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Heterogeneity of the aerial concentration and deposition of ascospores of *Venturia inaequalis* within a tree canopy during the rain

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Abstract

Scab is an important disease of apple and its control depends almost exclusively on frequent use of fungicides. Primary scab infection in the spring assumes several steps: ascospore maturation, liberation of ascospores that become airborne, deposition on susceptible tissues, and infection. However, the spatial heterogeneity of ascospores within the tree canopy is unknown. Aerial concentration of ascospore (ACA), ascospore concentration in rain water (ACR) and ascospore deposition (AD) were therefore measured at six heights (20–257 cm from the ground) with rotating-arm air samplers, funnels, and greased glass slides, respectively, during five rain events in 2001 and in 2002. In addition, ACR and AD were measured at eight locations within tree canopy at 196 cm height. Apple scab was assessed at the end of the primary infection period in each sampling location within the apple tree. A similar experimental design was used in 2003 to study the spatial heterogeneity of both AD and primary scab lesions. ACA and AD decreased with increasing height, while ACR increased with increasing height. Based on both variance to mean ratio and the power law relationship in both years, the ACR was heterogeneous, while AD was heterogeneous only during the peaks of ascospore release. The ACR was significantly higher at the centre of the trees and the AD was significantly higher at the centre and at the western edge of the trees. Only the cumulative AD was significantly correlated with apple scab lesions at the same location ($r = 0.83$). In 2003, a similar pattern of spatial heterogeneity within the tree canopy was observed for AD and primary scab lesion counts and there was a linear relationship ($R^2 = 0.84$) between these two variables. It was concluded that ACR and AD within the tree canopy are not randomly distributed at least during peaks of ascospore release and that AD is a good estimate of primary scab lesion development. This spatial heterogeneity should be considered when estimating ascospore deposition using mathematical models or when quantifying ascosporic inoculum using spore samplers.

Introduction

Apple scab, caused by *Venturia inaequalis* (Cke.), is of major economic importance in several apple (*Malus* × *domestica*) producing areas of the world (MacHardy, 1996). The disease can cause extensive yield and economic losses in regions where springs are cold and humid (MacHardy, 1996).

Although a tremendous amount of effort has been deployed to develop resistant cultivars

(Crosby et al., 1992) they have yet to gain popularity, mainly in North America. Fungicides, if properly timed, generally provide acceptable control of apple scab (O'Leary and Sutton, 1986). In North America, *V. inaequalis* overwinters as immature pseudothecia in apple leaf litter. Consequently, the main source of primary inoculum consists of ascospores that are produced within pseudothecia that develop during the spring. In north-eastern North America, the strategy to manage apple scab is mainly based on a good control of primary infections, in order to avoid epidemic build-up caused by secondary infections and subsequent fungicide applications during the summer months. During the primary infection period, fungicides are applied on a calendar basis, or based on risk of scab development (MacHardy, 1996). In the latter case, the risk for primary infection is estimated mainly from the stage of ascospore maturation and climatic conditions associated with risk of infection (MacHardy and Jeger, 1983). The risk associated with infection periods is still most often estimated using the Mills table in its original (Mills, 1944), or in one of its modified versions (MacHardy and Gadoury, 1989). Several models of ascospore maturation were developed over the years and for different regions (Gadoury and MacHardy, 1982; James and Sutton, 1982; St-Arnaud and Neumann, 1990). Most of these models are degree-day based and, in practice, they are mainly used to predict the beginning and end of the ascospore ejection period, regardless of the amount of ascosporic inoculum present in the orchard. Consequently, orchards with low inoculum may be treated too often and orchards with high levels of inoculum may not be sufficiently protected.

The potential ascospore dose (PAD) was developed to estimate the amount of ascospores produced per m^2 of orchard. It is derived from the amount of leaf scab present in the orchard the previous fall (Gadoury and MacHardy, 1986; MacHardy et al., 1993). MacHardy et al. (1993) showed that it is possible to delay a fungicide programme until the pink bud phenological stage in orchards with low PAD and keep fruit scab at an acceptable level. Despite the obvious interest of classifying orchards based on PAD, there are limits to the use of this criterion. PAD provides an overall estimation of the inoculum potential and is based on the assumption that the primary inoculum (ascospores per m^2) is uniform within the orchard as it provides one risk value per orchard. Monitoring of airborne ascospores has been suggested as one means to more precisely estimate scab risk for a specific infection period (Aylor, 1993; Charest et al., 2002). The possibility of using quantification of airborne ascospore concentration as an aid to manage apple scab have motivated researches on detection technologies (Ribber, 2005; Sholberg et al., 2005). Recently, Ribber (2005) proposed the use of biosensors for the in situ detection of airborne ascospores of *V. inaequalis*. Regardless of the detection technology, the problems of proper action threshold and reliability of sampling remain. Because the use of several samplers might not be practical for growers, the reliability of measurements assumes homogeneity of airborne ascospores within an orchard. However, in a recent study on spatial distribution of airborne ascospore concentration, Charest et al. (2002) demonstrated that ascospores were not randomly distributed but distinctly aggregated. The authors suggested that airborne ascospore concentration could be monitored using only one sampler from a representative sample of leaves collected and overwintered in a given orchard block. Nevertheless, the use of ascospore concentration measured at the ground level (40 cm from the ground) to estimate the risk of scab development is based on the assumption that this measurement is representative of the risk of scab development and that heterogeneity of ascospore concentrations within the apple canopy is low.

