Comparison of the Seasonal Pattern of Airborne *Venturia inaequalis* Ascospores with the Release Potential of *V. inaequalis* Ascospores from a Source

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ABSTRACT

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Release of *Venturia inaequalis* ascospores was monitored during four ascospore release seasons by two independent methods. In the first method, airborne ascospores were monitored in the field with a Burkard volumetric spore sampler located in the center of the source area and operated continuously during each season. In the second method, the potential release of ascospores was determined every 3 to 5 days during each season by measuring spore release from scabbed apple leaves freshly collected from the field and tested under standard conditions in spore release towers in the laboratory. In addition, the number of scabbed leaves per unit of ground area in the source area was determined periodically during each season. The date when half the season's total ascospores was released in the towers lagged behind the Burkard sampler by 7, 18, 11, and 14 days (mean = 12.3 days) in 1992, 1993, 1994, and 1995, respectively. This lag suggests a late-season decrease in the dispersal potential of *V. inaequalis* ascospores. Much of the lag between ascospores released in the towers and those airborne in the field can be explained by a natural decline in the number of source leaves per unit of ground area as the season progressed.

Additional keywords: apple scab, ascospore maturation.

Strategies for controlling apple scab, caused by the fungus *Venturia inaequalis* (Cooke) G. Wint., center on applications of fungicides during a few weeks in the spring when *V. inaequalis* ascospores are actively released into the air. The inoculum potentially available within leaf litter varies with time as ascospores mature and are released during the spring (8,9,15). It is of considerable practical importance in planning disease control measures to be able to determine the time course of ascospore release and, in particular, to pinpoint the beginning and end of the ascospore-release season.

Spore release towers (12,14) are often used alone or in conjunction with pseudothecial squash mounts (8,20) to monitor the seasonal development and release potential of *V. inaequalis* ascospores from leaf litter collected in the field. Although field measurement of ascospore concentration in the air above a source with a continuously recording volumetric sampler, such as a Burkard sampler, is a more direct method of monitoring ascospore release, it requires considerably more effort than the above-mentioned methods and is used less frequently. Spore release towers measure the release of physiologically mature ascospores and, thereby, should offer a direct method for measuring ascospore release potential. To properly interpret spore tower results when evaluating potential airborne inoculum, however, it is necessary to have knowledge of possible sources of attrition of airborne ascospore inoculum in the field. This attrition can be influenced by various factors, including ascospore removal from the air by rain (3,16), mechanical and biological breakdown of inoculum-harboring leaf litter (11), and entrapment of released ascospores in ground cover (1,5).

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In this paper, I compare the time course of the release of *V. inaequalis* ascospores above a source in the field with the release of ascospores from source leaves collected from the field and placed in a spore release tower in the laboratory. This comparison was repeated in four consecutive years. Results of these comparisons are interpreted with respect to measured attrition of source leaf litter and potential loss of ascospores in ground cover.

MATERIALS AND METHODS

Experimental sites. Scabbed apple leaves harboring *V. inaequalis* were overwintered on the ground in test sites at Lockwood Farm in Mt. Carmel, CT, during the 1992 to 1995 seasons. In 1992 and 1993, the test site was a 0.2-ha orchard of dwarf apple trees (including cvs. McIntosh, Empire, Liberty, Ida Red, and experimental scab-resistant varieties grafted onto dwarfing rootstocks Mark and M9) described elsewhere (2). The trees were in their fourth and fifth seasons and were about 2.0 and 2.3 m tall in 1992 and 1993, respectively. In 1994 and 1995, the test site was a 0.4-ha grass field (4,5) located about 300 m from the orchard site. The two sites were distinct in their exposure to wind, because of the presence or absence of apple trees and in the presence (orchard) or absence (field) of mowed-grass ground cover.

Apple leaves with scab lesions were collected from an orchard of McIntosh trees and were placed in the test sites during early December of 1991, 1992, 1993, and 1994 for the 1992, 1993, 1994, and 1995 tests, respectively. The scabbed leaves were distributed on the ground surface uniformly by hand. In the orchard site, leaves were placed predominantly within the grass aisles. Relatively few leaves from the orchard trees remained in the orchard when the source leaves were placed out because of late-season mowing and exposure to wind. In the grass field site, the leaves were spread over a 30 × 30-m area in the middle of the field. Bird netting was

placed on the ground over the source leaves in the grass field site to help keep the leaves from being blown away. The grass in the orchard source area was mowed twice during the test period in 1992 (on day of year [DOY] 126 and 135, measured from 1 January) and twice during 1993 (on DOY 126 and 145). The grass in the field plot used in 1994 and 1995 was not mowed.

Meteorological measurements. Air temperature, rainfall amounts, occurrence of wet periods, and wind speed at the height of the Burkard sampling orifice were monitored at the test sites with a data logger (model 21X, Campbell Scientific, Logan, UT). Air temperature was sensed with a thermistor (model 207, Campbell Scientific) shielded from the sun and located at a height of 1.5 m. A leaf wetness resistance grid (model 731, Campbell Scientific) was used to monitor wet periods, which included periods with dew, trace amounts of rain, and recorded rain. Wind speed was measured with a cup anemometer (model 014A, Met-One, Grants Pass, OR) located at a height of 0.6 m during 1992 and at a height of 0.5 m during 1993 to 1995. Rainfall amounts were measured with a tipping bucket rain gauge (model RG2501, Sierra Misco, Sacramento, CA [1 mm per tip], during 1992 and model TR-525, Texas Electronics, Dallas [0.1 mm per tip], during 1993 to 1995). Instrument signals were sampled every 10 s and averaged to yield 2-h (1992 to 1994) or 1-h (1995) average values. Rainfall amounts were totaled for these same periods. Daily mean temperatures and temperature variances were calculated from the recorded average temperatures. Average wind speeds, U (meters per second), were calculated for each ascospore release event with data from the cup anemometer. Averaging periods for U were chosen to coincide with the collection of airborne ascospores by the Burkard sampler (described below). The dates of the occurrence of the green tip, 1-cm of green, tight cluster, open cluster, pink, bloom, and petal fall stages of developing apple fruit buds were recorded each season for McIntosh apple trees in an orchard located within 300 m of the test sites.

