Disparity Between Morphological Maturity of Ascospores and Physiological Maturity of Asci in *Venturia inaequalis*

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ABSTRACT

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Records from 1984 to 1990 of the morphological maturity of ascospores of *Venturia inaequalis* in New York, New Hampshire, and Vermont indicated that discharged asci occurred rarely in pseudothecia until approximately 10–15% of asci contained ascospores rated as mature, even though numerous rain events occurred, in most years, before the time that this level of maturity was reached. Furthermore, laboratory discharge tests and field trapping of ascospores showed that few or no ascospores were detected before 10% of the asci contained morphologically mature ascospores and that a substantial number of asci containing morphologically mature ascospores were retained in pseudothecia after rain. The number of ascospores discharged per ascus containing morphologically mature ascospores increased through early spring, thus indicating that the ability of asci to release ascospores (physiological maturity of the ascus) lags behind morphological maturity of the ascospores by several days. In subsequent laboratory tests, nearly all ascospore release occurred within 90 min of the initial wetting of overwintered leaves. However, even after 5 hr of continuous wetting, over half of the pseudothecia examined contained between 10 and 50 asci with morphologically mature ascospores. The impact of these findings on the development and evaluation of predictive models of ascocarp development, development of apple scab epidemics, and the use of morphological assessments of ascospore maturity in grower advisory programs are discussed.

Venturia inaequalis (Cooke) G. Wint., the causal organism of apple scab, overwinters in North America as pseudothecia in fallen infected leaves. Ascospores, the primary inoculum, mature shortly after apple buds begin to grow in the spring and are discharged during rain. Apple scab is controlled by an intensive fungicide spray program aimed at limiting primary infection. Once the supply of ascospores is exhausted, the spray program is relaxed or may even be terminated if other fungal diseases do not require control (6). Because of the relationship between the maturity and release of ascospores of *V. inaequalis* and the use of fungicides to control apple scab, a significant and substantial effort is made each year to estimate or forecast ascospore maturity and discharge. This effort may involve the use of spore traps (4), wind tunnels (8), and weather-driven models (3,10,13,14), but the most common method used to estimate ascospore maturity and release is the microscopic examination of crushed pseudothecia or squash mounts (2,15). Pseudothecia are selected, crushed on glass slides, and examined microscopically. The asci are rated as immature, mature, or discharged based on the morphology of the ascus contents (2,15). Criteria used to judge the stage of development of asci include delimitation of ascospores, ascospore shape and color, and extrusion of the endoascus in discharged asci (2,15).

Although squash mounts have been used in advisory programs for apple scab management for many years (2,15), it has never been demonstrated that the morphological criteria used to identify mature ascospores are accurate indicators of the ability of an ascus to release ascospores under suitable conditions. The lack of this information also is of concern in developing or validating models of ascospore maturity and release, most of which have been developed from morphological assessments of ascospore maturity (10,13). Our objective in this study was to examine the relationship between morphological ratings of ascospore maturity and actual release of ascospores under field and laboratory conditions.

MATERIALS AND METHODS

Ascospore maturity and release. Squash mount assessments of ascospore maturity and discharge were made at approximately weekly intervals at the following locations: Geneva, in the Finger Lakes region of New York; Highland, in New York's Hudson River Valley; Burlington, in the Lake Champlain Valley of Vermont; and Durham, near Great Bay in New Hampshire. All of these assessments were prepared using a method described by Gadoury and MacHardy (2). Ascospores were rated as mature if they met all of the following criteria: 1) ascospores olive green in color under bright-field illumination, 2) apical cells of ascospores within an ascus broader than basal cells, and 3) epiplasm absent from the ascus containing the spores. Assessments were made during 1987–1990 in Geneva and 1986–1990 in Highland, NY; 1984–1987 in Burlington, VT; and 1987–1990 in Durham, NH. Weather instruments at each site provided records of temperature and rainfall, and the phenological stages of fruit buds were recorded at each site in each year of the study.

At Geneva, ascospore release from the leaf sample that was used to prepare the squash mounts also was measured. Ascospores were harvested from the leaf samples and enumerated using a method developed by Szkolnik (15). One-centimeter squares were cut from each of 20 leaves. The squares were air-dried for 1–2 hr and then immersed in distilled water for 5 min. The wetted squares then were placed on the inside of a petri plate lid, with the abaxial leaf surface exposed. The lid was inverted for 1 hr over a petri plate bottom containing 20 ml of distilled water and then removed. Ascospores on the petri plate bottom then were counted by examining 40 random microscopic fields at ×100.