Although apple scab has been intensively studied (MacHardy, 1996), little is known about its spatial distribution within an orchard or within the tree canopy. This study was part of a broader study on spatial heterogeneity of apple scab (Charest et al., 2002). The aims of this study were therefore twofold; first to acquire fundamental knowledge on heterogeneity of aerial concentrations of ascospores in rain water and deposition of ascospores of *V. inaequalis* within the apple canopy. Second, to study the relationship between ascospores released in rain water and ascospore deposition and primary scab lesions.

Materials and methods

Spatial heterogeneity of ascospores within a tree canopy

The experiment was conducted at the Agriculture Canada Experimental Farm, in Frelighsburg, Québec, Canada. In the selected orchard of 0.80 ha, the dwarf trees grafted on M.26 rootstock were planted in 1984 with distances of 4.5 m between rows and 2.5 m between trees. The canopy height varied between 3 and 3.5 m. This orchard was composed of the scab susceptible cv. McIntosh. Fall foliar scab was estimated on September 28, September 26 and October 2 in 2000, 2001, and 2002, respectively, using an adaptation of the method described by MacHardy et al. (1993). Scab was severe the previous years and the potential ascospore dose was 169, 181, and 156 scabbed leaves per 100 shoots, in the fall of 2000, 2001 and 2002, respectively. The aerial concentration of ascospores and ascospore deposition were measured during five selected rain events in the spring of 2001 and in 2002. In early spring, before the first ascospore ejection, estimated from the degree-day model developed by Gadoury and MacHardy (1982), the different spore trap devices were installed in one tree located in the centre of the orchard (a different tree each year). The aerial concentration of ascospores (ACA) was measured with home-made rotating-arm impaction spore samplers (Rotorod type). Each sampler consisted of two vertical arms (1.65 mm square cross section and 20 mm long), 83 mm apart and rotated at 2400 rpm. Airborne particles were impacted onto the leading edges of the rotation rods coated with a thin layer of silicone (High vacuum grease, Dow Corning corporation, Midland, Mich. 48640, USA). The effective sampling rate was 20.65 l of air min^{-1}. The samplers were sheltered from rain. The samplers were turned on at the beginning of each selected rainfall event and turned off when it ended. The number of ascospores per rod were later counted under a microscope (250×) and counts were converted to ascospores m^{-3} (number of spores per rod $\times$ 1000 l m^{-3})/(20.65 l min^{-1} rod $\times$ 60 min). Ascospore concentration in rain water (ACR) was measured with plastic funnels (model 40411605, Coopérative fédérée du Québec, Montreal, Canada). Funnels, with openings of 6.75 cm^2 were placed vertically in the tree within 1 h before the beginning of each selected rainfall event and removed immediately at the end of the rain. Their contents were transferred into 50 ml Falcon test tubes and water volumes measured. Preliminary experiments showed that ascospores did not adhere to the plastic of the funnels and that they were all (> 98%) transferred to the Falcon test tubes. A few drops of Lugol's iodine solution were added to each tube to stop ascospore germination. Ascospore concentration in each suspension was determined using a Fuchs Rosenthal haemocytometer (C.A. Hausser & Son, Max Levy Inc. USA) and expressed as the number of ascospores mm^{-1} of rain. Ascospore deposition (AD) was measured with microscope glass slides (25 $\times$ 75 mm) greased on the upper or on lower surface with a thin layer of silicone. The slides were placed in the tree within 1 h before the beginning of each selected rainfall event and removed immediately after the end of the rain. The slides were not shielded from the rain. The number of ascospores present on 12% of the slide surface was counted under a microscope at a magnification of 250 ×. Ascospore deposition was expressed as the number of ascospores cm^{-2} of slide.

The location of samplers within the tree canopy is presented in Figure 1. Impaction spore samplers were placed on a pole located 1 m from the trunk of the apple tree at heights of 20, 40, 80, 109, 196 and 257 cm above the ground. Glass slides and funnels were placed next to each other in the centre of the tree at the same heights as the samplers and at 196 cm from the ground on two horizontal axes oriented north-south and east-west at a distance of 75 cm between each device. ACR and AD were measured in a total of 14 locations for each of the 10 rain events (five events per year). The number of apple scab lesions was assessed in early July on 10 leaves of approximately the same age located within 20–30 cm from each sampling device (funnels or microscope slides) except for those located below the tree canopy.

Relationship between ascospore deposition and primary scab lesions

To study the relationship between AD and primary scab lesions within tree canopy, a similar experiment was conducted in 2003 in the same orchard with microscope slides greased on the

upper surface only. The microscope slides were placed as described previously except for the two locations below the tree canopy for a total of 12 locations (Figure 1). The data were collected in three different apple trees selected at random within the orchard block. The trees were distanced by 25–30 m. Leaf scab was assessed at the end of the primary infection period, when virtually all season's ascospores were released and all potential primary scab lesions appeared. For each tree, 10 leaves of approximately the same age located within 20 cm from each microscope slide were assessed for the number of scab lesions per leaf.

