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Investigation into effective control of powdery mildew with strobilurins in viticulture - a case study

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An illustrative example of what kind of difficulties pesticide resistance of pathogens can cause in plant protection is the rapid spread of strobilurin resistance of powdery mildew in a growing number of grape producing areas. Powdery mildew, caused by Erysiphe necator *Schwein., is one of the most important diseases of grapes worldwide. In the present study four different spraying plans were tested against powdery mildew on* Vitis vinifera *cv. 'Merlot' CI 181 and cv. 'Italian Riesling' GK1' in a three years period. The aim of the study was to examine the effect of the different spraying plans, in which the primary compounds were the strobilurins, called Quinone outside Inhibitors (QoI). QoI had never been used before in that experimental vineyard or in any nearby vineyards. For each spraying plan powdery mildew infection levels were monitored. Our results revealed significant chemical compound and compound combination dependent differences in the infection levels. When strobilurins alone were applied, little or no control on powdery mildew infections was observed, and the severity of the infection was not significantly different from the untreated vines. This indicates that strobilurin resistance was already present in this experimental vineyard, although the origin of it is unclear. By molecular genetic means we proved that the strobilurin resistant genotype is different from the sensitive one. When strobilurins were applied in combinations with other compounds the level of powdery mildew infection was either significantly lower than in the untreated vines or there was no infection at all. Based on our results, it is advised not to use only strobilurin or analogue compounds alone in blocks. Instead of that, combinations of different compounds are thought to provide efficient protection against grape powdery mildew, and this would also delay the development of resistance against the applied compounds.*

Keywords: powdery mildew, *Erysiphe necator*, spraying plan, fungicide resistance, *Vitis vinifera*, strobilurin

Untersuchung über die wirksame Bekämpfung von Oidium mittels Strobilurinen im Weinbau – eine Fallstudie. *Ein anschauliches Beispiel dafür, welche Art von Problemen Pestizidresistenzen von Krankheitserregern im Pflanzenschutz verursachen können, ist die rasche Ausbreitung der Strobilurinresistenz von Oidium in einer wachsenden Zahl von Weinbaugebieten. Oidium, das verursacht wird durch* Erysiphe necator *Schwein., ist weltweit eine der wichtigsten Rebkrankheiten. In dieser Untersuchung wurden vier verschiedene Spritzpläne gegen Oidium bei Reben der Sorten 'Merlot' CI 181 und 'Welschriesling' GK1 über drei Jahre getestet. Ziel der Untersuchung war es, den Effekt verschiedener Spritzpläne zu prüfen, deren Hauptwirkstoffe Strobilurine, auch Quinone outside inhibitors (QoI) genannt, waren. QoI waren in den Versuchsweingärten oder den anliegenden Weingärten nie vorher eingesetzt worden. Für jeden Spritzplan wurden die Infektionsraten aufgezeichnet. Die Ergebnisse zeigten signifikante Unterschiede in diesen Infektionsraten, abhängig von den Wirkstoffen bzw. ihrer Kombinationen. Wenn Stroliburine alleine angewendet wurden, wurde nur ein geringer oder gar kein Einfluss auf die Oidiuminfektion beobachtet, und die Infektionsstärke war nicht signifikant unterschiedlich im Vergleich zu den unbehandelten Reben. Das zeigt, dass in diesem Versuchsweingarten bereits eine Stroliburinresistenz vorhanden war, wenn auch deren Ursprung nicht klar ist. Mittels molekulargenetischer Untersuchungen wurde nachgewiesen, dass sich die stroliburinresistenten Genotypen von den nichtresistenten unterschieden. Wenn Stroliburine in Kombination mit anderen Wirkstoffen ausgebracht wurden, war die Infektionsrate entweder signifikant niedriger als in der unbehandelten Variante, oder es gab gar keine Infektion. Basierend auf unseren Ergebnissen wird empfohlen, Stroliburine oder analoge Wirkstoffe nicht alleine in Blöcken*

anzuwenden. Es wird angenommen, dass Kombinationen verschiedener Wirkstoffe einen effizienten Schutz gegen Oidium bieten, und das würde auch die Ausbildung von Resistenzen gegen die angewandten Verbindungen verlangsamen.
Schlagwörter: Oidium, *Erysiphe necator*, Spritzplan, Fungizidresistenz, *Vitis vinifera*, Strobilurin

