Sensitivity of *Uncinula necator* Populations Following DMI-Fungicide Usage in Austrian Vineyards

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Sensitivitätsveränderungen von *Uncinula necator*-Populationen bei Einsatz von DMI-Fungiziden in österreichischen Rebanlagen

1. Introduction

Fungicide treatments for plant disease control are crucial even in organically oriented farming systems. The introduction of modern, highly efficient fungicides, claiming to entail tolerable or no side effects, have made the problem of mycological selectivity more strongly evident (LYR, 1995). Highly effective, selective demethylation inhibition fungicides (DMI) have been used in viticulture for more than twenty years (OGAWA et al., 1988) to control *Uncinula necator* (Schwein.) Burr. (anamorph *Oidium tuckeri* Berk.), the causal agent of grapevine powdery mildew. In contrast to sulfur, the oldest fungicide against *U. necator*, classified as a low risk fungicide, DMIs were estimated as moderate risk fungicides (GEORGOPOULOS, 1985).

The development of *U. necator* strains resistant to DMIs occurred a few years after the introduction of these fungicides. First indications of a reduced, insufficient effect of DMIs against *U. necator* were found in southern European viticultural areas in the late eighties. In the following years, several worldwide investigations proved the development of resistant *U. necator* populations to DMIs (REDL and

Zusammenfassung

Im Rahmen eines Monitorings wurde die Resistenzentwicklung von *Uncinula necator*-Populationen gegen Demethylierungsinhibitoren (DMI) in österreichischen Rebanlagen untersucht. Nach mehreren Jahren mit unbefriedigendem Bekämpfungserfolg wurde von 1995 bis 1996 ein deutlicher Rückgang des Krankheitsdrucks durch den Echten Rebenmehltau verzeichnet und der Einsatz von DMI führte in den Jahren 1996 bis 1998 wieder zu einer zufriedenstellenden Reduktion des Befalls. Trotz des besseren Bekämpfungserfolges im Freiland wurde in den Laboruntersuchungen über den gesamten Untersuchungszeitraum eine reduzierte Sensitivität aller *U. necator*-Populationen gegen alle getesteten DMI nachgewiesen. Alle Proben zeigten zudem Kreuzresistenz zwischen allen getesteten DMI. In einigen Fällen war dieses Resistenzniveau sogar für jene DMI am höchsten, die nie in den Versuchsanlagen eingesetzt worden waren.

Schlagworte: *Uncinula necator*, Echter Rebenmehltau, *Oidium tuckeri*, Fungizidresistenz, Demethylierungsinhibitoren (DMI).

Summary

Monitoring of the development of *Uncinula necator* strains resistant to demethylation inhibitor fungicides (DMI) has been carried out in Austrian vineyards. After several years of unsuccessful attempts at control of grapevine powdery mildew in the field disease incidence and disease severity declined considerably from 1995 to 1996 and DMI application resulted in an evident reduction of the fungus from 1996 to 1998. Despite better results in the field, all *Uncinula necator* populations showed reduced sensitivity to all tested DMIs in the laboratory study. Moreover, all samples showed cross resistance among all tested DMIs. In some cases this resistance level was even higher against DMIs which had never been used in the plots the samples came from.

Key words: *Uncinula necator*, grapevine powdery mildew, *Oidium tuckeri*, fungicide resistance, Demethylation Inhibitor fungicide (DMI).

STEINKELLNER, 1996). In Europe, first in the southern regions and followed by the northern grape growing regions, this change has been connected to the unexpected strong infection levels of *U. necator* (STEVA et al., 1988; ALOI et al., 1991; STEDEN et al., 1994). Past experience has shown that the extensive performance problems with *U. necator* control are based on numerous factors, e.g. meterological conditions, mode of fungicide application or almost exclusive usage of DMI fungicides and shifting of sensitivities (ANONYMOUS, 1992; QUIMETTE and GUBLER ,1990; REDL et al., 1994; GUBLER et al., 1996), and various strategies have been proposed for an effective control of the fungus.

Based upon the first evidence about the shifting in sensitivity to DMI fungicides in Austrian vineyards from years with massive powdery mildew infections (REDL and STEINKELLNER, 1996), the object of this study was to monitor the further development of fungicide sensitivity over three consecutive years.

