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Monitoring of ascospore density of *Erysiphe necator* in the air in relation to weather factors and powdery mildew development

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Abstract In a 6-year Hungarian study, ascospore density of *Erysiphe necator* in the air was monitored and related to three weather variables (temperature, relative humidity, and rainfall) and powdery mildew disease progress in two commercial vineyards. Temporal pattern in aerial density was also quantified. In total, 71 ascospore trapping periods were detected over the 6-year period from early April until end June. Across all years, 6.6 % of the total ascospores (0.5 % mean ascospore percent per day) were caught between the initiation of sampling in April and bud break, 62.2 % (1.6 %) from bud break to bloom, and 31.2 % (0.3 %) between bloom and the conclusion of sampling at the end of June. Hourly proportions of ascospores caught did not reveal diurnal patterns of spore release. All three weather factors (in the order of rainfall, relative humidity and temperature) correlated significantly with mean ascospore catches in each year. Mean hourly rainfall correlated best with mean hourly ascospore catches (correlation coefficient, *r*, ranged from 0.43 to 0.78) in both vineyards and in all years. First leaf and berry symptoms appeared between 7 and 24 May and between 25 May and 19 June, respectively, during the 6-year study. Disease started to progress slowly after the appearance of the first infected leaf followed by an exponential increase from early June. By the end of June, leaf and berry disease incidences ranged from 4.1 to 98.2 % and from 0.9 to 6.8 %, respectively, over the 6-year period. Leaf incidences showed significant relationship with corresponding cumulative numbers of trapped ascospore in five out of 6 years, which was described by three-parameter Gompertz functions in each year. Results were compared and discussed with previous observations.

Keywords Aerial ascospore density · Disease incidence · Epidemiology · *Erysiphe necator* · Grape powdery mildew · Spore dispersal · Weather variables

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Introduction

Grape powdery mildew caused by the obligate biotroph *Erysiphe necator* (Schwein.) Burrill (syn. *Uncinula necator*) is the most serious fungal disease of grapevine throughout the world (Pearson 1988). The pathogen can survive the winter as ascospores in overwintering chasmothecia and/or as mycelium in infected buds providing primary inoculum for vineyards (Pearson and Goheen 1988). In cold-climate regions, *E. necator* overwinters as chasmothecia mainly on the exfoliating bark of the vine trunk (Gadoury and Pearson 1988, 1990) as it predominantly happens in the Szekszárd grape growing region of Hungary which belongs to Central

European continental climate region (Füzi 1999; Füzi and Holb 2007). Ascospores are released in spring and the first-formed basal leaves of shoots growing near bark are infected first, presumably due to their proximity to the overwintered chasmothecia (Gadoury and Pearson 1990; Halleen and Holz 2001).

Previous studies monitored aerial density of *E. necator* ascospores on exposed vaseline slides (e.g., Jailloux et al. 1998; Rossi et al. 2010) and with Burkard volumetric spore trap (e.g., Pearson and Gadoury 1987; Gadoury and Pearson 1990). Release of *E. necator* ascospores was clearly shown to be dependent on free water and/or rain events (Pearson and Gadoury 1987; Jailloux et al. 1998; Gadoury and Pearson 1990; Rossi et al. 2010). Water promotes the spread of ascospores from bark to first basal leaves; therefore, short range transport of ascopores was considered by splash dispersal (Pearson and Gadoury 1987). Pearson and Gadoury (1987) reported that ascospore release occurred during rain or during a leaf wetness period after rain and ascospores were trapped whenever rainfall exceeded 2.5 mm (Gadoury and Pearson 1990) or 2 mm (Jailloux et al. 1998). Recently, Rossi et al. (2010) confirmed that≈ 90 % of the ascospores were trapped during periods when these minimum requirements for ascospore discharge occurred. Relationship between weather parameters and aerial ascospore density of *E. necator* were partially investigated previously. Jailloux et al. (1998) performed correspondence analyses amongst ascospore release, temperature and rainfall and showed a significant relationship between aerial ascospore content and rainfall but the level of relationship was not further studied. Rossi et al. (2010) showed that the numbers of ascospores trapped strongly associated with rainfall periods but the relationship was not further quantified by statistical analyses.

Previous studies indicated that temporal pattern of *E. necator* ascospores may differ in various rain periods depending on the amount of rainfall and/or the duration of leaf wetness periods (Pearson and Gadoury 1987; Gadoury and Pearson 1990; Jailloux et al. 1998; Rossi et al. 2010). However, temporal pattern in aerial ascospore density of *E. necator* including diurnal periodicity has been poorly investigated in previous studies.

Previous studies demonstrated that ascospore release occurs under field conditions from early bud break until shortly after bloom (Pearson and Gadoury 1987; Gadoury and Pearson 1990; Grove 2004; Rossi et al. 2010; Moyer et al. 2014) but previous year fall and midwinter releases are also known from warmer grape growing regions (Gee et al. 2000; Rossi et al. 2010). The first infected leaf can appear before bloom in vineyards (e.g., Jailloux et al. 1999; Füzi and Holb 2007) and progresses continuously on the foliage which might be associated with the amount of ascospores dispersed in the air. However, the relationships among aerial density of *E. necator* ascospore and temporal disease development or annual disease pressure have received little attention in long-term multiyear studies.

The goals of this 6-year study were to: i) monitor aerial ascospore density of *E. necator* from early April until end June; ii) quantify the temporal pattern in aerial density; iii) correlate ascospore density to three weather variables (temperature, relative humidity, and rainfall); and iv) determine the relationship between spore data and disease development. The study was performed in two vineyards on two mildew-susceptible grape cultivars.

