Effects of Temperature on the Development of Pseudothecia of *Venturia inaequalis*

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ABSTRACT

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The ascigerous state of *Venturia inaequalis* was studied under laboratory and field conditions to determine the effects of temperature on the development of pseudothecia. Leaves were incubated at 4, 10, 15, and 20 C. Pseudothecia increased in diameter most rapidly at 10 C, but the number that formed per unit of leaf area was inversely proportional to temperature. Measurement of pseudothecial growth and productivity in field-stored leaves yielded similar results. Early leaf fall was associated with increased leaf decay and a resultant reduction in leaf weight and leaf area, but the date of leaf abscission per se did not affect pseudothecial development. Most pseudothecia were formed within 28 days of leaf fall. There was an inverse relationship between temperature and the number of asci that developed per pseudothecium. In pseudothecia from field-stored leaves, the mean number of asci per pseudothecium rose in early spring as asci matured and then declined as empty asci disintegrated. The thermal requirement of pseudothecia shifted during winter from 10 C for early diameter increase to 20 C for ascospore maturation. When the cumulative percentage of matured ascospores was plotted against time, the curves approximated cumulative normal distributions. There was a linear relationship between the probit of matured ascospores and degree day accumulation beginning with the date of the first discharge of ascospores.

Additional key words: apple scab, disease management

Venturia inaequalis (Cke.) Wint., which causes apple scab, overwinters as immature pseudothecia in fallen leaves of apple (*Malus* sp.). Development of the ascigerous state begins after leaf fall, when hyphae from a subcuticular stroma grow into the palisade and mesophyll regions of the leaf and there form pseudothecial initials. In spring the pseudothecia mature and ascospores are forcibly discharged when the leaves are wet by rain. In North America, a supply of ascosporic inoculum is a requisite for the development of apple scab epidemics (7).

The abundance or scarcity of primary inoculum affects the severity of scab infections (2,6,10), but in commercial apple orchards the timing of fungicide sprays has not been significantly based on or equated to the supply of primary inoculum throughout the primary scab season (1,9). Of course, spraying is encouraged early in the season once it has been demonstrated that mature spores are being released from overwintered leaves; spray intervals are increased and fungicide rates are decreased with confidence only when the ascospore depletion from overwintered leaves is very high. The factors affecting development of pseudothecia must be better understood before a working knowledge of inoculum development can be used in disease management.

Temperature has a marked effect on pseudothecial development, ie, growth of pseudothecia (15), numbers of pseudothecia produced per unit of infected leaf (13), and the rate of ascospore maturation (8). However, previous controlled environment studies have generally involved single assessment of pseudothecial maturity and productivity. There is no evidence that the relationships noted between temperature and pseudothecial development, under laboratory and field conditions, are consistent throughout development.

The time of leaf fall affects the number of pseudothecia that form per unit of leaf area (3) and the rate at which they develop (15). However, it is unclear whether the effect of the time of leaf fall is due to innate differences between leaves that fall at various times in autumn or due to environmental conditions after leaf abscission.

We have determined the effects of temperature on pseudothecial maturation and productivity under laboratory and field conditions from the time of ascocarp initiation to the completion of ascospore discharge.

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MATERIALS AND METHODS

Distribution of time of leaf fall. Abscission of spur and terminal leaves was monitored on five unsprayed McIntosh trees in the Mast Road Research Orchard in Durham, NH, at weekly intervals from 1 August 1979 until defoliation reached 97% on 1 November 1979.

Decomposition of overwintering leaves. Infected, freshly abscised leaves were collected from beneath unsprayed McIntosh trees at the research orchard on 15 August, 29 August, 12 September, and 25 October 1979 and were stored in wire mesh trays on the orchard floor. On 1 January 1980, three samples of 50 leaves from each collection were dried at 70 C for 24 hr and then weighed. The area of three samples of 20 leaves from each collection was also measured.

