Environmental Factors Influencing Pseudothecial Development and Ascospore Maturation of *Venturia inaequalis*

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ABSTRACT

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Pseudothecial ontogeny of *Venturia inaequalis* in apple leaves could be separated into two distinct phases. Ascogonia developed after leaf fall until the lumina of the pseudothecia were filled with pseudoparaphyses. Development of asci and ascospores was initiated in the spring only after a dormant or rest period during which no observable development was detected in the lumina of the pseudothecia regardless of temperature or moisture treatments. Laboratory and field studies made during a 3-yr period indicated that moisture was a limiting factor. Pseudothecia did not develop in air-dried apple leaves, but they did reach maturity during exposure to 100% relative humidity. The optimum temperature range for ascogonial development was 8–12 C and for ascospore maturation it was 16–18 C. Cultivar and date of leaf fall had no effect on date of ascospore maturation in the spring.

Additional key words: apple scab, epidemiology, *Malus sylvestris*.

Apple scab, which is caused by *Venturia inaequalis* (Cke.) Wint., is considered the major disease of apple (*Malus sylvestris* Mill.) trees in the United States. Control is based on the use of protectant or eradicant fungicidal sprays to prevent the primary disease cycle, which is initiated by ascospores ejected from pseudothecia that overwinter and develop in infected leaves on the orchard floor. Timing of fungicidal sprays to coincide with ascospore maturity and discharge is critical to disease control.

Wilson (12) indicated that temperature, leaf moisture, time of leaf fall, time of infection, and apple cultivar were major factors affecting development of pseudothecia in Wisconsin. Keitt and Jones (6) reported that average temperature during January, February, and March was more closely related to development of pseudothecia and time of ascospore discharge than was mean temperature and rainfall during April and May preceding discharge. Miller and Waggoner (9) observed that pseudothecia matured at approximately the same time each year regardless of spring temperatures and suggested that the average temperature during the winter was more important than spring temperature for their development. Hirst and Stedman (3) and Burchill (1) found that leaf fall date had no effect on the maturation of pseudothecia.

Several methods have been developed to determine ascospore maturity and discharge (8,11). Massie and Szkolnik (8) developed an equation to predict ascospore maturity based on accumulated degree days (using a 0 C degree-day base) and rainfall (in 2.54 cm) from the time of 50% leaf fall. The equation is not accurate under North Carolina environmental conditions, predicting ascospore maturity much too early (10).

The purpose of this study was to quantify the influence of environmental factors on pseudothecial development of *V. inaequalis*.

MATERIALS AND METHODS

Laboratory incubation studies, 1977–1978 and 1978–1979. Apple leaves of the Delicious cultivar infected with *V. inaequalis* were removed from orchard trees in Henderson County, NC, at 10% leaf fall (12 October 1977 and 19 October 1978). Leaf disks (1.3 cm in diameter) were punched from the leaves, placed in cheesecloth bags, and overwintered in Saran cloth (Chicopee Mfg. Co., Cornella, GA 30531) cages beneath apple trees at the Mountain Horticultural Crops Research Station (MHCRS), Fletcher, NC. At 2-wk intervals, from 31 October 1977 to 1 May 1978 and from 6 November 1978 to 27 March 1979, leaf disks (10 disks per treatment) were brought into the laboratory and subjected to a factorial design of 10 temperature and four moisture treatments. The temperatures were 0, 4, 8, 12, 16, 18, 20, 24, 28, and 32 C. The moisture treatments were: wet (leaves fully pliable; 40% leaf moisture); dry (10% leaf moisture); 1 wk wet/1 wk dry; and 1 wk dry/1 wk wet. Leaf disks in wet treatments were soaked in distilled water for 90 min, and placed in a single layer on moist laboratory towels; leaf disks in dry treatments were allowed to air-dry for 2 hr and placed on dry laboratory towels. Leaf disks were incubated in the dark in molded plastic boxes. During the winter of 1978–1979, the 18 and 32 C temperature treatments and the dry-wet moisture treatments were not included.

At the end of each incubation period, leaves were fixed in a mixture of 2-propanol, water, propionic acid, and formaldehyde (45:45:5:5, v/v) (FPP), dehydrated, embedded in Tissue Prep (MP = 63.5 C, Fisher Scientific Co., Fair Lawn, NJ 07410), sectioned at 12 μm, and stained according to a modified Conant's staining schedule (5). Pseudothecia were rated according to internal stage (st) of development as follows: st 1, subcuticular stroma; st 2, pseudothecial initial showing coiling of hyphae; st 3, formation of the ascogonium from the initial; st 4, pseudoparaphyses beginning to appear in the lumen of a pseudothecium as the ascogonium disappears; st 5, lumen of the pseudothecium filled with pseudoparaphyses; st 6, appearance of asci; st 7, asci one-half mature size; st 8, asci formed but contents not differentiated; st 9, asci with spores being formed, but not yet septate; st 10, asci with ascospores being formed, usually septate; st 11, asci with ascospores formed, but not pigmented; st 12, ascospores pigmented and mature; st 13, ascospores discharged (ie, asci empty); and st 14, asci aborted (Figs. 1–15). Serial sections of pseudothecia were observed and 50 pseudothecia per treatment (if available) were rated from the leaf disks. Pseudothecia with asci in different stages of development were given average ratings. Diameters of

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pseudothecia were also measured.

Regression analyses were used to describe pseudothecial development during each 2-wk incubation period (from 31 October 1977 to 1 May 1978 and 6 November 1978 to 27 March 1979) as a function of temperature for each moisture treatment. The large number of observations per treatment was relied on to compensate for expected non-normality stemming from the categorical nature of the data.

Relative humidity study, 1979–1980. Delicious apple leaves infected with *V. inaequalis* were removed from orchard trees in Henderson County, NC, at 10% leaf fall (15 October 1979). Leaf disks (1.3 cm in diameter) were punched from the leaves, placed in cheesecloth bags, and overwintered in Saran cloth cages beneath apple trees at MHCRS. On 4 and 18 February and 5 and 17 March, leaf disks were brought into the laboratory and subjected to various relative humidity (RH) and temperature treatments for 2-wk periods.

