Factors Affecting Maturation and Release of Ascospores of *Venturia pirina* in Oregon

R. A. Spotts and L. A. Cervantes

Oregon State University, Mid-Columbia Agricultural Research and Extension Center, Hood River 97031.
Use of trade names in this article does not imply endorsement by Oregon State University of the products named or criticism of similar products not mentioned.
Oregon Agricultural Experiment Station Technical Paper 9629.
Accepted for publication 30 November 1993.

ABSTRACT

Spotts, R. A., and Cervantes, L. A. 1994. Factors affecting maturation and release of ascospores of *Venturia pirina* in Oregon. Phytopathology 84:260-264.

The first mature ascospores of *Venturia pirina,* the causal agent of pear scab, were observed at bud swell in late February to late March. The percentage of asci that contained mature ascospores within a pseudothecium reached a maximum during the bloom period. Ascospore maturity during three seasons was described with linear regression. The accumulated degree-days with a base temperature of 0 C was the independent variable, and ln(1/1 − proportion of mature asci) was the dependent variable. The maturity model was validated at two diverse locations in 1993. Ascospore release usually was associated with rain (nine of 19 events) or dew (eight of 19 events). Light stimulated the discharge of ascospores, but night release of ascospores occurred in all 3 yr of the study. In only three release events did the percentage of ascospores trapped at night exceed the percentage of the wetness duration that occurred at night.

Additional keywords: epidemiology, *Pyrus communis*.

Pear scab caused by *Venturia pirina* Aderhold is a serious disease of pear leaves, shoots, and fruit in all pear-growing areas of the world (21). The disease has been an economic problem in Oregon since 1932 (12).

Mature ascospores of *V. pirina* usually are present at bud swell in infected leaves that have overwintered on the orchard floor. These spores are released over a period of 3 mo (5,9,12) to 4 mo (3,13,14). Maximum release usually occurs between early bloom and petal fall (2,3,12,13). Discharge of ascospores occurs during or soon after periods of rain or dew (14,24). In California (2), Australia (23), and Chile (14), the number of ascospores trapped at night was a small percentage of those trapped in daylight. However, some ascospores at all locations were trapped during darkness. Studies have been conducted that show distinct effects of temperature on maturation of ascospores of *V. inaequalis* (Cooke) G. Wint. (7,8,10,11), but comparable research has not been done with *V. pirina.*

The objectives of this research were to study the relationships, under orchard conditions, 1) between maturation of *V. pirina* ascospores and temperature and 2) between ascospore release and the environmental factors of moisture and light.

MATERIALS AND METHODS

Pear (*Pyrus communis* L. 'Bartlett') leaves with scab lesions were collected from trees at early leaf drop on 9 November 1979, 4 November 1980, 4 November 1981, and 18 September 1986. On the day of collection, leaves were inserted between two 1- × 1-m layers of nylon mesh and placed on the orchard floor at the Mid-Columbia Agricultural Research and Extension Center (MCAREC), Hood River, Oregon. In November 1980 and 1981, leaves were also placed in a pear orchard near Parkdale, Oregon, in the upper Hood River Valley.

Six leaves were removed at weekly intervals beginning in late February prior to bud swell and continuing through mid-June 1981, 1982, and 1987. Ascospore development was assessed in five pseudothecia per leaf with the differential count method of Gadoury and MacHardy (6), in which asci are classified as immature, mature, or empty. The average number of asci per pseudothecium was determined weekly, and the percentage of asci in each maturity class was corrected for undeveloped asci in early spring and exclusion of disintegrated asci in late spring (6). On each sampling date, the growth stage of pear trees within 50 m of the scab-infected leaf sites was estimated visually and classified according to a standard bud and flower phenology guide (20).

Concentrations of ascospores in the air were monitored at MCAREC in the spring of 1980, 1981, and 1987 with a volumetric spore trap (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, U.K.) placed in the center of the scab-infected leaves on the orchard floor. Orchard air was sampled continuously each year from the end of March until early June. The sampler orifice was located 1 m above the ground. Air was sampled at 10.5 L/min, and ascospores were trapped on a silicone grease-coated Melinex tape. The tape was examined for ascospores at 250×. Ascospores of *V. pirina* were identified on the basis of morphology, color, and size (22). The number of ascospores per cubic meter of air was calculated on an hourly basis by dividing the ratio of the spore count on the total area of the tape exposed to that on the area examined by the volume of air sampled.

