Preparation and Interpretation of Squash Mounts of Pseudothecia of *Venturia inaequalis*

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ABSTRACT

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Management of apple scab requires information on the development of pseudothecia of *Venturia inaequalis*. One method often used to assess ascospore maturity and discharge is the examination of crushed pseudothecia. Previously described techniques for the preparation and interpretation of pseudothecial squash mounts have been based on the assumption that asci examined at various times in the spring represent the season's total production of asci. Results presented in this paper show that the number of asci per pseudothecium increases in early spring and then decreases in subsequent weeks. A new technique is described that used the maximum number of asci recorded per pseudothecium to correct for exclusion of undeveloped asci in early spring and exclusion of disintegrated asci in late spring. Corrected assessments more accurately depict the seasonal development of *V. inaequalis* and can be used to more accurately detect the depletion of the supply of ascospores.

Additional key words: disease management.

The management of apple scab requires accurate information on the development of the pathogen (*Venturia inaequalis* (Cke.) Wint.) population. The disease is normally controlled by repeated fungicide applications, most of which are directed against the primary inoculum. Different management strategies have been developed to more efficiently schedule these applications. A common feature of the various management strategies is that the intervals between fungicide applications are lengthened once the supply of ascospores, the primary inoculum, is exhausted. The more precisely the initiation and length of the primary infection season can be defined, the more efficiently fungicide applications can be scheduled.

Examination of crushed pseudothecia is commonly used to assess ascospore maturity and discharge (2,4,6–10). There are differences in the techniques used by various research workers, but they involve categorization of asci as immature, mature, or empty (spores discharged). A percentage of the season's primary inoculum in a particular maturity class could be calculated from this categorization. The only detailed description of a procedure for the preparation and interpretation of squash mounts of pseudothecia of *V. inaequalis* is that of Szkolnik (8), who examined 20 asci from each of 20–25 pseudothecia. Massie and Gilpatrick (6) formulated a mathematical model of ascospore maturation by using data collected by Szkolnik, but this model has not accurately described ascospore maturation in Michigan (6) or New Hampshire (3). In New Hampshire, the model has consistently overestimated ascospore maturity in early spring and underestimated it in late spring.

Previously described methods for assessing ascospore maturity and discharge (2,4,6–10) have been based on the tacit assumption that asci examined at various times during the primary infection season represent the season's total production of asci; ie, all of the asci in a pseudothecium. We have shown that the number of asci per pseudothecium increases in early spring as the earliest developing asci mature, and then declines as empty asci disintegrate. Thus, previously described methods provide an estimate of ascospore maturity and discharge *only* in that portion of the population that is present at the time of assessment. Early spring assessments of ascospore maturity and discharge are biased by the exclusion of unseen asci. Assessments in late spring are affected by the exclusion of disintegrated empty asci.

This paper describes a technique for the preparation and interpretation of pseudothecial squash mounts that uses the maximum number of asci recorded per pseudothecium to adjust assessments for exclusion of unseen asci in early spring and the exclusion of disintegrated asci in late spring.

MATERIALS AND METHODS

Infected leaves collected from beneath unsprayed apple trees (*Malus pumila* Miller 'McIntosh') during the autumns of 1978 and 1979 were overwintered in wire mesh trays at a research orchard in Durham, NH. Beginning in early spring and at weekly intervals thereafter, 10 leaves were selected for examination and were fixed in water at 90 C as described by Szkolnik (8). A total of 20 pseudothecia were removed from the leaves with a fine sewing needle fitted to a wooden handle. Pseudothecia were randomly selected from the leaves under a dissecting microscope (×25) equipped with an ocular pointer. After a leaf was placed on the microscope stage, the two pseudothecia closest to the pointer were removed and transferred to a drop of lactophenol on a glass slide. A coverslip was placed over the drop, and pressure was applied over each pseudothecium to split the ascocarp wall while the pseudothecia were observed under low-power magnification ×100). The coverslip was then moved from side to side to spread the asci in a thin layer. The number of asci in each of the following maturity classes (Fig. 1) was recorded: immature asci, either without spores or with delimited, uncolored spores; mature asci containing septate, olivaceous spores; and empty asci (spores discharged). For lack of other criteria, ascospores were assumed to be mature when septate and olive green in color. Many pseudothecia examined in early spring contained only ascus initials and pseudoparaphyses (Fig. 2).

