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Research Note

Suppression of grapevine powdery mildew (*Uncinula necator*) by acibenzolar-S-methyl

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K e y w o r d s : Acibenzolar-S-methyl, systemic acquired resistance, *Uncinula necator, Vitis vinifera.*

Introduction: Powdery mildew (*Uncinula necator* [Schwein.] Burr.) is one of the most important diseases of grapevine (*Vitis vinifera* L.) in Chile. More than 10 fungicide treatments per year, applied until *véraison*, are commonly needed to control this disease. Growers use sulfur and organic compounds, mainly triazole and strobilurine fungicides alternatively. However, there is a need for new strategies due to the development of resistance that has already been reported elsewhere as well (Erickson and Wilcox 1997).

The systemic acquired resistance (SAR), an inducible physiological mechanism, has been investigated as a possible strategy for disease control on several crops. SAR is a broad physiological response, induced mainly by necrogenic pathogens, that results in the overexpression of certain enzymes with antimicrobial activity (Kessmann *et al.* 1994, Oostendorp *et al.* 2001).

Acibenzolar-S-methyl (1,2,3,-benzothiadiazole-7-thiocarboxylic acid S-methyl ester) is a promising defense system applied exogenously to activate resistance in many crop plants against fungi, bacteria, viruses and nematodes (Kessmann *et al.* 1994, Terry and Daryl 2000, Csinos *et al.* 2001, Oostendorp *et al.* 2001, Matheron and Porchas 2002, Maxson-Stein *et al.* 2002, Eikemo *et al.* 2003). However, evidence for this compound to induce resistance in grapevine is limited and, to our knowledge, there is no report with regard to grapevine powdery mildew. The objectives of this study were to determine if acibenzolar-S-methyl induces resistance against *U. necator* on wine cultivars of *V. vinifera,* and, if so, to compare the effect of enhanced resistance with two controls, untreated or treated with a strobilurine fungicide.

Material and Methods: Acibenzolar-S-methyl (250 mg·l^{-1}, Bion 50 WG, Syngenta, Swiss) was tested as elicitor of resistance against *U. necator* in one-year-old cvs. Carmenere, Chardonnay and Merlot. Kresoxim methyl (70 mg·l^{-1}, Stroby 50 SC, BASF, Germany) was included for comparison. Actively growing one-year-old plants were inoculated with *U. necator* by transferring conidia from colonies developed on Chardonnay grapevines to the upper side of the 5 youngest leaves of a plant. Inoculations were performed 4 and 7 d after being treated with kresoxim methyl and acibenzolar-S-methyl, respectively. An equal number of inoculated, but untreated, plants were left in each trial as control. Plants were then incubated for 18 d in the greenhouse.

Disease incidence and severity were determined 9, 14 and 18 d after inoculation. Disease severity was determined on a 0-4 scale, where 0 was healthy and 1-4 diseased (1 = <10 %, 2 = 11-30 %, 3= 31-60 % and 4= >61 % of the leaf blade covered with powdery mildew).

Treatments were distributed according to a complete randomized design, with 5 replicates, using one plant as experimental unit. Data were analyzed following the two way analysis of variance (SigmaStat 2.0 program, SPSS, Inc., Chicago, IL) with cultivar and treatment as independent variables. Significant differences were separated by the Duncan-Waller K-Ratio test. Data were also analyzed by regression analysis between days after inoculation (T) and disease incidence (I) and between T and disease severity (S). The infection rates were also analyzed for variance and mean significant differences were compared according to Tukey (Zar 1998).

Results and Discussion: The results obtained demonstrated that acibenzolar-S-methyl as a defense activator enhanced resistance to powdery mildew on grapevines, reducing disease incidence and severity. The number of leaves with powdery mildew decreased significantly ($p<0.001$) from 4.8, 3.6, and 3.8 leaves per plant on untreated cvs. Carmenere, Chardonnay, and Merlot, respectively, to an average of less than 0.6 diseased leaves per plant on plants treated only once with acibenzolar-S-methyl. Similarly, disease severity decreased from 12-46 % on untreated plants to 1-2 % on plants treated with acibenzolar-S-methyl. No significant differences were found between disease incidence and severity obtained with acibenzolar-S-methyl and kresoxim methyl. A similar SAR mechanism appeared to exist in Carmenere, Chardonnay and Merlot, suggesting that the mechanism of SAR is independent of the grapevine cultivar (Table).

The analysis of variance showed a non-significant interaction between grapevine cultivar and protection treatments ($p=0.263$). Therefore disease progress curves were obtained from the mean values of the three grapevine cultivars. For untreated plants, the time after inoculation (T) and disease incidence (I) ($I = 3.09T - 7.48$, $R^2 = 0.92$) were linearly related while an exponential relationship was established between T and disease severity (S) ($S = 0.082e^{0.32T}$, $R^2 = 0.90$) (Figure). The progress of powdery mildew was significantly reduced over time on plants treated with acibenzolar-S-methyl ($I = 0.59T-2.0$, $R^2 = 0.73$ and $S = 0.06T-0.2$, $R^2 = 0.63$) or kresoxim methyl ($I = 0.3T- 1.2$, $R^2 = 0.72$ and $0.04T-0.5$, $R^2 = 0.35$) (Figure).