A Burkhard volumetric spore sampler (Burkhard Scientific Sales, Ltd., Rickmansworth, England) was operated continuously in the research orchard at Highland, NY, from 1986 to 1989 during the period of ascospore release. The tapes from these traps were prepared and examined as described by Gadoury and MacHardy (4).

Retention of morphologically mature ascospores after wetting. Leaves bearing numerous scab lesions were collected from beneath unsprayed McIntosh trees in Geneva, NY, in April 1990, before the development of mature ascospores. The leaves were stored at −15 C until 3 January 1991. Approximately 50 leaves

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then were removed from storage and soaked in distilled water for 10 min. The leaves then were blotted to remove surface moisture and incubated in moist chambers at 16 C for 14 days to promote the development of mature ascospores. On 17 January, a single pseudothecium was removed from each of 20 leaves, the pseudothecia were crushed on glass slides, and the number of asci in each pseudothecium that contained morphologically mature ascospores was determined by use of the previously described criteria (2). Additionally, two 2.5-cm squares were cut from each of the same leaves. One square from each leaf was placed atop a spore discharge tower as described by Gilpatrick et al (8). Ascospores released into the airstream of the tower were collected on a glass microscope slide located beneath the orifice at the base of tower. The leaf sample was misted with distilled water every 5 min during the test. To ensure that ascospore release would not be affected by light quantity or quality in the laboratory (1,12), the spore discharge tower was illuminated by a 150W, daylight balanced, quartz-halogen lamp. The lamp was directed at the upper and lower leaf surfaces through a bifurcated fiber-optic bundle. The microscope slide was changed at 5- to 30-min intervals, and the test was terminated after 310 min. Ascospores of *V. inaequalis* collected on slides were enumerated by counting the number of ascospores in a single transect across the densest portion of the deposit at ×1,260.

The second 2.5-cm square from each of the leaves was wetted at 5-min intervals as above. At 30 min, 1 hr, and 5 hr after the start of the test, a 1-cm square was removed from each 2.5-cm square and fixed in 2% formaldehyde. A single pseudothecium then was removed from each 1-cm square, crushed on a glass slide, and the number of morphologically mature asci was determined as described by Gadoury and MacHardy (2). The experiment was repeated on 18 January.

RESULTS AND DISCUSSION

In each year, and at each site, asci containing morphologically mature ascospores usually were present for several days before empty asci were detected in squash mounts (Figs. 1–4). Few empty asci were seen in early-season assessments, despite the presence of large numbers of asci containing morphologically mature ascospores and the occurrence of rain events that were favorable for ascospore release (Figs. 1–4). Thereafter, large numbers of asci containing morphologically mature spores were retained in pseudothecia after rain events at each site in each year of the study (Figs. 1–4). Although several assessments performed during the major weeks of ascospore production fell on days that immediately followed extended wet periods, depletion of the supply of asci containing morphologically mature ascospores was not observed (Figs. 1–4).

Ascospores are released from leaves within minutes of the start of rain (1,12), and the time required for a single ascus to discharge has been measured at 3.5–120 s (1). During daylight hours, more than 90% of the ascospores trapped during a wetting period usually are trapped within 6 hr of the onset of rain (12). Although smaller secondary peak releases may occur on consecutive days if rain is continuous, these probably indicate the continued maturation of ascospores and not the delayed release of previously matured ascospores (12). Thus, it seems unlikely in the present study that the consistent presence of asci that contained morphologically mature ascospores resulted from insufficient opportunities for release of the ascospores by rain (Figs. 1–4).

