Indirect Effect of Fungicide Treatments on Chasmothecia of *Erysiphe necator* Schwein Overwintering on Grapevine Bark

Péter HOFFMANN[1], István FÜZI[1] and Ferenc VIRÁNYI[2]

[1]BASF Hungária Kft., Budapest, Hungary;
[2]Plant Protection Institute, Szent István University, Gödöllő, Hungary

Abstract

Hoffmann P., Füzi I., Virányi F. (2012): **Indirect effect of fungicide treatments on chasmothecia of** *Erysiphe necator* Schwein **overwintering on grapevine bark**. Plant Protect. Sci., **48**: 21–30.

Erysiphe necator Schwein overwinters in Hungary primarily as large numbers of chasmothecia providing primary inoculum for grapevine (*Vitis vinifera* L.) infection in the next year. In two field trials, the effect of fungicide treatments on powdery mildew severity and the number of chasmothecia produced on leaves and washed to the bark were studied. In 2005, the number of chasmothecia harvested from the bark showed a limited correlation with disease severity (r = 0.553) and number of chasmothecia on leaves (r = 0.600). In 2006, using a refined sieving technique, a substantial increase in the number of harvested chasmothecia could be achieved resulting in a much closer correlation between the variables above (r = 0.750 and r = 0.886, respectively). Among the fungicides applied, boscalid and fluquinconazole (SC formulation) showed the most significant activity by decreasing both the autumn leaf infection and the formation of chasmothecia on the leaves and providing the lowest number of chasmothecia on bark. The research underlined the importance of fungicide applications made in the previous year to decrease the amount of overwintering inoculum and to help protect grapevines from powdery mildew infection in the next year.

Keywords: grapevine powdery mildew; *Vitis vinifera*; chemical control; perennation

In-depth investigations into the sexual fruiting bodies of grapevine powdery mildew (*Erysiphe necator* Schwein) began by research conducted by Pearson and Gadoury (1987). Their work demonstrated for the first time that ascocarps, known as chasmothecia, survive winter and release ascospores the next spring, and that these ascospores function as primary inoculum. Afterwards, numerous studies reported that chasmothecia have a significant role in the overwintering of the fungus and the outbreak of powdery mildew epidemics worldwide (Stapleton *et al.* 1988; Cortesi *et al.* 1997; Steinkellner 1998; Grove *et al.* 1999; Jailloux *et al.* 1999; Grove 2004). However, in some regions *E. necator* is known to overwinter mainly as mycelium in infected dormant buds, where the flag shoots serve as primary inoculum (Ypema & Gubler 2000; Rügner *et al.* 2002). In Hungary, previous research has demonstrated that the initiation of a grapevine powdery mildew epidemic is usually the release of ascospores from chasmothecia (Lehoczky *et al.* 1991; Füzi 1999b, 2001; Hoffmann & Virányi 2007).

Under Hungarian conditions, *E. necator* forms a large quantity of chasmothecia on grapevine (*Vitis vinifera* L.) leaves beginning in August. Although at this time, after the growth stage of berry touch, berries are no longer susceptible to the disease, *E. necator* is capable of colonising the leaves until as late as leaf fall (Füzi 1999a). On severely infected leaves, millions of chasmothecia can be produced and washed onto the grapevine bark surface during autumn rains (Hoffmann & Virányi 2007).

Warm and dry conditions in late summer and early autumn are favourable for the formation of chasmothecia, while long and rainy autumns are ideal for their dispersal (Hill *et al.* 1995). Most chasmothecia are washed away by rain or fall with leaves onto the soil, while just a small number is deposited on the bark fissures of trunks, primarily on the horizontal cordons and the upper half of the trunks (Cortesi *et al.* 1995).

The epidemic role of the ascospores released from chasmothecia overwintered on fallen leaves on the soil surface is controversial. According to some researchers, these chasmothecia are very unlikely to survive during winter, and are unable to release viable ascospores in spring (Pearson & Gadoury 1987; Gadoury & Pearson 1988). In contrast, other studies have documented successful perennation on grapevine leaves (Cortesi *et al.* 1997) from which chasmothecia with viable ascospores could be isolated (Grove *et al.* 1999; Grove 2004). However, the location of chasmothecia that overwinter on the soil surface may negatively influence their epidemiological significance. Even if they are viable, the dispersion of ascospores from these chasmothecia will be less significant compared to those overwintering on the trunk, the latter being closer to the young leaves in spring and as a result the liberated ascospores will be able to infect the leaves more effectively (Füzi 2003).

In addition to weather conditions, fungicide applications have significant effects on the dynamics of powdery mildew epidemics. Vineyards are regularly sprayed from the 4–6 leaf stage (BBCH 14–16) to berry touch (BBCH 79) to control powdery mildew. The focus of the fungicide applications is mainly on protecting berries rather than leaves, where the chasmothecia are mostly formed. Previous research conducted in Hungary confirmed that fungicide applications carried out in the growing season affected the development of chasmothecia on the leaves of grapevine (Füzi 1999b).

Three important groups of fungicides with different modes of action have so far lost their efficacy in the control of *E. necator* worldwide as a result of fungicide resistance. Benzimidazole resistant strains of grapevine powdery mildew were first identified in New York (Pearson & Taschenberg 1980), while a decade later, resistance to sterol biosynthesis inhibitors was reported in Portugal (Steva *et al.* 1990). In addition, field resistance to QoI fungicides was detected first in California (Bartlett *et al.* 2002).

