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# UNIVERSITÀ DEGLI STUDI DI TORINO

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***This is an author version of the contribution published on:***

*Questa è la versione dell'autore dell'opera:*

*[Gabriolotto C., Monchiero M., Nègre M., Spadaro D., Gullino M.L. (2009) -  
Effectiveness of control strategies against Botrytis cinerea in vineyard and evaluation  
of the residual fungicide concentrations. Journal of Environmental Science and  
Health: Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 44 (4),  
389-396, DOI: 10.1080/03601230902801117]*

***The definitive version is available at:***

*La versione definitiva è disponibile alla URL:*

*[<http://www.tandfonline.com/doi/full/10.1080/03601230902801117#.U4smmyjm6eA>]*

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

19

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34

35 **ABSTRACT**

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

40 The tested fungicides were based on pyrimethanil, fludioxonil + cyprodinil, iprodione, and boscalid,  
41 a new carboxamide compound. An integrated strategy including a chemical (pyrimethanil) and a  
42 biocontrol agent (*Trichoderma* spp. t2/4ph1) was also included. At harvest, the percentage of

43 bunches and berries attacked by *B. cinerea* and the concentration of the chemical fungicides were  
44 determined. All the pesticide residues at harvest were below the MRL (maximum residue level),  
45 except when two applications of pyrimethanil per season were applied. Boscalid was the most  
46 effective active ingredient against *B. cinerea* among the tested ones. Its efficacy, when its  
47 application was followed by a treatment with pyrimethanil, was similar to the efficacy shown by  
48 two treatments of pyrimethanil. This second strategy anyway is not feasible, due to the risks of  
49 resistance development in the pathogen and to the residue accumulation, as the analysis showed.

50  
51 **Keywords:** boscalid, fungicide, grapevine, grey mould, residue, wine.

## 53 INTRODUCTION

54 In Italy, grapes represent the most widespread fruit crop (1.5 million tonnes of table grapes and 47.1  
55 millions hL of wine <sup>[1]</sup>). *Botrytis cinerea* Pers. Ex Fr. (teleomorph *Botryotinia fuckeliana* (de Bary)  
56 Whetzel), the causal agent of grey mould, causes severe losses on grapevine (*Vitis vinifera* L.),  
57 affecting wine quality. Control is achieved by integrating canopy and cluster management with  
58 fungicide treatments, generally applied twice per season, at touching of berries and veraison <sup>[2]</sup>.

59 Several families of synthetic chemicals are available for the control of *B. cinerea*. They include  
60 specific botryticides, such as dicarboximides which inhibit the lipid and membrane synthesis. For  
61 over 25 years, dicarboximides – chlozolate, iprodione, procymidone and vinclozolin – have been  
62 the most popular class of specific fungicides against grey mould. Vinclozolin has been banned for  
63 toxicological safety reasons. The repeated use of this fungicide class has caused the development of  
64 strains of *B. cinerea* resistant to dicarboximides, prevalent in the Italian and French vineyards <sup>[3, 4]</sup>.

65 During the last years, new molecules with specific action against *B. cinerea*, such as the  
66 anilinopyrimidines, including cyprodinil and pyrimethanil, the phenylpyrrole fludioxonil, the  
67 hydroxyanilide fenhexamid, and the carboxamide boscalid, have been introduced into the market.  
68 These active ingredients exploit novel mechanisms of action, allowing new strategies of

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

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

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

92 Studies about the fate of the major pesticides used in vineyard have been reviewed by Cabras and  
93 Angioni <sup>[14]</sup>. Among the fungicides, pyrimethanil seemed the most persistent with residue levels  
94 constant up to harvest, whereas fluazinam, cyprodinil, mepanipyrim, azoxystrobin, and fludioxonil