When squash mounts were prepared immediately after early-season rain events during 1986–1989 in Highland, NY, 18.7–49.1% of the asci contained morphologically mature ascospores (Table 1). In 3 of the 4 yr, 5–6 days of

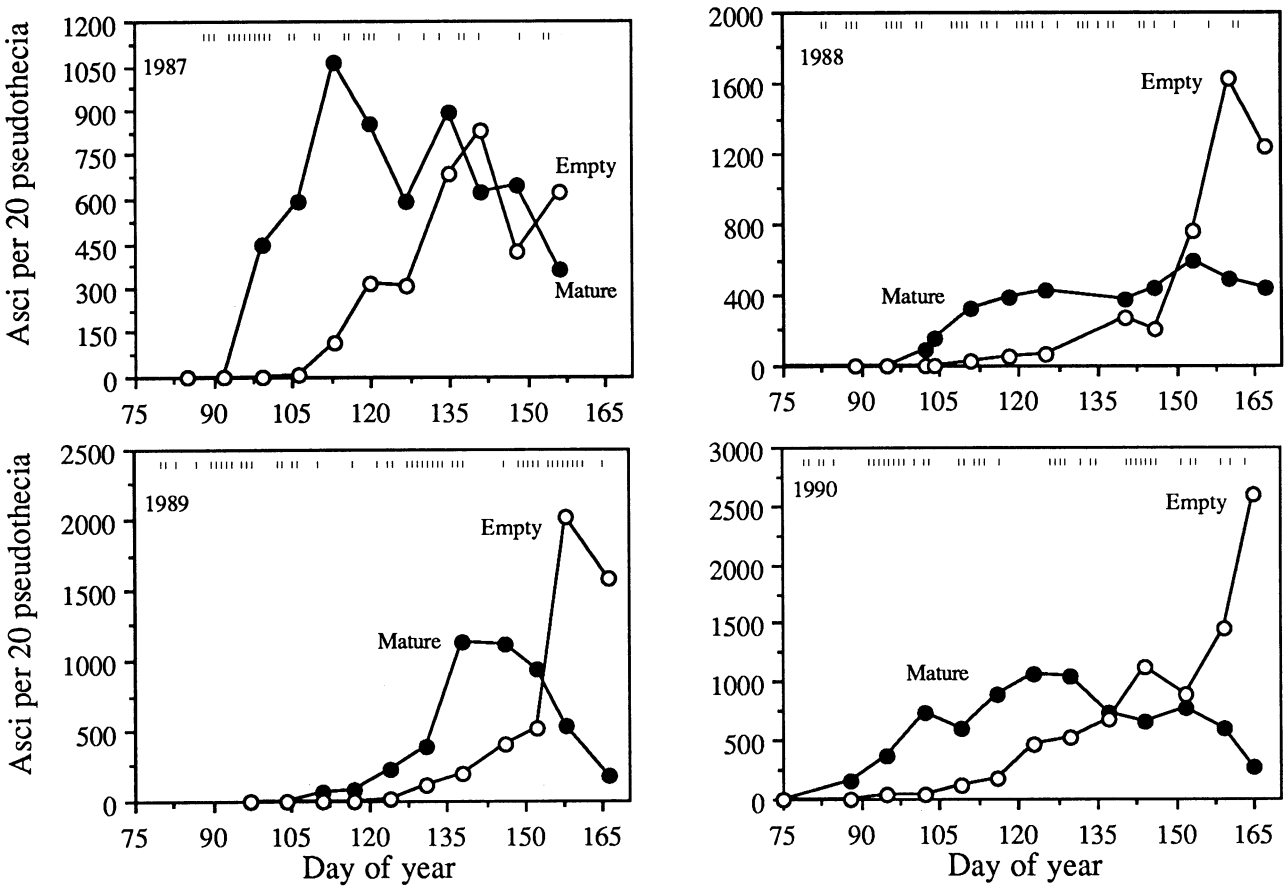

Fig. 1. Number of asci containing morphologically mature ascospores and number of discharged asci in 20 pseudothecia of *Venturia inaequalis* from Geneva, NY, for the years 1987–1990. Vertical bars indicate days on which measurable rain fell.