Relative humidities were maintained with aqueous supersaturated salt solutions in 1-L glass jars (13). Leaf disks (10 disks per treatment) were suspended 4 cm above the salt solution in Saran cloth baskets. Relative humidities maintained by saturated salt solutions in distilled water were: 100% RH, distilled water; 98% RH, potassium sulfate; 95% RH, potassium nitrate; 88% RH, potassium chloride; 80% RH, ammonium sulfate; and 75% RH, sodium chloride. Additionally, leaf disks were also kept wet (fully pliable), air-dried, or wet 1 wk/dry 1 wk. Leaf disks were incubated at 4, 8, 12, or 16 C. At the end of each incubation period, leaves were fixed in FPP, prepared for microscopic examination, and pseudothecia were rated as in the laboratory study.

Storage RH was checked by using a Hygrometer Indicator (Hygrodynamics Inc., Silver Springs, MD 20907). All salt solutions maintained RH within 1.5% of desired levels except for potassium chloride which was within 4%.

The experimental results were subjected to an analysis of variance with a factorial arrangement of temperature and moisture treatments. Incubation periods were analyzed both separately and combined.

Leaf moisture content. To determine leaf water content, on 5 March, five apple leaf disks (2.6 cm in diameter) were placed in the same moisture and temperature treatments as those used in the RH study. Water saturation deficit (WSD) was used as a measure of leaf water content (2). WSD is the ratio of the amount of actual water in tissue to the potential amount of water the tissue can absorb and is determined by the equation:

$$\text{WSD} = \frac{(\text{fully turgid weight}) - (\text{fresh weight})}{(\text{fully turgid weight}) - (\text{oven-dry weight})} \times 100\% \quad (1)$$

in which fully turgid weight = the weight of leaf disks after being soaked in distilled water for 4 hr; fresh weight = weight of leaf disks when they were removed from the relative humidity chambers; and oven-dry weight = weight of leaf disks after drying for 24 hr at 85 C.

Field study, 1977–1980. Delicious apple leaves infected with *V. inaequalis* were removed from trees at 10% leaf fall (12 October 1977, 19 October 1978, and 15 October 1979) from orchards in Henderson County, NC. Leaf disks (1.3 cm in diameter) were punched from the leaves, placed in cheesecloth bags, and

TABLE 1. Temperature dependent regression equations for stage of pseudothecial development of *Venturia inaequalis* during 2-wk incubation periods during 1978–1979[a]

Incubation period[b]	Field stage[c]	Moisture treatment[d]	Regression equations[e]	R^2[f]	Probability >F
6 November	1.0	1		0.611	0.01
		2	$y = 2.4 + 0.113\,(\text{TEMP}) - 0.006\,(\text{TEMP})^2$		
		3	$y = 1.2 + 0.135\,(\text{TEMP}) - 0.006\,(\text{TEMP})^2$		
20 November	2.0	1		0.453	0.01
		2	$y = 2.6 + 0.235\,(\text{TEMP}) - 0.012\,(\text{TEMP})^2$		
		3	$y = 2.4 + 0.169\,(\text{TEMP}) - 0.008\,(\text{TEMP})^2$		
4 December	3.5	1		0.222	0.01
		2	$y = 4.1 + 0.120\,(\text{TEMP}) - 0.006\,(\text{TEMP})^2$		
		3	$y = 3.2 + 0.125\,(\text{TEMP}) - 0.005\,(\text{TEMP})^2$		
18 December	3.4	1	$y = 4.04 + 0.066\,(\text{TEMP}) - 0.002\,(\text{TEMP})^2$	0.274	0.01
		2			
		3			
2 January	5.0	1			0.76
		2			
		3			
15 January	5.0	1		0.035	0.01
		2	$y = 5.0 + 0.043\,(\text{TEMP}) - 0.002\,(\text{TEMP})^2$		
		3			
29 January	5.0	1		0.250	0.01
		2	$y = 4.8 + 0.150\,(\text{TEMP}) - 0.005\,(\text{TEMP})^2$		
		3	$y = 4.9 + 0.029\,(\text{TEMP}) - 0.001\,(\text{TEMP})^2$		
12 February	5.0	1		0.411	0.01
		2	$y = 4.9 + 0.433\,(\text{TEMP}) - 0.017\,(\text{TEMP})^2$		
		3	$y = 4.8 + 0.117\,(\text{TEMP}) - 0.004\,(\text{TEMP})^2$		
26 February	6.0	1		0.656	0.01
		2	$y = 6.5 + 0.839\,(\text{TEMP}) - 0.035\,(\text{TEMP})^2$		
		3	$y = 5.4 + 0.590\,(\text{TEMP}) - 0.022\,(\text{TEMP})^2$		
12 March	8.3	1		0.697	0.01
		2	$y = 10.6 + 0.217\,(\text{TEMP}) - 0.008\,(\text{TEMP})^2$		
		3	$y = 8.6 + 0.406\,(\text{TEMP}) - 0.013\,(\text{TEMP})^2$		
26 March	9.9	1		0.581	0.01
		2	$y = 10.9 + 0.181\,(\text{TEMP}) - 0.005\,(\text{TEMP})^2$		
		3	$y = 9.7 + 0.343\,(\text{TEMP}) - 0.011\,(\text{TEMP})^2$		

[a] Leaves were collected on 19 October 1978, and overwintered beneath apple trees at MHCRS until brought into the laboratory. See Figs. 1–15 for definition of stages.

[b] Beginning date of each 2-wk incubation period.

[c] Field check at the beginning of each incubation period.

[d] Moisture treatment 1 = dry for 2 wk; moisture treatment 2 = wet for 2 wk; and moisture treatment 3 = wet 1 wk/dry 1 wk.

[e] Only significant ($P = 0.01$) beta values are included. y = stage of pseudothecial development.

[f] R^2 value for all equations in the incubation period.

overwintered in Saran cloth cages placed on the ground.

Each year, leaves were overwintered at four different locations. During the winter of 1977–1978, the locations were: MHCRS; Boone, NC (BOONE); the Walter Pace Orchard at Saluda, NC (PACE); and Central Crops Research Station at Clayton, NC (CC). During the winter of 1978–1979, the locations were MHCRS, BOONE, CC, and the Horticultural Farm on the Michigan State University Campus in East Lansing, MI (EL). During the winter of 1979–1980, locations were MHCRS, BOONE, EL, and near the North Carolina State University campus, Raleigh (NCSU). Leaf sampling began on 31 October 1977, 6 November 1978, and 12 November 1979, and continued at 2-wk intervals until mature ascospores were observed the following spring. Two replications (10 disks per replication) were collected on each date. Leaves were fixed in FPP, prepared for microscopic examination, and pseudothecia were rated for developmental stages as in the laboratory study.

