Phytopathol. Mediterr. (2000) 39, 433–446

Sensitivity to anilinopyrimidines and phenylpyrroles in *Botrytis cinerea* in north-Italian vineyards

M. Lodovica Gullino, Domenico Bertetti, Matteo Monchiero and Angelo Garibaldi

DI.VA.P.R.A. - Patologia vegetale, Via L. da Vinci 44, 10095 Grugliasco, Italy

Summary. Several commercial vineyards, located in Piedmont (Northern Italy), were monitored in order to evaluate the sensitivity of *Botrytis cinerea* Pers., the causal agent of grey mould, to five classes of botryticides: benzimidazoles, dicarboximides, phenylcarbamates, anilinopyrimidines and phenylpyrroles. Strains of *B. cinerea* resistant to anilinopyrimidines were easily detected, particularly in 1999, a year characterized by high disease pressure, even in vineyards not sprayed with that class of fungicides. Fludioxonil-resistance, on the contrary, was not detected. Resistance to benzimidazoles and dicarboximides was at previous observed levels. For the first time, resistance to phenylcarbamates was detected in the field. Strains of *B. cinerea* showing multiple resistance to benzimidazoles, dicarboximides and anilinopyrimidines and maintaining a good level of virulence, as shown by tests carried out on wounded apples, are present in Italian vineyards. Strategies in the use of the botryticides are discussed, in order to avoid a loss of disease control.

Key words: *Botrytis cinerea*, fungicide resistance, grapevine, anilinopyrimidines.

Introduction

Botrytis cinerea Pers., the causal agent of grey mould, causes severe losses on grapevine, particularly on the cultivars Moscato and Chardonnay, and also affects wine quality. In Italy, chemicals are regularly sprayed at least twice per season (at touching of berries and changing of colour) on these cultivars. In years with weather conditions highly favourable to grey mould, a third spraying is often carried out before harvest (Gullino, 1992). Several families of synthetic chemicals are now available for the control of *B. cinerea*. They include non-specific fungicides (dichlofluanid, thiram, chlorothalonil), which are only partially effective, and a number of specific botryticides, such as benzimidazoles, dicarboximides, phenylcarbamates. More recently, anilinopyrimidines, phenylpyrroles and the hydroxanilide fenhexamid have also been developed to control grey mould.

The anilinopyrimidines inhibit the biosynthesis of methionine, affecting cystathionine-ß-lyase (Fritz *et al*., 1997), and block the excretion of hydrolytic enzymes involved in the pathogenic process (Milling and Richardson, 1995). The phenylpyrroles affect cell wall synthesis and cause the accumulation of glycerol in mycelial cells: their primary target sites are possibly protein kynases involved in the regulation of polyol biosynthesis (Pillonel and Meyer, 1997). Cross-resistance to phenylpyrroles has been observed in strains of *B. cinerea*

Corresponding author: M.L. Gullino
Fax: 39 011 6708541
E-mail: gullino@agraria.unito.it

selected *in vitro* for their high resistance to dicarboximides, but has not been found in field isolates (Leroux *et al*. 1992). The mode of action of hydroxanilide fenhexamid is still unknown (Gullino *et al.*, 2000).

Taken together, these groups of chemicals give Italian growers a good range of choice. Among the new botryticides, the anilinopyrimidines pyrimethanil and cyprodinil and the phenylpyrrole fludioxonil are registered. However, fludioxonil and cyprodinil can only be applied on grapevine in mixtures with other agents.

Anilinopyrimidines started to be applied in Italy in 1997. Pyrimethanil, the first registered anilinopyrimidine, has often replaced dicarboximides, on cultivars, such as Moscato, where dicarboximide-resistance was widespread (Gullino, 1992; Gullino *et al.*, 1998). Fenhexamid was registered in Italy only in 2000.

It is well known that repeated applications of fungicides having a specific mode of action lead to the development of resistance with target strains. This phenomenon has been particularly serious on grapevine in several regions, leading in some cases to failure of control of grey mould in the field (Faretra and Pollastro, 1991; Gullino, 1992; Hilber *et al.*, 1995; Leroux and Descotes, 1996).

Recently, in some vineyards located in Piedmont (Northern Italy) strains of *B. cinerea* resistant to anilinopyrimidines were detected, although at low frequency (Gullino *et al.*, 1998). The anilinopyrimidine-resistant strains of *B. cinerea* developed in those vineyards where this group of fungicides had been widely used (Hilber and Hilber-Bodmer, 1998; Leroux *et al.*, 1998).

During the past two years the state of resistance of *B. cinerea* in Italian vineyards has continued to be evaluated, as has the effectiveness shown by new groups of botryticides in field trials.

Materials and methods

Isolates of *Botrytis cinerea*

Field strains of *B. cinerea* were isolated from diseased berries in 1998 and 1999, at harvest (September) in botryticide-treated vineyards located in Piedmont.

One laboratory mutant, highly resistant to dicarboximides (V 21), one strain of known sensitivity to both benzimidazoles and dicarboximides (Gr 70/89 S) and one strain with double resistance to benzimidazoles and dicarboximides, (Gr 146/86 RBD) were also included in this study. One strain of *B. cinerea*, isolated from rotted apples (Ro 1), was used as reference in tests carried out for evaluating the virulence of any resistant strain. All strains were maintained on potato-dextrose-agar PDA (Merck, Darmstadt, Germany) in tubes at 4 °C. Cultures were prepared on PDA and 7-10 day old colonies were used as inoculum for the different experiments.

Monitoring

A total of 42 and 30 commercial vineyards, all located in Piedmont, were monitored, respectively, in 1998 and 1999. The fungicides applied during the same seasons to control grey mould were recorded. Grey mould infected bunches were sampled randomly and transferred to the laboratory in paper bags. Moreover, a monitoring was carried out in two field trials, in which infected bunches were sampled randomly from different plots. Conidia collected from grey mould lesions were transferred to sterile water and processed following a previously described method (Gullino and Garibaldi, 1986). If sufficient conidia were not available, diseased bunches were incubated in a moist chamber at 20 °C for 24–48 h, to enhance sporulation. Three subsamples/vineyard (or three subsamples/plot in the case of the field trials) were prepared. Spore suspensions were transferred to a medium containing glucose, 10 g; KH_2PO_4, 2 g; K_2HPO_4 2 g; agar, 12.5 g; distilled water, 1 l (Leroux and Gredt, 1996). Benomyl, vinclozolin, fludioxonil and pyrimethanil were used as representative of the benzimidazoles, dicarboximides, phenylpyrroles and anilinopyrimidines respectively. Benomyl, vinclozolin, fludioxonil were used in the form of technical grade compounds kindly provided by the manufacturers, pyrimethanil as commercial formulation (Scala, Aventis, 37.4% a.i.). The compounds were dissolved in ethanol or acetone and added as 100 fold concentrated suspensions to the molten medium (50 °C) after autoclaving. The concentration of solvents (ethanol or acetone) in all media, including those lacking fungicides, was 1 ml/l. The final concentrations tested were 10 mg/l for benomyl, vinchlozolin and fludioxonil and 3 mg/l for pyrimethanil. The concentrations were higher than the MIC (Minimal Inhibitory Concentration) baseline values. Conidia germination and germ tube elongation were evaluated after 24–36 h of incubation at 20 °C in the dark. The percent germination of 200 conidia/treatment and the length of 50–100 germ tubes/treatment were determined under the microscope.