Materials and methods

Vineyard sites

A 6-year study (2004–2009) was carried out in two commercial vineyards in the Szekszárd grape growing region, in south-west Hungary (Table 1). The first plantation, located in Strázsa-hegy (lat. 46°31'15"N, long. 18°67'65"E), was 4.2 ha and consisted of a mixed stand of grape cultivars planted in alternating two-row strips. Cultivar (cv.) Portugieser, the highly powdery mildew-susceptible grape cultivar (Lisek 2013), was assessed from 2004 to 2006 in this location. Between-row and within-row distances were 3.5 and 1 m, respectively, maintained in a Moser cordon training system. Soil type of the vineyard was brown forest soil with alternating layers of clay.

The other vineyard, located in Faluhely (2 km northwest of the Strázsa-hegy site) was 4.8 ha (Table 1). It consisted of a mixed stand of grape cultivars planted in alternating two-row strips. Cultivar Limberger, the highly powdery mildew-susceptible grape cultivar (Hartman and Beale 2008), was assessed from 2007 to 2009 in this location. Between-row and within-row distances were 3.5 and 1 m, respectively, maintained in a Moser cordon training system. Soil type of the vineyard was brown forest soil.

Table 1 Location, cultivar, ascospore monitoring period, date of bud break, beginning of bloom, and first leaf and berry symptoms during a 6-year period (2004–2009)

Year	Location[a]	Cultivar	Ascopore monitoring period (start and end date)	Date of bud break (BBCH7)[b]	Date of beginning of bloom (BBCH61)[b]	Date of first leaf symptom	Date of first berry symptom
2004	Strázsa-hegy	Portugieser	9 April and 22 June	9 April	25 May	17 May (BBCH53)	5 June (BBCH57)
2005	Strázsa-hegy	Portugieser	17 April and 19 June	19 April	9 June	22 May (BBCH55)	17 June (BBCH71)
2006	Strázsa-hegy	Portugieser	11 April and 18 June	16 April	4 June	24 May (BBCH57)	19 June (BBCH73)
2007	Faluhely	Limberger	4 April and 10 June	20 April	30 May	15 May (BBCH57)	7 June (BBCH71)
2008	Faluhely	Limberger	2 April and 15 June	5 April	19 May	11 May (BBCH53)	25 May (BBCH57)
2009	Faluhely	Limberger	4 April and 7 June	10 April	28 May	7 May (BBCH55)	7 June (BBCH71)

[a] Location is within the Szekszárd grape growing region in Hungary

[b] For grapevine phenological stages, the BBCH-scale was used according to Lorenz et al. (1994)

Both vineyards relied on annual application of synthetic fertilizers for nutrient supply. A winter pruning before bud break was carried out each year. Bare soil, 0.3 m wide, was maintained in the rows, and grass was grown in the row middles. Vineyards were not irrigated. In both vineyards the disease and pest management program (data not shown) followed the Hungarian integrated production (IP) guidelines derived from the European IP guidelines (Malavolta and Boller 1999) since the planting of vineyards in 2001. All sprays were applied with a Kertitox Bora axial blower spray machine (Farmgép Ltd., Debrecen, Hungary) with a ceramic hollow cone at 1.1–1.2 MPa with a volume of 600 l ha^{-1}. In both fields and each year, untreated plots were prepared with a minimum of 100 grapevine plants which were used for all assessments. Plants in the untreated plot were protected with plastic foil coverages against fungicide spray drift during each spray event in the vineyards. The untreated plots were treated only against insects 5 times annually.

Quantifying ascospore in the air

The presence of ascospores in the air was monitored with a Burkard 7-day recording volumetric spore trap (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, UK) that was placed in the centre of the vineyard which consisted of grape cvs Portugieser and Limberger in Strázsa-hegy and Faluhely, respectively. In each year, the spore trap inlet orifice was about 1.3 m above ground level and was carefully placed to less than 5 cm close to the mass chasmothecia source. This mass chasmothecia source was a natural source on grapevine cordon near the trap inlet orifice estimated to include several thousand chasmothecia of *E. necator* on the sampled cordon arm. Spore trap was operated in each year from early bud break (April) until the first symptoms on berry (June) (Table 1). The reason why spore trap was operated until the first symptoms on berry was that this was the date before an efficient control can be performed against the pathogen in order to ensure high berry quality for vine production. Spore trap was operated at a flow rate of 12 l min^{-1}, as is used in similar studies (Gadoury and Pearson 1990). The flow rate of the trap was checked weekly by a calibrated Burkard flowmeter. The trap collected airborne particles on Melinex tape which was coated with a thin layer of the solution of vaseline (75 ml) and paraffin wax (9 g) (Burkard Scientific Sales Ltd., Rickmansworth, Hertfordshire, UK) and then attached to a slowly rotating drum. The tapes of spore traps that have been exposed for 7 days were cut into seven 48-mm long sections, representing 1-day exposure periods. Each piece of the tape was mounted on glass microscope slides in a very thin layer of the mixture of gelvatol (Monsanto Chemical Co., St. Louis, USA) and lactic acid (Anonymous 1980; Holb 2008). Each piece of tape was examined at hourly intervals of deposition (2 mm) in traverses perpendicular to the direction of movement and conidia were counted using a Zeiss Jenamed microscope (Carl Zeiss Jena GmbH, Jena, Germany) at a 400× magnification. All traverses were examined. *Erysiphe necator* ascospores in the size with 15–25×10–14 μm and with the shape of ovate to ellipsoid were counted according to the description of Kapoor (1967). Hourly spore counts were made during the whole period of spore sampling.

Firstly, the numbers per unit volume of air (m^3) per day was calculated according to the manufacturer's instructions (Anonymous 1980) and mean daily numbers of *E. necator* ascospores in the air was plotted against time and time was expressed in days starting from 1 April until 30 June.