2. Materials and Methods

Field trials were carried out in vineyards in Vienna and Lower Austria, which had a history of poor control of grapevine powdery mildew in the early nineties.

The experimental plots in Vienna (Mitterberg, Pisenkopf) were designed as blocks, each containing between 12 and 18 vines (*Vitis vinifera* cv. Grüner Veltliner) and were, with exception of Währing, located in large grape growing areas. In Lower Austria (Krems-Holzgasse, Krems-Heide), commercial vineyards with 160 to 240 vines (*Vitis vinifera* cv. Grüner Veltliner resp. Müller Thurgau) were investigated.

The plots were sprayed with several demethylation inhibitors (DMI), either pure or combined with other fungicides, all of them were officially registered in Austria for controlling grapevine powdery mildew. The DMI-fungicide schedule is shown in Table 1. Water treated control plots were sprayed with 300 l to 800 l (dependent on growth stage) of water and a fungicide against *Plasmopara viticola* was added to the tank. The efficacy evaluation of *U. necator* in the field was carried out on grapes after beginning of ripening (growth stage 81–83 BBCH). Analysis of variance was done after variance check by BARTLETT's test and in the case of disease severity data transformation x' = log (1/3+x).

Isolates of *U. necator* were collected from the vineyards, wrapped in a thick covering of healthy leaves, placed between sheets of newspaper inside a paper bag and sent to BIORIZON (limited company, Bordeaux Montesquieu, France) who was charged with the laboratory investigations. Each sample consisted of numerous infested leafs from the cluster zone of several plants. The method (STEVA, 1992) used to characterize the sensitivity, was based upon the sporulation of the fungus on treated or untreated leaf discs. Laboratory studies were done with a preliminary subculture of each sample to untreated hole leaves if it needs. Several active ingredients, according to the DMIs regularly sprayed in the vineyards, were applicated to the upper surface of leaf discs in petri dishes. 24 hours later the leaf discs were inoculated by deposition of conidia in a settling tower and placed in climatic chambers. A visual assessment of colonization and spore production was done after 12 days incubation. Three doses of several active ingredients were used for each population, corresponding to the concentration of, approximately, the minimal inhibitory concentration (MIC) for the reference strain (monoconidial

Table 1: DMI-fungicide schedule
Tabelle 1: DMI-Fungizid-Spritzplan

Fungicide	Formulated as	Registration in Austria	Formulated product/ha	Volume (litre ha^{-1})	Number of applications 1995	1996	1997	1998
Triadimefon	Bayleton 25	1981	60 – 150 g	300–600	6–8	6	5	5
Triadimenol	Bayfidan 050E	1995	800 – 1200 ml	300–600	6–8	6	5	5
Myclobutanil	Prothane	1989	120 – 300 ml	300–800	6–8	6	5	5
Penconazole	Topas 100EC	1989	140 – 375 ml	300–800	6–8	6	5	5
Pyrifenox	Dorado	1990	120 – 300 ml	300–800	6–8	6	5	5
Penconazole alternating	Topas 100EC	1989	140 – 375 ml	300–800	6–8	6		
with pyrifenox	Dorado	1990	120 – 300 ml					
Penconazole combined	Topas 100EC	1989	140 – 375 ml	300–800	6–8	6		
with sulfur	Thiovit	1951	0,6 – 1,3 kg					
Penconazole combined	Topas 100EC	1989	140 – 375 ml	300–800	6–8	6		
with dinocap	Karathane LC	1961	200 – 300 ml					
after flowering								

strain, isolated from a vineyard in the south of France that was never exposed to DMIs). A range of 0,3 mg/l , 1 mg/l and 3 mg/l a.i. was selected for pyrifenox and 0,6 mg/l, 1 mg/l and 3 mg/l a.i. for myclobutanil in 1996. All other investigations were carried out with 1 mg/l, 3 mg/l and 10 mg/l a.i.

A few samples reached BIORIZON in poor conditions and thus could not be tested.