Number of source leaves per unit of ground area in the test sites. To compare the time course of cumulative ascospore release from collected leaves in a spore tower in the laboratory with the time course for the cumulative number of airborne spores in the field, it was necessary to account for the loss of potential inoculum due to the reduction in the amount of source leaves during the season. This reduction can be due to biological degradation, mechanical breakage of leaves during mowing, and leaves being blown from the site by the wind.

The number of source leaves per m^2 of ground surface at the test site was determined several times during the ascospore-release season by counting the number of apple leaves in 3.0 × 0.1 m strips at 14 to 40 randomly selected locations in the source areas. Near the end of the season, inoculum-containing apple leaves had become fragmented in some sample areas. When this was the case, the number of leaves in a sample was estimated by conceptually combining fragments (usually no more than two to three fragments) into a whole-leaf equivalent.

The measured number density of apple leaves on the ground, N_L (leaves per m^2), was fitted to the following functions by nonlinear regression. In 1994, the equation fitted was

$$N_L(t) = N_{L0} \exp[-a(t - t_0)] \quad (1)$$

where t is the day of the year, t_0 is the date of the first leaf litter density measurement, and parameters N_{L0} (leaves per m^2), which represents the number density of source leaves at the beginning of the season, and a (decrease per day), which represents the rate of decrease of leaf number density, were determined by nonlinear regression analysis. In 1992, 1993, and 1995, the equation fitted was

$$N_L(t) = B_1\left(1 - 1/\{1 + \exp[-B_2(t - B_3)]\}\right) \quad (2)$$

where t is the day of the year, and parameters B_1, B_2, and B_3 were determined by nonlinear regression analysis. In 1992 and 1993, the values of N_L were multiplied by 0.6 to account for the fact that essentially all of the source leaves were contained in the grass aisles in the orchard, which comprised 60% of the area of the orchard floor (2).

Inoculum sources. The total number of ascospores potentially released from the source leaves over the entire season was estimated for each year in terms of potential ascospore dose (*PAD*) (ascospores per m^2 per year) (11). In the current studies, the *PAD* was estimated by

$$PAD = 8 N_A N_P A_L N_L(t_0) \quad (3)$$

where N_A is the average number of asci per pseudothecium, N_P is the average pseudothecial density on source leaves, A_L is the average area of a source leaf, and $N_L(t_0)$ is the number of apple leaves per unit of ground area in the source at the beginning of the season. The pseudothecial density on diseased leaves, N_P (pseudothecia per cm^2), was estimated by counting the pseudothecia at 50× in 15 randomly selected 5-mm-diameter fields per leaf on each of 61 leaves in 1992, 150 leaves in 1993 and in 1994, and 100 leaves in 1995. The leaves were randomly selected from the test sites during the ascospore-release season. The multiplier of 8 in equation 3 represents the number of ascospores per ascus. N_A was assumed to be 122 (SE = 7.9) asci per pseudothecium (11).

The estimated *PAD* during early spring was diminished by attrition of the source leaf litter. To account for this, an adjusted seasonal potential ascospore release (PAD_{adj}), was defined as

$$PAD_{adj} = PAD\left\{\int_{t_0}^{t_f} N_L(t)dt / [N_L(t_0)(t_f - t_0)]\right\} \quad (4)$$

where t_f is the end of the ascospore-release season, as indicated by the spore release towers (DOY = 170 in 1992, 1993, and 1995, and 175 in 1994). The effect of leaf attrition is represented by the terms inside the curly brackets in equation 4. These terms express PAD_{adj} as a proportion of *PAD*, in which the numerator represents the area under the curve for the number density of apple leaves given by equation 1 or 2 and the denominator represents a comparable area in the absence of leaf attrition. A value of PAD_{adj} was obtained from equation 4 for each year of the test.

Aerial ascospore concentrations. The airborne concentration of *V. inaequalis* ascospores, C (ascospores per m^3), was monitored continuously in the test sites with a 7-day Burkard volumetric spore trap (Burkard Scientific Sales, Ltd., Rickmansworth, Hertfordshire, England). The Burkard spore trap was located near the center of the source area each season, and its sampling orifice was at a height of 0.5 m. The surface of the spore-collecting tape was made sticky with a thin layer of high-vacuum grease (Dow Corning, Midland, MI). Time marks were made by a needle inserted through the trap orifice to identify the beginning and end of a sampling period. The tape was changed weekly. The clock of the spore trap kept accurate time to within 30 min or less during the 7-day sampling periods. *V. inaequalis* ascospores were counted in two 0.5-mm-wide strips located on either side of the center line and parallel to the length of the sampling tape (i.e., in the direction of the time axis on the sampling tape). Counts were recorded for each 15-min segment along the length of the tape. The counts from both strips were combined, and the individually recorded values along the time axis were summed to obtain hourly or daily totals. Counts were made during each wet period, which included several hours before and after each wet period. The length of time of the extended period for counting was determined by the criterion that no ascospores were counted for at least 2 h on either side of the wet period. Ascospore concentrations, C, were derived from these counts by accounting for the air volume sampling rate of the Burkard spore trap and for the fraction of the sample counted (3).

A normalized cumulative distribution for the daily average ascospore concentrations indicated by the Burkard sampler, S_{BC}, was defined by

$$S_{BC}(t_j) = \sum_{i=1}^{j} C(t_i) / \sum_{i=1}^{n} C(t_i) \quad (5a)$$

where n is the total number of days with ascospores counted on the Burkard tapes for each season (n = 41 in 1992, 31 in 1993, 35 in 1994, and 25 in 1995). The function, S_{BC}, has values in the range of 0 to 1. The number of days with daily average concentrations of ascospores >5 ascospores per m^3 was 20, 18, 29, and 16 days in 1992, 1993, 1994, and 1995, respectively.

