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FULL RESEARCH PAPER

Monitoring conidial density of *Monilinia fructigena* in the air in relation to brown rot development in integrated and organic apple orchards

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Abstract In a three-year Hungarian study, conidial density of *Monilinia fructigena* in the air determined from mid-May until harvest was related to brown rot disease progress in integrated and organic apple orchards. Conidia of *M. fructigena* were first trapped in late May in both orchards in all years. Number of conidial density greatly increased after the appearance of first infected fruit, from early July in the organic and from early August in the integrated orchard. Conidial number continuously increased until harvest in both orchards. Final brown rot incidence reached 4.3–6.6% and 19.8–24.5% in the integrated and organic orchards, respectively. Disease incidence showed a significant relationship with corresponding cumulative numbers of trapped conidia both in integrated and organic orchards, and was described by separate three-parameter Gompertz functions for the two orchards. Time series analyses, using auto-regressive integrated moving average (ARIMA) models, revealed that the temporal patterns of the number of airborne conidia was similar in all years in both integrated and organic orchards. Conidia caught over a 24-h period showed distinct diurnal periodicity, with peak spore density occurring in the afternoon between 13.00 and 18.00. Percent viability of *M. fructigena* conidia ranged from 48.8 to 70.1% with lower viability in dry compared to wet days in both orchards and all years. Temperature and relative humidity correlated best with mean hourly conidial catches in both integrated and organic apple orchards in each year. Correlations between aerial spore density and wind speed were significant only in the organic orchard over the 3-year period. Mean hourly rainfall was negatively but poorly correlated with mean hourly conidial catches. Results were compared and discussed with previous observations.

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Introduction

Monilinia fructigena is the causative agent of fruit rot in pome fruit crops in the temperate regions of the world (Byrde and Willetts 1977). Dispersal of *M. fructigena* conidia can occur by wind, water, insects, birds and man (Byrde and Willetts 1977). Water has been shown to be an important factor for spreading conidia within the tree; therefore, short-range transport of conidia was considered to be by splash dispersal (Byrde and Willetts 1977). Long-range dispersal probably relies on vector-borne or airborne mechanisms (Byrde and Willetts 1977). Insects were shown to be able to transport conidia from one fruit to another (Croxall et al. 1951; Lack 1989) and they were the major wounding agents in pome fruit orchards acting as prerequisite factors for infection (Xu et al. 2001b). Only a few studies have monitored aerial spore density of *M. fructigena* conidia. Airborne conidia have been trapped on exposed dishes and vaseline slides (Horne 1933; Bucksteeg 1939) but the authors did not quantify the aerial spore density in *M. fructigena*-infected orchards. Recently two studies reported a low aerial density of *M. fructigena* conidia in integrated apple orchards (Van Leeuwen 2000; Xu et al. 2001b). Xu et al. (2001b) in the UK recorded the presence but not the number of *M. fructigena* conidia. Van Leeuwen (2000) in The Netherlands did quantify aerial spore density of *M. fructigena*, but it was assessed only in a low-inoculum apple orchard and only from mid-July (appearance of first infected fruit) until mid-September (harvest date). The first infected fruit can appear before mid-July in organic apple orchards with high disease pressure and/or with early ripening cultivars (Holb 2003) which might be associated with the amount of conidia dispersed in the orchard air. In addition, season-long aerial density of *M. fructigena* conidia has not been monitored in orchards with either low or high inoculum sources of *M. fructigena*.

Previous studies demonstrated that density of *M. fructigena* conidia in apple orchards markedly increased from the fruit ripening stage (Van Leeuwen 2000; Van Leeuwen et al. 2000). The authors concluded that the amount of aerial inoculum and brown rot incidence may be correlated; however, quantification of this relationship was limited due to low inoculum density and low brown rot incidence of *M. fructigena* in these studies. Similarly to several airborne plant pathogens, a diurnal pattern in aerial spore density of *M. fructigena* was revealed (Van Leeuwen 2000), although this was again investigated in low-inoculum orchards restricted to late summer periods of the year. In the same study, a 60% viability of trapped conidia was noted based on a moderately dry 10-day period of one-year assessment (Van Leeuwen 2000). Viability of *M. fructigena* conidia may vary in dry and rainy periods, as the viability of aerially-dispersed spores can differ greatly depending on relative humidity (RH) and U.V. radiation (Rotem et al. 1985). However, changes in the viability of trapped *M. fructigena* conidia have not been investigated during dry or rainy periods in multi-year assessments.

Only the study of Van Leeuwen (2000) analysed the relationship between weather parameters and aerial spore density of *M. fructigena* focusing on a model-building aspect and showed peak spore density during the dry period of the season when air humidity was relatively low and temperature was highest. The study concluded that RH, mean temperature, wind speed and wind direction, at varying time lags, are important in explaining the variation in hourly spore counts of *M. fructigena* conidia (Van Leeuwen 2000). However, importance of these weather factors in multivariate models was determined only for one summer spore catch and only in a well-managed integrated apple orchard where spore dispersal was limited.

