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

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Summary

Erysiphe necator is favorably influenced by the increase in temperatures and remains the main pathogen threatening grape in Northern Italy. In the present work the efficacy of laminarin and calcium oxide in the control of grape powdery mildew was evaluated, comparing them to sulphur. Two tests were carried out on potted grape "Moscato" plants in 2017 and two field trials were carried out in Italy during 2016 and 2017 on a vineyard of the "Moscato" variety. Laminarin and calcium oxide showed, for the first time, to significantly reduce powdery mildew and this occurred in all trials. However, laminarin was less effective than sulphur in potted trials and in 2016 field trial, and both laminarin and calcium oxide were less effective than sulphur in 2017 field trial.

Keywords: *Erysiphe necator*, resistance induction, organic farming, sulphur.

Introduction

The current climate changes can affect the biology of plants and pathogens and, consequently, the epidemiology of plant pathogens causing an increase or a reduction of plant diseases. Powdery mildew remains the main phytosanitary threat in most wine-growing areas in North Italy (Bebber, 2015; Bois *et al.*, 2017), since the development of its causal agent, *Erysiphe necator*, is favorably influenced by the increase in average summer temperatures and also winter conditions (Delp, 1953; Gadoury *et al.*, 2012). Also in Italy and in Piedmont, infections by powdery mildew are increasing because of the weather conditions of recent years, with high temperatures for prolonged periods, high atmospheric humidity and rain reduction (Pugliese *et al.*, 2011), conditions that favor the development of the pathogen (Peduto *et al.*, 2013).

This could lead to the need for an increase in the number of treatments with fungicides with greater risks to human health and greater impact on the environment (Komarek *et al.*, 2010; Tsakirakis *et al.*, 2014). The European regulation on crop protection products and, in particular, the rules established by EC Directive 2009/128 of 21 October 2009, have imposed numerous restrictions on 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.

Materials and methods

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 flowering to avoid damages to flowers and another fertilizer containing amminoacids (Help N, AgriNewTech srl, Italy) was applied instead. The control of downy mildew was carried out every 7-8 days using a product based on metiram (Polyram DF 70%, BASF Italy) up to the pre-flowering phase, then with a product based on metiram and dimethomorph (Slogan TOP, DOW Agrosciences) up to the phase of pre-closing of the bunch and then with a product based on copper hydroxide (Coprantol Hi Bio 2.0, Syngenta) until the end of the test.

In 2017 the distribution of products began on May 4th and continued until the second half of July, after that in some theses it was no longer possible to assess the disease on the bunches because environmental conditions lead to an early harvesting and bunches started to fall off. The treatments were carried out every 8-10 days, according to weather conditions. The test protocol and treatment dates are shown in table 6. The control of downy mildew was carried out every 7-8 days using a product based on metiram (Polyram DF 70%, BASF Italy) up to the phase of pre-closure of the bunch and then with a product based on copper hydroxide (Coprantol Hi Bio 2.0, Syngenta) until the end of the test.

The efficacy was evaluated for both trials at starting of visible symptoms, in the phase of closure of the cluster, at the beginning of veraison and at the end of the trial, evaluating the percentage of affected leaves and grape clusters and the percentage of affected leaf surface and berries, i.e. both incidence and severity.

Statistical analysis

Analysis of variance was carried out with the statistical programme SPSS 22.0. After ANOVA, Tukey's "Honestly Significantly Different" was used as post-hoc analysis, with a significance defined at the $P<0.05$ level unless stated otherwise.

Abbott's formula was also applied to calculate corrected efficacy % (Abbott, 1925).

Results

Trials on potted plants

All products used significantly reduced the development of the disease on the leaves, especially with regard to the percentage of leaf area affected, with an efficacy higher than 90% in both trials (Table 3). Regarding the disease incidence, sulphur was the most effective with a reduction of the percentage of affected leaves by more than 85% compared to the untreated control in both trials (13% and 13.8% of affected leaves, respectively, in trial 1 and trial 2) (Table 3).

