Effect of Temperature and Wetness on Infection of Pear by *Venturia pirina* and the Relationship Between Preharvest Inoculation and Storage Scab

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ABSTRACT

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The effects of conidial inoculum dose and selected combinations of temperature and wetness duration on the incidence and severity of pear scab on fruit and foliage were studied. The relationship between the time of preharvest infection of pear fruit and the length of storage at −1 C before symptoms developed also was determined. Incidence and severity of scab on leaves and shoots of Bartlett pear seedlings increased linearly as inoculum concentration increased from 5×10^2 to 5×10^4 conidia per milliliter. Minimum wetness duration for foliar infection varied from 10 hr at 23.9 C to 25 hr at 7.2 C and was similar to apple scab infection periods determined by Mills. Fruit infection was minimal at 7.2 and 10 C but increased to more than 20% at 15.5 C for 15 hr of wetness and 21.1 C for 14 hr of wetness. Anjou and Bartlett fruit inoculated within 2 wk before harvest developed symptoms in storage at −1 C after 2–6.5 mo, but inoculation 4 wk before harvest required less than 2 mo of incubation. These relationships were described with second-order polynomial regression equations. Dodine application 1 day before or after preharvest fruit inoculation gave good control of scab in cold storage.

Additional keywords: *Pyrus communis*

Pear scab caused by *Venturia pirina* Aderhold is an economically important disease of pear throughout the world (16). In some areas, ascospores from leaves overwintered on the orchard floor are considered the main source of primary inoculum (3,19). However, other studies consider conidia from twig lesions more important than ascospores from overwintered leaves (4,5). Infection of pear foliage and fruit is thought to occur under conditions similar to those required for infection of apple by *V. inaequalis* (Cooke) G. Wint. (1) as described by Mills (9), but few data are available to verify this assumption. Shabi et al (17) reported that infection of pear foliage occurred at 5–28 C, but the study included only 24- and 64-hr wetness periods. Kienholz and Childs (5) stated that infection at 23.9 and 4.4 C occurred after 5 and 48 hr of wetness, respectively, but they presented no experimental methods or data.

Scab that appears on fruit in cold storage is known as "pinpoint" scab (12). This phase of the disease is not well understood, but it appears to be related to late-season, preharvest infection (5). However, quantitative data concerning infection and incubation are not available.

This study was conducted to determine the relationship between density of conidial inoculum and incidence and severity of foliar scab, the combinations of temperature and wetness duration required for conidial infection, and the relationship between time of infection of fruit before harvest and time of appearance of scab in cold storage at −1 C.

MATERIALS AND METHODS

Plant material. Dormant pear seedlings (*Pyrus communis* L. 'Bartlett') were planted in 15-cm-diameter plastic pots containing a 2:1:1 mix of Van Horne sandy loam soil/sand/peat. Seedlings were cut to three buds, fertilized with 15 g of 18-6-12 (NPK) slow-release fertilizer per pot, and kept in a greenhouse at 20 ± 5 C, 80 ± 20% RH, and a 14-hr photoperiod. Insects were controlled with 0.9 g of endosulfan (Thiodan 50WP, FMC Corporation, Philadelphia, PA) and 0.6 ml of fenbutatin oxide (Vendex 4L, E. I. du Pont de Nemours & Co., Wilmington, DE) per liter applied to foliage as needed. Seedlings used in each experiment had two or three shoots, each about 15 cm long.

Inoculum preparation. Conidia on sporulating scab lesions were washed from infected leaves of Bartlett pear with distilled water, filtered through four layers of cheesecloth, and used immediately or frozen at −20 C in water (13) until needed. Germination of conidia was determined at 25 C 1 day before each experiment on Difco potato-dextrose agar acidified with 1.5 ml/L of 85% lactic acid. Inoculum concentration was adjusted to give the desired level of viable spores. The average germination of conidia in these experiments was 67%.

Inoculum dose vs. disease incidence and severity. Inoculum was applied to leaves with a hand-pump sprayer to runoff. Inoculum concentrations of 0, 500, 2,500, 5,000, 12,500, 25,000, and 50,000 viable conidia per milliliter were used. Each inoculated seedling was enclosed in a plastic bag to maintain wetness and overlaid with aluminum foil to maintain temperature at about 20 C. After 24 hr, the bags were removed and the seedlings were kept in the greenhouse under conditions described earlier. Leaf and stem lesions were counted 2 wk after inoculation. Each inoculum dose was applied to nine seedlings, and the experiment was done twice.