Fungicide sensitivity

Twelve isolates of *B. cinerea* isolated from grapevine during in 1998 and fifty in 1999 were tested for sensitivity to benzimidazoles, dicarboximides, diethofencarb, anilinopyrimidines and phenylpyrroles, and compared to strains of known sensitivity to the benzimidazoles and dicarboximides. The strains used, as well as their origin, are given in Tables 2 and 4. Most of the tested strains were selected from those isolated in vineyards where the surveys permitted to detect resistance to some of the botryticides under evaluation.

The EC_{50} (Effective Concentration 50) and MIC of the following fungicides (technical grade, except pyrimethanil) were determined: benomyl, diethofencarb, fludioxonil, pyrimethanil and vinclozolin. Fungicides were added as methanol or acetone suspensions to a medium containing glucose, 10 g; KH_2PO_4, 2 g, K_2HPO_4, 1.5 g; $(NH_4)_2SO_4$, 1 g; $MgSO_47H_2O$, 0.5 g; agar, 15 g; distilled water, 1 l (Leroux and Gredt, 1995). Fungicide suspensions were added to the molten medium at 50 °C, after autoclaving. The final concentrations tested ranged from 0.1 to 300 mg/ml^{-1}. The concentration of solvents (ethanol or acetone) in all media, including those lacking fungicides, was 1 ml/l. The plates were inoculated with mycelial disks (6 mm diameter) of *B. cinerea*, taken from colonies actively growing on PDA. Three disks/plate were inoculated and three replicates were used. Incubation took place at 20 °C in the dark. The diameter of fungal colonies was measured daily for a week. The data are expressed as EC_{50} and MIC values, calculated on the fourth day of growth. All experiments were repeated at least twice.

Virulence

The virulence of the selected strains was evaluated on apples (cv. Golden delicious), disinfected with sodium hypochloride (8% chlorine), rinsed under tap water and punctured, when dry, at the equatorial region (3 punctures/apple) with a sterile needle (3-4 mm deep). Each apple was inoculated with a 30 ml/puncture of a conidial suspension of one of the strains, at a concentration of $1x10^5$ conidia/ml. After the inoculation, fruits were kept at 20–22 °C for 7 days, when the diameter of the lesions was measured. Rot caused by the different strains was expressed as percent of rot in comparison to that caused by reference strain Ro 1 of *B. cinerea*. The virulence tests were repeated twice.

Field trials

Four experimental trials (two each year) were carried out in Northern Italy in commercial vineyards planted with cv. Moscato, which is among the most susceptible to *B. cinerea*. Individual replicate plots were 10 m long and consisted of 10 vines each, with an untreated row marking the border between plots. All treatments were arranged in a randomized complete block design with four replicates.

The following fungicides were applied at the rates and phenological stages B: touching of berries; C: changing of colour, as indicated in Tables 8-10: cyprodinil+fludioxonil (Switch®, Novartis, Basel, Switzerland, 37.5+25% a.i.), fenhexamid (Teldor®, Bayer, Milano, Italy, 50% a.i.), pyrimethanil (Scala, Aventis, Milano, Italy 37.4% a.i.). Spray suspensions were applied with a knapsack sprayer, using 400 l/ha. Treatments against powdery and downy mildew were carried out by using the equipment, spray practices and timing regimes of the co-operating farms.

At harvest, the percentage of infected bunches and infected berries in 100 bunches carried by the eight vines in the middle of each plot was evaluated and the data obtained were arcsin-transformed and analysed with Duncan's multiple range test (P=0.05).

Results

Monitoring

In 1998 grey mould was not very severe, as shown by the disease incidence in the control plots of the field trials (Table 9). In 5 out of 42 commercial vineyards, more than 50% of the conidia of *B. cinerea* were resistant to the benzimidazoles, and in 10 more than 50% of conidia germinated on a vinchlozolin-amended medium (Table 1). In the case of the anilinopyrimidines, conidia able to germinate and showing normal germ tube elongation

Table 1. Results of a survey in Italian vineyards of the cultivar Moscato in 1998 to detect resistance of *Botrytis cinerea* populations to different classes of botryticides.

No.	Farm and location	Sprayed in 1998 with	% of conidia resistant to:		
			Benomyl	Vinclozolin	Pyrimethanil
			10[a]	10[a]	3[a]
1	Borello, Valdivilla	Procymidone	3	51	5
2	Saglietti, Valdivilla	Pyrimethanil	0	0	2
3	Filante, Cossano Belbo	Pyrimethanil	0	13	4
4	Rivetti, Neive	Vinclozolin	0	0	14
5	Giachino, Valdivilla	Procymidone	0	0	1
6	Giachino, Valdivilla	Pyrimethanil	33	80	1
7	Aimasso, Valdivilla	Pyrimethanil	57	46	2
8	Galletto, Valdivilla	Cyprodinil+fludioxonil[b]	0	0	2
9	Saracco, Castiglione Tinella	Iprodione	0	99	0
10	Donadio, Valdivilla	Iprodione	0	0	1
11	Efflandrin, Valdivilla	Procymidone	3	44	0
12	Amerio, S. Stefano Belbo	Procymidone	0	17	1
13	Bussi, S. Stefano Belbo	Procymidone	0	1	21
14	Alcalino, Cossano Belbo	Pyrimethanil	0	13	4
15	Masoero, Neive	Procymidone	0	1	2
16	Rivetti, Neive	Procymidone	0	0	1
17	Tropea, Neive	Iprodione	0	20	1
18	Ressia, Neive	Procymidone	3	4	2
19	Rivetti, Neive	Procymidone	0	26	1
20	Giachino (Carretta), Valdivilla	Procymidone	18	15	0
21	Morando, Castiglione Tinella	Procymidone	0	0	1
22	Soria, Castiglione Tinella	Iprodione	20	33	2
23	Saracco, Castiglione T.	Iprodione	0	19	0
24	Soave, Castiglione Tinella	Vinclozolin	0	0	2
25	Scarrone, Valdivilla	Iprodione	41	90	34
26	Zizzi, Moncucco	Procymidone	0	0	1
27	Bussi, S. Stefano Belbo	Iprodione	6	72	1
28	Boma, S. Stefano Belbo	Iprodione	81	69	0
29	Boma, S. Stefano Belbo	Iprodione	0	28	3
30	Alcalino, Cossano Belbo	Procymidone	35	94	4
31	Ghignone, Cossano Belbo	Procymidone	0	0	1
32	Balbo, Cossano Belbo	Procymidone	0	41	1
33	Molinari, S. Stefano Belbo	Iprodione	5	4	0
34	Colli, Cossano Belbo	Iprodione	100	100	0
35	Cauda, Trezzo Tinella	Procymidone	95	24	2
36	Bazzano, Trezzo Tinella	Procymidone/Pyrimethanil[c]	3	36	0
37	Bazzano, Trezzo Tinella	Procymidone/Pyrimethanil[c]	0	6	1
38	Meistro, S. Donato Mango	Procymidone	0	0	0
39	Came 1, S. Donato Mango	Procymidone	0	31	1
40	Came 2, S. Donato Mango	Unknown	0	51	0
41	Aimasso, Trezzo Tinella	Procymidone	0	0	0
42	Aimasso, Trezzo Tinella	Procymidone	70	74	1

