

Active Discharge Distance of Ascospores of *Venturia inaequalis*

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ABSTRACT

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Ascospores of *Venturia inaequalis* were discharged into still air inside small chambers from pseudothecia on small wetted bits of leaf cut from diseased apple leaves that had overwintered on the orchard floor. Ascospores were actively projected away from the leaf surface over distances ranging from 0.1 to 8.1 mm (one reached 13.2 mm). Three-quarters

of the ascospores were projected less than 4.1 mm from the surface (mean distance for all ascospores was 3.0 mm). Only 1% were projected as far as 6.6 mm. The observed discharge distances were shown to be consistent with those expected from the mechanical forces acting on the ascospores.

Additional keyword: apple scab.

To calculate the likelihood of primary apple scab infections on developing apple (*Malus domestica* Borkh.) tissue, it is necessary to know, among other things, the airborne dose of ascospores of *Venturia inaequalis* (Cooke) G. Wint. in the orchard. Ascospores are released from overwintering leaves on the orchard floor (15,16,18). The potential ascospore dose (PAD) of *V. inaequalis* can be estimated in terms of pseudothecial and ascus densities on fallen leaves and on leaf litter density on the orchard floor (11). The actual airborne dose (AAD) of ascospores depends on the fraction of the PAD that becomes airborne. Estimates of PAD are uncertain because of natural variation in the densities that go into its calculation. In estimating AAD from PAD, it is tempting to assume that eight ascospores per ascus are projected sufficiently far from the leaf surface to become entrained into the turbulent air and, thus, potentially carried to infection sites (11). There are few references to studies on the distance of active

discharge of *V. inaequalis* ascospores. Mills and Dewey (19) reported that ascospores shoot out about 6.4 mm from leaves and Aderhold (1) reported a maximum distance of 15 mm.

If only one or two of the ascospores in an ascus are projected far enough to escape the relatively still layer of air near the surface of the leaf, then values of AAD may be only one-fourth to one-eighth of PAD because of this factor alone. Apple leaves that are nestled down inside a grass canopy will be exposed to a much reduced wind speed as compared with leaves on bare ground (3). In addition, leaves will be exposed to highly intermittent wind, consisting of relatively long quiescent periods and occasional gusts (3). The settling speed of ascospores of *V. inaequalis* is about 2 mm/s (12). Thus, if the lulls between gusts last 3 s or longer, then ascospores that are projected less than about 6 mm from the leaf will have a high probability of being deposited on the ground during these lulls in the wind. Models of spore transport that incorporate these features of the wind are currently under development (3).

In this paper, we report measured discharge distances of

ascospores of *V. inaequalis* and relate these distances to mechanical forces acting on the spores.

MATERIALS AND METHODS

Diseased apple (cultivar MacIntosh) leaves that had overwintered on the orchard floor were brought into the laboratory on 26 April 1990, and stored dry at 4 C until used (within the next 4–40 days). Small pieces, about 3-mm square, containing one to several pseudothecia were excised from these leaves and used in the discharge studies described.

Ascospore discharge took place in small closed chambers that were 1 cm high, 2.5 cm wide, and 5 cm long. One end was open until the introduction of the sample, whereupon it was closed. These dimensions assured that the Rayleigh number (23) was well below the critical value for which free convection air currents are initiated. To further insure against the possibility of air currents in the chambers, leaves were placed on a heat reservoir kept at 1.5–2.0 C below ambient temperature. Good thermal contact between the discharge chambers and the reservoir was maintained by a thin film of water under the chambers. Thus, temperature gradients in the chambers were stable, and they suppressed any tendency for free convection (23). Two similar chambers were constructed, one made from glass slides and one from 1.7-mm thick pieces of aluminum (the end where the sample was placed was glass). There were no differences in the results with the two chambers.