Good results were also obtained using calcium oxide (21.3 and 33.3% of leaves affected, respectively, in trial 1 and trial 2) and laminarin (32.0 and 33.0% of leaves affected, respectively, in

trial 1 and 2). The two products, although differing from sulphur, significantly reduced the incidence of the disease compared to the untreated control (Table 3).
Disease severity was significantly reduced by the 3 products (Table 3). Sulphur (0.2 and 0.3 % of leaf surface affected, respectively, in trial 1 and trial 2) and calcium oxide (1.1 and 0.7 % of leaf surface affected, respectively, in trial 1 and trial 2) were the most effective, with a reduction higher than 90% compared to the untreated control in both trials (Table 3). Laminarine was also effective as sulphur and calcium oxide in the first trial, but less effective than sulphur in the second trial (Table 3).

Field trials

The two years showed different meteorological conditions: 2016 was more rainy and with a lower infection pressure of powdery mildew, while 2017 was warmer and drier, with an increased presence of the disease. Indeed in 2016 disease incidence and severity in the untreated control were, respectively, 70.5 and 9.3%, while in 2017 they were 100 and 41.5 % (Tables 5 and 7).
In 2016, sulphur was the most effective product and reduced the disease severity by more than 87%, both on leaves and on clusters. Calcium oxide reduced the disease incidence by 68.4% on leaves and by 58.3% on clusters, and disease severity by 89% on leaves and 74.3%, respectively, compared to the untreated control. Laminarin reduced the disease incidence by 56% on leaves and 30.9% on clusters, and disease severity by 77.1% and 49.3%, respectively, compared to the untreated control (Table 5).
In 2017, calcium oxide reduced the disease incidence by 40% on leaves and 35.1% on clusters, and disease severity by 86.1% and 82.5%, respectively, compared to the untreated control. Laminarin reduced the disease incidence by 54.3% on leaves and 52% on clusters, and disease severity by 94.5 and 86.9%, respectively, compared to the untreated control. Sulphur reduced the disease incidence on leaves by 70% and on bunches by 53.7%, while it reduced the severity by 97.3% and 89.2%, respectively, compared to the control (Table 7).

Discussion

From the results obtained we can deduce that it is possible, even in the presence of a very high infection pressure of the disease in the field, like it happened in 2017, to adopt strategies that are alternative to the use of conventional chemical fungicides, which allow to control powdery mildew effectively, significantly reducing its spread. However compared to sulphur, results obtained using laminarin and calcium oxide were less constant and effective, in particular with very suitable conditions for the pathogen such as higher temperatures and low relative humidity that occurred in 2017.

Oligosaccharides derived from algae of the genus *Laminaria* have the ability to act as elicitors (Labarre and Orieux, 2010; Vera *et al.,* 2011), thanks to their capacity to stimulate plant cells to produce active defense substances effective against the pathogens of grapevine, *Plasmopara viticola* and *Botrytis cinerea* (Aziz *et al.,* 2003; Gauthier *et al.,* 2014; Chalal *et al*., 2015). Laminarin has recently shown to be effective against powdery mildew on strawberry (Melis *et al.,* 2017); this is the first case of efficacy demonstrated on grape powdery mildew and in the present manuscript the elicitation ability was very good on the leaves but lower on bunches, especially in the presence of a strong disease pressure. Due to its already demonstrated efficacy against downy mildew (Romanazzi *et al.,* 2016), laminarin may play an interesting role in integrated and biological management of diseases on grape.

The efficacy shown by calcium oxide in reducing the disease confirms the importance of this nutrient in the response of plants to biotic and abiotic stresses, probably for its role in the production of salicylic acid, the main chemical signal involved in acquired systemic resistance (SAR) (Deliopoulos *et al.,* 2010; Chen *et al.,* 2015; Dubrovina *et al.,* 2017). This is the first case of demonstrated efficacy of calcium oxide on the control of powdery mildew of grape.