[a] mg/l.
[b] Mixture.
[c] Rotation.

Table 2. Results of a survey in 1998, to detect resistance of *Botrytis cinerea* to different classes of botryticides in 2 vineyards (cv. Moscato) where experimental trials were carried out.

No.	Farm and location	Sprayed in 1998 with	% of conidia resistant to:		
			Benomyl	Vinclozolin	Pyrimethanil
			10[a]	10[a]	3[a]
S 1/98	Saracco, Castiglione Tinella	Untreated control	0	0	0
S 2/98	Saracco, Castiglione Tinella	Untreated control	41	0	3
S 3/98	Saracco, Castiglione Tinella	Untreated control	0	0	0
S 4/98	Saracco, Castiglione Tinella	Pyrimethanil/Fenhexamid[b]	0	0	0
S 5/98	Saracco, Castiglione Tinella	Pyrimethanil/Fenhexamid	0	0	1
S 6/98	Saracco, Castiglione Tinella	Pyrimethanil/Fenhexamid	0	0	1
S 7/98	Saracco, Castiglione Tinella	Pyrimethanil	0	0	0
S 8/98	Saracco, Castiglione Tinella	Pyrimethanil	0	0	1
S 9/98	Saracco, Castiglione Tinella	Procymidone	0	14	1
S 10/98	Saracco, Castiglione Tinella	Procymidone	24	0	1
S 11/98	Saracco, Castiglione Tinella	Procymidone	64	35	3
S 12/98	Saracco, Castiglione Tinella	Procymidone	0	0	0
S 13/98	Saracco, Castiglione Tinella	Cyprodinil+fludioxonil[c]	0	0	0
S 14/98	Saracco, Castiglione Tinella	Cyprodinil+fludioxonil	0	0	0
S 15/98	Saracco, Castiglione Tinella	Iprodione	0	3	3
S 16/98	Saracco, Castiglione Tinella	Pyrimethanil	0	0	1
S 17/98	Saracco, Castiglione Tinella	Pyrimethanil	22	0	2
A 1/98	Aimasso, Valdivilla	Untreated control	7	6	1
A 2/98	Aimasso, Valdivilla	Untreated control	6	43	2
A 3/98	Aimasso, Valdivilla	Untreated control	16	14	4
A 4/98	Aimasso, Valdivilla	Fenhexamid	44	0	1
A 5/98	Aimasso, Valdivilla	Fenhexamid	94	39	0
A 6/98	Aimasso, Valdivilla	Fenhexamid	3	3	6
A 7/98	Aimasso, Valdivilla	Pyrimethanil/Fenhexamid	23	13	2
A 8/98	Aimasso, Valdivilla	Pyrimethanil/Fenhexamid	15	8	5
A 9/98	Aimasso, Valdivilla	Pyrimethanil/Fenhexamid	3	7	0
A 10/98	Aimasso, Valdivilla	Pyrimethanil/Fenhexamid	57	24	1
A 11/98	Aimasso, Valdivilla	Pyrimethanil	0	0	0
A 12/98	Aimasso, Valdivilla	Procymidone	0	0	1
A 13/98	Aimasso, Valdivilla	Procymidone	20	20	1
A 14/98	Aimasso, Valdivilla	Procymidone	49	0	0
A 15/98	Aimasso, Valdivilla	Cyprodinil+fludioxonil	25	0	1
A 16/98	Aimasso, Valdivilla	Cyprodinil+fludioxonil	0	0	0
A 17/98	Aimasso, Valdivilla	Cyprodinil+fludioxonil	2	1	2

[a, b, c] See Table 1.

on pyrimethanil-amended medium, were found in 31 vineyards, but at a very low frequency except for vineyards No. 13 and 25, where more than 20% of conidia were resistant (Table 1). No germination was observed on fludioxonil-amended medium (Table 1).

In the experimental vineyards, the same trend was observed. The treatments carried out in the different plots did not seem to influence the response to fungicides (Table 2).

In 1999, grey mould development was favoured by frequent rains during the month of August, as indicated by the high disease incidence in the untreated plots of the two experimental trials (Ta-

Table 3. Results of a survey in 1999 to detect resistance of Botrytis cinerea to different classes of botryticides.

No.	Farm and location	Cultivar	Sprayed in 1999 with	% of conidia resistant to:		
				Benomyl	Vinclozolin	Pyrimethanil
				10[a]	10[a]	3[a]
1	Scagliola, Neive	Moscato	Procymidone/Pyrimethanil[b]	0	0	17
2	Rivetti, Neive	Moscato	Procymidone	87	94	50
3	Ressia, Neive	Moscato	Procymidone	0	63	73
4	Rivetti, Neive	Moscato	Pyrimethanil	1	1	29
5	Barbero, Neive	Moscato	Procymidone	44	100	100
6	Perrone, Valdivilla	Moscato	Cyprodinil+fludioxonil[c]	76	62	18
7	Giachino, Valdivilla	Moscato	Iprodione	11	4	80
8	Donadio, Valdivilla	Moscato	Procymidone	11	100	96
9	Efflandrin, Valdivilla	Moscato	Iprodione	3	11	29
10	Saglietti, Valdivilla	Moscato	Pyrimethanil	5	3	70
11	Saglietti, Valdivilla	Moscato	Pyrimethanil	27	14	37
12	Lolli, Cossano Belbo	Moscato	Procymidone/Pyrimethanil	1	0	4
13	Alossa, Moncucco	Moscato	Procymidone/Pyrimethanil	0	100	100
14	Morando, Castiglione T.	Moscato	Iprodione	49	82	35
15	Soria, Castiglione Tinella	Moscato	Iprodione	0	100	94
16	Saracco, Castiglione Tinella	Moscato	Iprodione/Pyrimethanil	4	73	24
17	Rabino, S. Vittoria d'Alba	Barbera	Procymidone	0	100	1
18	Tarasco, S. Vittoria d'Alba	Barbera	Procymidone	0	0	35
19	Oricco, S. Vittoria d'Alba	Moscato	Procymidone/Pyrimethanil	1	2	4
20	Borsa, Alba	Moscato	Procymidone	19	96	15
21	Bazzano, Trezzo Tinella	Moscato	Procymidone	27	80	74
22	Monchiero, Pocapaglia	Arneis	Cyprodinil+fludioxonil	0	0	0
23	Rabino, S. Vittoria d'Alba	Nebbiolo	Iprodione	0	88	96
24	Benevelli, Monforte d'Alba	Barbera	Procymidone	0	0	2
25	Rabino, S. Vittoria d'Alba	Barbera	Iprodione	77	91	55
26	Dallorto, Pocapaglia	Barbera	Pyrimethanil	0	0	49
27	Messa, Pocapaglia	Barbera	Procymidone	0	0	23
28	Monchiero, Pocapaglia	Barbera	Cyprodinil+fludioxonil	0	0	0
29	Tibaldi, Pocapaglia	Barbera	Pyrimethanil	0	97	0
30	Bosio, Pocapaglia	Barbera	Procymidone	0	0	57