At each location temperature (TEMP) and RH were measured hourly with a hygrothermograph enclosed in a standard instrument shelter. At MHCRS and PACE, rainfall (RAIN) was measured with a recording top-weighing rain gauge; at CC it was measured with a tipping-bucket rain gauge; and at BOONE and EL it was measured with a fence-post rain gauge. Meteorological data obtained from the Asheville Airport (AV) were used as an historical base for seasonal comparisons in the Henderson County area (MHCRS and PACE orchards).

To determine the relationship of pseudothecial development to the meteorological variables of TEMP, RH, and RAIN, developmental stages for each 2-wk period were correlated with: mean of daily temperature maxima (MAXT), minima (MINT), and average TEMP (AVGT); accumulated degree days using a 0 C base (DEGREE DAYS); amount of RAIN, number of days in which RAIN $\geqslant$ 0.25 mm (NRAIN); and accumulated hours of RH = 100% (RH100), $\geqslant$ 95% (RH95), $\geqslant$ 90% (RH90), and $\geqslant$ 80% (RH80).

A stepwise multiple linear regression analysis was performed on the data by using the meteorological variables listed above as independent variables and pseudothecial development stage as the dependent variable. The best three-variable equation was retained.

Leaf fall and cultivar study, 1978–1979 and 1979–1980. During the fall of 1978, Delicious apple leaves infected with *V. inaequalis* were collected in nets beneath apple trees on 22 and 29 September, 6, 13, 20, and 27 October, and 3 November. Leaf disks (1.3 cm in diameter) were punched from the leaves, placed in cheesecloth bags and overwintered in a Saran cloth cage beneath an apple tree at MHCRS. On 4 December, 12 and 26 February, 12 and 26 March, and 9 April, two replications (10 disks per replicate) were collected from samples collected on each leaf fall date. Leaves were fixed in FPP, prepared for microscopic examination, and pseudothecia were rated as in the laboratory study.

During the fall of 1979, leaves of cultivars Delicious, Golden Delicious, Rome Beauty, and McIntosh infected with *V. inaequalis* were collected during leaf fall on 10 and 26 October and 9 November. Leaf disks (1.3 cm in diameter) were punched from the leaves, placed in cheesecloth bags, and overwintered in a Saran cloth cage beneath an apple tree at MHCRS. A subsample of the leaf disks collected on 26 October was suspended in an apple tree until 2 January 1980, when it was placed on the ground. Delicious leaves were sampled on 4 December, 4 and 18 February, 5 and 17 March, and 1 and 14 April. Other cultivars were sampled on 5 and 31 March. Leaves were fixed in FPP, prepared for microscopic examination, and development of pseudothecia was rated as in the laboratory study.

RESULTS

Laboratory incubation study. The ontogeny of pseudothecia of *V. inaequalis* could be separated into two distinct phases. In the fall, ascogonial initials (st 2) developed until the lumina of the pseudothecia were filled with pseudoparaphyses (st 5). Development of asci and ascospores (st 6 to 12) was initiated only after a dormant period during which no development was detected in the lumina of pseudothecia regardless of temperature or moisture treatments.

In wet treatments, the optimum temperature range for ascogonial development was 8–12 C; 16–18 C was the optimum temperature range for ascospore maturation (Fig. 16). Little development occurred at 0 C. Some abortion of pseudothecia occurred at 24 C; at 28 and 32 C, all pseudothecia aborted. In aborted pseudothecia, there was a breakdown of the internal contents of the lumina. Less abortion of pseudothecia occurred in dry than in wet treatments.

During the winter of 1978–1979, pseudothecial development was best described by quadratic equations for temperature before development reached st 5 or st 12 (Table 1). In the fall of 1978, R^2 values of 0.611, 0.453, and 0.222 were obtained between stages of pseudothecial development and quadratic temperature equations for incubation periods beginning on 6 and 20 November and 4 December, respectively (Table 1). There were no linear or quadratic temperature effects in the wet or wet/dry treatments during the incubation period beginning on 18 December because pseudothecia matured to st 5 at all temperatures (Fig. 16). Temperature and moisture had no effect (P = 0.77) on pseudothecial development during the period from 2 to 15 January 1979, when all pseudothecia were in st 5 (Table 1) (Fig. 16). An R^2 value of 0.035 was obtained for quadratic equations in the incubation period beginning 15 January (Table 1).

Asci appeared in pseudothecia in wet treatments during incubation periods beginning on 23 January 1978 and 15 January 1979; mature ascospores were observed in pseudothecia in 16 C wet treatments during incubation periods beginning on 6 February 1978 and 12 February 1979.

R^2 values of 0.25, 0.411, 0.656, 0.697, and 0.581 were obtained between stages of pseudothecial development and quadratic temperature equations for incubation periods beginning on 29 January, 12 and 26 February, and 12 and 26 March 1979, respectively (Table 1). No significant temperature effect was observed in wet/dry treatments during the incubation period beginning on 15 January, and rate of development in wet treatments was less than in later periods (Table 1).

Little or no pseudothecial development occurred in air-dried apple leaves during 1978–1979. There was no significant (P = 0.05) linear or quadratic temperature effect on pseudothecial development except during the incubation period beginning on 18 December (Table 1). Pseudothecial development in wet/dry treatments was intermediate between wet and dry treatments (Table 1).

Regression equations similar to those presented in Table 1 were obtained for incubation periods during 1977–1978. Little difference was observed between wet/dry and dry/wet treatments.

The correlation between pseudothecial diameters and stages of

TABLE 2. Influence of relative humidity, moisture, and temperature on stages (st) of pseudothecial development of *Venturia inaequalis* in apple leaves incubated from 17 March to 31 March 1980[w]

Percent RH or moisture level[x]	°C				
	4	8	12	16	Mean[y]
100	11.3[z]	11.5	12.0	12.1	11.6 a
98	7.3	7.5	7.6	9.2	7.8 b
95	7.7	10.6	7.5	9.5	8.8 b
88	7.0	8.5	7.2	7.3	7.5 b
80	9.2	10.3	6.8	8.2	8.7 b
75	6.8	7.0	9.7	7.4	7.7 b
Wet	11.6	12.0	11.9	11.9	11.9 a
Dry	7.5	7.6	7.8	7.5	7.6 b
Wet/Dry	11.9	11.5	12.0	12.2	11.9 a
Mean	9.3 a	9.8 a	8.7 a	9.5 a	

[w] Field check on 17 March was st 8.0.