The goals of this three-year study were to (1) monitor aerial spore density of *M. fructigena* conidia from mid-May until harvest; (2) determine the relationship between spore data and disease development; (3) quantify the temporal pattern and viability of trapped spores in dry and wet periods of the seasons; and (4) correlate spore density to four weather variables (RH, temperature, wind speed and rainfall). The study was performed in a highly brown rot-infested (organic) and well-managed (integrated) apple orchard.

Materials and methods

Orchard sites

A three-year study (2004–2006) was carried out in an integrated and organic commercial apple orchard in eastern Hungary. The integrated orchard, located in Sárospatak (48°19′10″N, 21°34′00″E), was 10 ha and consisted of a mixed stand of apple cultivars planted in alternating two-row strips. Between-row and within-row distances were 4 and 1.5 m, respectively, and trees were ca. 2.7 m tall. Orchard soil type was meadow soil. The disease and pest management programme followed the Hungarian integrated fruit production (IFP) guidelines derived from the European IFP guidelines (Cross and Dickler 1994) since the planting of the orchard in 1996. Fungicides and insecticides used in the integrated orchard are listed in Table 1; application schedules were similar to those reported in a previous paper (Holb et al. 2005). The orchard relied on annual applications of synthetic fertilizers for nutrient supply.

Table 1 Fungicide, insecticide, and herbicide active ingredients used in integrated and organic apple orchards in Sárospatak and Eperjeske, Hungary, from 2004 to 2006

Integrated	Organic
Fungicides	
captan, 50%	calcium polysulphides, 29%
copper hydroxide, 77%	copper hydroxide, 77%
difenoconazole, 250 g l^{-1}	copper sulphate, 350 g l^{-1}
dithianon, 70%	elemental sulphur, 80%
dodine, 500 g l^{-1}	elemental sulphur, 900 g l^{-1}
kresoxim-methyl, 50%	
pyrimethanil, 300 g l^{-1}	
trifloxistrobin, 50%	
Insecticides	
acetampirid, 20%	*Bacillus thuringiensis*, 3.2%
fenoxicarb, 25%	mineral oil, 90%
flufenzin, 200 g l^{-1}	plant oil extract, 50%
hexytiazox, 10%	
lufenuron, 50 g l^{-1}	
mineral oil, 90%	
tiacloprid, 480 g l^{-1}	
triflumuron, 25%	

Application schedules similar to those reported by Holb et al. (2005).

The organic orchard, located in Eperjeske (52 km northeast of the Sárospatak site) was 6.8 ha. It consisted of eight apple cultivars, in a random row arrangement, with a minimum of 1,500 trees i.e. at least four rows of each cultivar. This orchard consisted of 48 rows, with a distance between rows of 5 m and within row of 2 m, and trees were ca. 3.3 m tall. Orchard soil type was brown forest soil with alternating layers of clay. Hungarian organic production guidelines derived from the IFOAM standards (Anon. 1998) have been applied since the planting of the orchard in 1996. Fungicides and insecticides used in the organic orchard are listed in Table 1; application schedules were similar to those reported in a previous paper (Holb et al. 2005). Stable manure and compost were applied every other year for nutrient supply.

Trees in both orchards had been planted in 1996 on M26 rootstock and were pruned to spindle shape. A winter pruning before bud break and two summer prunings at the beginning of June and August were carried out each year. Bare soil, 0.5 m wide, was maintained in the rows, and grass was grown in the middle of the rows. Orchards were not irrigated. In both orchards, all sprays were applied with a Kertitox 2000 axial blower spray machine (Debreceni Gépgyár B.V., Debrecen, Hungary) with a ceramic hollow cone at 1.1–1.2 MPa with a volume of 1,000 l ha^{-1}.

Monitoring environmental variables

Temperature (°C), RH (%), rainfall (mm), and wind speed (m s^{-1}) were recorded at 12-min intervals using a Metos Compact agrometeorological station (Pessl Instrument GmbH, Weiz, Austria) at Eperjeske and a Lufft HP100 agrometeorological station (G. Lufft Mess- und Regeltechnik GmbH, Fellbach, Germany) at Sárospatak from 20 May until 10 October 2004, 2005 and 2006. The agrometeorological stations were located at a distance of 50 and 30 m from the spore trap in the orchards at Sáropatak and Eperjeske, respectively. Sensors were mounted 1 m above the ground in the centre of the canopy of a tree during each season at each site. Data of each environmental variable were summarized as hourly averages.