Analysis

To compare aerial ascospore concentration, concentration of ascospores in rain water and deposition of ascospores monitored at different sampling dates, data were transformed to ascospores m^{-3} h^{-1}; ascospores mm^{-1} h^{-1}; and ascospores cm^{-2} h^{-1}, for ACA, ACR, and AD, respectively, where h is the duration of the rain event (Mandrioli et al., 1998). The effect of sampling location on ACR and AD of *V. inaequalis* were analyzed with a repeated measures analysis of variance (ANOVA) (Sokal and Rohlf, 1995). In this case, the measurement of ascospore concentrations at all sampling locations over sampling dates provides a restriction on randomization over time.

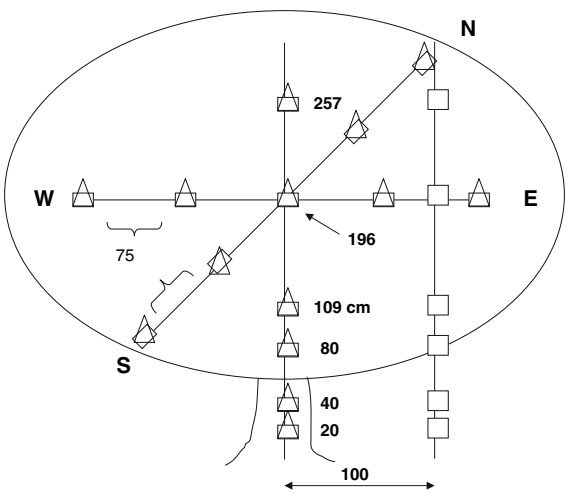

Figure 1. Sampling location within and below the apple tree canopy. The squares, triangles and rectangles represent the impaction samplers, funnels, and glass slides, respectively.

To estimate spatial heterogeneity, the variance (v) to mean (m) ratio (v/m), was calculated as an index of heterogeneity (Campbell and Madden, 1990). When the ratio is <1, $=1$, or >1, the pattern of spatial distribution is considered regular, random, or aggregated, respectively (Campbell and Madden, 1990). Significant aggregation or departure from randomness was determined based on a chi-square test where the test statistic is $(N-1)*(v/m)$, where N is the number of sampling locations. If there is a random distribution, $(N-1)*(v/m)$ follows a χ^2 distribution with $(N-1)$ degree of freedom (Campbell and Madden, 1990).

The Taylor power law (Equation 1) was used to describe the relationship between means (m) and variance (v) (Taylor, 1961) for the data collected with different devices and at different times. The linear form of the power law was used to estimate the values of parameters $\log(A)$ and b (Equation 2):

$$v = Am^b \tag{1}$$

$$\ln(v) = \ln(A) + b\ln(m) \tag{2}$$

where v is the sample variance, m is the sample mean, and $\log(A)$ and b are the intercept and the slope of the regression line, respectively. Estimates of $\log(A)$ and b were obtained with ordinary least square regression, significance of estimates was determined with F test and goodness of fit determined based on coefficient of determination (R^2) and mean square error (MSE). A random distribution is indicated by $\log(A) = 0$ or $A = 1$ and $b = 1$, and values of b could be used as an index of aggregation with $b > 1$ indicating that there is heterogeneity (Campbell and Madden, 1990). Correlation and regression analyses were used to measure the degree of association between the concentration of ascospores in rain water or ascospores deposition cumulated over sampling dates and primary scab lesions. Statistical analyses were done using the Statistical Analysis System version Windows 9.1 (SAS Institute inc. Cary, NC, USA).

Results

Spatial heterogeneity of ascospores within a tree canopy

Tree phenological stages and total amounts of rainfall and duration of rain events are given in Table 1. Overall, rainfall varied from 5.6 to 16.6 mm with durations of 4–13.5 h (Table 1). The amount of rain water collected in funnels positioned at different heights during each rain event in both 2001 and 2002 is given in Table 1. Regardless of the devices used, the amount of ascospores collected over sampling dates was low for the first sampling, increased to reach the maximum at the second and third samplings, and then decreased on the last samplings in both years (Table 2). In general there was a large variation between sampling location as denoted by the minimum and maximum ACR and AD (Table 2). Both the ACA and AD decreased with increasing height from 20 to 275 cm above the ground (Figure 2a–c). However, the highest ACR values were observed at the tree canopy level (109, 196 or at 257 cm from the

Table 1. Rainfall and amount of water collected with funnels as a function of height within the tree canopy for each rain event of 2001 and 2002

	Rain event in 2001					Rain event in 2002				
	1	2	3	4	5	1	2	3	4	5
Rainfall (mm)	5.6	16.6	10.6	12.6	8.4	6.4	11.2	15.4	13.8	6.6
Duration (h)	6	12	5	9	5	4	6.3	13.3	15.5	4.5
Height (cm)	Amount of rain water collected (ml)									
20	2.5	8.5	12	9.9	6.1	4.2	8.9	14.8	8.9	7.9
40	3.5	7.9	10	9	8	2.9	8.3	8.7	6.1	1.2
80	2.7	7.3	3.5	6	1.2	1.4	7.9	13.3	7.8	4.4
109	1.4	7.9	8	7.6	4.5	3	9.3	15.8	8	5.7
196	2.9	8.5	12.5	8.9	5.8	6.1	9.3	21	7.8	8.5
257	5.1	7.9	12.5	6.5	8.6	3.4	8.7	5.3	6.3	3.5

Table 2. Summary of mean, minimum, maximum and variance to mean ratio for concentration of ascospores in rain water and ascospore deposition of *Venturia inaequalis* observed in 2001 and 2002

