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UNIVERSITY

**THE ROLE OF SEXUAL FRUITING BODIES OF *ERYSIPHE*  
*NECATOR* SCHWEIN. IN THE GRAPEVINE POWDERY MILDEW  
EPIDEMIOLOGY**

MAIN POINTS OF THE PhD THESIS

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## BACKGROUND AND OBJECTIVES

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Powdery mildew is one of the most important diseases of grapevine worldwide. In Hungary, one has to take into account that an outbreak of mildew epidemics will follow in every second year. Though complete management programs, including efficient fungicides are available for controlling the disease, their success fails in several cases. There must be certain deficiencies behind the lack of positive results whose overcoming requires additional studies of the biology of *Erysiphe necator* Schwein. in the frame of new research programs.

It is not by chance that significant new knowledge has come to light, at both international and national levels, concerning the fungus causing powdery mildew for the last twenty-five years. When PEARSON and GADOURY (1987) confirmed for the first time that the ascospores released from chasmothecia were able to infect grapevine at spring, studies started to focus on the sexual form of this pathogen. As a result, dominance of either sexual or asexual overwintering forms were investigated and clarified in several grapevine growing regions of the world (GADOURY and PEARSON 1988, STAPLETON *et al.* 1988, MAGAREY *et al.* 1994, CORTESI *et al.* 1997, STEINKELLNER 1998, STEINKELLNER and REDL 1998, GROVE *et al.* 1999, JAILLOUX *et al.* 1999, HALLEEN and HOLZ 2000, YPEMA and GUBLER 2000, RÜGNER *et al.* 2002, GROVE 2004, MILADINOVIC *et al.* 2007).

In Hungary, field surveys made by FÜZI (1999a, 1999b) in the southern Transdanubian vineyards, and by DULA and SCHMIDT (2001) nation-wide, showed that the powdery mildew fungus survived mostly in sexual form, and the overwintering asexually was sporadic. At the same time, however, several studies uniformly confirmed that the ascospores released from the sexual fruiting bodies had a decisive role both in the initiation of spring infection and in the establishment of cluster damage (GADOURY *et al.* 1994, FÜZI 1999b, MAGAREY *et al.* 2000, MOYER *et al.* 2008).

István Füzi has been studying the environmental factors influencing the dynamics of *E. necator* epidemics, the role of the sexual overwintering form and the possibility of using fungicides for controlling the disease since the beginning of the 1990s. It was in the frame of my dissertation work that I participated in his research at the beginning of the 2000s. As several additional questions raised during our joint

research work, particularly related to the sexual overwintering of the pathogen, he encouraged me to start with this subject but in more details.

Keeping in mind the results of previous investigators, as well as the gaps of knowledge on the subject, we decided to focus our work on the sexually produced chasmothecia of *E. necator*, as of primary source of inoculum in the vineyards. Therefore, the following objectives were put forward:

1. Investigating the sexual overwintering form of *E. necator* under field conditions on various grapevine varieties and in different wine growing regions of Hungary, in different years.
2. Studying the infectivity of ascospores in the chasmothecia in the year of their production and in the next spring, at the beginning of the growing season.
3. Studying the relationship between the extent of powdery mildew colonization of the grapevine canopy and the quantity of fruiting bodies produced on the leaves that later was washed off onto the barks, with particular attention to their epidemiological significance.
4. Investigating the connection between primary infection and level of cluster infection.
5. Comparative assessment of the efficacy of fungicides used to control powdery mildew with regard to the relation of powdery mildew appearance on leaves and clusters, the inhibition of chasmothecia production, as well as the initial infection of the next year.
6. Working out a laboratory method to survey the quantities of sexual fruiting bodies overwintering on the bark surface of grapevine.

## MATERIALS AND METHODS

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### FIELD TRIALS

#### FEATURES OF THE SITES OF SMALL-PLOT TRIALS AND THE EXPERIMENTAL CONDITIONS

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The small-plot field trials were carried out in the wine growing region of Szekszárd: in 2005 in a 1.4 ha plantation of the cultivar Nosztori Riesling at Görögszó, and in 2006, 2007 and 2008 in a vineyard (0.2 ha of each) of the cv. Blue Frankish at Faluhely near Szekszárd.

