Optimization of copper applications using Laminarine for the sustainable control of grape downy mildew

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1 Introduction

Grapevine Downy mildew (GDM) is caused by the oomycete *Plasmopara viticola*, affecting the leaves and berries under warm and humid environmental conditions. P. viticola may reduce grape yield and affects the aroma and flavours in wine, with organoleptic impacts on berry quality and vinification. GDM is mainly controlled using chemical fungicides in conventional vineyards, where problems with pesticide residues and resistance to active ingredients are increasing, while copper and sulphur are used in organic farms (Gessler et al., 2011; Gadoury et al., 2012). Due to the high number of treatments carried out in viticulture restrictions have been applied by the European Commission on the number of pesticide treatments (Directive 2009/128/EC) and on the maximum quantity per year of copper fungicides (Regulation 2002/473/EC). Recently, copper, which is still considered the most important antifungal product in organic viticulture, has been added to the list of candidates for substitution (European Commission Implementing Regulation 2018/84) and limited to 4 kg per hectare/year spread over 7 years (European Commission Implementing Regulation 2018/1981 of 13 December 2018). Therefore, new strategies have been developed to prevent grapevine downy mildew (GDM) in a more sustainable way. 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 semi-field and field trials were carried out to evaluate the efficacy of laminarine in combination to a reduced amount of copper-based product to contain the GDM in a sustainable viticulture.

2 Materials and Methods

In 2019 and 2020 3 efficacy trials were carried out as following with the aim to evaluate the efficacy of laminarine in combination with a reduced amount of copper-based product. The characteristics of employed products are shown in table 1.

Product	a.i.	Conc. % or g/L	Formulation
Poltiglia Dissperss®	Copper - Bordeaux mixture	20	WG
Vacciplant®	Laminarine	45	SL

Table 1 Characteristics of employed products

Field Trials. In 2019, field efficacy trial was accrued out on Cv. Trebbiano Romagnolo on Kober 5BB rootstock planted in 2003, trained as “Casarsa” and spaced at 1.5 x 3.5 m. In 2020, the trial was carried out Cv. Sangiovese on Kober 5BB rootstock planted in 1999, trained as spour cordon and spaced at 1.2 x 3.0 m. Both trials were experimentally designed as Randomized Complete Block (RCB) with 4 replications in 2019 and 5 replications in 2020. Sprays and volumes were applied as shown in table 1 & 2.

Semi-field trial. Potted grapevine plants) cv Pinot Grigio, were previously moved inside the greenhouse for acclimation. After the experimental application with the test products (performed outside the greenhouse) the plants were kept inside the climatic chamber to avoid any runoff due to rain events. The artificial infection was performed outside the greenhouse and then the plants were kept in the climatic chamber as previously described. A total of 1 litre per treatment, corresponding to 0.33 litres per plant, was applied in a single experimental application. The plants were brought outside the greenhouse only for the fungicide application: they were moved just before the spray and were brought back inside the greenhouse after the complete dry-off of the application (when the canopy was dried). Application was carried out 2 days before the artificial inoculum at three different dosages. Artificial inoculation was carried out with a hand-pump using a water suspension containing 5 x 104 sporangium/mL of Plasmopara viticola. A total of 2.5 litres of solution was prepared and inoculated (having on average 30-32 mL of inoculum per plant). The selected pathogen strain was characterized by complete sensitivity to the fungicides included in the trial.

In field trials, disease evaluation was performed by visual observation of 100 leaves and 50 bunches per plot estimating disease incidence (percentage of affected leaves or bunches) and severity (percentage of injured leaf or bunch area) using the following score: 0 = absence of symptoms; 1 = 0.1 ÷ 5.0% of surface with damage; 2 = 5.1 ÷ 15.0% of surface with damage; 3 = 15.1 ÷ 40.0% of surface with damage; 4 = 40.1 ÷ 70.0% of surface with damage; 5 = 70.1 ÷ 100.0% of

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surface with damage. For the trial in semi field condition visual observation of 100 leaves, with the same assessment procedure above described, was performed. Statistical analysis Analysis of Variance (ANOVA) and Duncan's New MRT ($p \leq 0.05$) was used in 2019 trail, while SNK test ($p \leq 0.05$) and Abbott's formula were used to calculate the efficacy in 2020 trials

2019 Efficacy trial open filed

The final scope in the trail was to evaluate the combined efficacy of Vacciplan (laminarine 45 g/L) at the dosage of 1,5 L/ha with a copper compound at half dosage, to obtain an annual copper amount distributed within the European limit. The sequence of 4 or 3 applications (tank mix) were carried out in a specific growth period as reported in table 2: at the beginning ABCD corresponding from 3 leaves developed to pre flowering; in the core EFGH from flowering to berry set up; at the end of growth cycle from berry pea size to bunch closing ILM. The standard was a full copper dosage rate for the whole cycle and compared with a total amount of copper per hectar respect the EU rules (4 kg Cu/ha). Spray interval was fixed at 7 days.