In Hungary, the resistance of *E. necator* against QoI fungicides was recently verified in two field trials in 2006 (Hoffmann *et al.* 2009). Subsequently, this type of resistance was also found in several other European countries (FRAC 2007, 2008, 2009). However, under field conditions, all other fungicides provided satisfactory efficacy in the control of the powdery mildew pathogen throughout Hungary (Dula 2007).

The objectives of this research were: (1) to more fully understand how fungicide applications influence the development of chasmothecia on leaves, and (2) to determine the effect of fungicide applications on the number of chasmothecia dispersed and accumulated on the bark of grapevines. A preliminary account of portions of this work has already been published (Hoffmann *et al.* 2007, 2009).

MATERIAL AND METHODS

Field trials. Fungicide trials were carried out within the wine-growing region of Szekszárd, located in southern Hungary. Experiments were conducted in 2005 in a vineyard planted with the cultivar Nosztori Riesling (trial 1) and in 2006 in another vineyard of cv. Bluefrankish (trial 2). The size of trials was approx. 1000 m^2 each. Trial 1 included eight treatments plus non-treated control while in trial 2 there were nine treatments plus control (Table 1). Each plot included 10 plants. A randomised plot design with three replicates was used for both experiments. Furthermore, six (A– F) and five (A–E) fungicide applications per treatment were carried out with the same active substance at approx. 10–14 days intervals from the beginning of flowering (BBCH 61) to the beginning of ripening (BBCH 81) in 2005 and in 2006, respectively. The applied fungicides are registered products for controlling grapevine powdery mildew in Hungary (Szabadi 2010). Applications were made with knapsack sprayers using 500 l/ha water.

Vineyard survey and sample collection. In the second half of August 2005 and 2006, 14 days after the last application, the severity of final fruit infection was determined by evaluating 50 clusters/plot. Leaf infection was assessed at the end of the growing season by collecting thirty leaves per plot at random in the first half of October, considering the whole canopy to be represented in the sampling. The severity of powdery mildew

Table 1. Fungicide applications carried out in trials 1 and 2

Treatment	Dose rate (g ai/ha)		Application date	
	trial 1	trial 2	trial 1 – 2005	trial 2 –2006
Untreated	–	–	A 3 June (BBCH 61)	A 6 June (BBCH 61)
Metrafenone	100	100	B 16 June (BBCH 69)	B 20 June (BBCH 69)
Proquinazid	40	40	C 28 June (BBCH 73)	C 6 July (BBCH 75)
Boscalid	600	600	D 8 July (BBCH 75)	D 20 July (BBCH 77)
Pyraclostrobin	100	100	E 21 July (BBCH 77)	E 10 August (BBCH 81)
Kresoxim-methyl	100	100	F 11 August (BBCH 81)	
Azoxystrobin	200	200		
Fluquinconazole*	50	50		
Fluquinconazole**	50	–		
Trifloxystrobin	–	75		
Tebuconazole+ triadimenol+ spiroxamine		50.1 12.9 75		

*SC formulation; **WG formulation

colonisation on the adaxial surface of leaves was determined and the number of chasmothecia on leaves was recorded using the methodology described by Füzi (2003).

The efficacy of fungicide treatments was further evaluated by determining the number of overwintering chasmothecia. In January after both growing seasons bark samples were collected from each plot. Five to ten cm long exfoliated bark pieces from the horizontal cordons and the upper half of the trunks (five vines per plot) were put into paper bags and stored in a dry place at 15–20°C.

Harvesting chasmothecia from bark. Samples from trial 1 were examined in February 2006. 10 g of bark per sample were placed into a beaker containing 150 ml of distilled water and the beakers were put in an ultrasonic cleaner (35 kHz) for eight minutes. The agitated suspension was poured over a 1500 µm mesh size sieve into an Erlenmeyer flask. The accumulated bark sample was rinsed again with an additional 500 ml of water and poured over the same sieve as above into the Erlenmeyer flask. The content of the flask was then poured over an 800 µm 4-layer sieve and rinsed with 200 ml of water. Finally, the resultant 850 ml suspension was poured over a 55 µm mesh size sieve. The number of chasmothecia retained on this sieve was determined microscopically using 40× magnification. The application of the first two mesh size sieves was aimed to separate coarse size parts of bark and any dirt. By using the 55 µm mesh size sieve the highest proportion of chasmothecia could be collected from the suspension, since practically all chasmothecia can be trapped by the last sieving (Cortesi *et al.* 1995).

Refining the harvest method. For samples collected from trial 2 in January 2007, the sieving methodology was refined. In the first series of experiments, five out of 30 samples were selected that originated from treatments differing in powdery mildew infection on the leaves. These bark samples were sonicated in 150 ml of water similarly to the method used in 2006. They were then rinsed three times with 150 ml of water each in a 1000 ml beaker and the 600 ml suspension obtained was sieved by the above described method except that the quantity of rinsing water after both the first and second sieves was 500 ml each instead of 500 ml and 200 ml like in 2006. The chasmothecia that remained on the 55 µm mesh size sieve were counted microscopically. In order to increase the efficiency of harvesting chasmothecia from bark debris of the five selected samples trapped on the 1500 µm mesh size sieve, the whole sieving process was repeated eight times.