The maximum number of asci recorded per pseudothecium (A/P_{max}) was not known until midway through the ascospore discharge season. In the weeks prior to the occurrence of A/P_{max}, the percentage of asci in the various maturity classes was calculated as a percentage of the weekly average number of asci per

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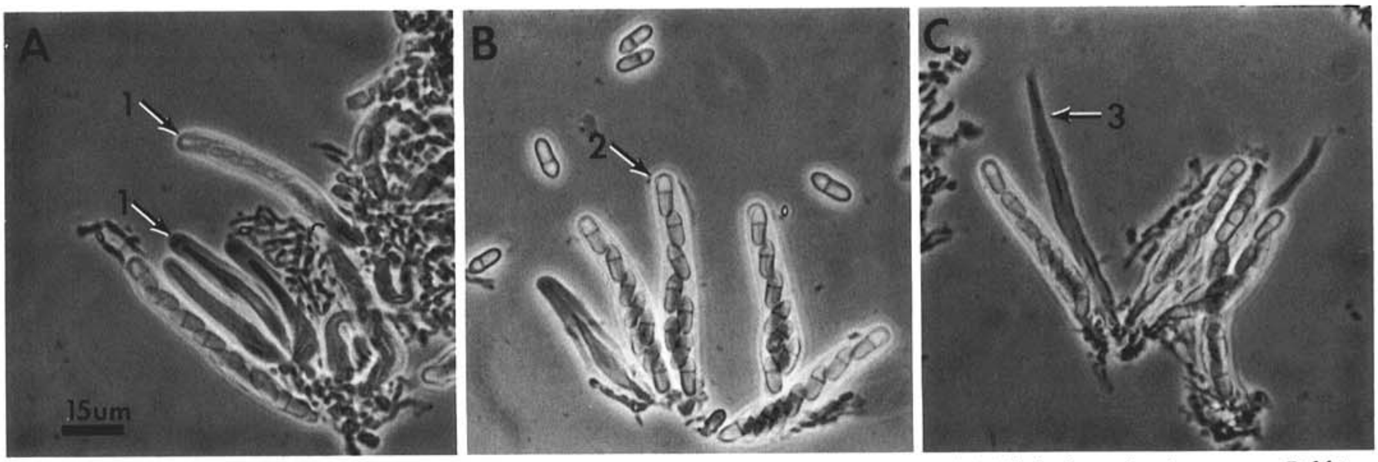

Fig. 1. Maturity classes of asci of *Venturia inaequalis*. **A,** Immature asci (1), either without ascospores or with delimited, uncolored ascospores. **B,** Mature asci (2) containing septate, olivaceous ascospores. **C,** Empty asci (3) after ascospore discharge.

pseudothecium (A/P_t). Once A/P_{max} was known, an estimate of the percentage of the season's primary inoculum in any of the maturity classes could be calculated as a percentage of A/P_{max} after the use of one of the following correction factors: assessments made prior to the occurrence of A/P_{max} were adjusted by adding the difference between A/P_{max} and A/P_t to the average number of immature asci per pseudothecium counted in $week_{(t)}$ (pseudothecia that contained only ascus initials and pseudoparaphyses were counted as having contained A/P_{max} immature asci); in the weeks after the occurrence of A/P_{max}, the difference between A/P_{max} and A/P_t was added to the average number of empty asci counted per pseudothecium in $week_{(t)}$. We assumed that empty asci did not disintegrate prior to the occurrence of A/P_{max} and that after A/P_{max} had been reached, no additional asci were formed.

