Effectiveness of control strategies against Botrytis cinerea in vineyard and evaluation of the residual fungicide concentrations

Effectiveness of control strategies against *Botrytis cinerea* in vineyard and evaluation of the residual fungicide concentrations.

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ABSTRACT

Different control strategies against *Botrytis cinerea* have been tested in vineyards. The pesticide residues at harvest and the control efficacy of each strategy, have been determined. Two commercial vineyards – one “Barbera” and one “Moscato” – located in Piedmont (Northern Italy) were divided in seven plots and treated with different combinations of fungicides.

The tested fungicides were based on pyrimethanil, fludioxonil + cyprodinil, iprodione, and boscalid, a new carboxamide compound. An integrated strategy including a chemical (pyrimethanil) and a biocontrol agent (*Trichoderma* spp. t2/4ph1) was also included. At harvest, the percentage of bunches and berries attacked by *B. cinerea* and the concentration of the chemical fungicides were determined. All the pesticide residues at harvest were below the MRL (maximum residue level), except when two applications of pyrimethanil per season were applied. Boscalid was the most effective active ingredient against *B. cinerea* among the tested ones. Its efficacy, when its application was followed by a treatment with pyrimethanil, was similar to the efficacy shown by two treatments of pyrimethanil. This second strategy anyway is not feasible, due to the risks of resistance development in the pathogen and to the residue accumulation, as the analysis showed.

Keywords*:* boscalid, fungicide, grapevine, grey mould, residue, wine.

INTRODUCTION

In Italy, grapes represent the most widespread fruit crop (1.5 million tonnes of table grapes and 47.1 millions hL of wine [1]). *Botrytis cinerea* Pers. Ex Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel), the causal agent of grey mould, causes severe losses on grapevine (*Vitis vinifera* L.), affecting wine quality. Control is achieved by integrating canopy and cluster management with fungicide treatments, generally applied twice per season, at touching of berries and veraison [2].
Several families of synthetic chemicals are available for the control of *B. cinerea*. They include specific botryticides, such as dicarboximides which inhibit the lipid and membrane synthesis. For over 25 years, dicarboximides – chlozolinate, iprodione, procymidone and vinclozolin – have been the most popular class of specific fungicides against grey mould. Vinclozolin has been banned for toxicological safety reasons. The repeated use of this fungicide class has caused the development of strains of *B. cinerea* resistant to dicarboximides, prevalent in the Italian and French vineyards [3, 4].
During the last years, new molecules with specific action against *B. cinerea*, such as the anilinopyrimidines, including cyprodinil and pyrimethanil, the phenylpyrrole fludioxonil, the hydroxyanilide fenhexamid, and the carboxamide boscalid, have been introduced into the market. These active ingredients exploit novel mechanisms of action, allowing new strategies of

intervention against resistance. Excellent control was obtained with anilinopyrimidines, that inhibit the biosynthesis of methionine, affecting the cystathionine-β-lyase [5, 6] and block the excretion of hydrolytic enzymes involved in the pathogenic process [7]. The phenylpyrroles affect cell wall synthesis and cause the accumulation of glycerol in mycelial cells: their primary target sites are protein kinases involved in the regulation of polyol biosynthesis [8]. Fenhexamid is able to inhibit the sterol biosynthesis, belonging to the class III of sterol biosynthesis inhibitors [9]. The carboxamides block the energy production in the fungal cells, by inhibiting the succinate ubiquinone reductases containing sulphur [10].

In most grapevine growing areas, populations of *B. cinerea* resistant to benzimidazoles and/or dicarboximides are widespread [11], but their incidence is decreasing, thanks to the introduction of anilinopyrimidines and phenylpyrroles that provide good levels of disease control [12]. The availability of novel classes of compounds does not imply the total replacement of the older fungicides, but represents a new powerful tool for the growers. Against grey mould, two sprays per season are applied, and such strategy permits an effective alternation of dicarboximides, anilinopyrimidines, phenylpyrroles, hydroxyanilides and carboxamides.

Consumers more and more request foodstuffs, including wine, with low levels of pesticide residues. The alternation of different fungicides and the introduction of more efficient and less persistent compounds should contribute to the reduction of pesticide residues at harvest, permitting to improve of the quality of the grapes. According to an Italian Residue Monitoring Programme conducted by the Ministry of Agriculture on grape samples collected in the field, 7.9%, 6.5%, and 2.5% samples were irregular in 1996, 1998, and 1999 respectively [13]. Most of the irregular levels found were caused by poor compliance of the pre-harvest interval, especially after repeated treatments with the same active ingredients.