Secondly, the numbers per unit volume of air (m^3) caught per hour was calculated according to the manufacturer's instructions (Anonymous 1980) and air dispersal pattern of ascospores was shown by plotting the mean proportion of spores caught each hour for all 24-h periods (24.00 to 24.00 h) during the trapping period in each year. Temporal pattern of the hourly number of *E. necator* ascospores caught was analysed by the time-series analysis method of autoregressive integrated moving-average (ARIMA) model using the procedure as described by Guerin et al. (2001) and Holb (2008). Numbers of ascospores were transformed to natural logarithms for ARIMA. ARIMA models were fitted to the hourly number of spore data set for each year (including all days of observations) and location and then the classical parameter tests and Akaike's Information Criterion (AIC) was used to select the possible (appropriate) model. Genstat Release 9.1 (Lawes Agricultural Trust, IACR, Rothamsted, UK) was used for the analyses.

Finally, the cumulative percentage of ascospores was calculated and, similarly to mean daily numbers of *E. necator* ascospores, it was plotted against time and time was expressed in days starting from 1 April until 30 June.

Environmental variables and their relation to ascospore caught

Temperature (°C), relative humidity (%), rainfall (mm), and wet periods of >3.5 h not initiated by rain (i.e., dew) were recorded at 12-min intervals using a Metos Compact agrometeorological station (Pessl Instrument GmbH, Weiz, Austria) at both locations from 1 April until 30 June in each year. The agrometeorological stations were located at a distance of 100 and 120 m from the spore trap in the vineyards at Strázsa-hegy and Faluhely, respectively. Sensors including the rainfall and leaf wetness sensors were mounted 1.3 m above the ground in the centre of the canopy of a grape plant during each season at each site and all environmental data were obtained inside the plant canopy. Data of each environmental variable were summarized as hourly averages.

Mean values of environmental variables (temperature and rainfall) were plotted against time. Relationship among hourly summaries of environmental variables (hourly temperature value, hourly relative humidity value and hourly amount of rain in mm) and hourly values of ascospore trapped were analysed by calculating Pearson correlation coefficients and associated significance levels for each location in each year. Genstat Release 9.1 (Lawes Agricultural Trust, IACR, Rothamsted, UK) was used for the analyses.

Disease assessment and relationship between aerial spore density and disease incidence

Dates of the first leaf and berry symptoms were assessed on previously selected nontreated plants on cv Portugieser in Strázsa-hegy from 2004 to 2006 and on cv Limberger in Faluhely from 2007 to 2009. Twenty plants were assessed for temporal disease assessment every 10 to 15 days from 1 April until end June. Fifty leaves and twenty five berries (flower) typical of the given phenological stage were observed on each of the selected plant part and assessed [diseased (+) or healthy (−)]. The disease was determined on randomly selected leaves and berries at each assessment date. Symptoms caused by ascospores on a leaf or a berry was considered if the symptom constricted to the abaxial surface of the leaves, and only if a pale yellowish spot was presented on the upper surface (Pearson and Gadoury 1987; Caffi et al. 2011). Leaf and berry incidences were calculated as the percentage of diseased leaf and berry. On each leaf powdery mildew severity was also estimated based on percent leaf area diseased. Leaf severity was calculated as the percentage of diseased leaf area. Mean values of leaf and berry incidences and leaf severity of grape powdery mildew were plotted against time and time was expressed in days starting from 1 April until 30 June.

In order to investigate the relationship between ascospores in the air and temporal disease dynamics, cumulative numbers of trapped ascospores were calculated and plotted against mean percentage of disease in each year. Only leaf incidence was retained for this analysis due to poor fit of berry incidence and leaf severity values to the cumulative numbers of trapped ascospores. Before any analyses, incidence data were transformed using angular transformation (arcsine of the square root

of the incidence). In a preliminary analysis, non-linear growth functions (three-parameter logistic, Gompertz, Mitscherlich and Bertalanffy, and Weibull) were fitted separately for each year data set by a nonlinear mixed-effect modelling approach using R version 2.0.0 with the 'nlme' statistical package (Pinheiro et al. 2004). The best-fitted model was selected based on the overall goodness-of-fit, visual examination of standardized residuals versus predicted values, and Akaike's Information Criterion (Burnham and Anderson 2002). The goodness-of-fit of the best-fitted model to the data set was evaluated using standard deviation of the residual obtained from the fitted random effects model (SD*rrem*) and the P values of the parameters of the model tested by a *t*-test.

Results

Daily, hourly and cumulative numbers of ascospores in the air

In total, 71 ascospore trapping periods were detected over the 6 years. Mean daily numbers of *E. necator* ascospores varied in all years (Figs. 1 and 2). Numbers of trapped ascospores also varied during the assessment periods and considerable increase in the numbers of trapped ascospore was detected mainly in rainy days in both vineyards and in all years. The total numbers of ascospores trapped were 1736, 1499, 1470, 644, 476, and 388; and the maximum number of trapped ascospores was 125, 141, 121, 98, 118, and 56 per m^3 in a day in 2004, 2005, 2006, 2007, 2008, and 2009, respectively.

Total ascospores trapped during a 24-hour period and hourly proportions of ascospore caught did not reveal any distinct diurnal patterns of release during the 6-year trapping period (Fig. 3). Patterns of hourly proportion ranged between 1.1 and 8.1 % and were not significantly different among vineyards and years. In addition, time-series analysis (ARIMA) showed that autocorrelation of the hourly numbers of *E. necator* ascospores were variable over time lags and no significant partial autocorrelation was found for the years (*data not shown*). Furthermore, no significant ($\alpha<0.05$) autoregressive parameters were found for ARIMA models sufficient to describe the autocorrelation of ascospore numbers in the air for each year. As a consequence ARIMA also confirmed no diurnal patterns of the ascospores caught.