The trial was carried out in the countryside of Ravenna, Emilia-Romagna, a region in the northeast of Italy, in a typical wine production area, where Downy mildew (Plasmopara viticola - PLASVI) often causes heavy yield losses.

The trial was made in a commercial vineyard of Trebbiano Romagnolo on Kober 5BB rootstock planted in 2003, trained as "Casarsa" and spaced at 1.5 x 3.5 m. The five-vine plots were arranged in four blocks using a randomized complete block design. An untreated plot per block was present.

Trt No.	Treatment Name	Rate kg/hl	Application Code	Spray Volume L/ha
1	Untreated Check			
2	Poltiglia Disperss	0,4	ABCD	400
	Poltiglia Disperss	0,4	EFGH	600
	Poltigia Disperss	0,4	ILM	1000
3	Poltiglia Disperss + Vacciplant	0,2 + 1,5 L/ha	ABCD	400
	Poltiglia Disperss	0,4	EFGH	600
	Poltiglia Disperss	0,4	ILM	1000
4	Poltiglia Disperss	0,4	ABCD	400
	Poltiglia Disperss + Vacciplant	0,2 + 1,5 L/ha	EFGH	600
	Poltiglia Disperss	0,4	ILM	1000
5	Poltiglia Disperss	0,4	ABCD	400
	Poltiglia Disperss	0,4	EFGH	600
	Poltiglia Disperss+ Vacciplant	0,2 + 1,5L/ha	ILM	1000
6	Poltiglia Disperss	0.8 kg/ha	AB	400
	Poltiglia Disperss	1.2 kg/ha	CD	400
	Poltiglia Disperss	1.6 kg/ha	EFGH	600
	Poltiglia Disperss	2.4 kg/ha	ILM	1000

Table 2 Treatments detail

Application code

A = BBCH13 19th April 2019; B = BBCH14 25th April 2019; C = BBCH16 2nd May 2019; D = BBCH16 8th May 2019; E = BBCH53 15th May 2019; F = BBCH55 22nd May 2019; G = BBCH57 28th May 2019; H = BBCH60 5th Jun 2019; I = BBCH71 13th June 2019; L = BBCH73 21st June 2019; M = BBCH77 28th June 2019

Assessments:

BBCH57 27th May 2019 Leaves (evaluation efficacy ABCD applications); BBCH57 10th June 2019 leaves and bunches (evaluation efficacy EFGH applications); BBCH77 3rd July 2019 leaves and bunches (evaluation efficacy ILM applications)

2020 efficacy trial open filed

The trial site was selected in Ravenna province (Emilia Romagna region); it was located in the area near Faenza, an area particularly suitable to vine growing and downy mildew infections. The trial was made in a commercial vineyard of Cv. Sangiovese on Kober 5BB rootstock planted in 1999, trained as spour cordon and spaced at 1.2 x 3.0 m. The trial has been set up as randomized Complete Block (RCB), with 5 replications, single control randomized in each block. The treatments details are reported in table 3. Spray interval was fixed at 7 days.

Trt No.	Treatment Name	Rate kg/hl	Application Code	Spray Volume L/ha
1	Untreated Check			
2	Poltiglia Disperss	0,4	AB	555
	Poltiglia Disperss + Vacciplant	0,2 + 1,5 L/ha	CD	667
	Poltiglia Disperss	0,2 + 1,5 L/ha	E	889
	Poltiglia Disperss	0,4	F	889
	Poltiglia Disperss + Vacciplant	0,2 + 1,5 L/ha	GHI	1000
	Poltiglia Disperss	0,4	LM	1000
3	Poltiglia Disperss	0,4	AB	555
	Poltiglia Disperss + Vacciplant	0,2 + 1,5 L/ha	CD	667
	Poltiglia Disperss + Vacciplant	0,2 + 1,5 L/ha	E	889
	Poltiglia Disperss	0,4	F	889
	Poltiglia Disperss	0,4	G	1000
	Poltiglia Disperss+ Vacciplant	0,2 + 1,5 L/ha	HIL	1000
	Poltiglia Disperss	0,4	M	1000
4	Poltiglia Disperss	0,4	AB	555
	Poltiglia Disperss	0,4	CD	667
	Poltiglia Disperss	0,4	EF	889
	Poltiglia Disperss	0,4	GHILM	1000