Studies about the fate of the major pesticides used in vineyard have been reviewed by Cabras and Angioni [14]. Among the fungicides, pyrimethanil seemed the most persistent with residue levels constant up to harvest, whereas fluazinam, cyprodinil, mepanipyrim, azoxystrobin, and fludioxonil showed higher disappearance rates ($t_{1/2}$ were 4.3, 12, 12.8, 15.2, and 24 days, respectively). Pesticide residues in wine were always smaller than those on the grapes and in the must, except for those without a preferential partition between liquid and solid phase (azoxystrobin, dimethoate, and pyrimethanil) present in wine at the same concentration than on the grapes.

The aim of this work was to test the effectiveness against grey mould of different fungicide control strategies on two experimental vineyards belonging to the cultivars “Barbera” and “Moscato”. The tested fungicides were boscalid (2-chloro-N-(4’-chlorobiphenyl-2-yl)-nicotinamide), a new carboxamide compound, cyprodinil (4-cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine), fenhexamid (2’,3’-dichloro-4’-hydroxy-1-methylcyclohexanecarboxanilide), fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-pyrrole-3-carbonitrile), iprodione (3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboximide), and pyrimethanil N-(4,6-dimethylpyrimidin-2-yl)aniline). An integrated control strategy including a chemical (pyrimethanil) and a biocontrol agent (*Trichoderma* spp. t2/4ph1) was also included. A second aim was to determine the residual concentration of the fungicides on the grapes at harvest, to understand if the treatment strategies were able to keep the residues within the MRLs (maximum residue levels).

MATERIALS AND METHODS

Experimental design

Two experimental trials were carried out in two commercial vineyards located in Piedmont (Northern Italy): one planted with the white cultivar “Moscato” (Valdivilla, Asti Province) and the other one planted with the red cultivar “Barbera” (Vezza d’Alba, Cuneo Province). Every replicate plot was 10 m long and consisted of 10 vines each, with an untreated row marking the border between the plots. All the treatments were arranged in a randomized block design with four replicate plots per treatment. Six different disease strategies were performed in each vineyard, applying the fungicides at the rates and in the dates indicated in Table 1. Five treatment schemes included two fungicide sprays at two phenological stages crucial for grey mould control during the cropping season: before bunch closure (B stage) and between veraison and preharvest interval (C stage). The integrated control scheme included a chemical treatment (pyrimethanil) and four biocontrol agent (*Trichoderma* spp. t2/4ph1) applications, at flowering (A stage), before bunch closure (B stage), at veraison (C stage) and three weeks before harvesting (D stage). The *Trichoderma* strain was a biocontrol agent isolated and studied by AGROINNOVA – University of Torino for its efficacy against *B. cinerea* on grapes [15]. The strain was produced in liquid hydrolyzed casein without shaking for 30 days at 26°C. The fungal mycelium was filtered and resuspended in water to 10^8 conidia mL^{-1}. The fungicides and the biocontrol agent were applied by a motor knapsack sprayer, by using 400 l/ha of water. Four untreated plots were used as control.

Efficacy against *Botrytis cinerea*

At harvest time (18 September 2006 for "Moscato" cultivar, and 2 October 2006 for "Barbera" cultivar), a survey on the incidence of *Botrytis cinerea* was performed, evaluating the percentage of grapes with symptoms and the percentage of berries attacked in every bunch. Samples of rotten bunches were brought to the laboratory to confirm the pathogen identification through plating on Potato Dextrose Agar (PDA; Merck) with 50 mg L^{-1} of streptomycin Merck. The Duncan's Multiple Range Test was employed at $P<0.05$ for the analysis of the data and the SPSS-WIN 13.0 program was used.

Reagents

All reagents were analytical or HPLC grade. The analytical standards of boscalid, cyprodinil, fenhexamid, fludioxonil, iprodione, and pyrimethanil were provided by Sigma-Aldrich (Milano, Italy). The Supelclean LC18 columns (Supelco, 1g, 6 mL) were provided by Sigma-Aldrich.