In 2005, the Görögszó trial included nine treatments with three replicates (27 plots). According to similar principles, trials containing ten treatments with three replicates (30 plots), eleven treatments with three replicates (33 plots), and ten treatments with four replicates (40 plots) were conducted at Faluhely in 2006, 2007 and 2008, respectively. The size of these trials was 1 000 m<sup>2</sup> each and there was an average of 15 plants in each plot.

In the experiments there was a non-treated control during the whole growing season. In the other plots, serial fungicide applications per treatment were made to control powdery mildew infection on clusters and leaves in 3 and 4 replicates and the preparations were used according to a particular spraying program. From the beginning of flowering to early berry touch, six and seven fungicide applications were made in 2005, 2006, 2007, and 2008, respectively. Applications were made with knapsack sprayers using 500 l/ha water.

#### FEATURES OF THE SITES OF LARGE-PLOT TRIALS AND THE EXPERIMENTAL CONDITIONS

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Large-plot field trials were carried out in 2004 and 2005 in the wine growing regions of Szekszárd, Mecsekalja, Kunság and Eger, while in 2006 in the wine growing regions of Szekszárd and Mecsekalja. Uniform sprayings on the sampling sites of 0.1-1.5 ha were conducted by the owners according to a previously determined management program. To study the natural increase of powdery mildew population, 20-50 plants/cultivar were designated for untreated control in each experiment. In the three years, treated and untreated places were designated in 9 different grapevine cultivars.

In the small-plot experiments, the date of the appearance of the first powdery mildew symptoms was recorded from the 4-6 leaf-stage of grapevine and it was determined whether they originated from an infection by sexually produced ascospores or were produced by conidial infection from in-bud overwintered mycelium. Then, the powdery mildew infection was continuously observed under both treated and untreated conditions. In October, 30 leaves/site were collected, the powdery mildew colonies on the leaf surface was determined and the leaves were grouped into categories accordingly. Taking the colonisation into consideration, fifteen out of 30 leaves/sample were selected and stored in 5-10 °C until they were further examined.

In the spring of 2008, the plots of trials conducted in 2007 were thoroughly studied at Faluhely, and the colonies and frequency of powdery mildew were surveyed on the underneath of the leaves and the budded clusters before the first farm-scale spraying in order to determine the initial infection. In 2009 similar survey was made on the young leaves close to the trunk surface in the experimental plots where trials had been carried out in the previous year.

In the large-plot trials the vineyards were studied at regular intervals from the 4-6 leaf stage. In each survey, 500 leaves/sample were studied, the date of the appearance of the first powdery mildew symptoms was recorded and it was determined whether the symptoms were resulted from infections caused by ascospores or mycelium. Then, the development of powdery mildew infection was observed in both treated and untreated sites. In October, 50 leaves/plot were collected, the powdery mildew level on the leaf surface was evaluated under artificial light. From the leaf samples, reduced number (25 pcs) of samples was produced and stored at 5-10 °C until they were further examined.

## LABORATORY INVESTIGATIONS

### DETERMINATION OF THE QUANTITY OF CHASMOTHECIA ON THE GRAPEVINE CANOPY

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For processing the leaf samples originating from small-plot and large-plot field trials, I used the method of FÜZI (2003). I cut discs of 11 mm in diameter from 5 determined places of all leaves. I examined the discs under stereomicroscope with 25-35x magnification. I counted the chasmothecia on both sides of the leaf discs. Taken the data of all examined leaves, I calculated an average per sample. The obtained results were converted for unit leaf surface (pcs/cm<sup>2</sup>).

### HARVESTING CHASMOTHECIA FROM THE BARK OF GRAPEVINE PLANTS BY USING A SIEVING PROCESS

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In January 2006, I collected bark samples from the plots of small-plot trials carried out at Görögyszó in 2005. The samples were taken separately from the two-year-old woody parts, and from the upper half of the trunks and the horizontal cordons. I repeated the sampling in July 2006, taking bark only from the old parts. Then I stored the samples in paper bags under dry conditions, at room temperature until processing.

During laboratory processing, I weighed 10 g of barks from each air-dried sample on an analytical balance. The samples were separately placed into a beaker containing 150 ml of tap water on an ultrasonic bath (35 kHz) for 8 minutes. The agitated suspension was poured over a 1 500 µm mesh size sieve into an Erlenmeyer flask in order to separate coarse size parts of bark. The content of the flask was then poured over an 800 µm 4-layer sieve into another Erlenmeyer flask and rinsed with additional 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 40-45x magnification. The obtained results were expressed in unit of pieces/10 g.

