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Evaluation of a CAA-based Management Strategy for the Downy Mildew Control in a Vineyard with CAA Resistance

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ABSTRACT

The effectiveness of two treatment strategies with or without the CAA fungicide mandipropamid against the grapevine downy mildew agent *Plasmopara viticola* has been evaluated during three grapevine growing seasons in a vineyard with a high disease pressure. CAAs resistance was reported in this location before the study. Compared to the untreated plot (disease severity higher than 65% on leaves and 95% on bunches), both the CAA and NO-CAA strategies adequately and analogously protected the plants from heavy infections, avoiding yield losses (severity <15% on leaves and <4% on bunches). Biological and molecular assays on *P. viticola* populations collected from the untreated and treated plots showed that resistance in the field is mainly associated with G1105V mutation in the target *Ces*A3 gene and that resistance frequency did not increase in the populations when applying the CAA mandipropamid following an anti-resistance strategy.

INTRODUCTION

Plasmopara viticola (Berk. *et* Curt.) Berlese and De Toni, is an oomycete causing grapevine downy mildew, one of the most devastating diseases of *Vitis vinifera* L. in regions characterized by high precipitation rate and mild temperatures. Chemical control is performed to obtain adequate yields at the quantitative and qualitative levels. Active ingredients belonging to CAA fungicide class (FRAC) are often used in spray programs to control *P. viticola*. The active substances belonging to CAAs are cross resistant and considered by FRAC as medium risk to evolve fungicide resistance. Studies carried out on mandipropamid demonstrated that CAAs inhibit cellulose biosynthesis in Oomycetes and that the mechanism of resistance is mainly based on G1105S mutation in the *Ces*A3 gene but also G1105V was found (Blum et *al.* 2010; Sierotzki et *al.* 2011). Resistance inheritance is recessive (Gisi et *al.*

2007): the homozygous strains carrying the wild type G1105 allele and the heterozygous strains are phenotypically sensitive to CAAs, whereas the homozygous strains with either S1105 or V1105 allele are resistant. Sensitivity monitoring activity is required as a consequence of the combined risk derived from the interaction of the pathogen, fungicide and agronomic components. Anti-resistance strategies are recommended for CAA. However up until now no data on the efficacy of the disease control programs in relationship with the sensitivity levels of the populations have not been reported.

In the present study, disease intensity and level of CAA's sensitivity of *P. viticola* populations have been evaluated over a three-year period (2013-2015) in a commercial vineyard located in a region of Northern Italy characterized by severe downy mildew epidemics. At the beginning of the project, *P. viticola* strains resistant to CAAs were already present in the vineyard, due to the high utilization of this class in previous years.

MATERIALS AND METHODS

Field assay

The Pinot gris vineyard was divided into three plots: the first plot (0.1 ha) was not treated against downy mildew, the other plots (1 ha each) were treated according to an identical strategy differing only for treatments three and four (Table 1): at 5-6 leaves unfolded, mandipropamid was applied in the strategy A, whereas cymoxanil was used in the strategy B; between fruit set and berry touch, 2/3 mandipropamid applications were carried out in strategy A, whereas ametoctradin was used in strategy B. The active substances were applied in mixture with an anti-resistance partner using the farmer's equipment.

Table 1 List of the treatments carried out at each phenological stage in the A and B plots

Treatment n.	Phenological stage	Strategy A	Strategy B
1	Shoot length 10 cm	metiram	metiram
2	5-6 leaves unfolded	mandipropamid+mancozeb	cymoxanil+mancozeb
3	Inflorescence development	metalaxyl M+mancozeb	metalaxyl M+mancozeb
4	Pre-bloom/bloom	metalaxyl M+mancozeb	metalaxyl M+mancozeb
5	Fruit set	mandipropamid+zoxamide[1]	ametoctradin+metiram[1]
6	Fruits swelling	mandipropamid+zoxamide[2]	ametoctradin+metiram
7	Before berry touch	mandipropamid+zoxamide[2]	ametoctradin+metiram
8	Berry touch	copper oxychloride	copper oxychloride
9	Berry touch	copper oxychloride	copper oxychloride

[1]Replaced by cyazofamid in 2014 and 2015

[2]Zoxamide was replaced by folpet in 2014 and 2015

Disease evaluation

Each year at berry touch (between beginning and mid July), disease severity was assessed. The disease severity was evaluated by scoring four replicates of 100 leaves and bunches for the percentage of symptomatic area of each organ and calculating the percentage infection index

(I%I) as described by Toffolatti and coworkers (2016). The index of effectiveness (I%E) of the two treatment strategies was calculated by the Abbott's formula (Abbott 1925). ANOVA and multiple comparison (REGW-F) of the mean I%I and I%E values were carried out in order to evaluate the existence of significant differences between the different plots. Statistical analysis was carried out using SPSS v. 23.