Recherche relative à la lutte contre l'oïdium à l'aide de strobilurines dans la viticulture – une étude de cas.
Un exemple illustrant le genre de problèmes pouvant être occasionnés par les résistances d'agents pathogènes aux pesticides dans le domaine de la protection de plantes est la propagation rapide de la résistance de l'oïdium aux strobilurines dans un nombre croissant de vignobles. L'oïdium, qui est provoqué par Erysiphe necator *Schwein., est l'une des principales maladies de la vigne à l'échelle mondiale. Dans le cadre de la présente recherche, des vignes des cépages 'Merlot' CI 181 et 'Welschriesling' GK1 ont fait l'objet d'un test de quatre plans de pulvérisation différents contre l'oïdium durant trois ans. Le but de la recherche a été d'examiner les effets des différents plans de pulvérisation dont les substances actives étaient des strobilurines, également appelés Quinone outside inhibitors (QoI). Les QoI n'ont encore jamais été utilisés dans les vignobles d'essai ou dans les vignobles avoisinants. Les taux de contamination ont été enregistrés pour chaque plan de pulvérisation. Les résultats ont fait ressortir qu'il existe des différences significatives dans ces taux de contamination en fonction des substances actives et/ou des combinaisons de celles-ci. Dans les cas où seules les stroliburines avaient été appliquées, on n'a observé qu'un effet faible ou aucun effet du tout sur la contamination par l'oïdium, et l'ampleur de la contamination n'était pas significativement différente de celle des vignes non traitées. Ceci prouve qu'une résistance aux stroliburines était déjà présente dans ce vignoble d'essai, même si son origine n'est pas claire. Des études génétiques moléculaires ont permis de prouver que les génotypes résistants aux stroliburines se distinguaient des génotypes non résistants. Dans les cas où les stroliburines ont été épandues en combinaison avec d'autres substances actives, le taux de contamination était significativement inférieur à celui de la variante non traitée, ou bien il n'y avait aucune contamination du tout. Sur la base de nos résultats, il est recommandé de ne pas utiliser les stroliburines ou des substances actives analogues seules par blocs. On suppose que les combinaisons de différentes substances actives assurent une protection efficace contre l'oïdium et que cela permettrait également de freiner la formation de résistances contre les composés appliqués.*
Mots clés : oïdium, *Erysiphe necator*, plan de pulvérisation, résistance aux fongicides, *Vitis vinifera*, strobilurines

Powdery mildew was introduced into Europe in the 19th century probably through the purchase of infected canes from North-America. The first official note of the fungus was taken by a gardener named Tucker among on glasshouse-grown grapes in England in 1845 (Bulit and Lafon, 1978).
The Erysiphe necator Schwein. is an obligate parasitic fungus (Halleen and Holz, 2001), which can infect all green parts of sensitive plants of the Vitaceae family. Without any protection the yield loss can be even 100 % (Füzi, 1994). Sulphur was the first fungicide used to control powdery mildew on grapes (Bulit and Lafon, 1978), but later several non-systemic and systemic fungicides were developed to enhance the effectiveness of chemical compounds. Amongst them strobilurins were developed from a natural molecule, strobilurin A, which was isolated from Strobilurus tenacellus (Anke et al., 1977). Strobilurins belong to the Quinone outside Inhibitors (QoI) fungicide group, which inhibit mitochondrial respiration (Bartlett et al., 2002) by blocking the electron transfer at the cytochrome bc1-complex (Joseph-Horne and Hollomon, 2000; Fernandez-Ortuno et al., 2008). Strobilurins are important tools for the control of several other fungi also and have a different mode of action from other available fungicides (Taksonyi et al., 2009). The chosen chemical compounds and the applied method strongly influence the development and intensity of powdery mildew epidemic (Füzi, 1994). While QoI fungicides may effectively be used against powdery mildew, it should be noted, that due to their single-site mode of action, there is a high risk of resistance development against them (Dula, 2007). In 2006 several surveys of Erysiphe necator revealed the presence of resistant strains in Hungary. The resistant populations were found in two of the wine growing regions of Hungary (there are 22 wine growing regions in Hungary): one in Southern Hungary (wine region Szekszárd) and the other in northern Hungary (wine region Eger) (Taksonyi et al., 2009).
The aim of the present study was to analyze the effectivity of strobilurins against powdery mildew in different spraying plans, to contribute to a more effective plant protection in grape production.