Table 3 Treatments detail

Application code

BBCH53 27th April 2020; BBCH57 6th May 2020; BBCH60 13th May 2020; BBCH61 20th May 2020; BBCH69 26th May 2020; BBCH71 3rd June 2020; BBCH73 9th June 2020; BBCH75 16th June 2020; BBCH75/77 23rd June 2020; BBCH77 1st July 2020; BBCH79 8th July 2020

Assessments:

BBCH75/77 22 June 2020 Leaves and bunches; BBCH79 16th July 2020 leaves and bunches.

In figure 6 – 7 – 8- 9 thesis 2 and 3 are respectively reported as thesis 2 = Modality 1 and thesis 3 = Modality 2

2020 efficacy semi field trial

The trial was performed in semi-field conditions in the greenhouse. The trial detail is reported in table 3. Potted grapevine plants, cv Pinot Grigio, were previously moved inside greenhouse for acclimation. After the experimental application with the test product (performed outside the greenhouse) the plants were kept inside the climatic chamber to avoid any runoff due to rain events. A total of 1 litre per treatment, corresponding to 0.33 litres per plant, was applied in a single experimental application. The plants were brought outside the greenhouse only for the fungicide application: they were moved just before the spray and were brought back inside the greenhouse after the complete dry-off of the application (when the canopy was dried). Treatment was performed on May 2nd 2020 using a backpack sprayer Stihl SR 430. The artificial infection was performed 2 days after, on May 4th 2020. Artificial inoculation was performed with a solution containing 5 x 10^4 sporangium/mL of *Plasmopara viticola* A total of 2.5 litres of solution was prepared and inoculated (having on average 30-32 mL of inoculum per plant). The selected pathogen strain was characterized by complete sensitivity to the fungicides included in the trial.

The artificial infection was performed with a hand-pump on May 4th on all the plants. Plants were then closed in a plastic bag, kept in the climatic chamber and incubated for 24 hours. Evaluation of the disease was performed 1 week after symptom occurrence.

Trt No.	Treatment Name	Rate ml/hl	Application Code
1	Untreated Check		
2	Vacciplant	100	A
3	Vacciplant	150	A
4	Vacciplant	200	A

Table 4 Protocol trial
A = 2 days before the artificial inoculum
Assessment 15th May

3 Results and discussion

Efficacy trials open field 2019

3 assessments were carried out, to evaluate the activity of Vacciplant in tank mix with a half dosage rate of copper (Politglia Disperss) compared with a full dose rate. Treatment 6 follow the rules about EU copper limitation at 4 kgCu/ha.

To focus the attention for each block of application the table below reported the follow treatments: Poltiglia disperss full dosage rate; Poltiglia disperss half dosage rate + Vacciplant; Poltiglia disperss complaining to 4 kg Cu/ha.

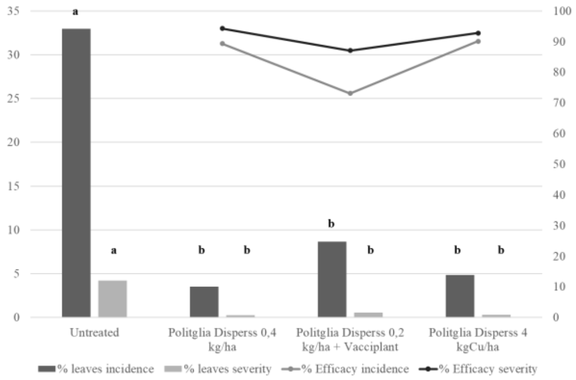

Figure 1: Evaluation of Vacciplant applications in ABCD Assessment BBCH57 27th May 2019. Disease Incidence and severity on leaves (bars) and tested products efficacy (lines) %