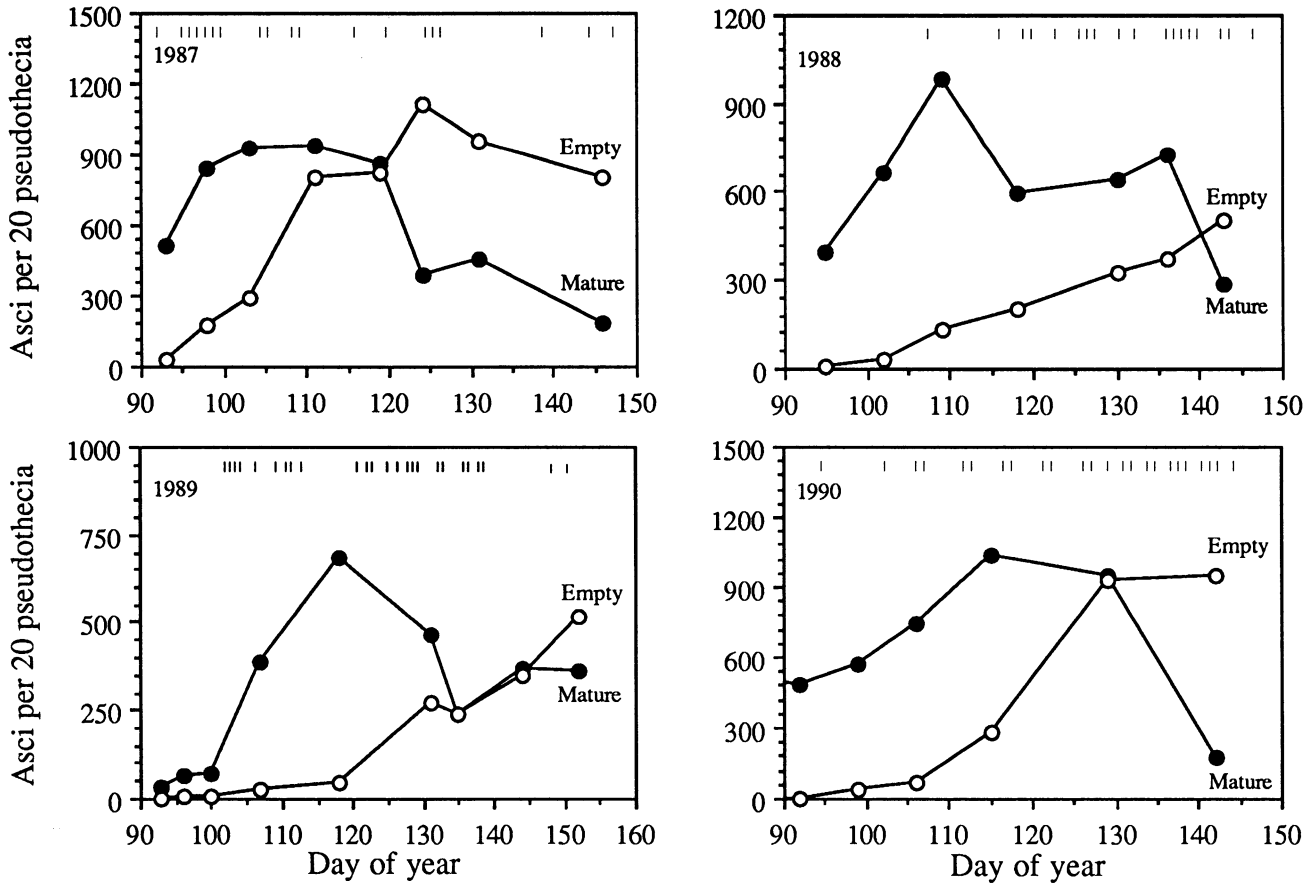

Fig. 2. Number of asci containing morphologically mature ascospores and number of discharged asci in 20 pseudothecia of *Venturia inaequalis* from Highland, NY, for the years 1987–1990. Vertical bars indicate days on which measurable rain fell.