Materials and methods

Plant material

The experiment was set up at the experimental vineyard of the Georgikon Faculty, University of Pannonia, in Cserszegtomaj, which belongs to the Balaton wine-grape growing region (GPS coordinates: 33°55'12"S 18°51'36"E / 33.92000°S 18.86000°E). In the present study during from 2008 to 2010 four spraying plans (Table 1) were tested against powdery mildew on *Vitis vinifera* cv. 'Merlot' CI 181 and cv. 'Italian Riesling' GK1. The selected plantations were of different age. The 'Italian Riesling' GK1 was established on 'Teleki 5C' rootstock in 1993, it is cordon cultivation, short spur pruned (10 to 12 buds/vine), with 3 x 1 m spacing (3 m between rows and 1 m between vines). The 'Merlot' CI 181 was established in 1996 on 'Teleki Kober 125AA' rootstock, it is also cordon cultivation and short spur pruned, with 2.3 x 1 m spacing.

Spraying plans

Spraying was done with a SOLO 450, 10 l tank capacity (Solo, Sindelfingen, Germany) motorized knapsack sprayer. Spraying was performed always depending on the weather conditions, and the weather data were collected with Metos (by Pessl Instruments, Weiz, Austria), which was placed in the middle of the vineyard.
In each spraying plan plots were created, and one plot consisted of 23 to 32 plants. Each spraying plan was performed in seven replicates (seven plots/spraying plan), while the untreated control was in three replicates. Timing of spraying was adjusted to the developmental stage of grapes (Lorenz et al., 1994) (Table 1). During the three years (2008 to 2010) of the experiment each plot always got the same treatment. To eliminate fungicide drift from surrounding plots, between treatments and replicates a buffer zone of six rows was used. In the buffer zone the vines were sprayed with fungicides containing no QoI-fungicides.
Treatments were divided into four plans according to the active ingredients (Table 2a, 2b). In the first spraying plan (I), only QoI fungicides were used in all three years, 7 to 8 times per year. In the second spraying plan (II), commercially available formulations of fungicides were used, where strobilurins were combined with active ingredients against *Plasmopara viticola* (Berk. and Curt.). Spraying plan III was like spraying plan II but the fungicides were supplemented also either with sulphur (IIIa and IIIb) or triazol (IIIc). In the fourth spraying plan (IV) strobilurins were applied in rotation with other fungicides in a program according to the technological recommendations of the manufacturer's representatives. During the sprayings, the used chemicals were distributed by the companies in compliance with their proposed order and dose. The dose of spraying was calculated by the molar-concentration with the following formula (Fig. 1):

Fig.1

$$\rho_B = \frac{m_B}{V_\circ} \; g/l; g/dm^3$$

The relationship between Figure 1 and Table 2: The applied doses for spraying plan I, II and III were calculated with the formula in Figure 1 (see values in Table 2a) , but for spraying plan IV (Table 2b) dose recommendations of the manufacturers were applied.

Table 1: Timing of protection in the experimental years

Protection 2008			Protection 2009			Protection 2010		
Number	Date	BBCH	Number	Date	BBCH	Number	Date	BBCH
1	2008.05.31	19	1	2009.05.16	15	1	2010.05.13	16
2	2008.06.14	65	2	2009.05.25	19	2	2010.05.21	19
3	2008.06.25	71	3	2009.06.06	55	3	2010.06.10	55
4	2008.07.18	75	4	.2009.06.15	65	4	2010.06.15	65
5	2008.07.27	77	5	2009.06.30	71	5	2010.06.26	71
6	2008.08.04	79	6	2009.07.09	75	6	2010.07.07	75
7	2008.08.19	81	7	2009.07.22	77	7	2010.07.28	77
-	-	-	8	2009.08.11	79	8	2010.08.10	79