Sensitivity assays

The sensitivity to CAA of *P. viticola* populations was evaluated by both biological and molecular assays after randomly collecting 100 leaves showing downy mildew symptoms in each plot at berry touch. The leaves were washed under running tap water and incubated overnight in humid chamber at 20-22°C. The next day, the fresh sporangia were resuspended in sterile distilled water obtaining a bulk suspension, half of which was stored at -20°C for molecular assays. The sporangia bulk was immediately used for inoculations (Toffolatti *et al.* 2016) of leaf discs (cv Cabernet sauvignon) previously sprayed with different concentrations of mandipropamid (0, 0.1, 1, 5, 10, 100 mg/L a.i.) and dimethomorph (0, 0.1, 1, 10, 100 and 300 mg/L a.i.). Commercial formulates containing mandipropamid (Pergado SC, 250 g/L a.s.) and dimethomorph (Forum 50 WP) were used. Six leaf discs (Ø 1.5 cm) derived from different leaves were sprayed with each fungicide concentration and placed, lower surface upwards, in growth chamber for 7-10 days at 20-22°C. The EC_{50} values were calculated by probit analysis (SPSS v. 23) using the percentages of sporulation inhibition (IS) at each fungicide concentration. EC_{50} values higher than 10 mg/L indicate the presence of resistance (Sierotzki et *al.* 2011). Following DNA extraction (Toffolatti et *al.* 2007), the percentages of the allelic variants associated to sensitivity (G1105) or resistance (S1105 or V1105) in sporangia bulks were estimated by the allele-specific real time PCR (Eppendorf Mastercycler Realplex) method developed by Sierotzki and coworkers (2011).

RESULTS

Field trials

The meteorological conditions in vineyard were highly favourable to the pathogen epidemics in the three grapevine seasons of investigation, even if differences could be found (Figure 1).

The 2013 spring period was characterized by moderate average daily temperatures and high rain amount (400 mm between April and May), whereas the summer period was particularly dry (36 mm in June-July) (Figure 1A). The mandipropamid treatments were applied on May 1 (in mixture with mancozeb) and on June 2, 11 and 19 (in mixture with zoxamide). The first oil spots were observed on 15th of May, probably due to the contaminations occurred from 6th to 8th May 2013. Heavy contaminations occurred on leaves (I%I= 66%) and bunches (I%I= 97.4%) on bunches in the untreated plot (Table 2). The disease severity was significantly lower on A and B plots, which showed analogous I%E.

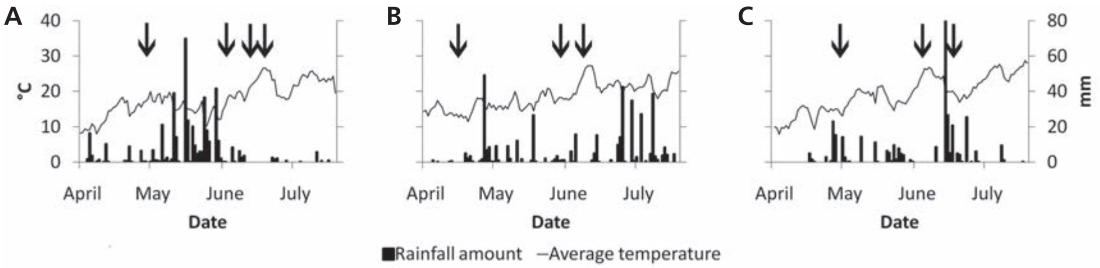

Figure 1 Daily rainfall amount (mm) and average temperatures (°C) recorded in Casarsa della Delizia (PN) during spring-early summer 2013 (A), 2014 (B) and 2015 (C). The arrows indicate the date of the mandipropamid treatment.

In 2014, compared to the mean values of the area, the average daily temperatures were particularly high in spring and low in summer (Figure 1B). Frequent rainfall led to a high disease pressure in summer. Due to the high temperatures occurring in spring and to the early phenological stages of grapevine, the first mandipropamid treatment was applied on April 23 (in mixture with mancozeb) and the others on May 29 and June 8 (in mixture with folpet). The first sporadic symptoms of the disease were observed on May 20. At bunch closure the disease severity on both leaves and bunches was higher than 92% in the untreated plot (Table 2). The two treatment strategies analogously protected grapevine from *P. viticola*, as demonstrated by the particularly low disease severity (I%I< 2 %) and by the high effectiveness (I%E>98%) indexes of the two plots.