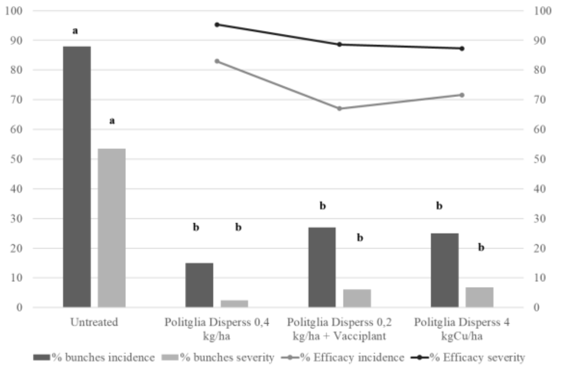

Figure 2: Evaluation of Vacciplant applications in EFGH Assessment BBCH57 10th June 2019. Disease Incidence and severity on bunches (bars) and tested products % efficacy (lines).

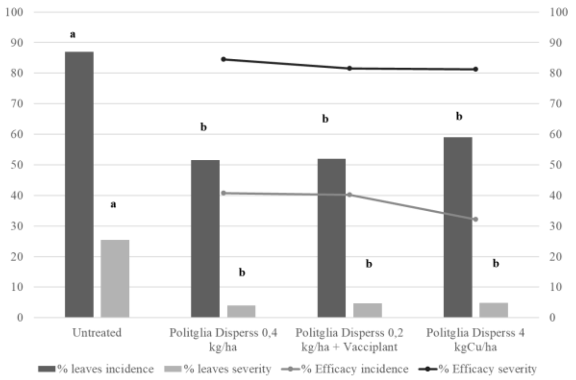

Figure 3: Evaluation Vacciplant applications ILM Assessment BBCH77 3rd July 2019. Disease Incidence and severity on leaves (histograms) and tested products efficacy (lines) %
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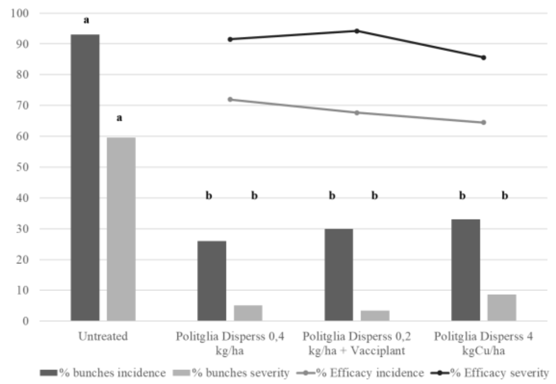

Figure 4: Evaluation Vacciplant applications at ILM Assessment BBCH77 3rd July 2019. Disease Incidence and severity on bunches (histograms) and tested products efficacy (lines) %

Efficacy trials open field 2020

The experimental design adopted was the randomized block with 5 replications and each plot consisted of 5 contiguous plants on the rows. The applications were performed with a pneumatic shoulder-supported nebuliser (mod. Stihl SR 430) distributing a variable volume of water between about 555 and 1000 L/Ha, depending on the phenological stage of the plant. The assessment to evaluate the efficacy of the products, the incidence (% affected leaves and bunches) and the severity (% affected area) of the disease was performed observing 100 leaves and 50 bunches per plot.

In the vineyard where the trial plots were located, the first symptoms of the disease were observed on the leaves on May 26th as consequence of the rains occurred on May 17th and May 19th and of the first overcanopy irrigation executed on May 16th.

The disease appeared slight late in the season with a natural infection. Weather in the spring 2020 was not favourable for downy mildew characterized by a drought climate with a significantly low rainfall. From mid-May to June, canopy irrigations were done twice a week to create favourable conditions to the disease. A total of 13 artificial overcanopy irrigations were carried out (15 mm each). Irrigations were done utilizing micro sprinkler that works at 2,2 bar. During the trial, the crop condition was normal in accordance with local practices for commercial production purposes and allowed to evaluate the activity of plant protection products under test. The assessment performed on June 22nd (figure 6) showed in the untreated check an incidence of 20.4% on leaves with a severity of 3.40%. All strategies applied demonstrated to obtain, on leaves very good results with a control ranging from 97% to 100%. No statistical differences among the different strategies were detected.

