Germination and Appressorium Formation by *Venturia inaequalis* During Infection of Apple Seedling Leaves

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ABSTRACT

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The rate of germination and appressorium formation of both conidia and ascospores of *Venturia inaequalis* was directly proportional to temperatures from 5 to 20 C, but condia germinated and formed appressoria more quickly than ascospores. Multiple-regression equations were developed to describe the processes of germination and appressorium formation on the basis of time and temperature.

Additional key words: apple scab, epidemiology

Ascospores and conidia of *Venturia inaequalis* (Cke.) Wint. are the primary and secondary inoculum, respectively, in apple scab. Both types of spores require free water to infect susceptible tissue (4). Although Clayton (2) and Louw (6) reported that a small percentage of spores germinated in the absence of free water at relative humidities near saturation, infection has not occurred under these conditions. The number of hours of leaf wetness required for infection is dependent on temperature. Mills (9) published a graph in 1944 and Mills and LaPlante (10) published a table in 1951 that defined the conditions of temperature and hours of leaf wetness needed for infection in an orchard with an abundance of primary inoculum. Preece and Smith (13) reported that under field conditions, hours of relative humidity above 90% could be substituted for hours of leaf wetness, because such high humidity usually coincided with rain, dew, and leaf wetness. At any temperature, increasing the hours of leaf wetness increased the incidence of infection. Mills and LaPlante's table (10) dealt only with primary infection. Secondary (conidial) infection was reported to occur in about two-thirds of the time required for primary infection (10). In laboratory studies, however, Keitt and Jones (4), Moore (11), Roosje (14), and Schwabe (16) reported that ascospores initiate infections in less time than conidia.

Suprisingly little is known of the effects of temperature on germination and appressorium formation of *V. inaequalis* on its host. Although several researchers have attempted to determine the minimum time required for infection at various temperatures (7,11,14,16,17), no one has attempted to observe and quantify the processes of germination and appressorium formation by *V. inaequalis* on apple foliage. We are currently developing a system to forecast lesion development in commercial apple orchards (8), and models to describe the effects of temperature on the infection process of *V. inaequalis* are an important part of this system. In the present study, we have observed and quantified the effects of temperature on the processes of germination and appressorium formation of ascospores and conidia of *V. inaequalis* during infection of apple seedling leaves.

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MATERIALS AND METHODS

Apple seedlings. McIntosh apples were harvested in September and stored for 16 wk at 0 C. The seeds were then removed and planted in flats of moistened perlite. After 3 wk, the seedlings were transplanted to 10-cm-diameter pots of sterilized soil and peat. The seedlings were fertilized with Osmocote 14-14-14 (Sierra Chemical Co., Milpitas, CA) at the rate of 5 ml/pot about 7 days after transplanting. Insect and mite infestation was avoided by treating the soil with aldicarb (Temik 10G) at the rate of 0.1 g/pot at transplanting and at 4- to 6-wk intervals thereafter.

Inoculum. Leaves bearing prominent scab lesions were collected from unsprayed McIntosh trees at the Mast Road Research Orchard in Durham, NH, just before leaf fall in 1981 and 1982. The leaves were placed in wire cages on the orchard floor over the winter, retrieved from the orchard in early spring, and stored at −10 C until needed. Leaves were thawed and incubated at 15–20 C and 90% relative humidity to produce inoculum.

Conidia were obtained as needed from young lesions on McIntosh leaves collected from unsprayed trees at the research orchard. The conidia were suspended in chilled, distilled water, adjusted to a concentration of 72,000 spores per milliliter, and used immediately.

Inoculation and incubation of apple seedlings. Apple leaves bearing pseudothecia with mature ascospores were immersed in water for 10 min, then placed for 1 hr on a wire frame suspended about 25 cm above seedlings to be inoculated. Conidial inoculum was applied using a precision spray chamber similar to Szkolnik's (18). The inoculated seedlings were placed in a fog chamber maintained at 5, 10, 15, or 20 C ± 1.5 C. The fog was produced by combining water and compressed air in a mist nozzle (Spraying Systems Inc., Manchester, NH), thereby creating a saturated atmosphere that maintained a film of water on the leaves without water running off the leaves.