[x] Leaves in wet treatments were kept moist for 2 wk; leaves in dry treatment were kept air-dried for 2 wk; leaves in wet/dry treatments were kept moist 1 wk and dry 1 wk.

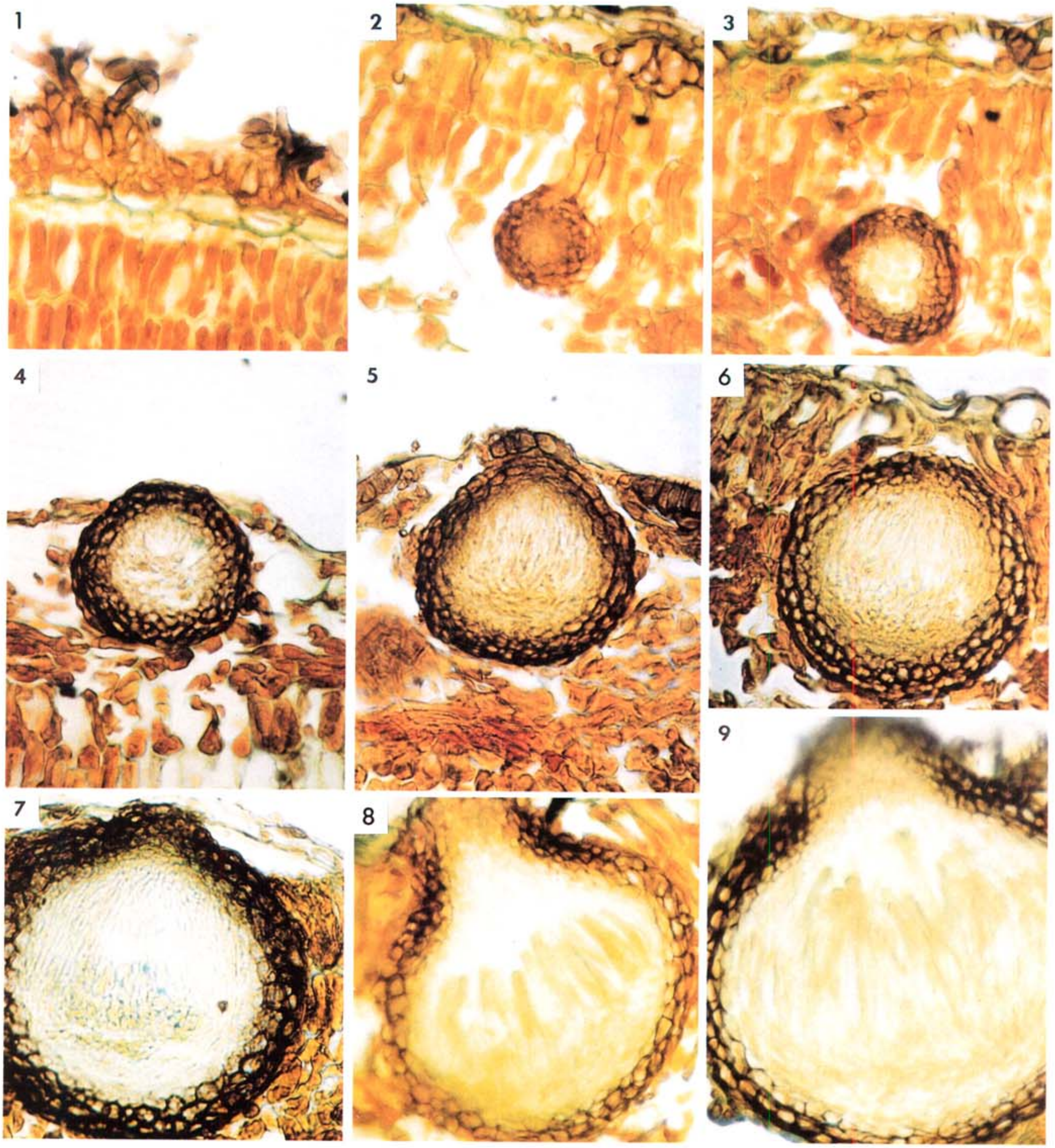
[y] Different letters denote differences in significance, P = 0.05.

[z] See Figs. 1–15 for definition of stages.

development was greater during st 1 to 4 ($r = 0.77$, $P = 0.01$) than st 6–12 ($r = 0.54$, $P = 0.01$). Ascogonial initials (st 2) were approximately 22 μm, and ascogonia (st 3), 58 μm. Although pseudothecia remained in st 5 (Fig. 5) during the winter, they increased in diameter (Fig. 6) and were approximately 88 μm when asci began to appear. Mature pseudothecia (st 12) were approximately 120 μm in diameter.

Relative humidity study. In the combined analysis of the four RH incubation periods, 100% RH was the only relative humidity treatment significantly different ($P = 0.01$) from the 2-wk dry treatments. No difference ($P = 0.01$) was observed among the 98, 95, 90, 88, 80, and 75% RH treatments. Wet treatments were optimum for pseudothecial development for all incubation periods except the period from 17–31 March when mature ascospores (st 12) were also observed in all 100% RH treatments (Table 2). No significant difference ($P = 0.01$) was observed between 100% RH and wet-dry treatment during the four incubation periods.

At 100% RH, pseudothecial development at 8, 12, and 16 C was significantly greater ($P = 0.01$) than at 4 C during the first three incubation periods. However, no difference was observed during



Figs. 1–9. Pseudothecial development of *Venturia inaequalis* in various stages: **1,** (stage [st] 1) subcuticular stroma; **2,** (st 2) pseudothecial initial showing coiling of the hyphae; **3,** (st 3) formation of the ascogonium from the initial; **4,** (st 4) pseudoparaphyses beginning to appear in the lumen of pseudothecium as the ascogonium disappears; **5,** (early st 5) pseudoparaphyses fill the lumen and the pseudothecium has increased in diameter; **6,** (later st 5) pseudothecium that has increased in diameter; **7,** (st 6) appearance of asci; **8,** (st 7) asci about one-half mature size; **9,** (st 8) asci formed, but contents not differentiated. All figures ×400.