According to the data on the assessment of these five samples, at least 80% of chasmothecia were collected by the first three agitations, the other 25 bark samples were subjected to the whole sieving procedure only three times.

Data analysis. The significance of differences between means was determined by Tukey's HSD test ($P \leq 0.05$). To measure the degree of association between variables, the Pearson product-moment coefficient was used.

RESULTS

Trial 1

There was a severe powdery mildew outbreak in the vineyards in 2005. The level of cluster infection by August 27 reached 86.27% on the untreated grape plants compared to a maximum of 18.37% of the berries affected on plants that received fungicide treatments (Table 2). The best disease control (exceeding 99%) was obtained with pyraclostrobin, boscalid and proquinazid, while metrafenone and kresoxim-methyl showed moderate efficacy and finally, fluquinconazole (both formulations) and azoxystrobin were the least effective fungicides.

Pyraclostrobin, fluquinconazole (SC formulation) and boscalid were the most effective treatments for managing powdery mildew on leaves based on the assessment made on October 16, when average severity was 4.23%, 7.29%, and 8.64%, respectively, in comparison with 73.36% on the untreated plots (Table 2). The leaf infection exceeded 10% in the case of applications with metrafenone, fluquinconazole (WG) and kresoxim-methyl and, by mid-October, the severity of powdery mildew colonisation increased to 30.23% and 40.84% in the plots treated with proquinazid and azoxystrobin, respectively.

The number of chasmothecia on the leaves showed a large standard deviation between the applications (Table 2). On the leaf samples taken from untreated plots, 115.2 sexual fruiting bodies/10 cm^2 were counted, whereas the lowest numbers (0.30–5.10/10 cm^2) were found on samples taken after the application of pyraclostrobin, fluquinconazole (SC), boscalid or metrafenone. In contrast, the highest numbers of chasmothecia from the treated plots developed in the case of applications with proquinazid and azoxystrobin (67.80 and 76.70/10 cm^2).

When the bark samples taken in January 2006 were examined, on average 183.96 chasmothecia per 10 g of air-dried bark were harvested (Table 2). The lowest number of chasmothecia (65/10 g) was isolated from the plots treated with fluquinconazole (SC), and the applications of pyraclostrobin, boscalid, proquinazid and metrafenone resulted in 89.33, 104, 109.67 and 120.33 fruiting bodies/10 g, respectively. Large numbers of chasmothecia were counted on plots treated with fluquinconazole (WG) (245/10 g) and kresoxim-methyl (197/10 g), while the application of azoxystrobin led to the highest number of chasmothecia (383.33/10 g), the latter exceeding even the average number of samples obtained from the untreated plots (342 fruiting bodies/10 g).

Table 3 includes the correlation coefficients of the parameters studied, while Figure 1 illustrates

Table 2. Disease assessment data in trial 1 (2005)

Disease parameter	Pyraclostrobin	Fluquinconazole (SC)	Boscalid	Metrafenone	Fluquinconazole (WG)	Kresoxim-methyl	Proquinazid	Azoxystrobin	Untreated
Disease severity on berries (%)	0.30^{d*}	10.33^{bc**}	0.60^{d}	5.13^{cd}	18.37^{b}	6.27^{cd}	0.53^{d}	12.10^{bc}	86.27^{a}
Calculated control on berries (%)	99.66^{a}	87.98^{b}	99.30^{a}	94.14^{ab}	78.64^{c}	92.76^{ab}	99.38^{a}	85.79^{bc}	0.00^{d}
Disease severity on leaves (%)	4.23^{d}	7.29^{cd}	8.64^{cd}	11.97^{cd}	17.82^{bcd}	19.42^{bcd}	30.23^{bc}	40.84^{b}	73.36^{a}
Calculated control on leaves (%)	93.68^{a}	89.40^{ab}	87.05^{ab}	83.21^{ab}	76.04^{abc}	72.35^{abc}	60.88^{bc}	45.78^{c}	0.00^{d}
Number of chasmothecia on leaves per 10 cm^2	0.30^{d}	1.58^{d}	0.04^{d}	5.10^{d}	12.40^{d}	27.80^{cd}	67.80^{bc}	76.70^{ab}	115.20^{a}
Number of chasmothecia on bark per 10 g	89.33^{de}	65^{e}	104^{de}	120.33^{cde}	245^{bc}	197^{cd}	109.67^{de}	383.33^{a}	342^{ab}

*average of four replicates; **letters indicate the significance of differences between means (Tukey's HSD test – $P \leq 0.05$)

Table 3. Correlation coefficients (r) between the disease parameters assessed in both trials