Sampling	Phenological	Sampling devices											
		Funnel				Greased slide (upper side)				Greased slide (lower side)			
Date	Stage[a]	Mean	Min.	Max.	v/m[b]	Mean	Min.	Max.	v/m	Mean	Min.	Max.	v/m
2001													
May 05	HG	0.76	0.00	3.87	1.80*	0.48	0.10	1.85	0.53	0.49	0.07	2.10	0.63
May 10	EP	4.75	0.21	17.86	7.15*	3.82	0.78	13.15	2.56*	2.67	0.23	9.00	2.43*
May 12	EP	11.97	2.19	38.54	14.68*	2.99	0.72	8.72	1.64*	1.40	0.00	5.32	1.47*
May 18	FP	0.95	0.13	7.21	3.07*	0.99	0.02	3.57	1.28*	0.55	0.05	2.37	0.65
May 27	C	0.19	0.00	0.97	0.31	0.40	0.05	2.23	0.68	0.39	0.05	1.70	0.62
2002													
May 02	GT	1.22	0.05	5.35	1.52*	0.29	0.05	1.22	0.32	0.53	0.08	1.75	0.60
May 10	HG	10.35	2.36	21.43	5.86*	1.60	0.00	4.93	1.38*	2.42	0.13	7.37	2.32*
May 14	TC	14.16	2.48	31.05	8.22*	3.30	0.50	7.50	1.81*	3.53	0.47	8.13	1.49*
May 21	EP	0.76	0.11	5.39	2.20*	1.15	0.12	3.68	1.13*	0.62	0.02	1.72	0.41
May 26	FP	0.17	0.02	0.67	0.16	0.41	0.07	1.62	0.47	0.29	0.05	1.40	0.38

[a]Phenological stages: GT, green tip; HG, half inch green; TC, tight clusters; EP, early pink; FP, full pink; C, calyx.
[b]Variance to mean ratio = v/m where v is the sample variance and m is the sample mean.
*Significantly >1 based on the χ^2 test at $\alpha = 0.05$.

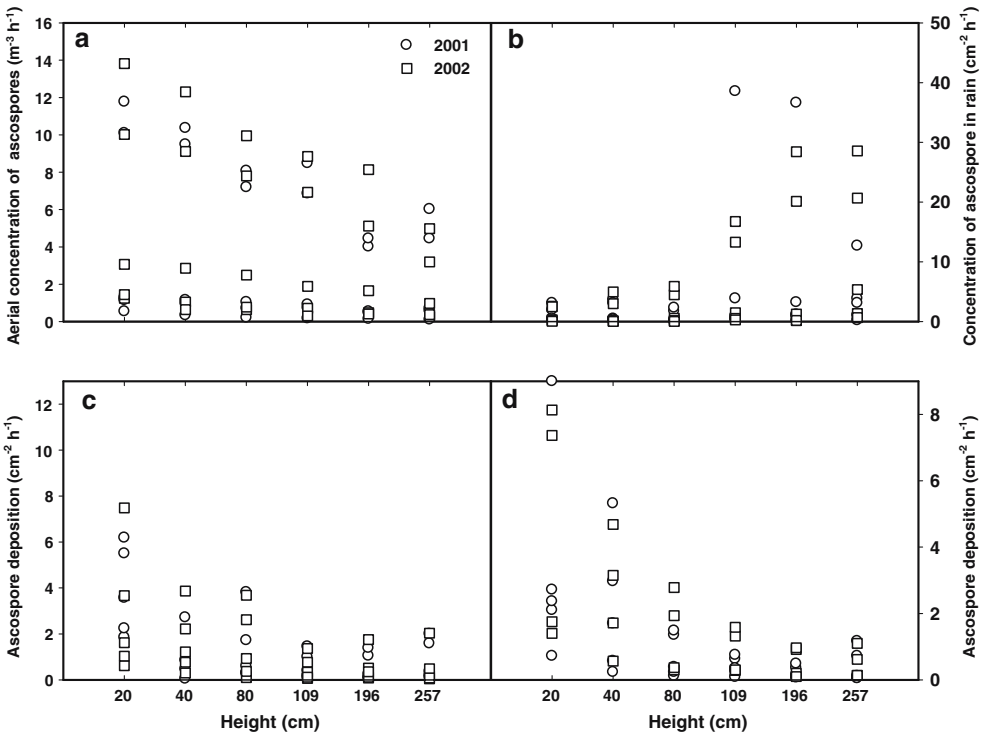

Figure 2. Aerial concentration of ascospores, concentration of ascospores in rain water and ascospore deposition of *Venturia inaequalis* as a function of height monitored during five rain events in 2001 (circles) and in 2002 (squares). Ascospores in the air were monitored with impaction samplers located at one meter from the trunk of the tree (a); ascospores in rain water were monitored by collecting rain water with funnels (b), deposition was monitored with glass slides greased on the upper (c) or lower (d) surface (see Figure 1).

ground). Lower numbers of ascospores were collected under the tree canopy at heights of 20–80 cm from the ground (Figure 2b). In general, there was less rain collected in the centre of the tree canopy (Table 1). There was a significant effect of sampling height on the ACA, ACR, and AD ($P < 0.001$). In both 2001 and 2002, there was a significant effect of sampling location on ACR ($P = 0.02$ and $P < 0.001$, for 2001 and 2002, respectively). Significantly higher ACR were observed in the centre of the trees (Figures 3 and 4). In both 2001 and 2002, there was a significant effect of sampling location on AD ($P = 0.02$ and $P < 0.001$, for 2001 and 2002, respectively). The AD was significantly higher at the centre and at the western edge of trees (Figures 3 and 4).

