THE SENSITIVITY OF *Botrytis cinerea Pers.*:Fr. TO NEW BOTRITICIDES IN THE VINEYARDS

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ABSTRACT

The grey mould caused by *Botrytis cinerea* is one of the economically important grapevine diseases. Chemical control remains the only way to reduce the incidence of grey mould in grapevine but the problem of *B. cinerea* resistance phenomena restricted the use of many fungicides. In order to overcome the problem of resistance there are continuous world efforts to develop new active ingredients. During the last 5 years new active ingredients have been developed in the world, of which pyrimethanil and cyprodinil from anilinopyrimidine group were acknowledged in Croatia in 1997 and fenhexamide (hidroxianilide) in 1998. In 1998 and '99 we conducted trials in order to monitor the risk of *B. cinerea* resistance build up to the mentioned ingredients in vineyards in which those ingredients had been applied intensively, but also in vineyards in which they had never been used. Strains of *B. cinerea* that are inherently resistant to pyrimethanil, cyprodinil and fenhexamide were determined both in vineyards with the performed intensive control and in vineyards in which these ingredients had never been applied, but there is still no danger of the appearance of practical resistance.
Key words: *Botrytis cinerea*, cyprodinil, fenhexamide, grapevine, pyrimethanil, resistant biotypes

IZVLEČEK

OBČUTLJIVOST GLIVE Botrytis cinerea Pers.:Fr. NA NOVE BOTRITICIDE UPORABLJANE V VINOGRADIH

Siva plesen (*Botrytis cinerea*) je ena od gospodarsko pomembnih bolezni vinske trte. Uporaba fungicidov ostaja edini način za zmanjšanje pojava sive plesni v vinogradih. Težave povezane z njeno odpornostjo na fungicide omejuje uporabo le-teh. Z namenom, da bi rešili problem z njeno odpornostjo, si po vsem svetu prizadevajo, da bi razvili nove učinkovine. V zadnjih letih so v svetu razvili nekaj učinkovin, od katerih so bile v Hrvaški leta 1997 potrjene pirimetanil in ciprodinil iz skupine anilinpirimidinov, ter fenheksamid (hidroksianilid) v letu 1998. V letih 1998 in 1999 smo delali poskuse z namenom ugotavljanja povečane odpornosti na omenjene aktivne snovi v vinogradih, kjer so bile le-te pogosto uporabljane, pa tudi v tistih kjer niso bile nikoli uporabljane. Rase glive *B. cinerea*, ki so dedno odporne na pirimetanil in ciprodinil so bile ugotovljene tako v vinogradih, kjer so pogosto uporabljali te učinkovine, kot v tistih, kjer niso bile nikoli uporabljena. Toda tam kljub temu še vedno ni nevarnosti za pojav praktične odpornosti.

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Ključne besede: *Botrytis cinerea*, ciprodinil, fenheksamid, pirimetanil, vinska trta, rezistentni biotipi

1. INTRODUCTION

Botrytis cinerea, the cause of grey mould, is a fungus well known for the fast development of fungicides resistance. The problem of *B. cinerea* resistance is especially pronounced in the protection of vineyards because grey mould is one of the economically significant grape vine diseases. In the continental part of our country the disease inflicts damages of 50 to 60 %, and in the Mediterranean part 3 to 5 %, which continue in the wine making process (Cvjetković, 1987). In order to overcome the problem of resistance there are continuous world efforts to develop new active ingredients. New ingredients, in addition to having a good efficacy against *B. cinerea* should enable the control of biotypes resistant to existing fungicides and have such a mode of action that does not selects new resistant biotypes. Within such research, methods are being developed for testing the sensitivity of *B. cinerea.* By performing tests in field trials during the pre-registration phase of a new fungicide it is possible in *B. cinerea* populations to detect strains that are naturally resistant to the active ingredient that has not been applied yet. These can be signs for the rapid appearance of resistance if the active ingredient were to be applied intensively. During the last 5 years new active ingredients have been developed in the world (Leroux, 1995; Leroux, 1996), of which pyrimethanil and cyprodinil from the anilinopyrimidine group were acknowledged in Croatia in 1997 and fenhexamide (hidroksianilde) in 1998 (Glasnik zaštite bilja, 1998). Until then the palette of botryticides was based on dicarboximides, the use of which is limited to once or twice during vegetation due to resistance. Dicarboximide-resistance was determined in Croatia in 1993 (Cvjetković *et al.*, 1994; Topolovec-Pintarić, 1995). In 1998 and '99 we conducted trials in order to monitor the presence of strains resistant to the mentioned ingredients in field populations of *B. cinerea* in vineyards in which those ingredients had been applied intensively, and for pyrimethanil and cyprodinil also in vineyards in which they had never been used.