Effect of temperature after leaf fall on early development of pseudothecia. Infected leaves were collected beneath unsprayed McIntosh trees on 12 September 1979. Squares 2.5 × 2.5 cm were cut from these leaves and were fastened between two rectangular pieces of fiberglass screen. The screen was then rolled and two ends fastened to form a cylinder (Fig. 1A). The leaves were incubated at 4, 10, 15, and 20 C at 90% RH. Leaf pieces stored under these conditions remained pliable without the presence of surface moisture. To eliminate moisture stress as a factor affecting development, we immersed screened leaf cylinders with the leaf pieces in distilled water for 2 hr every 7 days. Surface moisture was allowed to evaporate from the leaf pieces before they were returned to the incubators.

At 14, 28, 56, and 110 days after leaf fall, a 1-cm diameter disk was cut from each of four leaf squares for each incubation temperature. The disks were cleared in sodium hydroxide and chloral hydrate (11) and examined microscopically for number of pseudothecia per disk, and diameter of 25 pseudothecia per disk. Data were recorded only on pseudothecia that bore hyphal connections to the subcuticular stroma.

Similar studies were conducted with leaves stored in wire mesh trays at the research orchard from collections made on 15 August, 12 September, and 10 October 1979. A thermograph in a standard U.S. Weather Service instrument shelter provided a record of temperature at the leaf storage site. Specimens were

cleared and examined 28 days after the leaves were collected.

Leaves from each collection were incubated at 10 C at 90% RH to determine whether differences in pseudothecial growth and productivity were due to innate differences in leaves from various collections. Moisture was supplied as above. Leaf disks from each collection were cleared 110 days after leaf fall, and the number of pseudothecia per disk and the diameter of 25 pseudothecia per disk were recorded.

Effects of temperature on ascus development and ascospore maturation. Infected McIntosh leaves collected in December 1978 were stored over winter in wire mesh trays at the research orchard. These leaves were incorporated into screened leaf cylinders and were incubated at 10, 15, and 20 C at 90% RH on 15 March, when the most advanced pseudothecia in the leaf samples were in early stages of ascus formation. Moisture was supplied as above.

At weekly intervals, two pseudothecia were removed from each of 10 leaf pieces at each incubation temperature. The pseudothecia were crushed on glass slides and examined under magnification (×430), and the number of asci per pseudothecium was recorded. Weekly observations were discontinued once the mean number of asci per pseudothecium began to decline as empty asci disintegrated. Treatment means were recorded as the mean number of asci per pseudothecium.

Similar studies were conducted to determine the effects of temperature on ascus development under natural conditions. Leaves from the December 1978 collection and leaves collected in October 1979 that had overwintered at the research orchard were stored at the orchard in wire mesh trays during the springs of 1979 and 1980, respectively. At approximately weekly intervals, beginning 15 March 1979 and 1 April 1980, two pseudothecia from each of 10 leaves were removed and examined. The temperature from the first appearance of asci (15 March 1979 and 1 April 1980) to the maximum mean number (7 May 1979 and 18 May 1980) was recorded by a thermograph at the leaf storage site. Weekly observations were discontinued on 1 July 1979 and 27 June 1980.

On 13 April 1980, overwintered leaves from the research orchard were incorporated into screened leaf cylinders. The composition of each leaf sample reflected the distribution of time of leaf fall; ie, 25% of the leaf pieces were from the 10 October collection and the remaining 75% were from the 25 October collection. Leaves were incubated at 6, 10, 15, and 20 C at 90% RH. Screened leaf cylinders were also stored in moist chambers housed in the U.S. Weather Service instrument shelter at the research orchard. The moist chamber was a covered plastic container measuring 30 × 30 × 11.2 cm. A 2.5-cm thick pad of polyurethane foam was placed in the bottom of the container and five 1-cm holes were punched in the lid in an X pattern. The polyurethane foam was saturated with water to provide a humid atmosphere. Periodic inspection of the leaf cylinders at the orchard showed that the moist chamber maintained the leaf pieces in a pliable condition without surface moisture.