Cumulative numbers of trapped ascospores continuously increased form early April until sampling was concluded near the end June in all years and the percentage of the ascospores trapped (relative to the total number trapped in the trapping period) changed among years (Fig. 4). In separate years, 5.9, 16.4, 12.5, 2.1, 0.9, and 2.1 % of the ascospores were trapped between the initiation of sampling in April and bud beak, 53.9, 66.7, 67.6, 72.9, 42.8, and 69.3 % between bud break and bloom, and 40.2, 16.9, 19.9, 25.0, 56.3, and 28.6 % and the conclusion of sampling at the end of June in order from 2004 to 2009, respectively. Because the above ascospore percentages involve different numbers of days in each of the three phenological periods in each year, we also calculated the rate of trapped ascospores per day for comparison. The mean ascospore percent per day was 0.8, 1.1, 0.8, 0.1, 0.2, and 0.2 % between the initiation of sampling in April and bud break, 1.7, 1.3, 1.4, 1.9, 1.7, and 1.5 % between bud break and bloom, and 0.5, 0.2, 0.2, 0.4, 0.3, and 0.3 % and the conclusion of sampling at the end of June in the order from 2004 to 2009, respectively. Across all years, 6.6 % of the total ascospores (0.5 % mean ascospore percent per day) were caught between the initiation of sampling in April and bud break, 62.2 % (1.6 %) from bud break to bloom, and 31.2 % (0.3 %) between bloom and the conclusion of sampling at the end of June.

Relationship between ascospore density and environmental variables

Ascospores were trapped in 71 trapping periods, 58 of these (81.7 % of the periods) being when rainfall was >2.5 mm with the mean daily temperature ranged from 4 to 25 °C (Figs. 1 and 2). In the remaining 13 periods, ascospores were trapped when there was rainfall <2.5 mm or in wet periods of >3.5 h that were not initiated by rain; these periods occurred on 18, 20, 25 April 2004, 5 May 2005, 5 June 2005, 21 April 2006, and 1 May 2006 (Fig. 1); 6, 11, 14, 31 May 2008, 15 May 2009 and 2 June 2009 (Fig. 2).

Correlation between ascospore density and environmental variables was similar in both locations. Of the environmental variables measured, mean hourly rainfall correlated best with mean hourly ascospores catches in both vineyards in all years (Table 2). The best correlation for rainfall ($r=0.78$, $P<0.001$) were found at Strázsa-hegy site in 2004. Mean hourly relative humidity had lower correlation coefficient values (ranged from

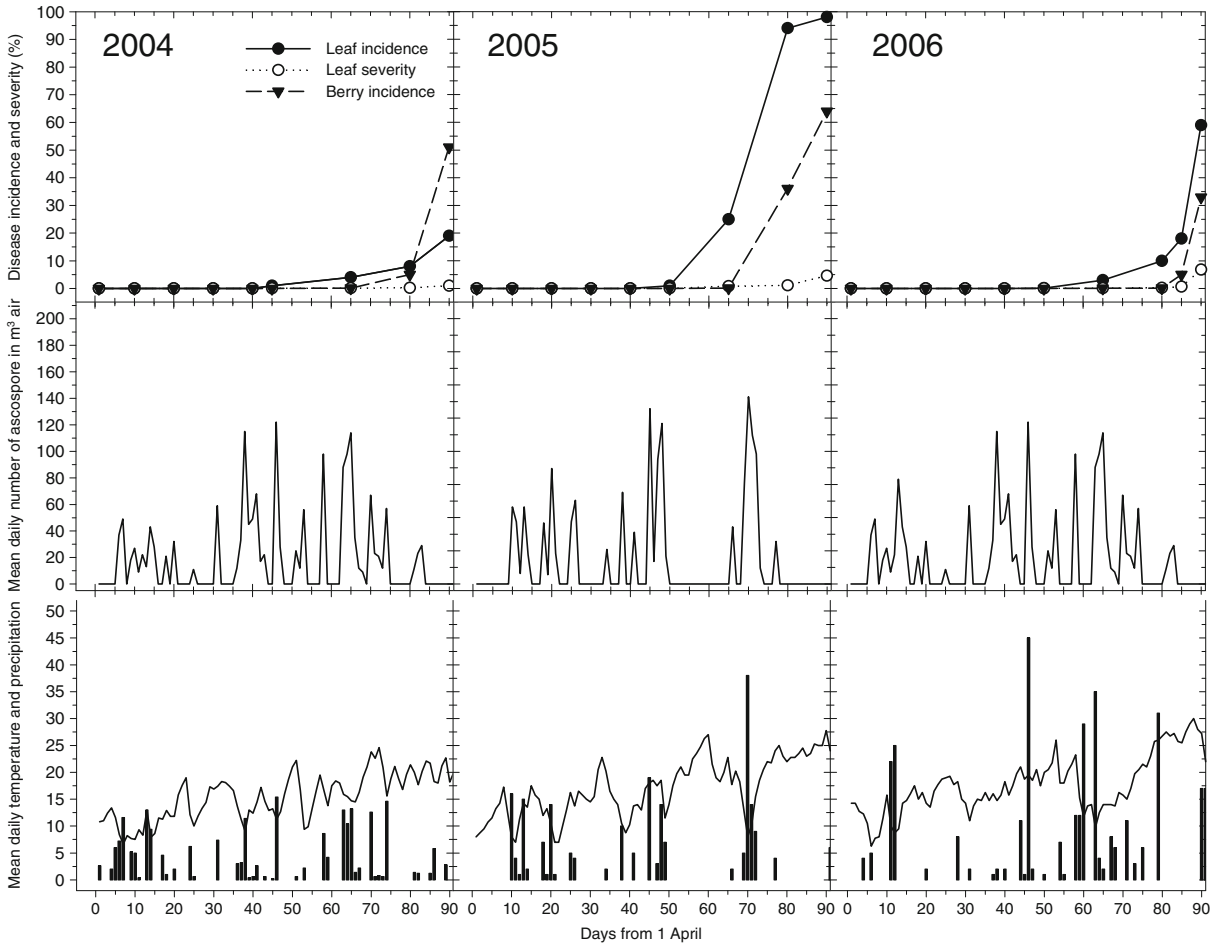

Fig. 1 Leaf and berry incidence and leaf severity of grape powdery mildew, mean daily numbers of *Erysiphe necator* ascospores in the air, and mean daily temperature (°C, *black line*) and precipitation (mm, *black column*) in a vineyard from 1 April until 30 June on cultivar Portugieser (2004–2006, Strázsa-hegy, Hungary)

0.30 to 0.44) in both vineyards over the 6-year period. Mean hourly temperature had negative and the lowest correlation coefficient values (ranged from −0.31 to 0.36) with the exception of Faluhely site in 2007.