Quantifying airborne conidia

The presence of airborne conidia was monitored with a Burkard 7-day recording volumetric spore trap (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, UK) that was placed in the centre of an orchard plot which consisted of apple cv. Mutsu in both orchards and all years. The spore trap inlet orifices were about 1.5 m above ground level as was suggested by previous studies on *Monilinia* spp. (Kable 1965; Corbin et al. 1968; Van Leeuwen 2000). Spore traps were operated from 20 May until mid-October in each year. Spore traps were operated at a flow rate of 10 l min^{-1}, as was commonly used in a similar study (Holb et al. 2004). The flow rate of each trap was checked weekly by a calibrated Burkard flowmeter. The trap collected airborne particles on Melinex tape (Burkard Scientific Sales Ltd., Rickmansworth, Hertfordshire, UK) attached to a slowly rotating drum. The tapes of spore traps exposed for 7 days were cut into seven 48-mm long sections, representing 1-day exposure periods. Each piece of tape was mounted on a glass microscope slide in a mixture of Gelvatol (Monsanto Chemical Co., St. Louis, USA) and lactic acid (Anon. 1980), and examined at hourly intervals of deposition (2 mm) in traverses perpendicular to the direction of movement; conidia were counted using a Zeiss Jenamed microscope (Carl Zeiss Jena GmbH, Jena, Germany). *M. fructigena* conidia vary in size and shape, ranging from elongate-ellipsoid to ovoid and limoniform. Only limoniform-shaped conidia with a length >20 µm could be identified definitely as *M. fructigena* conidia and therefore only these were counted according to the description of Van Leeuwen et al. (2002a). Hourly spore counts were made during the whole period of spore sampling. The numbers per unit volume of air (m^3) caught per hour and per day were calculated according to the manufacturer's instructions (Anon.1980) and plotted against time, but in all analyses (described below) the original hourly count data were analysed.

Spore viability test

A Rotorod sampler (Perkins 1957) was also operated in each orchard for 30 days in order to determine the viability of trapped conidia. The spore trap was placed about 1.2 m above ground level in each year during the peak of the conidial trapping period from 11 September until 10 October. Strips of tape on the sampling face of rotorods were coated with a solution of vaseline (75 ml) and paraffin wax (9 g) dissolved in hexane (400 ml). After a one-day exposure, rotorods were placed in a sealed container until the two strips were submerged in a 1:10 solution of fluorescein diacetate (FDA; Sigma Chemical Co., St. Louis, USA), of which the stock solution contained 1 mg FDA ml^{-1} acetone. After incubation for 20 min at 25°C in darkness, the two strips were mounted side by side on a microscope slide (76 × 26 mm) in clear gelvatol. A coverslip (64 × 22 mm) was placed on the slide and the gelvatol was allowed to dry. The slides were examined for viable and dead *M. fructigena* conidia. Viable conidia stained bright yellow with FDA when viewed under a Zeiss Jenamed microscope equipped with an incident-light fluorescence illuminator, a 450–490 nm excitation filter, a 510-nm dichroic mirror, and a 520-nm barrier filter.

Disease assessment

The presence of brown rot fruit was assessed on the late season cv. Mutsu in both orchards and all years. Twenty trees were selected randomly for disease assessment every 7 to 9 days from 20 May until harvest (mid-October). Fifty fruit typical of the given phenological stage were observed on each of the selected 20 trees and assessed [diseased (+) or healthy (−)]. A fruit was considered to be diseased if at least one visible and sporulating brown rot lesion was present on the fruit. Brown rot incidence was calculated as the percentage of diseased fruit.

Data analyses

Seasonal patterns of conidial density were examined by plotting mean daily spore catch. Mean values of brown rot incidence were also plotted against time. In order to investigate the relationship between airborne conidia and the incidence of disease, mean disease incidence and corresponding cumulative numbers of trapped conidia were calculated and non-linear growth functions were fitted separately for each management system by a non-linear mixed-effect modelling approach using R version 2.0.0 with the 'nlme' statistical package (Pinheiro et al. 2004). Management system was treated as fix and year as random factor. 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. A three-parameter Gompertz function was deemed most appropriate for describing data. The function is given by $y = y_f + A \bullet \exp(-\exp(-\beta(x - M)))$, where y is the disease incidence (%) at corresponding cumulative numbers of trapped conidia 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 ($spore^{-1}$); and M the inflection point, i.e., the number of cumulative trapped spores when the absolute rate dy/dx is at a maximum. Then, t-tests were used to determine if y_f, β , and M values were significantly different between the integrated and organic production systems.

Temporal pattern of the hourly number of *M. fructigena* conidia caught was characterised by the time-series analysis method of autoregressive integrated moving-average (ARIMA) model using the procedure as described by Xu et al. (1995) and Guerin et al. (2001). ARIMA models were fitted to the hourly number of spore data set for each year (including 142 days, i.e. 142 periods of observations) and management system and then the classical parameter tests and Akaike's Information Criterion (AIC) were used to select the best (apppropriate) model. Diurnal periodicity was shown by plotting the mean proportion of spores caught each hour for all 24-h periods (24.00–24.00 h) during the trapping period in each year.

Sampled days for the conidium viability test were sorted into three classification categories: (1) dry, with precipitation less than 1 mm day^{-1}; (2) moderately wet, with precipitation of 1–5 mm day^{-1}; and (3) wet, with precipitation >5 mm day^{-1}. A minimum of a five-day sample was used for each category containing a minimum of 50 spores in a sample day. In each year and each orchard, percent viability was calculated as percentage of living spores for each day category. LSD tests (α=0.05) were applied to compare percent viability values estimated for each day category.