A coverslip (22 × 50 mm) was placed on the bottom of the chamber so that its end was flush with the open end of the chamber, where the sample was introduced. A small piece of leaf was wetted and attached near the end of a microscope slide with a small drop of water. This slide was placed vertically against the open (1 × 2.5 cm) end of the chamber (leaf side in) and held in place with a rubberband. Thus, ascospores were projected away from the sample in a horizontal direction above the coverslip and allowed to settle onto it in the still air inside the chamber.

One to two hours after introducing the sample into the chamber, the chamber was opened and the coverslip was removed. The number of ascospores deposited at various distances from the end of the coverslip was determined by counting (at ×250) all ascospores in 0.25-mm wide transects across the width of the coverslip. The distance of each transect from the end was measured with the stage micrometer on the microscope. In addition, the coverslip was carefully scanned to measure the distance of the ascospore farthest from the end. Twenty replicates of this experiment were done and more than 5,000 spores were counted.

A limited number of observations were made of the discharge of ascospores into water. Single pseudothecia were excised and placed in water in the counting chamber (0.1 mm deep) of a hemacytometer. Asci extruded from the pseudothecium and were watched until ascospores were discharged. The orientation and distance of the ascospore farthest from the opening of the pseudothecium was measured with an ocular micrometer. The temperature of the water in the well was warmed above ambient by the microscope light and was estimated by measuring the temperature in the loading notch as close as possible to the well by using a fine thermocouple (0.12-mm copper-constantan wire). This measurement was made after a length of time that corresponded to the time it usually took for discharge to occur.

To obtain an estimate of the osmotic potential inside the asci, we examined at least two pseudothecia in each of 13 sucrose solutions with molalities ranging from 0.1 to 4.0 and in water as a control. Leaf pieces that had been stored dry at 4 C and contained pseudothecia of *V. inaequalis* were placed in the solutions to allow hydration. Individual pseudothecia were carefully removed, placed in a drop of sucrose solution of the same molality on either a microscope slide or a hemacytometer, and examined at ×500 with and without phase contrast. Some of the pseudothecia were gently crushed open by applying slight pressure to the cover glass. Ascospores were forcibly discharged only when the pseudothecia were intact and the pseudothecial opening was not obstructed. Asci that were free of the pseudo-

thecium or exposed by a break in the body sometimes expanded, and occasionally oozed out ascospores, but never forcibly discharged them.

RESULTS

The cumulative number of ascospores with distance (Fig. 1) suggests that 99% of the ascospores were projected less than 6.6 mm from the sample surface. The mean discharge distance of all ascospores observed was 3.0 mm. The farthest distance an ascospore was projected, d_F , in individual experiments ranged from 5.1 to 13.2 mm (mean = 7.1 mm; SD = 1.7 mm). The 5,071 ascospores counted represented about half of the total ascospores deposited on the coverslips. Cumulative distributions for individual experiments and comparison with our model calculations are shown in Figure 2.

In water, d_F ranged from 0.20 to 0.25 mm. The temperature of the water in the observation well averaged about 28 C. When asci were placed in both 2.5 and 3.0 molal sucrose solutions, the endotunica appeared to be form-fitted or “shrink-wrapped” around the ascospores; whereas, at 2.0 molal sucrose solution, it did not appear to be shrink-wrapped. The molality at which this change occurred was taken as an estimate of the internal osmotic potential of the asci.

DISCUSSION

Distance of farthest projection. The paths of the horizontally discharged ascospores in the present experiment were expected to follow the “sporobolic” path described by Buller (5,17). The farthest distance to which an ascospore is actively discharged (projected) from the ascus before it settles onto the coverslip is the stop distance, d_S (cm), which can be obtained by integrating Newton's second law of motion (10):

$$m (du/dt) = -F_D \quad (1)$$

in which, u is the instantaneous horizontal speed of the ascospore at time t , m is the mass of the ascospore, and F_D is the aerodynamic drag acting on the spore.