Disease progress

First leaf and berry symptoms appeared between 7 and 24 May and between 25 May and 19 June, respectively, during the 6-year study period from 2004 to 2009 (Table 1). These days represent T_0 dates for starting of the temporal powdery mildew developments on leaf and berry. Disease started to progress slowly after the appearance of the first infected leaf followed by fast increase of the disease from early June in the years of 2004, 2005, 2006 and 2008. Final leaf and berry disease incidences were 19.3 and 51.1 % in 2004, 98.2 and 62.1 % in 2005, 59.3 and 35.2 % in 2006, and 64.5 and 90.3 % in 2008 which were generally 2–15 times higher than in 2007 and 2009 when final leaf and berry disease incidences were 8.4 and 11.7 % in 2007, and 4.1 and 2.1 % in 2009, respectively (Figs. 1 and 2). However, leaf severity values were generally low in all years (ranged from 0.9 to 6.8 %).

Relationship between aerial spore density and disease incidence

Leaf incidence data corresponded well with the cumulative numbers of trapped ascospore in 2004, 2005, 2006, 2007 and 2008 (Fig. 5). The three-parameter Gompertz function was deemed most appropriate for describing all year data set with the exception for 2009 when low incidence data were not suitable for curve

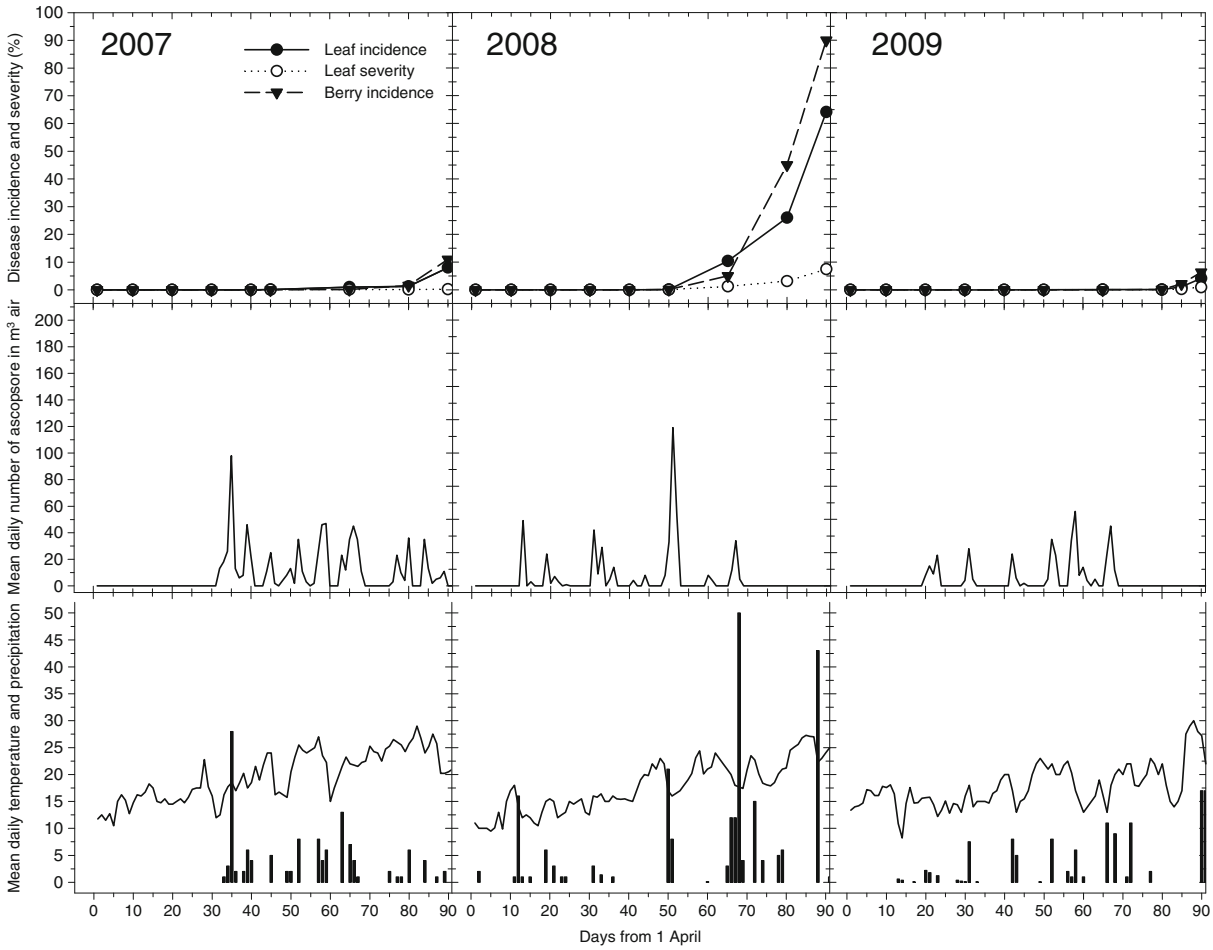

Fig. 2 Leaf and berry incidence and leaf severity of grape powdery mildew, mean daily numbers of *Erysiphe necator* ascospores in the air, and mean daily temperature (°C, *black line*) and precipitation (mm, *black column*) in a vineyard from 1 April until 30 June on cultivar Limberger (2007–2009, Faluhely, Hungary)