		Trial 1 (n = 27)	Trial 2 (n = 30)
Disease severity on berries	disease severity on leaves	0.749	–
Calculated control on berries	calculated control on leaves	0.763	–
Disease severity on berries	number of chasmothecia on leaves	0.637	–
Calculated control on berries	number of chasmothecia on leaves	0.639	–
Disease severity on berries	number of chasmothecia on bark	0.623	–
Calculated control on berries	number of chasmothecia on bark	0.633	–
Disease severity on leaves	number of chasmothecia on leaves	0.916	0.905
Calculated control on leaves	number of chasmothecia on leaves	0.900	0.838
Number of chasmothecia on leaves	number of chasmothecia on bark	0.600	0.886
Disease severity on leaves	number of chasmothecia on bark	0.553	0.750
Calculated control on leaves	number of chasmothecia on bark	0.538	0.706
$r_{0.05}$		0.367	0.349

the trends of correlations between the data. It is clear from Figure 1 that an increase in powdery mildew infection resulted in significant changes in the number of chasmothecia developed on the leaves and washed off onto the bark. The correlation coefficients indicate that cluster infection had a medium correlation with leaf infection and with the number of chasmothecia counted on the leaves and overwintered on bark. The closest correlation occurred between the powdery mildew colonisation of the leaves and the number of chasmothecia produced on leaf surfaces. A moderate correlation was evident between leaf infection and the number of overwintered chasmothecia, while

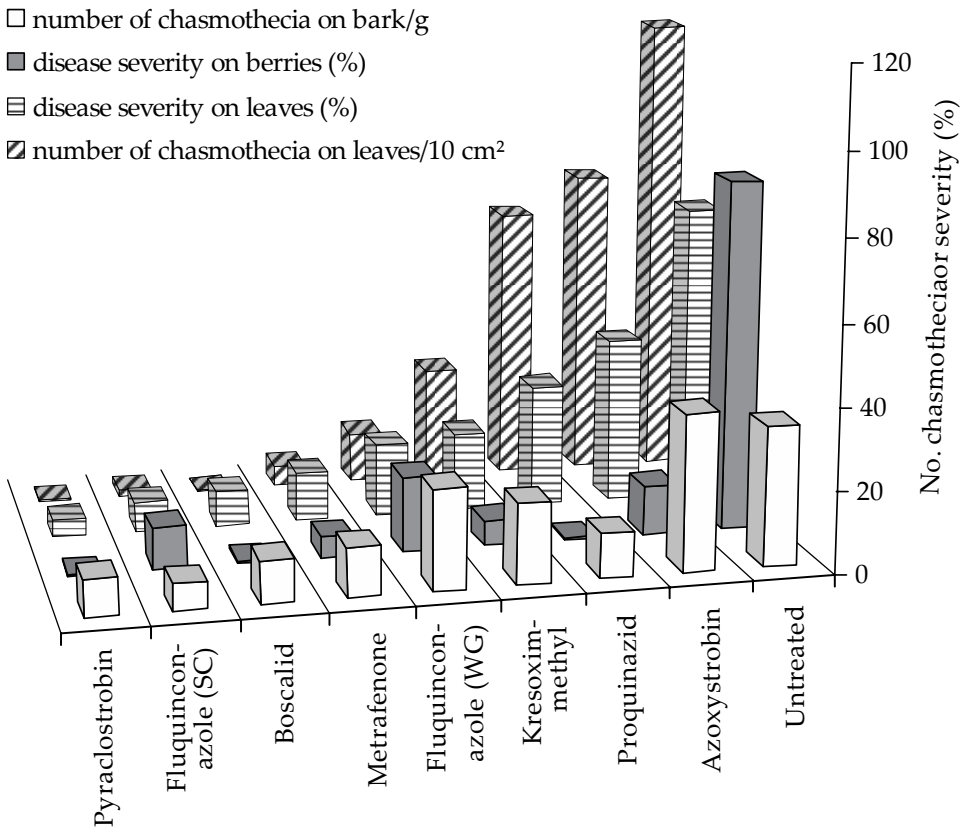

Figure 1. Effect of fungicide treatments on the disease parameters in trial 1 (2005)

Table 4. Disease assessment data in trial 2 (2006)

Disease parameter	Boscalid	Fluquinconazole (SC)	Proquinazid	Metrafenone	Tebuconazole+triadimenol+spiroxamine	Pyraclostrobin	Trifloxystrobin	Kresoxim-methyl	Untreated	Azoxystrobin
Disease severity on leaves (%)	1.07^{d*}	4.01^{d**}	46.33^{c}	56.24^{b}	60.67^{b}	64.68^{b}	75.56^{a}	80.67^{a}	82.11^{a}	82.22^{a}
Calculated control on leaves (%)	98.68^{a}	95.13^{a}	43.13^{b}	31.26^{c}	26.24^{cd}	20.75^{d}	8.86^{e}	3.82^{e}	0.00^{e}	2.21^{e}
Number of chasmothecia on leaves per 10 cm^2	0.1^{e}	0.4^{e}	106.5 ^{de}	105.2^{de}	188.0^{cde}	267.3^{bcd}	526.7^{a}	414.6^{abc}	486.8^{ab}	539.4^{a}
Number of chasmothecia on bark per 10 g	28.00^{c}	46.33^{c}	408.67^{c}	143.00^{c}	443.67^{c}	1156.00^{bc}	3120.67^{a}	1853.33^{ab}	1319.33^{bc}	2668.33^{a}

*average of four replicates; **letters indicate the significance of differences between means (Tukey's HSD test – $P \leq 0.05$)

the latter showed a medium correlation with the number of chasmothecia counted on the leaves.