The variance to mean ratios for ACR were significantly greater than one for most sampling dates in both 2001 and 2002, indicating heterogeneity within the tree canopy (Table 2). Similarly, for most sampling dates, the variance to mean ratios were significantly greater than one for AD measured with microscope slides greased on the upper surface (Table 2). Conversely, the variance to mean ratios for the AD measured with microscope slides greased on the lower surface were smaller or near to one at most sampling dates in both 2001 and 2002, indicating a regular or random distribution within the tree canopy (Table 2). However, the variance to mean ratios were significantly greater than one on May 10 and May 12, in 2001 and on May 10 and May 14, 2002, indicating an aggregated pattern of distribution. These dates corresponded to peaks in the number of ascospores released (Table 2).

The linear form of the Taylor's power law adequately described the relationship between the mean and variance over the sampling period in both years, for all devices (Table 3). The coefficient of determination (R^2) varied from 0.94

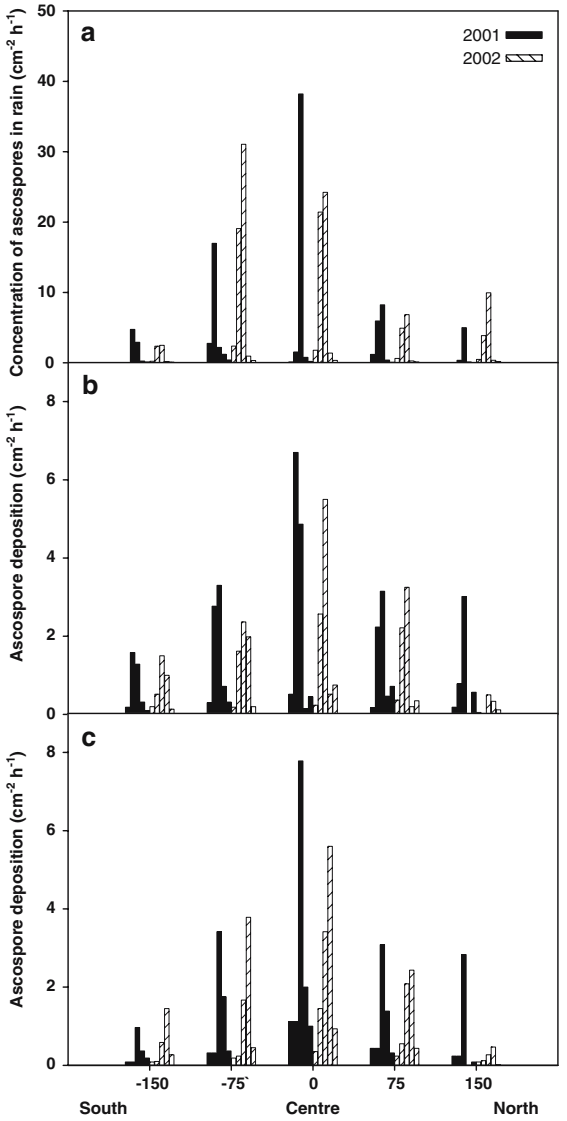

Figure 3. Concentration of ascospores in rain water and ascospore deposition of *Venturia inaequalis* during five rain events in 2001 (solid bars) and in 2002 (dashed bars) as a function of the position within the tree canopy on a south–north axis. Ascospores in rain water were monitored by collecting rain water with funnels (a). Deposition was monitored with glass slides greased on the upper (b) or lower (c) surface (see Figure 1).