Relationships among hourly summaries of environmental variables and conidial numbers were analysed by calculating Pearson correlation coefficients and associated significance levels for each management system (orchard) in each year. Numbers of spores were transformed to natural logarithms for ARIMA and correlation analyses in which residual plots showed it necessary to stabilize variance. Genstat Release 9.1 (Lawes Agricultural Trust, IACR, Rothamsted, UK) was used for all analyses.

Results

Seasonal density of conidia and disease progress

In each year, both seasonal density of conidia and brown rot incidences were 2–10 times higher in the organic orchard than in the integrated one. Seasonal density of conidia followed temporal progress of brown rot development in both orchards and in all years. Considerable drop in the numbers of trapped conidia was detected in both orchards and all years, when weather was rainy for longer than four consecutive days (Figs. 1 and 2).

Although conidia of *M. fructigena* were first trapped in late May of all years in both orchards (Figs. 1 and 2), mean daily numbers of airborne conidia in the integrated orchard were very low in all years until the appearance of first infected fruit (early August) (Fig. 1). Then, number of airborne spores continuously increased until harvest. Maximum mean daily density of conidia recorded was 387, 304, and 395 conidia m^{-3} air in 2004, 2005, and 2006, respectively. First infected fruit was detected in early August in the integrated orchard in all years (Fig. 1). Disease started to progress slowly after the appearance of the first infected fruit followed by an exponential increase from early-September until harvest. Final disease incidence ranged from 4.5 to 6.6% over the 3-year period.

Conidia were trapped continuously in relatively low numbers between end-May and end-June in the organic orchard (Fig. 2). From end-June, numbers of trapped conidia continuously increased until harvest. Maximum mean daily density of conidia recorded was 2,556, 1,874, and 2,775 conidia m^{-3} air in 2004, 2005, and 2006, respectively. First infected fruit was detected in early July in the organic orchards, in all years (Fig. 2). Disease started to progress slowly and increased exponentially from mid-August until har-

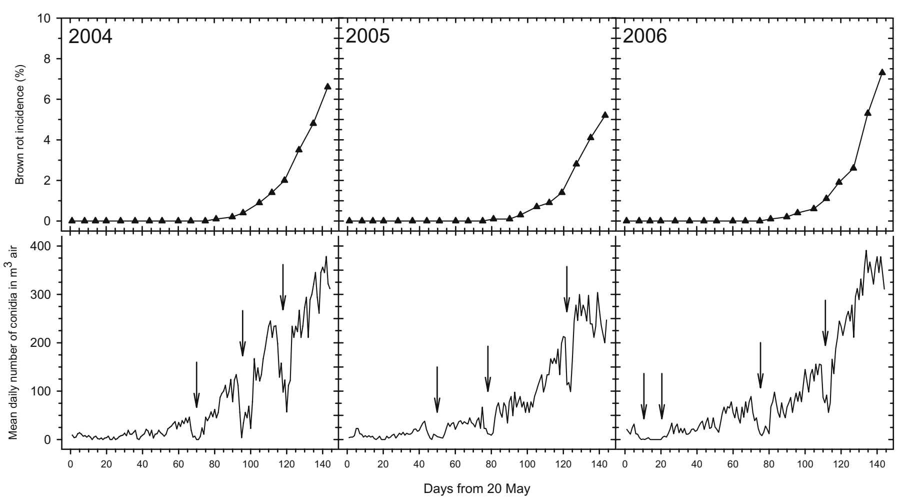

Fig. 1 Fruit brown rot incidence and mean daily density of *Monilinia fructigena* conidia in the air measured in an integrated apple orchard from 20 May until 10 October on cv. Mutsu (Sárospatak, Hungary, 2004–2006). *Arrows* represent rain periods longer than 4 days

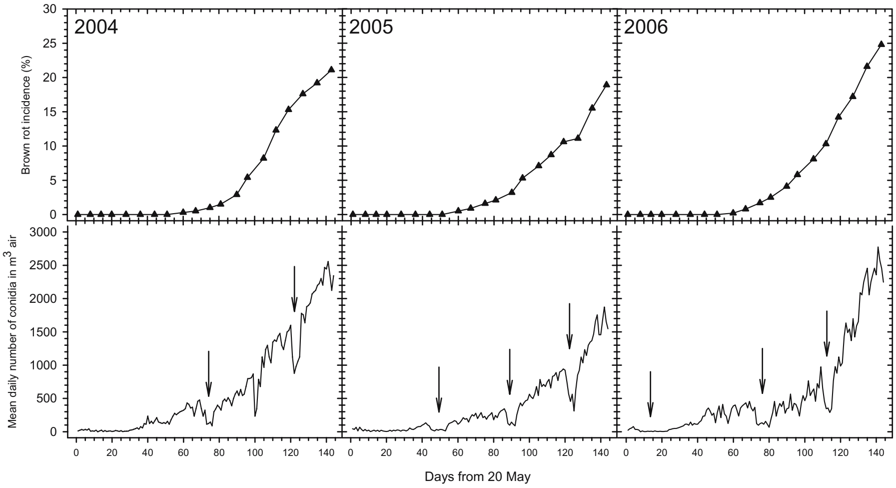

Fig. 2 Fruit brown rot incidence and mean daily density of *Monilinia fructigena* conidia in the air measured in an organic apple orchard from 20 May until 10 October on cv. Mutsu (Eperjeske, Hungary, 2004–2006). *Arrows* represent rain periods longer than 4 days

vest. Final disease incidence ranged from 19.5 to 24.5% in the organic orchard over the 3-year period.