Table 2a: Tested spraying plans (I – III) in Cserszegtomaj during 2008 to 2010

Spraying plan	Mark of plot	Year	Number of protection 1	2	3	4	5	6	7	8	ρB (g/l)	Replicate (plot number)	Number of vines/plot
			Active ingredient (Trade name)										
I.	a	2008 2009 2010	Pyraklostrobin								0.8 / 1.6	7	32
	b	2008 2009 2010	Azoxystrobin (Quadris)								1.2 / 2.4	7	31
	c	2008 2009 2010	Trifloxystrobin (Zato)								0.3 / 0.6	7	32
		Protection	x	x	x	x	x	x	x	*			Σ 95
II.	a	2008 2009 2010	Pyraklostrobin + metiram (Cabrio Top)								2 / 4	7	28
	b	2008 2009 2010	Azoxystrobin + folpet (Quadris Max)								2 / 4	7	30
	c	2008 2009 2010	Trifloxystrobin + cymoxanil (Eclair)								0.5 / 1	7	23
	d	2008 2009 2010	Cymoxanil + famoxadone (Tanos)								0.4 / 0.8	7	29
		Protection	x	x	x	x	x	x	x	*			Σ 110
III.	a	2008 2009 2010	Pyraklostrobin + metiram + sulphur (Cabrio Top + Kumulus S)								(2) + 4 / (4) + 8	7	32
	b	2008 2009 2010	Trifloxyistrobin + cymoxanil + sulphur (Eclair + Kumulus S)								(0.5) + 4 / (1) + 8	7	32
	c	2008 2009 2010	Azoxystrobin + folpet + penconazole (Quadris Max + Topaz 100 EC)								(2) + 3 / (4) + 6	7	32
		Protection	x	x	x	x	x	x	x	*			Σ 96

* Only in 2009 to 2010

x: Treated

Table 2b (cont.): Tested spraying plan IV in Cserszegtomaj during 2008 to 2010

Mark of plot	Year	Number of protection 1	2	3	4	5	6	7	8	Replicate (plot number)	Number of vines/plot
		Active ingredient (Trade name)									
c SYN- GENTA	2008	Thiovit Jet 10 kg/ha	Bravo 500 2.5 l/ha Topas 0.3 l/ha	Ridomil G. 2.5 kg/ha Topas 0.3 l/ha	Quadris Max 2 l/ha	Quadris Max 2 l/ha	Quadris Max 2 l/ha	Ridomil 2.5 kg/ha Topas 0.3 l/ha Chorus 75WG 0.6 kg/ha	Thiovit Jet 10 kg/ha Ridomil G. 2.5 kg/ha Chorus 75WG 0.6 kg/ha	7	30
	2009	(Topas 0.3 l/ha Ridomil MZ 2.5 kg/ha)	(Quadris Max 2 l/ha)	(Topas 0.3 l/ha Pergado F 2.5kg/ha)	(Quadris Max 2 l/ha)	(Quadris Max 2 l/ha)	(Topas 0.3 l/ha Pergado F 2.5kg/ha)	Not sprayed	(Thiovit Jet 10 kg/ha Ridomil Plus 4 kg/ha Chorus 75WG 0.6 kg/ha)		
	2010	(Topas 0.3 l/ha Ridomil MZ 2.5 kg/ha)	(Quadris Max 2 l/ha Thiovit Jet 8 kg/ha)	(Topas 0.3 l/ha Pergado F 2.5 kg/ha)	(Quadris Max 2 l/ha Thiovit Jet 8 kg/ha)	(Quadris Max 2 l/ha Thiovit Jet 8 kg/ha)	(Topas 0.3 l/ha Pergado F 2.5 kg/ha)	(Thiovit Jet 10 kg/ha Ridomil Plus 4 kg/ha)	(Thiovit Jet 10 kg/ha Ridomil Plus 4 kg/ha Chorus 75WG 0.6 kg/ha)		
											Σ 87
C	2008/2009/2010 – Untreated									3	15

Molecular identification of strobilurin resistance

For molecular analysis *E. necator* infected leaves were collected. Two samples were collected from 'Italian Riesling', of which one was from an azoxystrobin-treated plant, while the other was from a trifloxystrobin-treated plant. Also two samples were collected from 'Merlot', of which one was from a pyraclostrobin-treated plant and the other from a trifloxystrobin-treated plant. For comparison wild E. necator genotypes were collected from four plants from a strobilurin treatment-free area.
DNA was extracted by the Doyle & Doyle method (Doyle and Doyle, 1987) from 50 mg infected leaves and primers were designed based on the cytochrome b1 sequence of *Blumeria graminis*, since sequence data were not available for this gene of *E. necator* in the open databases (NCBI, DDBJ). Those primers amplified the expected 653 bp fragment that contains also the mutation site responsible for strobilurin resistance in other fungi. Sequencing of this fragment from the resistant and sensitive genotypes was performed. After comparison of these *E. necator* sequences a primer specific for the mutation was designed too, and was used in PCR together with the previous two primers. Due to the mutation specificity of this third primer a 206 bp band was expected in the strobilurin resistant *E. necator* genotypes.