Trial 2

In 2006, powdery mildew started developing very late, therefore cluster infection was low. Large numbers of fungal colonies developed on leaves during the second half of the growing season. By October 9 severity exceeded 82% on untreated plots (Table 4). However, powdery mildew was also severe on leaves on plots treated with proquinazid, metrafenone, tebuconazole+triadimenol+spiroxamine or pyraclostrobin, and it was also high on those plots treated with trifloxystrobin, kresoxim-methyl or azoxystrobin, the disease severity reaching 45–65% and 75–82%, respectively. Severity on the leaves of plants treated with boscalid or fluquinconazole (SC) was only 1.07% and 4.01%, respectively.

On average 263.5 chasmothecia/10 cm^2 were counted on the leaves in trial 2, while 486.8 chasmothecia/10 cm^2 were found on the untreated plots (Table 4). A significant number of chasmothecia developed on the leaves treated with strobilurins. Thus, treatments with azoxystrobin or trifloxystrobin showed a higher number of chasmothecia than that found on untreated plots, and in the case of kresoxim-methyl or pyraclostrobin no significant differences between the treated and non-treated plots could be seen. The lowest number of fruiting bodies (0.1 and 0.4/10 cm^2) was produced on plots treated with boscalid and fluquinconazole SC, respectively.

In January 2007, the bark collected from the experimental plots was used to improve the sieving technique on five selected samples with eight

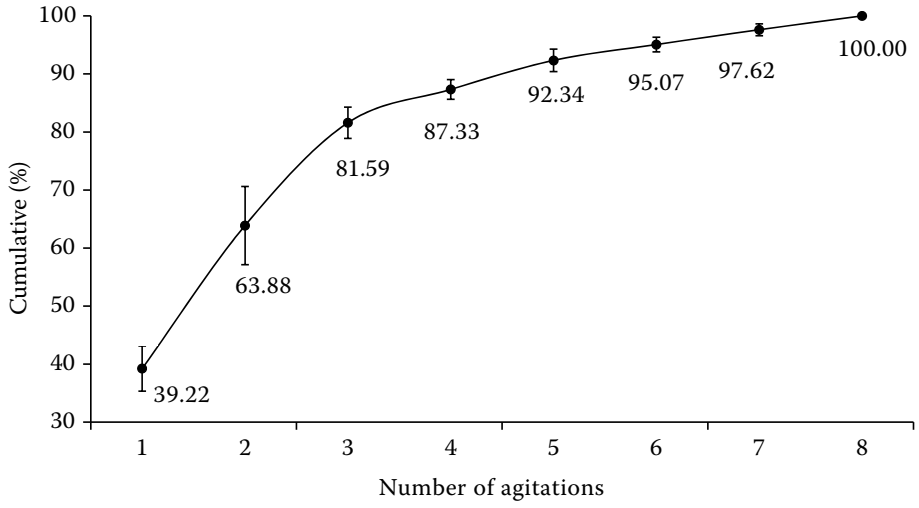

Figure 2. Average cumulative percentages of counted *E. necator* chasmothecia for the five selected samples from trial 2

Table 5 The number and proportion of *E. necator* chasmothecia on 10 g of bark obtained after using a sieving technique repeatedly on the five selected samples (I–V) from trial 2

Agitation	I		II		III		IV		V	
	no.	(%)	no.	(%)	no.	(%)	no.	(%)	no.	(%)
1st	756	43.45	350	32.92	1051	38.90	1855	40.66	95	40.20
2nd	442	25.40	211	19.80	637	23.57	1189	26.07	68	28.43
3rd	266	15.29	303	28.47	417	15.43	624	13.67	37	15.69
4th	98	5.63	55	5.20	240	8.88	232	5.07	9	3.92
5th	94	5.40	47	4.46	183	6.77	250	5.48	7	2.94
6th	36	2.07	39	3.71	20	0.74	147	3.23	9	3.92
7th	34	1.95	24	2.23	66	2.43	145	3.17	7	2.94
8th	14	0.80	34	3.22	89	3.28	121	2.65	5	1.96
Σ 1–3	1464	84.14	864	81.19	2105	77.90	3668	80.40	200	84.32
Σ 4–8	276	15.86	199	18.81	598	22.10	895	19.60	37	15.68
Σ 1–8	1740	100	1063	100	2703	100	4563	100	237	100

repetitions. Most of the chasmothecia were collected after the first agitation, and by further repetitions the number of harvested fruiting bodies continuously decreased (Table 5). Thus, 39.22%, 63.88%, and 81.59% of chasmothecia were obtained from the bark by the first, second and third sonication, respectively, relative to the total number of chasmothecia harvested after eight sieving cycles (Figure 2). Since additional applications after the third cycle did not significantly increase the number of removed