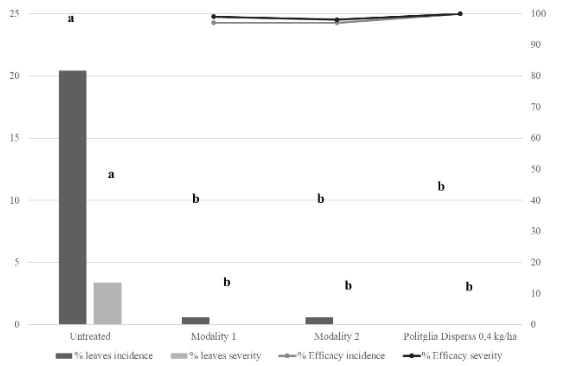

Figure 5: Assessment on leaves 22nd June Disease Incidence and severity on leaves (histograms) and tested products efficacy (lines) %

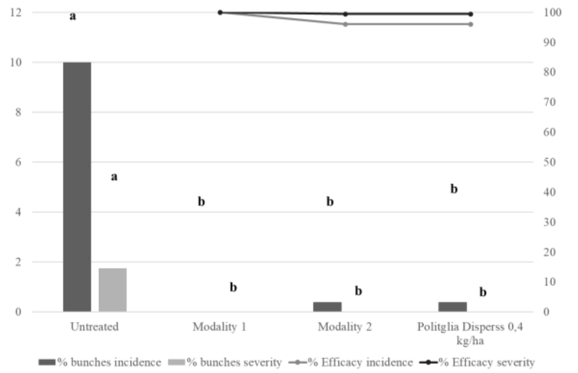

Figure 6: Assessment on bunches 22nd June Disease Incidence and severity on bunches (histograms) and tested products efficacy (lines) %

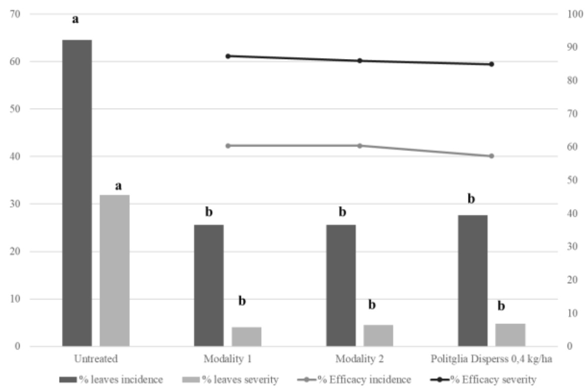

Figure 7: Assessment on leaves 16th July Disease Incidence and severity on leaves (histograms) and tested products efficacy (lines) %

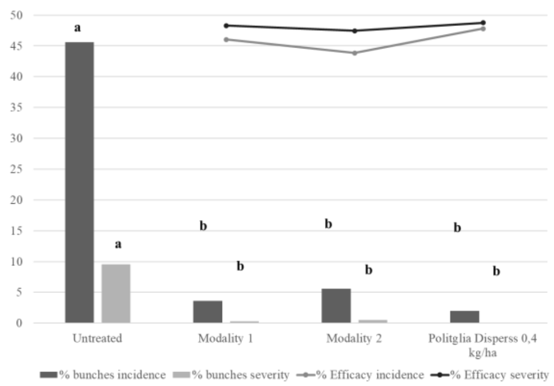

Figure 8: Assessment on bunches 16th July.
Disease Incidence and severity on bunches (histograms) and tested products efficacy (lines) %

The assessment on bunches (figure 7) showed the untreated check an incidence of 10.0% with a severity of 1.75%. As seen previously, all strategies obtained an excellent efficacy around 100% of control for disease incidence and severity.
The last assessment, was carried out on July 16th (figure 8 and 9) on leaves and bunches, showed in the untreated check an incidence on leaves of 64.6% with a severity of 31.85% and on bunches of 45.6% with a severity of 9.52%. The trial highlighted as the strategies in which Poltiglia disperss was used at low dosage (200 g/hL) and in tank mixtures with Laminarine (in phase B and D) obtained similar results (60-87% of efficacy on disease incidence and severity on leaves respectively and 90-95% of disease control on bunches) respect to that achieved by the Poltiglia disperss used at higher dosage (400 g/hL) during all the season (57-85% of efficacy on disease incidence and severity on leaves respectively and 95-99% of disease control on bunches).
No symptoms of phytotoxicity occurred during the trials.