3. Results

3.1 Development of *Uncinula necator* in the vineyards

Disease incidence and disease severity of *U. necator* in Table 2 demonstrate the development of the fungus in the vineyards over the years 1995 to 1998. For this purpose, only data from untreated and penconazole treated research plots in the Viennese vineyards have been outlined. Disease intensities, both incidence and severity, declined considerably from 1995 to 1996. While in 1995, with penconazole treatment, no adequate control of *U. necator* could be obtained, in 1996 to 1998 the fungicide application resulted in a clear reduction of the fungus and the infestation decreased to an acceptable level.

3.2 Laboratory studies

Over the period of the laboratory study (1996 to 1998) 25 isolates of *U. necator*, 20 collected in Viennese and 5 in Lower Austrian vineyards, were investigated. All populations showed reduced sensitivity to all tested DMIs over the investigation period, in comparison with the reference strain (Table 3 and 4). Most populations from untreated plots (Table 3) were characterized by a more than 3 times higher MIC (minimal inhibitory concentration) of DMIs than the reference strain. Only a few populations could be controlled with just 1–3 mg/l a.i. For myclobutanil, an increase in resistance has been noted in both vineyards investigated in 1998: The MIC was of the order of over 10 mg/l.

Although in 1996 to 1998 adequate control of *U. necator* in the vineyards was reached after application of penconazole and/or pyrifenox or myclobutanil (disease incidence in

Table 2: Effect of penconazole treatment on *Uncinula necator* development in Viennese vineyards in 1995 to 1998
Tabelle 2: Auswirkungen von Penconazole auf die Entwicklung von *Uncinula necator* in Wiener Weingärten in den Jahren 1995–1998

Location	Fungicide management	1995	1996	1997	1998
		Diseases incidence (%)			
Mitterberg					
	Control	100,0	46,3	56,0	27,9
	Penconazole	100,0	3,2	1,4	0,7
Pisenkopf					
	Control	87,8	88,8	55,7	69,5
	Penconazole	56,5	7,2	0,0	26,4
		Diseases severity (%)			
Mitterberg					
	Control	100,0	26,2	22,8	21,9
	Penconazole	70,0	5,0	5,8	10,0
Pisenkopf					
	Control	59,9	58,0	52,7	59,8
	Penconazole	35,1	5,0	0,0	27,6

Disease incidence			
Main effects		Interactions	
F-Ratio year	47,38 ***	F-Ratio year x location	5,97 **
F-Ratio location	5,55 *	F-Ratio location x management	2,72 n.s.
F-Ratio management	74,92 ***	F-Ratio management x year	9,03 ***
Disease severity			
Main effects		Interactions	
F-Ratio year	10,45 ***	F-Ratio year x location	3,88 *
F-Ratio location	0,12 n.s.	F-Ratio location x management	1,62 n.s.
F-Ratio management	31,45 ***	F-Ratio management x year	3,90 *

Table 3: Sensitivity of *Uncinula necator* populations from control plots in Viennese vineyards to DMI-fungicides in 1996 to 1998
Tabelle 3: Sensitivität von *Uncinula necator*-Populationen aus Wiener Kontrollparzellen gegenüber DMI-Fungiziden in den Jahren 1996–1998

Location	Sampling date	Minimal inhibition concentration (mg/l)		
		penconazole	pyrifenox	myclobutanil
1996				
Mitterberg	01.08.	3–10	>3	>3
Pisenkopf	16.08.	3–10	1–3	–
1997				
Mitterberg	18.08.	3–10	3–10	3–10
Pisenkopf	18.08.	3–10	1–3	3–10
1998				
Mitterberg	17.08.	1–3	3–10	>10
Pisenkopf	17.08.	3–10	3–10	>10
Reference strain		<1	<1	<1

the range of 0–26,4 %, disease severity of 0–27,6 %), most of the populations investigated were characterized by a MIC between 3 and 10 mg/l (Table 4). The admixture of sulfur or dinocap lead to no improvement in the vineyard but appeared to result in a slight decrease in resistant populations in the laboratory.

A few populations indicated a higher level of resistance to penconazole and myclobutanil and could not be controlled by 10 mg/l. In 1996, the application of an older charge of penconazole (a product from 1995) at Mitterberg resulted in a MIC over 10 mg/l while populations treated with the identical new formulation in the vineyard were characterized by a MIC of 1–3 mg/l, and also 3–10 mg/l.