In January 2007, I collected bark samples from the plots of small-plot trials carried out at Faluhely in 2006. This time no samples were taken from the young woody parts, they were only collected from the upper half of the trunks and the horizontal cordons. I stored the isolated bark parts in paper bags under dry conditions, at room temperature until processing.

Then I took five of the samples and examined separately in order to refine the sieving methodology. I weighed 10 g bark from each of the five samples then placed them into a beaker containing 150 ml of tap water on an ultrasonic bath (35 kHz) for 8 minutes. The agitated sample and the water were poured in a 1 000 ml beaker and the agitated beaker was rinsed with 3x150 ml of water. The resultant (600 ml) suspension was poured over a 1 500 µm mesh size sieve placed in a funnel, into a larger Erlenmeyer flask. To separate the coarse parts of bark I used a 800 µm 4-layer sieve. Finally, the suspension was poured by sections over a 55 µm mesh size sieve, forming sub-samples. The quantity of rinsing water for each sieve was 500 ml. The sub-samples were examined microscopically and the number of chasmothecia retained on the 55 µm mesh size sieve was determined using 40-45x magnification. In order to increase the efficiency of harvesting chasmothecia from bark debris on the five selected samples trapped on the 1 500 µm mesh size sieve, the whole sieving process was repeated eight times. Based on the obtained results, the other 25 bark samples originating from the trial were examined by the sieving process only three times.

In January 2009, I collected bark samples from the plots of small-plot trials carried out with cv. Blue Frankish in 2008. This time the samples were taken from the upper half of the trunks and the horizontal cordons and processed with the refined methodology (with three sieving processes).

#### ARTIFICIAL INOCULATION WITH ASCOSPORES

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In 2004 and 2005 samples of 8 grapevine cultivars containing chasmothecia were collected at different dates in 6 production places. The primary objective was to study under laboratory conditions the infectivity of ascospores released from the fruiting bodies.

First, the artificial inoculation was made on 6 May 2004 for which the samples were taken from cv. Chardonnay at Kecskemét. On 16 February 2004, parts of bark were collected from the vine surfaces then, on 3 May, three days before the artificial inoculation, leaf debris were taken from the fallen leaves originating from the previous growing season. In autumn 2004, leaves infected by powdery mildew were twice collected from different points in the leaf canopy from different cultivars at two different places of production. On 30 September and 14 October, artificial inoculations were made by using the leaves collected between 6 and 8 September and between 30 September and 5 October, respectively. One year later, on 15 September 2005 the

experiment was repeated with the leaf samples taken between 31 August and 14 September.

For the artificial inoculation made in spring 2004, discs were cut from the leaf debris in the fallen leaves, then the number of fruiting bodies on them were determined and fixed on filter papers, on the one hand, and the chasmothecia harvested from parts of bark and the debris were directly placed on the filter papers, on the other. In the autumn infection, we used in all cases the discs cut from the leaves originating from the vines and fixed on the filter papers.

We used the young leaves of 3-5 cm of grapevine cuttings grown in the greenhouse for the *in vitro* inoculations. We used grapevine leaves of the cultivars Leányka and Blue Frankish in 2004 and 2005, respectively. Agar growth medium containing benzimidazole was placed in the top and bottom of the Petri dishes. The young leaves disinfected twice with distilled water and once with sodium-hypochlorite solution were placed on the growth medium in the bottom of Petri dish. The isolated chasmothecia and the filter papers with the leaf discs were placed on the growth medium in the top. The covered Petri-dishes were incubated at room temperature for 8-14 days, and then the powdery mildew colonies produced on the leaves were counted.

#### METEOROLOGICAL OBSERVATIONS

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For the evaluation of results of the trials carried out in the wine growing region of Szekszárd, meteorological data of the weather station of the former Plant Protection and Soil Conservation Service of county Tolna was used, whereas for those carried out in the wine growing regions of Mecsekalja, Kunság and Eger, data obtained by the local research institutes were considered.

#### STATISTICAL ANALYSIS

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For evaluating the data obtained in the small-plot trials of multiple replicates I used the statistical program package “ARM 8.3.2” where the differences between means were determined by LSD-test and expressed at  $p=0.05$ . To measure the degree of association between variables, the Pearson product-moment coefficient ( $r$ ) was used.