Effect of temperature and wetness duration on disease incidence and severity. Bartlett seedlings were inoculated with 5×10^4 viable conidia per milliliter as described earlier. Seedlings were placed in incubators in the dark at 7.2, 10.0, 12.8, 15.5, 18.3, 21.1, and 23.9 C for wetness durations that ranged from 7 hr at 23.9 C to 29 hr at 7.2 C. Wetness duration included the time required for leaves to dry after removal of the bags. Drying times determined visually and with a leaf wetness sensor connected to a 21X datalogger (Campbell Scientific, Logan, UT) ranged from 15 min at 23.9 C to 1 hr at 7.2 C. Five seedlings were inoculated for each combination of temperature and wetness duration, and all combinations were done at least twice. Seedlings were incubated in the greenhouse after leaves were dry. Leaf lesions were counted after 3 wk of incubation.

The effects of temperature and wetness duration on disease incidence and severity also were evaluated using scaffold limbs on mature (60-yr-old) Bartlett pear trees in an orchard at the Mid-Columbia Agricultural Research and Extension Center, Hood River. Leaves and fruitlets on limbs were inoculated with 5×10^4 conidia per milliliter and then covered with plastic bags overlaid with aluminum foil. A 2-m length of each limb was enclosed in a portable cage equipped with temperature control (8). Limb cages were maintained at 7.2, 10.0, 12.8, 15.5, and 21.1 ± 1 C. Temperatures were moni-

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tored using a 21X datalogger equipped with thermocouples attached to inoculated shoots covered with bags and foil. Wetness duration varied from 10 hr at 21.1 C to 29 hr at 7.2 C. Five shoots with attached fruitlets were included in each temperature-wetness duration combination. The experiment was done on 20 April (90% petal fall) and 17 May 1988 and 27 April (90% petal fall) and 2 May 1989. Leaf and fruit infections were evaluated 4–6 wk after inoculation.

Effect of preharvest inoculation on development of fruit scab in cold storage. Pear fruits on Anjou trees were inoculated weekly beginning 6 wk before harvest in 1980, 1981, 1988, and 1989. Pear fruits on Bartlett trees were inoculated weekly beginning 4 wk before harvest in 1989. Fruits were inoculated with 5×10^4 viable conidia per milliliter using the method described earlier. Foil and plastic bags were removed after 48 hr. At 2 wk before harvest, dodine (Cyprex 65WP, American Cyanamid Co., Princeton, NJ) at 579 μg a.i./ml was applied to fruits at 1 day before and 1, 2, and 7 days after inoculation. Each dodine treatment and each weekly inoculation was applied to 20 fruits in 1980 and 1981 and to 12 fruits per tree on each of three trees in 1988 and 1989. Fruits were harvested at the initial date each year for commercial harvest as determined by fruit firmness of 62.3 N (6.36 kg) (2). Fruits were stored at −1 C in boxes lined with polyethylene and evaluated for scab once every 2 wk for 7 mo.

RESULTS

Inoculum dose vs. disease incidence and severity. The number of infected leaves and the number of stem lesions per shoot increased with increasing inoculum dose, and the relationships between disease and inoculum dose were described best with linear regression (Table 1). The relationship between the number of lesions per infected leaf and inoculum dose was also significant ($P = 0.01$) (Table 1). No infection occurred on control seedlings sprayed with water. Typical scab lesions appeared on leaves and shoots of inoculated seedlings after about a 10-day incubation period.

Effect of temperature and wetness duration on disease incidence and severity. The incidence and severity of infection on leaves of potted seedlings was affected by temperature and wetness duration. Infection was greatest at 15.5, 18.3, and 21.1 C with 14–15 hr of wetness and averaged about 1.1 infected leaves per shoot (Fig. 1). At 7.2 and 23.9 C, severity was less than at other temperatures and averaged about 0.2 infected leaves per shoot when all wetness durations were combined. At 23.9 C, 0.25 and 0.50 infected leaves per shoot occurred at 12 and 14 hr of wetness, respectively (values hidden in Fig. 1). Minimum wetness duration for infection was 25, 15, 14, 11, 10, 10, and 10 hr at 7.2, 10, 12.8, 15.5, 18.3, 21.1, and 23.9 C, respectively (Figs. 1 and 2). At 7.2 and 10.0 C, infection appeared to occur at shorter wetness times than indicated earlier (Fig. 1), but at each temperature, only one leaf was infected on 13 shoots. The number of lesions per infected leaf was greatest at 21.1 C with 4.3 lesions per leaf at 14 hr of wetness and an average over all wetness durations of 2.3 lesions per leaf. Severity was least at 23.9 C with an average of 0.8 lesions per infected leaf over all wetness durations (Fig. 3). Number of lesions per infected leaf at other temperatures was similar and varied from 1.1 to 1.7 when wetness durations were combined (Fig. 3). At 23.9 C and 14 hr of wetness, 1.7 lesions per leaf occurred (value hidden in Fig. 3). Atypical, chlorotic lesions occasionally developed at wetness durations shorter than indicated in Figures 1 and 3. However, these lesions remained chlorotic without any further symptom progression, and no sporulation was observed.