All screened leaf cylinders were tested at approximately weekly intervals for the presence of mature ascospores. One-hundred milliliters of distilled water was added to a 250-ml beaker containing a leaf cylinder. A 100-ml plastic bottle filled with water was used as ballast to raise the water level above the leaf pieces (Fig. 1B) and the beaker was agitated in a shaker bath at 20 C for 2 hr. The plastic bottles and cylinders were then removed and rinsed with approximately 100 ml of water. The rinse water and spore suspensions were transferred to 200-ml berzelius beakers. One milliliter of Lugol's solution (aqueous iodine) was added to each suspension to preserve the spores and to raise their specific gravity. The spore suspensions were allowed to settle undisturbed for 24 hr. A Pasteur pipet attached to a pump set at a vacuum of 125 mm Hg was then used to draw off 125 ml of water from each beaker without disturbing the ascospores. The remaining suspensions were transferred to 100-ml graduated cylinders, the berzelius beakers were rinsed with 25 ml of water, and the rinse water was added to the suspensions. The suspensions were again allowed to settle for 24 hr and were then reduced to 30 ml by drawing off excess water as described.

Aliquots of each spore suspension were transferred to 5-ml plankton counting chambers, allowed to settle for 12 hr, and examined with a Wild M40 Inverted

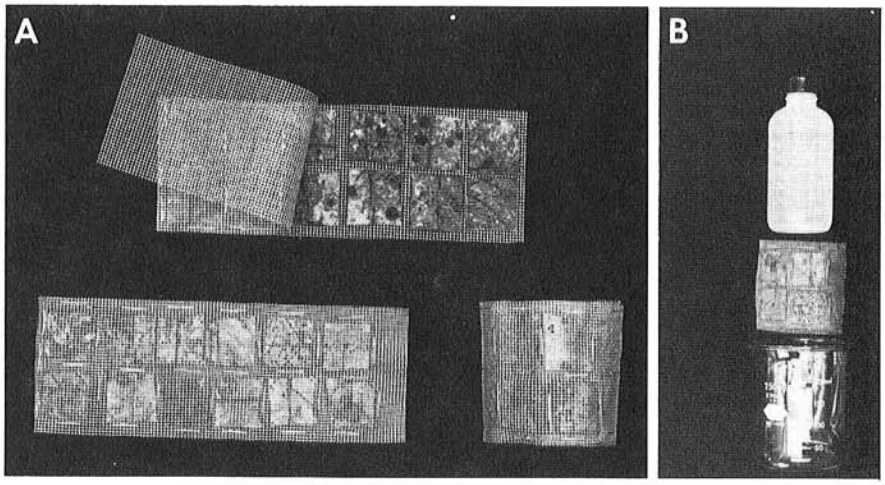

Fig. 1. (**A**) Fabrication of screened leaf cylinders. (**B**) Plastic ballast bottle, screened leaf cylinder, and beaker for inducing ascospore discharge.

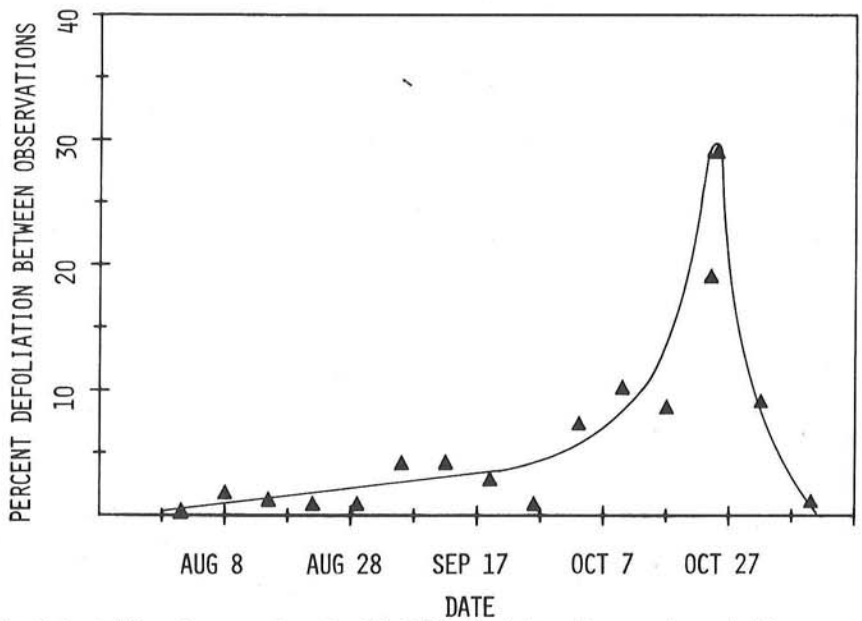

Fig. 2. Leaf fall at the research orchard in 1979. Abscission of spur and terminal leaves was monitored from 1 August to 1 November.

