Effects of Temperature During Ascus Formation and Frequency of Ascospore Discharge on Pseudothecial Development of *Venturia inaequalis*

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ABSTRACT

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The number of asci that developed in pseudothecia of *Venturia inaequalis* was determined by the temperature between the appearance of ascus initials and the appearance of ascospores. Varying the interval between ascospore discharges had no significant effect on production or maturation of asci when leaves were kept moist and pliable between discharges. There was no evidence of ascospore discharge by means of circumscissile dehiscence or bursting of pseudothecia as noted in some earlier reports. When ruptured pseudothecia were examined, the rupture of the ascocarp wall appeared to be the result, rather than the cause, of senescence.

Additional key words: apple scab

Apple scab is a fungal disease of the foliage and fruit of apple (*Malus pumila* Mill.). The causal organism, *Venturia inaequalis* (Cke.) Wint., overwinters as immature pseudothecia in fallen, infected leaves. In spring, ascospores are discharged from the pseudothecia when the leaves are wet by rain. Control of apple scab is usually achieved by repeated fungicide applications that prevent or stop infections. When the supply of primary inoculum is thought to be exhausted, the interval between fungicide applications is increased and fungicide rates are decreased.

Recently, several mathematical models have been described that can be used to estimate (4,6,9,14) or forecast (12) relative inoculum maturity, ie, the proportion of the season's ascospores that have matured. We are currently developing a system to predict absolute inoculum levels or ascospore dose based on a summary model described by MacHardy and Jeger (13). In their model, potential ascospore dose is defined as the number of ascospores produced per square meter of orchard floor. So defined, potential ascospore dose becomes the product of lesion density (lesions per square meter of leaf tissue), pseudothecial density (pseudothecia per lesion), ascal density (asci per pseudothecium), a constant (eight ascospores per ascus), and leaf litter density (the proportion of the orchard floor covered by leaf litter).

Preliminary studies of ascus formation by Gadoury (4), who incubated pseudothecia at temperatures from 6 to 20 C, showed that the number of asci formed per pseudothecium was inversely proportional to temperature. One objective of this study was to determine exactly when in pseudothecial development this influence of temperature on ascus production is most pronounced.

Xeric conditions have been shown to inhibit maturation of pseudothecia of *V. inaequalis* (8,10,11,17). However, there have been no previous studies to determine the effects of the frequency of ascospore discharge when moisture between discharges is not a limiting factor for maturation. Moisture supplied between the rains necessary for ascospore discharge is common. For example, in Durham, NH, leaves on the orchard floor were wet by dew for at least 6 hr during every night of May from 1979 to 1983 (D. M. Gadoury, *unpublished*).

Discharge of ascospores from wetted pseudothecia of *V. inaequalis* has been described differently by various authors: as an orderly process of ascus emergence through the ostiole followed by discharge (1), a violent explosion of the pseudothecium (2), a rupture of the pseudothecium (3), and a circumscissile dehiscence of the pseudothecium (16). Only Brook (1) observed ascospore discharge in situ. Discharge of ascospores by rupture or dehiscence of pseudothecia could affect the apparent maturation rate of a population of pseudothecia because ruptured ascocarps would effectively be removed from the population regardless of whether or not all of the asci had matured. Our second objective was to determine whether or not the frequency of ascospore discharge affected the rate of pseudothecial maturation or the incidence of pseudothecial dehiscence.

MATERIALS AND METHODS

Effect of temperature. Infected leaves were collected from beneath unsprayed McIntosh trees at the Mast Road Research Orchard in Durham, NH, on 6 February 1981. At this time, the lumina of pseudothecia were filled primarily with pseudoparaphyses and ascal initials were beginning to develop (Fig. 1). Squares 2.5 × 2.5 cm were cut from these leaves and fastened between two pieces of fiberglass screen, which was then rolled and the two ends fastened to form a cylinder as described previously (7). Leaf squares were incubated at 6 and 12 C at 90% RH. Leaf pieces stored under these conditions remained pliable without surface moisture. Presence of mature ascospore was detected by immersing the leaf samples in water at 5- to 7-day intervals and examining the resultant suspensions as described by Gadoury and MacHardy (7). When mature ascospores were found at an incubation temperature, the leaf sample at that temperature was divided into two groups, one of which was moved to either 6 or 12 C. Thus, there were four temperature treatments: constant storage at 6 C, constant storage at 12 C, storage at 6 C until initial ascospore maturation and then at 12 C, and storage at 12 C until initial ascospore maturation and then at 6 C. Ascigerous productivity and the rate of ascospore maturation were assessed at weekly intervals by examining 20 crushed pseudothecia from each treatment as described by Gadoury and MacHardy (5). The maximum number of asci per pseudothecium (mean of 20 replicates) and the cumulative proportion of ascospores matured were recorded.