Infection rates were reduced 5 and 10 times on plants treated with acibenzolar S-methyl and kresoxim methyl, respectively. Analysis of variance showed a significant effect of treatment on infection rates, with lower infection rates on treated plants in comparison with untreated control. The infection rates on plants treated with either acibenzolar-S-

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Table

Effectiveness of acibenzolar-S-methyl and kresoxim methyl to control grape powdery mildew (*Uncinula necator*) on one-year old grapevine plants 18 d after inoculation under greenhouse conditions. I-disease incidence, S-disease severity

Protection treatments	Rate	Grapevine cultivars[1]					
		Chardonnay		Carmenere		Merlot	
	mg·l[-1]	I (nº)	S (%)	I (nº)	S (%)	I (nº)	S (%)
Acibenzolar-S- methyl (Bion 50 WG)	250	0.4 a	1.0 a	0.6 a	2.0 a	0.4 a	1.0 a
Kresoxim methyl (Stroby 50 SC)	70	0.0 a	0.0 a	0.4 a	1.0 a	0.6 a	1.0 a
Untreated	0	3.6 b	12.0 b	4.8 b	46.0 b	3.8 b	20.0 b

[1] Mean followed by the same letter in each column are not significantly different at p = 0.05 (Duncan-Waller K-Ratio test).

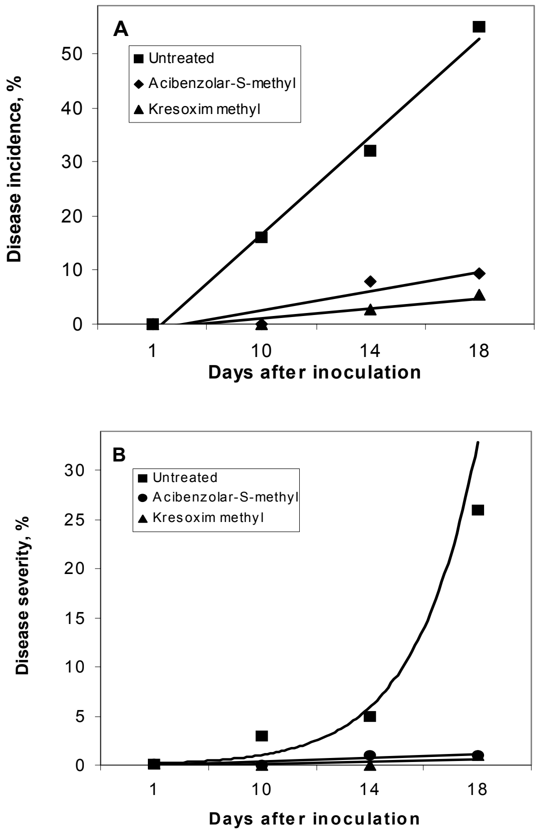

Figure: The effect of acibenzolar-S-methyl and kresoxim methyl on the development of powdery mildew caused by *Uncinula necator* over time (T). A. Disease incidence (I): untreated plants ($I = 3.09T - 7.48$, $R^2 = 0.92$), acibenzolar-S-methyl ($I = 0.59T - 2.0$, $R^2 = 0.73$) and kresoxim methyl ($I = 0.3T - 1.2$, $R^2 = 0.72$). B. Disease severity (S): untreated plants ($S = 0.082e^{0.32T}$, $R^2 = 0.90$), acibenzolar-S-methyl ($S = 0.06T - 0.2$, $R^2 = 0.63$) and kresoxim methyl ($0.04T - 0.5$, $R^2 = 0.35$).

methyl or kresoxim methyl were significantly different ($p<0.005$) from the infection rates obtained for untreated control plants.

Although disease incidence and severity increased with time, differences between treated and non-treated plants were still significant 18 d after inoculation. This period is within the protection period of 30-45 d previously reported for other crops (YAMAGUCHI 1998). Similar to other studies, a 7-d-period between treatment and inoculation was satisfactory to induce resistance under our experimental conditions (CSINOS *et al.* 2001, MATHERON and PORCHAS 2002, MAXSON-STEIN 2002, EIKEMO *et al.* 2003).

The present study confirms that acibenzolar-S-methyl induces resistance of grapevine conferring protection against *U. necator*, similar to the protection achieved with a single application of kresoxim methyl. Therefore, the use of SAR mechanisms on grapevines appears to be a promising alternative to control powdery mildew. Further research is necessary, however, to establish a general recommendation.

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