Apparatus and operating conditions

Chromatographic analyses of sample extracts were performed with a Spectra System 2000 equipped with a SupelcoSil column ™ LC-ABZ (25cm x 4.6mm; 5µm), and a Spectra Series UV 100 detector. The mobile phase was water acidified to pH 3 with phosphoric acid (A) and acetonitrile (B). The composition of the mobile phase (% A: %B, V/V) and the detection wavelength were as follows: boscalid 50:50, 230 nm; cyprodinil and fludioxonil 58:42, 270 nm; fenhexamid 55:45, 230 nm; iprodione 45:55, 240 nm; pyrimethanil 45:55, 270 nm. The retention times were as follows: 7.0 min for boscalid, 4.7 min for cyprodinil, 7.6 min for fenhexamid, 6.2 min for fludioxonil, 2.7 min for iprodione, and 9.5 min for pyrimethanil. Typical chromatograms of the pesticides (analytical standards and samples) are illustrated in figure 1.

Mass spectrometry analysis

The identity of each peak was confirmed by LC-MS/MS using a Varian HPLC–MS/MS system consisted of a ProStar 410 autosampler, two ProStar 212 pumps, and a 310 MS triple quadrupole mass spectrometer equipped with an electrospray ionization source, the ESI-MS interface was operated in the positive mode at 200°C. The transitions used were m/z 343 → 140 for boscalid, m/z 226 → 209 for cyprodinil, m/z 303 → 142 for fenehexamid, m/z 249 → 183 for fludioxonil, m/z 331 → 163 for iprodione, and m/z 200 → 107 for pyrimethanil.

Extraction procedure

Each plot was separately harvested and the grapes – 1 Kg randomly per plot - were stored at -2'°C up to the determination of the fungicide residues. Each sample was defrosted at around 0°C for 12 h, then homogenised with a food cutter (Princess 2080). 100 mL of an acetone-methanol solution (50:50, V/V) were added to 25 g sub-sample in a 250 mL bottle. The suspension was shaken on a mechanical stirrer for 15 min, then centrifuged at 3000 rpm for 15 minutes. The extraction was repeated, after the removal of the supernatant, with 50 mL of extracting solution, 5 min stirring and 5 min centrifugation. The two supernatants were filtrated on hydrophilic cotton and collected in a

250 mL volumetric flask. The solution was brought to volume with water. A 25 mL aliquot of this solution, diluted with 200 mL of water, was eluted on Supelclean LC18 columns, previously conditioned with 6 mL methanol, then with 6 mL water. The eluate was discarded and the column was eluted with 5 mL methanol, collected in a volumetric flask and analysed by LC.

Recovery experiments

In order to determine the recovery of the analytical procedure, samples of 25 g of grapes from an untreated vineyard were spiked with 1 mL acetone solutions of the fungicides at different concentrations. After solvent evaporation (about 30 min), the fungicide concentrations were measured according to the above described procedure. The percentage of recovery for each fungicide is indicated in table 2.

RESULTS AND DISCUSSION

One of the aims of the work was to compare the efficacy of boscalid, belonging to the newborn chemical group of carboxamides, with the effectiveness of other products registered on the market. By designing the disease control strategies, particular attention was paid to choose fungicides belonging to different chemical groups for the two treatments, in order to reduce the risk of pathogen resistance. To compare the effectiveness of boscalid with pyrimethanil, two treatments with such anilinopyrimidine were applied in treatment 4 (Table 1).

The weather conditions during 2006 contributed to produce heavy attacks of grey mould: 68.2% of the bunches were rotted in the commercial vineyard of Moscato and 88.0% in the Barbera vineyard (table 3). During April, May and June, approximately 50 mm of rain felt – about a quarter of the average precipitation for the period. July was characterized by heavy rainfalls (such as 63.6 mm on July 4, 2006), spaced by hot and dry periods, so the Botrytis attacks, normally occurring from the veraison, were not present. Since the beginning of August and for all month long, continued and low intensity rains favoured the attacks of *B. cinerea*. In September, heavy rainfalls (about 125 mm in 48 hours) caused the explosion of the disease.

The white cultivar Moscato was chosen because of its susceptibility to *B. cinerea*. The cultivar Barbera was chosen as a representative one among the red varieties and for its importance in the Piedmont wine production. In presence of strong grey mould attacks, the best results on both varieties were obtained by treating with boscalid at phenological stage B and pyrimethanil at stage C, with pyrimethanil both at stages B and C, and with fenhexamid at stage B and a mixture of cyprodinil and fludioxonil at stage C (Table 3). Lower efficacy results were obtained by using a mixture of cyprodinil and fludioxonil at stage B and fenhexamid in C, or fenhexamid at stage B followed by iprodione in C. The results obtained by applying *Trichoderma* spp. t2/4ph1 at stages A, B and D and pyrimethanil at stage C were not statistically different from the untreated control, except for the number of bunches attacked in the Moscato vineyard.