95 showed **higher** disappearance rates ( $t_{1/2}$  were 4.3, 12, 12.8, 15.2, and 24 days, respectively).  
96 Pesticide residues in wine were always smaller than those on the grapes and in the must, except for  
97 those without a preferential partition between liquid and solid phase (azoxystrobin, dimethoate, and  
98 pyrimethanil) present in wine at the same concentration than on the grapes.  
99 The aim of this work was to test the effectiveness against grey mould of different fungicide control  
100 strategies on two experimental vineyards belonging to the cultivars “Barbera” and “Moscato”. The  
101 tested fungicides were boscalid (2-chloro-N-(4'-chlorobiphenyl-2-yl)-nicotinamide), a new  
102 carboxamide compound, cyprodinil (4-cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine),  
103 fenhexamid (2',3'-dichloro-4'-hydroxy-1-methylcyclohexanecarboxanilide), fludioxonil (4-(2,2-  
104 difluoro-1,3-benzodioxol-4-yl)-pyrrole-3-carbonitrile), iprodione (3-(3,5-dichlorophenyl)-N-  
105 isopropyl-2,4-dioxoimidazolidine-1-carboximide), and pyrimethanil N-(4,6-dimethylpyrimidin-2-  
106 yl)aniline). An integrated control strategy including a chemical (pyrimethanil) and a biocontrol  
107 agent (*Trichoderma* spp. t2/4ph1) was also included. A second aim was to determine the residual  
108 concentration of the fungicides on the grapes at harvest, to understand if the treatment strategies  
109 were able to keep the residues within the MRLs (maximum residue levels).

110

## 111 **MATERIALS AND METHODS**

112

### 113 **Experimental design**

114 Two experimental trials were carried out in two commercial vineyards located in Piedmont  
115 (Northern Italy): one planted with the white cultivar “Moscato” (Valdivilla, Asti Province) and the  
116 other one planted with the red cultivar “Barbera” (Vezza d'Alba, Cuneo Province). Every replicate  
117 plot was 10 m long and consisted of 10 vines each, with an untreated row marking the border  
118 between the plots. All the treatments were arranged in a randomized block design with four  
119 replicate plots per treatment. Six different disease strategies were performed in each vineyard,  
120 applying the fungicides at the rates and in the dates indicated in Table 1. Five treatment schemes

121 included two fungicide sprays at two phenological stages crucial for grey mould control during the  
122 cropping season: before bunch closure (B stage) and between veraison and preharvest interval (C  
123 stage). The integrated control scheme included a chemical treatment (pyrimethanil) and four  
124 biocontrol agent (*Trichoderma* spp. t2/4ph1) applications, at flowering (A stage), before bunch  
125 closure (B stage), at veraison (C stage) and three weeks before harvesting (D stage). The  
126 *Trichoderma* strain was a biocontrol agent isolated and studied by AGROINNOVA – University of  
127 Torino for its efficacy against *B. cinerea* on grapes <sup>[15]</sup>. The strain was produced in liquid  
128 hydrolyzed casein without shaking for 30 days at 26°C. The fungal mycelium was filtered and  
129 resuspended in water to 10<sup>8</sup> conidia mL<sup>-1</sup>. The fungicides and the biocontrol agent were applied by  
130 a motor knapsack sprayer, by using 400 l/ha of water. Four untreated plots were used as control.

131

### 132 **Efficacy against *Botrytis cinerea***

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

140

### 141 **Reagents**

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

145

### 146 **Apparatus and operating conditions**

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

156

### 157 **Mass spectrometry analysis**

158 The identity of each peak was confirmed by LC-MS/MS using a Varian HPLC–MS/MS system  
159 consisted of a ProStar 410 autosampler, two ProStar 212 pumps, and a 310 MS triple quadrupole  
160 mass spectrometer equipped with an electrospray ionization source, the ESI-MS interface was  
161 operated in the positive mode at 200°C. The transitions used were m/z 343  $\rightarrow$  140 for boscalid, m/z  
162 226  $\rightarrow$  209 for cyprodinil, m/z 303  $\rightarrow$  142 for fenhexamid, m/z 249  $\rightarrow$  183 for fludioxonil, m/z  
163 331  $\rightarrow$  163 for iprodione, and m/z 200  $\rightarrow$  107 for pyrimethanil.