Relationship between aerial spore density and disease incidence

Disease incidence correlated with the corresponding cumulative numbers of trapped conidia both in integrated and organic orchards (Fig. 3). Yearly data were combined in both orchards due to lack of significant differences among years. A three parameter Gompertz function gave the best fit to the data with SD_{rrem} values of 1.529 ($P<0.001$) and of 4.236 ($P=0.011$) for the integrated and organic plots, respectively. Values of y_f, β , and M were significantly different between the two management systems ($P\leq0.001$).

ARIMA models and diurnal periodicity of airborne conidia

Autocorrelation of the hourly numbers of *M. fructigena* conidia declined gradually over time lags and partial autocorrelation was significant at lag 6 for each year and management system (*data not shown*). AR (6) models without a constant parameter were sufficient to describe the autocorrelation of conidial numbers for each year and management system, i.e.:

$$y_t = \sum_{i=1}^{6} \Phi_i y_{t-1} + \alpha_t$$

where $y_t=x_t-x_{t-24}$ is the difference between the current number of conidia, x_t, and the number of the equivalent number of conidia the previous day, x_{t-24}, (this difference is used to remove diurnal periodicity at 24 h level as suggested in Xu et al. (1995); parameter Φ_i represents autoregressive parameters from 1 to 6;

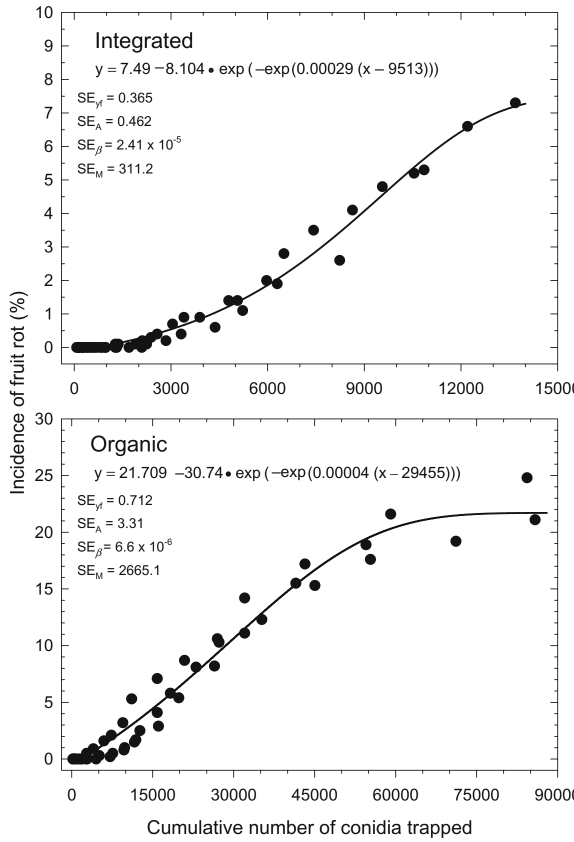

Fig. 3 Mean brown rot incidence in relation to corresponding cumulative numbers of trapped conidia in integrated and organic apple orchards over the three-year period from 2004 to 2006

Table 2 Parameter estimates in the ARIMA models for characterizing mean hourly density of *Monilinia fructigena* conidia in integrated and organic apple orchards (Sárospatak and Eperjeske, Hungary, 2004–2006)

Parameter[a]	Year		
	2004	2005	2006
Sárospatak (integrated)			
Φ_1	0.084±0.013***[b,c]	0.067±0.011***	0.062±0.011***
Φ_2	0.062±0.011***	0.131±0.022***	0.970±0.015***
Φ_3	0.045±0.009**	0.070±0.011***	0.030±0.003*
Φ_4	0.126±0.025***	−0.002±0.000*	0.136±0.024***
Φ_5	0.012±0.002 ns	0.157±0.030***	0.138±0.025***
Φ_6	0.098±0.018***	0.073±0.012***	0.047±0.009**
δ_a^2	3.276	2.834	2.913
Eperjeske (organic)			
Φ_1	0.215±0.034***	0.808±0.092***	0.189±0.027***
Φ_2	0.087±0.011***	0.267±0.032***	0.103±0.017***
Φ_3	0.134±0.026***	0.038±0.004***	0.128±0.022***
Φ_4	0.124±0.024***	−0.037±0.006**	0.066±0.009***
Φ_5	−0.027±0.004*	−0.477±0.051*	0.018±0.001 ns
Φ_6	0.029±0.004*	0.306±0.041***	0.075±0.010***
δ_a^2	12.228	3.504	11.085

[a] Φ, autoregressive parameter and δ_a^2, the variance of the error series.