[a, b, c] See Table 1.

bles 10 and 11). Under such rainy conditions, resistance to the benzimidazoles, dicarboximides and anilinopyrimidines became much more frequent. In commercial trials, in 3 (No. 2, 6, 25) out of 30 vineyards more than 50% of conidia germinated on a benomyl-amended medium. In 15 vineyards more than 50% of conidia germinated on a vinchlozolin-amended medium and in 11 vineyards more than 50% of conidia germinated on a pyrimethanil-amended medium. In 1999 there was no germination on the fludioxonil-amended medium (Table 3).

Resistance to anilinopyrimidines was quite widespread in the two vineyards where the field trials were carried out (Table 4). A high percentage of anilinopyrimidine-resistant conidia was detected even in the control plots. Dicarboximide resistance was more widespread on the Aimasso farm (Table 4).

Sensitivity to fungicides

Benzimidazoles.

As regards *in vitro* sensitivity to benomyl, two phenotypes of *B. cinerea* were prevalent in this survey in both growing seasons: benomyl-sensitive strains and highly resistant strains. Their respec-

Table 4. Results of a survey carried out in 1999 to detect resistance of *Botrytis cinerea* to different classes of botryticides in two vineyards, cv. Moscato, where experimental trials were carried out.

No.	Farm and location	Plot sprayed with	% of conidia resistanto to:		
			Benomyl	Vinclozolin	Pyrimethanil
			10[a]	10[a]	3[a]
S 1/99	Saglietti, Valdivilla	Untreated control	1	1	99
S 2/99	Saglietti, Valdivilla	Untreated control	0	0	91
S 3/99	Saglietti, Valdivilla	Untreated control	0	11	0
S 4/99	Saglietti, Valdivilla	Pyrimethanil	0	0	100
S 5/99	Saglietti, Valdivilla	Pyrimethanil	0	0	35
S 6/99	Saglietti, Valdivilla	Pyrimethanil	0	0	44
S 7/99	Saglietti, Valdivilla	Pyrimethanil	12	15	52
A 1/99	Aimasso, Valdivilla	Pyrimethanil	1	2	92
A 2/99	Aimasso, Valdivilla	Pyrimethanil	0	89	26
A 3/99	Aimasso, Valdivilla	Pyrimethanil	35	70	100
A 4/99	Aimasso, Valdivilla	Pyrimethanil	19	82	56
A 5/99	Aimasso, Valdivilla	Untreated control	0	0	100
A 6/99	Aimasso, Valdivilla	Untreated control	5	6	95
A 7/99	Aimasso, Valdivilla	Untreated control	6	28	13
A 8/99	Aimasso, Valdivilla	Procymidone	13	62	77
A 9/99	Aimasso, Valdivilla	Procymidone	0	99	61
A 10/99	Aimasso, Valdivilla	Procymidone	82	73	1
A 11/99	Aimasso, Valdivilla	Pyrimethanil	1	1	85
A 12/99	Aimasso, Valdivilla	Cyprodinil+fludioxonil[b]	0	0	100
A 13/99	Aimasso, Valdivilla	Cyprodinil+fludioxonil	1	61	100
A 14/99	Aimasso, Valdivilla	Cyprodinil+fludioxonil	0	0	0
A 15/99	Aimasso, Valdivilla	Fenhexamid	94	91	0
A 16/99	Aimasso, Valdivilla	Fenhexamid	0	0	77
A 17/99	Aimasso, Valdivilla	Fenhexamid	1	0	95
A 18/99	Aimasso, Valdivilla	Fenhexamid	0	10	28

[a] mg/l .
[b] Mixture.

tive EC_{50} values for mycelial growth were 0.2 mg/l or below for the sensitive group, higher than 70 mg/l for the resistant one (Tables 5 and 6). Four strains (Gr 157/98, 118/99, 141/99 and 166/99) seemed to belong to a third group, that was characterized by low sensitivity to both the benzimidazoles and phenylcarbamate. While negative cross resistance to the phenylcarbamate diethofencarb characterized all the strains belonging to the first two groups, the last four strains, moderately resistant to benzimidazoles were not hypersensitive to diethofencarb (Tables 5 and 6), and thus did not show the negative-cross resistance pattern.

Dicarboximides.

The laboratory mutant (V 21), highly resistant to the dicarboximides was confirmed to have positive cross-resistance to the phenylpyrroles. However, such a phenotype of *B. cinerea*, with positive cross resistance to both dicarboximides and phenylpyrroles was not detected in the tested vineyards. All dicarboximide-resistant strains detected exhibited a moderate level of resistance (EC_{50} between 0.7 and 7 mg/l) (Tables 5 and 6). Such strains were not cross-resistant to the anilinopyrimidines.

Anilinopyrimidines.

Eleven isolates out of 12 in 1998, and 44 out of 50 in 1999 showed ED_{50} values equal or higher than 0.2 mg/l and MIC values equal to or higher than 3 mg/l. In several cases, resistance to the anilinopy-

Table 5. Efficacy of botryticides of different classes against isolates of *Botrytis cinerea* isolated from Italian vineyards in 1998. Results are expressed as Effective Concentration 50 (EC_{50}) and Minimal Inhibitory Concentration (MIC) in mg/l.