Effect of frequency of ascospore discharge. Leaves collected from unsprayed McIntosh trees on 25 October 1979 were stored over winter in wire-mesh trays at the research orchard until 4

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January 1980. Leaf samples were then prepared as before and incubated at 15 C and 90% RH. Ascospore discharge was induced at 7-, 14-, or 21-day intervals by immersing the leaf samples in water. The resultant spore suspensions were examined microscopically and the cumulative proportion of matured ascospores was determined as described by Gadoury and MacHardy (7). Treatments were replicated three times.

Twenty pseudothecia were removed each week from leaves wetted at each interval. Pseudothecia were crushed on glass slides, examined microscopically, and the number of asci per pseudothecium was recorded. Every 7 days, four 1-cm disks were cut from leaves tested at each wetting interval. The disks were cleared in sodium hydroxide and chloral hydrate (15) and examined under magnification. The proportion of pseudothecia that was ruptured or dehiscent was recorded. Data were recorded only for pseudothecia that bore visible hyphal connections to the subcuticular stromata of *V. inaequalis*.

RESULTS AND DISCUSSION

Pseudothecia that were initially incubated at 6 C produced more asci than did pseudothecia initially incubated at 12 C (Table 1). However, temperature had no significant effect upon ascus production after the first ascospores had matured. Data indicate that temperature influences the number of asci produced in the early stages of ascus development, ie, during the period between the appearance of ascal initials and the development of the first mature ascospores.

Of what significance are the above results in determinations of ascospore dose? Prediction of ascospore dose sensu MacHardy and Jeger (13) requires that a value be assigned to ascal density (the number of asci formed per pseudothecium). 15 March to 15 April is the period when asci are normally produced in New Hampshire apple orchards. The mean temperature in Durham, during this time, averaged for 1959–1981, was 4 C and ranged from a low of 2 C to a high of 7 C. Gadoury and MacHardy (7) found that the number of asci per pseudothecium was reduced by about six for each Celsius degree that incubation temperatures were increased. The mean number of asci per pseudothecium for three orchard sites in New Hampshire (6) for 1979–1982 was 119 (±12, $P=0.10$). Hence, in most years, *V. inaequalis* will produce about 100–130 asci per pseudothecium in New Hampshire and perhaps correspondingly more or fewer asci in other areas, depending on early spring temperatures. The number of asci produced per pseudothecium is also an important component in estimations of relative inoculum maturity. For example, we have used the mean number of asci per pseudothecium to adjust squash-mount assessments of ascospore maturity and discharge for decomposition of empty asci (5). The adjusted assessments are then used by New Hampshire apple growers in planning fungicide schedules to control apple scab.

Pseudothecial ontogeny of *V. inaequalis* is now well understood (4–9,11,17). Ascospore discharge is assumed to occur through a well-developed ostiole (1). However, early studies of ascospore discharge (2,3,16) repeatedly refer to aberrant forms of spore discharge. We could not discount the early reports because pseudothecial dehiscence would profoundly affect maturation rates of populations. We had found ruptured pseudothecia in leaves collected from various New Hampshire orchards but only in late spring, at a time when nearly all ascospores had matured.