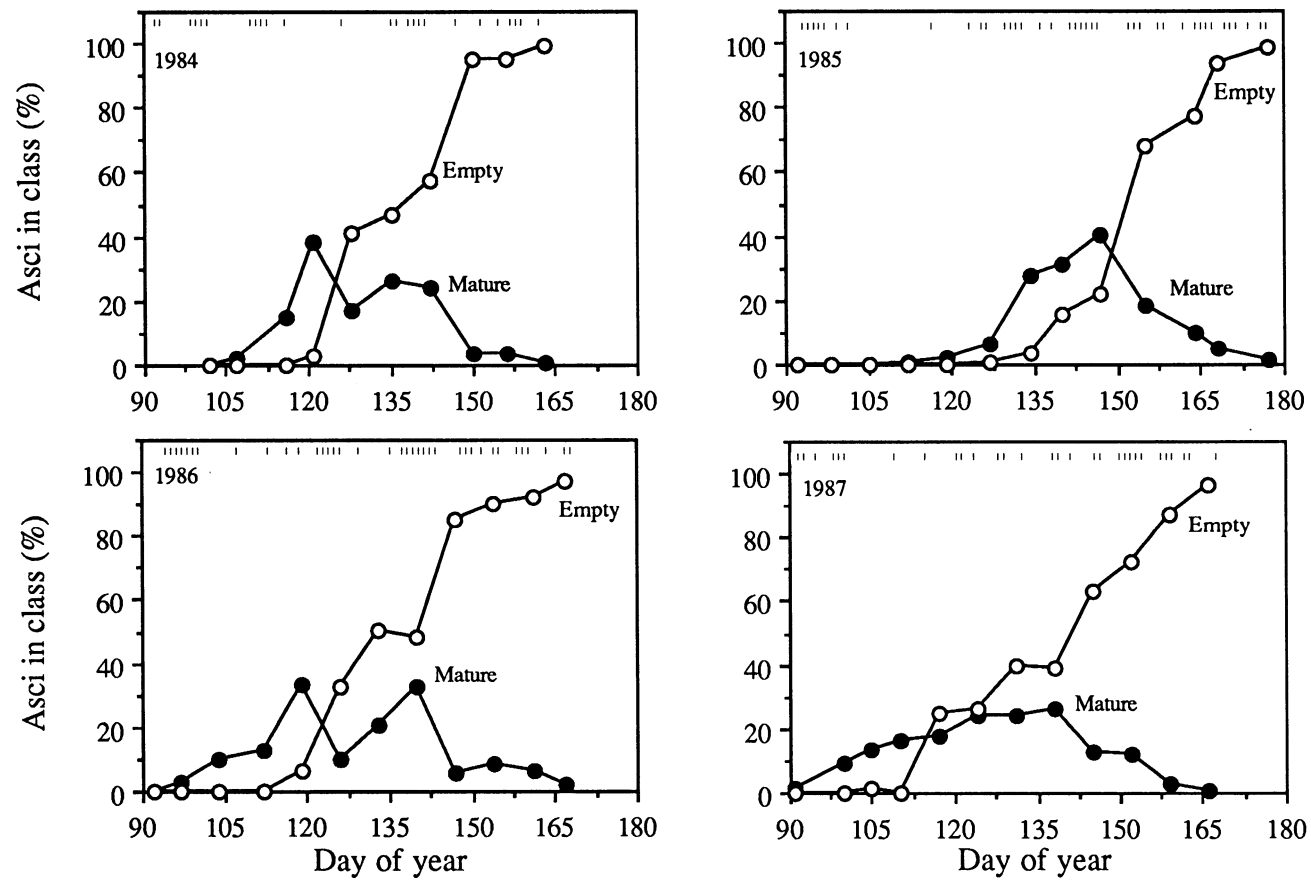

Fig. 3. Percentage of asci containing morphologically mature ascospores and percentage of discharged asci in 20 pseudothecia of *Venturia inaequalis* from Burlington, VT, for the years 1984–1987. Vertical bars indicate days on which measurable rain fell.