Strain	Origin	Fungicide							
		Vinclozolin		Benomyl		Diethophencarb		Pyrimethanil	
		EC_{50}	MIC	EC_{50}	MIC	EC_{50}	MIC	EC_{50}	MIC
V 21	Bari Collection	>300	>300	<0.1	0.1	>300	>300	<0.1	300
Gr 70/89 S	Di.Va.P.R.A. collection	0.7	10	<0.1	0.3	>300	>300	<0.1	0.1
Gr 146/86 RBD	Di.Va.P.R.A. collection	2	>300	70	>300	<0.1	0.3	<0.1	300
Gr 8/98	Borello, Valdivilla	2	>300	>300	>300	0.2	1	0.2	30
Gr 9/98	Scarrone, Valdivilla	7	>300	0.2	0.3	>300	>300	20	300
Gr 21/98	Saglietti, Valdivilla	0.7	3	<0.1	10	>300	>300	0.2	300
Gr 37/98	Filante, Cossano Belbo	0.7	3	<0.1	0.1	>300	>300	0.2	300
Gr 58/98	Rivetti, Neive	2	>300	<0.1	1	>300	>300	0.2	>300
Gr 72/98	Giachino, Valdivilla	0.7	3	<0.1	1	>300	>300	0.2	30
Gr 78/98	Giachino, Valdivilla	2	>300	0.7	>300	<0.1	1	0.2	100
Gr 90/98	Saracco, Castiglione T.	0.7	3	<0.1	0.3	70	>300	0.2	100
Gr 133/98	Saracco, Castiglione T.[a]	0.7	3	0.2	1	>300	>300	0.7	>300
Gr 157/98	Saracco, Castiglione T.[a]	0.7	>300	0.7	>300	>300	>300	0.2	>300
Gr 223/98	Aimasso, Valdivilla[a]	7	>300	>300	>300	0.2	1	0.2	30
Gr 333/98	Galletto, Valdivilla	0.7	3	0.2	0.3	>300	>300	<0.1	1

[a] Strain isolated in one of the vineyards subject to the experimental trials of Di.Va.P.R.A. in 1998.

rimidines developed in strains already resistant to the dicarboximides. Strains Gr 78/98, Gr 157/98, Gr 142/99, Gr 151/99, and Gr 167/99 showed resistance to the anilinopyrimidines as well as to the benzimidazoles and dicarboximides (Tables 5 and 6).

Phenylpyrroles.

Most of the tested isolates, were sensitive to this group of fungicides in both growing seasons, with EC_{50} values <0.1 mg/l. Three strains (Gr 22/99 Gr 166/99 and Gr 124/99) showing a still low ED_{50} value, but a higher MIC (10–30 mg/l) than the others, were detected in 1999 in vineyards that had not been not treated with this class of fungicides (Table 6).

Virulence

Most of the strains showed a good level of virulence when tested on artificially wounded apples. In 1998, only three strains (Gr 8/98, Gr 9/98 and 90/98) showed reduced ability to cause rot. Also strain V 21, which is *in vitro* resistant to the dicarboximides showed a only limited ability to infect apples (Table 7).

In 1999, most strains (except V 21, 161/99 and 162/99) were highly virulent on apple (Table 8).

The strains showing multiple resistance to benzimidazoles, dicarboximides and anilinopyrimidines infected wounded apples (Tables 7 and 8).

Field trials

In 1998, grey mould incidence was low, as shown by the low level of disease in the control plots (4.5 and 11.2% infected berries in trials 1 and 2 respectively). The best control of grey mould was with a mixture of cyprodinil+fludioxonil or with an alternation of pyrimethanil (applied at B) and fenhexamid (applied at C). Two treatments with only fenhexamid (at either tested dosage) or only pyrimethanil were slightly less effective (Table 9). In 1999, the rainy month of August favoured grey mould development: 57.6% of berries were infected in trial 3 (Table 10) and 33.5% in trial 4 (Table 11) in the control plots. Pyrimethanil, applied in two treatments (B and C), showed an efficacy ranging from 40% (trial 3, Table 10) to 64% (Trial 4, Table 11). But fenhexamid, applied twice, at B and C, did not provide complete control of grey mould (Table 10).

Table 6. Efficacy of botryticides of different classes against isolates of *Botrytis cinerea* isolated from Italian vineyards in 1999. Results are expressed as Effective Concentration 50 (EC_{50}) and Minimal Inhibitory Concentration (MIC) in mg/l.