In this study, the frequency of ascospore discharge had no significant effect on the number of asci that developed per pseudothecium, the rate of ascospore maturation, or the incidence of rupture or dehiscence of pseudothecia (Table 2). Rupture of the ascocarp wall was associated with a depletion of the ascospore supply; however, there was no evidence of ascospore discharge by means of dehiscence or explosion of pseudothecia as described by Cunningham (2), Curtis (3), or Wallace (16). Asci were never found in spore suspensions. When ruptured ascocarps were removed from leaves, crushed, and examined under magnification, the internal contents were invariably in an advanced stage of decomposition. Generally, ruptured pseudothecia contained the remnants of 10–15 asci. Of these asci, about half were empty. The remaining asci contained delimited, pale-colored spores. Rupture of the ascocarp wall was never circumscissile. A fissure would often form between the ostiole and the base of the pseudothecium (Fig. 2). Ruptured pseudothecia were rarely found until the supply of ascospores was nearly exhausted (Table 2). Rupture of the ascocarp wall appeared to be the result of senescence of pseudothecia rather than the cause of this senescence.

Ascospores matured independently of

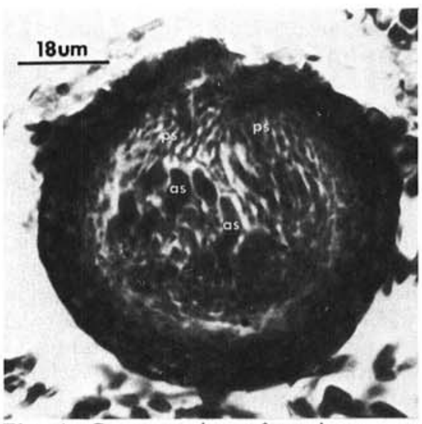

Fig. 1. Cross section of an immature pseudothecium of *Venturia inaequalis*. The lumen of the ascocarp is filled with pseudoparaphyses (ps). The newly formed ascus initials (as) are visible at the base of the centrum.

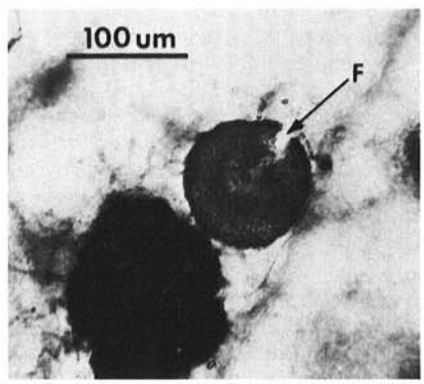

Fig. 2. Pseudothecium of *Venturia inaequalis* 42 days after initial ascospore discharge. The ascocarp wall is ruptured, though not circumscissally. The fissure (F) runs from the ostiole to the base of the ascocarp. Subsequent removal and examination of this pseudothecium showed that the ascogenous contents had degenerated, leaving only the remnants of empty asci.

Table 1. Effects of temperature before and after initial ascospore maturation on the number of asci formed per pseudothecium of *Venturia inaequalis*

Incubation temperature (C)		Asci per
Initial	Final	pseudothecium[z]
6	6	125 a
6	12	116 a
12	12	73 b
12	6	83 b

[x]Means followed by the same letter do not differ significantly at $P=0.05$. Infected leaf pieces were incubated at either a constant temperature of 6 or 12 C or were moved from 6 to 12 C or from 12 to 6 C once mature ascospores were observed.

Table 2. Effect of frequency of ascospore discharge on asci formed per pseudothecium, ascospore maturation, and rupture or dehiscence of pseudothecia of *Venturia inaequalis*

Frequency of ascospore discharge (days)	Asci per pseudothecium	Percentage of spores mature		Percentage of ruptured pseudothecia	
		Day 21	Day 42	Day 21	Day 42
7	98[a]	78[a]	99[a]	0[a]	12[a]
14	90	65	97	0	7
21	86	71	99	1	10

[a]Differences within columns are not significant at $P=0.05$. Infected leaf pieces were incubated at 15 C, 90% RH.

ascospore discharge. Pseudothecia matured at the same rate whether the ascospores were discharged every 7, 14, or 21 days (Table 2). Accumulation of mature ascospores between spore discharges caused no inhibition of ascospore maturation and the interval between discharges was not related to the rupture of pseudothecia. Our results are in accordance with those of Brook (1). Observations of ruptured or dehiscent pseudothecia of *V. inaequalis* in apple leaves (2,3,16) were probably accurate but were made after depletion of the ascospore supply was nearly complete. Therefore, the length of the interval between ascospore discharges and pseudothecial dehiscence can be safely omitted from models of ascospore maturation.

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