For comparison with the release of ascospores from field-collected leaves in laboratory spore towers, the concentration values should be weighted by the wind speed to produce an airborne flux (2,4). The horizontal flux of airborne ascospores carried by the wind past the Burkard spore trap sampling orifice during an ascospore release event was calculated as the product UC. A normalized cumulative distribution for the airborne ascospore fluxes indicated by the Burkard sampler, S_{BF}, was defined by

$$S_{BF}(t_j) = \sum_{i=1}^{j} C(t_i)\,U(t_i) \Big/ \sum_{i=1}^{n} C(t_i)\,U(t_i) \qquad (5b)$$

where n has the same values as indicated for equation 5a. The denominator on the right side of equation 5b represents the season-total airborne flux of ascospores carried past the Burkard sampler by the wind. The function, S_{BF}, takes on values in the range of 0 and 1 and gives a measure of the seasonal progress of ascospore release as indicated by the Burkard sampler.

Spore release tower. The number of *V. inaequalis* ascospores released from samples of scabbed apple leaves collected from the ground during each season was determined with a spore tower in the laboratory. The spore tower was essentially a vertical wind tunnel. Scabbed leaves were placed at the top of the tower and the released ascospores were drawn downward through the tower with a fan. It was similar to the spore tower described by Gilpatrick et al. (12), except the current one was modified by incorporating a small volumetric suction spore sampler (13) above (upstream of) the air distribution plate (4). The flow through the suction sampler was controlled by a critical orifice to ensure a standard spore collection rate (13). Two duplicate towers were operated simultaneously with replicate samples of diseased leaves to obtain two independent estimates of ascospore release for each sampling date.

Leaves were placed in the spore release towers within 40 to 60 min of being collected from the field. Leaves were sandwiched between two screens made of hardware cloth. If leaves were dry when they were collected, they were moistened by submerging them in water for 5 min before placing them in the spore release tower. Excess water was removed by gently shaking the screens. Care was taken to place leaves in the screens so they did not overlap and so the faded upper surface of the leaf that faced skyward in the field was facing downward (in the direction of the air flow) in the towers (10). During operation of the spore towers, leaves were wetted about every 6 min by briefly (5 s) removing the screens from the towers and misting the leaves with water from a spray bottle. The duration of each spore release tower test was 2 h. The results obtained with the two spore release towers were compared for differences in magnitude by a paired t test and for differences in the shapes of the cumulative distribution of ascospores released in each tower by a Kolmogorov-Smirnov 2-sample 2-sided test (7). Differences between the towers were not significant ($P > 0.1$) based on either test in any of the years. The results of the two towers were averaged and further comparisons were made with these average values.

The number of ascospores released from leaves in the towers, R (ascospores per leaf), was determined from counts of ascospores deposited on sticky surfaces in the suction traps (13). A normalized cumulative distribution of the number of ascospores collected in the towers, S_T, was derived from these results by

$$S_T(t_j) = \sum_{i=1}^{j} R(t_i) \Big/ \sum_{i=1}^{n} R(t_i) \qquad (6)$$

where n is the total number of tower release tests for each season (n = 24 in 1992 and 1993, 26 in 1994, and 23 in 1995). The function, S_T, has values in the range of 0 to 1. The normalized cumulative distribution of ascospores released in the towers, S_T, was compared to the normalized cumulative distribution for the number of airborne ascospores trapped by the Burkard sampler, S_B, by a Kolmogorov-Smirnov 2-sample 2-sided test (7).

Potential ascospore release per unit of ground area of source. To account for the decrease with time in the number of inoculum-containing leaves in the source, the observed tower release, R, on day t_i (i.e., $R(t_i)$) was multiplied by $N_L(t_i)$, obtained from either equation 1 or 2 as appropriate for the year of the test. This yielded an estimate of the potential release of ascospores per unit of ground area in the field on day t_i, i.e., $R(t_i)N_L(t_i)$, with units of ascospores per m^2. The normalized cumulative release of ascospores per unit of ground area, S_G, was defined as

$$S_G(t_j) = \sum_{i=1}^{j} R(t_i)\,N_L(t_i) \Big/ \sum_{i=1}^{n} R(t_i)\,N_L(t_i) \qquad (7)$$

where the summation is taken over the total number, n, of tower release tests during a season. The denominator on the right side of equation 7 represents the season-total release of ascospores per ground area, indicated by the sum of the products of the release of ascospores in the laboratory spore tower and the number density of leaves in the field source. The function, S_G, has values in the range of 0 to 1. The normalized cumulative release of ascospores per unit of ground area, S_G, was compared to the normalized cumulative distribution for the number of airborne ascospores trapped by the Burkard sampler, S_B, by a Kolmogorov-Smirnov 2-sample 2-sided test (7). The seasonal total release of ascospores obtained from the semiweekly laboratory spore tower release experiments (given by the denominator on the right side of equation 7) is compared (below) with the corresponding values of PAD_{adj} (derived from equation 4) to help put the spore tower results in perspective with the total ascospore supply in the leaf litter and to serve as a rough check of the tower method.

The results for S_{BC}, S_{BF}, S_T, and S_G obtained from equations 5 through 7 were fitted to a logistic function of the form

$$S = 1/\{1 + \exp[-r_L(t - t_{1/2})]\} \qquad (8)$$

by nonlinear regression. The rate and location parameters of the logistic function, r_L and $t_{1/2}$, respectively, are compared below.

RESULTS

Weather conditions and apple development. Daily average air temperatures over the ascospore-sampling season were similar in all 4 years (Fig. 1). For the period between apple green tip and petal fall (Table 1), temperatures were warmer in 1993 than in the other years. The total rainfall for the 1992 sampling season was much higher than for the other years, mostly due to 15.1 cm of rain that fell during a 2-day period at 42 to 43 days after green tip. Rain fell frequently during each season, but rainfall during 1994 and 1995 was more evenly distributed over the season than during the other 2 years. Fruit bud development on McIntosh apple trees at the test location advanced fastest in 1993 and slowest in 1994 (Table 1).