fitting; therefore, only the observed data were presented (Fig. 5). The function is given by $y=y_f+A \bullet \exp(-\exp(-\beta(x-M)))$, where y is the disease incidence (%) at corresponding cumulative number of trapped ascospore x; y_f the estimated final disease incidence or upper asymptote; A is a constant with a value< 0; β the estimated relative rate of progress curves (ascospore^{-1}); and M the inflection point, i.e., the cumulative percentage of trapped ascospore when the absolute rate dy/dx is at a maximum. Because the best fits were obtained using this Gompertz function, results of the other functions are not shown in this work. Parameter estimates and corresponding standard errors of the obtained three-parameter Gompertz functions were y_f= 126.4±2.341, 96.1±2.081, 134.8±3.012, 30.83± 1.038, and 491.2±23.12; A=−125.9±2.579, −96.0± 2.574, −138.8±3.626, − 30.46±1.283, and −491.2± 24.37; β = −0.01142±0.00228, −0.012±0.00434, −0.01215±0.00473, −0.0322±0.00578, and −0.02949 ±0.00422; and M=1634±9.376, 1151±7.592, 1833± 9.671, 682.6±5.279, and 555.4±5.541 with the SD_{rrem} value of 0.1287, 0.1365, 0.1422, 0.1301, and 0.1295 (P=0.0218, 0.0308, 0.0317, 0.0202, and 0.0220) in 2004, 2005, 2006, 2007, and 2008, respectively. Therefore, the goodness-of-fit of functions may be considered high.

Discussion

Surprisingly, very little is known about ascosporic infections in powdery mildew fungi in general, and in

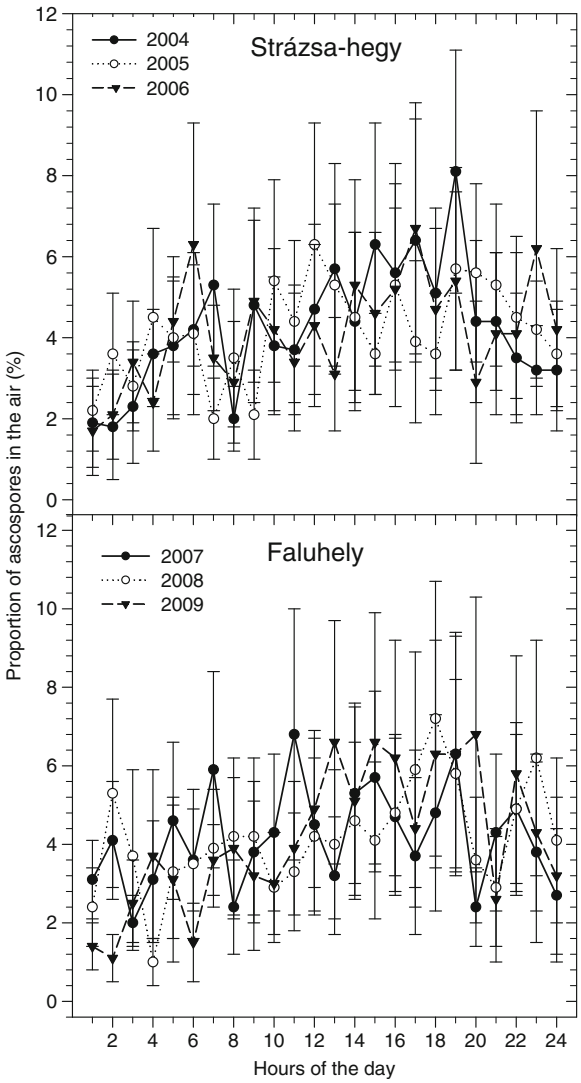

Fig. 3 Hourly proportion of *Erysiphe necator* ascospores in the air in vineyards at Strázsa-hegy and Faluhely from 2004 to 2009. Data are mean hourly proportion of ascospores caught each day with a volumetric spore trap from 1 April until 30 June. *Error bars* represent SD values

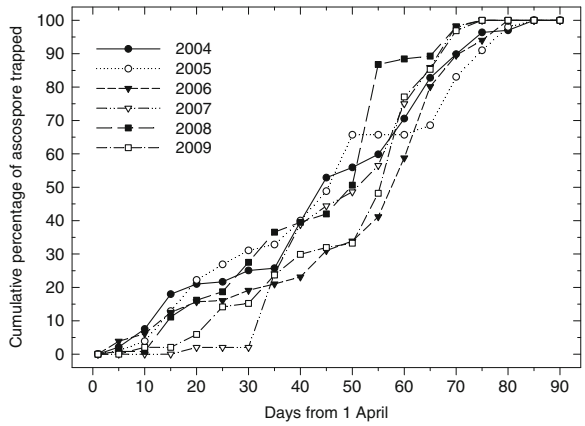

Fig. 4 Cumulative percentage of trapped ascospore of *Erysiphe necator* from 1 April until 30 June in two vineyards (Strázsa-hegy and Faluhely) over a 6-year period from 2004 to 2009

economically important species in particular (Jankovics et al. 2015). *Erysiphe necator* is probably the best studied powdery mildew species in this respect though until the 1980s it was thought that the pathogen ascospores were non-functional due to the fact that attempts to germinate ascospores were unsuccessful. The release of *E. necator* airborne ascospores in spring has been studied in the eastern and western United States (Pearson and Gadoury 1987; Gadoury and Pearson 1990; Grove 2004; Moyer et al. 2014), France (Jailloux et al. 1998, 1999), southern Australia (Gee et al. 2000) and Italy (Rossi et al. 2010). In this study the main ascospore releasing period in spring (accounting for an average of 62.2 %) was between bud break and bloom in agreement with previous studies of Pearson and Gadoury (1987), Gadoury and Pearson (1990) and Rossi et al. (2010). Proportion of ascospore release was relatively high (average of 31.2 %) between bloom and early summer which was comparable with results from warmer regions (e.g., Rossi et al. 2010) but opposite with results from cold-climate regions (e.g., Pearson and Gadoury 1987; Gadoury and Pearson 1990; Moyer et al. 2014). However, number of trapped ascospore before bud break was relatively low (average