Assessment of spore germination and appressorium development. Three seedlings were removed from the incubation chamber each hour during the first 6 hr of incubation and at 2-hr intervals during the remaining 6–24 hr of incubation. The three youngest leaves were removed from each seedling, cleared in ethanol and acetic acid (20), stained with acid fuchsin in lactophenol (12), and mounted on glass slides. Spores, germ tubes, and appressoria were stained bright pink, whereas the leaf tissue remained colorless. The leaves were scanned at ×200 using transmitted light. Spores were categorized as 1) ungerminated; 2) germinated, with a germ tube at least one-half the length of the spore but without an appressorium; and 3) germinated with appressoria. A total of 100 conidia or ascospores were examined per leaf, and the number of

spores in each of the three categories was recorded. The study was repeated six times at each incubation temperature.

RESULTS AND DISCUSSION

Keitt and Jones (4) reported that ascospores of *V. inaequalis* sometimes penetrated leaves directly without forming appressoria, but we did not observe this phenomenon in our study. Conidia and ascospores typically formed short germ tubes, from one-half to twice the length of the spore, that terminated in clavate appressoria separated from the germ tube by a single septum. We assessed the effects of temperature on the initial steps in the infection process by following the progress of conidia and ascospores through discrete stages, i.e., germination and appressorium formation. Earlier workers (4,6) had measured germ tube growth. Germ tube length may offer some indication of the favorability of certain temperatures for infection, but the results could be confounded by formation of appressoria in response to tactile stimuli (21), particularly when such germination studies are conducted on glass slides rather than on host tissue. We believe that the most valuable information can be gained by observing the time required for a spore to reach a discrete stage of development on host tissue at temperatures approximating those encountered in the field.

The rate of germination and appressorium formation of both conidia and ascospores was directly proportional to temperatures from 5 to 20 C (Figs. 1 and 2), but conidia germinated and formed appressoria more quickly than did ascospores at all temperatures (Table 1). Various combinations of regressors were used to describe the relationship between temperature, germination, and appressorium formation. The highest coefficients of determination and the lowest standard errors were obtained in multiple regressions using time, temperature, and degree-hours as the independent variables.

The following equations describe the relationship between temperature and germination of conidia and ascospores, respectively: $Y_0 = 10.7 - 0.132\,X_1 + 0.051\,X_2 - 0.0448\,X_3$, $R^2 = 0.93$ (equation 1) and $Y_0 = 12.2 - 0.164\,X_1 - 0.075\,X_2 - 0.0119\,X_3$, $R^2 = 0.95$ (equation 2), where $Y_0 = \sqrt{\%}$ spores ungerminated, X_1 = hours after inoculation, X_2 = temperature (C), and X_3 = degree-hours (base = 0 C).

The following equations describe the relationship between temperature and appressorium formation of conidia and ascospores, respectively: $Y_2 = -42.1 + 4.52\,X_1 + 1.70\,X_2 + 0.0512\,X_3$, $R^2 = 0.90$ (equation 3) and $Y_2 = -47.8 + 2.91\,X_1 + 0.924\,X_2 + 0.132\,X_3$, $R^2 = 0.97$ (equation 4), where Y_2 = % spores with appressoria, X_1 = hours after inoculation, X_2 = temperature (C), and X_3 = degree-hours (base = 0 C).