2. MATERIALS AND METHODS

The field trials were carried out in a vineyard at Kutjevo during 1998 and 1999. The grape variety "Graševina bijela" was used. The aim was to analyse the efficacy of new active ingredients and to monitor their influence on *B. cinerea* sensitivity. The trials were set up according to a randomised complete block design in three repetitions. Each plot consisted of three rows with 8 plants per row (a total of 24 plants). Fungicides were applied at recommended concentrations in 1000 l ha -1 of water with Knapsack sprayer. The treatments were conducted according to phenological method (Table 1). Prior to the vintage (28 days after the last treatment) the evaluation of the bunch infection was conducted on a 0-5 scale recommended by EPPO (Anonymous, 1982). The percentage of the infection was calculated according to the Townsend-Heuberger formula (Table 1). At the same time the ripe grape berries with sporulating *B. cinerea* were collected on each repetition. Each sample consisted of at least 10 infected berries, which were placed in plastic tubes and transferred to the laboratory. In vineyard at locations Jastrebarsko and Božjakovina the trials were not conducted and the new active ingredients pyrimethanil and cyprodinil, to which was tested resistance of *B. cinerea*, were never used. The samples were collected prior to the vintage by random choice. At Jastrebarsko in 1998 the grape variety "Rajnski rizling" was used and in 1999 the infected berries of different grape varieties were collected:

Rajnski riling, Pinot sivi, Traminac and Graševina. At location Božjakovina the variety "Pinot sivi" was used in both years.
For each repetition of trials at Kutjevo three isolates of *B. cinerea* were isolated from the collected samples on malt agar and incubated at 18°C in the dark. Ten days later, conidia were harvested and suspensions were prepared in sterile destiled water, conc. 2 x 105. The resistance of *B. cinerea* was tested *in vitro* by germ-tube assay described by Leroux and Gredt (1996). Technical grade pyrimethanil (AgrEVO) or cyprodinil (Novartis) was incorporated into test medium as an ethanolic solution in concentration 1 mg l -1. The fenhexamide was prepared from fungicide Teldor (50% fenhexamide) and incorpored into a test medium in the same concentration.

3. RESULTS AND DISCUSSION

There were no statistically significant differences between the efficacy of pyrimethanil, cyprodinil and fenhexamide that gave very good control. Alternations Mikal-Mythos-Switch-Kidan and Mikal-Mythos-Switch came second and provided disease control superior to the standard alternation Folicur E-Mikal-Kidan-Kidan. Standard alternation gave satisfactory control and it was equal to the fungicides from the dicarboximide group, Kidan and Ronilan. Last according to efficacy was biofungicid Trichodex. The results of the evaluation of fungicides performance are shown in Table 1.

Table 1: Treatment schedules and performance against *B. cinerea* in the field trials at Kutjevo, 1998 - 1999.

Treatment	Active ingredient	(%)	Conc. (%)	Spray shedule +				Diseased bunch surface (%)		Efficacy of fungicides (%)			Stat. sig.**
				A	B	C	D	1998	1999	1998	1999	x	
SWITCH	CIPRODINIL	35 %		+	+	+	+	5,78	3,60	87,65	90,95	89,30	a
	+FLUDIOKSONIL	35 %	0,08										
TELDOR	FENHEKSAMID	50 %	0,15	+	+	+	+	11,09	3,67	76,29	90,78	83,53	a b
MYTHOS	PIRIMETANIL	30 %	0,25	+	+	+	+	13,79	5,10	70,52	87,19	78,85	b
MIKAL	Al-EFOSIT	50%											
	+ FOLPET	25 %	0,3	+	-	-	-						
MYTHOS	PIRIMETANIL	30 %	0,25	-	+	-	-	16,87	9,52	63,94	75,41	69,67	c
SWITCH	CIPRODINIL	35 %											
	+FLUDIOKSONIL	35 %	0,08	-	-	+	-						
KIDAN	IPRODION	25 %	0,2	-	-	-	+						
MIKAL	Al-EFOSIT	50%											
	+ FOLPET	25 %	0,3	+	-	-	-						
MYTHOS	PIRIMETANIL	30 %	0,25	-	+	-	-	22,00	9,79	52,97	76,09	64,53	c
SWITCH	CIPRODINIL	35 %											
	+FLUDIOKSONIL	35 %	0,08	-	-	+	-						
KIDAN	IPRODION	25 %	0,2	+	+	+	+	29,54	13,41	35,81	66,32	51,06	d
FOLICUR E	TEBUKONAZOL	10 %											
	+ DIKLOFLUANID	40 %	0,3	+	-	-	-						
MIKAL	Al-EFOSIT	50%						30,03	16,21	36,85	49,82	43,33	d e
	+ FOLPET	25 %	0,3	-	+	-	-						
KIDAN	IPRODION	25 %	0,2	-	-	+	-						
KIDAN	IPRODION	25 %	0,2	-	-	-	+						
RONILAN	VINKLOZOLIN	50 %	0,1	+	+	+	+	35,20	19,98	24,76	59,29	42,02	e
TRICHODEX	*T.harzianum*	*	0,4	+	+	+	+	40,86	22,66	12,65	43,09	27,87	f
KONTROLA	-		-	-	-	-	-	46,78	39,82	-	-	-	-