Data collection and evaluation

In each treatment 50 randomly chosen leaves were examined and the rating of surface infection was determined on a scale from 0 to 5, and the Townsend-Heuberger infection level was determined (Gartner, 1971). On the established scale the 0 represented the uninfected leaves, rating 1 represented surface infection under 5 %, rating 2 surface infection between 5 and 25 %, rating 3 from 25 % to 50 %, rating 4 from 50 % to 75 % and rating 5 represented a surface infection of more than 75 %. Data of each replicates were averaged and evaluated by the SPSS 13.0 (SPSS Chicago, USA) statistical analysis and data management software. The method of the statistical analysis was two-way analysis of variance.

Results

Weather conditions and infections

Weather conditions during the infection period of the three experimental years (2008 to 2010) are shown in Table 3. In May, June and July, the temperature showed the usual rising tendency, where the hottest month was July. The humidity varied between 70 % and 78.4 % in all three years. We measured the highest humidity in June in all three years. For the monthly precipitation considerable differences were registered each year. While the amount of precipitation was average in 2008, the season in 2009 was dry, and in 2010 it was rainy. In 2008 the rainiest month was June, while in 2009 and 2010, it was May.
2008 was an average year considering precipitation, relative humidity and temperature in Hungary (Table 3). The first infections in this year were observed only in July. There were no, or only a low number of chasmoteciums – which is the general overwintering form of powdery mildew in Hungary – in the experimental vineyard in spring time, so the infections came from outside of the experimental field.
In 2009 the majority of the precipitation was in the first half of the growing season, and this was followed by a longer drier period. The initial high moisture and high humidity resulted in high downy mildew infection in the test area. However, in the middle of the growing season, the amount of precipitation was low, but rainfall occurred relatively frequently, resulting in a strong powdery mildew infection. The relatively late emergence of powdery mildew was possibly influenced by the earlier occurring heavy downy mildew infection in the first half of June.
The year 2010 brought an outstanding amount of rain in May accompanied by long-lasting, cold, wet wea-

Table 3: Average weather conditions in the experimental field during the infection period

Weather indicators	2008			2009			2010		
	May	June	July	May	June	July	May	June	July
Average air temperature (°C)	15.8	19.8	20.2	16.0	17.6	21.0	15.5	18.6	21.8
Average rel. humidity (%)	70.0	77.4	74.2	74.8	78.4	73.6	70.5	78.0	72.3
Average precipitation (mm)	38.2	104.0	84.6	60.0	24.2	1.0	152.4	97.8	44.0

ther. Rainy periods were followed by 10 to 14 days long dry periods. The intensity of the precipitation was extreme and it was often accompanied by high force wind, which decreased the wetness of the leaf surface forming perfect conditions for powdery mildew infection. The first infections were observed at the end of June.

Effect of spraying plans on powdery mildew infections

In this study, the powdery mildew infection values were between 1.40 to 2.75 in 2008, 0.07 to 1.45 in 2009 and 0.07 to 2.49 in 2010 (Table 4). It means that the lowest infection value was close to 0 % and the highest was about 37.5 % of the total leaf area. No significant differences could be detected among the exclusively strobilurin treated plots (spraying plan I.) and the untreated controls. However, the control plots showed generally higher infection rates, and in 2008 and 2009 on 'Italian Riesling' the infection rates were even higher in the strobilurin treated plots than in the untreated control. The highest infection rate, 2.75, was observed in 2008 on 'Italian Riesling' GK1 (Table 4). This result is astonishing since no strobilurins had been used before 2008 in the experimental field and in the surrounding vineyards. Both in the control and