Table 2 Correlation coefficients (*r*) between mean hourly concentration of *Erysiphe necator* ascospores and temperature, relative humidity, and rainfall analysed on a mean hourly basis in two vineyards from 1 April until 30 June (Strázsa-hegy, Hungary, 2004–2006; Faluhely, Hungary, 2007–2009)

Site and year	Temperature	Relative humidity	Rainfall
Strázsa-hegy			
2004	0.13 [a]	0.42** [b]	0.78***
2005	−0.24*	0.31*	0.67***
2006	0.32*	0.42**	0.64***
Faluhely			
2007	0.36**	0.30*	0.61***
2008	−0.31*	0.44**	0.43**
2009	0.21	0.34**	0.47**

[a] correlation coefficient

[b] *, **, and *** are significantly different at 0.05, 0.01, and 0.001, respectively

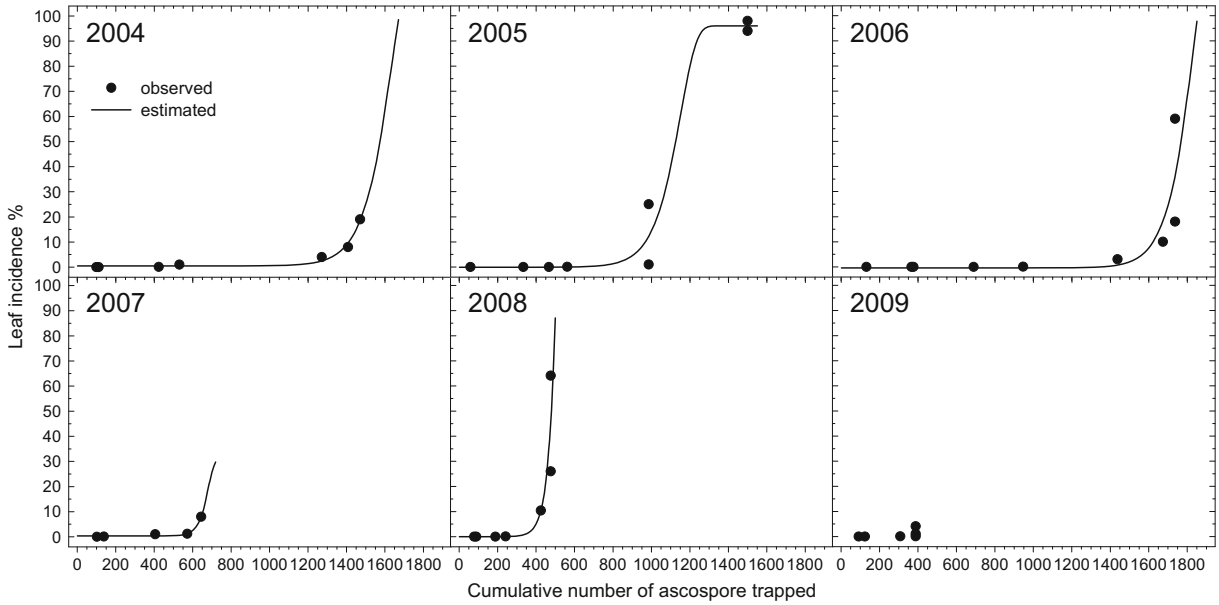

Fig. 5 Mean leaf incidence of grape powdery mildew in relation to corresponding cumulative numbers of trapped ascospores in two vineyards (Strázsa-hegy and Faluhely) over a 6-year period from 2004 to 2009. Each year observed data were fitted the best to the three-parameter Gompertz function prepared by a nonlinear mixed-effect modelling approach using R version 2.0.0 with the 'nlme' statistical package. Parameter estimates and corresponding standard errors of the obtained three-parameter Gompertz functions were y_f=126.4±2.341, 96.1±2.081, 134.8±3.012, 30.83±1.038, and 491.2±23.12; A=−125.9±2.579, −96.0±2.574, −138.8±3.626, − 30.46±1.283, and −491.2±24.37; β=−0.01142±0.00228, −0.012±0.00434, −0.01215±0.00473, −0.0322±0.00578, and −0.02949±0.00422; and M=1634±9.376, 1151±7.592, 1833±9.671, 682.6±5.279, and 555.4±5.541 with the SD_{rrem} value of 0.1287, 0.1365, 0.1422, 0.1301, and 0.1295 (P=0.0218, 0.0308, 0.0317, 0.0202, and 0.0220) in 2004, 2005, 2006, 2007, and 2008, respectively. Low incidence data for 2009 were not suitable for curve fitting; therefore, only the observed data were presented

of 6.6 %) and no midwinter release occurred (Füzi and Holb 2007) in contrast with results of Moyer et al. (2014), Rossi et al. (2010) and Gee et al. (2000). It needs to note that investigations on ascospore release before leaf fall in the previous season have not been included in this study but partial ascospore release is likely to occur in previous autumn due to the ripening of chasmothecia at late summer in the Szekszárd region (Füzi and Holb 2007). In most cases, lower mean temperatures occur while grapevines are dormant, and inoculum availability is irrelevant for the development of the disease. However, chasmothecia can release ascospores before bud break continuously if favourable wet *vs* temperature periods occur early in the growing season and ascospore release can nearly complete before bud break of grapevines (Moyer et al. 2008, 2010, 2014). Even if ascospore releases occur after bud break, they can cause very few or no symptoms in the early season, as for instance in this study, disease incidence was ≤5 % when cumulative trapped ascospores were ≤1400 in 2004 and 2006 (Fig. 5). As a consequence, from a disease management standpoint, estimations of remaining primary inoculum at bud break can be useful to predict severity of early powdery mildew epidemics between bud break and fruit set when grape leaves and berries are susceptible to infection.