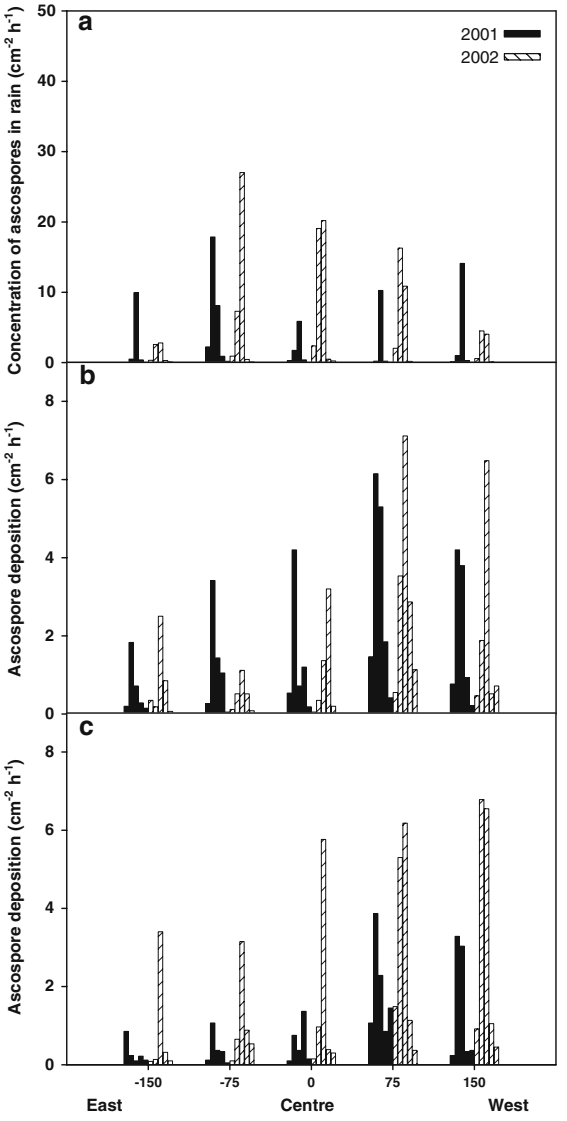

Figure 4. Concentration of ascospores in rain water and ascospore deposition of *Venturia inaequalis* during five rain events in 2001 (solid black bars) and 2002 (dashed bars) as a function of the position within the tree canopy on an east–west axis. Ascospores in rain water were monitored by collecting rain water with funnels (a). Deposition was monitored with glass slides greased on the upper (b) or lower (c) surface (see Figure 1).

to 0.99, and the estimates of b, the slope were significantly ($P < 0.05$) greater than one for both years for all devices, indicating the presence of heterogeneity (Table 3). When data from both sampling years were pooled, the estimate of b was significantly greater than one ($P < 0.05$). Similar results were also obtained for the ACR and AD, (Figure 5a–c) indicating that heterogeneity was greater when more ascospores were collected.

When cumulative ACA (ΣACA_i, where i is the ith rain event), ACR (ΣACR_i) and cumulative AD ($\sum AD_i$) (lower or upper greased slides) were correlated to the average number of lesions for the

Table 3. Parameter estimates and associated statistics for Taylor's power law used to describe the relationship between the mean and variance concentration of ascospores in rain water and ascospores deposition of *Venturia inaequalis* measured during 10 rain events in 2001 and 2002

Sampling device	Year	df	R^2	Log (A) (SE)	B (SE)	P of $b > 1$
Funnel	2001	4	0.99	0.2185 (0.0784)	1.9366 (0.0928)	0.0021
	2002	4	0.97	−0.0247 (0.0689)	1.8763 (0.0706)	0.0011
	Pooled	9	0.98	−0.1057 (0.0795)	1.8895 (0.0871)	<0.0001
Upper side greased slides	2001	4	0.94	−0.0288 (0.0697)	1.5047(0.1760)	0.0034
	2002	4	0.98	−0.0763 (0.0310)	1.6826 (0.0826)	0.0037
	Pooled	9	0.96	−0.0562 (0.0360)	1.5952 (0.0934)	0.0002
Lower side greased slides	2001	4	0.96	0.0534 (0.0177)	1.7595 (0.0545)	0.0008
	2002	4	0.95	−0.0850 (0.0723)	1.6928 (0.1739)	0.0283
	Pooled	9	0.96	−0.0179 (0.0405)	1.7026 (0.1084)	0.0002

same location within the tree canopy, the coefficients of correlation r were −0.42 ($P = 0.17$), −0.16 ($P = 0.41$), 0.83 ($P < 0.001$) and 0.30 ($P = 0.11$), respectively.

Relationship between ascospore deposition and primary scab lesions

In 2003, heterogeneity of the AD measured with the microscope slides greased on the upper surface was similar for the different trees sampled. A repeated measures ANOVA revealed a significant effect of sampling location on the AD ($P = 0.003$) and of the sampling date ($P < 0.0001$). The AD was significantly ($P = 0.001$) higher in the centre of the trees (0 and 75 cm from the tree trunk) than at the edges of the tree (150 cm from the trunk). Similar to the 2001 and 2002 observations, the variance to mean ratios were >1 for the sampling dates with high AD values indicating an aggregated distribution within the tree canopy (Table 4). The linear form of Taylor's power law adequately described the relationship between the mean and variance over the sampling period ($R^2 = 0.97$). The estimated slope was >1 ($b = 1.91$, $P < 0.001$) indicating the presence of heterogeneity. The variance to mean ratios for mean number of lesions per leaf were significantly >1 with values of 13.62, 13.45, and 19.77 for the different trees sampled. There was a linear relationship ($R^2 = 0.84$) between the cumulative AD and the mean number of lesions per leaf at the same sampling location within the tree canopy (Figure 6).

Discussion

In the present study, heterogeneity of ascospore concentration in rain water and ascospore deposition was measured at different heights and at different locations within an apple tree canopy. Large variations in the concentration of ascospores in rain water or ascospore deposition were observed between sampling locations. The ratio between the maximum and the minimum varied from 2.4 to 114.5 and from 4.9 to 216.0 for ascospores in rain water or ascospore deposition (glass slides greased on the upper surface), respectively. During the peaks of ascospore discharge, the concentration of ascospores in rain water and ascospore deposition were heterogeneous within the apple canopy, but were homogenous during the periods when low numbers of ascospores were discharged. This phenomenon could be explained at least in part by the narrow range of values (and hence the small variation between sampling locations) observed for the number of ascospores released during the non-peak discharge periods as oppose to the wide range of values observed under the peak-discharge periods. These results suggest that the location of the sampler is important to minimize this variability and hence to increase reliability of estimation of spore concentrations.

Cumulative aerial ascospore concentration, ascospore concentration in rain water, and ascospore deposition measured with glass slides greased on the under surface were not correlated with the average number of scab lesions per leaf.