RESULTS

When plotted against time, A/P_t rose throughout April and early May and then declined (Fig. 3). A/P_{max} occurred on 7 May in 1979 and on 18 May in 1980. The increase in A/P_t in early spring was due to the continuous development of new asci, and the subsequent decline in A/P_t was due to the disintegration of empty asci. In early spring, the proportions of the season's ascospores matured or discharged were overestimated, because many asci had not yet become visible (Fig. 4A). Late in the ascospore discharge season, the proportion of mature ascospores was overestimated and the proportion of discharged ascospores was underestimated because many empty asci had disintegrated and were no longer visible (Fig. 4D). The previously described correction factors were used to adjust assessments to compensate for unseen asci in early spring and disintegrated asci in late spring. For example, on 28 April 1980 the mean number of asci counted per pseudothecium (A/P_t) was 47. Of these 47 asci, an average of 29 (62%) were immature, 16 (34%) contained mature ascospores, and two (4%) were empty. The maximum number of asci per pseudothecium, A/P_{max}, was 102 (Fig. 3); therefore, $102-47=55$ asci were added to the 29 immature asci and the proportion of each category based on 102 total asci was calculated as 82% immature, 16% mature, and 2% empty (Table 1). On 19 June 1979, A/P_t was 38. Of these 38 asci, 11 (29%) contained mature ascospores and 27 (71%) were empty. Since A/P_{max} was 155, $155-38=117$ asci were added to the 27 empty asci and the proportions of mature and empty asci, based on 155 total asci, were calculated as 7 and 93%, respectively (Table 1). The dates of 95% (±2%) ascospore depletion, as determined by corrected assessments, were 19 June in 1979 and 5 June in 1980. The comparable dates for uncorrected assessments were 1 July 1979 and 27 June 1980.

Our assumption that ascus formation is complete by the date of A/P_{max} is supported by assessments made on 21 May and 19 June 1979 (Fig. 4C–D). Less than 2% of the asci examined on 21 May were immature. No immature asci were found in pseudothecia examined after 28 May 1979. No significant disintegration of empty asci occurred prior to A/P_{max} in either 1979 or 1980 except from 13 to 20 April 1979 (Table 2), when the mean number of empty asci per pseudothecium decreased from 6.05 to 0.55. The disintegration of empty asci was a phenomenon that primarily occurred in late spring. Therefore, A/P_{max} will usually be a valid estimate of the total number of asci produced during the primary infection season.

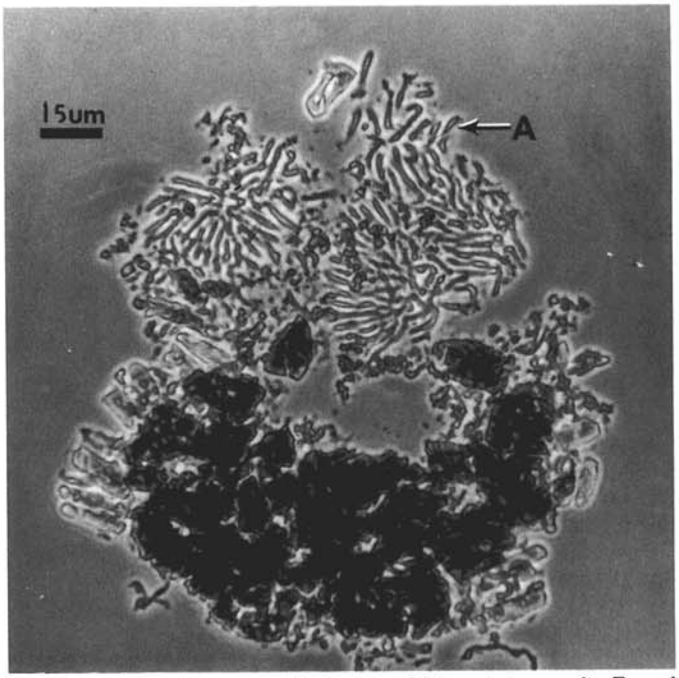

Fig. 2. Crushed immature pseudothecium of *Venturia inaequalis*. Fungal material (A) is composed primarily of ascus initials and pseudoparaphyses.