[b] Mean values ± standard error.

[c] ns, *, **, and *** are non-significant, significantly different at 0.05, 0.01, and 0.001, respectively.

and a_t is a normally distributed random variable with a variance of δ_a^2. Estimates of the autoregressive parameters (Φ_1–Φ_6) are shown in Table 2. All parameter estimates were significant ($P\leq0.05$) for each year and management system, except for parameter estimates of Φ_5 for integrated and organic orchards in 2004 and 2006, respectively (Table 2). Possible further autoregressive parameters, i.e. Φ, where $i\geq7$, were found not to be significant, which coincides with the values of partial autocorrelations.

Hourly proportions of conidia caught over a 24-h period showed distinct diurnal periodicity in both integrated and organic orchards (Fig. 4). Patterns of diurnal periodicity were similar in both orchards over the 3-year period except for the integrated orchard in 2006 when diurnal periodicity was not as obvious as that for all other year and orchard combinations. Most spores were caught between 11.00 and 20.00 h in all years and both orchards, with peak aerial spore density in the afternoon. In the integrated orchard the maximum spore catch occurred at 15.00, 17.00, and 18.00 h, whereas in the organic orchard, it occurred at 13.00, 17.00, and 14.00 h in 2004, 2005, and 2006, respectively.

Viability of conidia

Percent viability of *M. fructigena* conidia ranged from 48.8 to 61.8 and from 54.3 to 70.1% in the integrated and organic apple orchard, respectively, from early September until harvest over the 3 years (Table 3). Spore viability was lowest in the dry and the highest in the wet days; however, such differences in spore viability were significant at $P<0.05$ only in 2006 in both orchards. Among years, largest differences of RH and temperature values were in 2006 between dry and wet days (*data not shown*).

Correlation between spore density and environmental variables

Correlation between spore density and environmental variables was consistently higher in the organic orchard than in the integrated one. Of the environmental variables measured, temperatures and RH correlated best with mean hourly conidial catches in both integrated and organic orchards in each year (Table 4). The best correlations for temperature (r=0.64; $P<0.001$) and for RH (r=−0.59; $P<0.001$)

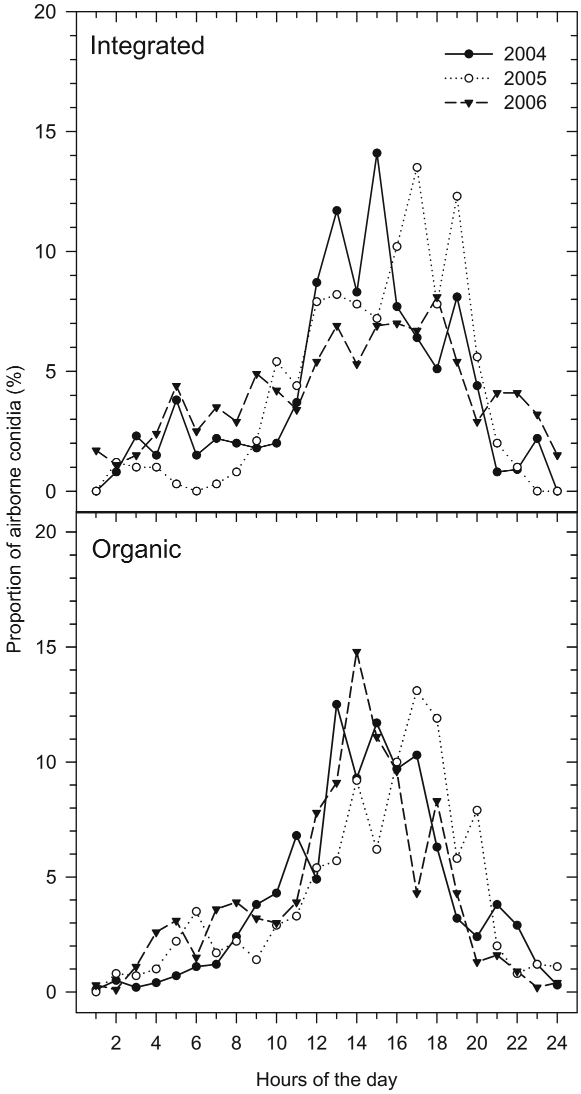

Fig. 4 Diurnal periodicity of conidia of *Monilinia fructigena* in integrated and organic apple orchards in Sárospatak and Eperjeske, respectively, from 2004 to 2006. Data are mean hourly proportion of spores caught each day with a volumetric spore trap from 20 May until 10 October

were found for the organic orchard in 2006. Mean hourly wind speed had lower correlation coefficient values (ranged from 0.11 to 0.44) in both orchards over the 3-year period. Correlation coefficients between spore density and wind speed were higher in the organic orchard than in the integrated one, and were significant only in the organic orchard over the 3-year period. Mean hourly rainfall was negatively but poorly correlated with mean hourly conidial catches and correlations were significant only in the organic orchard in 2004.