Biological Microscope. The number of ascospores of *V. inaequalis* in the counting chamber was recorded and the total number of discharged ascospores was calculated. All treatments were replicated three times.

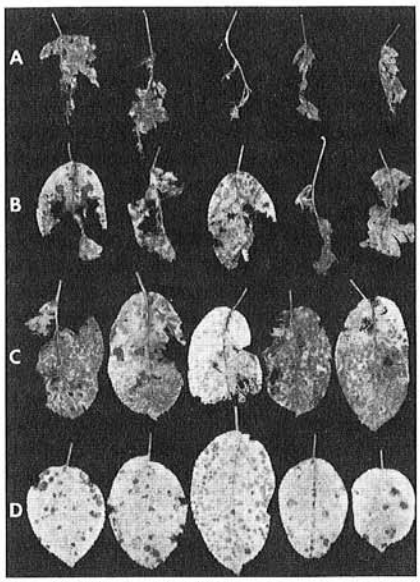

Fig. 3. Effect of leaf fall date on survival of leaves: (**A**) 15 August, (**B**) 29 August, (**C**) 12 September, (**D**) 25 October 1979. (Photographed 1 January 1980.)

RESULTS AND DISCUSSION

Effects of distribution of time of leaf fall, survival of leaves, and temperature on early development of pseudothecia. The distribution of time of leaf fall at the research orchard in 1979 was leptokurtic and negatively skewed (Fig. 2). Defoliation progressed from 33% on 10 October to 61% on 24 October, 89% on 25 October, and 97% on 1 November.

On 1 January 1980, leaves that had fallen in August and September 1979 were more decomposed than leaves that had fallen in October (Fig. 3). Early leaf fall was associated with reductions in leaf weight (Fig. 4A) and in leaf area (Fig. 4B), which was most pronounced in the August collections, presumably as a result of increased activity of detritivores and decay organisms during late summer (12).

The number of pseudothecia that formed per unit of leaf area was inversely proportional to temperature from 4 to 20 C (Fig. 5). Ross and Hamlin (13) reported a similar relationship between temperature and pseudothecial productivity. The trend of increased pseudothecial productivity at lower temperatures was evident by 14 days after leaf fall (Fig. 5B).

Although the productivity of pseudothecia was inversely proportional to temperature from 4 to 20 C, a different relationship was found between temperature and rate of growth of pseudothecia. The increase in diameter of pseudothecia was most rapid at 10 C and progressively slower at 15, 4, and 20 C (Fig. 6A). These results are similar to those reported by Wilson (15). The relationship between temperature and rate of growth was consistent from 14 to 56 days after leaf fall (Fig. 6B).

When leaves were stored in the field, the effects of temperature on pseudothecial productivity and rate of growth were similar to those observed under controlled conditions (Figs. 5A and 6A). As the temperature of the 28-day period after leaf fall decreased, the number of pseudothecia formed per unit of leaf area increased (Fig. 5A). The rate of growth, measured as the average pseudothecial diameter, was greatest in the September collection, where the average temperature of the 28-day period after leaf fall was 12.3 C (Fig. 6A).