DISCUSSION

Numerous workers (2,4,6–10) have examined crushed pseudothecia of *V. inaequalis* to assess ascospore maturity and discharge, but none has corrected assessments for changes in the number of asci per pseudothecium. Failure to do so can present a distorted picture of the patterns of ascospore maturation. Compared to corrected assessments, a mathematical model of ascospore maturation (6) based on uncorrected assessments consistently overestimated ascospore maturity in early spring and underestimated ascospore maturity in late spring (3). Miller and

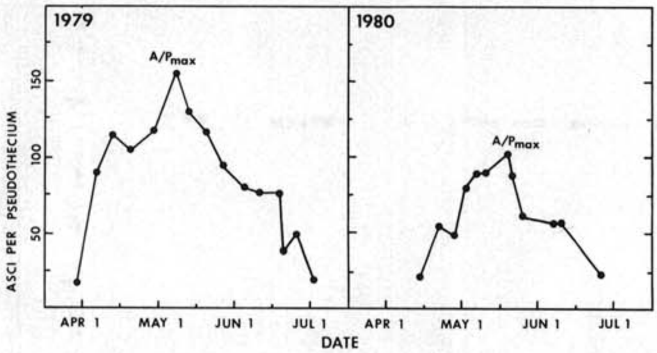

Fig. 3. Asci per pseudothecium in Durham, NH, during 1979 and 1980. Twenty pseudothecia of *Venturia inaequalis* were crushed and examined at weekly intervals. The number of asci per pseudothecium reached a maximum of 155 on 7 May 1979 and a maximum of 102 on 18 May 1980.