Temperature and relative humidity were monitored at MCAREC and Parkdale with a 7-day recording hygrothermograph (Belfort Instrument Co., Baltimore, MD) situated 1.5 m above the ground in a standard weather shelter. Rainfall was monitored with a 7-day recording rain gauge (Weather Measure Corp., Sacramento, CA) at ground level. Sensitivity of the gauge was 0.25 mm of precipitation. Leaf wetness was determined with a 7-day recording leaf wetness meter (M. DeWit, Hengelo, Holland) located 1.5 m above the ground. Wetness was defined as approximately 20% or more of the full scale deflection. In the spring and fall of 1992 and the spring of 1993, a second wetness meter was operated at MCAREC; the sensor was placed about 2 mm above the pear leaves on the ground. Leaf wetness that occurred in the early morning in the absence of recorded rain was attributed to the formation of dew. To determine the hours of day and night, total solar radiation was measured at 15-min intervals with a precision pyranometer (model PSP, The Eppley Laboratory, Inc., Newport, RI) located 3 m above the

ground. The first 15-min interval during which any solar radiation was measured was considered the beginning of the day period. The first interval in the evening during which no solar radiation was measured was considered the beginning of night.

Degree-days were determined with a computer program that used daily maximum and minimum temperatures to calculate the area under a sine curve (1). In preliminary calculations, base temperatures from −10 to 10 C were used to determine degree-days, and a base of 0 C was selected as the temperature most closely related to ascospore maturity on the basis of coefficient of determination (R^2) values of regression analyses. The relationship between ascospore maturity and degree-days was described with linear regression (NWA Statpak 4.1, Portland, OR) and a monomolecular model (17).

Validation of the model relating ascospore maturity to degree-days was done at two locations in 1993. Infected Bartlett leaves were collected on 30 September 1992 from an orchard near Parkdale. The leaves were placed between nylon mesh on the orchard floor as described above. Infected Bosc pear leaves in an orchard near Hood River were allowed to overwinter naturally. On 29 March 1993, they were carefully moved, without inverting the leaves, to nylon mesh as described above. Leaves were removed weekly from March through June 1993 and assessed for ascospore maturity as described above.