The results of infection described for potted seedlings were similar for leaves and fruit on scaffold limbs of mature trees. Infection of leaves was greater at 15.5 and 21.1 C and longer wetness durations (up to 18% infection) than at cooler temperatures, whereas less than 2% infection was observed at 7.2 C and 25–29 hr of wetness (Fig. 4). Fruit infection varied from 0 at 7.2 C to 3.6% at 10 C but increased to 20.5 and 29.5% at the longer wetness periods at 15.5 and 21.1 C, respectively (Fig. 5). No atypical lesions developed on leaves in the limb cage experiments.

Incubation time for lesions on leaves of potted seedlings was 14.5 ± 3.5 days at an average temperature of 19 C and

Table 1. Effect on inoculum dose of conidia of *Venturia pirina* on severity of leaf and shoot infection of Bartlett pear seedlings

Conidia/ml (no. × 1,000)	Infected leaves/shoot (no.)	Lesions/ infected leaf (no.)	Stem lesions/shoot (no.)
0.0	0.00	0.00	0.00
0.5	0.80	1.47	0.00
2.5	1.88	2.66	0.59
5.0	1.06	2.44	0.06
12.5	2.73	6.62	1.47
25.0	2.67	7.57	0.73
50.0	3.81	11.38	1.81
Intercept[a]	6.2×10^{-5}	1.2×10^{-4}	3.25×10^{-5}
Slope[a]	1.008	1.7262	0.2222
r[b]	0.86*	0.95**	0.81*

[a]Linear regression based on the formula $Y = mX + b$ where Y = disease severity, X = number of conidia per milliliter, b = Y intercept, and m = slope.
[b]r = Correlation coefficient. ** And * indicate significance at $P = 0.01$ and 0.05, respectively. Disease values represent the means of two experiments, each with nine replications.

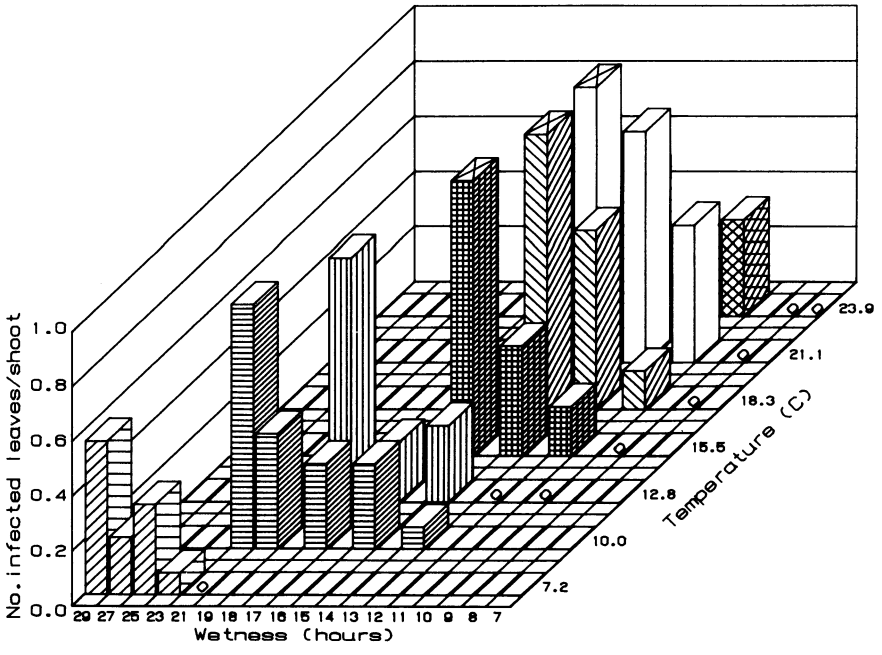

Fig. 1. Effect of temperature and wetness duration on number of infected leaves per shoot of Bartlett pear seedlings inoculated with 5.0×10^4 conidia of *Venturia pirina* per milliliter. Combinations without bars or zeros were not tested.