## RESULTS

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### DOMINANCES OF THE OVERWINTERING SEXUAL AND ASEQUAL FORMS OF POWDERY MILDEW FUNGUS

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In the trial years between 2004 and 2006, chasmothecia of *E. necator* were produced in large numbers every year in all the 9 grapevine cultivars, in 11 plantations, in 6 different places of production in the wine growing regions of Eger, Kunság, Mecsekalja and Szekszárd. The flag shoot of the pathogen appeared only in the vineyard of cv. Blue Frankish in Sióagárd among all the observation sites in 2004 and 2005. In contrast, during the six years the symptoms originating from the infection by ascospores could be identified in 27 cases independently from the wine growing regions, places of production and grapevine cultivars.

In our studies, neither the flag shoots nor the powdery mildew colonies originating from the infection by ascospores were found in 19 cases. In these cases, the disease symptoms always appeared late, at the end of June or in July, and must have been the results of conidial infections. Conidia causing late infection could be formed from the sporadically occurring powdery mildew colonies not observed at the survey of infection by ascospores or could be transported by winds from other plantations to the area in question.

### CORRELATION OF CLUSTER DAMAGE WITH THE DATE OF APPEARANCE OF THE FIRST SYMPTOMS

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In our studies the date of the appearance of the symptoms of primary infection significantly influenced the extent of cluster damage established by the end of the susceptible period of berries. The earlier of the first symptoms of the pathogen appeared, the more severe powdery mildew infection was observed on the clusters. In the studied plantations the beginning of flowering and the susceptibility of clusters of grapevine was between the last decade of May and the first half of June, depending on spring weather, cultivars and features of production sites. This initial date of flowering is considered a “demarcation line” because the berries may be diseased from this date on. In cases if the symptoms of primary infection appeared much before the beginning of flowering the cluster damage was always stronger than medium. But if the appearance of the first symptoms was delayed compared to the plant growth, the

severity of cluster disease was reduced. Earliness of the appearance of symptoms had significant influence on the establishment of infection pressure independently from the production place, grapevine cultivars and the weather conditions during the susceptible period of clusters.

#### LOCATION OF CHASMOTHECIA OVER SPACE AND THE INFECTIVITY OF ASCOSPORES RELEASED FROM THEM

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In spring 2004, successful artificial inoculation was made under laboratory conditions using the chasmothecia remaining both in the bark cracks of the plants and on the surface of leaf debris in the fallen leaves. In field trials we found that chasmothecia overwintering in the plant parts played primary role in the initial infection because the symptoms caused by ascospores always appeared on the leaves near the plants almost covering them.

The question whether the ascospores are able to release from the sexual fruiting bodies without overwintering after their formation and to cause infection was answered by the *in vitro* experiments made in autumn 2004 and 2005. It was observed that even the ascospores released from the samples that were taken in autumn before washing off, infected the young grapevine leaves on which, as a consequence, powdery mildew colonies developed. Another question raised: to what extent did the release of ascospores decrease the number of primary inocula at the beginning of the next growing season. Our studies revealed that, in January 2007, significantly less chasmothecia were available on the vines of the untreated areas than on those of treated sites on which leaf infection of similar severity had established in October of the previous year. In spring 2009, significantly less powdery mildew colonies originating from ascospore infection appeared in the plots where no chemical control was made in the previous year than in the areas treated with mephyl-dinocap and proquinazid.

#### EFFECT OF FUNGICIDES ON THE OCCURRENCE OF CLUSTER AND LEAF POWDERY MILDEW AND THE PRODUCTION OF CHASMOTHECIA

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In the small-plot trials carried out in four years, the different active substances and mixtures of active substances were compared under significantly different infection pressure. In 2005 and 2008 the epidemic outbreak was significant, in 2006 the clusters remained almost healthy but the canopy became significantly attacked in autumn and in

2007 the cluster infection was moderate. The results justified that certain fungicides should be evaluated separately according to their efficacy against powdery mildew infection on clusters and leaves. The reason is that most preparations are not similar in this respect. As fungicides were applied from the beginning of flowering to early berry touch, it was actually the direct efficacy of the preparations that played a role in preventing the cluster damages, while the duration of their action prevailed in inhibiting the late leaf infection and the production of chasmothecia.