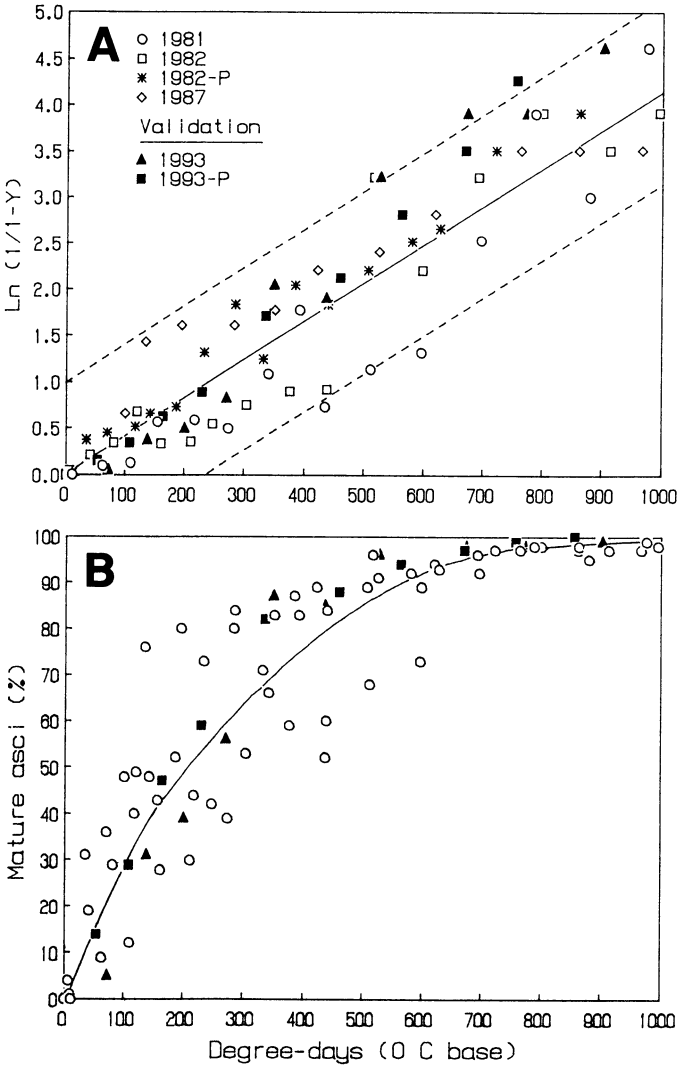

Fig. 1. Effect of cumulative degree-days on maturation of ascospores of *Venturia pirina* beginning at detection of the first mature ascospores. Regression is significant at $P = 0.01$. **A,** Y = proportion of mature asci, where 100% = 1.0; **B,** percentage of pseudothecia containing asci with mature ascospores. Cumulative degree-days were calculated with 0 C as the base temperature. Data were combined from 1981, 1982 (P = Parkdale), and 1987. Validation data points for 1993 are indicated for Hood River (▲) and Parkdale (■).

RESULTS

The first mature ascospores in pseudothecia on leaves that had overwintered were observed at or before bud swell in late February to late March. Considerable variation in the maturation of ascospores occurred between years, but a general pattern in which the percentage of asci with mature ascospores reached a maximum during the bloom period was observed. Full bloom occurred 6, 7, 9, and 10 wk after bud swell in 1987, 1981 (MCAREC), 1981 (Parkdale), and 1982, respectively. Ascospores continued to mature and to be discharged over a period of about 3–4 mo until the middle of June. Mature ascospores in about 5% of the asci were not discharged but appeared to shrivel and disintegrate in June.

Ascospore maturity was closely related to accumulated degree-days with a base temperature of 0 C (Fig. 1). Only three of 60 data points (two in 1981 and one in 1982) fell outside the 95% confidence intervals. The combined data were described with the equation

$$\ln(1/1 - Y) = -0.00797 + 0.00415X$$

where Y = the proportion of mature asci and X = degree-days (Fig. 1A); $R^2 = 0.86$. Degree-day accumulation was calculated from a biofix defined as the date on which the first mature ascospores were observed. Ascospore maturity values were corrected on the basis of the maximum number of asci per pseudothecium (6), which varied from 138 in 1987 to 151 in 1981. The 3-yr average value for *V. pirina* in our study was 143 ± 7 and exceeded the value of 119 ± 12 reported for *V. inaequalis* (8). The maximum number of asci per pseudothecium was observed between the tight cluster floral stage in 1981 and 1987 and petal fall in 1982. The relationship between the cumulative percentage of pseudothecia with mature asci and degree-days also is presented (Fig. 1B) to allow comparison with the pattern of ascospore maturation of *V. inaequalis* (7,15). In 1993, nine of 10 validation data points from Parkdale and 10 of 11 from Hood River fell within the 95% confidence interval (Fig. 1A).

During the 3-yr study, eight ascospore release events were associated with dew, nine with rain, and two with no detectable rain or dew (Table 1). The average duration of ascospore release per wetness event was similar for dew (6.4 h) and rain (7.1 h). More ascospores were trapped per hour and per event during rain-related releases than from dew-related releases in 1980, but the opposite occurred in 1981 and 1987 (Table 1). Ascospore releases during the two periods with no measurable wetness occurred in the late afternoon and were preceded by a morning dew.