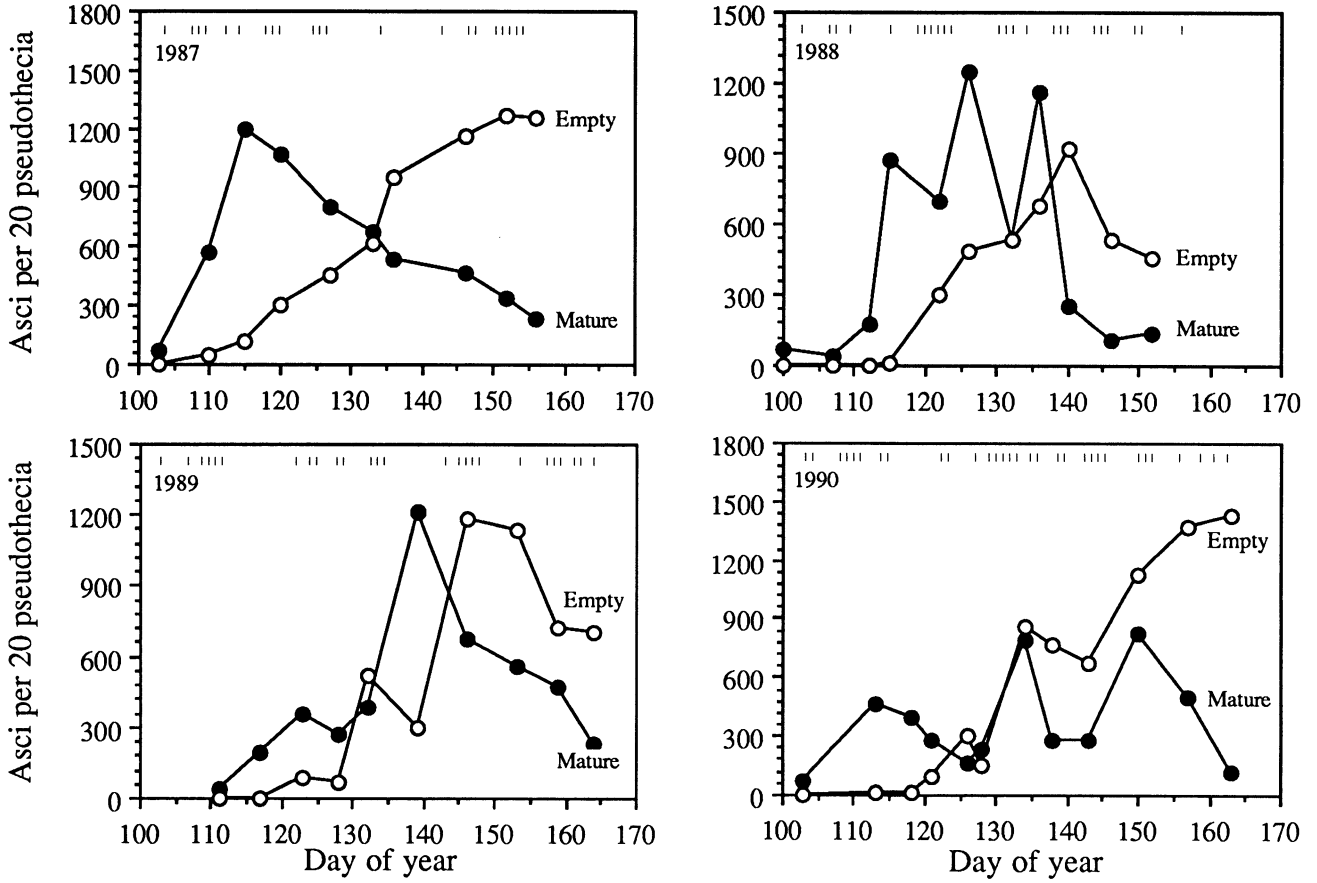

Fig. 4. Number of asci containing morphologically mature ascospores and number of discharged asci in 20 pseudothecia of *Venturia inaequalis* from Durham, NH, for the years 1987–1990. Vertical bars indicate days on which measurable rain fell.

Table 1. Retention of morphologically mature ascospores of *Venturia inaequalis* after rain in Highland, NY

Year	Tree phenophases[a]	Days with rain[b]	Total rain[c] (cm)	Percent asci with mature ascospores[d]	Percent discharged asci[d]	Percent ascospores trapped[e]
1986	GT - 2 cm	6	1.2	31.0	4.2	4.0
1987	GT - 1 cm	6	9.7	33.1	6.8	3.9
1988	GT - 2 cm	1	0.7	49.1	6.7	9.3
1989	GT - 1 cm	5	2.8	18.7	0.8	3.2

[a] Phenophases of McIntosh fruit buds at the beginning and end of the interval. GT = green tip, 1 cm = 1 cm green, and 2 cm = 2 cm green.
[b] Number of days on which rain fell between the initial detection of morphologically mature ascospores and the end of the wet period.
[c] Total rain during the interval.
[d] The percentage of asci that had discharged ascospores or contained morphologically mature ascospores at the end of the wet period, determined immediately after the first interval of rain that followed the initial detection of morphologically mature ascospores.
[e] The percentage of the season's total catch of ascospores trapped in a Burkhard volumetric spore sampler during the wet period.