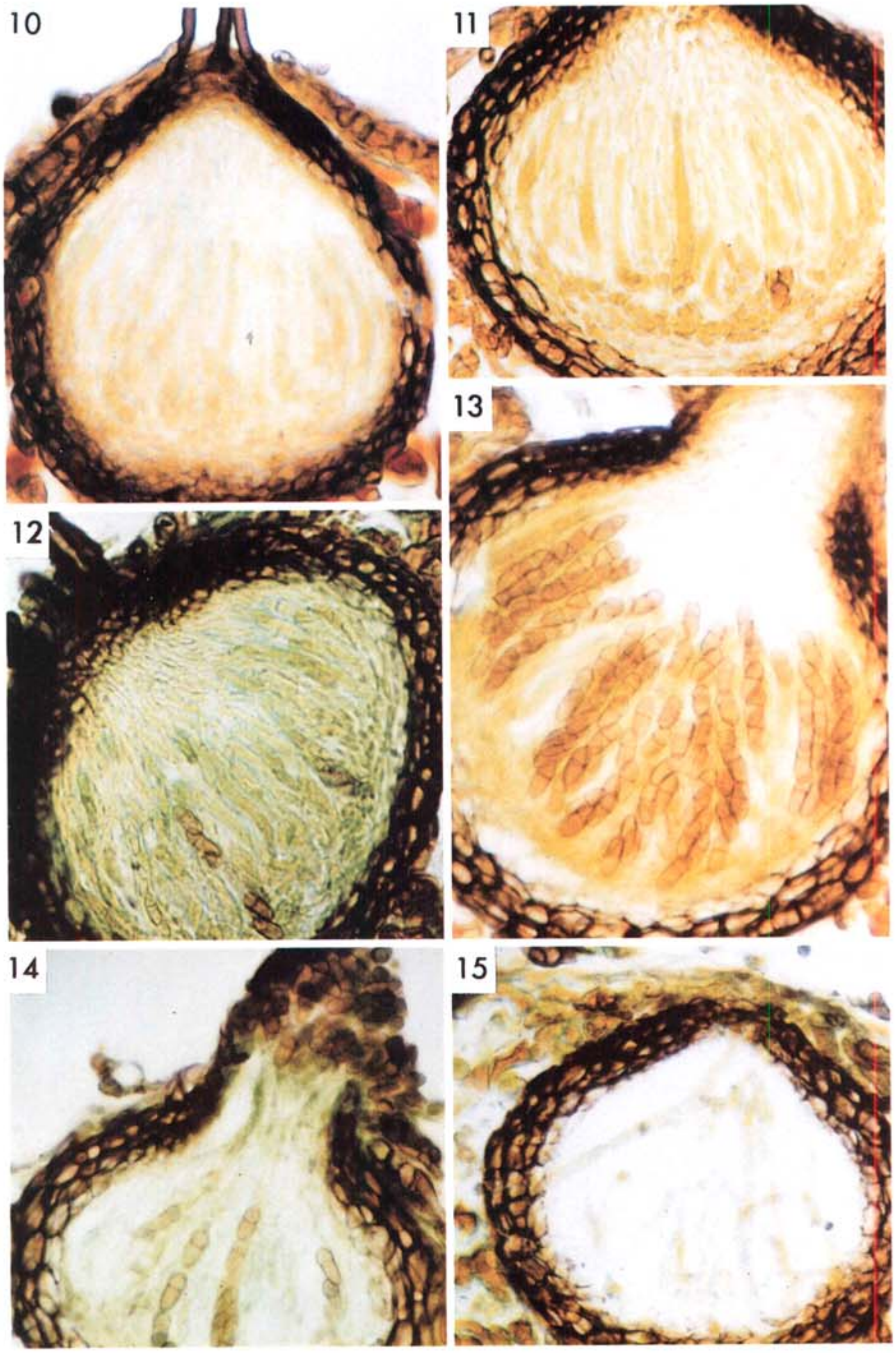
the incubation period from 17–31 March as mature ascospores (st 12) formed in all wet, wet/dry, and 100% RH treatments (Table 2).

Average WSD recorded during the incubation period from 5–17 March for 100, 98, 95, 88, 80, and 75% RH were 70, 83, 85, 89, 90, and 92%, respectively. Average WSD for wet and dry moisture treatments were 39 and 96, respectively. No consistent differences in WSD were observed at the different temperatures. Pseudothecial development of *V. inaequalis* was retarded at WSD less than ~85%.

Field study. During the 3 yr of the study, considerable differences were observed in date of ascospore maturation (Tables 3–5). During the fall of 1977, ascogonial initials developed within 2 wk of leaf fall and pseudothecia matured to st 5 by 12 December (Table 3). Below-normal rainfall was recorded during February and April of the following spring (Table 6). At MHCRS asci (st 6) were not observed until 20 March and ascospores matured near 1 May (Fig. 17). Little difference in the date of pseudothecial maturation was observed at MHCRS, BOONE, or PACE during 1977–1978 (Table 3). *V. inaequalis* did not overwinter well at CC. Few ascogonia initials formed in November and only a few pseudothecia matured the following spring (Table 3).

During the fall of 1978, below-normal rainfall was recorded during October and November (Table 6) and ascogonia initials developed later in November (Table 4). Asci began to appear on 12 February, and ascospores matured about 9 April at all locations in NC (Table 4). At MHCRS, above-normal rainfall was recorded in February, March, and April (Table 6), and pseudothecia matured 3–4 wk earlier than the previous spring (Fig. 18).

Pseudothecial development at EL occurred approximately 3 wk later than at locations in NC. Ascogonia (st 3) were formed by 18 December, st 5 by 29 January, st 6 by 12 March, and mature ascospores were formed by 7 May (Table 4).



Figs. 10–15. Pseudothecial development of *Venturia inaequalis* in various stages: **10,** (stage 9) asci with spores in the process of formation; **11,** (stage 10) asci with ascospores being formed, usually septate; **12,** (st 11) asci with ascospores formed but not pigmented; **13,** (st 12) ascospores pigmented and mature; **14,** (st 13) asci empty; and **15,** (st 14) asci aborted. All figures ×400.