Date of leaf fall did not significantly affect either pseudothecial productivity

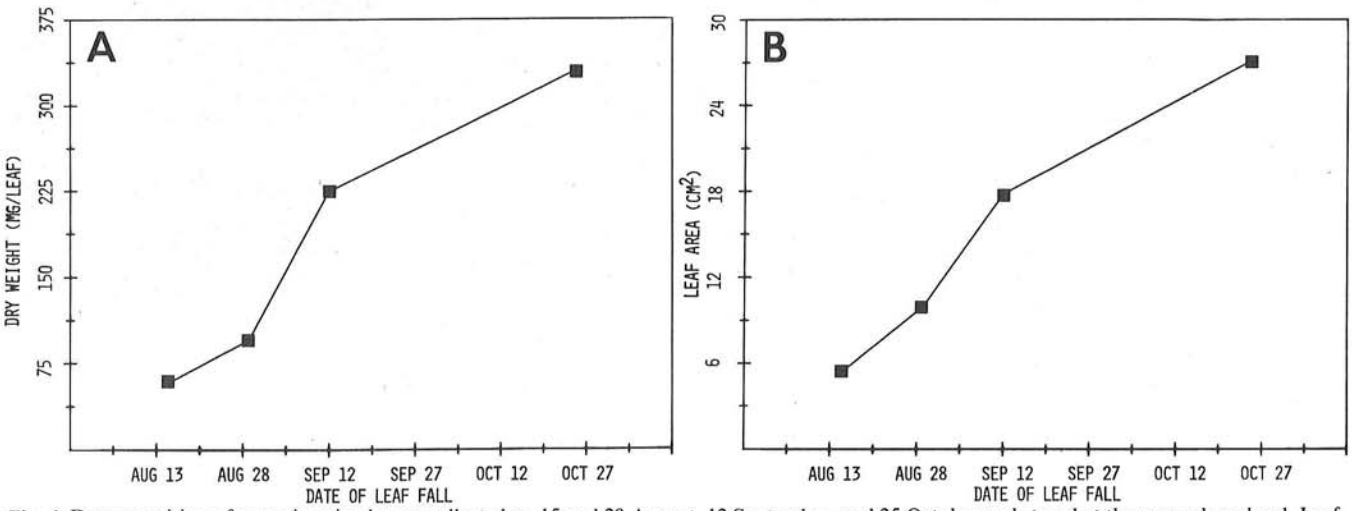

Fig. 4. Decomposition of overwintering leaves collected on 15 and 29 August, 12 September, and 25 October and stored at the research orchard. Leaf weights (**A**) and leaf areas (**B**) were determined on 1 January. Differences between treatment means are significant at $P = 0.10$.

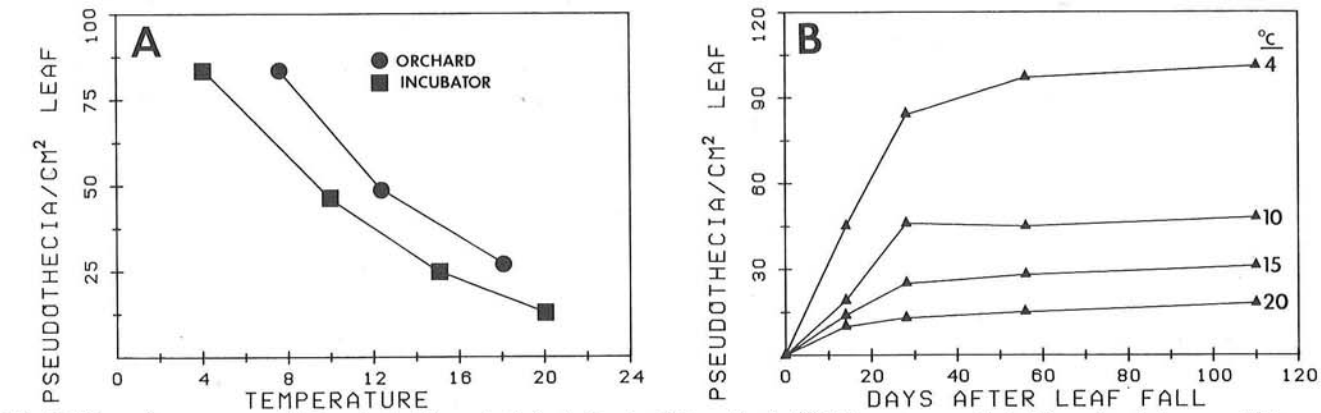

Fig. 5. Effects of temperature: (**A**) Pseudothecial productivity during the 28 days after leaf fall. Leaves were collected for orchard storage on 15 August, 12 September, and 10 October. (**B**) Pseudothecial productivity 14–110 days after leaf fall. Differences between treatment means are significant at $P = 0.10$.

or the rate of growth in leaves collected in August, September, or October and incubated at 10 C (Table 1).