Discussion

In this study, in agreement with Van Leeuwen (2000), aerial density of *M. fructigena* conidia was low in integrated apple orchards. However, maximum spore densities were higher in this study (395 conidia m^{-3} day^{-1}) compared to that of Van Leeuwen (2000) (120 conidia m^{-3} day^{-1}). Higher final disease incidence (5.1–7.6%) on cv. Mutsu in this study compared with the 2.7–4.2% incidence of cv. James Grieve in the Dutch study (Van Leeuwen, 2000) may relate to the observed higher aerial density of *M. fructigena* conidia.

During the entire trapped periods, conidial numbers were 2–10 times higher in the organic orchard than in the integrated one, which might be associated with larger sporulating sources in the organic orchard. This can be explained with low control efficacy against insects and brown rot in organic orchards (Anon. 1998; Tamm et al. 2004). Due to less effective insecticides used in organic production, insects, mainly codling moth caterpillars, cause wounds on numerous fruit resulting in infection and subsequent production of airborne conidia by *M. fructigena* (Lack 1989; Van Leeuwen et al. 2000; Xu et al. 2001b). In addition, most insect-injured fruit drop on to the ground and soil provides moist conditions for severe infection and subsequent profuse sporulation on dropped fruit. Furthermore, neither fruit infection nor mass production of conidia can be suppressed effectively by fungicides used in organic orchards, such as copper- and sulphur-based compounds (Tamm et al. 2004; Holb and Schnabel 2005). This rarely occurs in integrated orchards due to availability of more effective insecticides and frequent use of systemic fungicides against apple scab, which also control brown rot of apple (Van Leeuwen et al. 2000).

In late spring and early summer, larger amounts of *M. fructigena* conidia with an earlier increase in air were detected in the organic orchard than in the integrated one (Figs. 1 and 2). Previous studies on *M. fructicola* demonstrated that late spring and early summer dispersal of conidia originated from mummified fruit, blighted blossoms and spurs (Corbin et al. 1968; Kable 1965). However, *M. fructigena* does not cause blossom and/or twig blight in apple in the Central-European region (Holb 2004) and most overwintered mummified fruit are destroyed or not able to sporulate any longer by the occurrence of first

Table 3 Percent viability of *Monilinia fructigena* conidia in dry, moderately wet and wet days sampled from mid-September until harvest in integrated and organic apple orchards (Sárospatak and Eperjeske, Hungary, 2004–2006)

Spore viability (%)

Site and year	Weather condition on sampling day			*F*-test[c]
	Dry	Moderately wet	Wet	
Sárospatak (integrated)				
2004	56.1[a]	61.0	61.2	ns
2005	57.3	56.4	58.1	ns
2006	48.8 a[b]	58.1 b	61.8b	*
Eperjeske (organic)				
2004	57.5 a	60.2 ab	64.6 bc	+
2005	54.8	55.6	58.1	ns
2006	54.3 a	60.7 b	70.1 c	*

[a] Values are mean data of each sampled day with a minimum of 5 days for each classification category of sampled days.
[b] Values within rows followed by different letters are significantly different. Fishers' protected LSD *t*-test was used for comparing sampled periods.
[c] ns, +, and *, are not significant, significantly different at 0.1, and 0.05, respectively.

infected fruit on the tree. Therefore, early summer releases of *M. fructigena* conidia can originate mainly from other inoculum sources (Xu et al. 2001b; Van Leeuwen et al. 2002b). Large numbers of fruit fell in early summer (June drop) and become infected on the ground of organic orchards (Holb 2003). These fruit sporulate earlier than fruit on the tree (Holb and Scherm 2007), which may result in early summer increases of trapped *Monilinia* spores in organic orchards. However, early summer increase of airborne conidia was only sporadically detected in the integrated orchard (Fig. 1), which is probably due to the low numbers of sporulating fruit on the ground and subsequent low spore density in the air. Therefore, any increase in aerial spore contents of this orchard was difficult to detect with a volumetric spore trap. Burkard spore trap is able to detect about 10 spores m^{-3} at low wind speed in order to detect one spore in the routine method of scanning at 1-h intervals (Hirst 1953).

Trapped conidia markedly increased by harvest in both integrated and organic orchards and were strongly correlated with brown rot incidence (Figs. 1, 2 and 3). Previous studies on *M. fructicola* (Kable 1965) reported a maximum of 5,000 conidia m^{-3} at 5% disease incidence of peach fruit and an exponential increase in the number of spores (138,000 conidia m^{-3}) above 20% disease incidence. Such conidial numbers

Table 4 Correlation coefficients (*r*) between mean hourly density of *Monilinia fructigena* conidia and temperature, relative humidity, wind speed, and rain analysed on a mean hourly basis in integrated and organic apple orchard from 20 May until mid-October (Sárospatak and Eperjeske, Hungary, 2004–2006)

Site and year	Temperature	Relative humidity	Wind speed	Rain
Sárospatak (integrated)				
2004	0.43**[a,b]	−0.39**	0.11	−0.09
2005	0.26*	−0.29*	0.15	−0.11
2006	0.38**	−0.40**	0.22	0.06
Eperjeske (organic)				
2004	0.53***	−0.46**	0.39**	−0.27*
2005	0.49**	−0.39**	0.37**	−0.21
2006	0.64***	−0.59***	0.44**	−0.19

[a] correlation coefficient.