Strain	Origin	Fungicide							
		Vinclozolin		Benomyl		Diethophencarb		Pyrimethanil	
		EC_{50}	MIC	EC_{50}	MIC	EC_{50}	MIC	EC_{50}	MIC
V 21	Bari Collection	>300	>300	0.07	0.1	>300	>300	0.2	300
Gr 70/89 S	Di.Va.P.R.A. Collection	2	3	0.2	1	>300	>300	0.2	1
Gr 146/86 RBD	Di.Va.P.R.A. Collection	7	>300	>300	>300	0.07	1	0.7	100
Gr 17/99	Saglietti, Valdivilla[a]	2	3	0.07	0.1	>300	>300	0.07	1
Gr 22/99	Saglietti, Valdivilla[a]	2	3	0.2	0.3	>300	>300	0.2	100
Gr 25/99	Saglietti, Valdivilla[a]	2	3	0.07	1	200	>300	0.7	10
Gr 67/99	Aimasso, Valdivilla[a]	2	3	0.2	0.3	>300	>300	0.2	1
Gr 73/99	Aimasso, Valdivilla[a]	7	>300	0.07	1	200	>300	0.2	>300
Gr 77/99	Aimasso, Valdivilla[a]	2	3	0.07	0.3	>300	>300	0.2	10
Gr 113/99	Scagliola, Neive	0.7	10	0.07	1	>300	>300	0.07	>300
Gr 116/99	Scagliola, Neive	2	3	0.07	1	200	>300	0.2	30
Gr 118/99	Rivetti, Neive	20	>300	200	>300	>300	>300	0.2	1
Gr 119/99	Rivetti, Neive	7	>300	200	>300	0.07	0.3	0.2	30
Gr 121/99	Ressia, Neive	0.2	3	0.2	0.3	20	>300	0.2	100
Gr 124/99	Ressia, Neive	0.7	>300	0.07	0.3	200	>300	0.07	1
Gr 125/99	Rivetti, Neive	2	10	<0.01	0.3	200	>300	0.2	3
Gr 127/99	Rivetti, Neive	0.7	1	0.07	0.3	>300	>300	0.07	>300
Gr 129/99	Barbero, Neive	0.7	>300	0.07	0.1	>300	>300	0.7	10
Gr 131/99	Barbero, Neive	7	>300	200	>300	0.07	1	0.7	30
Gr 132/99	Barbero, Neive	7	>300	0.2	1	>300	>300	0.7	1
Gr 133/99	Perrone, Valdivilla	7	>300	>300	>300	0.2	1	0.2	1
Gr 134/99	Perrone, Valdivilla	2	3	0.2	1	200	>300	0.2	10
Gr 137/99	Giacchino, Valdivilla	2	3	0.07	1	200	>300	0.2	3
Gr 141/99	Donadio, Valdivilla	2	>300	>300	>300	>300	>300	0.2	3
Gr 142/99	Donadio, Valdivilla	7	>300	0.07	1	>300	>300	0.7	>300
Gr 143/99	Donadio, Valdivilla	7	>300	0.07	1	>300	>300	0.2	3
Gr 144/99	Donadio, Valdivilla	7	>300	0.07	0.3	>300	>300	0.7	30
Gr 145/99	Efflandrin, Valdivilla	2	10	0.07	0.3	70	>300	0.07	30
Gr 146/99	Efflandrin, Valdivilla	2	10	0.07	1	200	>300	0.2	3
Gr 147/99	Efflandrin, Valdivilla	2	10	0.07	1	>300	>300	0.7	10
Gr 148/99	Efflandrin, Valdivilla	0.7	3	0.07	1	0.7	>300	0.7	>300
Gr 150/99	Saglietti, Valdivilla	0.7	10	0.07	0.3	>300	>300	0.2	30
Gr 151/99	Saglietti, Valdivilla	2	10	0.2	1	>300	>300	0.2	>300
Gr 153/99	Saglietti, Valdivilla	0.7	3	0.07	1	>300	>300	0.2	>300
Gr 154/99	Saglietti, Valdivilla	0.7	10	0.07	3	>300	>300	0.2	30
Gr 157/99	Lolli, Cossano Belbo	0.7	10	0.2	1	>300	>300	0.7	>300
Gr 159/99	Lolli, Cossano Belbo	2	10	0.2	3	>300	>300	0.7	3
Gr 161/99	Alossa, Moncucco	7	>300	0.07	1	>300	>300	0.2	>300
Gr 162/99	Alossa, Moncucco	7	>300	0.07	1	>300	>300	>300	>300
Gr 163/99	Alossa, Moncucco	0.7	>300	0.07	0.3	>300	>300	0.7	300
Gr 164/99	Alossa, Moncucco	0.7	10	0.07	1	200	>300	0.2	>300
Gr 166/99	Morando, Castiglione T.	0.7	300	0.07	100	>300	>300	0.07	10
Gr 167/99	Morando, Castiglione T.	7	>300	>300	>300	0.7	1	0.7	300
Gr 169/99	Soria, Castiglione Tinella	2	>300	0.07	1	>300	>300	0.2	300
Gr 170/99	Soria, Castiglione Tinella	2	10	0.7	1	>300	>300	0.7	>300
Gr 173/99	Saracco, Castiglione T.	0.2	3	0.07	1	20	>300	0.2	>300
Gr 193/99	Aimasso, Valdivilla[a]	0.07	3	0.07	0.1	>300	>300	0.2	3
Gr 206/99	Rabino, S. Vittoria d'Alba	7	>300	0.07	1	200	>300	0.2	100
Gr 209/99	Tarasco, S. Vittoria d'Alba	2	3	0.2	1	>300	>300	0.2	>300
Gr 211/99	Tarasco, S. Vittoria d'Alba	2	10	0.07	0.3	200	>300	0.2	>300
Gr 229/99	Monchiero, Pocapaglia	2	10	0.07	1	>300	>300	0.2	>300
Gr 237/99	Benevelli, Monforte d'Alba	2	100	0.07	1	200	>300	0.2	>300
Gr 257/99	Tibaldi, Pocapaglia	7	>300	0.07	0.3	>300	>300	0.2	100

[a] Strain isolated in one of the vineyards subject to the experimental trials of Di.Va.P.R.A. in 1999.

Table 7. Reaction of strains of *Botrytis cinerea* to different classes of botryticides, and virulence of the strains as evaluated on apple (cv. Golden delicious), tested in 1998.

Strain	Reaction to[a]				
	Dicarboximides	Benzimidazoles	Phenylcarbamates	Anilinopypirimidines.	% rot[b]
Gr 8/98	R	R	S	S	40
Gr 9/98	R	S	R	R	38
Gr 21/98	S	S	R	R	71
Gr 37/98	S	S	R	R	117
Gr 58/98	R	S	R	R	118
Gr 72/98	S	S	R	S	94
Gr 78/98	R	R	S	R	118
Gr 90/98	S	S	R	R	18
Gr 133/98	S	S	R	R	119
Gr 157/98	R	R	R	R	94
Gr 223/98	R	R	S	S	77
Gr 333/98	S	S	R	S	172

[a] R: resistant; S: sensitive.
[b] As compared to rot in apple fruits inoculated with strain Ro 1.

Table 8. Reaction of *Botrytis cinerea* strains to different classes of botryticides, and virulence of the strains, as evaluated on apple (cv. Golden delicious), tested in 1999.

Strain	Reaction to[a]				
	Dicarboximides	Benzimidazoles	Phenylcarbamates	Anilinopypirimidines.	% rot[b]
Gr 17/99	S	S	R	S	120
Gr 22/99	S	S	R	R	124
Gr 25/99	S	S	R	S	136
Gr 67/99	S	S	R	S	141
Gr 73/99	R	S	R	R	219
Gr 77/99	S	S	R	S	168
Gr 113/99	R	S	R	R	125
Gr 116/99	S	S	R	S	78
Gr 118/99	R	R	R	S	257
Gr 119/99	R	R	S	S	115
Gr 121/99	S	S	R	S	87
Gr 124/99	R	S	R	S	114
Gr 125/99	R	S	R	S	121
Gr 127/99	S	S	R	R	59
Gr 129/99	R	S	R	S	138
Gr 131/99	R	R	S	S	130
Gr 132/99	R	S	R	S	165
Gr 133/99	R	R	S	S	113
Gr 134/99	S	S	R	S	122
Gr 137/99	S	S	R	S	125
Gr 141/99	R	R	R	S	107
Gr 142/99	R	S	R	R	92
Gr 143/99	R	S	R	S	121
Gr 144/99	R	S	R	S	153

(*continued on the next page*)

Table 8. (*continued from the preceding page*)

Strain	Reaction to[a]				
	Dicarboximides	Benzimidazoles	Phenylcarbamates	Anilinopypirimidines.	% rot[b]
Gr 145/99	R	S	R	S	90
Gr 146/99	R	S	R	S	163
Gr 147/99	R	S	R	S	178
Gr 148/99	S	S	R	R	116
Gr 150/99	R	S	R	S	111
Gr 151/99	R	S	R	R	113
Gr 153/99	S	S	R	R	217
Gr 154/99	R	S	R	S	128
Gr 157/99	R	S	R	R	122
Gr 159/99	S	S	R	S	129
Gr 161/99	R	S	R	R	0
Gr 162/99	R	S	R	R	6
Gr 163/99	R	S	R	R	124
Gr 164/99	R	S	R	R	124
Gr 166/99	R	R	R	S	104
Gr 167/99	R	R	S	R	256
Gr 169/99	R	S	R	R	122
Gr 170/99	R	S	R	R	175
Gr 173/99	S	S	R	R	127
Gr 193/99	S	S	R	R	197
Gr 206/99	R	S	R	R	139
Gr 209/99	S	S	R	R	154
Gr 211/99	R	S	R	R	237
Gr 229/99	R	S	R	R	212
Gr 237/99	R	S	R	R	220
Gr 257/99	R	S	R	R	311

[a, b] See Table 7.