Aerial ascospore concentrations. The highest daily value for average aerial concentration of *V. inaequalis* ascospores occurred during the early part of the season in each of the 4 years (Fig. 2). This was most noticeable in 1993, when about 80% of the season's total ascospores collected by the Burkard sampler occurred before DOY 125. The Burkard sampler collected ascospores during 178, 122, 256, and 157 1-h periods in 1992, 1993, 1994, and 1995, respectively. Maximum observed hourly concentrations were 1,377, 5,600, 13,836, and 3,897 ascospores per m^3 in 1992, 1993, 1994, and 1995, respectively. The majority (>72%) of the ascospores were collected by the Burkard sampler before the petal fall stage of McIntosh apple trees at the test location in all 4 years. The percentages of the seasonal total of ascospores captured before petal fall were 88, 92, 72, and 95% in 1992, 1993, 1994, and 1995, respectively.

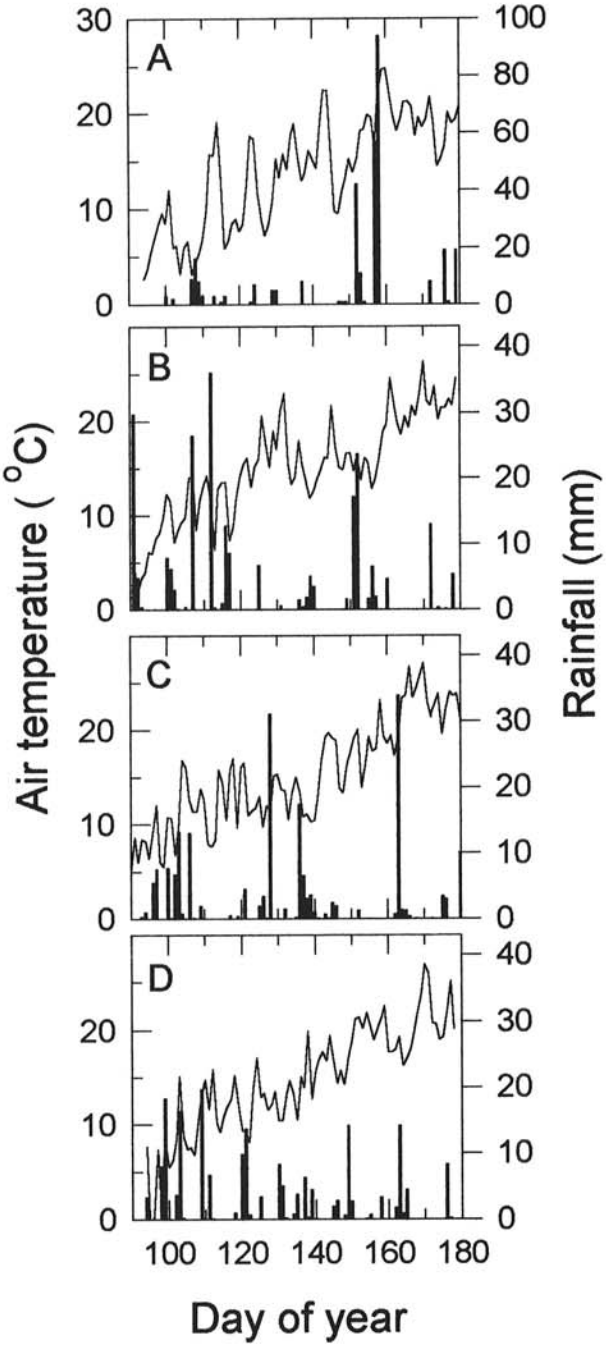

Fig. 1. Average air temperatures (line) and daily rainfall amounts (bars) measured at the test sites in Connecticut during **A to D,** the 1992 to 1995 sampling seasons, respectively.

TABLE 1. Summary of selected weather conditions at test sites in Connecticut during the principal part of the period during which ascospores of *Venturia inaequalis* were released during 1992 to 1995

Year	Green tip[a]	Petal fall[a]	T_{air}[b] (°C)	Growing-degree-days[c]	Rainfall[b] (mm)
1992	115	142	12.8	100	30
1993	108	131	13.9	105	66
1994	102	131	12.5	98	78
1995	103	135	11.8	90	90

[a] Day of year (measured from 1 January) when the green tip and petal fall stages of apple fruit bud development were observed on McIntosh trees at the test location.

[b] Average temperature and rainfall amount during the period between apple green tip and petal fall during each season.

[c] Number of growing-degree-days (base = 10°C) accumulated between green tip and petal fall.