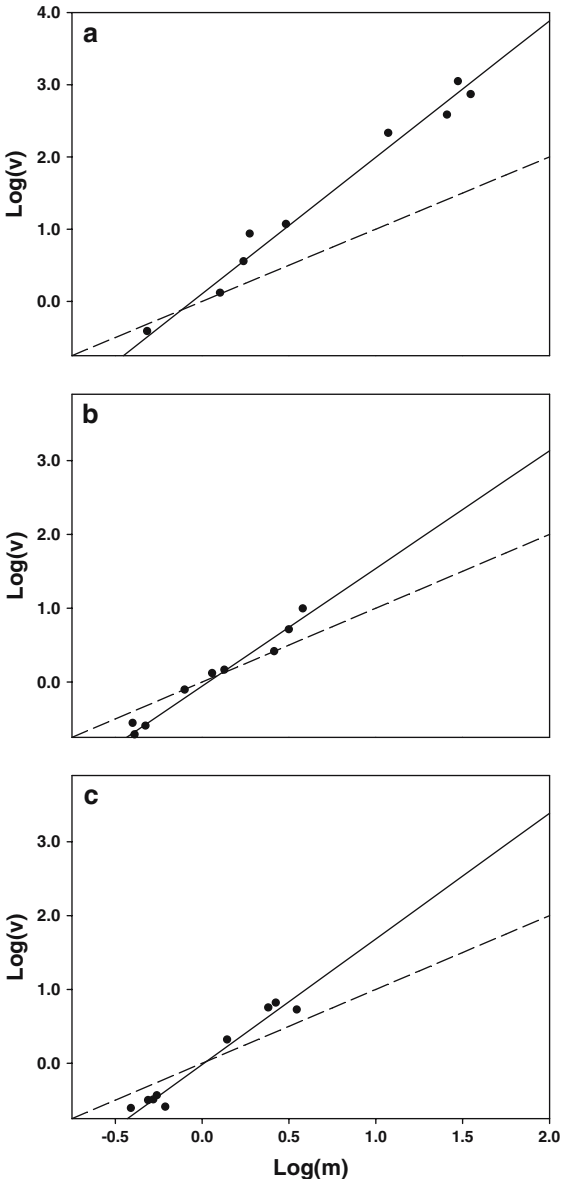

Figure 5. Regression of the logarithm of the variance (v) as a function of the logarithm of the sample mean (m) for concentration of ascospores in rain water (a) and ascospore deposition monitored with glass slides greased on the upper (b) or lower (c) surface (see Figure 1). Data from 2001 and 2002 were pooled.

However, significant correlations were observed with cumulative ascospore deposition measured with glass slides greased on the upper surface. This suggests that ascospore deposition measured with the greased glass slides adequately reflected ascospore deposition on apple leaves. Ascospore concentration in rain water represents all ascospores including those that will adhere to the leaves and cause infection as well as those that will be washed off by raindrops. The lack of correlation between ascospore deposition measured with glass slides greased on the under surface and average number of scab lesions per leaf could be explained, at least in part, by the very low number of ascospores collected at some sampling locations.

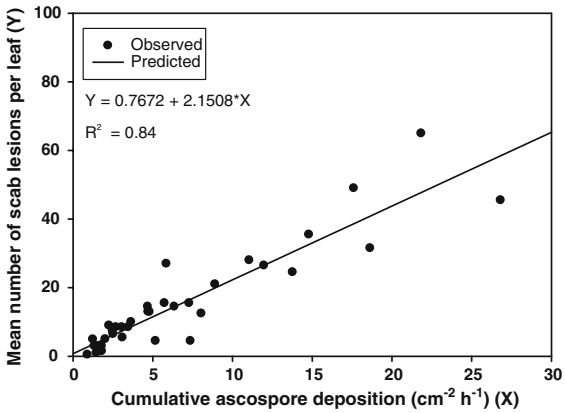

Figure 6. Relationship between the mean number of scab lesions per leaf and the cumulative ascospore deposition of *Venturia inaequalis* assessed at the same location within a tree canopy.

In this study, the aerial concentration of ascospores and ascospore deposition decreased with increasing height from 20 to 257 cm above the ground. These observations are similar to those reported by Aylor (1995). However, the concentration of ascospores in rain water was highest at the tree level (109, 196 or at 257 cm from the

Table 4. Variance to mean ratio for ascospore deposition of *Venturia inaequalis* measured within the tree canopy in 2003 with greased microscope slides

Sampling	Phenological	v/m ratio[b]		
Date	Stage[a]	Tree 1	Tree 2	Tree 3
May 1	GT	0.78	0.77	0.86
May 6	HG	3.92*	2.85*	5.05*
May 12	TC	2.53*	2.00*	3.47*
May 21	FP	0.92	0.69	1.20*
May 26	B	0.65	0.68	0.84

[a]Phenological stages: GT, green tip; HG, half inch green; TC, tight clusters; FP, full pink; B, bloom.
[b]Variance to mean ratio = v/m where v is the sample variance and m is the sample mean.
*Significantly >1 based on the χ^2 test at $\alpha = 0.05$.

ground). This phenomenon might be explained by the interception of ascospores by leaves and branches. To our knowledge, the variation in ascospore concentrations within an apple tree canopy has not been previously studied. In this study, we observed higher concentrations of ascospores in the centre of the tree as compared to the edges, and at the western edge for ascospore deposition. This might be due to the presence of a herbicide strip under the rows and of a herbicide-free zone between the rows. In the herbicide strip, the absence of grass allows more ascospores to become airborne (Aylor, 1994).