Efficacy trials semi field 2020
The trial was conducted adopting the experimental design, with 3 repetitions/treatment. Every repetition was based on one single potted grapevine plant. The experimental application was performed with a backpacked nebulizer sprayer Mod Stihl SR430. A solution containing 5 x 104 sporangium/mL of *Plasmopara viticola* was sprayed on the plants treated 2 days before the artificial infection. The results were evaluated 11 days from the infection in terms of disease incidence and severity on the leaves of the potted grapevine plants. The assessment performed on May 15th (at 11 days after the application of the inoculum – Figure 10) showed how in the untreated check the inoculum originated infections on 54,7% of the leaves present, affecting a leaf area estimated close to 5%. The results of the trial showed that Vacciplant obtained a good control of the disease (82,6-95,3% of efficacy respectively on the incidence and the severity of the disease). Vacciplant when used at 200 mL/100L increasing efficacy although not statistically evident. Trial demonstrated that two days between application and the artificial inoculum were enough to induce an elicitor effect (self-plants protection). The selectivity assessments were performed just before the artificial infection, to be sure that the plants were all asymptomatic and no problem of phytotoxicity could interfere with the experiment. No phytotoxicity symptom occurred during the experimental application.

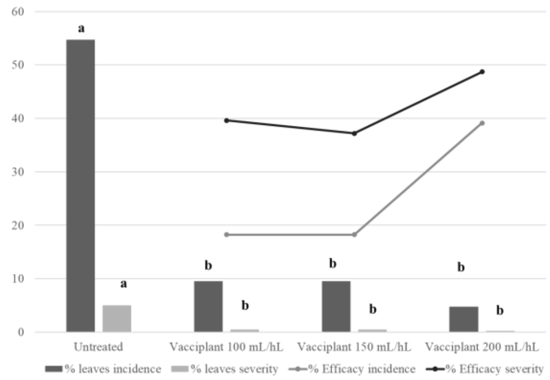

Figure 9: Efficacy of Laminarine application at 2 days before the artificial inoculum

4 Conclusions

In 2 years, experimentation trials indoor and outdoor, it was demonstrated that laminarine based product (Vacciplant), can be effectively included in a strategy for the control of grapevine downy mildew in tank mix with copper to optimize the copper rate to be complaint on EU rules. The elicitor laminarine activity express its activity within one day of the application and maintain a good activity until 9 – 10 days (Taibi O. et al.). The laminarine behavioir and activity could be synchronized with the copper contact product in a sustainable spray interval, with low dosage rate of copper and a very good bunches protection.

References

1. Aziz A, Poinssot B, Daire X, Adrian M, Bézier A, Lambert B, Joubert J-M, Pugin A (2003) Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. Mol Plant Microbe Interact 16:1118–1128
2. Chalal M, Winkler JB, Gourrat K, Trouvelot S, Adrian M, Schnitzler J-P, Jamois F, Daire X (2015) Sesquiterpene volatile organic compounds (VOCs) are markers of elicitation by sulfated laminarine in grapevine. Front Plant Sci 6:1-9
3. Delaunois B, Farace G, Jeandet P, Clément C, Baillieul F, Dorey S, Cordelier S (2014) Elicitors as alternative strategy to pesticides in grapevine? Current knowledge on their mode of action from controlled conditions to vineyard. Environ Sci Pollut R 21:4837-4846
4. Labarre A, Orieux R (2010) Plant defences stimulation: Laminarin, a natural compound for the plant protection. Julius-Kühn-Archive 428:117
5. Ménard R, Alban S, de Ruffray P, Jamois F, Franz G, Fritig B, Yvin J-C, Kauffmann S (2004) β-1,3 Glucan Sulfate, but not β-1,3 Glucan, induces the Salicylic Acid signaling pathway in Tobacco and Arabidopsis. Plant Cell 16:3020–3032
6. Romanazzi G, Mancini V, Feliziani E, Servili A, Endeshaw S, Neri D (2016) Impact of alternative

fungicides on grape downy mildew control and vine growth and development. Plant Disease 100:739-748

7. Taibi O, Bardelloni V, Bove F. Scaglia F. 2, Caffi T. Rossi V. Attività fungicida di induttori di resistenza nei confronti di *Plasmopara viticola* Atti Giornate Fitopatologiche
8. Vera J, Castro J, Gonzalez A, Moenne A (2011) Seaweed polysaccharides and derived oligosaccharides stimulate defense responses and protection against pathogens in plants. Mar Drugs, 9:2514-2525