, p.426-434, 2017.

ATAK, A.; AKKURT, M.; POLAT, Z.; ÇELIK, H.; KAHRAMAN, K.A.; AKGÜL, D.S.; ÖZER, N.; SÖYLEMEZOĞLU, G.; ŞIRE, G.G.; EIBACH, R. Susceptibility to downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) of different *Vitis* cultivars and genotypes. **Ciência e Técnica Vitivinícola**, Oeiras, v.32, n.1, p.23-32, 2017.

BUONASSISI, D.; COLOMBO, M.; MIGLIARO, D.; DOLZANI, C., PERESSOTTI, E.; MIZZOTTI, C.; VELASCO, R.; MASIERO, S.; PERAZZOLLI, M.; VEZZULLI, S. Breeding for grapevine downy mildew resistance: a review of "omics" approaches. **Euphytica**, Dordrecht, v.213, n.103, p.1-21, 2017.

DE BEM, B.P.; BOGO, A.; EVERHART, S.E.; CASA, R.T.; GONÇALVES, M.J.; MARCON FILHO, J.L.; RUFATO, L.; SILVA, F.N. da; ALLEBRANDT, R.; CUNHA, I.C. da. Effect of four training systems on the temporal dynamics of downy mildew in two grapevine cultivars in southern Brazil. **Tropical Plant Pathology**, Brasília, DF, v.41, p.370-379, 2016.

FERREIRA, D.F. Sisvar: A computer statistical analysis system. **Ciência e Agrotecnologia**, Lavras, v.35, n.6, p.1039-1042, 2011.

FÜRSTENBERGER, A.L.F.; CANTERI, M.G. **AACPD** - Software para cálculo da área abaixo da curva de progresso da doença no ambiente Windows. 1999.

GENTA, W.; TESSMANN, D.J.; ROBERTO, S.R.; VIDA, J.B.; COLOMBO, L.A.; SCAPIN, C.R.; RICCI, W.S.; CLOVIS, L.R. Manejo de míldio no cultivo protegido de videira de mesa 'BRS Clara'. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.45, n.12, p.1388-1395, 2010.

HOLCMAN, E. **Sistemas de alerta fitossanitário para o controle do míldio em vinhedos conduzidos sob coberturas plásticas no Noroeste Paulista**. 2014. 236 f. Tese (Doutorado em Ciências – Física do Ambiente Agrícola) - Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 2014.

KRANZ, J. Epidemiology, concepts and scope. In: RAYCHAUDHURI, S.P.; VERMA, J.P. (Ed.). **Current trends in plant pathology**. Lucknow: University of Lucknow, 1974. p.26-32.

MAIA, J.D.G.; RITSCHEL, P.S.; SOUZA, R.T. de; GARRIDO, L.da R. '**BRS Vitória' - uva para mesa, sem sementes, de sabor especial e tolerante ao míldio: recomendações agronômicas para a região de Campinas, São Paulo**. Bento Gonçalves: Embrapa Uva e Vinho, 2016. 28p. (Comunicado Técnico, 129).

MELLO, L.M.R.; MAIA, J.D.G. Custos e rentabilidade. In: EMBRAPA. **Cultivo da videira 'Niágara Rosada' em regiões tropicais do Brasil**. Bento Gonçalves: Embrapa Uva e Vinho, 2003. (Sistema de Produção, 5). Disponível em: <http://www.cnpuv.embrapa.br/publica/sprod/UvaNiagaraRosadaRegioesTropicais/custo.htm>. Acesso em: 15 nov. 2015.

NAVES, R.L.; SANTANA, A.P.S.; RITSCHEL, P.S.; CAMARGO, U.A.; MAIA, J.D.G.; HENRIQUE, C.R.; LACERDA, L.A. Avaliação da resistência ao míldio em cultivares e seleções de uvas de mesa apirênicas. **Tropical Plant Pathology**, Brasília, DF, v.33, p.S201, 2008. Suplemento.

NAVES, R.L.; SILVA, K.F.B.; RITSCHEL, P.S.; MAIA, J.D.G. Resistência de cultivares e seleções de uvas de mesa ao míldio. In: CONGRESSO BRASILEIRO DE FITOPATOLOGIA, 47., 2014. Londrina. **Anais...** Brasília: Sociedade Brasileira de Fitopatologia, 2014. CD-ROM

PEREIRA, C.B. **Patogenicidade de *Alternaria* spp. em videira ‘Niágara Rosada’ e severidade do míldio em ‘BRS Vitória’**. 2015. 88f. Dissertação (Mestrado em Agronomia) – Universidade Estadual de Maringá, Maringá, 2015.

RITSCHEL, P. S.; GIRARDI, C. L.; ZANUS, M.C.; FAJARDO, T. V. M.; MAIA, J. D. G.; SOUZA, R. T.; NAVES, R. de L.; CAMARGO, U. A. Novel Brazilian grape cultivars**. Acta Horticulturae**, Leuven, v.1082, p.157-164, 2015.

SÁNCHEZ-MORA, F.D.; SAIFERT, L.; ZANGHELINI, J.; ASSUMPÇÃO, W.T.; GUGINSKI-PIVA, C.A.; GIACOMETTI, R.; NOVAK, E.I.; KLABUNDE, G.H.; EIBACH, R.; DAL VESCO, L.; NODARI, R.O.; WELTER, L.J. Behavior of grape breeding lines with distinct resistance alleles to downy mildew (Plasmopara viticola). **Crop Breeding and Applied Biotechnology**, Viçosa, MG, v.17, p.141-149, 2017.

SCOTT, A.; KNOTT, M.A. Cluster analysis method for grouping means in the analysis of variance. **Biometrics**, Raleigh, v.30, n.3, p.507-512, 1974.

SÔNEGO, O.R.; GARRIDO, L. da R.; GRIGOLETTI JÚNIOR, A. **Principais doenças fúngicas da videira no Sul do Brasi**l. Bento Gonçalves: Embrapa Uva e Vinho, 2006. 34p. (Circular Técnica, 56)

SÔNEGO, O.R.; GARRIDO, L.da R.; GRIGOLETTI JÚNIOR, A.Doenças fúngicas.In: FAJARDO, T.V.M. **Uva para processamento**: fitossanidade. Brasília, DF: Embrapa Informação Tecnológica, 2003. p.1-44. (Série Frutas do Brasil, 35)

TESSMANN, D. J.; VIDA, J. B.; GENTA, W.; KISHINO, A. Y. Doenças e seu manejo. In: KISHINO, A.Y.; CARVALHO, S.L.C.; ROBERTO, S.R. (Org.). **Viticultura tropical – o sistema de orodução do Paraná**. Londrina: IAPAR, 2007. p.25-293.