Contact active substances were studied alone only in 2008, the year of the most severe infection pressure. Under such conditions, the preparations of sulphur, dinocap and mepthyl-dinocap used alone were not able to control either the cluster infection or the autumn leaf infection and to prevent, at the same time, the mass production of chasmothecia. The active substances of triazole and the mixtures containing triazole showed medium efficacy in controlling the cluster powdery mildew. In controlling the autumn leaf infection, only the liquid formulation of fluquinconazole showed acceptable results as it prevented the most significantly the increase of powdery mildew population on the canopy and the production of chasmothecia. The other triazoles showed very low efficacy. Proquinazid showed good efficacy in protecting the clusters though it had rather low efficacy in controlling either the autumn leaf infection in two out of four years or the production of chasmothecia in three years. Efficacy of metrafenone was slightly behind that of proquinazid on clusters, but it was significantly better in protecting the canopy and in reducing the number of fruiting bodies. Throughout the four years and of all the studied compounds only boscalid controlled both the cluster and the leaf infection and prevented production of chasmothecia.

The group of QoI-fungicides have to be considered separately. The key element of the pest management practice used in the large-plot trials was the three applications of pyraclostrobin between 2004 and 2006. This technique offered adequate efficacy both in preventing the cluster damages and in inhibiting the autumn leaf infection and the mass production of chasmothecia. The efficacy of pyraclostrobin slightly exceeded that of boscalid in the small-plot trial carried out in 2005 at Görögyszó. Efficacy of kresoxim-methyl was slightly behind these preparations but with no significant difference. With no account of inhibition of powdery mildew of clusters, azoxystrobin showed only minimal efficacy for several parameters already in 2005. All the applied QoI-fungicides showed no or very low efficacy in controlling the autumn leaf infection by powdery mildew in 2006 at Faluhely. Then the laboratory analyses showed

resistance of *E. necator* to these preparations (Taksonyi *et al.* 2009). In summary, it is found that the widely used QoI-fungicides successfully inhibited the population increase of the pathogen on the canopy together with the production of chasmothecia before the development of resistance, in addition to their excellent efficacy in controlling the powdery mildew on the clusters. However, their practical use continuously decreases because of the building up and spread of resistance to these preparations.

Among the active substances tested in the experiments, only boscalid could jointly prevent the cluster damages and the production of sexual inoculums.

#### IMPORTANCE OF THE AUTUMN INFECTIVITY OF CANOPY

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In almost all of our trials, the leaf canopy, under untreated conditions, was severely diseased by the end of the growing season, though this did not correlate with the previous level of powdery mildew on the clusters. In plots with no chemical treatments the cluster damages were severe at Görögszó in 2005 and Faluhely in 2008, medium in 2007 and low in 2006. However, the incidence of powdery mildew turned out to be significant, between 73 % and 96 % by the end of the growing season in all years of the experiment.

A statistically confirmed positive correlation could be proved between the number of chasmothecia produced on the leaves and the severity of leaf infection. This correlation prevailed also if, independently from the place of production, the year and the grapevine cultivars, I analysed data of all large-plot trials and also if I compared the results/year of the samples taken from the same grapevine cultivars from the same place of production in the same plantation.

#### HARVESTING THE CHASMOTHECIA FROM THE BARK

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In the investigations, I made agitation of bark samples using ultrasonic bath during a given period and used three sieves of different mesh sizes (1 500, 800 and 55  $\mu\text{m}$ ). I made the sieving process only once with the bark samples (with three different series of samples) originating from small-plot trials carried out in 2005. On average, 184 chasmothecia could be harvested from the older bark parts collected in January 2006, while 57 chasmothecia were obtained from the young two-year-old woody parts, expressed in the mass of 10 g bark. The number of fruiting bodies collected from the young two-year-old woody parts were lower in all plots than the number taken from the

old barks, the difference ranging from 2 to 4.5 times. The old bark samples taken in July 2006 contained an average of 67.71 chasmothecia/10 g.

In the following experimental series I refined the sieving method. In doing so I collected samples in January 2007 from the surface of old plant parts in the small-plot trials designed in 2006. Using five samples I made the sieving process and obtained, on average, 2 061 chasmothecia/10g per sample. As I harvested over 80 % of the total number of the obtained chasmothecia by using the sieving process three times, I examined the bark samples by means of the refined sieving method with three agitations in 2007 and 2009. I obtained, on average, 1 118 chasmothecia/10 g and 3 523 chasmothecia/10 g per sample from the bark parts in January 2007 and in January 2009, respectively.