Equation 1 was solved with the initial condition $u(t = 0) = U_0$, in which U_0 is the initial horizontal speed of the spore. The drag force on a spore is given by (8,21):

$$F_D = 0.5 \rho_a C_D A u^2 \quad (2)$$

in which A is the projected cross-sectional area of the spore, ρ_a is the density of air, and C_D is the drag coefficient. Over the

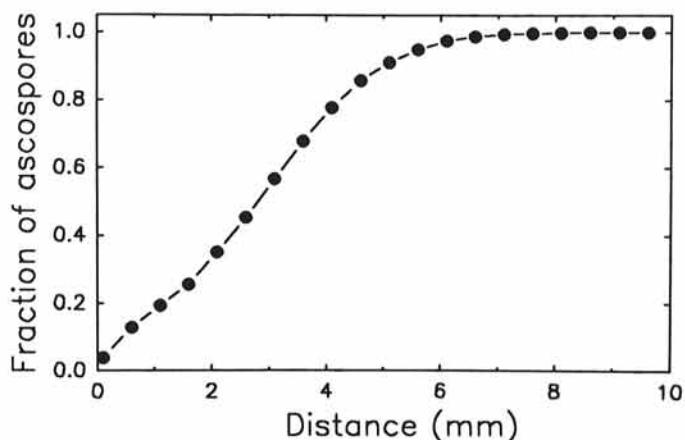


Fig. 1. The cumulative fraction of ascospores of *Venturia inaequalis* actively projected to various distances in still air for the combined totals for 20 replicate experiments. Numbers have been normalized by dividing by the total counted (5,071).

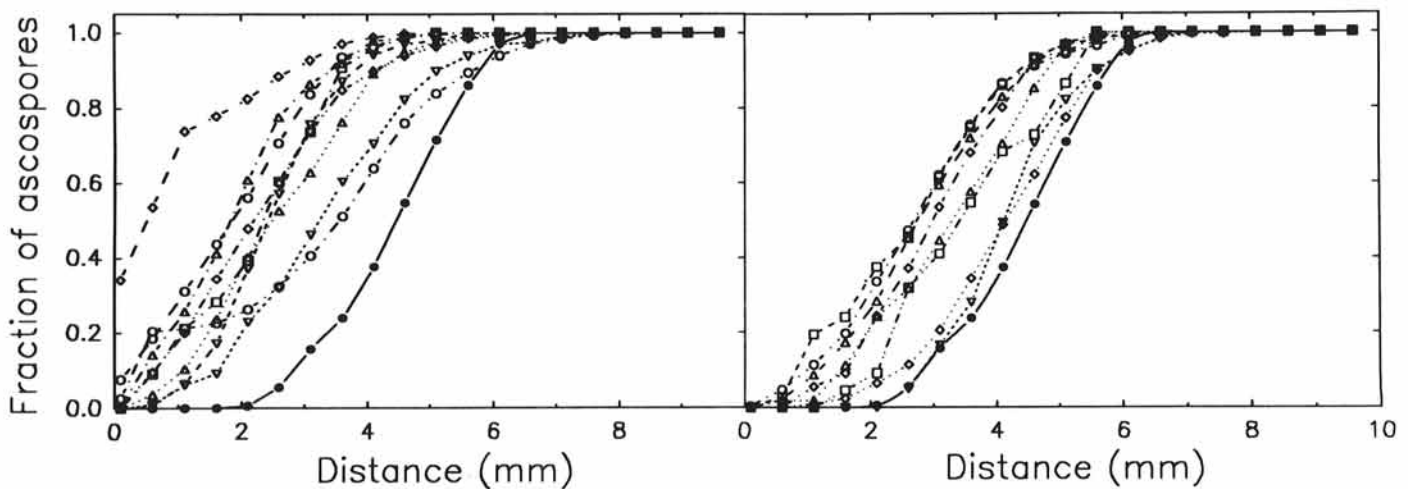


Fig. 2. The cumulative fraction of ascospores of *Venturia inaequalis* actively projected to various distances in still air for individual experiments (open symbols and broken lines) and the results of the model calculation (dots and corresponding solid line). Results have been plotted in two panels for clarity.