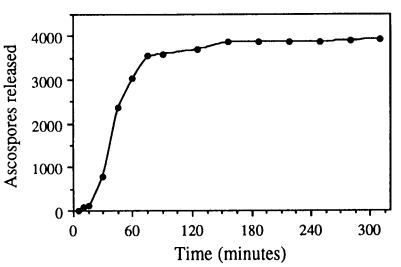

Fig. 5. Release of ascospores of *Venturia inaequalis* in a discharge tower during continuous leaf wetness.

rain had preceded the assessments (Table 1). The percentage of asci that discharged during the rain events corresponded to the percentage of the season's total catch of ascospores caught by the volumetric trap during the rainy period (Table 1). The retention of ascospores by such a large percentage of asci after extended periods that were favorable for ascospore release suggests that the asci, though containing morphologically mature ascospores, were not physiologically mature or able to release those spores.

Ascospores were released primarily within the first 75 min of wetting in the laboratory (Fig. 5), and little additional release occurred after 2 hr (Fig. 5). Likewise, the greatest changes in the number of pseudothecia containing 100–150 asci with morphologically mature ascospores occurred in the first 30–60 min of wetting (Fig. 6). However, a substantial proportion of pseudothecia contained 10–49 morphologically mature asci after 5 hr of wetting (Fig. 6). The near cessation of ascospore release after 90 min of wetting and the retention of morphologically mature asci during 5 hr of wetting together indicate a disparity between morphological maturity of ascospores and physiological maturity of asci.

At Geneva, NY, ascospores were not released from field samples of overwintered leaves until 2–14 days after asci contained morphologically mature ascospores during 1987–1990 (Fig. 1). When the number of ascospores counted in the discharge test (15) was divided by the mean number of asci containing morphologically mature spores per pseudothecium, this ratio increased progressively and substantially from green tip through the bloom or petal fall stages of fruit bud development (Table 2), indicating that an increasing number of ascospores were being released per ascus. The relationship between the proportion of the

asci that contained morphologically mature ascospores and the proportion of the asci that were physiologically mature apparently changed as the season progressed. Because the ratio on any given sampling date would be affected by previous environmental conditions governing ascospore release in the field, we cannot determine the precise nature of this relationship; i.e., whether the number of physiologically mature asci is a constant proportion of the number of asci containing morphologically mature ascospores or whether the relationship is more complex.

Apparently not all asci containing morphologically mature ascospores of *V. inaequalis* are capable of discharge, even under the most favorable conditions of release. In the case of squash mounts, the net effect of this disparity between morphological maturity of ascospores and physiological maturity of asci is a consistent overestimation of the proportion of the inoculum that is available for release. This overestimation in squash mount assessments has a minor influence on disease control measures in midseason, primarily because fungicides are scheduled as protectant or postinfection sprays once a substantial proportion of the inoculum has matured. It matters little in the timing of these sprays if the percentage of matured ascospores is 40 or 70%; the decision to apply a fungicide would likely be the same in either instance. However, the disparity between morphological maturity of ascospores and physiological maturity of asci is more significant early in the primary infection season. Between the bud break and tight cluster phenophases, a significant percentage of the asci may contain morphologically mature ascospores. However, our results indicate that release of ascospores is unlikely to occur until approximately 10% of the asci contain ascospores that are morphologically mature. If an assessment of ascospore morphology is used to determine when the first ascospores are available for release, the initiation of the primary infection season may be anticipated up to 14 days before asci are physiologically mature and inoculum is available for release.

The lag between morphological maturity of ascospores and physiological maturity of asci also may, in part, provide an alternative explanation for the success of disease management programs in which fungicide applications are delayed until the tight cluster to pink phenophases (6). The success of these programs has been attributed tentatively to forecasts of ascospore dose and the delay of apple scab epidemics that start from extremely small quantities of primary inoculum (5,6). Our results indicate that asci often are not physiologically mature until several days after bud break. This delay of inoculum release may indicate that fungicide application could be postponed beyond the date computed based only on ascospore dose (5,6). Alternatively, fungicide applications could be postponed in orchards, irrespective of ascospore dose, until asci are physiologically mature and capable of releasing ascospores.

Morphological characteristics of ascospores have long been used to determine the effects of experimental treatments on ascospore maturation in *V. inaequalis* (3,9,11,16). While the responses noted may be reproducible, the consistency and reproducibility of the relationship between morphological maturity of ascospores and physiological maturity of asci is unknown. Thus, it is uncertain whether two pseudothecia containing the same number of asci with morphologically mature ascospores actually contain the same number of physiologically mature asci.