During the fall of 1979, ascogonia (st 3) developed by 12 November and pseudothecia developed to st 5 by 24 December at all locations in NC (Table 5). Asci (st 6) appeared near 1 February and ascospores matured on approximately 31 March at NCSU and BOONE and 14 April at MHCRS. At EL, asci (st 6) began to appear on 17 March and ascospores (st 12) matured on approximately 5 May.

Pseudothecial development during st 1 to 4 was significantly correlated ($P = 0.01$) with all environmental variables considered (Table 7). Correlations were highest with NRAIN ($r = 0.696$), RH100 ($r = 0.542$), RH95 ($r = 0.555$), RH90 ($r = 0.573$), and DEGREE DAYS ($r = 0.475$). Pseudothecial development during st 6 to 12 was also significantly correlated ($P = 0.01$) with all environmental variables. Correlations were highest with RAIN ($r = 0.694$), RH90 ($r = 0.706$), RH95 ($r = 0.661$), DEGREE DAYS ($r = 0.662$), and NRAIN ($r = 0.632$) (Table 7).

Stepwise multiple linear regression calculated by using data from all 3 yr yielded the following three-variable equation for pseudothecial development during st 1 to 4:

$$\text{st} = 3.27 + 0.097\,(\text{NRAIN}) = 0.002\,(\text{DEGREE DAYS}) - 0.030\,(\text{MAXT}) \quad (2)$$

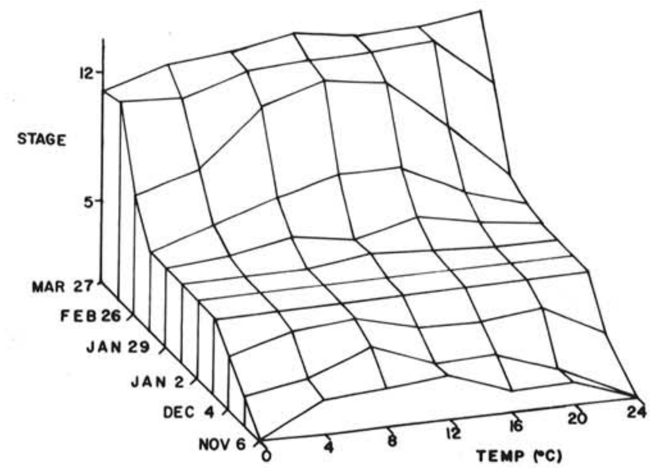


Fig. 16. Effect of temperature on mean stage of pseudothecial development of *Venturia inaequalis* in 2-wk wet incubation periods during 1978–1979. Temperature is on the X axis, time is on the Y axis, and stages of pseudothecial development are on the Z axis.

TABLE 3. Pseudothecial development of *Venturia inaequalis* in apple leaves placed at CC, BOONE, MHCRS, and PACE during the winter of 1977–1978. Leaves were collected at leaf fall on 12 October

Sample date	Location			
	CC	BOONE	MHCRS	PACE
31 October	...[a]	2.0[b]	2.1	1.7
14 November	2.0(2)[c]	2.8	3.2	2.3
28 November	2.5(2)	3.8	4.5	3.7
12 December	...	5.0	4.8	4.7
26 December	...	5.0	4.9	5.0
9 January	...	5.0	5.0	5.0
23 January	...	5.0	5.0	5.0
6 February	5.0(12)	5.3	5.0	5.1
20 February	5.0(3)	5.2	5.0	5.0
6 March	5.0(2)	5.1	5.0	5.3
20 March	...	5.5	5.3	6.1
3 April	5.0(3)	6.8	6.3	6.3
17 April	8.0(6)	9.9	8.2	8.4
1 May	...	11.1	10.1	12.1
15 May	11.6(5)	13.1	12.8	12.6
29 May	12.8(3)	...	...	...

[a] No pseudothecia observed in samples.
[b] Average of two replications. See Figs. 1–15 for definition of stages.
[c] Numbers in parentheses represent the total number of pseudothecia observed.

The R^2 value for the equation is 0.648 and all terms are significant, $P = 0.01$.

Stepwise multiple linear regression using data for all 3 yr yielded the following three-variable equation for pseudothecial development during st 6 to 12:

$$\text{st} = 3.70 + 0.186\,(\text{RAIN}) + 0.029\,(\text{RH90}) + 0.0002\,(\text{DEGREE DAYS}) \quad (3)$$

The R^2 value for the equation is 0.747 and all terms are significant, $P = 0.01$.

Variation in pseudothecial maturity. Variation in the sample populations was related to time and the stage of development. Standard means and standard deviations of stage of pseudothecial development at MHCRS during 1978–1979 (Table 8) indicate that variation increased within the population as ascogonia (st 3) formed after leaf fall. On 4 December, 66% of the population was within st 3.5 ± 0.7, and 99% of the population was within st 3.5 ± 1.4. There was no variation in stage of development during January and early February after pseudothecia matured to st 5. When asci began to develop (st 6), variation in the population increased. On 27 March, 4.1% of the pseudothecia were at st 6, 4.1% at st 7, 16.2% at st 8, 13.5% at st 9, 16.3% at st 10, 42.1% at st 11, and 2.7% at st 12.

TABLE 4. Stages of pseudothecial development of *Venturia inaequalis* in apple leaves placed at CC, BOONE, MHCRS, and EL during the winter of 1978–1979. Leaves were collected at leaf-fall on 18 October

Sample date	Location			
	CC	BOONE	MHCRS	EL
6 November	1.0[a]	1.0	1.0	1.0
20 November	3.4	3.0	2.0	1.0
4 December	4.9	4.9	3.5	2.7
18 December	5.0	5.0	3.4	2.9
2 January	5.0	5.0	5.0	4.0
15 January	5.1	5.0	5.0	3.7
29 January	5.0	5.3	5.0	4.9
12 February	5.1	5.9	5.0	5.0
26 February	5.2	7.5	6.0	5.0
12 March	7.0	8.7	8.3	5.1
26 March	10.8	10.0	9.9	6.5
9 April	11.4	11.1	11.6	9.3
23 April	11.7	12.9	13.0	8.8
7 May	...[b]	...	...	11.3
21 May	...	...	...	12.3
2 June	...	...	...	12.9

[a] Average of two replications. See Figs. 1–15 for definition of stages.
[b] ... Samples not taken.