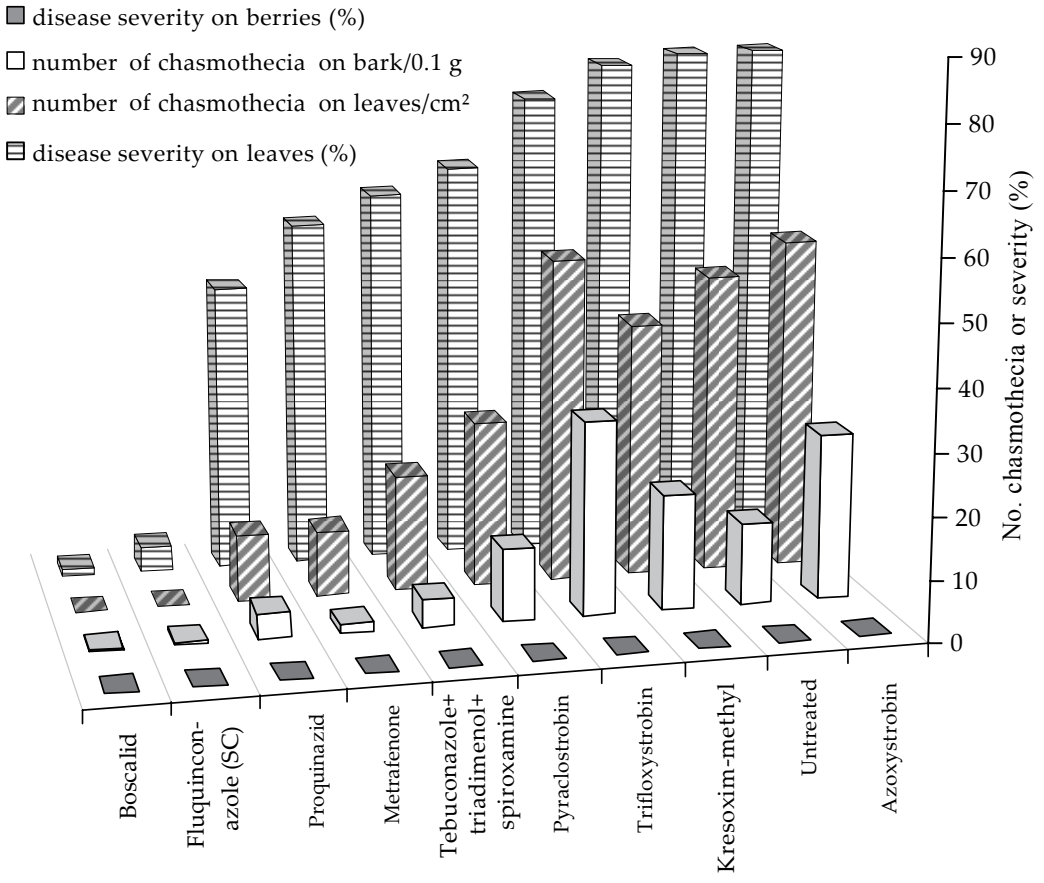

Figure 3. Effect of fungicide treatments on the disease parameters in trial 2 (2006)

chasmothecia, the remaining 25 bark samples were evaluated after the third sieving cycle only.

A total of 1118.73 chasmothecia per 10 g bark tissue on average could be harvested during this procedure. The lowest number (28/10 g) was removed from bark samples taken from the plots treated with boscalid (Table 4). In contrast, the number of chasmothecia was 2-, 5-, 15- and 16-fold higher on plots treated with fluquinconazole, metrafenone, proquinazid and tebuconazole+triadimenol+spiroxamine, respectively. The number of chasmothecia on bark samples taken from the plots treated with strobilurins was similar to (1156/10 g and 1853.33/10 g) or significantly higher (2668.33/10 g and 3120.67/10 g) than the quantity harvested from the untreated plots (1319.33/10 g).

The experimental data revealed that the more intensive powdery mildew infection resulted in an increase in the number of chasmothecia; more chasmothecia were produced per unit leaf area and a larger population accumulated on the bark as well (Figure 3). Table 3 illustrates a very close correlation between leaf infection and the number of chasmothecia formed on the leaves, close correlation between the numbers of produced and overwintered chasmothecia, and a medium correlation between the colonisation of the leaves and the number of chasmothecia isolated from the bark.

DISCUSSION

Grape powdery mildew infection takes place at different periods on clusters and leaves, with berries becoming heavily infected from early flowering (BBCH 60–61) to pea-sized berries (BBCH 75), while leaves are mostly attacked during the second half of the growing season. In the two-year research project, the development of epidemics differed greatly. In 2005, the first symptoms appeared two weeks prior to flowering, and the infection pressure steadily increased. Therefore, both the clusters and the leaves were significantly infected. In contrast, in 2006, the berries remained healthy because of the late appearance of the fungus so that only the leaves became diseased in the second half of the growing season. However, the number of chasmothecia developed in 2006 was much higher than that in the previous year. Studying the epidemic dynamics of this disease, Füzi and Holb (2007) underlined that in 2005, in spite of the powdery mildew epidemics, the cold rainy weather of late summer negatively influenced the spread of infection to the leaves and, as a consequence, the development of chasmothecia. In contrast, the hot and dry late summer and early autumn periods in 2006 were more suitable for the development of *E. necator*, therefore significantly higher numbers of chasmothecia were formed as compared to the previous year.