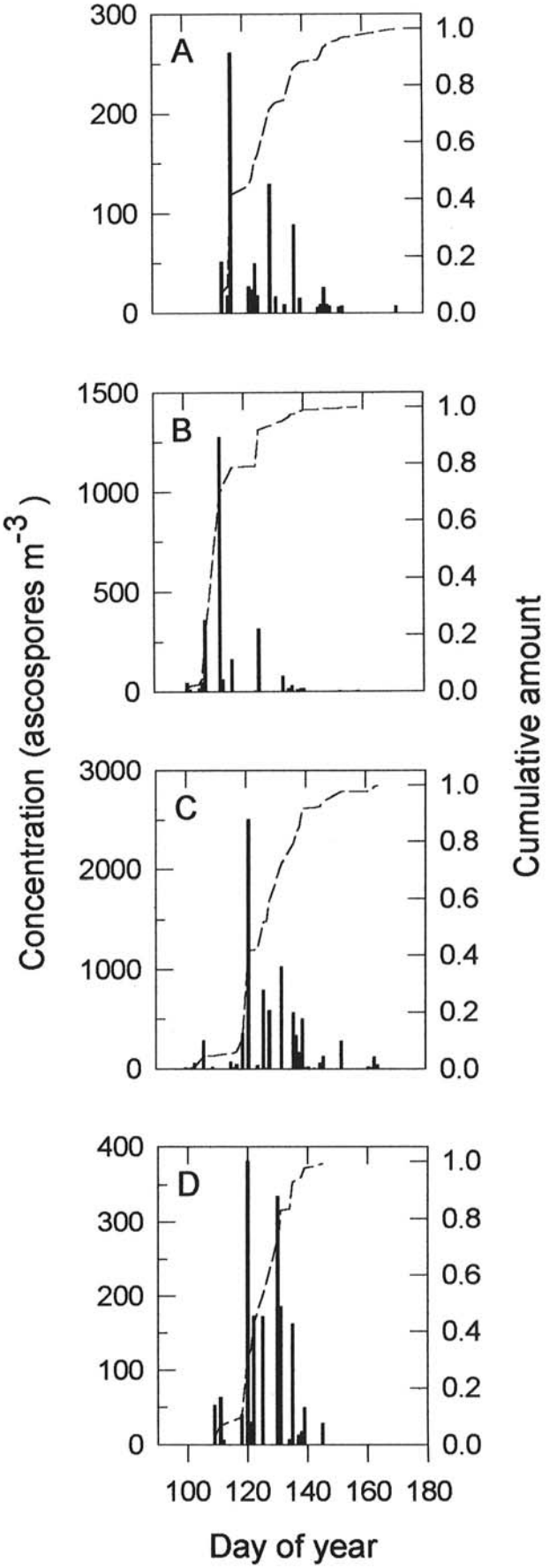

Fig. 2. Daily average concentrations of *Venturia inaequalis* ascospores (ascospores per m^3) measured in the field with a Burkard spore sampler during **A to D,** the 1992 to 1995 sampling seasons, respectively. The dashed lines indicate the relative cumulative number of spores counted for each season.

Ascospore release in a spore tower. The first sizable ascospore release in the laboratory spore towers occurred at about the time when trees were in green tip in 1992, somewhat earlier than green tip in 1993, and several days after green tip in 1994 and 1995 (Fig. 3, Table 1). The overall pattern of ascospore release during each year was marked by one or more release events that accounted for 15% or more of the seasonal total collected in the spore release towers.

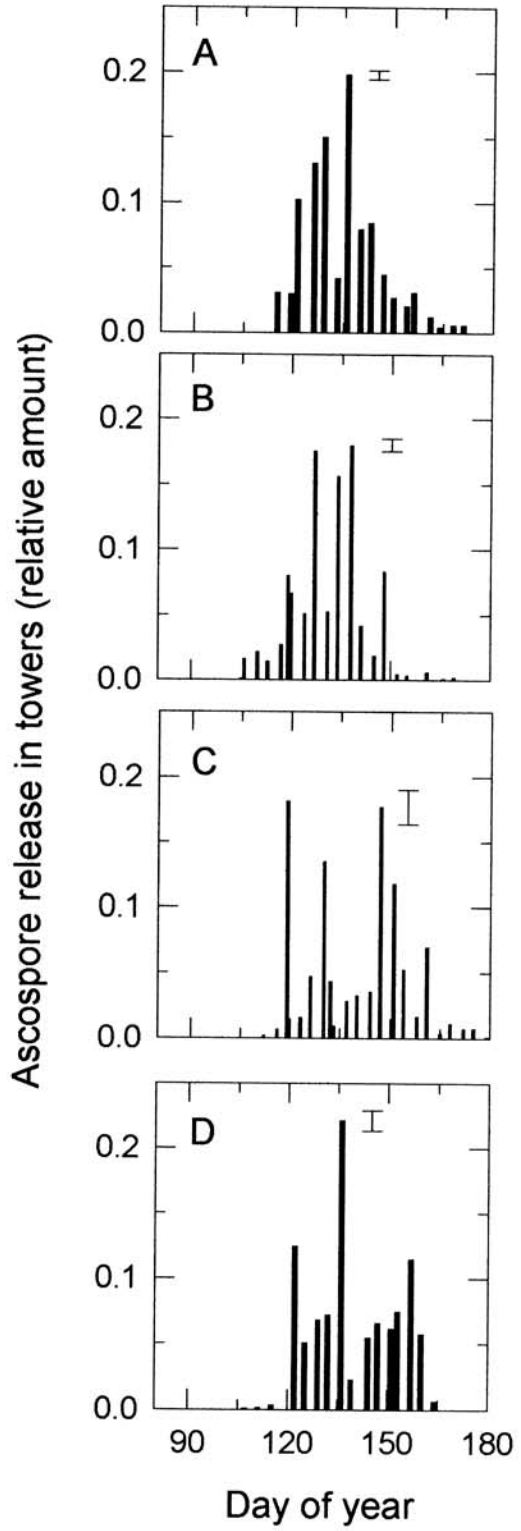

Fig. 3. Relative number (fraction of the season's total) of *Venturia inaequalis* ascospores collected in spore release towers from leaves collected from the field on the indicated days during **A to D,** the 1992 to 1995 ascospore-release seasons, respectively. The "I" bar in each panel represents twice the standard error of the differences between the towers.

Number density of source leaves and potential ascospore dose. The decrease in the number of scabbed apple leaves per unit of ground area, N_L, in the test sites was described by equation 1 in 1994 ($r^2 = 0.77$) and by equation 2 in 1992, 1993, and 1995 ($r^2 =$ 0.66, 0.75, and 0.78, respectively; Fig. 4). N_L decreased with time most rapidly in 1993 and least rapidly in 1994. At season's end, values of N_L were very similar in the 1992, 1993, and 1995 seasons. *PAD* was estimated by equation 1 to be about 8.0, 15.0, 27.0, and 9.9×10^7 in 1992, 1993, 1994, and 1995, respectively. Values of potential ascospore dose adjusted for attrition of source leaves during the season, PAD_{adj} (equation 4), were 0.34, 0.32, 0.64, and 0.45 as large as *PAD* in 1992, 1993, 1994, and 1995, respectively.