TABLE 5. Stages of pseudothecial development of *Venturia inaequalis* in apple leaves placed at NCSU, BOONE, MHCRS, and EL during the winter of 1979–1980. Leaves were collected at leaf fall on 15 October

Sample date	Location			
	NCSU	BOONE	MHCRS	EL
12 November	2.0[a]	3.2	3.0	...[b]
26 November	4.7	4.2	4.6	...
10 December	5.0	5.0	4.6	4.9
24 December	5.0	5.0	5.0	...
6 January	5.0	5.0	5.0	5.0
21 January	5.0	5.2	5.0	...
4 February	5.2	7.1	5.1	...
18 February	5.8	7.4	5.3	...
5 March	9.2	7.5	6.1	5.0
17 March	10.8	11.8	8.0	5.5
31 March	12.0	12.1	9.1	8.0
14 April	...[b]	12.3	12.1	10.6
28 April	...	...	13.0	11.3
5 May	...	...	...	12.2
26 May	...	...	...	12.8

[a] Average of two replications. See Figs 1–15 for definition of stages.
[b] ... Samples not taken.

Variation decreased on 9 April as the majority of the pseudothecia matured to st 12 (Table 8).

Leaf fall and cultivar study, 1978–1980. The only significant ($P=0.01$) leaf fall effect during 1978–1979 was observed on 4 December (Table 9). In apple leaves collected from 22 September to 6 October, pseudothecia had developed to st 5, while ascogonia (st 3) were forming in leaves collected at all later dates. On 12 February and later sample dates, there was no detectable leaf fall effect.

Similar results were obtained in the leaf fall study during 1979–1980. Pseudothecial development in leaves which fell on 9 November or were placed on the ground on 2 January was significantly ($P=0.01$) later for all sample dates except 14 April when there was no difference ($P=0.01$) in ascospore maturation at all leaf fall dates. No difference was observed between leaf fall dates of 12 and 26 October at any sample date.

There was no significant difference ($P=0.01$) in pseudothecial development in leaves of cultivars Delicious, Golden Delicious, Rome Beauty, or McIntosh in samples collected on 17 and 31 March.

DISCUSSION

In the fall, ascogonial initials (st 2) of *V. inaequalis* developed until the lumina of the pseudothecia were filled with pseudoparaphyses (st 5). Development of asci (st 6) was initiated only after a dormant period during which there was no observable development in the lumina of the pseudothecia. This apparent dormant period has not been previously reported for *V. inaequalis*. Luttrell (7) reported that pseudothecia of *Pyrenophora* spp. developed slowly and required low temperatures for development. During the 3-yr field study, no relationship was observed between accumulated degree days (using a 0 C base) and the length of time pseudothecia of *V. inaequalis* remained in the dormancy period. Temperature and moisture had no effect on length of the dormancy period. Laboratory and field observations indicated that the dormant period lasts approximately 45 days, and dormancy requirements were met at approximately 1 February in NC. Pseudothecia were capable of rapid maturation through st 6 to 12 during periods of favorable temperature and moisture after that date.

The existence of this dormant period helps explain why the Massie and Szkolnik model (8) works satisfactorily in NY, but predicts ascospore maturity (st 12) much earlier than it occurs in nature under NC conditions (11). The NY model is based on the premise that *V. inaequalis* develops continuously through the winter at temperatures greater than 0 C. Because few degree days (0 C base) accumulate in Geneva, NY, during the winter, little development is predicted and low temperatures apparently mask the dormant period. However, in NC, degree days (0 C base) frequently accumulate rapidly during the winter months, and unless the dormant period is taken into account, ascospore maturity will be predicted much earlier than it actually occurs in nature.

Of the five variables that Wilson (12) indicated had the most effect on pseudothecial development in Wisconsin, temperature and leaf moisture appear to be most important in NC. Experiments during this study indicate that cultivar and time of leaf fall had no effect on date of ascospore maturation. Time of infection was not investigated in this study. Wilson (12) showed that definite-

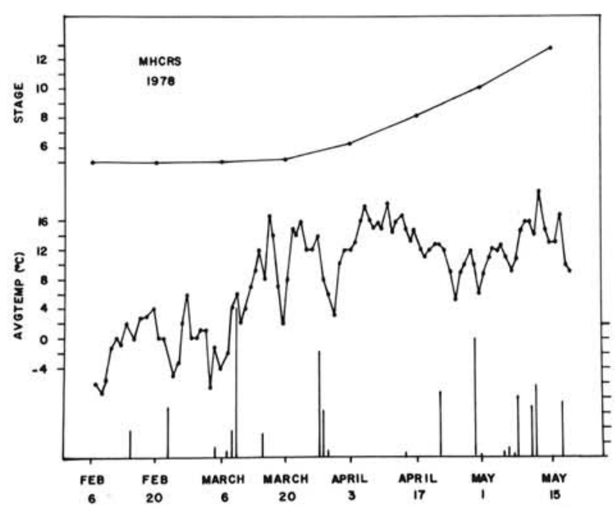


Fig. 17. Mean stage of pseudothecial development of *Venturia inaequalis* during February, March, April, and May 1978 at MHCRS, compared with daily AVGTEMP and RAIN.

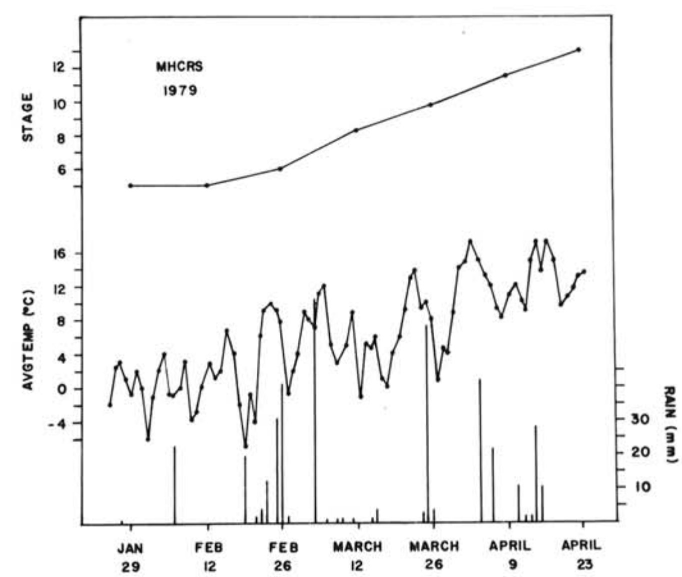


Fig. 18. Mean stage of pseudothecial development of *Venturia inaequalis* during February, March, and April 1979 at MHCRS compared with daily AVGTEMP and RAIN.