From the spring of 1992 to the spring of 1993, the wetness meter on the ground recorded 66 and 27 wetness events associated with dew and rain, respectively. During this time, the wetness sensor at 1.5 m above the ground recorded 55 and 25 events

TABLE 1. Ascospore releases during rain, dew, or periods of no detectable moisture

	Moisture condition		
Year	Dew	Rain	Dry
1980			
Number of release events	2	2	0
Release duration (h)	15	17	...
Average number of ascospores/m^3/h	57	73	...
Spores trapped/m^3/event	834	1,249	...
1981			
Number of release events	3	4	0
Release duration (h)	5	6	...
Average number of ascospores/m^3/h	81	45	...
Spores trapped/m^3/event	428	261	...
1987			
Number of release events	3	3	2
Release duration (h)	2	2	3
Average number of ascospores/m^3/h	120	58	47
Spores trapped/m^3/event	201	133	165

related to dew and rain, respectively. Thus, the sensor at ground level indicated 17 and 7% more dew- and rain-related wetness events, respectively, than did the sensor at the 1.5-m level.

The pyranometer recorded light energy from 0545 to 1845 on 1 April of each year (13.0-h photoperiod). Day length increased to include the time from 0430 to 1945 (15.25 h) by May 31. Pyranometer values indicating daylight were 0.2–14.8 kJ/m^2/15 min.

Several combinations of dew- and rain-related ascospore release events were selected to illustrate the relationships between ascospore release, moisture, and light (Fig. 2). Ascospore release began early in the dew periods during darkness (Fig. 2A, C, and D). Dew-associated ascospore releases occurred during all night hours, but the maximum release occurred during the 4 h before dawn. No wetness or spore releases were detected during the 2 days preceding these dew-associated ascospore releases. A second ascospore release occurred shortly after daylight while the leaves were still wet with dew (Fig. 2C and D).

Additional data on each of the 19 ascospore release events are presented in Table 2. In only three of the release events (14 May 1980, 4 April 1981, and 16 April 1987) was the percentage of ascospores trapped during the day less than the percentage of wetness that occurred during that day. On 14 May 1980, wetness from dew started at 2300, and the main ascospore release was at 0200 the following day (Fig. 2A). On 4 April 1981, wetness from dew started at 2200. Maximum ascospore release occurred at 0300 the following day, and all ascospores were trapped within 3 h (Table 2). On 16 April 1987, rain started at 2000, and all ascospores were trapped during the first hour after the start of the rain (Table 2). In the other 14 wetness-related events, the percentage of ascospores trapped during the day was proportional to or exceeded the percentage of time that wetness occurred during that day. An event that shows evidence of light-stimulated release took place on 9 April 1987 when wetness from dew started at 0100, but no ascospores were trapped until between 0600 and 0700, just after daylight (Table 2). Peak numbers of ascospores trapped just after the beginning of daylight also occurred in several other wetness events (Fig. 2A–D).

DISCUSSION

Mature ascospores were observed at bud swell in late February to late March and were present until the middle of June 1981, 1982, and 1987. Peak ascospore maturation occurred during the bloom period. This pattern is similar to that described in Chile (13), New Zealand (19), Poland (3), and Oregon (12). Shriveled, disintegrating ascospores were observed in about 5% of the asci in early June and were not discharged. Miller and Waggoner (18) reported that mature ascospores of *V. inaequalis* remained in 25% of the asci in late spring as a result of a weakening of the discharge mechanism. However, Gadoury and MacHardy (6) suggested that the quantity of residual ascospores is actually lower when adjustment is made for ascus disintegration. They reported only 2% had not discharged by 1 July.

Ascospore maturity was described as a function of cumulative degree-days with a base temperature of 0 C. The description of ascospore maturity for *V. pirina* in relation to degree-day accumulation lacked the initial lag observed for *V. inaequalis* (7,15). Approximately 98% of the ascospores had matured after an accumulation of 1,000 degree-days, which usually was reached