EFFECT OF POWDERY MILDEW INFECTION AND THE NUMBER OF INOCULUM  
OVERWINTERED ON THE BARKS OF THE PREVIOUS YEAR ON THE INITIAL INFECTION BY  
POWDERY MILDEW FUNGUS OF THE NEXT YEAR

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In the untreated plots of the small-plot trial series, a significant powdery mildew colonisation between 73 % and 96 % established by the end of the growing season in all years, nevertheless the chemical treatments carried out with the fungicides of different efficacy greatly differentiated the canopy infection. Thus, it was possible to study the number of chasmothecia washed off to the bark parts in a system where, under the effect of the treatments, the variables were expressed by the severity of autumn leaf infection and the number of fruiting bodies produced, though all other conditions were the same in the areas (cultivars, cultivation mode, age, row spacing, exposure, climatic conditions, with special attention to the wash-off precipitation). As a result, the differences in the number of chasmothecia harvested from the bark samples collected in January among the different trials could be explained by the fact that the number of inocula produced on the leaves was different already in autumn.

Our studies confirmed that statistically positive correlation could be demonstrated between the severity of autumn leaf infection and the number of chasmothecia produced on the leaves and remained on the barks.

We studied also in spring of 2008 and 2009 if the chemical treatments made in the previous year and resulting in different severity of leaf infection and number of fruiting bodies both on the leaf canopy and the barks, had an effect on the initial

infection of the next year. On 26 May 2008, the frequency of the occurrence of symptoms on the clusters at budding stage and the leaves immediately prior to flowering and, at the same time, the powdery mildew colonies were the most important in plots that had heavy powdery mildew infection on the canopy in autumn of the previous year. In contrast, the vines that had healthier canopy in autumn showed lower number of diseased clusters and leaves in the spring (Figure 1).

A year later, in May 2009, it was also demonstrated that there was a close correlation between the autumn leaf infection and the fruiting bodies produced on the leaves, the number of chasmothecia overwintering on the bark parts and the number of powdery mildew colonies caused by ascospore infection in spring (Figure 2). The starting point of the powdery mildew infection was by all probability the fruiting bodies that remained on the bark surface of grapevine. The more fruiting bodies overwintered on the barks, the higher number of symptoms appeared on the underneath of young leaves near the vine.