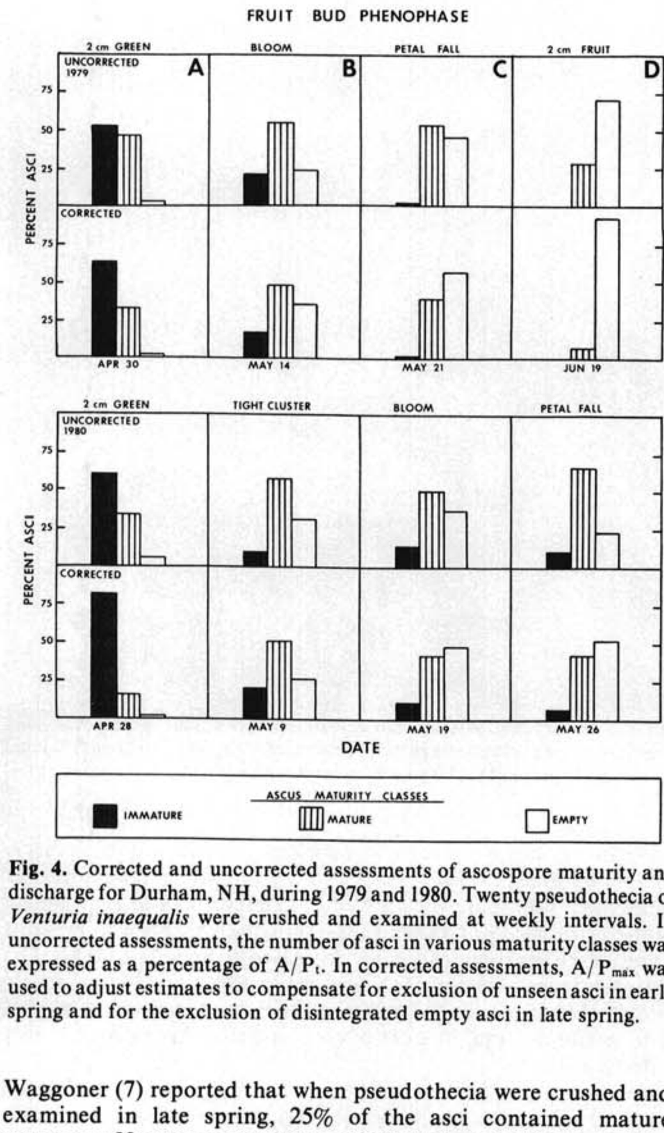

Fig. 4. Corrected and uncorrected assessments of ascospore maturity and discharge for Durham, NH, during 1979 and 1980. Twenty pseudothecia of *Venturia inaequalis* were crushed and examined at weekly intervals. In uncorrected assessments, the number of asci in various maturity classes was expressed as a percentage of A/P_t. In corrected assessments, A/P_{max} was used to adjust estimates to compensate for exclusion of unseen asci in early spring and for the exclusion of disintegrated empty asci in late spring.

Waggoner (7) reported that when pseudothecia were crushed and examined in late spring, 25% of the asci contained mature ascospore. No ascospores were collected by volumetric traps at the leaf storage site, even though weather was favorable for ascospore discharge. They concluded that the absence of airborne ascospores was not due to a depletion of the inoculum supply, but rather to a weakening of the discharge mechanism. Based on the results of our study, we suggest a different explanation for this phenomenon. The procedure used by Miller and Waggoner (7) underestimated the percentage of discharged ascospores. For example, when we examined pseudothecia on 1 July 1979, only 84% of the asci present were empty, but when the number of empty asci was adjusted for ascus disintegration and expressed as a percentage of A/P_{max}, we found that 98% of the ascospores had been discharged.

TABLE 1. Effects of correction factors on assessments of ascospore maturity and discharge of *Venturia inaequalis*

	Ascospore stage (%)[a]			
	Early spring		Late spring	
	Uncorrected	Corrected	Uncorrected	Corrected
Immature	62	82	0[b]	0
Mature	34	16	29	7
Discharged	4	2	78	93

[a] Early-spring (28 April) assessment was adjusted to compensate for exclusion of unseen immature asci. Late-spring (19 June) assessment was adjusted to compensate for exclusion of disintegrated empty asci.
[b] Uncorrected percentage is not significantly different from the corrected percentage at $P = 0.10$.

TABLE 2. Changes in the number of empty asci per pseudothecium of *Venturia inaequalis* between observations. Weather conditions between paired observation dates were not suitable for ascospore discharge

1979		1980	
Date	Empty asci	Date	Empty asci
31 March	0.35[a]	23 April	1.65[a]
6 April	0.50	28 April	2.20
13 April	6.05[b]	1 May	6.10[a]
20 April	0.55	5 May	5.70
30 April	2.85[a]	9 May	27.90[a]
7 May	2.80	18 May	29.00

[a] Differences between means are not significant at $P = 0.10$.
[b] Differences between means are significant at $P = 0.10$.

In 1979 and 1980, growers in the Durham area were advised to lengthen the interval between fungicide applications once 95% of the ascospores had been discharged. The use of uncorrected estimates of ascospore discharge would have delayed this advisement by 12 days in 1979 and by 22 days in 1980. Reduction in the number of fungicide sprays varied depending on whether a protectant (1) or eradicant (5) fungicide program was in use, but from one to two sprays were saved in 1979 and two sprays were saved in 1980.

Changes in A/P_t can be monitored and used to adjust ascospore maturity and discharge assessments to more accurately depict the development of the pathogen population. Adjustments of early-season assessments are made ex post facto, since A/P_{max} is not known until midseason. However, corrections made after the fact are preferable to uncorrected errors and prove useful in a retrospective analysis of data. It may be possible to predict A/P_{max} based on late-winter and early-spring temperatures (3), and this would allow the immediate adjustment of early-spring assessments. Assessments made after the occurrence of A/P_{max} can be corrected immediately. Late-season assessments of ascospore maturity and discharge are perhaps the most critical in disease management programs since the depletion of the supply of primary inoculum is an event that indicates that the time interval between fungicide applications directed against apple scab can be lengthened. This depletion of inoculum can be more accurately detected when assessments of ascospore maturity and discharge are adjusted for changes in A/P_t rather than expressed as a percentage of the asci sampled.

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