Due to differences in the infection pressure and its dynamics, the efficacy of fungicides applied to control powdery mildew could be compared on clusters and leaves only in 2005 (trial 1). Though applications from early flowering (BBCH 61) to pea-sized berries (BBCH 75) played an important role in controlling cluster infection, in the case of autumn leaf infection the application at the beginning of ripening (BBCH 81) was the most significant in both years. Because of the long period between the last application and disease evaluation (66 and 60 days in 2005 and 2006, respectively), the control of leaf infection was less dependent on the activity of fungicides than on their persistence. Based on the results of 2005, the application of pyraclostrobin, boscalid and fluquinconazole (both the liquid and solid formulations) had nearly the same effect on both clusters and leaves, whereas metrafenone, kresoxim-methyl, proquinazid and azoxystrobin were significantly less effective for controlling powdery mildew on leaves than on clusters. Thus, our results are in accordance with those reported by Füzi (2003), who confirmed that there might be significant differences in the efficacy provided by different compounds in controlling infection either on clusters or on leaves.

There was a very close relationship found between powdery mildew leaf colonization and the number of chasmothecia produced both in 2005 and 2006 (r = 0.916 and r = 0.905, respectively). As a result, fungicide treatments which resulted in the lowest number of chasmothecia on grapevine leaves were, at the same time, able to protect the canopy with the highest efficacy, confirming what was observed by Füzi (1999a, 2003) in previous years. In the research described here, the leaf infection and the formation of chasmothecia were most successfully inhibited by the use of fluquinconazole (SC) and boscalid in both years.

In 2005, the applications of pyraclostrobin and azoxystrobin showed excellent and moderate efficacy on leaves and clusters, respectively, while in 2006, all strobilurin fungicides provided only poor efficacy. In order to clarify the reason for low efficacy, leaf samples were collected and sent

to a laboratory in Germany for resistance testing. The evaluation of the samples taken from trial 2 revealed a high level of resistance of the pathogen to QoI fungicides (Hoffmann *et al*. 2009).

When considering the methodology used in our study to collect chasmothecia from grapevine bark samples, there was a large difference found between the sieving techniques for their ability to collect chasmothecia. For example, in 2005 the samples were washed and sieved only once, which resulted in significantly fewer chasmothecia/unit weight than a year later, when they were washed and sieved several times. If this technique was used eight times with each of the five preselected samples and considering the cumulative ratio of chasmothecia harvested in each step, the obtained results were similar to those published by Cortesi *et al*. (1995). Furthermore, in comparison with the eight steps, applying the technique three times resulted in more than 80% harvest of chasmothecia and was sufficient to compare the results of different fungicide treatments, as further repetitions did not significantly increase the number of chasmothecia collected. In the leaf infection and chasmothecia on the bark relationship, a close correlation was found between the number of chasmothecia isolated from bark samples and the efficacy of the various fungicides evaluated. Apart from this, other factors such as the accuracy of the sieving methodology used as well as the occurrence of a fungal hyperparasite, *Ampelomyces* spp., in the trials could affect this relationship in our two-year study.

From a practical point of view, it can be of interest for farmers to understand how chemical control regimes in a vineyard might influence the occurrence and severity of powdery mildew attack during the season and to what extent the fungicide applications may affect the epidemics and the impact in the next year. Cortesi *et al*. (1995) found no correlation between the infection level caused by powdery mildew in the previous season and the number of chasmothecia isolated from the bark next spring. They assumed that differences in the precipitation measured at the different sampling sites of various regions, i.e. the amount, distribution and intensity of rainfall, had a significant effect on the dispersal of chasmothecia. In the present study, experimental plots could be characterised by similar environmental conditions (relief, weather conditions, cultivation and pruning methods, age, etc.), and only the applied pest management practices were different. The results indicated that the applications of fungicides from the start of the growing season to the beginning of the fruit ripening period did not only affect the disease level on the clusters but also determined the incidence of the autumn leaf infection. Furthermore, the level of leaf infection altered the number of chasmothecia formed on the leaves and their dispersal and accumulation on the bark of vines. Since the spread of *E. necator* over long distances is thought to be limited (Steva & Cazenave 1996), the role of the inoculum produced in a particular vineyard is decisive for the outbreak of the disease. On the basis of the research reported here, it can be concluded that fungicide applications carried out during the growing season will have significant effects on the infection pressure in the next year.

References

Bartlett D.W., Clough J.M., Godwin J.R., Hall A.A., Hamer M., Parr-Dobrzanski R. (2002): The strobilurin fungicides. Pest Management Sciences, **58:** 649–662.

Cortesi P., Gadoury D.M., Seem R.C., Pearson R.C. (1995): Distribution and retention of cleistothecia of *Uncinula necator* on the bark of grapevines. Plant Disease, **79**: 15–19.

Cortesi P., Bisiach M., Ricciolini M., Gadoury D.M. (1997): Cleistothecia of *Uncinula necator* – An additional source of inoculum in Italian vineyards. Plant Disease, **81**: 922–926.

Dula T. (2007): The issue of fungicide resistance, particularly powdery mildew fungi. Növényvédelem, **43**: 253–260. (in Hungarian)

FRAC (2007): Minutes of the meeting. Available at http://www.frac.info/frac/meeting/qoi/FRAC_QoI_Minutes_2007_Final_print_version_eb.pdf (accessed 20. 2. 2011).

FRAC (2008): Minutes of the meeting. Available at http://www.frac.info/frac/meeting/qoi/FRAC_QoI_Minutes_2008.pdf (accessed 20. 2. 2011).