Because development of the ascigerous state of *V. inaequalis* begins at leaf fall, the distribution of time of leaf fall will, in part, determine the variance in maturity of a population of pseudothecia. Leaf fall at the research orchard in 1979 began in mid-August and was 97% complete by 1 November. Most of the leaves fell in a much shorter time span, however; 64% of the total leaf fall occurred between 10 October and 1 November. The distribution of leaf fall was negatively skewed, but warmer temperatures during August and September acted as stabilizing selection pressures; ie, not only did fewer leaves fall before 10 October, but fewer survived, and those that did survive bore fewer pseudothecia per unit of leaf area than leaves that fell after 10 October.

The effect of this distribution of time of leaf fall and the temperatures after leaf fall was that most pseudothecia began development in a 3-wk period, under similar environmental conditions, and could be expected to mature at approximately the same time in spring. The uniformity among pseudothecia with respect to maturity in spring was evident in the pattern of ascospore maturation at the research orchard. Eighty-eight percent of the ascospores matured between 25 April and 16 May 1980 (Fig. 7A).

The productivity of pseudothecia was maximized at a lower temperature than was the growth rate of pseudothecia. The number of pseudothecia formed per unit of leaf area, both in field and laboratory studies, was inversely proportional to temperature. Pseudothecial diameter increased most rapidly at 10 C under controlled conditions and at 12.3 C at the research orchard. The relationship between temperature and pseudothecial productivity was consistent from 14 to 110 days after leaf fall. However, temperature during the first 28 days after leaf fall had the most pronounced effect on pseudothecial productivity, since at all incubation temperatures approximately 90% of the pseudothecia formed within 28 days of leaf fall (Fig. 5B).

The date of leaf fall had no significant effect on the number of pseudothecia that formed per unit of leaf area or on the rate of diameter increase. Pseudothecia developed at the same rate and were produced in the same numbers in leaves collected in August, September, and October and incubated at 10 C. Ross and Hamlin (13) obtained similar results when leaves from various collection dates were incubated at 4 C. Pseudothecial productivity and rate of development is governed by the environmental conditions after leaf fall rather than by innate

Table 1. Effect of leaf fall date on pseudothecial growth and productivity[a]

Leaf collection	Pseudothecia Diameter (μm)	Pseudothecia (no./cm² of leaf)
15 August	153[b]	41[b]
12 September	142	48
10 October	169	43

[a] Leaf pieces in screened cylinders were incubated at 90% RH for 110 days.
[b] Differences within columns are not significant at $P = 0.10$.