80% RH. Incubation time for lesions on fruit and leaves of scaffold limbs of mature trees in the orchard was 19 days at 14 C and 57% RH.

Effect of preharvest inoculation time on development of fruit scab in cold storage. When Bartlett fruit were inoculated more than 4 wk and Anjou fruit more than 6 wk before harvest, scab symptoms were visible at harvest. However, when inoculations were made at several times closer to harvest, no symptoms were observed at harvest. The relationship between the length of the incubation period in cold storage at −1 C and the number of weeks before harvest that fruit were inoculated was described with second-order polynomial regression (Fig. 6). The regression equation for Anjou fruit was $Y = 6.53 - 2.01X + 0.19X^2$ and for Bartlett fruit was $Y = 6.34 - 2.70X + 0.30X^2$ where Y = incubation time in months at −1 C and X = time of inoculation in weeks before harvest. Both regressions were significant ($P = 0.01$). Thus, the longer the period between inoculation and harvest, the shorter the period required for development of visible symptoms in storage. For example, when Anjou fruit were inoculated 4 wk before harvest, the incubation period at −1 C was 1.5 mo; when inoculation was done 1 wk before harvest, the incubation period was 4.7 mo (Fig. 6).

Application of dodine to fruit at 1 day before or 1 day after inoculation resulted in 7 and 12%, respectively, of fruit infected, and infection was significantly ($P = 0.01$) less than that of unsprayed fruit (74% infected). Dodine application 2 or 7 days after inoculation resulted in 59 and 63% infection, respectively. Disease incidence was not significantly different from the unsprayed control. Dodine application 1 day before or after inoculation caused a 2- to 4-wk increase in the incubation period at −1 C.

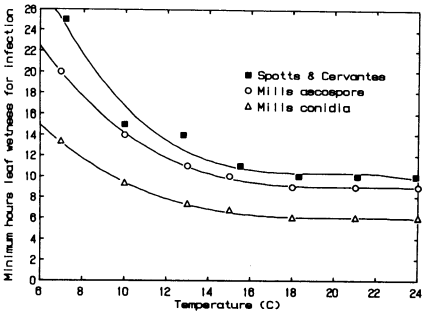

Fig. 2. Comparison of minimum hours of wetness for infection of pear leaves with *Venturia pirina* conidia with requirements for infection of apple foliage with *V. inaequalis* according to Mills and LaPlante (11). Regression of Y (minimum hours wetness for infection) on X (temperature) Spotts and Cervantes: $Y = 66.82 - 8.72X + 0.44X^2 - 0.0076X^3$. $R^2 = 0.967$.

DISCUSSION

Conidia of *V. pirina* are considered by some researchers to be an important source of primary inoculum (4,5,7). A positive linear relationship between conidial concentrations up to 5×10^4 spores per milliliter and incidence of scab infection of both leaves and stems was detected herein. However, in one experiment (R. Spotts, *unpublished*), inoculum at 1×10^5 per milliliter did not increase infection compared with 5×10^4 per milliliter. Research on host resistance and physiological races of *V. pirina* have been done with inoculum doses ranging from 1×10^3 (18) to 2.8×10^5 conidia per milliliter (17). Because disease incidence depends on inoculum dose, future studies with *V. pirina* should include a range of concentrations of inoculum from 10^3 to 10^5 conidia per milliliter.

The minimum wetness durations required for foliar infection by conidia of *V. pirina* ranged from 25 hr at 7.2 C to 10 hr at 23.9 C. These wetness durations all were between the values required for "light" and "moderate" infection of apple by *V. inaequalis* ascospores according to Mills (9). Thus, when using the Mills table for pear scab, the hours