The use of laminarin and calcium oxide is expected to support the adoption of integrated strategies against *E. necator* (Delaunois *et al*., 2014). Using this alternative products is expected to reduce the number of chemical fungicides that have restrictions on use and sometimes high risks of resistance. Further research should better clarify the mechanisms involved on the efficacy of calcium oxide and its effect on downy mildew and other vine disease, as well as the efficacy of integrated strategies, applying them alone ore mixed with sulphur, as well as evaluations on different cultivars are necessary.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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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
1	Healthy control	-	-	-	-	-
2	Inoculated untreated control	-	-	-	-	-
3	Laminarin	VACCIPLANT	45 g/l	9	200	7-9
4	Sulphur	TIOVIT JET	80 %	320	400	7-9
5	Calcium oxide	CALIFOL	22.1 %	88.4	400	7-9

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

Thesis	Active ingredient (a. i.)	Dates		First inoculation	Dates		Second inoculation	Dates	
1	Healthy control	-	-	**-**	-	-	**-**	-	-
2	Inoculated untreated control	-	-	**17/08/2017**	-	-	**05/09/2017**	-	-
3	Laminarin	10/08	17/08	**17/08/2017**	26/08	04/09	**05/09/2017**	11/09	19/09
4	Sulphur	10/08	17/08	**17/08/2017**	26/08	04/09	**05/09/2017**	11/09	19/09
5	Calcium oxide	10/08	17/08	**17/08/2017**	26/08	04/09	**05/09/2017**	11/09	19/09

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
1	Healthy control	7.0	b*	-	75.8	c*	-	11.0	c*	-	85.3	c*	-
2	Inoculated untreated control	12.0	c	**0**	90.5	d	**0**	14.8	d	**0**	94.0	d	**0**
3	Laminarin	0.8	a	**93.7**	32.0	b	**64.5**	1.0	b	**93.4**	33.0	b	**64.9**
4	Sulphur	0.2	a	**98.4**	13.0	a	**85.6**	0.3	a	**97.9**	13.8	a	**85.4**
5	Calcium oxide	1.1	a	**91.3**	21.3	ab	**76.5**	0.7	ab	**95.1**	33.3	b	**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.	
1	Untreated control	-	-	-	-
2	Laminarin	Vacciplant	100	2000	13, 20, 30/5; 6, 16, 27/6; 7, 18, 27/7
3	Sulphur	Tiovit Jet	4000	5000	13, 20, 30/5; 6, 16, 27/6; 7, 18, 27/7
4	Calcium oxide	Califol	884	4000	13, 20, 30/5;
	Amminoacids	Help N		3000	6, 16/6
	Calcium oxide	Califol	884	4000	27/6; 7, 18, 27/7

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
1	Untreated control	9.3	c*	**0**	70.5	c*	**0**	20.0	c*	**0**	89.0	c*	**0**
2	Laminarin	2.0	b	**77.1**	31.0	b	**56.0**	10.5	b	**49.3**	62.0	b	**30.9**
3	Sulphur	1.1	a	**87.5**	22.0	a	**69.1**	2.4	a	**87.1**	28.0	a	**68.5**
4	Calcium oxide	0.9	a	**89.1**	22.5	a	**68.4**	5.1	ab	**74.3**	37.0	a	**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.	
1	(Untreated control)	-	-	-	-
2	Laminarin	Vacciplant	100	2000	4, 12, 22, 31/5; 8, 15, 24/6; 3, 11, 19/7
3	Sulphur	Tiovit Jet	4000	5000	4, 12, 22, 31/5; 8, 15, 24/6; 3, 11, 19/7
4	Calcium oxide	Califol	884	4000	4, 12, 22, 31/5; 8, 15, 24/6; 3, 11, 19/7

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
1	Untreated control	41.5	c*	**0**	100.0	d*	**0**	29.1	b*	**0**	89.0	c*	**0**
2	Laminarin	2.3	ab	**94.5**	45.8	b	**54.3**	3.8	a	**86.9**	42.8	a	**52.0**
3	Sulphur	1.1	a	**97.3**	26.5	a	**70.0**	3.1	a	**89.2**	41.3	a	**53.7**
4	Calcium oxide	5.8	b	**86.1**	60.0	c	**40.0**	5.2	a	**82.5**	57.8	b	**35.1**

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