Several weather-driven models of ascospore maturity have been proposed (3,10,13). Two of these models have been developed from squash mount assessments (13) or from examinations of sectioned pseudothecia (10). A meaningful validation of these models will be difficult unless some predictable relationship can be established between morphological assessments and physiological maturity. The model developed by Gadoury and MacHardy (3) was based primarily on ascospore maturity as assessed by harvesting discharged ascospores (3) and should not be affected by the disparity between morphological maturity of ascospores and physiological maturity of asci. The model developed by St.-Arnaud et al (14) was developed from squash mount assessments, but it is designed to estimate the proportion of discharged asci, independent of morphological maturity of ascospores and, thus, should also be unaffected by the disparity.

Examinations of the morphology of the contents of pseudothecia, either through squash mounts or through more detailed examinations, will continue to be valuable in research on *V. inaequalis* and in management of apple scab. However, the morphological criteria presently used to gauge ascospore maturity are poor indicators of physiological maturity of asci. Squash mount assessments still can provide accurate information on the

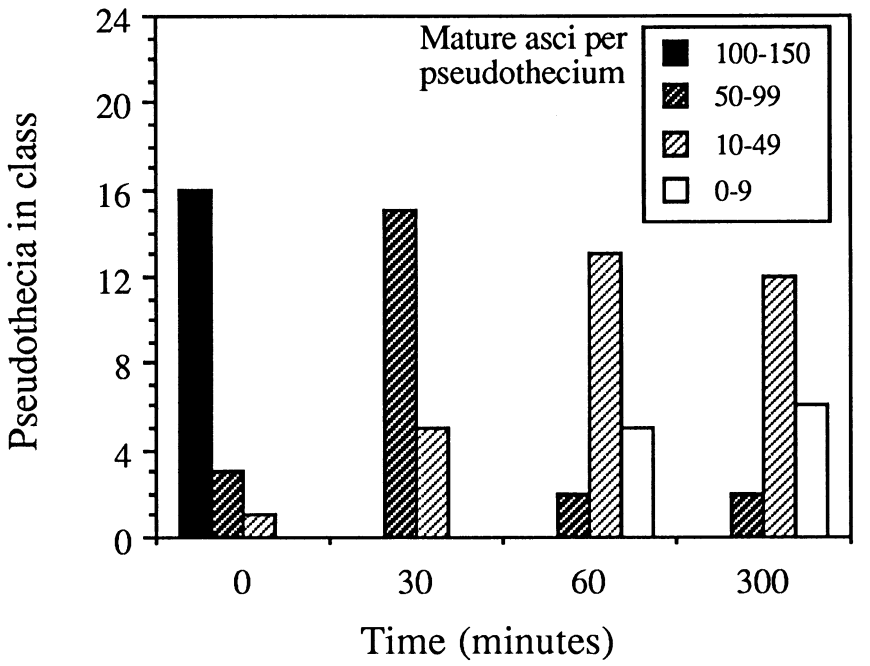

Fig. 6. Changes in the numbers of asci containing morphologically mature ascospores in 20 pseudothecia subjected to continuous leaf wetness. Pseudothecia of *Venturia inaequalis* were assigned to classes based on the number of asci containing morphologically mature ascospores observed when the ascocarps were crushed and examined microscopically.

Table 2. Seasonal changes in the ratio of the number of ascospores captured from 20 1-cm squares of overwintered apple leaf to the mean number of morphologically mature asci per pseudothecium of *Venturia inaequalis*

Year	Green tip[a]	1 cm green	Tight cluster	Pink	Bloom	Petal fall
1987	0.02	0.04	0.98	2.00	7.16	4.24
1988	0.02	0.22	1.36	...	4.44	5.30
1989	0.00	0.04	1.04	2.64	4.16	2.06
1990	0.76	1.46	...	4.06	4.12	3.44

[a] Phenological stage of McIntosh fruit buds at time of observations. Missing values indicate that no test was performed at this stage of development.

total number of asci, the number of asci without delimited spores, and the number of asci that have released spores. However, it will be necessary to change the application of this information in disease management programs to focus more on discharged asci as an indicator of availability of inoculum and less on the numbers of asci containing morphologically mature ascospores. The sampling aspects of preparing and interpreting squash mounts also have been neglected in previous studies (2,15). Sampling aspects of squash mount assessments (7) and our recommendations for the use of squash mount assessments in research and disease management will be presented in a future article.

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