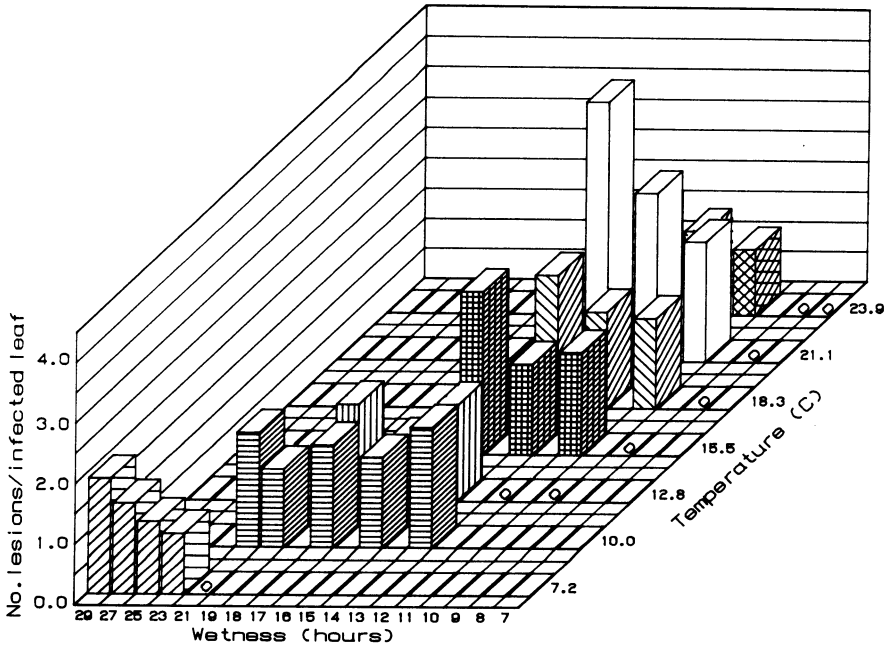

Fig. 3. Effect of temperature and wetness duration on the number of scab lesions per infected leaf of Bartlett pear seedlings inoculated with 5.0×10^4 conidia per milliliter of *Venturia pirina*. Combinations without bars or zeros were not tested.

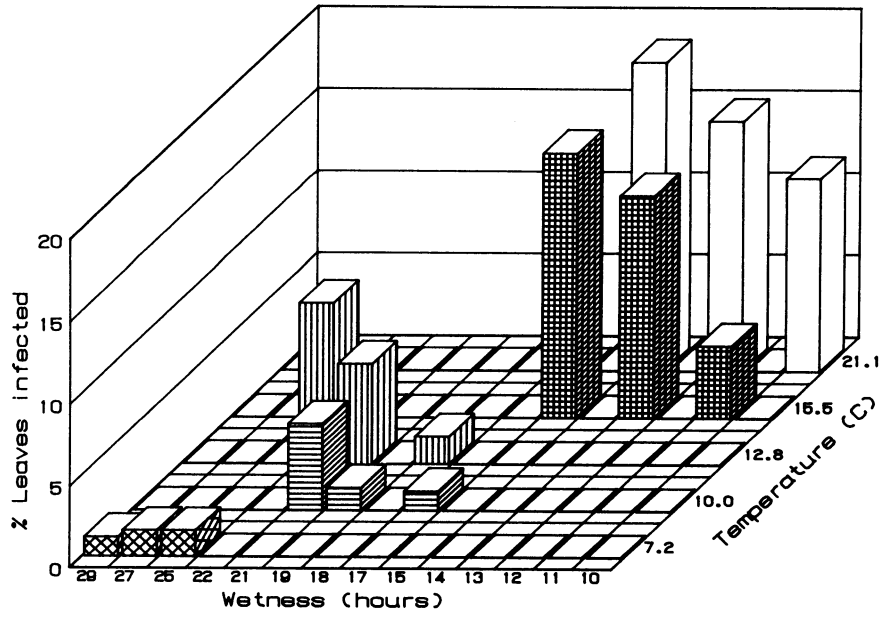

Fig. 4. Percent leaves infected on scaffold limbs of mature Bartlett pear trees after inoculation with 5×10^4 conidia per milliliter of *Venturia pirina*, then exposed to selected combinations of temperature and wetness duration. Combinations without bars or zeros were not tested.