Comparison of tower data with Burkard sampler data. Functions S_{BC}, S_{BF}, S_T, and S_G were described by a logistic function of time (Table 2). The rate parameters were larger, and the values of $t_{1/2}$ were smaller for both S_{BC} and S_{BF} than for S_T in all 4 years. Wind speed had a relatively minor effect on the fitted parameters for the results obtained by the Burkard sampler, as shown by the comparison between S_{BC} and S_{BF}. Function S_{BF} reached a final plateau value equal to 1.0 earlier than S_T in all 4 years (Fig. 5).

After accounting for the temporal decrease in the leaf litter density by equation 7 (Fig. 5, dotted lines), the agreement between the tower and Burkard sampler results was improved. The distributions for the Burkard sampler and tower results were significantly different ($P = 0.032$, 0.015, and 0.008) in three (1992, 1993, and 1995) of the four years (Table 3). When the attrition of source leaf area was included, the differences between the cumulative distributions for the Burkard sampler and tower results were no longer significant ($P > 0.1$), indicating that the attrition of source leaves could account for at least part of these differences.

The season-total airborne flux of ascospores indicated by the Burkard sampler, the $\Sigma(CU)$, and the release of ascospores from the source indicated by the laboratory spore towers, $\Sigma(RN_L)$, were highly correlated (Table 4). Moreover, the magnitudes of PAD_{adj} and $\Sigma(RN_L)$ were close, indicating that *PAD*, adjusted for attrition of leaf litter, provided a reasonable measure of seasonal ascospore release.

DISCUSSION

There was a strong tendency in all 4 years (Fig. 5) for a smaller percentage of the season-total ascospores to be airborne in the field later in the season than might be suggested by the results of the laboratory spore tower experiments. The lag between the cumulative

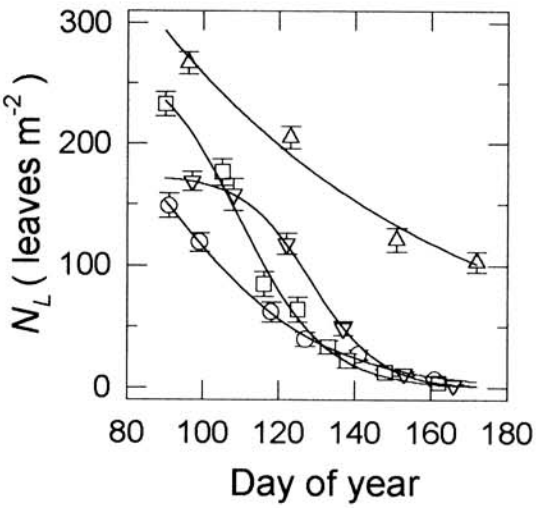

Fig. 4. Time course of the number of inoculum source leaves per m^2 of ground area, N_L, in the test sites during the 1992 (○), 1993 (□), 1994 (△), and 1995 (▽) ascospore-release seasons. The "I" bars represent the standard errors, and the lines through the data represent equation 1 (1994) or equation 2 (1992, 1993, and 1995).

curves for the results from the Burkard sampler (S_{BF}) and the spore tower (S_T) indicated that most ascospores were airborne in the field during the first half of the season, suggesting there is seasonal attrition of the potential supply of ascospores. The lag was most dramatic in 1993 (18 days, Table 2), which may reflect the generally higher average temperatures (Fig. 1, Table 1) during that year.

There are at least four effects that could contribute to the observed lags between the Burkard sampler and tower results. The first is due to inherent differences in the way the Burkard sampler and spore towers sample the release of ascospores, and the other three are due to physical effects: attrition of source leaves over the course of the season, changes in leaf orientation due to mowing, and deposition of ascospores in ground cover. The relative importance of these effects will be discussed below.

Tower results are expected to give a generally good indication of the ascospore maturation rate and release potential because ascospores that are physiologically ready-to-be-released in the field will be released, providing temperature and light conditions are conducive and there is sufficient wetting of the inoculum-harboring leaves (15,18). However, a certain amount of lag between Burkard sampler and laboratory spore tower results can be artificially introduced by the sampling procedure. The reason for the lag is purely mathematical and due to the accumulation of two or more laboratory spore tower release results from leaves collected in the field during the period between ascospore release (rainfall) events. If this is the case, a certain portion of the ascospore supply will be counted twice and can introduce an artificial lag into the cumulative curve for S_T.

The magnitude of the lag introduced by the sampling procedure was estimated by a simple model for the time course of ready-to-be-released ascospores. The model is written as a balance between the maturation of ascospores and their release during rainfall events. Specifically, the time course for the fraction, $F(t)$, of the total number of ascospores that are ready-to-be-released when triggered by moisture was modeled by

$$F(t_n) = \int_0^{t_n} \frac{dY(t)}{dt}\, dt - \sum_{i=1}^{n} Q(\tau_i)\, u(t - \tau_i) \qquad (9)$$

where $Y(t)$ is the fraction of the total ascospore pool ($0 \le Y \le 1$) matured by time, t, $Q(\tau_i)$ is the fraction of the total pool of ascospores released into the air at time τ_i, and u represents the unit step response function (19) defined by

TABLE 2. Parameter values obtained by fitting the logistic function, $S = 1/\{1 + \exp[-r_L(t - t_{1/2})]\}$, to the normalized cumulative release of *Venturia inaequalis* ascospores in spore towers in the laboratory, S_T; the normalized cumulative release of ascospores/unit of ground area in the field, indicated by the product of the release of ascospores in the laboratory spore towers and the number of leaves/unit of ground area in the field, S_G; the normalized cumulative number of ascospores collected by the Burkard volumetric spore sampler in the field, S_{BC}; and the normalized cumulative flux of ascospores indicated by the Burkard sampler in the field, S_{BF}

Year	Function[a]	r_L[b] (per day)	$t_{1/2}$[b] (days)	r^2[c]
1992	S_T	0.156	132.1	0.996
	S_G	0.183	127.5	0.996
	S_{BC}	0.149	124.9	0.973
	S_{BF}	0.201	122.7	0.969
1993	S_T	0.165	129.4	0.997
	S_G	0.181	122.2	0.995
	S_{BC}	0.349	111.9	0.956
	S_{BF}	0.414	111.6	0.975
1994	S_T	0.104	137.3	0.984
	S_G	0.106	134.1	0.981
	S_{BC}	0.171	126.8	0.989
	S_{BF}	0.188	125.5	0.989
1995	S_T	0.120	138.2	0.986
	S_G	0.197	128.0	0.992
	S_{BC}	0.210	124.5	0.996
	S_{BF}	0.217	123.9	0.996

[a] Functions S_{BC}, S_{BF}, S_T, and S_G are defined in the text by equations 5a, 5b, 6, and 7, respectively.