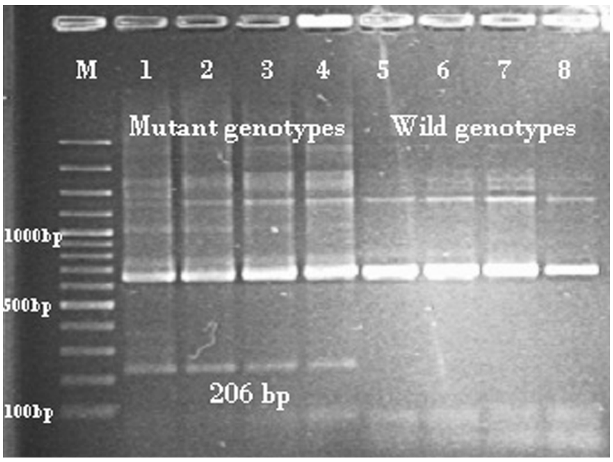

Fig. 2: An electrophoretogram demonstrating the detection technique with 4-4 strobilurin resistant and sensible *Erysiphe necator* genotypes. M: DNA ladder; 1 - 4: treated genotypes, 1: 'Italian Riesling' with azoxystrobin, 2: 'Italian Riesling' trifloxystrobin, 3: 'Merlot' pyraclostrobin, 4: 'Merlot' trifloxystrobin, 5 - 8: wild genotypes from strobilurin treatment-free area. The presence of the 206 bp size fragment indicates the strobilurin resistance in *E. necator.*

treated plots the severity of powdery mildew infection was the most serious in 2008. Samples that showed resistance to strobilurins were compared by molecular genetic analysis with sensitive samples. Based on differences detected in the DNA of the two genotypes we elaborated a detection technique for the fast and simple detection of strobilurin resistance in E. necator. Here we present an electrophoretogram showing typical banding pattern of strobilurin resistant and sensitive genotypes (Fig. 2). (Detailed description of the detection technique will be published elsewhere.)

The QoI-fungicides in combination with specific active ingredients against powdery mildew (plan III. and IV.) were significantly more effective than spraying plans I. and II. (Table 4).

The powdery mildew infection cycles were delayed in 2009 and the infection rates were lower than in the other two years because of the initially rainy and later dry season. These conditions are more favourable to downy mildew infection. After the arrival of the warm dry period, spot-like powdery mildew infections formed in that year, but the infection rates in the control and in all treated plots were significantly lower than in the other two years.

In 2009 and 2010 the spraying plans proposed by the manufacturers (IV.) were the most effective against powdery mildew. Although in 2008 the program IV. compared to the I. and II. spraying plans was significantly more effective, high infection rates were observed. In 2008 the spraying plan III., where strobilurins were combined with E. necator specific agents were somewhat more effective than the programs proposed by the manufacturers (IV.).

From the two compared varieties 'Italian Riesling' seemed to be more sensitive to powdery mildew than the variety 'Merlot' and for 'Italian Riesling' in each treatment higher infection rates were registered than for 'Merlot'.

At the beginning of the analysis we did not find any chasmoteciums in the experimental area, but during the experiment we had more and more of them year after year. In Hungary powdery mildew overwinters in chasmotecium form. Where the spraying plan had no effect there the number of chasmoteciums was higher. Powdery mildew infection was two to four times higher in 2010 than in 2009. The highest infection level was recorded in the control, untreated plants in both varieties, 'Merlot' Cl 181 and 'Italian Riesling' GK1, the Townsend-Heuberger index indicated 2.03 and 2.49 values, respectively (Table 4).

Table 4: Results of two-way analysis of variance in the four spraying programs and control on two grape varieties in 2008 – 2010

Treatments	Year	Level of infections		SE	Fixed effect (P value = ?)		
		Ital. Riesling (n = 140)	Merlot (n = 140)		Trt[9]	Var[10]	Trt x Var[11]
					< 0.01	0.66	0.90
Strobilurin agents (I.)	2008	2.75[a]	2.19[a]	± 0.26			
Strobilurin agents and agents to *P. viticola* (II.)	2008	2.15[a]	2.20[a]	± 0.22			
Strobilurin agents and agents to *E. necator* (III.)	2008	1.40[b]	1.51[b]	± 0.26			
Spraying plans proposed by manufacturers (IV.)	2008	1.64[b]	1.56[b]	± 0.26			
Untreated (Control)	2008	2.73[a]	2.62[a]	± 0.45			
					<0.001	0.78	0.39
Strobilurin agents (I.)	2009	0.90[abe]	0.67[abe]	± 0.14			
Strobilurin agents and agents to *P. viticola* (II.)	2009	0.68[abc]	0.26[abc]	± 0.12			
Strobilurin agents and agents to *E. necator* (III.)	2009	0.30[cd]	0.20[cd]	± 0.14			
Spraying plans proposed by manufacturers (IV.)	2009	0.11[d]	0.07[d]	± 0.14			
Untreated (Control)	2009	0.86[e]	1.45[e]	± 0.23			
					<0.001	0.17	0.93
Strobilurin agents (I.)	2010	1.98[a]	1.70[a]	± 0.20			
Strobilurin agents and agents to *P. viticola* (II.)	2010	1.56[b]	1.07[b]	± 0.17			
Strobilurin agents and agents to *E. necator* (III.)	2010	0.62[c]	0.53[c]	± 0.20			
Spraying plans proposed by manufacturers (IV.)	2010	0.18[cd]	0.07[cd]	± 0.20			
Untreated (Control)	2010	2.49[ae]	2.03[ae]	± 0.34			