FRAC (2009): Minutes of the meeting. Available at http://www.frac.info/frac/work/FRAC_QoI_Minutes2009Final.pdf (accessed 20. 2. 2011).

Füzi I. (1999a): The occurrence of the cleistothecial form of grape powdery mildew (*Uncinula necator* /Schw./ Burr.) and the process of formation of cleistothecia in Trans-Danubian vineyards. Növényvédelem, **35**: 137–145. (in Hungarian)

Füzi I. (1999b): Role of the cleistothecium form of grapevine powdery mildew in the development of epidemics in the vine-growing region of Szekszárd. Növényvédelem, **35**: 215–221. (in Hungarian)

Füzi I. (2001): Importance of cleistothecia of grapevine powdery mildew in Hungary. Növényvédelem, **37**: 241–248. (in Hungarian)

Füzi I. (2003): The role of environmental conditions in the dynamics of epidemics of *Uncinula necator* (Schw.) Burr. [PhD Thesis.] University of Pannonia, Keszthely. Available at http://twilight.vein.hu/phd_dolgozatok/fuzi-istvan/fuziistvan.pdf (accessed at 20. 2. 2011).

Füzi I., Holb I. (2007): The epidemiological role of the overwintering forms of grapevine powdery mildew fungus. Növényvédelem, **43**: 237–245. (in Hungarian)

Gadoury D.M., Pearson R.C. (1988): Initiation, development, dispersal and survival of cleistothecia of *Uncinula necator* in New York vineyards. Phytopathology, **78**: 1413–1421.

Grove G.G. (2004): Perennation of *Uncinula necator* in vineyards of Eastern Washington. Plant Disease, **88**: 242–247.

Grove G.G., Davis G. H., Boal R.J. (1999): Powdery mildew of grape: Perennation of *Uncinula necator* in eastern Washington. Phytopathology, **89**: 30.

Hill G.K., Baumberger I., Spies S. (1995): Studies on the occurrence of the cleistothecia of *Uncinula necator* (Schw) Burr. in two vine-growing areas of Germany. Wein-Wissenschaft, **50**: 3–8.

Hoffmann P., Virányi F. (2007): The occurrence of cleistothecia of *Erysiphe necator* (grapevine powdery mildew) and their epidemiological significance in some vine-growing regions of Hungary. Acta Phytopathologica et Entomologica Hungarica, **42**: 9–16.

Hoffmann P., Füzi I., Virányi F. (2007): Investigation of cleistothecia of *Erysiphe necator* Schwein with laboratory methods. Növényvédelem, **43**: 265–272. (in Hungarian)

Hoffmann P., Füzi I., Virányi F. (2009): New results about the sexual overwintering form of *Erysiphe necator* Schwein in Hungary. Növényvédelem, **45**: 63–68. (in Hungarian)

Jailloux F., Willocquet L., Chapuis L., Froidefond G. (1999): Effect of weather factors on the release of ascospores of *Uncinula necator*, the cause of grape powdery mildew, in the Bordeaux region. Canadian Journal of Botany, **77**: 1044–1051.

Lehoczky J., Makó Sz., Kiss J. (1991): Role of the sexual reproductive body (fruiting body, cleistothecia) of grapevine powdery mildew fungus in the overwintering and the development of initial infection at spring. Kertgazdaság, **23**: 46–58. (in Hungarian)

Pearson R.C., Gadoury D.M. (1987): Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. Phytopatology, **77**: 1509–1514.

Pearson R.C., Taschenberg E.F. (1980): Benomyl-resistant strains of *Uncinula necator* on grapes. Plant Disease, **64**: 677–680.

Rügner A., Rumbolz J., Huber B., Bleyer G., Gisi U., Kassemeyer H.-H., Guggenheim R. (2002): Formation of overwintering structures of *Uncinula necator* and colonization of grapevine under field conditions. Plant Pathology, **51**: 322–330.

Stapleton J.J., Gubler W.D., Fogle D., Chellemi D., Bettiga L., Leavitt G. (1988): Relationships among climate, primary inoculum source, dormant and post-emergence control sprays, and grape powdery mildew in California. Phytopathology, **78**: 1531.

Steinkellner S. (1998): Overwintering of *Uncinula necator* in Austrian vineyards. Vitis, **37**: 193–194.

Steva H., Cartolaro P., Gomes da Silva M.T. (1990): Tolerance of powdery mildew of SBI fungicides: situation for 1989. Phytoma, **419**: 41–44.

Steva H., Cazenave C. (1996): Evolution of grape powdery mildew sensitivity to DMI fungicides. In: Brighton Crop Protection Conference – Pests & Diseases: 725–730.

Szabadi G. (ed.) (2010): Növényvédő szerek, termésnövelő anyagok I. Agrinex Bt., Budapest.

Ypema H.L., Gubler W.D. (2000): The distribution of early season grapevine shoots infected by *Uncinula necator* from year to year: a case study in two California vineyards. American Journal of Enology and Viticulture, **51**: 1–6.

Received for publication December 2, 2010
Accepted after corrections February 4, 2012

Corresponding author:

Péter Hoffmann, MSc, Orgona utca 16., H-7761 Kozármisleny, Hungary
tel. + 36 30 906 57 35, e-mail: peter.hoffmannb@basf.com