Total numbers of trapped ascospores ranged from 388 to 1736 ascospores per m^3 air (Figs. 1 and 2). Relatively low total ascospore release can partially explained by that fact that spore trapping areas used in this study were partially sprayed which reduced the inoculum sources (such as production of chasmothecia) in previous year resulting in a lower ascospore release in the following spring. Low ascospore number may also associate with the lower trapping efficacy of Burkard volumetric spore trap compared to catches on silicon coated slides. In early studies of Pearson and Gadoury (1987) and Gadoury and Pearson (1990) noted that airborne ascospores of *E. necator* was relatively low in the field, near the threshold detection of the spore sampler. In order to avoid efficacy discrepancies of spore trapping methods with previous studies, spore trap inlet

orifice was placed less than 5 cm to the source of overwintered inoculum in this study.

Neither specific hourly temporal pattern nor diurnal periodicity of *E. necator* ascospore was found in this study (Fig. 3). Previously, diurnal periodicity has been shown for asexual spores (conidia) of powdery mildew fungus in many taxa including *E. necator* (e.g., Paddy 1972; Xu et al. 1995; Willocquet and Clerjeau 1998) but this phenomenon has not been shown for sexual spores (ascospore) of *E. necator*. Diurnal periodicity is commonly occurs with a peak spore release during daylight (Hirst 1953). The possible reason of the lack of diurnal periodicity for airborne ascospores of *E. necator* is that occurrence of other environmental factors such as definitive rainy events (Pearson and Gadoury 1987; Gadoury and Pearson 1990; Jailloux et al. 1999; Rossi et al. 2010) are more essential for successful ascospore release than a 12-h alternation of light and darkness during a day.

In earlier studies, the release of ascospores has been related to rainy periods with cumulative rainfall >2 mm (Jailloux et al. 1999) or >2.5 mm (Pearson and Gadoury 1987; Gadoury and Pearson 1990) within the temperature range from 4 to 25 °C (Gadoury and Pearson 1990). In this study, most ascospores (≈80 %) were trapped when rainfall was >2.5 mm and temperature was >4 °C (Figs. 1 and 2) which agrees with the study of Rossi et al. (2010). In addition, strong positive correlations were found between rainfall and the hourly concentration of airborne ascospores over the 6-year period of this study (Table 2) which also supported the essential feature of free water for ascospore release (Pearson and Gadoury 1987; Gadoury and Pearson 1990; Jailloux et al. 1999). However, similarly to previous studies of Jailloux et al. (1999) and Rossi et al. (2010), ascospores were also trapped in≈20 % of the release periods when there was <2.5 mm of rainfall. In a few cases of this work, ascospore release was also detected in wet periods of >3.5 h that were not initiated by rain. Our environmental data supported that these periods occurred during night and the conditions including low enough temperature and high enough RH were definitely suitable for formation of dew. Since the availability of free water is necessary for chasmothecia to dehisce (Gadoury and Pearson 1988), overnight dew exposure may explain the detection of ascospores on days with no precipitation. However, water is not only critical for ascospore release but also for ascospore viability, germination and infection, as does a saturated atmosphere (Gadoury and Pearson 1990; Jailloux et al. 1998; this study). For instance, *E. necator* ascospores were shown to be more sensitive to dry conditions than conidia because ascospore germination and appressorium formation were significantly reduced when air humidity decreased from 100 to 54 % at 25 °C while conidia were relatively insensitive to air humidity reduction (Gadoury and Pearson 1990). In this work, the role of air humidity for airborne ascospores was further supported by demonstrating positive significant correlations between relative humidity and aerial content of trapped ascospores (Table 2). Overall, results suggested that wet circumstances enable ascospore dispersal of *E. necator*, as well as germination and infection (Gadoury and Pearson 1990; Jailloux et al. 1998); thus, if relatively dry conditions occur in the field, only short survival periods are available for ascospores.

In this study, three parameter Gomperz functions described the best relationships between cumulative numbers of trapped ascospores and leaf incidences of grape powdery mildew (Fig. 5). The fitted curves showed that relationships between disease incidence and cumulative trapped ascospores were highly dependent on year. Of the 6 years studied, this relationship was strongest in 2004–2006 and 2008 when larger ascospore numbers corresponded to larger disease incidences. In the previous study by Gadoury et al. (1997), authors showed that periods of ascospore release between bud break and blossom to be almost twofold in years of high disease severity. However, Jailloux et al. (1999) were not able to show a relationship between the number of released periods and disease severity on grapes. In spite of contradicting results, authors agrees that reducing the risk of ascosporic infections could not only delay the onset of powdery mildew but it could also slow the disease progress rate later in the season and reduce the disease severity in fruit clusters (e.g., Gadoury and Pearson 1990; Gee et al. 2000; Rossi et al. 2010). In fact, lime sulfur applications to dormant grapevines were proven to kill the ascocarps of *E. necator* and delayed the development of powdery mildew epidemics (Gadoury et al. 1994). As a consequence, forecasting options for ascosporic (primary) infections have been proposed as tools for early-season strategy that reduces establishment of the disease and, thereby, reduces the probability of powdery mildew epidemics later in the summer (e.g., Caffi et al. 2011, 2012, 2013; Legler et al. 2012; Moyer et al. 2010, 2014). However, such forecasting models for

E. necator may require validation and subsequent adjustment of parameters to the local environmental scenario in order to keep the disease pressure below an economic threshold.

In summary, this in-depth study demonstrated the temporal pattern of aerial density of *E. necator* ascospores under field conditions and disease incidence and severity of grape powdery mildew until early summer. In addition, relationships were determined between aerial ascospore density and three weather parameters, and between cumulative ascospore numbers and disease incidences of grape powdery mildew in a long-term multiyear study under Central European continental climate conditions.

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