Moreover, all samples showed cross resistance to all DMIs tested. In some cases that resistance-level was even higher against DMIs that had never been used in the plots the samples came from (such as triadimefon 1996, pyrifenox 1998 and myclobutanil 1997, 1998).

Table 4: Effect of the application of demethylation inhibitors in field on sensitivity of *Uncinula necator* populations
Tabelle 4: Auswirkungen von DMI-Applikationen im Freiland auf die Sensitivität von *Uncinula necator*-Populationen

Location	Fungicide management	Disease incidence (%)	Disease severity (%)	Sampling date	Minimal inhibitory concentration (mg/l)				
					penconazole	pyrifenox	myclobutanil	triadimenol	triadimefon
1996									
Mitterberg	penconazole (charge 1995)	6,3	5,0	12.09.	>10	–	–	–	–
Mitterberg	penconazole	5,9	9,4	12.09.	1–3	–	–	–	–
Mitterberg	penconazole	3,4	6,7	12.09.	3–10	>3	–	–	–
Mitterberg	penconazole/pyrifenox	4,5	5,0	12.09.	3–10	>3	1–3	–	–
								–	–
Pisenkopf	penconazole	7,2	5,0	16.08.	>10	1–3	–	–	–
Pisenkopf	penconazole & sulfur	20,0	13,0	16.08.	3–10	1–3	>3	–	–
Pisenkopf	penconazole & dinocap	18,7	5,8	16.08.	3–10	>3	>3	–	–
Währing	triadimenol	6,0	5,0	01.08.	3–10	>3	–	3–10	>10
Währing	triadimefon	18,2	6,3	12.09.	3–10	>3	–	3–10	–
Holzgasse	penconazole/pyrifenox	0,1	5,0	01.08.	1–3	>3	–	–	>10
1997									
Mitterberg	penconazole	1,4	5,8	02.09.	3–10	1–3	3–10	–	–
Mitterberg	pyrifenox	6,9	6,1	02.09.	1–3	3–10	>10	–	–
Mitterberg	myclobutanil	1,7	5,0	18.08.	3–10	–	>10	–	–
Holzgasse	penconazole	0,0	0,0	02.09.	3–10	1–3	–	–	–
1998									
Pisenkopf	penconazole	14,0	11,7	17.08.	3–10	>10	>10	–	–
Pisenkopf	penconazole	18,2	15,3	17.08.	3–10	3–10	3–10	–	–
Heide*	penconazole	0,2	5,0	17.08.	3–10	3–10	–	–	–
Holzgasse	penconazole	0,1	5,0	17.08.	1–3	3–10	–	–	–
Holzgasse*	penconazole	0,1	5,0	17.08.	1–3	1–3	–	–	–
Reference strain					*<1*	*<1*	*<1*	*<1*	*1–3*

* *Vitis vinifera* cv. Müller Thurgau

Underlayed values mark cross resistance

4. Discussion

Despite strict adherence to recommendations for controlling *U. necator* through the use of DMIs, repeated high degrees of infestation with the fungus have been ascertained in the early nineties. Particularly in warm years with low precipitation, a high threat of disease has to be expected (HILL, 1990) and, primarily, temperature can influence reproduction rate of *U. necator* and thereby the rate of resistance development (GUBLER et al., 1994). Moreover, high temperatures can reduce the effect of fungicides against grapevine powdery mildew (CHELLEMI and MAROIS, 1991). Therefore the meterological conditions have been optimal for the development of the fungus. Only since 1996, under cooler and more rainy conditions, have an evident reduction in the threat of disease and better effects of DMIs been noticed in various vineyards.

A direct link between the infection of grapes or leaves by *U. necator* in the field and the results of the laboratory tests could not be proven. Whereas first investigations (REDL and STEINKELLNER, 1996) were done against a background of poor control of *U. necator* in the field, the current studies were based on acceptable disease control efficacy. Nevertheless MIC about >10 mg/l a.i. in the laboratory were obtained from plots with negligible disease intensities. Similar experience exists from a number of European vineyards (STEDEN et al., 1994).

In contrast to better results in the vineyard, the investigation in the laboratory showed the development of resistance to DMIs very well.

ERICKSON and WILCOX (1997) linked the effect of DMIs (myclobutanil) in vineyards with sensitivity distribution: 65 % of isolates categorized as resistant may lead to the development of practical resistance to the fungicide and easily result in poor disease control.

