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# Spore dispersal, diurnal pattern and viability of *Monilinia* spp. conidia and the relationship with weather components in an organic apple orchard

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**Summary:** In a two-year Hungarian study, spore dispersal diurnal periodicity and viability of *Monilinia* spp. and their relation to weather components were determined in an organic apple orchard. Conidia of *Monilinia* spp. were first trapped in late May in both years. Low number of conidia were trapped until end-June. Thereafter, number of conidia continuously increased until harvest. Conidia in a 24-h period showed diurnal periodicity pattern, with the highest concentration in the afternoon hours. Spore viability with FDA staining showed that viability of conidia ranged from 45 to 70% with showing lower viability in the dry than in the wet days in both years. Temperature and relative humidity correlated positively with mean hourly conidia numbers in both years. Mean hourly rainfall was negatively but poorly correlated with conidia catches in both years. Results were compared and discussed with previous observations.

**Keywords:** aerial dispersal, brown rot, epidemiology, viability, diurnal periodicity, organic, apple

## Introduction

*Monilinia* spp is the causative genus of fruit rot in fruit crops in the temperate regions of the world (Byrde & Willetts, 1977; Holb, 2006, 2008a). Dispersal of *Monilinia* spp. conidia can occur mainly by wind, water, insects, birds and man. Short range transport of conidia could be considered by splash dispersal (Byrde & Willetts, 1977). Few studies monitored dispersal of *M. fructigena* conidia. Airborne conidia have been trapped on exposed dishes and vaseline slides (Horne, 1933). Water was shown to be an important factor for spreading conidia within the tree (Pauvert *et al.*, 1969). Insect dispersal of conidia has also been an important factor from one fruit to another (Croxall *et al.*, 1951; Lack, 1989). Studies demonstrated a low aerial concentration of *M. fructigena* in integrated apple orchards (Van Leeuwen *et al.*, 2000; Xu *et al.*, 2001; Holb, 2008b) while in organic apple orchards high conidial concentration were recorded (Holb, 2008b; Holb *et al.*, 2011). Previous studies showed that viability of trapped conidia was 60 % (Holb, 2008b). Relative humidity, mean temperature, and wind were shown to be important in explaining the variation in hourly spore counts of *M. fructigena* conidia (Holb, 2008b; Bannon *et al.*, 2009).

The aims of this two-year study were to i) monitor conidial content of *Monilinia* spp. in the air from mid-May until harvest; ii) determine diurnal periodicity of trapped conidia iii) evaluate viability of trapped spores; and iv) correlate spore data with weather variables of relative humidity, temperature and rainfall in an organic apple orchard.

## Materials and methods

### Orchard sites

A two-year study (2013 and 2014) was done in an organic apple orchard in eastern Hungary. The orchard, located in Eperjeske consisted of eight apple cultivars, with a distance between rows of 5 m and within a row of 2 m. Orchard soil type was brown forest soil and Hungarian organic production guidelines have been applied since the planting of the orchard. Trees were planted in 1996 on M26 rootstock and were pruned to spindle shape. All sprays were applied with a Kertitox 2000 axial blower spray machine.

### Monitoring environmental variables

Temperature (°C), relative humidity (%), and rainfall (mm) were detected with a Metos Compact agrometeorological station from 20 May until 10 October in 2013 and 2014. Detecting sensors were mounted 1 m above the ground in the centre of the canopy of a tree during.

### Quantifying airborne conidia

Conidia was monitored with a Burkard 7-day recording volumetric spore trap which was placed in the centre of an orchard. The spore trap were placed about 1.5 m above ground level (Kable, 1965; Corbin *et al.*, 1968; Leeuwen *et al.*, 2000; Holb, 2008). Spore traps were operated from 20 May until mid-October in both years. Spore trap was operated at a flow

rate of 10 L min<sup>-1</sup>. (Wilocquet & Clerjeau, 1998; Holb *et al.*, 2004). The tapes of spore traps were cut into seven 48-mm long sections. Each piece of tape was examined and conidia were counted with a microscope. Only limoniform-shaped conidia were identified as *Monilinia* spp. van Leeuwen *et al.* (2002). Hourly spore counts were also made.

### Spore viability test

30 days spore trap data were used for determining the viability of trapped conidia. Strips were submerged in a 1:10 solution of fluorescein diacetate. After incubation for 20 min strips were mounted in gelvatol. The strips were examined for living and dead *M. fructigena* conidia. Viable conidia stained bright yellow with FDA under a Zeiss Jenamed fluorescence microscope.

### Data analyses

Conidial dispersal was examined by plotting daily totals of spore caught. Diurnal periodicity was evaluated by plotting the mean proportion of spores caught each hour (24.00 to 24.00 hour) during the 30-day period. Days for viability test were sorted into two classification categories: i) dry, with less precipitation than 1 mm day<sup>-1</sup> and iii) wet, with larger precipitation than 5 mm day<sup>-1</sup>. LSD tests ( $P = 0.05$ ) were applied to compare percent viability values estimated for the two categories. Relationship among environmental variables and conidia numbers were analysed by calculating Pearson correlation coefficients and associated significance levels.

## Results

### Seasonal conidial concentration

Seasonal concentrations of conidia increased with brown rot incidence. Decrease in the numbers of conidia was detected when weather was rainy longer than four subsequent days. Conidia of *Monilinia* spp. were first trapped in late May. Conidia were trapped with a relatively low numbers but continuously between end-May and end-June. From end-June numbers of conidia continuously increased until harvest.