For all values of Y_0^2 and Y_2 between 0

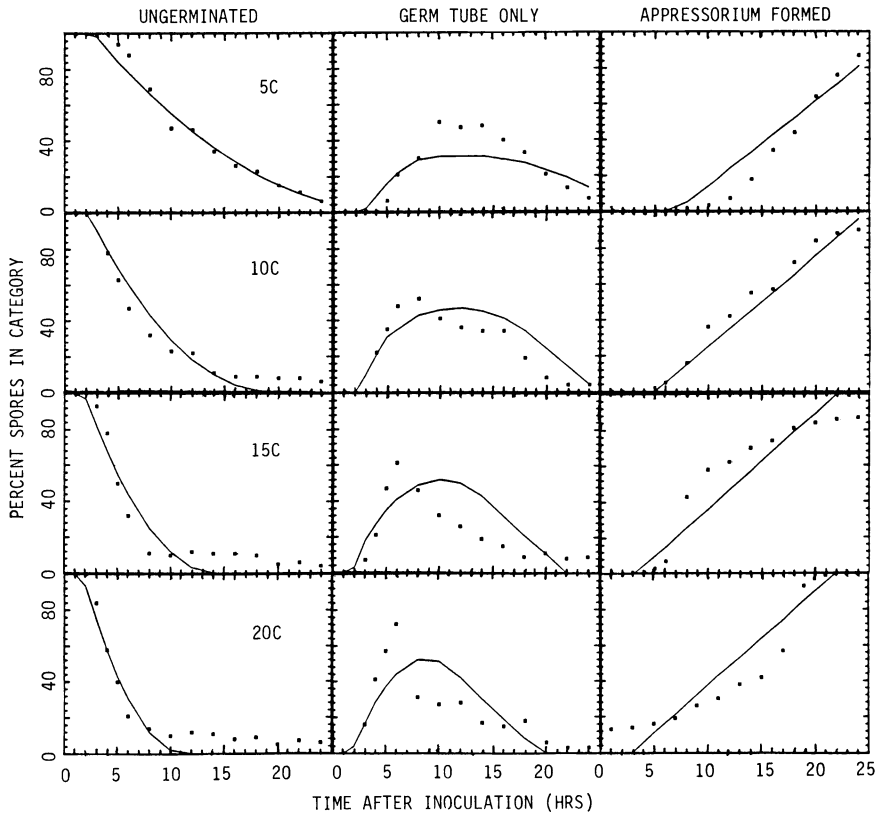

Fig. 1. Germination and appressorium formation of conidia of *Venturia inaequalis* at 5, 10, 15, and 20 C on apple seedling leaves. Data points indicate the mean percentage of spores that were ungerminated, germinated but without appressoria, and germinated with appressoria. Solid lines indicate the percentage of spores in each category as predicted by equations 1 (ungerminated), 5 (germ tube only), and 3 (appressorium formed).

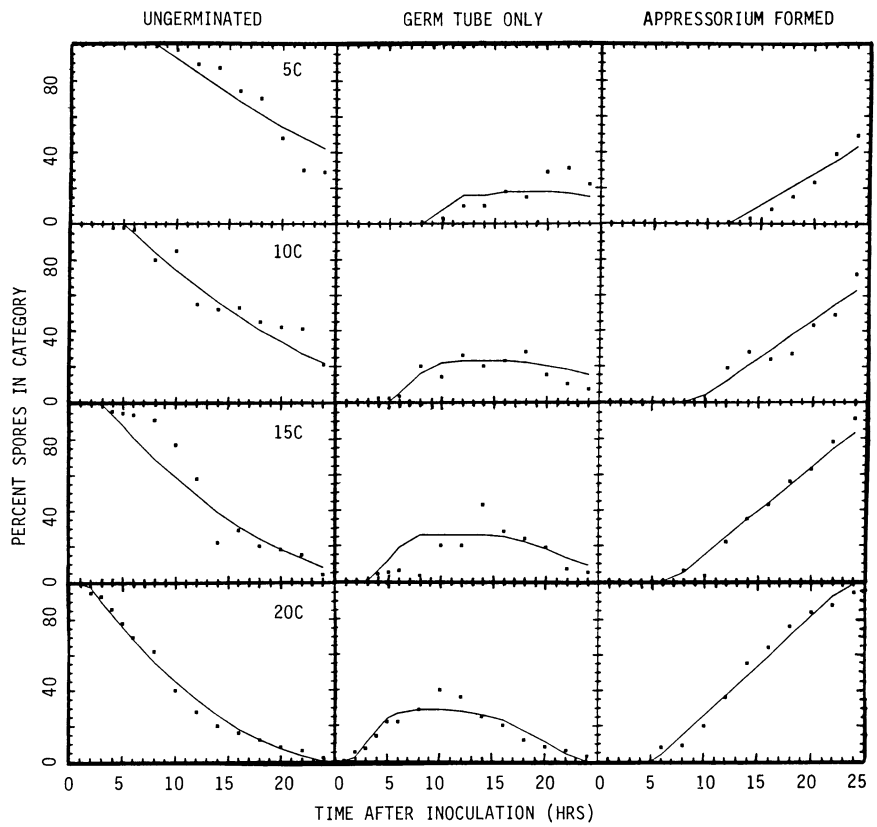

Fig. 2. Germination and appressorium formation of ascospores of *Venturia inaequalis* at 5, 10, 15, and 20 C on apple seedling leaves. Data points indicate the mean percentage of spores that were ungerminated, germinated but without appressoria, and germinated with appressoria. Solid lines indicate the percentage of spores in each category as predicted by equations 2 (ungerminated), 5 (germ tube only), and 4 (appressorium formed).

and 100, the percentage of spores that had formed germ tubes but not appressoria (Y_1) was $100 - (Y_0^2 + Y_2)$ (equation 5).