Table 9. Efficacy of different fungicides against *Botrytis cinerea* on grapevine, cv. Moscato. Trial 1: Saracco Farm, Castiglione Tinella; Trial 2: Aimasso Farm, Valdivilla, 1998.

Treatment	Dosage g a. i. /ha	Phenological stage[a]	% infected bunches[b]		% infected berries[b]	
			Trial 1	Trial 2	Trial 1	Trial 2
Untreated	-	-	38.4 a	52.7 a	4.5 a	11.2 b
Fenhexamid	500	BC	27.9 a	37.2 a	1.3 a	6.1 ab
Fenhexamid	750	BC	29.4 a	34.6 a	1.7 a	5.7 ab
Pyrimethanil	748	B	19.9 a	36.8 a	1.8 a	1.8 a
Fenhexamid	750	C				
Pyrimethanil	748	BC	26.9 a	29.1 a	1.3 a	7.1 ab
Cyprodinil+fludioxonil	300+200	BC	18.3 a	22.0 a	1.6 a	2.3 a

[a] Trial 1: dates of treatments: B, 8 July; C, 7 August; assessment 7 September. Trial 2: dates of treatments: B, 8 July; C, 7 August; assessment 3 September.
[b] Values of the same column followed by the same letter do not differ significantly according Duncan's multiple range test (P=0.05).

Table 10. Trial 3. Efficacy of different fungicides against *Botrytis cinerea* on grapevine, cv. Moscato, Aimasso farm, Valdivilla, 1999.

Treatment	Dosage g a. i. /ha	Phenological stage[a]	% infected[b]			
			Assessment 1[c]		Assessment 2[c]	
			Bunches	Berries	Bunches	Berries
Untreated	-	-	91 c	44.5 b	93 c	57.6 b
Pyrimethanil	748	BC	72 b	25.3 a	76 ab	28.2 ab
Cyprodinil+fludioxonil	300+200	BC	56 a	13.8 a	67 a	20.8 a
Fenhexamid	750	BC	70 b	24.7 a	81 bc	38.2 ab

[a] Dates of treatments: B, 9 July; C, 11 August.
[b] See Table 9.
[c] Dates of assessments: I, 6 September; II, 13 September.

Table 11. Trial 4. Efficacy of different fungicides against *Botrytis cinerea* on grapevine, cv. Moscato, Saglietti Farm, Valdivilla, 1999.

Treatment	Dosage g a. i. /ha	Phenological stage[a]	% infected[b]			
			Assessment 1[c]		Assessment 2[c]	
			Bunches	Berries	Bunches	Berries
Untreated	-	-	66 b	21.7 b	76 b	33.5 b
Pyrimethanil	748	BC	45 a	7.8 a	58 a	12.5 a

[a] Dates of treatments: B, 9 July; C, 13 August.
[b] See Table 9.
[c] Dates of assessments: I, 3 September; II, 9 September.

Discussion

Monitoring in 1998 and particularly in 1999, when disease pressure was high, confirmed the existence of dicarboximide resistance in North-Italian vineyards. This resistance remained stable, compared with levels reported in Gullino *et al*. (1998). Dicarboximide-resistant strains in the field were not cross-resistant to the phenylpyrroles, contrary to what was observed with laboratory strains (Faretra and Pollastro, 1993). Phenotypes of *B. cinerea* with positive cross-resistance to the dicarboximides and phenylpyrroles were not found, confirming what has been reported from Italy (Faretra and Pollastro, 1993) and France (Leroux *et al*., 1999).

Benzimidazole-resistance was stable, when compared with previous levels (Gullino, 1992). The benzimidazoles, never widely applied on grapevine, have not been in use for many years. The phenylcarbamates too have only very rarely been sprayed in Italy, in mixture with benzimidazoles, contrary to what occurs in France, where they are widely applied in anti-resistance strategies (Leroux *et al*., 1999). For the first time, strains of *B. cinerea* resistant to the benzimidazoles and not hypersensitive to the phenylcarbamates have now been detected in North-Italian vineyards, after being observed on Table grape in southern-Italy (Faretra and Pollastro, 1992).

In 1999, resistance to the anilinopyrimidines spread to Piedmont, apparently following the same trend as that observed in 1986 and 1987 with the dicarboximides: resistance was observed first on cv. Moscato, a cultivar frequently sprayed because of its high susceptibility to *B. cinerea* (Gullino, 1992). The presence of strains of *B. cinerea* able to germinate and grow on pyrimethanil-amended medium, already noted in surveys in 1996 and 1997 (Gullino *et al*., 1998), increased rapidly in 1999 and is now quite widespread in the area under study. Strains of *B. cinerea* that grow in the presence of the anilinopyrimidines were also detected in vineyards that had not been sprayed with that group of fungicides and in the control plots of experimental fields, confirming previous observations (Gullino *et al*., 1998). All the anilinopyrimidines-resistant strains showed a low level of resistance, as indicated by the relative low EC_{50} values.

Observations in Switzerland indicate that, as with dicarboximide-resistant fungi, anilinopyrimidine-resistant isolates decrease substantially in winter (Hilber *et al*., 1998).

The risk of the development of resistance to the anilinopyrimidines was experimentally demonstrated in Switzerland (Forster and Staub, 1996). To reduce this risk, an anti-resistance strategy was agreed on by companies producing and/or distributing this class of fungicides. In Switzerland, where the first strains of *B. cinerea* resistant to the anilinopyrimidines were detected (Hilber and Schuepp, 1996), anti-resistance strategies have been developed in concert with the registration authorities: only one treatment per season with anilinopyrimidines, or two with a mixture anilinopyrimidine+fludioxonil are permitted. This anti-resistance strategy has proved successful (Hilber *et al*., 1998).

Surveys carried out in Italy in an area and on a cultivar where fungicide resistance generally develops quickly, clearly indicates the risk linked to the repeated use of anilinopyrimidines. This risk is confirmed by the fact that resistance already occurs in Switzerland and France, two countries where the anilinopyrimidines were first registered (Ruegg *et al*, 1997; Leroux *et al*., 1998). The degree of control achieved in our tests, with two sprays containing pyrimethanil in 1999, was already significantly lower than that shown by the same fungicide only a year earlier (Gullino and Monchiero, 1998): this poses yet more questions concerning the risk of repeated applications of the anilinopyrimidines in the field.