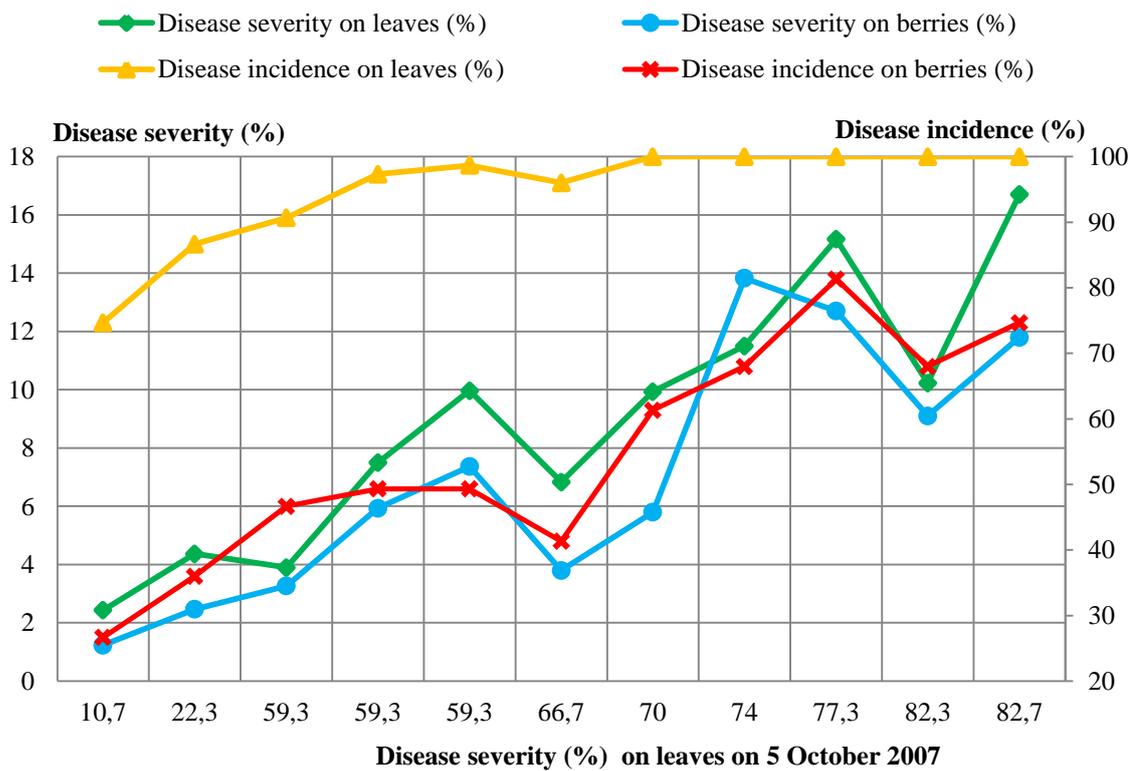


Figure 1 Disease incidence and severity on 26 May 2008 in function of the powdery mildew infection level on the canopy in the previous year (2007-08 Faluhely, cv. Blue Frankish, small-plot trial)

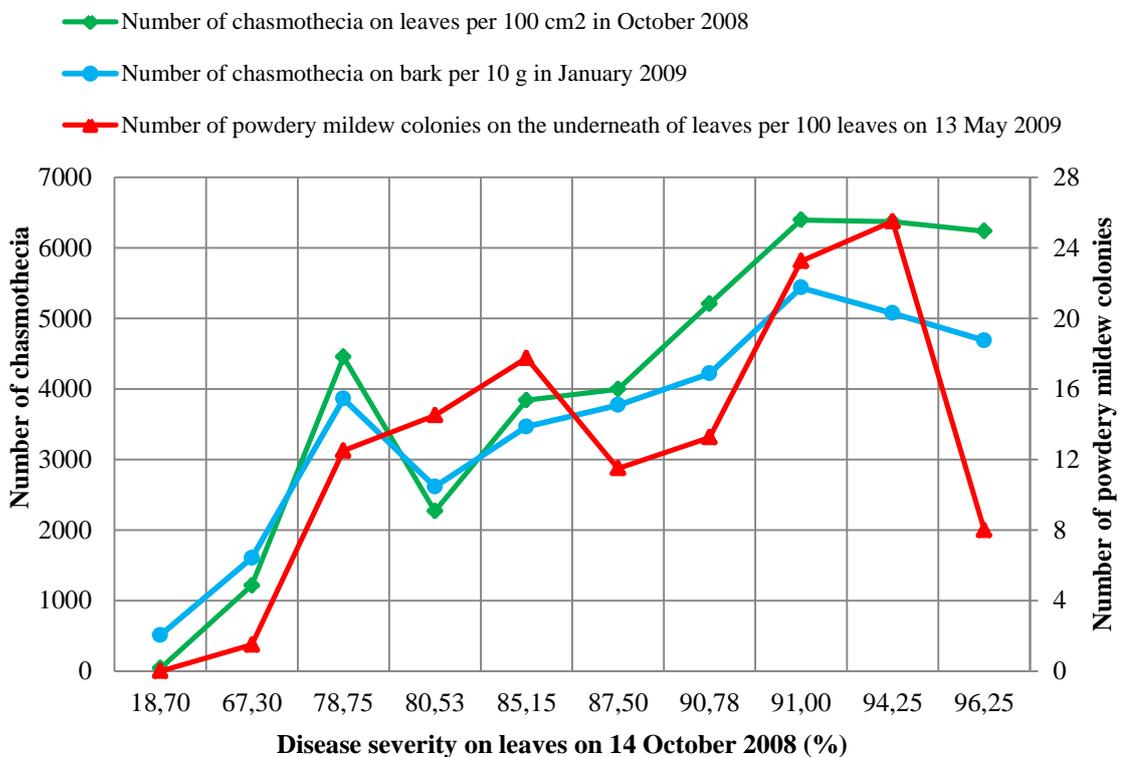


Figure 2 Effect of the previous year powdery mildew infection, the number of chasmothecia produced on the canopy and washed away to the barks on the initial infection of the next year (2008-09 Faluhely, cv. Blue Frankish, small-plot trial)