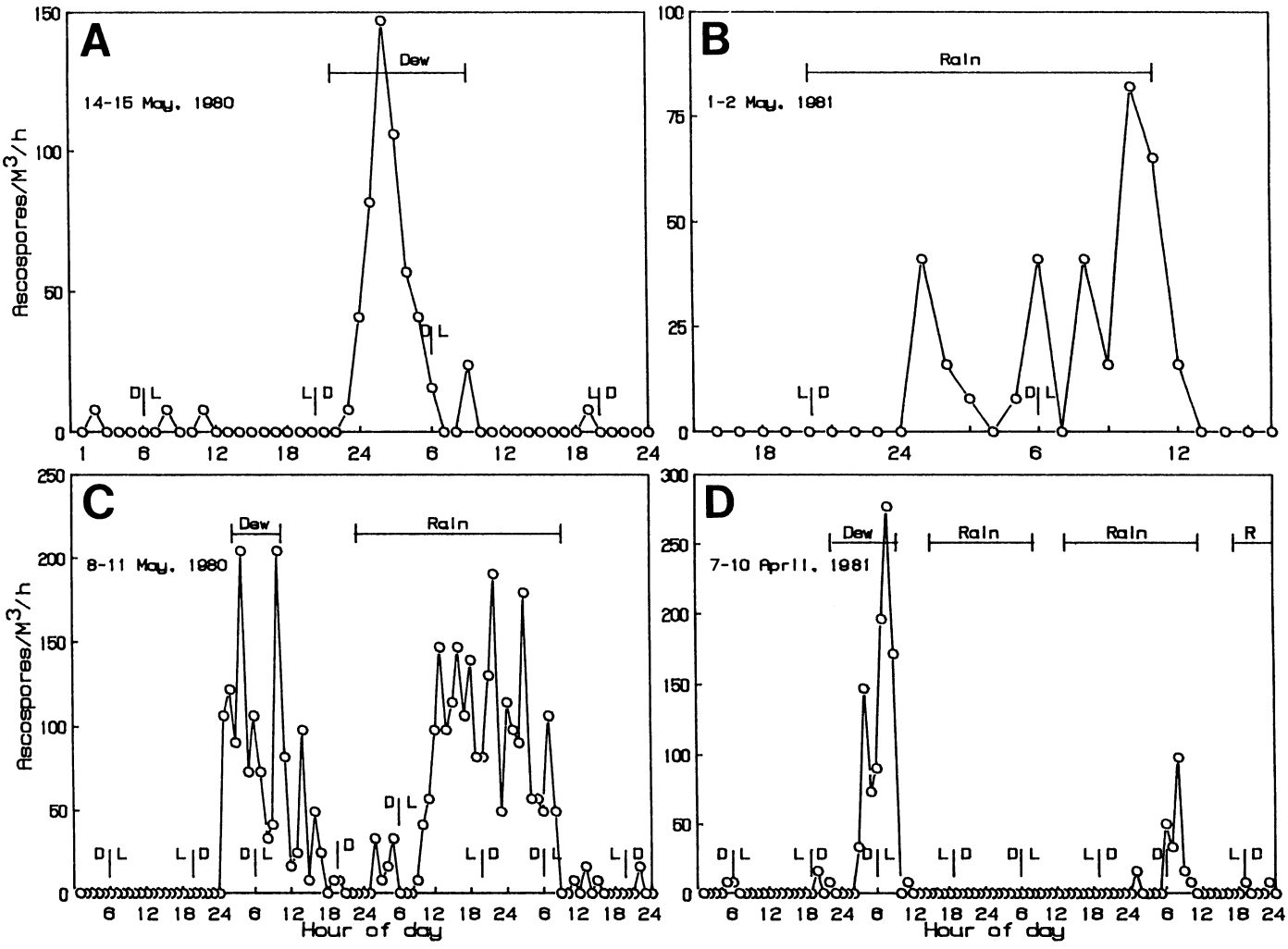

Fig. 2. Hourly trap counts of *Venturia pirina* ascospores. Duration of wetness due to rain (R) or dew is indicated at the top. Vertical lines above the x-axis indicate beginning and end of light (L) and dark (D) periods.

between late May and early June.

The ascospore maturity model was validated in 1993 at two diverse locations. The Hood River site had an elevation of 150 m, and bloom occurred on 26 April. The Parkdale site had an elevation of 600 m at the base of Mount Hood and had a bloom date of 12 May. Nevertheless, once the first mature ascospores were detected, maturity proceeded similarly at both locations in response to degree-days according to the model. Validation of this model outside Oregon remains to be done.