### Diurnal periodicity of airborne conidia

The pattern of mean hourly proportions of conidia caught over a 24-h period showed diurnal periodicity (Fig. 1). Most spores were caught between 10.00 and 19.00 hours. Peak aerial conidia concentration occurred in the afternoon. Maximum spore catch occurred at 16.00 and 17.00 hour.

### Viability of conidia

Viability of *Monilinia* spp. conidia ranged from 45.2 to 70.1 % from early September until harvest over the two years (Table 1). Spore viability was lowest in the dry and the highest in the wet days and spore viability was significant at  $P < 0.05$  between dry and wet days for both years.

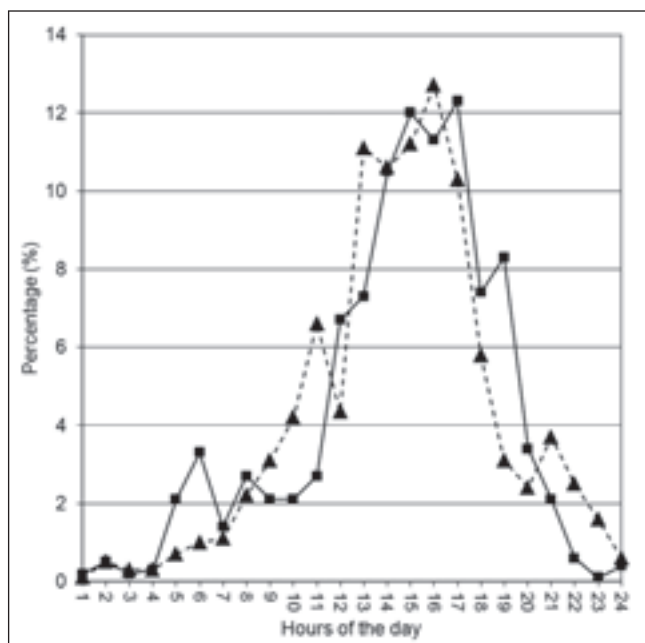


Figure 1. Patterns of diurnal periodicity of *Monilinia* spp. (Eperjeske, Hungary, 2013 and 2014)

Table 1. Percent viability of *Monilinia* spp. conidia in dry and wet days sampled from mid-September until harvest an organic apple orchard (Eperjeske, Hungary, 2013–2014)

Year	Dry	Wet	F-test <sup>a</sup>
2013	45.2 a	61.8 b	*
2014	53.8 a	70.1 b	*

<sup>a</sup> Values within rows followed by different letters are significantly different. LSD t-test was used for comparing sampled periods; \* is significantly different at 0.05, respectively.

Correlation between conidial content and environmental variables

Temperature and relative humidity correlated positively with mean hourly conidia numbers in both years (Table 2). Mean hourly rainfall was negatively but poorly correlated with conidia catches in both years.

Table 2. Correlation coefficients ( $r$ ) between mean hourly concentration of *Monilinia* spp. conidia and temperature, relative humidity and rain in an organic apple orchard from 20 May until mid-October (Eperjeske, Hungary, 2013–2014)

Site and year	Temperature	Relative humidity	Rain
2013	0.31 *	0.25 *	-0.13 ns
2014	0.36 **	0.36 **	-0.08 ns

<sup>a</sup> correlation coefficient.

<sup>b</sup> ns, \* and \*\* are significantly different at nonsignificant, 0.05 and 0.01, respectively.

## Discussion

Aerial spore contents of *Monilinia* spp. were demonstrated in an organic apple orchards. Diurnal pattern and high

viability percentage of trapped conidia were shown. Strong relationship was found among hourly spore counts and low relative humidity and high temperature.

Mean daily conidia started to continuously increase only when first infected and sporulating fruits in this study. Similarly to results of this study a previous Dutch and Hungarian studies (Van Leeuwen *et al.*, 2000; Holb, 2008b) demonstrated that dispersal of *M. fructigena* conidia increased after the appearance of first infected fruits. During the whole trapped periods, conidia were significantly in this study (organic orchard) compared with other studies in integrated orchard (van Leeuwen *et al.*, 2000; Xu *et al.*, 2001; Holb, 2008b). A previous study from the on *M. fructicola* (Corbin *et al.*, 1968) demonstrated that conidia dispersal period occurred between blossom and the appearance of first fruit. *M. fructigena* does not cause blossom and/or twig blight in apple in the Central-European region (Holb *et al.*, 2004); therefore, conidial inoculum is originated from other sources. Spring conidia can be originated from sporulating mummified fruits that overwintered in the tree canopy (Van Leeuwen *et al.*, 2002) and summer inoculum from the dopped fruit on the orchard floor (Holb & Scherm, 2007).

Viability of trapped conidia was the highest when it was collected under wet conditions with the lowest temperatures (Table 1). In the study of van Leeuwen (2000), 60% viability was similar to our results. Results also agreed with previous laboratory studies (Naqvi & Good, 1957; Xu *et al.*, 2001) as higher temperatures resulted in a reduction in conidial viability. Relationship between weather variables and viability data from field sampled spores are quite difficult to interpret as weather variables are fluctuating under field conditions.

A diurnal fluctuation in conidia concentration was observed, which approached similar type that was reported by Hirst (1953). Similarly to this study, previous studies (Kable, 1965; Jenkins, 1965; Corbin *et al.*, 1968; Holb, 2008b) on *Monilinia* spp. also demonstrated a significantly higher conidia in the afternoon and evening hours. The highest temperature mostly occurs from the early afternoon hours until late afternoon hours. As most spores also trapped during this period the positive association are also justified by Pearson correlation as there were strong positive correlations between hourly spore content and temperature.

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