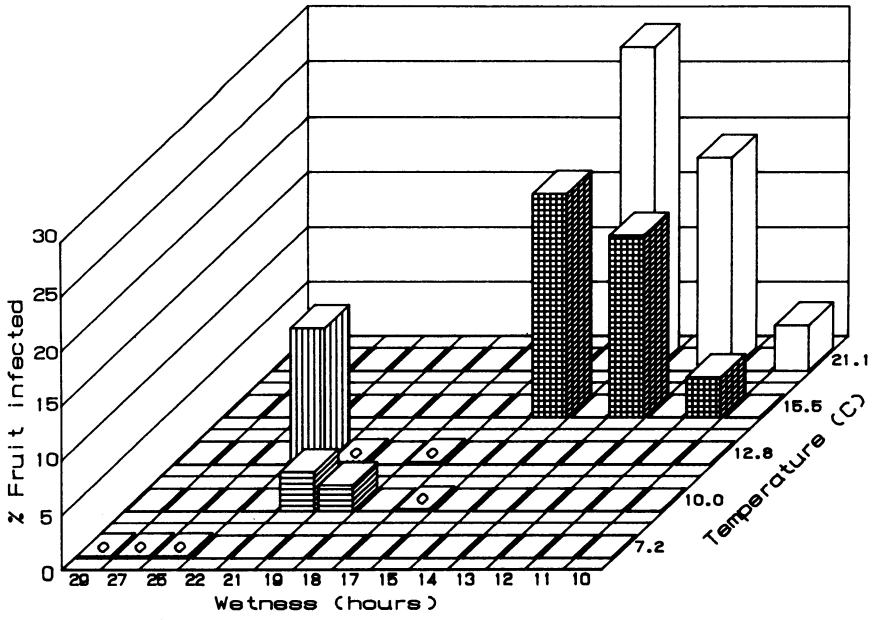

Fig. 5. Percent of fruit infected on scaffold limbs of mature Bartlett pear trees after inoculation with 5×10^4 conidia per milliliter of *Venturia pirina*, then exposed to selected combinations of temperature and wetness duration. Combinations without bars or zeros were not tested.

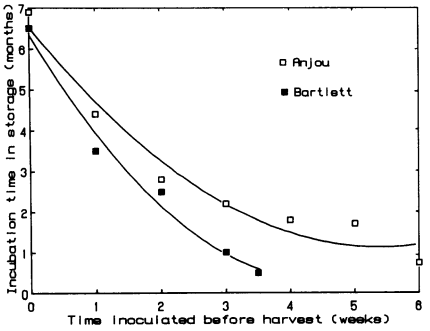

Fig. 6. Relationship between time of inoculation of Bartlett and Anjou pear fruit before harvest and time fruit were in cold storage at −1 C before scab lesions became visible. Inoculations at time 0 were made 2 days before harvest.

of wetness for "light" infection would be conservative because infection may occur before the number of hours for "moderate" infection are reached. A revision of Mills' criteria was proposed (6) that indicates infection of apple leaves by ascospores occurs in 3 hr less wetness duration than originally proposed by Mills. In addition, conidia require about 2.5 hr more wetness than ascospores to infect apple foliage (6). This report by MacHardy and Gadoury (6), as well as a study by Schwabe (15), indicates that the wetness times given by Mills actually fit more accurately the requirements for infection by conidia than by ascospores.

Because the Mills criteria appear close to those reported herein for infection by conidia of *V. pirina*, it can be concluded that conidia of both *V. pirina* and *V. inaequalis* are similar in the wetness requirements for leaf infection over a wide range of temperatures. In California, the Mills table appeared to serve as a reasonably accurate guideline for predicting pear scab infection (1). The durations of wetness needed for infection of pear foliage by ascospores of *V. pirina* may be different than those reported herein for conidia and require further study. Infection patterns on foliage of mature trees were similar to those of foliage of seedlings. However, fruit appeared more resistant than leaves, especially at temperatures below 10 C. Apple fruit are more resistant than leaves as fruit mature, but differences are not evident early in the season (20).

Incubation periods in our studies varied from 10 to 19 days and were longer than those for apple scab (10). An earlier report set the incubation period for pear scab at 12–25 days (5). The incubation period increases with increasing host resistance (17). The incubation period also is affected by RH. Symptoms appear 4–16 days later when apple seedlings are incubated at less than 70% RH for 10–24 days, then transferred to RH above 70% compared with seedlings held continuously at the high RH (21). The incubation period in our limb cage study was 6 days longer than that for apple scab at 14 C, and our average RH was 57%. Thus, long incubation periods in some fruit growing districts in the Pacific Northwest may be explained, in part, by the dry climate with low RH.

The relationship between the incubation period of fruit scab in cold storage and the time when infection occurs before harvest is important for several reasons. In the Pacific Northwest, fungicide applications for scab control usually are discontinued in early June because of dry weather. However, preharvest rain in mid-August has occurred in six of the last 10 yr, and additional fungicide applications may be necessary to prevent scab in storage. Dodine commonly is used to control pear scab in Oregon (14), but it must be applied within 24 hr after the start of an infection period to control fruit infection. Second, if infection occurs close to harvest, the incubation period at −1 C may exceed 4 mo for Bartlett and 5 mo for Anjou pears. This would allow time to market the infected fruit before symptoms appear.

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