Most of the ascospore release events were associated with rain (47%) and dew (42%). Thus, dew appears to be an important source of free moisture for the release of ascospores of *V. pirina* in Oregon. Latorre et al (14) reported that 20% of *V. pirina* ascospores were trapped after dew periods, and Williamson and Burchill (24) reported trapping ascospores following dew periods and after less than 0.25 mm of rainfall. In contrast, ascospores of *V. inaequalis* were not trapped when dew occurred unless the dew was closely associated with rain (16). Although dew was important for the release of ascospores of *V. pirina,* the length of wetness associated with dew during this study was not of sufficient duration for infection.

Two of the 19 ascospore release events observed in this study occurred at times when no rain or dew were recorded during at least a 6-h period prior to the release. However, during years when ascospore release was assessed, wetness was measured at 1.5 m above the ground. We later found that 17 and 7% of dew- and rain-related wetness events, respectively, at ground level were not detected by a sensor at 1.5 m above the ground. Thus, these two of 19 events (11%) fall within the range of detection failure associated with sensor position. It is interesting to note that ascospores of *V. inaequalis* occasionally are trapped during fair weather (4,16,18). Fair-weather releases in one study were closely associated with rain on the previous day (16). In these studies, the location of the wetness sensors either was not mentioned (4,18) or was also 1.5 m above the ground (16).

Between 18.5 and 49.8% of all ascospores of *V. pirina* were trapped during hours of darkness. Bearden et al (2) reported that *V. pirina* ascospore catches at night occurred frequently in California, but concentrations at night were lower than levels during daylight. Latorre et al (14) trapped less than 1% of the total ascospore catch during darkness in Chile, and Washington (23) trapped 2.4% of the ascospores at night in Victoria, Australia. In a parallel study (same location and time), we trapped 95.2% of the ascospores of *V. inaequalis* during daylight (R. A. Spotts, *unpublished*). The reasons for the different ascospore release responses for *V. pirina* and *V. inaequalis* are not known.

We conclude that mature ascospores of *V. pirina* are present in the orchard leaf litter when green tissue emerges from the buds. Ascospore release is favored by free moisture and light but frequently occurs in darkness, especially during dew periods. Because the percentage of ascospores trapped during daylight was equal to or exceeded the percentage of hours of wetness that occurred during the day in 14 of 17 release events, it appears that the magnitude of daytime releases is greater than that at night. However, because of the ubiquitous nature of mature ascospores of *V. pirina* in Oregon pear orchards throughout the spring and their complex release patterns, infection periods should be calculated from the start of rain- or dew-related wetness, even when that wetness begins at night.

LITERATURE CITED

1. Baskerville, G. L., and Emin, P. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. Ecology 50:514-517.
2. Bearden, B. E., Moller, W. N., and Reil, W. O. 1976. Monitoring pear scab in Mendocino County. Calif. Agric. 30:16-19.
3. Borecki, Z. 1957. Perithecial stage of *Venturia pirina* during winter and spring in field conditions. Acta Agrobot. 6:108-114.
4. Childs, L. 1917. New facts regarding the period of ascospore discharge of the apple scab fungus. Oreg. State Agric. Coll. Exp. Stn. Bull. 143.
5. Curtis, K. M. 1921. Ascospore ejection of the apple and pear black-spot fungi. N.Z. J. Sci. Technol. 5:83-90.
6. Gadoury, D. M., and MacHardy, W. E. 1982. Preparation and interpretation of squash mounts of pseudothecia of *Venturia inaequalis*. Phytopathology 72:92-95.
7. Gadoury, D. M., and MacHardy, W. E. 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. Phytopathology 72:901-904.
8. Gadoury, D. M., MacHardy, W. E., and Hu, C. 1984. Effects of temperature during ascus formation and frequency of ascospore discharge on pseudothecial development of *Venturia inaequalis*. Plant Dis. 68:223-225.
9. Hearman, J. 1933. Control of black spot of pears in Western Australia. J. Agric. West. Aust. 10:292-316.
10. James, J. R., and Sutton, T. B. 1982. Environmental factors influencing pseudothecial development and ascospore maturation of *Venturia inaequalis*. Phytopathology 72:1073-1080.
11. James, J. R., and Sutton, T. B. 1982. A model for predicting ascospore

