Overwintering of Conidia of *Venturia inaequalis* in Apple Buds in New York Orchards

C. M. BECKER, Graduate Research Assistant, T. J. BURR, Professor, and C. A. SMITH, Research Support Specialist, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456

ABSTRACT

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Viable conidia of *Venturia inaequalis* were detected in dormant apple buds and on early developing apple tissues during 1988–1990. Zero to 142 viable conidia per bud were detected from the inner tissues of flower buds. Apple scab lesions were observed on the adaxial bud scale surfaces of 12 of 1,000 dormant buds just before budbreak from one orchard, and 77% of the conidia arising from the lesions were viable. Viable conidia were rarely detected on the exterior surfaces of overwintered buds or lesions on infected shoots. They were never detected in lesions on overwintered foliage or fruit that were assayed from March to June. When apple tissues at early stages of bud growth were inoculated with conidia, an increase in sepal and fruit infection resulted.

Additional keyword: survival

It is well documented that pseudothecia of *Venturia inaequalis* (Cooke) G. Wint. are produced in infected, overwintering apple leaves and that ascospores serve as primary inoculum for infections (6,17). The disease is controlled mainly by the application of fungicides in association with measurements of ascospore maturity and release (24,36). By monitoring the duration of leaf wetness and temperature during the wetting period, the occurrence of infection periods can be determined according to the criteria of Mills (23,28).

Reports vary as to whether conidia of *V. inaequalis* (anamorph = *Spilocaea pomi* Fr.) overwinter within orchards. Conidia have been detected in the spring within scab lesions on shoots (9,14,25) and bud scales (10,16,27,33) and on seemingly uninfected foliage, shoots, or bud scales (12,15,26). However, in the United States, conidia have been reported to overwinter only in lesions on infected shoots (8,31) and it has been generally assumed that they are not important as inoculum for primary infections.

The objective of this research was to determine if conidia of *V. inaequalis* overwinter in lesions on fruit, foliage, and shoots or in dormant apple buds and, thus, serve as a source of primary inoculum.

MATERIALS AND METHODS

Orchards. Three orchards near Geneva, NY, designated A, B, and C; an orchard in Wayne County, NY, designated orchard D; and the Cornell Orchards in Ithaca, NY, designated orchard E were used during this study. Orchard A was at the New York State Agricultural Experiment Station (NYSAES); it was unsprayed in 1988, 1989, and 1990. Each year, the incidence of fruit and foliage scab was greater than 80%. Orchard B was a commercial orchard located 6 km west of the NYSAES and had less than 5% fruit and foliar scab each year. Orchard C was located 4 km west of the NYSAES. It received a full fungicide program in 1988 but was abandoned in 1989 and 1990. About 20% of the fruit and foliage was infected with scab in 1988, and 100% was infected in 1989 and 1990. Orchard D was unsprayed in 1988 and in 1989, only Delicious, Rome Beauty, and RI Greening received fungicides. The owner of orchard D reported >70% of the fruit and foliage was infected with scab in 1988 and we recorded <5% scab in 1989.

Overwintering of conidia in leaf, fruit, and shoot lesions. Approximately 600 apple leaves of the cultivar McIntosh infected with *V. inaequalis* were collected on 22 October 1986 from Orchard A. About 200 leaves were placed in each of three cages that were constructed out of two 40 × 60 cm sheets of 2.4-cm-mesh wire screen. The cages were placed either on the ground under an apple tree, within a tree, or within a weather shelter where they would not be subjected to wetting.

Infected McIntosh leaves and fruit were collected on 23 October 1987, divided into three groups, and placed between wire screens, as described earlier. Cages for leaves were similar to those used in 1986; cages for fruit were 5 × 40 × 75 cm and constructed from 1.2-cm-mesh wire screen. Cages of leaves and fruit were placed on the ground in three orchards, two at different blocks (separated by 5 km) in orchard A and one in orchard E.

Samples of 10 leaves and fruit were collected near the middle of each month from October through May during 1986–1987 and 1987–1988. Fifteen disks, 1 cm in diameter, were removed from lesions on leaf and fruit samples using a cork borer. Disks were placed in a test tube with 10 ml of distilled water and stirred vigorously on a vortex shaker for 1 min. The suspensions were filtered through two layers of cheesecloth, centrifuged to concentrate the spores, 9.5 ml of the supernatant was drawn off, and the pellet was resuspended in the remaining 0.5 ml of water. In the 1986–1987 season, three 50-µl drops of the spore suspension from each tube were placed on each of two microscope slides. Two 50-µl drops of the resuspended suspension were placed on slides in the 1987–1988 season. One set of slides was immediately viewed with a microscope, and the number of nongerminated conidia and germlings (germinated conidia, without or with appressoria) were recorded. The remaining slides were placed in a moist chamber at 20 C to allow viable conidia to germinate. After 24 hr, the number of conidia and the percent germinated conidia were recorded. The total number of conidia per disk was determined by averaging the mean number of conidia from observations before and after the 24-hr period in the moist chamber. The mean percentage of conidia that was viable was determined by the fraction of conidia that germinated in the moist chamber.