164

### 165 **Extraction procedure**

166 Each plot was separately harvested and the grapes – 1 Kg randomly per plot - were stored at -2°C  
167 up to the determination of the fungicide residues. Each sample was defrosted at around 0°C for 12  
168 h, then homogenised with a food cutter (Princess 2080). 100 mL of an acetone-methanol solution  
169 (50:50, V/V) were added to 25 g sub-sample in a 250 mL bottle. The suspension was shaken on a  
170 mechanical stirrer for 15 min, then centrifuged at 3000 rpm for 15 minutes. The extraction was  
171 repeated, after the removal of the supernatant, with 50 mL of extracting solution, 5 min stirring and  
172 5 min centrifugation. The two supernatants were filtrated on hydrophilic cotton and collected in a

173 250 mL volumetric flask. The solution was brought to volume with water. A 25 mL aliquot of this  
174 solution, diluted with 200 mL of water, was eluted on Supelclean LC18 columns, previously  
175 conditioned with 6 mL methanol, then with 6 mL water. The eluate was discarded and the column  
176 was eluted with 5 mL methanol, collected in a volumetric flask and analysed by LC.

177

### 178 **Recovery experiments**

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

184

## 185 **RESULTS AND DISCUSSION**

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

192 The weather conditions during 2006 contributed to produce heavy attacks of grey mould: 68.2% of  
193 the bunches were rotted in the commercial vineyard of Moscato and 88.0% in the Barbera vineyard  
194 (table 3). During April, May and June, approximately 50 mm of rain fell – about a quarter of the  
195 average precipitation for the period. July was characterized by heavy rainfalls (such as 63.6 mm on  
196 July 4, 2006), spaced by hot and dry periods, so the Botrytis attacks, normally occurring from the  
197 veraison, were not present. Since the beginning of August and for all month long, continued and

198 low intensity rains favoured the attacks of *B. cinerea*. In September, heavy rainfalls (about 125 mm  
199 in 48 hours) caused the explosion of the disease.

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

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

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

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

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

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

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

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

244 The concentration at harvest of the other fungicides tested were lower than the MRL in all the  
245 treatments, except in the treatment 4 where two subsequent treatments with Scala provoked an  
246 accumulation of pyrimethanil with residual concentrations higher than the MRL (3 mg Kg<sup>-1</sup>) in the  
247 grapes collected from both vineyards. Such result is in contrast with previous findings reported by  
248 Rabølle et al. <sup>[22]</sup> on strawberries where 0.15 mg Kg<sup>-1</sup> pyrimethanil were found after 42 and 29 days

249 from the treatments. When a single treatment was performed, the pyrimethanil concentration varied  
250 from 0.24 mg Kg<sup>-1</sup> (trial 3, 65 DAT) to 1.37 mg Kg<sup>-1</sup> (trial 1, 58 DAT). These results suggest that,  
251 although the same formulation and application rate were used, the dissipation rate of pyrimethanil  
252 varied among the trials. This is in agreement with the discrepancy between the results of Cabras et  
253 al. <sup>[23]</sup> who found 1.11 mg Kg<sup>-1</sup> pyrimethanil on grapes at 28 DAT and those of Angioni et al. <sup>[24]</sup>  
254 who found 0.45 mg Kg<sup>-1</sup> in similar conditions. In an experiment conducted on agar plates, Vaughan  
255 et al. <sup>[25]</sup> observed that the metabolic activity of the actively growing mycelium of *Botrytis cinerea*  
256 provoked the mobility of pyrimethanil within the agar medium. An interaction between the  
257 development of *B. cinerea* and the disappearance of pyrimethanil seems to be confirmed by our data  
258 since the lowest concentrations of residues (trial 3) corresponds to the highest percentage of  
259 bunches with grey mould. The potential persistence of pyrimethanil on grapes is of concern since it  
260 has been reported that the fungicide passes into the wine during wine-making <sup>[23]</sup>.

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

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

271 The residual concentration of iprodione was between 2.01 and 4.23 mg Kg<sup>-1</sup>, therefore below the  
272 MRL (10 mg Kg<sup>-1</sup>), but about one order of magnitude higher than the other tested fungicides except  
273 pyrimethanil. The behaviour of iprodione in plants is largely documented in literature, in particular  
274 in a review of Cabras et al. <sup>[28]</sup> concerning the fate of pesticides from vine to wine. The residues

275 reported in grapes varied between 0.46 and 8.3 mg Kg<sup>-1</sup> depending on the application conditions.  
276 Recent studies confirmed the high persistency of iprodione on the treated fruits: Cabras et al. [29]  
277 found 1.09 mg Kg<sup>-1</sup> on apricots 21 DAT and Stensvand and Christiansen [30] 2.2 mg Kg<sup>-1</sup> in green-  
278 house grown strawberries.