range of Reynolds numbers, Re , of importance here, C_D can be approximated by (9):

$$C_D = 24 Re^{-1}(1 + 0.158 Re^{2/3}) \quad (3)$$

in which $Re = uD_p/\nu$, in which D_p is the aerodynamic diameter (8) of an ascospore, and ν is the kinematic viscosity of air (21). Equations 1–3 can be solved to yield the following expression for the stop distance (10):

$$d_S = B(0.158^{1/2} Re_0^{1/3} + \tan^{-1}(0.158^{-1/2} Re_0^{-1/3}) - \pi/2) \quad (4)$$

in which $B = (D_p \rho_p / 0.158^{3/2} / 6 \rho_a)$. ρ_p is the density of an ascospore and Re_0 is the initial Reynolds number (i.e., $U_0 D_p / \nu$). To calculate Re_0 , we set D_p equal to the Stokes' equivalent diameter of the spore (14). The settling speed, ν_s , of ascospores of *V. inaequalis* is about 0.2 cm s^{-1} (12), which leads to a value of $D_p = 8.16 \mu\text{m}$. We used equation 4 in a model for the spatial distribution of deposited ascospores and adjusted the value of U_0 until the solution gave a value for the mean discharge distance, d_M , about equal to that for the experiment with the largest d_M (4.4 mm). This gave a value of U_0 of about 58 m s^{-1} and a value of d_S of about 6.7 mm.

In nature, many ascospores of *V. inaequalis* will be projected vertically. Their upward motion will be slowed by the force of gravity (i.e., $m g$, in which g is gravity) in addition to F_D . The weight of an ascospore is very small compared with F_D , except when its outward motion has essentially been arrested by the drag. Therefore, the distance of the farthest projection is not significantly different for an ascospore projected vertically or horizontally. We calculated that the force of gravity would reduce d_S for a vertically projected ascospore, by only about 0.04%.

The impulsive force, F (dynes), which must act on the spore to result in an initial speed (U_0) can be estimated again from Newton's law, written in a form applicable to impulsive motion (13):

$$F = m \delta u / \delta t \quad (5)$$

in which m is the mass of the spore (estimated from ascospore dimensions to be about 30 ng), and δ represents an incremental change in a quantity (13). To estimate F , we first estimated the time period δt over which the expulsion force acts on the ascospore. We assumed that this mechanical force acts during the time it takes for the length of the spore to pass the end of the ascus, i.e., $\delta t = L_S / U_0$, in which L_S is the length of the spore (taken in our calculations to be $14.7 \mu\text{m}$). Thus, we find that:

$$F = m U_0^2 / L_S \quad (6)$$

For the values of m , U_0 , and L_S given above, F is about 7 dyn for the first spore ejected.

The force acting on the outermost ascospore just before discharge should be approximately equal to the hydrostatic pressure inside the ascus multiplied by the projected area of the end of the ascospore. This projected area ranged between 19.6 and $28.3 \times 10^{-8} \text{ cm}^2$ for ascospores in our experiments. Thus, a force of 7 dyn corresponds to hydrostatic pressures ranging between 2.5 and 3.6 MPa. By analogy to the relationships between turgor pressure and incipient plasmolysis in plant cells (22), we assumed that the hydrostatic pressure inside a fully turgid ascus is approximately equal in magnitude to the osmotic potential of a solution that would cause the ascus to shrink around the ascospores. Thus, based on this, these pressures should correspond to sucrose solutions with molalities of about 1.0–1.3 (7,24). Our observations suggest that zero turgor pressure (marked by the transition to shrink-wrapping) inside the asci occurs for external sucrose solutions with molalities of between 2.0 and 2.5. Thus, our estimate of the force acting on the outermost ascospore in an ascus just before discharge seemed reasonably consistent with the water relations of the ascus. Our estimate of this force was less than that suggested by the osmotic potential, as it must be, because there were energy losses that occurred during the rupture of the endotunica that were not accounted for by our model.