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

were not obtained in this study even when disease incidence reached 25%. Lower densities of aerial conidia at harvest may be due to slower disease development and less profuse sporulation of *M. fructigena* in apple orchards compared to peach orchards. Lower temperature in autumn may delay sporulation of infected apple fruit more on late-season apple cultivars than on mature peach fruit in summer. In addition, uninjured fruit is hardly infected by *M. fructigena* in contrast with *M. fructicola* (Byrde and Willetts 1977; Xu and Robinson 2000), indicating a great injury-dependence of *M. fructigena* infection on pome fruits. This difference in the epidemiology of the two fungi may also influence the amount of aerially dispersed conidia of *M. fructigena* compared with *M. fructicola*.

Viability of *M. fructigena* conidia ranged between 48.8 and 70.1% depending on weather conditions of trapping periods (Table 3) while Van Leeuwen (2000) reported a 60% viability from an integrated orchard during a moderately dry period. Though only few significant differences were found among viability of conidia and different weather circumstances in the field, viability of spores trapped under wet conditions was higher compared with those trapped in dry periods. Previous studies in the laboratory (Xu et al. 2001a) also confirmed that higher temperatures reduced conidial viability, and conversely, higher RH reduced the rate of loss in viability.

Temporal patterns of the number of *M. fructigena* airborne conidia, characterised by ARIMA models, showed significant relationships among spore numbers within 6 h according to AR(6) models for hourly analysed data sets (Table 2). This corroborates with results of Xu et al. (1995) where a significant relationship was found between a 3-h and a previous 3-h data set of *Podosphaera leucotricha* conidial number in ARIMA(1) models, again covering a 6-h period. In addition, a diurnal fluctuation in temporal patterns of *M. fructigena* conidia was observed in all years and orchards (Fig. 4), which approached the typical pattern for the group of dry-air spore types (Hirst 1953). Previous studies on *Monilinia* spp. (Kable 1965; Jenkins 1965; Corbin et al. 1968; Sanderson and Jeffers 1992; Van Leeuwen 2000) also demonstrated significantly higher amounts of conidia in the afternoon hours though daily peak times varied considerably from 11.00 to 21.00 h. During Hungarian summer days, the highest temperature and the lowest RH mostly occur from 13.00 to 17.00 h. As most spores were also trapped during this period, certain associations may exist between conidial levels and the above environmental variables. This association was shown by significant positive correlations between hourly spore density and temperature, and by negative correlations between hourly spore density and RH (Table 4) in agreement with previous *Monilinia* studies (Corbin et al. 1968; Sanderson and Jeffers 1992; Van Leeuwen 2000). This study further demonstrated that correlations between aerial spore density and weather variables were stronger in the highly infected organic orchard than in the well-managed integrated one. This was clearly observed for wind speed as a significant correlation between aerial spore density and wind speed was detected only in the organic orchard. In summary, wind speed was found to be the most essential variable, followed by temperature and air humidity, in stone fruit orchards severely infected by *M. laxa* (Corbin et al. 1968) and by *M. fructicola* (Kable 1965; Jenkins 1965). These results suggest that the relationship between aerial spore density and wind speed might be strongly associated with different levels of inoculum sources in the orchards. Under low disease incidence values, the variability of conidial sources and airborne conidial density are high at different sites within the orchard. Thus, change in wind direction may result in no relationship between wind speed and trapped spores, as the air stream can be obtained from those sectors of the orchard where spore density may be below the detectable level. In highly infected orchards, inoculum sources are probably more dispersed and aerial spore density is more homogenous, which may explain significant correlations between aerial spore density and wind speed. Though negative correlations between spore catches and rainfall were significant only for one case, longer rainy periods appeared to disrupt aerial spore densities, which was probably due to the scrubbing of spores (Hirst 1953; Gregory 1973).

Results of correlations between aerial spore density and environmental variables suggest that relatively dry circumstances enable spore dispersal; however, conidia of *M. fructigena* can germinate and infect fruit at near saturation humidity (>97%) (Xu et al. 2001a). This contradiction suggests that viability of *M. fructigena* conidia may be long-lasting in the field and/or enough moisture exists at the infection site. Up to 20 days of viability of *M. fructigena* conidia (Xu et al. 2001a) and/or moisture on the wound surface of injured fruit (Xu and Robinson 2000) may be sufficient enough for conidial germination and infection in the field even if spore liberation occurs under relatively dry conditions.

In summary, this is the first study to demonstrate a relationship between the cumulative number of airborne conidia and brown rot development caused by *M. fructigena* in orchards with either low or high inoculum sources. This in-depth study clearly showed the temporal pattern of aerial spore density of *M. fructigena* as well as diurnal patterns and viability of trapped conidia in relation to four weather parameters in integrated and organic apple orchards.

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