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

288

## 289 **CONCLUSION**

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

297

## 298 **ACKNOWLEDGEMENTS**

299 This research was carried out within the framework of the projects “Risk evaluation, prevention  
300 and management of the presence of mycotoxins in the Piedmont grapes and wine chain”, funded by

301 the Piedmont Region, and “Evaluation and prevention of the risk of ochratoxin A in the Piemontese  
302 wine chain”, funded by the CRT Foundation. Moreover, the authors gratefully acknowledge Dr.  
303 Giovanna Gilardi for her help in the realization of the trials and Dr. Jeanne Griffin for her linguistic  
304 advice.

305

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

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Treatment	Active ingredient (%)	Commercial formulate	Application rate (g a.i. 100 l <sup>-1</sup> )	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	<i>Trichoderma</i> spp. t2/4ph1			13-06	17-06
	Pyrimethanil (37.4%)	Scala	74.8	08-07 (65d)	12-07 (98d)
	<i>Trichoderma</i> spp. t2/4ph1			31-07	05-08
4	<i>Trichoderma</i> spp. t2/4ph1			22-08	28-08
	Pyrimethanil (37.4%)	Scala	74.8	08-07 (65d)	12-07 (98d)
5	Pyrimethanil (37.4%)	Scala	74.8	08-08 (35d)	23-08 (58d)
	Fenhexamid (50.0%)	Teldor	75	08-07 (65d)	12-07 (98d)
6	Cyprodinil (37.5%) + fludioxonil (25.0%)	Switch	30+20	08-08 (35d)	23-08 (58d)
	Fenhexamid (50.0%)	Teldor	75	08-07 (65d)	12-07 (98d)
7 (control)	Iprodione (50.0%)	Rovral	75	08-08 (65d)	23-08 (58d)

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434 **Table 2.** Percentage of recovery of the pesticides from spiked grape samples  
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438	<b>Fungicide</b>	<b>Fortification level mg Kg<sup>-1</sup></b>	<b>% Recovery ± RSD (3 replicates)</b>
439	boscalid	1.2	89 ± 3
440		0.7	87 ± 2
440		0.2	87 ± 3
441	cyprodinil	1.0	86 ± 2
442		0.5	85 ± 2
442		0.1	87 ± 2
443	fenhexamid	1.2	89 ± 4
444		0.7	85 ± 3
444		0.1	90 ± 4
445	fludioxonil	1.0	92 ± 2
446		0,6	93 ± 3
446		0.1	92 ± 2
447	iprodione	1.0	92 ± 4
448		0.6	86 ± 3
448		0.1	87 ± 3
449	pyrimethanil	1.0	86 ± 3
450		0.5	89 ± 2
450		0.1	90 ± 3

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460 **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  
 461 two vineyards of Moscato and Barbera varieties.

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

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

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472 **FIGURE CAPTIONS**

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474 Figure 1. Typical chromatograms (A): boscalid (analytical standard at 1.1 mg Kg<sup>-1</sup>, sample of  
475 Barbera from trial 1), (B): cyprodinil (analytical standard at 0.5 mg Kg<sup>-1</sup>, sample of Barbera from  
476 trial 2), (C): fenexhamid (analytical standard at 0.96 mg Kg<sup>-1</sup>, sample of Barbera from trial 9), (D):  
477 fludioxonil (analytical standard at 0.5 mg Kg<sup>-1</sup>, sample of Barbera from trial 7), (E): iprodione  
478 (analytical standard at 1.0 mg Kg<sup>-1</sup>, sample of Moscato from trial 6), (F): pyrimethanil (analytical  
479 standard at 0.5 mg Kg<sup>-1</sup>, sample of Moscato from trial 4).