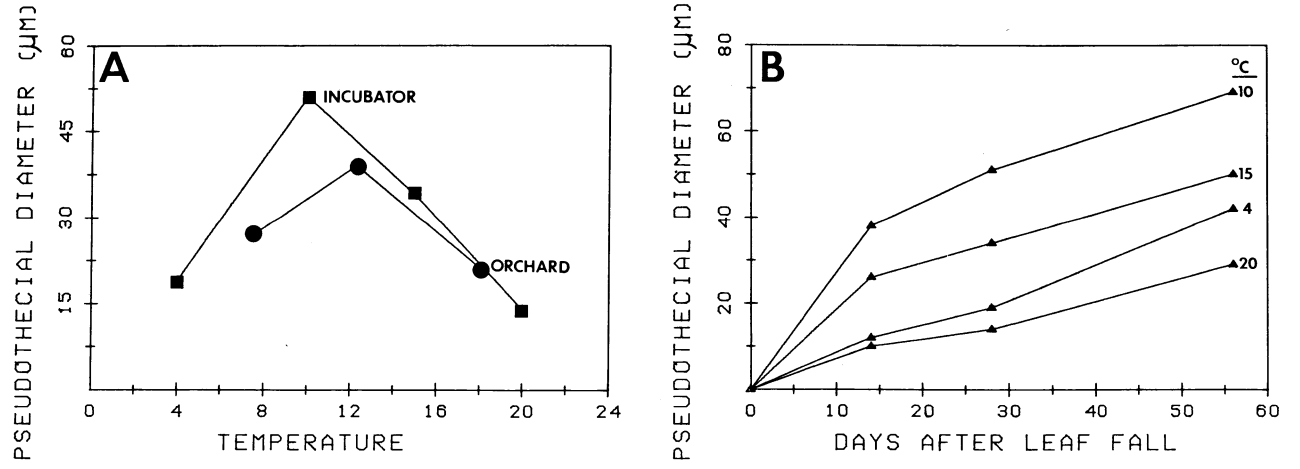

Fig. 6. Effects of temperature: (**A**) Growth of pseudothecia during the 28 days after leaf fall. Leaves were collected for orchard storage on 15 August, 12 September, and 10 October. (**B**) Growth of pseudothecia 14–56 days after leaf fall. Each point is the mean of the diameters of 25 pseudothecia on four 1-cm disks. Differences between treatment means are significant at $P = 0.10$.

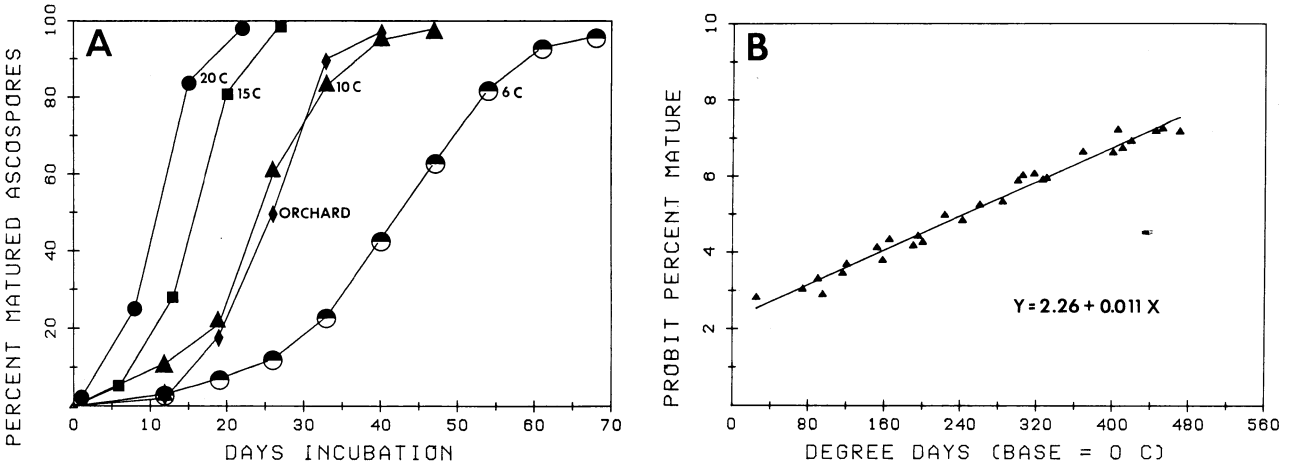

Fig. 7. (**A**) Maturation of ascospores at temperatures of 6–20 C and in leaves stored at the research orchard. Ascospore discharge was induced at weekly intervals by immersion of screened leaf cylinders in water. (**B**) Effect of temperature on ascospore maturation. The probit of the cumulative percentage of matured ascospores was plotted against degree day accumulation from the first discharge of ascospores. $R^2 = 0.98$.