## NEW SCIENTIFIC RESULTS

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1. In Hungary we are the first to thoroughly study the chasmothecia of *Erysiphe necator* overwintering on the bark surface of grapevine and to publish data on their epidemic importance.
2. We proved that under Hungarian conditions, the ascospores available in the chasmothecia that overwintered in autumn on the leaves, in spring in the fallen degrading leaves and on the woody barks of grapevines were infectious.
3. We worked out new and efficient procedure to harvest the chasmothecia from the barks; this method allows to precisely studying the quantity of actually overwintered primary inoculum.
4. We confirmed furthermore that there was a close correlation among the autumn leaf infection, the number of sexual fruiting bodies produced on leaves and remained in barks and the number of powdery mildew colonies originating from the spring ascospore infection and the extent of initial infection.
5. We stated that the date of the appearance of the first symptoms significantly influences the extent of cluster damages established up to the end of the susceptible period of berries independently from the place of production, the year and grapevine cultivars.
6. We also stated that any chemical control that cannot stop either the increase of pathogen population on the leaves or the formation of chasmothecia in the second half of the growing season might increase both the number of primary inocula of the following year and the risk of epidemics.
7. We stated furthermore that the chemical control which efficiently moderates the development of autumn infection, and, hence, the formation of chasmothecia and the number of overwintering primary inocula, might significantly decrease the extent of the initial infection of the following year.



## CONCLUSIONS

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In Hungary, the fungus causing grapevine powdery mildew overwinters mainly with chasmothecia produced during sexual reproduction, while the occurrence of the asexual form, i.e. the mycelium hiding in the buds is only sporadic. From an epidemic point of view, the released ascospores function as primary inoculum, their role being significantly more important than that of flag shoots bursting from the mycelium infected buds.

The highest number of chasmothecia is produced on grapevine leaves, their number being primarily determined by the powdery mildew colonisation of the canopy. The leaf infection in the second half of the growing season does not correlate with the severity of earlier cluster damage. Therefore, mass production of chasmothecia may take place even if the clusters have not been diseased.

Chasmothecia on the grapevine leaves contain infectious ascospores before their washing away. If fungicides are not applied, the production of chasmothecia begins much earlier and they are ripen sooner than under sprayed conditions. Thus, the ascospores are released with greater chance in non-treated grapevine even in autumn.

At spring, the ascospores of chasmothecia overwintered both in the fallen leaves and the woody bark of grapevine are infectious. As regards the initial infection, the chasmothecia which overwintered on the bark surface are the most important as they are nearer the canopy than those on the fallen leaves. It is therefore obvious that the first symptoms appear more frequently on the underneath of the young leaves tightly attached to the plant barks.

With the ageing of the plantation, the thickening of woody parts, and the increase of their surface, more chasmothecia can remain attached to the plant, therefore there is a greater chance of the development of powdery mildew epidemics in older plantations than just after the establishment of a new vineyard.

As regards the development of epidemic curve of *Erysiphe necator* during the growing season, the number of primary inocula is of great importance. At spring, the number of chasmothecia that overwintered on grapevine bark surface significantly influences both the frequency of primary infection and the severity of initial infection. Our research also confirmed that there was a close correlation among the autumn leaf infection, the number of sexual fruiting bodies produced on leaves and remained in

barks and the number of powdery mildew colonies originating from the spring ascospore infection and the extent of initial infection.

The date of the appearance of the first symptoms basically influences the extent of cluster damages established up to the end of the susceptible period of berries. Severe disease of the clusters may be accounted for if the appearance of the first symptoms well precedes grapevine flowering. If nevertheless the first powdery mildew colonies develop later, the chance for the epidemic outbreak is gradually decreased. The first symptoms appearing after flowering shall not generally result in heavier infection pressure than medium level.

The use of preparations for the control of powdery mildew has an effect on the formation of chasmothecia. Most fungicides applied between the growth stages of cluster elongation and berry touch in the first half of the growing season are not capable of significantly inhibiting the formation of sexual fruiting bodies which takes place in the second half of the growing season. As they, however, delay the increase of powdery mildew population on the leaves, they prolong the formation of chasmothecia over time. At the same time they probably reduce the possibility of autumn release of ascospores. As a result, both the number of ascospores causing spring infection and the risk of epidemics may eventually be increased as compared with the unsprayed control.

The refined sieving technique developed during our work can be used for determining the number of sexual fruiting bodies on the grapevine barks. Since the volume of primary inocula definitely influences the extent of initial infection and the date of the appearance of the first symptoms influences the status of cluster damages, the expectable infection risk can be determined with higher probability by surveying the actual number of overwintering inocula and detecting the appearance and frequency of the first powdery mildew colonies.

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