Concentration of ascospores in rain water was generally heterogeneous within the apple tree canopy. Typically, variance to mean ratio values were >1 for sampling dates corresponding to peaks in ascospore ejections. In both years, Taylor's power law adequately described the relationship between mean and variance over the sampling period for both concentration of ascospores in rain water and ascospore deposition. The estimated slope values were >1 indicating the presence of heterogeneity. When the experiment was repeated in 2003, similar spatiotemporal heterogeneity in ascospore deposition was observed. Thus, it was concluded that heterogeneity in the concentration of ascospores in rain water and ascospore deposition varies considerably during the primary infection period.

The question remains as to whether the spatial heterogeneity of ascospore distribution is reflected by the pattern of distribution of primary scab lesions. Aylor and Kiyomoto (1993) showed that the number of primary apple scab lesions is quantitatively related to the concentration of ascospores in the air surrounding the susceptible apple leaf tissues. In their experiments, potted trees were exposed to ascospore inoculum released under orchard conditions. The concentration of ascospores in the air was estimated using rotating-arm spore samplers (Aylor and Kiyomoto, 1993). The number of lesions that developed on the trees was quantitatively related to the exposure to ascospores ($r = 0.83$). In our study, similar correlation coefficients ($r = 0.83$ and 0.91) between the cumulative ascospore deposition within the apple canopy and the number of scab lesions were found. Furthermore, the spatial heterogeneity of ascospore deposition and primary lesions within the tree canopy was similar. Ascospore deposition thus appears to be a good predictor of apple scab risk.

Ascospore concentrations or deposition could be estimated using mathematical models such as those proposed by Aylor and collaborators (Aylor and Anagnostakis, 1991; Aylor and Sutton, 1992; Aylor, 1994, 1995), by Kaplan (1986) and more recently by Rossi et al. (2003). Prediction of aerial ascospore concentrations using mathematical models is complex as it depends on several physical and biological factors. The aerial concentration of ascospores of *V. inaequalis* is not directly related to the PAD because winter conditions will affect the amount of ascospores that will be produced within the pseudothecia (Jeger and Butt, 1983; Charest et al., 2002). During the spring, the rate of physical and biological breakdown of leaf litters will influence the pattern and amount of ascospores released (Aylor, 1998). Only a proportion of the mature ascospores will escape from ground cover and then a further proportion will be lost during aerial transportation due to wind shear, turbulent diffusion and washout by rain (Kaplan, 1986; Aylor, and Anagnostakis, 1991; Aylor and Sutton, 1992; Aylor, 1995). Moreover, because measurement of ascospore deposition is time-consuming, most simulation models were not validated against observed data. In the model recently developed by Rossi et al. (2003), ascospore deposition expressed as the number of ascospores deposited varied from 0 to a maximum of about 0.17 ascospores cm^{-2} h^{-1} in the simulation runs. The observed values from our study, which was conducted in an orchard with a high level of primary inoculum, varied from 0.02 to 3.82 (average per tree) and from 0.78 to 13.15 ascospores cm^{-2} h^{-1} depending on the sampling location within the apple canopy. In addition, prediction models provide a single value per orchard, or even per region, depending on weather data availability. In the present study, there were heterogeneities in spore concentrations measured at different sampling locations within the apple canopy regardless of the methods used. Thus, where to place spore traps becomes important in order to minimise this variability and hence to increase reliability of estimating average spore concentrations.

Depending on the regions and weather conditions during the spring, control of apple scab may require 6–14 fungicide treatments per season (MacHardy, 1996). These sprays represent

important costs to growers. In the United States, a total of 2 million kg of fungicides were applied on 140,667 ha of apple orchards surveyed in eight states in 2001 (Anon., 2002). In addition, fungicides may have various impacts, including adverse effects on predatory mites, and health concerns for both farmers and consumers (Schneider and Dickert, 1994; Bower et al., 1995). However, considering the low acceptable economic threshold of about 1% fruit scab at harvest (Seems et al., 1989; Van der Scheer, 1992), the strategies for controlling apple scab with less fungicide will require improved methods that may include monitoring ascosporic inoculum. The results of this study suggest that aerial ascospore concentration is not correlated with scab development while ascospore deposition was a good predictor of apple scab leaf infection. The results also suggested that spatial heterogeneity should be considered in predicting or measuring ascospore deposition. Because of the large variation in ascospore deposition within an apple tree canopy, several samplers may be required to account for heterogeneity. As a consequence, quantification of ascospore deposition as an extension tool for practical disease management is unlikely. Furthermore, measuring ascospore deposition implies examination of collection surfaces to obtain accurate spore counts, and this requires significant amounts of time and expertise. This can make it difficult to use the results in 'real time'. These limitations could be overcome by adopting new methods for quantifying airborne spores based on immunological (Kennedy et al., 2000; Ribber, 2005) or molecular techniques (Calderon et al., 2001). Such techniques are under development for *V. inaequalis* (Sholberg et al., 2005) and could result in standardized and rapid methods of spore quantification, making inoculum detection a more practical tool in disease management.

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