[b] r_L and $t_{1/2}$ are the rate and location parameters, respectively, for logistic function $S = 1/\{1 + \exp[-r_L(t - t_{1/2})]\}$ fitted to the cumulative distributions for S_{BC}, S_{BF}, S_T, and S_G.

[c] r^2 is the coefficient of determination for the nonlinear regression fits.

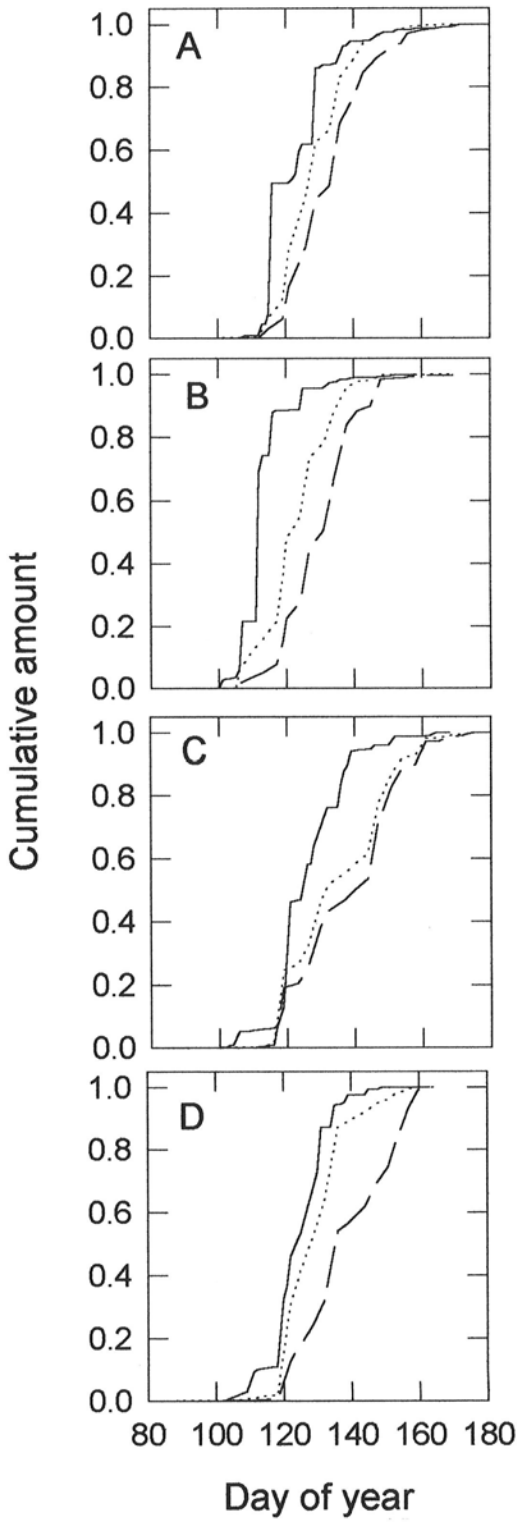

Fig. 5. Comparison of cumulative numbers of *Venturia inaequalis* ascospores collected with a Burkard volumetric spore sampler (S_{BF}, solid line) and spore release towers (S_T, long-dash line) during **A to D,** the 1992 to 1995 ascospore-release seasons, respectively. The short-dash lines, the middle lines, indicate S_G (described by equation 7), which accounts for the temporal decrease in the number of source leaves per unit of ground area.

$$u = 1 \text{ for } t \geq \tau_i \qquad i = 1,2\ldots n$$
$$u = 0 \text{ for } t < \tau_i \qquad (10)$$

τ_i marks the occurrence of the ith rain event during the ascospore-release season, and n is the number of rain events. Y was chosen to be a logistic function of time (17,18). The release, Q, during a rain event is equal to αF, where $\alpha(\cong 1)$ is the fraction of ready-to-be-released ascospores, F, discharged during a rain event. Thus, the second term on the right side of equation 9 represents the accumulation of ascospores released during successive rain events.

Equation 9 describes a "saw-tooth" pattern for F that increases gradually between rain events and then drops sharply when ascospore release occurs during a rain event (Fig. 6). A function comparable with S_T was obtained by integrating equation 9 with respect to time and dividing the result by the season-total ascospore release. This yields an equation for the normalized cumulative release of ascospores in the tower, S_M, analogous to equation 6:

$$S_M(t) = \int_0^t F(t)\,dt \Big/ \int_0^{t_f} F(t)\,dt \qquad (11)$$

where t_f marks the end of the ascospore-release season.

The general shapes of Y, F, and S_M are shown in Figure 6. The lag between Y and S_M is indicative of the lag introduced by the tower sampling method and depends on the length of time, Δt, between ascospore release (rainfall) events in the field. This lag was evaluated by performing the following steps: (i) equation 11 was evaluated to obtain S_M, (ii) S_M was fitted to a logistic function (equation 8) by nonlinear regression analysis, and (iii) the fitted function was compared to the function for Y. The lag was taken to be the difference in the $t_{1/2}$ values for the two functions. This procedure was carried out for a range of time periods between rain events, Δt. The lag between Y and S_M was estimated to be less than 1 day when $\Delta t \leq 2$ days and less than 5 days when $\Delta t \leq 15$ days. The mean number of days between measurable rainfall was 4.7 (range 1 to 13) in 1992, 3.6 (range 1 to 10) in 1993, 2.8 (range 1 to 7) in 1994, and 2.4 (range 1 to 5) in 1995 (Fig. 1). Because of the relatively frequent occurrence of ascospore release (rainfall) events during the experiments, any lag introduced by the sampling procedure is expected to be small compared to the lags observed (7 to 18 days, Table 2).