SE – Standard error
[9]Treatment effect; [10]Effect of varieties; [11]Treatment effect and varieties interaction
[abcde]Means in the same column with different superscript differ ($P < 0.05$)
I. - IV. – represent the different spraying plans
n – post data registration weighted average number of repeats

Discussion

In our study, the broadest possible spectrum of QoI-fungicides that are used in the viticultural practice in Hungary was covered. Spraying plans and fungicides with different chemical compounds and modes of action were tested in our study against Erysiphe necator. Most case-studies on powdery mildew resistance compared only the effect of one product for several years (Steva and Clerjeau, 1990; Redl and Steinkeller, 1996; Halleen and Holz, 2001; Miller and Gubler, 2004; Wong and Wilcox, 2002) in block sprayings. Our results showed that only a three- years-use of strobilurins – all types which are commercially available in Hungary – could be enough to select less sensitive powdery mildew genotypes. Most of the published results on strobilurin resistance in E. necator report only the detection of resistant strains (Fernandez-Ortuno et al., 2008; Ishii et al., 2001). The spraying plans (III; IV) in which the use of strobilurins with sulphur, penconazol or other combination partners are recommended to slow down the selection of strobilurin-resistant strains. Our experiment shows how the spraying strategy can influence the development of resistance. We have not observed any antagonistic effect of sulphur with strobilurins, like Steva (1992) did with triadimenol. The mixture with fungicides against downy mildew did not reduce the risk of strobilurin resistance development, but our results show some effect on powdery mildew (II). Nevertheless, these effects cannot protect the vines. The spraying schedules recommended by each of the three companies (Table 1) were the best among the treatments. It can be concluded that in the protection against powdery mildew manufacturers' recommendations have to be followed, so strobilurins should not constitute more than 1 to 2 sprayings of the total number of applications. We agree with the FRAC (1997) statement that a strobilurin treatment has to be followed by the application of a fungicide with different mode of action, but our results indicate that an addition of sulphur should be recommended.

No significant differences were found between the varieties 'Merlot' CI 181 and 'Italian Riesling' GK1 in powdery mildew infection levels in the untreated control. However, there were differences in the powdery mildew infection levels depending on the chemical compounds and the combinations of compounds tested. The strobilurins provided either little or no control of powdery mildew infections, and the infection rate was not significantly different from that of the untreated vines. This suggests that the population of powdery mildew in this experimental vineyard contained already resistant strains, which due to the selection pressure of strobilurin treatment rapidly proliferated in those plots. We sprayed the strobilurins in block spraying in all three years. The results show that block spraying cannot protect the vines against powdery mildew. In the other combinations of compounds, the level of powdery mildew infection was either significantly lower than in the untreated vines or there was no infection at all. We have not got significant differences between the first (I) spraying plan, untreated plan and the second (II) spraying plan. Also the use of the combination of the second (II) spraying plan could not protect the vine stocks against powdery mildew during the vegetation period. On the other hand, significant differences were obtained from the first two spraying plans, and the untreated groups when these were compared to the third (III) spraying plan. The combinations of strobilurins and sulfur have a high potential to protect the vine stocks during the vegetation period. During the experiment the best protection results were obtained with the fourth (IV) spraying plan. The IV. spraying plan was significantly better than the other spraying plans. These results show that the use of block spraying without any combination partners against powdery mildew is not effective, but the use of combination (plan III) and rotation (spraying plan IV) during the vegetation period can protect the vine stocks against a strong pressure of powdery mildew. With the use of combination and rotation of fungicides we can also exclude the development of strobilurin resistant strains in vineyards. These results show also that the used strobilurin increased the level of QoI-resistance in the powdery mildew population. As the infection level was as high (5 to 37.5 %) in the strobilurin-treated vines as it was in the control vines, these results provide a warning that in the future QoI-resistant powdery mildew strains are likely to be a problem. New types of fungicides which have a single-site mode of action should be carefully monitored, because the risk for the development of resistant strains is higher with them.

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