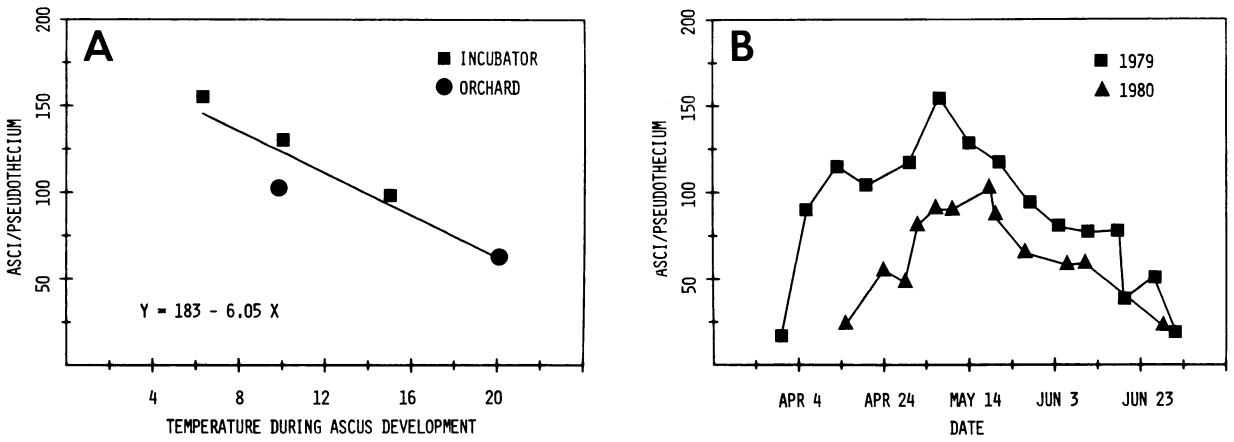

Fig. 8. (**A**) Effect of temperature on ascus development. Leaves bearing pseudothecia in early stages of ascus formation were incubated at 10, 15, or 20 C or were stored at the research orchard during the springs of 1979 and 1980. The average mean temperature at the orchard during ascus development was 6.3 C in 1979 and 9.8 C in 1980. $R^2 = 0.86$. (**B**) Mean number of asci per pseudothecium (20 replicates) at the research orchard in 1979 and 1980.

differences among leaves that fall at various times in autumn.

Effects of temperature on ascus development and ascospore maturation. The number of asci that matured per pseudothecium was inversely proportional to the temperature during the period of ascus maturation, under both controlled and field conditions. Linear regression analysis was used to formulate an equation that describes the relationship between temperature and ascus development (Fig. 8A).

Ascus development in leaves stored at the research orchard was continuous and proceeded for approximately 5 wk during 1979 and 1980 (Fig. 8B). The number of asci that developed per pseudothecium was inversely proportional to the temperature during this period (ie, the time from the first appearance of asci (15 March 1979 and 1 April 1980) until the mean number of asci per pseudothecium reached a maximum. Before ascus formation, pseudothecia contained only undifferentiated hyphae and pseudoparaphyses. The average number of asci per pseudothecium increased from approximately 20 on 24 March 1979 to 155 on 7 May 1979 as asci matured and declined thereafter as empty asci disintegrated. A similar trend was observed in 1980, but fewer asci matured, possibly because temperatures were higher during ascus development (Fig. 8A).

The optimum temperature for pseudothecial development shifted from 10 C for early diameter increase (Fig. 6) to 20 C for ascospore maturation (Fig. 7A). The rate of maturation of ascospores was directly proportional to temperature from 6 to 20 C. Louw (8) and Wilson (15) described a similar relationship.

The percentage of matured ascospores was plotted against incubation time (Fig. 7A). Curves of ascospore maturation under controlled and natural conditions were sigmoid and approximated cumulative normal distributions. Ascospore maturation at the research orchard closely approximated ascospore maturation at an incubation temperature of 10 C (Fig. 7A). The average mean temperature of the period of ascospore maturation at the research orchard was 10.5 C.

All data collected on ascospore maturation under field and laboratory conditions were combined and plotted against Celsius degree day accumulation using base temperatures from 0 to 6 C, beginning with the date of the first discharge of ascospores. A base temperature of 0 C yielded the highest correlation ($R^2 = 0.98$) between ascospore maturation and degree day accumulation (Fig. 7B). There was a linear relationship between the probit of ascospore maturity and degree day accumulation from the first discharge of ascospores. A linear statistical model of ascospore maturation based on this relationship has been described (D. M. Gadoury, M.S. thesis; 4).

The existence of a linear relationship between degree day accumulation and ascospore maturity is significant since it will allow the prediction of primary inoculum maturity based on daily temperatures. At the present time, inoculum levels are assessed repeatedly during the primary infection season by tedious visual methods (5,14). The predictive model of ascospore maturation is being tested at agricultural experiment stations in New Hampshire, New York, and Pennsylvania to evaluate its application in disease management programs for apple scab.

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