Differently from the past, when *B. cinerea* control mainly relied on the dicarboximides, new botryticides, belonging to the four different chemical groups (anilinopyrimidines, phenylpyrroles, hydroxyanilides and carboxamides) and based on four different modes of action, constitute effective options for grey mould control and anti-resistance management strategies [2].

Boscalid was one of the most effective products among the chosen ones. Its efficacy when followed by a treatment with pyrimethanil (trial 1) was similar to the efficacy shown by two treatments of pyrimethanil (trial 4). This second strategy anyway presents some constraints, related to the risk of resistance development [12, 16, 17] and to the residue accumulation, as the analysis showed.

The third strategy that permitted a high control of *B. cinerea* included a treatment with fenhexamid followed by a treatment by cyprodinil+fludioxonil (treatment 5). A normally used strategy, based first on a treatment with cyprodinil+fludioxonil and then one with fenhexamid (treatment 2), in order to exploit the preventive action of the anilinopyrimidines [18], showed to be less effective either on Moscato or on Barbera vineyards.

The efficacy data provided by the use of fenhexamid and iprodione were not satisfying, especially on the cultivar Barbera, probably for the high number of strains of *B. cinerea* resistant to dicarboximides [3]. Finally, the application of three treatments of a *Trichoderma* spp. was really ineffective against grey mould, even though in association with pyrimethanil (treatment 3).

The data of efficacy were compared with the residue analysis in the grapes harvested from different plots, to guarantee the possibility of practically using the designed strategies in vineyard, keeping the level of fungicide residues below the maximum residue level.

The maximum residue level (MRL) on grapes and the pre-harvest interval for each fungicide, according to the European regulation [19] are as follows: 5 mg Kg^{-1} and 21 days for cyprodinil, 5 mg Kg^{-1} and 7 days for fenhexamid, 2 mg Kg^{-1} and 21 days for fludioxonil, 10 mg Kg^{-1} and 28 days for iprodione, and 3 mg Kg^{-1} and 21 days for pyrimethanil. Grapes were harvested respectively 35 days and 58 days after the last fungicide application in the Valdivilla and Vezza vineyards, therefore largely beyond the pre-harvest intervals. Since boscalid is a newly registered fungicide, the European MRL is 5 mg Kg^{-1} but a worldwide harmonization is still not achieved [20]: MRLs on grapes are higher in Japan and other countries (10 mg Kg^{-1}).

The residual concentrations of the tested fungicides at harvest are reported in table 3. The concentration of boscalid was lower than 0.30 mg Kg^{-1} in grapes of both vineyards. To our knowledge, no data concerning boscalid residues in grapes have been published. Chen et al. [21] measured the dissipation rate of boscalid on cucumbers treated with a commercial WG BASF formulation at 0.50 and 0.83 Kg a.i/ha, observing a rapid dissipation of the a.i, leading to residues lower than 0.2 mg Kg^{-1} after 6 days.

The concentration at harvest of the other fungicides tested were lower than the MRL in all the treatments, except in the treatment 4 where two subsequent treatments with Scala provoked an accumulation of pyrimethanil with residual concentrations higher than the MRL (3 mg Kg^{-1}) in the grapes collected from both vineyards. Such result is in contrast with previous findings reported by Rabølle et al. [22] on strawberries where 0.15 mg Kg^{-1} pyrimethanil were found after 42 and 29 days from the treatments. When a single treatment was performed, the pyrimethanil concentration varied from 0.24 mg Kg^{-1} (trial 3, 65 DAT) to 1.37 mg Kg^{-1} (trial 1, 58 DAT). These results suggest that, although the same formulation and application rate were used, the dissipation rate of pyrimethanil varied among the trials. This is in agreement with the discrepancy between the results of Cabras et al. [23] who found 1.11 mg Kg^{-1} pyrimethanil on grapes at 28 DAT and those of Angioni et al. [24] who found 0.45 mg Kg^{-1} in similar conditions. In an experiment conducted on agar plates, Vaughan et al. [25] observed that the metabolic activity of the actively growing mycelium of *Botrytis cinerea* provoked the mobility of pyrimethanil within the agar medium. An interaction between the development of *B. cinerea* and the disappearance of pyrimethanil seems to be confirmed by our data since the lowest concentrations of residues (trial 3) corresponds to the highest percentage of bunches with grey mould. The potential persistence of pyrimethanil on grapes is of concern since it has been reported that the fungicide passes into the wine during wine-making [23].