+ Growth stages: A-end of flowering, B-before bunch closer, C-beginning of berry ripening, D-4 weeks before harvest.
* Biofungicide based on fungus *Trichoderma harzianum*, 10^{10} conidia/g
+ Application at that growth stage.
- Application omite at that growth stage.
** Number followed by the same letter within columns do not differ significantly; Duncan's multiple range test (p = 0,005)

The resistance of *B. cinerea* to pyrimethanil and cyprodinil was tested *in vitro* by germ-tube assay described by Leroux and Gredt. The main advantages of germ-tube assay are its speed and reliability. The same test was conducted for testing resistance of *B. cinerea* to fenhexamide and it was proven that it could also be used for such testing. Three phenotypes of *B. cinerea* can be distinguished according to their *in vitro*

responses to pyrimethanil, cyprodinil and fenhexamide. Most of the strains were sensitive - Sa type or exhibited low levels of resistance - Ra1 type. Highly resistant strains - Ra2 type were found in both years at Kutjevo, although their number increased in the second year of trials. At Jastrebarsko and Božjakovina Ra2 types were found only in 1999. The results of resistance testings are shown in Figures 1 and 2.

Figure 1: Resistance of *B. cinerea* to pyrimethanil, cyprodinil and fenhexamide at Kutjevo, 1998-1999.

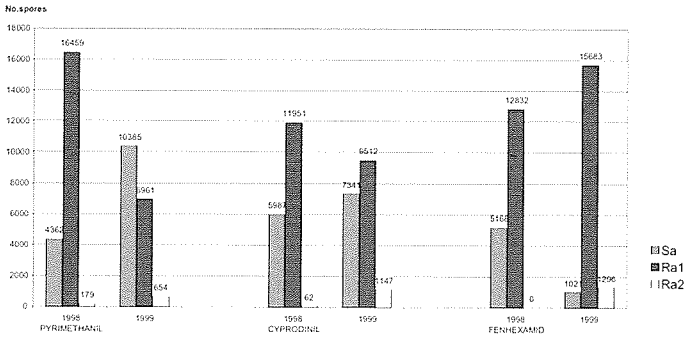
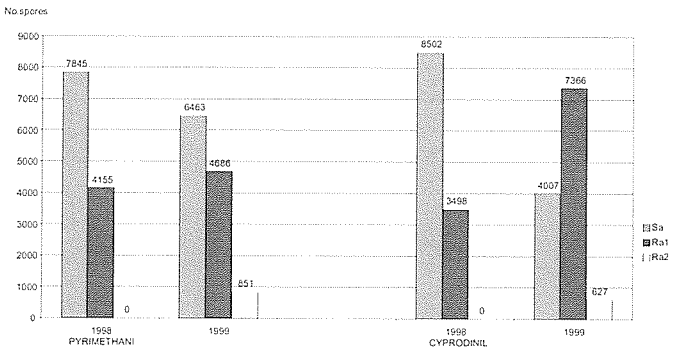


Figure 2: Resistance of *B. cinerea* to pyrimethanil and cyprodinil at locations Jastrebarsko and Božjakovina, 1998-1999.



The resistance of *B. cinerea* to pyrimethanil, cyprodinil and fenhexamide was determined for the first time in a vineyard at Kutjevo. Among the resistant strains two phenotypes were distinguished, Ra1 and Ra2. The reduced efficacies would occur only with Ra2 type. The number of Ra2 phenotype was grew from the first to the second year of trials that lead to the conclusion of the appearance of so called "acquired resistance". Examination of *B. cinerea* field isolates collected in vineyards at Božjakovina and Jastrebarsko showed that this fungi constitute a high "inherent resistance" to pyrimethanil and cyprodinil. This was the confirmation of the FRAC warning that anilinopyrimidines are a high risk for resistance development (Birchmor *et al.*, 1996). The obtained results, detection of Ra2 type, are a warning sign for the application of the mentioned ingredients, but there is still no danger of the appearance of practical resistance. Therefore, the alternations of different active ingredients should be recommended as the base of antiresistance strategy for *B. cinerea* control because they reduce the use of anlinopyrimidines to one or two times per season.