Application of laminarin and calcium oxide for the control of grape powdery mildew on Vitis vinifera cv. Moscato

the use of plant protection products and have progressively reduced the availability of active ingredients with good activity towards this pathogen. All these factors require a review of the strategies to reduce the use of classic synthetic products, which, above all, aims to improve the sustainability of crop protection, evaluating the efficacy of alternative products compared to conventional fungicides. This situation has led, in recent years, to the increased study of different products such as biological control agents, biostimulants, plant extracts, mineral substances and resistance inducers. These last two strategies, mineral substances and resistance inducers,, which aim to stimulate or strengthen the mechanisms of defense of the vine, seem to be promising (Delaunois *et al.*, 2014). The activity of mineral salts in modulating plant responses to stress is known since many years and calcium in particular plays an important role both in the production of salicylic acid and chitinase, involved in systemic acquired resistance (SAR), and in response of plants to thermal and water stresses (Deliopoulos *et al.*, 2010; Chen *et al.*, 2015; Dubrovina *et al*., 2017).

Algae extracts are known among the resistance inducers of natural origin (Vera *et al.*, 2011), and among these the laminarine, a glucanoligosaccharide extracted from the brown alga *Laminaria digitata*, is well known as an elicitor of the defense mechanisms of plants (Labarre and Orieux, 2010; Bernardon Mery and Joubert, 2012), including grape (Aziz *et al.*, 2003; Trouvelot *et al.*, 2008) on which the laminarin sulphate is active (Ménard *et al.*, 2004). Such activity has often been tested on grape against *Plasmopara viticola* and *Botrytis cinerea* (Aziz *et al.*, 2003; Gauthier *et al.*, 2014; Chalal *et al.*, 2015; Romanazzi *et al.*, 2016).

In the present work the results obtained using a calcium fertilizer and laminarin against powdery mildew in two trials carried out in Piedmont in 2016 and 2017, on a vineyard of the "Moscato" variety, and in two tests carried out on potted plants during 2017, on the same variety are reported.

The control of *E. necator* was evaluated comparing the efficacy of a foliar calcium fertilizer (Califol, AgriNewTech srl, Italy) and laminarin (Vacciplant, Arysta Lifescience, France) to sulphur (Tiovit Jet, Syngenta, Switzerland), used as standard product for managing powdery mildew also in organic farming. The list of tested products, dosages and applications are described in Tables 1, 4 and 6.

Trials on potted plants

The trials were carried out between August and September 2017, outdoors, in meteorological conditions similar to those of open field, at the University of Turin's Agroinnova Competence Center, located in Grugliasco, in the Northwest of Italy (GPS: 45° 03' 57.8" N, 7° 35' 29.5" E).

Plants used belong to white "Moscato" variety and were grown in 4 liters pots. One pot was used for one plant, starting from grape cuttings, and used for the trials nearly 60 days after transplanting the cuttings. A complete randomized system was adopted, using 5 plants for each replicated and 4 replicates for each treatment, corresponding to 20 plants for each treatment.

The plants were treated twice, starting one week before the artificial inoculation, to induce the activation of the resistance mechanisms and were then inoculated with a suspension of $1x10^5$ conidia/ml of the pathogen. Two treatments were carried out also after the first inoculation and, two weeks after, a second inoculation was done, again using a suspension containing $1x10^5$ conidia/ml. Another two treatments were still performed after the second round of inocula was distributed. The products used and the dates of treatments are shown in Tables 1 and 2. Two controls were used: healthy control, consisting of plants not inoculated with the pathogen and not treated; inoculated untreated control, consisting of plants inoculated with the pathogen and not treated.

Treatments with methiram (Polyram DF 70%, BASF Italy) were carried out with an interval of 7-8 days in order to protect all plants from downy mildew infections and being able to evaluate only the mildews caused by *E. necator*. The two controls were also treated with methiram.

The severity of the attacks (% of leaf surface affected) and the incidence of the disease (% of affected leaves) were evaluated every 10 days starting from the appearance of the symptoms on the leaves.

Field trials

The trials were carried in 2016 and 2017 in a vineyard of "Moscato" located in Piedmont, Italy, at the University of Turin's Agroinnova Competence Center, located in Grugliasco, in the Northwest of Italy (GPS: 45° 03' 51.4" N, 7° 35' 34.4" E). The vineyard is grown with espalier with "Guyot" pruning, in which the grass is kept inter-row, with periodic shredding, while the area under the row is chemically weeded. A motorized shoulder pump was used to carry out the treaments and 400-600 liters of irrigation water were distributed per ha on the leaves, depending on the development of the vegetation. A complete randomized system was adopted, using 8 plants for each replicated and 4 replicates for each treatment, corresponding to 32 plants for each treatment.

In 2016, products application started on May 13th and continued until the end of July. The treatments were carried out every 8-10 days, according to weather conditions. The test protocol and the dates of the treatments are shown in Table 4. In this trial, calcium oxide was suspended during

Table 1 – Tested products and experimental protocol used for trials on potted plants. Methiram was applied on all plants every 7-8 days in order to protect the plants from downy mildew infections.

Thesis	Active ingredient (a. i.)	Commercial product (c. p.)	A. I. concentration	Dose a.i. (g/hl)	Dose c.p. (g/hl)	Days between treatmens

Table 2 – Date of treatments of potted trials in 2017.

Thesis	Active ingredient (a. i.)			First inoculation			Second inoculation		
				-			-		
				17/08/2017			05/09/2017		
				17/08/2017			05/09/2017		
				17/08/2017			05/09/2017		
				17/08/2017			05/09/2017		

Table 3 – Efficacy of the treatments against *Erysiphe necator* on potted grape plants in 2017.

Treatments		Trial 1					Trial 2				
		% leaf surface affected	Abbott's	% leaves affected	Abbott's		% leaf surface affected	Abbott's	% leaves affected	Abbott's	
			0		0			0		0	
			93.7		64.5			93.4		64.9	
			98.4		85.6			97.9		85.4	
			91.3		76.5			95.1		64.6	

** Tukey's HSD (P<0,05).*

Table 4 – Tested products and experimental protocol used for the field trials in Grugliasco, 2016. Methiram, methiram plus dimethomorph and copper hydroxide were applied on all plants in order to protect them from downy mildew infections.

	Active ingredient	Commercial product	Dosages		Dates for treatments
			g/ha a.i.	g/ha c.p.	

Table 5 – Efficacy of the treatments on grape "Moscato" against *Erysiphe necator* in field trials (Grugliasco, 2016).

Treatments		% leaf surface affected		Abbott's	% leaves affected		Abbott's	% bunches affected		Abbott's	% clusters affected		Abbott's
				0			0			0			0
				77.1			56.0			49.3			30.9
				87.5			69.1			87.1			68.5
				89.1			68.4			74.3			58.3

** Tukey's HSD (P<0,05).*

Table 6 – Tested products and experimental protocol used for the field trials in Grugliasco, 2017. Methiram and copper hydroxide were applied on all plants in order to protect them from downy mildew infections.

	Active ingredient	Commercial product	Dosages		Dates for treatments
			g/ha a.i.	g/ha c.p.	

Table 7 – Efficacy of the treatments on grape "Moscato" against *Erysiphe necator* in field trials (Grugliasco, 2017).

Treatments		% leaf surface affected		Abbott's	% leaves affected		Abbott's	% bunches affected		Abbott's	% clusters affected		Abbott's
				0			0			0			0
				94.5			54.3			86.9			52.0
				97.3			70.0			89.2			53.7
				86.1			40.0			82.5			35.1

** Tukey's HSD (P<0,05).*