Both cyprodinil and fludioxonil treatments leaded to a final level of residues much lower than the MRLs in all the trials. The highest concentrations were 0.56 mg Kg^{-1} of fludioxonil and 0.42 mg Kg^{-1} cyprodinil (trial 5, moscato, 35 DAT). The low persistence of these two compounds on grapes was attested by Marin et al. [26] who report no detectable levels of fludioxonil and 0.030 mg Kg^{-1} cyprodinil 21 days after treatment. In contrast, higher residual concentrations of 1.03 mg Kg^{-1} cyprodinil and 0.78 mg Kg^{-1} fludioxonil were found by Cabras et al. [23] at 28 DAT.

Also the residues of fenhexamid were largely below the MRL (5 mg Kg^{-1}) in all the trials. The highest value was 0.56 mg Kg^{-1} (trial 3, 35 DAT). This result is in agreement with that of Cabras et al. [27] who found 0.80 mg Kg^{-1} 21 DAT while the residual concentration measured by Rabølle et al. [22] on strawberries was lower (0.041 mg Kg^{-1}), although the crop was treated twice.

The residual concentration of iprodione was between 2.01 and 4.23 mg Kg^{-1}, therefore below the MRL (10 mg Kg^{-1}), but about one order of magnitude higher than the other tested fungicides except pyrimethanil. The behaviour of iprodione in plants is largely documented in literature, in particular in a review of Cabras et al. [28] concerning the fate of pesticides from vine to wine. The residues reported in grapes varied between 0.46 and 8.3 mg Kg^{-1} depending on the application conditions. Recent studies confirmed the high persistency of iprodione on the treated fruits: Cabras et al. [29] found 1.09 mg Kg^{-1} on apricots 21 DAT and Stensvand and Christiansen [30] 2.2 mg Kg^{-1} in green-house grown strawberries.

At the moment, the MRL are fixed for single active ingredient but there is concern about the possible synergic effect on the human health due to the presence of residues of different fungicides on the same sample. In Italy, part of the large distribution is promoting high quality products with residues levels not higher than 30% of the MLR and also the cumulative residues start to be considered, by adopting the empirical equation: Cumulative index = $\Sigma(R_i/MRL_i)$x100. (R_i = residual concentration of pesticide i). To exclude any risk for the consumer health, the cumulative index should be lower than 100. In this experiment, the cumulative index was lower than 100 for all the treatments except for treatment 5, because of the high concentration of pyrimethanil already discussed.

CONCLUSION

Except in the case of two subsequent treatments with pyrimethanil, the chemical control of *B. cinerea* with the tested fungicides should not be dangerous for the human health, taking in consideration the residual concentrations found. The efficacy data provided by the use of fenxhexamid or iprodione were not satisfying, and the application of a formulation based on the biocontrol agent *Trichoderma* spp. t 2/4ph1 was ineffective against grey mould, even in association with pyrimethanil. The new carboxamide compound boscalid was more effective against grey mould than the other treatments.

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Table 1. Experimental design of the trials carried out during 2006 to evaluate the efficacy of different control strategies against *Botrytis cinerea* in two vineyards of Moscato and Barbera varieties.

Treatment	Active ingredient (%)	Commercial formulate	Application rate (g a.i. 100 l^{-1})	Application date (days between treatment and harvest).	
				cv. Moscato	cv. Barbera
1	Boscalid (50.0%)	Cactus	60	08-07 (65d)	12-07 (98d)
	Pyrimethanil (37.4%)	Scala	74.8	08-08 (35d)	23-08 (58d)
2	Cyprodinil (37.5%) + fludioxonil (25.0%)	Switch	30 + 20	08-07 (65d)	12-07 (98d)
	Fenhexamid (50.0%)	Teldor	75	08-08 (35d)	23-08 (58d)
3	*Trichoderma* spp. t2/4ph1			13-06	17-06
	Pyrimethanil (37.4%)	Scala	74.8	08-07 (65d)	12-07 (98d)
	Trichoderma spp. t2/4ph1			31-07	05-08
	Trichoderma spp. t2/4ph1			22-08	28-08
4	Pyrimethanil (37.4%)	Scala	74.8	08-07 (65d)	12-07 (98d)
	Pyrimethanil (37.4%)	Scala	74.8	08-08 (35d)	23-08 (58d)
5	Fenhexamid (50.0%)	Teldor	75	08-07 (65d)	12-07 (98d)
	Cyprodinil (37.5%) + fludioxonil (25.0%)	Switch	30+20	08-08 (35d)	23-08 (58d)
6	Fenhexamid (50.0%)	Teldor	75	08-07 (65d)	12-07 (98d)
	Iprodione (50.0%)	Rovral	75	08-08 (65d)	23-08 (58d)
7 (control)					