The frequent occurrence of disruptive selection towards resistance, the fact that anilinopyrimidine resistance is encoded in a single gene (Hilber and Hilber-Bodmer, 1998; Chapeland *et al*., 1999) and that resistant isolates have good maintenance of virulence, support the hypothesis of Hilber *et al*. (1998) that the anilinopyrimidines carry a high risk of selecting for resistance in *B. cinerea*.

The careful use of anilinopyrimidines is therefore strongly advised. This should not be difficult on grapevine in Italy, where, at least on wine grape, most growers spray only twice per season. Under such conditions, one spray per season with an anilinopyrimidine, fludioxonil or a mixture of the two should not create excessively high selection pressure. Moreover, since a wide range of compounds are already available to protect cultivars such as Moscato and to be effective in situations of high disease pressure, it may also be advisable not to rely on botryticides with the same mechanism of action two years in succession: an alternation of products could again bring resistant populations to below treshold levels.

The occurrence of isolates of *B. cinerea* with multiple resistance to the benzimidazoles, dicarboximides and anilinopyrimidines, and also coupled with a good level of virulence, threatens to reduce the effective control of grey mould in the field if strict anti-resistance strategies are not followed.

The anilinopyrimidines and the phenylpyrroles are important new chemical tools to control grey mould: their careful application including the observance of anti-resistance strategies will prolong their effectiveness under practical conditions.

Acknowledgements

Work carried out with a grant from Ministry of University (MURST 40%). We thank Prof. Francesco Faretra, University of Bari, for kindly providing strain V 21.

The first author planned the work and wrote the manuscript, the second author carried out the laboratory work, the third author conducted the field trials, the fourth author revised the text.

Literature cited

Chapeland F., R. Fritz, C. Lanen, M. Gredt and P. Leroux, 1999. Inheritance and mechanisms of resistance to anilinopyrimidine fungicides in *Botrytis cinerea*. *Pesticide Biochemistry and Physiology*, 64, 85–100.

Faretra F. and S. Pollastro, 1991. Genetic basis of resistance to benzimidazole and dicarboximide fungicides in

Botrytotinia fuckeliana (*Botrytis cinerea*). *Mycological Research*, 95, 943–951.

Faretra F. and S. Pollastro, 1993. Isolation, characterization and genetic analysis of laboratory mutants of *Botryotinia fuckeliana* (*Botrytis cinerea*) resistant to the phenylpyrrole fungicide CGA 173506. *Mycological Research*, 97, 620–624.

Forster B. and T. Staub, 1996. Basis for use strategies of anilinopyrimidine and phenylpyrrole fungicides against *Botrytis cinerea*. *Crop Protection*, 15, 529–537.

Fritz R., C. Lanen, V. Colas and P. Leroux, 1997. Inhibition of methionine biosynthesis in *Botrytis cinerea* by the anilinopyrimidine fungicide pyrimethanil. *Pesticide Science*, 49, 40–46.

Gullino M.L, 1992. Chemical control of grey mould of *Botrytis* spp. *In*: Recent Advances in Botrytis Research (K. Verhoeff, N.E. Malathrakis, B.Williamson. ed.), Pudoc Scientific Publishers, Wageningen, The Netherlands, 217–222.

Gullino M.L., D. Bertetti, M. Mocioni and A. Garibaldi, 1998. Sensitivity of populations of *Botrytis cinerea* Pers. to new fungicides. *Medelelingen Faculteit Landbouwkundige Universiteit Gent*, 63/3b, 1047–1056.

Gullino M.L. and A. Garibaldi, 1986. Fungicide resistance monitoring as an aid to tomato grey mould management. Proceedings Brighton Crop Protection Conference, Brighton, UK, 2, 499–505.

Gullino M.L., P. Leroux and C.M. Smith, 2000. Uses and challenges of novel compounds for plant disease control. *Crop Protection*, 19, 1–11.

Gullino M.L. and M. Monchiero, 1998. Prove di lotta contro la muffa grigia su vite in Piemonte con l'impiego di pyrimethanil. *Vignevini*, 25 (5 Suppl.), 8–21.

Hilber U.W., C. Baroffio., W. Siegfried and M. Hilber-Bodmer, 1998. Antiresistance strategy for anilinopyrimidines used to control *Botryotinia fuckeliana* in Switzerland. *In:* Modern Fungicides and Antifungal Compounds (H. Lyr *et al*. ed.), Gustav Fischer, Jena, Germany, 291–295.

Hilber U.W. and M. Hilber-Bodmer, 1998. Genetic basis and monitoring of resistance of *Botryotinia fuckeliana* to anilinopyrimidines. *Plant Disease*, 82, 496–500.

Hilber U.W., F.J. Schwinn and H. Schuepp, 1995. Comparative resistance patterns of fludioxonil and vinclozolin in *Botryotinia fuckeliana*. *Journal of Phytopathology*, 143, 423–428.

Leroux P., 1996. Recent developments in the mode of action of fungicides. *Pesticide Science*, 47, 191–197.

Leroux P., F. Chapeland, A. Arnold and M. Gredt, 1998. Résistance de *Botrytis cinerea* aux fongicides, du laboratoire au vignoble et vice versa. *Phytoma-La Défense des Végêtaux*, 504, 62–67.

Leroux P., F. Chapeland, D. Desbrosses and M. Gredt, 1999. Patterns of cross-resistance to fungicides in *Botryotinia fuckeliana* (*Botrytis cinerea*) isolates from French vineyards. *Crop Protection*, 18, 687–697.

Leroux P. and A. Descotes, 1996. Resistance of *Botrytis cinerea* to fungicides and strategies for its control in the Champagne vineyards. Proceedings Brighton Crop Protection Conference, Brighton, UK, 131–136.

Leroux P. and M. Gredt, 1996. *In vitro* methods for monitoring pyrimethanil resistance of *Botrytis cinerea* in grapevine. *EPPO Bulletin*, 26, 186–188.

Milling R.J. and C.J. Richardson, 1995. Mode of action of the anilino-pyrimidine fungicide pyrimethanil. 2. Effects on enzyme secretion in *Botrytis cinerea*. *Pesticide Science*, 45, 43–48.

Pillonel C. and T. Meyer, 1997. Effect of phenylpyrroles on glycerol accumulation and protein kinase activity of *Neurospora crassa*. *Pesticide Science*, 49, 229–236.

Pollastro S. and F. Faretra, 1992. Genetic characterization of *Botryotinia fuckeliana* (*Botrytis cinerea*) field isolates coupling high resistance to benzimidazoles to insensitivity toward the N-phenylcarbamate diethofencarb. *Phytopathologia Mediterranea*, 31, 148–153.

Ruegg J., H.P. Lauber, W. Siegfried, O. Viret and U. Hilber, 1997. Experiences with anilinopyrimidines in Switzerland. *Pesticide Outlook*, 6, 28–33.

Accepted for publication: November 17, 2000