Previous studies (REDL and STEINKELLNER , unpublished data) have shown that the use of older doses of DMIs (penconazole, product from 1995) have an unfavourable effect on disease intensities. This may be explained by a rapid loss of efficacy with advancing years of a product. Nevertheless, the reason for the high level of the MIC in this case should be further researched.

The 25 grapevine powdery mildew populations are a random choice from areas well known for a high degree of infestation and are not representative for all Austrian grape growing areas. A decrease in DMI sensitivity has been evident in Austria for a number of years (REDL and STEINKELLNER, 1996) and despite incomplete data, there is every indication of a very stable resistance against DMIs. First of all, *U. necator* populations from plots which had been untreated for several years were characterized, in most cases, by a MIC between 3 and 10 mg/l a.i. over all years of investigation. All untreated plots were situated in large vinegrowing areas treated against *U. necator*, hence spray drift carried by the wind might be a possible source of less sensible conidia.

Speculation upon Californian results (YPEMA and GUBLER, 1997), where the infrequent use of DMIs (triadimefon) may lead to a reduction in the frequency of organisms possessing the lowest sensitivity to triadimefon, could not be confirmed in our investigations. Only for myclobutanil, which has rarely been used in the vineyards, was a increase in resistance among populations from untreated plots evident in 1998.

Populations from plots treated with DMIs showed no evident difference in sensitivity against DMIs in comparison to populations from untreated plots. The level of reduced sensitivity is still very high for most DMIs at most locations in Austria and a few *U. necator* populations at some locations even showed progressive development. They were not eliminated by 10 mg/l a.i., the highest concentration in the test, while the reference strain was controlled with less than 1 mg/l a.i. These results correspond with the first Austrian investigations (REDL and STEINKELLNER, 1996) and were within the range of the first European values (STEVA et al., 1990; STEDEN et al., 1994). These results are similar to experiences reported for cereal powdery mildews (ANONYMOUS, 1995). The MIC values also indicate that the conducted fungicide measurements had little impact on reverting resistance and have stabilized the development of less sensitive populations as in California (GUBLER et al., 1994). Although, according to the qualitative assessment of the laboratory test only, a definite statement about the importance of resistant phenotypes inside the populations is not feasible, they should not be underestimated.

The reported definite differing sensitivity of *U. necator* to various DMIs (GUBLER et al., 1996; YPEMA et al., 1997) has not been corroborated.

Experiments in California indicated a decrease in resistance for myclobutanil used in alternation with sulfur (YPEMA et al., 1997) and also a better level of disease control than DMIs applied by themselves or than by tank-mixing DMIs and sulfur (GUBLER et al., 1996). In other field trials, admixtures with sulfur or dinocap gave good results as well (REDL et al., 1994; KAST, 1991). The unfavourable effect of such admixtures may be due to the different site of action (HILBER and BODMER, 1993) or have been explained by an antagonism with DMIs (STEVA, 1992) and they have been classified as a non effective anti-resistance strategy (STEVA, 1994). In the present study a slight advantage in the case of sensitivity could be observed due to the use of admixtures compared to that of penconazole alone.

In addition, a positive cross resistance has been proven among all DMIs investigated, e.g. penconazole, pyrifenox, myclobutanil, triadimefon and triadimenol. These results confirm first studies in Austrian vineyards (REDL and STEINKELLNER, 1996) and correspond with international results for *U. necator* (STEVA and CLERJEAU, 1990; YPEMA et al., 1997) or rather powdery mildew of barley and wheat (BUCHENAUER, 1995). It seems that both location and cultivation method influenced this situation, as established by MIC of about 3 mg/l a.i. and about 10 mg/l a.i. respectively. In Währing, cross resistance between triadimefon (triadimenol) and penconazole was detected, although penconazole had never been used in this isolated vineyard. Similar results exist about cross resistance in the case of *Venturia inaequalis* (KUNZ et al., 1997).

However, the vinegrowers are bound to pursue an efficient anti resistance strategy to avoid renewed problems in controlling *U. necator* in case of an increase in the threat of disease. For this reason, in spite of DMI resistance and the development of new fungicides, such as strobilurins or phenoxyquinoleines, DMIs are still of great importance.

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