Values of Y_0^2 and Y_2 of less than 0 or more than 100 were set equal to 0 and 100, respectively, for the purpose of computing Y_1 by equation 5.

Because conidia of *V. inaequalis* germinated and formed appressoria more quickly than did ascospores, conidia at any given temperature should be expected to require less time to infect the host than ascospores—an assumption that seems reasonable based on the extensive field studies of Mills (9). In laboratory studies (4,11,14,16), however, ascospores initiated infections in less time than conidia. We have three possible explanations for this dichotomy, none of which is mutually exclusive: 1) inoculation of the host in the field occcurs under conditions that differ from those in laboratory inoculations, 2) laboratory inoculations using ascospores are difficult to standardize and may result in consistently greater inoculum densities in ascospore inoculations than in conidial inoculations, and 3) ascospores may be more effective than conidia in colonizing host tissue after appressorium formation.

In laboratory inoculations of apple seedlings or potted trees, conidia are usually applied in an aqueous suspension and ascospores are discharged from overwintered leaves and allowed to settle onto the test plants (4,11). Thus, the time of inoculation is precisely known. A similar situation exists in the dispersal of condial inoculum under orchard conditions. Conidia float free from the conidiophores as they are wet by rain and are dispersed throughout the canopy by rain splash, runoff, and wind. The time of inoculation is approximately coincident with the onset of rain and leaf wetness. However, this is not the case with ascospores. Ascospores of *V. inaequalis* are discharged in significant numbers only during daylight hours (1,7). Thus, the interval between the onset of rain and the discharge of ascospores can be a matter of minutes during daytime rains or several hours if the rain begins at night. Mills (9) was apparently unaware of the diurnal periodicity of ascospore discharge, and his infection period table was based on several years of field data that included primary infection periods initiated by daytime and nighttime rains (5). The data Mills used to establish a relationship between temperature and infection probably included several primary infection periods that were initiated by night rains. Assuming that the onset of rainfall occurs randomly at various times of the day and night, the effect of the delayed release of ascospores during infection periods beginning at night, averaged over many observations, would be that ascospores would appear to require more time to infect the host than would conidia.

The above would explain the different results reported in the field studies of Mills (9) and several laboratory studies (4,11,14,16). However, it would not explain how ascospores could initiate infection in less time than conidia (4,11,14,16) in light of our findings that condia germinate and form appressoria much more rapidly than do ascospores (Table 1). For this to occur, either the inoculum dose would have to be higher in ascospore inoculations than in conidial inoculations, ascospores would have to be more effective than conidia in colonizing the host after appressorium formation, or both.

One difficulty encountered in controlling apple scab is the determination of the infection periods of Mills (9) when leaf wetness is discontinuous. Several studies have attempted to identify the maximum time that a spore can remain dry between two wet periods, but conclusions and recommendations vary widely (3,4,11, 15,17–19). The inconsistencies may be due to inconsistent experimental conditions. The effect of temperature during the test period was often not considered, thus the progress of spores in the infection process was unknown, except as measured by the eventual success of the spores in causing lesions. There is no information on the relative sensitivity of ungerminated, germinating, or appressoria-bearing conidia and ascospores to intermittent dryness under orchard conditions. Until such information is available, our ability to interpret the effects of discontinous wetting on infection will probably not improve.

Our results and those of a previous study of ascospore discharge in *V. inaequalis* (7) indicate that some revision of Mills's criteria for determining primary infection periods may be desirable based on the slower rate of development of ascospores during the infection process and the delayed release of ascospores when rain begins at night. Further research using carefully controlled inoculations is needed to answer the questions raised about the efficacy of conidia and ascospores as inoculum in the development of apple scab.

Table 1. Rate of germination and appressorium formation of *Venturia inaequalis* on apple seedling leaves

Event	Spore type	Time from inoculation to event (hr)[a] 5 C	10 C	15 C	20 C
10% Germination	Conidia	5.6	3.5	3.2	2.6
	Ascospores	11.8	6.8	8.1	3.4
50% Germination	Conidia	9.7	5.8	5.0	4.4
	Ascospores	19.8	16.8	12.4	9.1
10% Appressorium formation	Conidia	12.5	6.9	6.2	6.1
	Ascospores	16.6	11.0	10.7	8.2
50% Appressorium formation	Conidia	18.6	13.2	8.9	7.8
	Ascospores	24.2	22.1	17.1	13.5

[a] Values interpolated from Figures 1 and 2.

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