TABLE 6. Average temperatures and rainfall for AV and departures from normal for the period October–May for 1977–1978, 1978–1979, and 1979–1980

	Normal		Departure from average					
			1977–1978		1978–1979		1979–1980	
Month	TEMP[a]	RAIN[b]	TEMP	RAIN	TEMP	RAIN	TEMP	RAIN
October	13.2	105	−1.4	14	−0.6	−75	−0.8	47
November	7.5	75	1.7	100	3.1	−11	1.6	122
December	4.6	101	−1.0	− 29	1.3	19	1.8	− 65
January	2.9	76	−4.7	103	−2.0	87	1.5	− 14
February	3.6	92	−3.3	− 80	−2.0	39	−2.3	− 78
March	8.1	121	0.0	142	2.3	30	0.2	91
April	13.0	70	0.5	−142	0.0	95	0.4	31
May	16.8	136						

[a]Temperature C.
[b]Millimeters.

TABLE 7. Correlation coefficients between stage (st) of pseudothecial development of *Venturia inaequalis* and monitored environmental variables during the 3-yr field study

Environmental variable	Correlation coefficient[a]	
	st 1 to 4	st 6 to 12
MAXT	−0.456	0.416
MINT	−0.484	0.451
AVGT	−0.492	0.437
DEGREE DAYS	0.475	0.662
RAIN	0.271	0.694
NRAIN	0.696	0.632
RH100	0.542	0.625
RH95	0.555	0.661
RH90	0.573	0.706
RH80	0.394	0.605

[a] All correlations are significant at the $P = 0.0001$ level.

TABLE 8. Standard mean and standard deviation of stages of pseudothecial development of *Venturia inaequalis* at MHCRS during 1978–1979

Date	Standard mean	Standard deviation
6 November	1.0[a]	0.0
20 November	2.0	0.0
4 December	3.5	0.7
18 December	3.4	0.6
2 January	5.0	0.0
15 January	5.0	0.0
29 January	5.0	0.0
12 February	5.0	0.0
26 February	6.0	0.7
12 March	8.3	0.7
27 March	9.7	1.9
9 April	11.6	0.9

[a] See Figs. 1–15 for definition of stages.

margined lesions from early-season infections produce less pseudothecia than indefinitely margined lesions from late-season infections. This would, however, quantitatively affect ascospore productivity and would have little qualitative influence on overwintering.

Moisture appears to be the limiting factor for pseudothecial development of *V. inaequalis* in NC. In laboratory studies, no development of pseudothecia occurred in air-dried apple leaves, and in the field pseudothecial development was most highly correlated with measures of rainfall or high relative humidity. Stepwise multiple linear regression yielded number of rains (NRAIN) and total rain (RAIN) as the major environmental variables affecting pseudothecial development during st 1 to 4 and st 6 to 12, respectively. The importance of moisture to pseudothecial development was noted several times during the 3-yr field study. During November 1978, below-normal rainfall occurred at MHCRS and ascogonia (st 3) were not formed until approximately 4 December 1978 (Table 4), as compared with 14 November 1977 (Table 3), and 12 November 1979 (Table 5). In 1978, dry periods at all locations during the last 2 wk in March and the first 2 wk in April delayed ascospore maturation until early May. In 1979 at MHCRS, rainfall was more frequent, and ascospores matured approximately 1 mo earlier than in 1978.

When moisture was not limiting, temperature had a major influence on pseudothecial development. During 1978–1979, significant linear and quadratic coefficient values for temperature were obtained in wet and wet/dry treatments during all incubation periods except during the dormancy period. In wet treatments, pseudothecial abortion was observed at 24 C and was extensive at 28 and 32 C. Although laboratory experiments were conducted for 7- and 14-day intervals, similar abortion of *V. inaequalis* might occur in the field under warm moist conditions. For instance, during February and March 1976, temperatures above 20 C occurred on 23 days at MHCRS. During this season, ascospore

TABLE 9. Influence of leaf-fall date on stages of pseudothecial development of *Venturia inaequalis* during the winter of 1978–1979

Sample date	Date of leaf-fall						
	22 Sep	29 Sep	6 Oct	13 Oct	20 Oct	27 Oct	3 Nov
4 December	4.8[a]	5.0	5.0	3.1	3.0	3.0	3.3
12 February	5.0	5.0	5.2	5.1	5.0	5.0	5.0
26 February	6.9	5.9	7.3	6.1	6.5	6.0	6.3
12 March	8.3	6.8	7.0	6.2	6.4	5.5	6.9
26 March	8.9	7.0	8.7	9.6	9.0	9.0	8.4
9 Apr	11.7	11.2	10.5	9.5	11.3	10.4	11.4
LSD .05[b]	ns	ns	ns	ns	ns	ns	ns

[a] See Figs. 1–15 for definition of stages.
[b] Combined analysis of all sample dates; ns = not significant.

production and dose were low (T. B. Sutton, *unpublished*).

In NC, temperature and moisture during February, March, and April have more influence on pseudothecial maturation than temperature and moisture during the fall or early winter. In the fall, considerable differences were observed in date of ascogonial development through st 1 to 5 during the 3-yr field study and 2-yr leaf fall experiment; however, this delay had no effect on date of ascospore maturity the following spring. This might be explained by the lower temperature requirement for ascogonial development. If leaf fall was late or conditions following leaf fall were unfavorable for pseudothecial development, development could still occur during the late fall and early winter before temperatures averaged <0 C.

Relative humidity experiments indicate that pseudothecia were capable of development during periods of high relative humidity (Table 2). Such periods often follow rain, and could account for pseudothecial development during periods when no measureable rainfall occurs.

Field and laboratory data obtained in this study are currently being analyzed to develop a predictive model for ascospore maturation (4).

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