Breakdown of leaf litter can have a direct effect on reduction of airborne ascospores. A major portion of the lag between the Burkard sampler and tower results can be explained by seasonal attrition of inoculum source leaves (Fig. 5, dotted lines). In three of the four years, differences between the cumulative distributions for S_{BF} and S_T were significant, whereas differences between S_{BF} and S_G were not (Table 3).

It is possible that the potential number of airborne ascospores was reduced further when some of the source leaves were turned upside down and broken up during mowing. This disturbance could reduce the effective discharge of ascospores into the air because of the tendency for pseudothecia to be formed on the upper surface of overwintering leaves (10). In this way, mowing (which occurred after midseason) could have reduced late-season ascospore release in 1992 and 1993 but not in 1994 and 1995 when the grass in the source area was not mowed. Unfortunately, orientation of leaves on the ground as to which side was up after mowing was not recorded.

Finally, a certain fraction of the released ascospores could be trapped in the grass ground cover late in the season and contribute to the lag between S_{BF} and S_T, because these would not be accounted for by the Burkard sampler. Deposition of ascospores in ground cover would result in attrition of potentially airborne ascospores. Previous calculations of transport and escape of *V. inaequalis* ascospores from grass ground cover (1,5) indicate that most of the released ascospores (>50% of those released) are likely to escape from ground cover into the freely moving air above. Thus, deposition is expected to reduce airborne ascospores by, at most, a factor of 2. This reduction is relatively small compared with the nearly 100-fold decrease in N_L noted in three of the four seasons.

In summary, several factors could have contributed to the lag between the Burkard sampler and laboratory spore tower results (Fig. 5). Of those examined, the seasonal decrease in the number

TABLE 3. Comparisons of the cumulative distribution of fluxes of *Venturia inaequalis* ascospores measured by a Burkard volumetric spore sampler in the field, S_{BF} (equation 5b) with the distribution obtained for release of *V. inaequalis* ascospores in spore towers in the laboratory, S_T (equation 6); and S_{BF} with the distribution of release of ascospores in spore towers after correcting for attrition of source leaves in the field, S_G (equation 7), during the springs of 1992 through 1995

Year	Comparison	P[a]
1992	$S_{BF} * S_T$	0.032
	$S_{BF} * S_G$	0.183
1993	$S_{BF} * S_T$	0.015
	$S_{BF} * S_G$	0.150
1994	$S_{BF} * S_T$	0.128
	$S_{BF} * S_G$	0.247
1995	$S_{BF} * S_T$	0.008
	$S_{BF} * S_G$	0.338

[a] P value for the indicated comparison according to the Kolmogorov-Smirnov 2-sample 2-sided test (7).

TABLE 4. Correlations between summation over the season of airborne fluxes of *Venturia inaequalis* ascospores, $\Sigma(CU)$ (equal to the denominator of the right side of equation 5b); summation over the season of the potential release of ascospores/unit of ground area, $\Sigma(RN_L)$ (equal to the denominator on the right side of equation 7); and potential ascospore dose of ascospores in the field adjusted for attrition of source leaves, PAD_{adj} (equation 4), during the springs of 1992 through 1995

Year	$\Sigma(CU)$[a] (ascospores/m^2/s^1)	$\Sigma(RN_L)$[a] (ascospores/m^2)	PAD_{adj} (ascospores/m^2)
1992	1,141	3.8×10^7	2.7×10^7
1993	3,469	11.7×10^7	4.8×10^7
1994	13,634	30.4×10^7	17.3×10^7
1995	2,630	4.8×10^7	4.4×10^7
Pearson correlation matrix			
$\Sigma(CU)$	1.000		
$\Sigma(RN_L)$	0.985 ($P = 0.044$)[b]	1.000	
PAD_{adj}	0.999 ($P = 0.003$)	0.978 ($P = 0.066$)	1.000

[a] The summations are taken over the number of sample dates (described in text).
[b] Bonferroni adjusted probabilities.

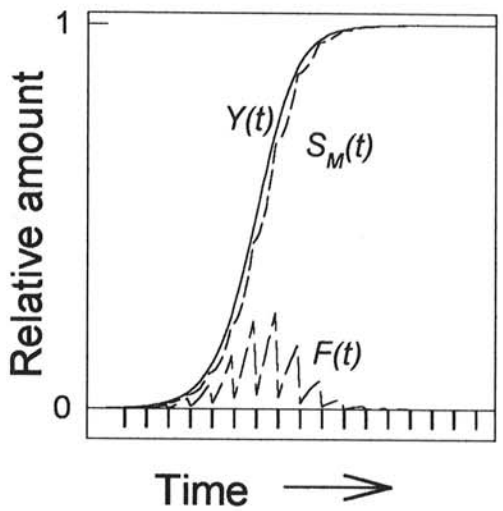

Fig. 6. Modeled seasonal variation of *Venturia inaequalis* ascospore maturation, $Y(t)$, potential ascospore release, $F(t)$, and the integral of $F(t)$ over the season, $S_M(t)$ (described by equations 9 through 11). The vertical bars below the x-axis indicate ascospore release (rainfall) events.

of source leaves appears to have contributed the most in the current experiments.

Spore release towers provide a relatively simple and direct laboratory method for determining the potential for ascospore release in the field prior to rain events. Their utility has recently been enhanced by methods that allow ascospores to be readily identified at low magnification under field conditions (6). The study reported here should aid in our understanding and interpretation of spore tower tests in relation to the potential number of airborne ascospores that could result from their release from a source of inoculum. The results also indicate that the breakup of leaf litter can have a significant effect on inoculum dose, especially late in the season.

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