Relative discharge distance in water and air. Because of the differences in the density and viscosity of water and air, the ratio of stopping distances in water and air ($d_{\text{Sair}}/d_{\text{Swater}}$) should be given approximately by (9,10):

$$d_{\text{Sair}}/d_{\text{Swater}} = ([\rho_p + \rho_a/2] \mu_w) / ([\rho_p + \rho_w/2] \mu_a) \quad (7)$$

in which ρ_w is the density of water, and μ_a and μ_w ($\text{g cm}^{-1} \text{ s}^{-1}$) are the viscosity of air and water, respectively. Taking ρ_p to be 1.05 g cm^{-3} , and evaluating the densities and viscosities of the fluids at the appropriate temperatures (18 C for air and 28 C for water) in the chambers, this ratio is about 32. Thus, our observations of the average distance of the farthest discharge in water and air (ratio $\sim 7.1/0.22 = 32$) were consistent with each other.

Spatial distribution of deposited ascospores. As each successive spore is discharged there is a decrease in pressure inside the ascus (and thus force on the leading ascospore) because of the reduction of internal volume equivalent to the ejected ascospore plus a small amount of accompanying fluid (5). Just before discharge, the endotunica (2,20) expands by increasing its length to about twice that in the relaxed state, while its diameter increases very little (unpublished data). Changes in the internal volume of asci are associated mainly with changes in their length. Thus, it is reasonable to expect that the internal volume (and thus the pressure

inside the endotunica) will effectively decrease linearly as ascospores are successively discharged.

This decrease in pressure inside an ascus will result in a smaller force acting on the ascospores discharged successively and, thus, they should be projected a shorter distance. This reduction in force can account for much of the spatial distribution of deposited ascospores (Fig. 2). There were at least three other factors that could have contributed to the observed spatial distribution of spores on the coverslips: the angle, with respect to the horizontal, at which the spores were discharged; random variation in the initial force acting on spores because of variations in pressure inside the ascus; and variations in the amount of force lost during rupture of the endotunica.

We constructed a simple model based on equation 4 and on the assumptions that the force acting on each successive ascospore discharged from an ascus decreased linearly, that the angle of discharge was a random variable with values between 0 and 45°, and that the initial force inside individual asci varied randomly by about 30%. The calculation of stopping distance was repeated for 700 asci (5,600 ascospores). From these results, we constructed a theoretical cumulative distribution (Fig. 2) for comparison with the data. Clearly, our simple model does not account for everything. However, considering that U_0 is the only parameter that was adjusted, it was encouraging that the calculated results tended to mimic the general shape for most and the magnitude for some of the observed results (Fig. 2).

Much of the observed variation between experiments was likely because of differences in the initial force inside asci or to differences in the energy lost during rupture of the endotunica. It is also possible that some asci did not quite penetrate a thin film of water that may have been on the outer surface of the wetted leaf pieces. In this case, most of the energy of release may have been dissipated in breaking through this thin film of water (4,6). Because of energy losses in moving through the water and in overcoming surface tension, the thickness of such a film of water must be less than 0.20–0.25 mm for ascospores to escape (see Results). This energy loss cannot readily be distinguished from that which occurs during rupture of the endotunica. To go much beyond the simple model presented here would require measurements of the strength and the elasticity of the ascus material and of the energy dissipation during release, which is beyond our present capabilities.

Mature ascospores are discharged once the leaf tissue is sufficiently wetted, regardless of whether atmospheric conditions are windy or calm. Our results suggest that the majority of the ascospores of *V. inaequalis* will be projected less than 4 mm from the leaf surface. Thus, if they are released during essentially calm periods lasting 2–3 s, as can occur close to the ground inside a ground-cover canopy (3), they will have a relatively high probability of being deposited on the ground and thus be lost to dispersal. Models of intermittent airflow in canopies are currently under development (3). The incorporation of the active discharge distance of ascospores into these canopy flow models should permit better estimates of airborne ascospore dose from potential ascospore dose.

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