TABLE 2. Time and duration of ascospore release events and associated moisture and light conditions in 1980, 1981, and 1987

Date	Day of year	Start of wetness	Wetness duration (h)	Temperature[a] (C)	Time of initial spore release	Release duration (h)	Ascospores per m^3/h (no.)	Wetness during day (%)	Spores trapped in day (%)
1980									
4/14	104	1400	4	12.8	1400	3	70	100	100
5/09	129	0200[b]	8	10.0	0200	18	68	50	51
5/09	129	2230	35	10.0	0200	31	77	49	56
5/14	134	2300[b]	10	7.8	2300	11	47	30	5
1981									
3/29	88	0200	32	6.7	1100	5	47	52	100
4/01	91	2200[b]	11	7.2	0100	6	68	27	36
4/04	94	2200[b]	11	6.7	0200	3	33	18	0
4/07	97	2200[b]	11	5.0	0200	7	141	18	70
4/09	99	1330	20	5.0	0500	5	41	47	76
5/01	121	2000	15	7.8	0000	12	28	37	66
5/03	123	1630	3	11.1	1630	1	65	100	100
1987									
3/31	90	...	...	17.2	1500	4	45	...	100
4/09	99	0100[b]	10	6.7	0600	3	52	50	100
4/09	99	2300[b]	11	5.0	1000	1	236	41	100
4/10	100	1200	7	10.5	1400	4	35	100	100
4/14	104	...	...	20.5	1400	3	49	...	100
4/16	106	2000	14	9.4	2000	1	98	36	0
4/20	110	0100[b]	9	2.2	0500	1	73	55	100
5/11	131	2300	19	15.0	1400	2	41	66	100

[a] Temperature during the wetness period.
[b] Wetness from dew.

maturation of *Venturia inaequalis*. Phytopathology 72:1081-1085.
12. Kienholz, J. R., and Childs, L. 1951. Pear scab in Oregon. Agric. Exp. Stn. Tech. Bull. 21.
13. Latorre, B. A., and Marín, G. 1982. Effect of bitertanol, fenarimol, and urea as fall treatments on *Venturia pirina* ascospore production. Plant Dis. 66:585-586.
14. Latorre, B. A., Yañez, P., and Rauld, E. 1985. Factors affecting release of ascospores by the pear scab fungus (*Venturia pirina*). Plant Dis. 69:213-216.
15. MacHardy, W. E., and Gadoury, D. M. 1985. Forecasting the seasonal maturation of ascospores of *Venturia inaequalis*. Phytopathology 75:381-385.
16. MacHardy, W. E., and Gadoury, D. M. 1986. Patterns of ascospore discharge by *Venturia inaequalis*. Phytopathology 76:985-990.
17. Madden, L. V. 1980. Quantification of disease progression. Prot. Ecol. 2:159-176.
18. Miller, P. M., and Waggoner, P. E. 1958. Dissemination of *Venturia inaequalis* ascospores. Phytopathology 48:416-419.
19. Parham, B. D. 1932. Apple and pear black spot (scab). N.Z. J. Sci. Technol. 14:184-192.
20. Riedl, H., Spotts, R. A., Mielke, E. A., Long, L. E., Fisher, G. C., Pscheidt, J. W., and William, R. 1992. Pest management guide for tree fruits in the Mid-Columbia area. Oreg. State Univ. Ext. Serv. EM 8293.
21. Shabi, E. 1990. Pear scab. Pages 22-23 in: Compendium of Apple and Pear Diseases. A. L. Jones and H. S. Aldwinckle, eds. American Phytopathological Society, St. Paul, MN.
22. Sivanesan, A., Waller, J. M., and Mordue, J. E. M. 1974. Descriptions of Pathogenic Fungi and Bacteria, set 41. Commonwealth Mycological Institute, Kew, England.
23. Washington, W. S. 1988. Diurnal periodicity of ascospore discharge of *Venturia pirina*. Trans. Br. Mycol. Soc. 90:112-114.
24. Williamson, C. J., and Burchill, R. T. 1974. The perennation and control of pear scab (*Venturia pirina* Aderh.). Plant Pathol. 23:67-73.