Table 2. Percentage of recovery of the pesticides from spiked grape samples

Fungicide	Fortification level mg Kg^{-1}	% Recovery ± RSD (3 replicates)
boscalid	1.2	89 ± 3
	0.7	87 ± 2
	0.2	87 ± 3
cyprodinil	1.0	86 ± 2
	0.5	85 ± 2
	0.1	87 ± 2
fenhexamid	1.2	89 ± 4
	0.7	85 ± 3
	0.1	90 ± 4
fludioxonil	1.0	92 ± 2
	0,6	93 ± 3
	0.1	92 ± 2
iprodione	1.0	92 ± 4
	0.6	86 ± 3
	0.1	87 ± 3
pyrimethanil	1.0	86 ± 3
	0.5	89 ± 2
	0.1	90 ± 3

Table 3 . *Botrytis cinerea* attacks on bunches and berries (%) and residues of the botryticides used at harvest in the trials carried out during 2006 in two vineyards of Moscato and Barbera varieties.

Treatment	Variety	Percentage of bunches attacked by *Botrytis cinerea*		Percentage of berries attacked by *Botrytis cinerea*		Fungicide concentration at harvest (mg kg^{-1} ± S.D.)					
						Boscalid	Cyprodinil	Fenhexamid	Fludioxonil	Iprodione	Pyrimethanil
1	Moscato	***22.9**	**a**	***2.4**	**a**	0.30 ± 0.15					1.26 ± 0.28
	Barbera	**30.3**	**a**	**7.3**	**a**	0.26 ± 0.18					1.37 ± 0.14
2	Moscato	**32.9**	**b**	**7.0**	**ab**		0.17 ± 0.01	0.56 ± 0.19	0.17 ± 0.01		
	Barbera	**68.0**	**b**	**20.9**	**ab**		0.20 ± 0.01	0.45 ± 0.07	0.16 ± 0.01		
3	Moscato	**51.5**	**c**	**9.3**	**bc**						0.24± 0.04
	Barbera	**81.3**	**b**	**22.9**	**ab**						0.47 ± 0.10
4	Moscato	**18.7**	**a**	**3.1**	**a**						**5.80 ± 1.50**
	Barbera	**35.3**	**a**	**3.8**	**a**						**3.81 ± 0.79**
5	Moscato	**23.1**	**a**	**5.7**	**ab**		0.42 ± 0.24	0.41 ± 0.24	0.56 ± 0.02		
	Barbera	**34.0**	**a**	**6.8**	**a**		0.37 ± 0.22	0.28 ± 0,04	0.44 ± 0.02		
6	Moscato	**29.5**	**ab**	**5.7**	**ab**			0.21 ± 0.02		4.23 ± 0.36	
	Barbera	**57.0**	**ab**	**27.3**	**ab**			0.18 ± 0.02		2.21 ± 0.31	
7	Moscato	**68.2**	**d**	**14.7**	**c**						
	Barbera	**88.0**	**b**	**48.4**	**b**						

*Values followed by the same letter within the same cultivar are not statistically different by Duncan's Multiple Range Test (*P* < 0,05).

FIGURE CAPTIONS

Figure 1. Typical chromatograms (A): boscalid (analytical standard at 1.1 mg Kg^{-1}, sample of Barbera from trial 1), (B): cyprodinil (analytical standard at 0.5 mg Kg^{-1}, sample of Barbera from trial 2), (C): fenexhamid (analytical standard at 0.96 mg Kg^{-1}, sample of Barbera from trial 9), (D): fludioxonil (analytical standard at 0.5 mg Kg^{-1}, sample of Barbera from trial 7), (E): iprodione (analytical standard at 1.0 mg Kg^{-1}, sample of Moscato from trial 6), (F): pyrimethanil (analytical standard at 0.5 mg Kg^{-1}, sample of Moscato from trial 4).

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