Table 2 Severity of downy mildew on leaves and bunches (I%I), effectiveness of the two treatment strategies (I%E) in field trials and results of statistical analysis*

Year	Plot	I%I leaves	I%I bunches	I%E leaves	I%E bunches
2013	Untreated	65.6 a	97.4 a	-	-
	A	12.7 b	4.1 b	80.7 a	95.8 a
	B	5.6 b	0.8 b	91.4 a	99.2 a
2014	Untreated	92 a	95 a	-	-
	A	2 b	1 b	97.9 a	99.2 a
	B	1 b	0.3 b	99.0 a	99.7 a
2015	Untreated	92 a	96 a	-	-
	A	15 b	2 b	86.6 a	97.1 a
	B	12 b	3 b	83.9 a	97.8 a

*different letters correspond to significant differences among I%I and I%E values of the three plots with P>95%

In 2015, the rainy events were particularly frequent in the second half of May and June (Figure 1C). The first downy mildew symptoms appeared later than the two previous years (June 3) but the disease showed a more rapid increase: in the untreated plot at the beginning of July the disease symptoms were observed on 100% of leaves and clusters, with I%I higher than 92%. The CAA treatments were carried out on May 1 (in mixture with mancozeb) and on June 6 and 17 (in mixture with folpet). An analogous and adequate protection, particularly of the bunches, was obtained in both the treated plots (Table 2).

Sensitivity assays

The I%I of the leaf discs treated with mandipropamid and inoculated with sporangia suspensions were similar at all fungicides concentrations (Figure 2) in almost all the *P. viticola* populations sampled from 2013 until 2015, apart from those of the 2015 untreated and B plots. As a consequence, the EC_{50} values were always higher than 100 mg/L mandipropamid, with the exception of the samples collected at from the untreated (EC_{50}=54 mg/L a.i.) and B plots (EC_{50}=27 mg/L a.i.) of 2015 (Table 3).

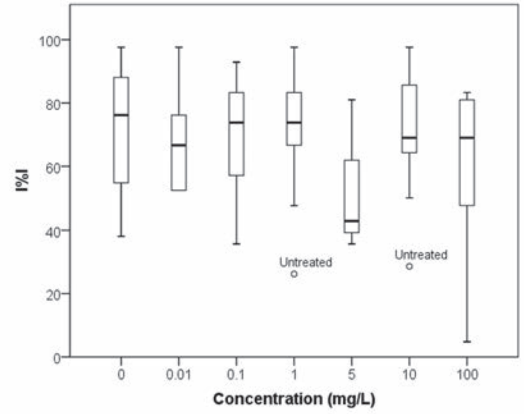

Figure 2 Box-plot distribution of I%I values at each mandipropamid concentration following *P. viticola* inoculation.The outlier is represented by the 2015 untreated plot.

The G1105 and S1105 alleles were seldomly found in most of the samples, whereas the V1105 allele was detected at the highest frequency (>53%), indicating the presence of numerous pathogen strains that are CAA resistant and probably homozygous for V1105 (Table 3). These results are consistent with the EC_{50} values of the biological assays, which are higher than 10 mg/L in all the plots. Interestingly, in the B plot the percentage of G1105 increased in 2015, indicating a shift towards sensitivity during the third year of CAAs suspension.

Table 3 EC_{50} values (mgL^{-1}) and percentages of the three allelic variants at codon 1105 associated with sensitivity (G1105) or resistance to mandipropamid (S/V1105) in *P. viticola* populations isolated from the untrated and treated plots in 2013-2015

Year	Plot	EC_{50}	G1105	S1105	V1105
2013	Untreated	>100	8	12	81
	A	>100	2	5	93
	B	>100	10	20	70
2014	Untreated	>100	6	2	92
	A	>100	6	5	89
	B	>100	6	2	92
2015	Untreated	54	12	8	81
	A	>100	16	5	79
	B	27	34	14	53

4. CONCLUSIONS

The field located in northern Italy was characterized by the presence of resistance to CAAs, mainly associated with the V1105 allele, throughout the trials. Despite this, the CAA based strategy applied in the A plot showed a good effectiveness, identical to that found in the no-CAA strategy (B plot). This indicates that sound disease management strategy based on CAA applications in mixture and in alternation with fungicides having other mode of actions, could be effectively employed in the protection of grapevine from the downy mildew agent also in a high disease pressure area and in presence of resistant strains, without particularly increasing the frequency of mutated alleles and therefore the risk of practical field resistance.

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