To determine if conidia of *V. inaequalis* overwinter in scab lesions that developed on apple shoots, shoots with typical scab lesions that formed in May 1988 were collected from orchards A and C during July and October 1988 and April 1989. Shoots with lesions that had developed in 1989 were collected on 27 April 1990. Ten lesions were excised from shoots from orchards A and C. Each lesion was placed in a separate Eppendorf microfuge tube with 1.5 ml of distilled water. The tubes were stirred on a vortex shaker for 1 min, submerged in the water bath of a Bransonic ultrasonic cleaner (model

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number B-8200R-1, Bransonic Cleaning Equipment Co., Shelton, CT), and sonicated for 10 min. Tubes were removed from the sonicator, lesions were removed, and the remaining suspensions were centrifuged on an Eppendorf 5414 microfuge (Brinkmann Instrument Inc., Westbury, CT) for 7 min. The pellets were resuspended in approximately 100 μl of water by stirring on a vortex shaker. The entire 100 μl was placed on a microscope slide and incubated in a moist chamber at 20 C for 24 hr before the number of conidia per drop and percent germination was recorded.

Viability of conidia associated with buds. Samples of buds (both flower and vegetative) were collected on 10, 13, and 14 May 1988 from orchard A when two to five small leaves had separated from the buds (early tight-cluster growth stage). Each sample contained 10 buds that were collected from two to four trees.

Samples of McIntosh flower buds were collected from 22 March (dormant buds) to 28 April 1989 (1-cm green phenological stage of growth) from orchards A, B, and C. McIntosh, Delicious, RI Greening, and Rome Beauty flower buds were sampled in orchard D from 7 April (silver-tip stage of growth) to 19 May (pink-bud stage of growth). Dormant flower buds were collected from orchards A and C on 28 March 1990. Samples consisted of three replicates of 100 flower buds excised approximately 2 mm below the bud scales. Flower buds were collected from orchard B on 18 April 1990 (1-cm green stage of growth); the three replicates each consisted of 300 buds.

To enumerate the presence of conidia associated with the flower buds, all leaves and petioles, if present, were first removed. Buds were then dissected by removing the outer bud scales, and, for the 1989 and 1990 samples, teasing the inner tissues apart. Dissected buds were placed in Ziploc bags with 20–100 ml of distilled water. The bags were then immersed in the water bath of the sonicator and sonicated for 10 min. Suspensions were then filtered through a double layer of cheesecloth into beakers. For each sample, 1.5 ml of the suspension was placed in each of six Eppendorf microfuge tubes and centrifuged for 7 min. The supernatant was discarded, and the pellet was processed as described earlier for enumeration of viable conidia.

The distribution of conidia within dormant buds was determined in 1989 and 1990. Three replicates of 100 flower buds were collected from orchard C on 22 March 1989 and from orchards A and C on 28 March 1990, respectively. Buds were evaluated first for the presence of conidia on the outer surfaces by placing intact buds in Ziploc bags with 10–50 ml of water and sonicating the bags for 10 min. The buds were then removed from the bags and dissected to separate the outer four bud scales from the remaining inner tissues. The outer and inner portions of the buds from each replicate were placed into separate Ziploc bags for sonication and subsequent enumeration of conidia as described earlier. In all assays where conidia were placed in water for 24 hr, the percentage that germinated was reported as the percentage that was viable; however, these numbers may be conservative because it is likely that there were some viable spores that failed to germinate.

Scab lesions on the adaxial surface of bud scales. Ten replicates of 100 dormant buds were collected from orchard A on 5 April 1990 to determine if scab lesions could be found on the inner bud tissues. Each bud scale was removed and the number of lesions on the adaxial surfaces was recorded. The viability of the conidia within lesions was determined by scraping spores from the surface of six lesions with a scalpel blade and suspending them in drops of distilled water. Three drops of the suspension from each lesion were placed onto microscope slides. The slides were incubated 24 hr in moist chambers at 20 C, after which the percent germination was recorded.

Three bud scales with lesions were prepared for viewing with scanning electron microscopy (SEM). Lesions with approximately 0.5 mm of the surrounding healthy tissues were fixed by immersion in 2% aqueous osmium tetroxide for 2 hr, washed in two changes of distilled water, and dehydrated in a graduated ethanol series in 30-min steps. They were critical-point dried, sputter-coated with gold/palladium, and examined with a Hitachi S-530 scanning electron microscope (Hitachi, Ltd., Tokyo, Japan) operating at 25 kV. Photographs were taken with Polaroid 55 film.

Inoculation of early apple growth stages with conidia. In 1989, McIntosh flower buds in orchard A were inoculated with conidia of *V. inaequalis* on 19 April, when green tissue was just expanding beyond the bud scales (green-tip growth stage); on 1 May when two to four small leaves had separated from the expanding bud (early tight cluster); and on 10 May, when five to seven leaves had separated from the bud (tight cluster). In 1990, flower buds were inoculated on 9 April (green tip), 19 April (0.5-cm green), and 24 April (early tight cluster). Conidia for inoculations were collected in water from infected leaves that had been dried and refrigerated at 3 C since the previous July (>70% of the conidia were found to be viable after storage). Buds were sprayed to runoff with a suspension of 5×10^4 conidia per milliliter using a No. 15 DeVilbiss atomizer (DeVilbiss Inc., Somerset, PA). Approximately 0.3 ml of the suspension was sprayed on each bud. Inoculated tissues were individually covered with a 150-ml waxed paper cup with a plastic lid containing a moistened paper towel. Inoculated buds and foliage were inserted through enlarged slits in the lids of the cups, and the cups were attached to the lids and to the adjacent branch with tape. They were left closed for 48 hr. The mean temperatures for the 24-hr periods after inoculations in 1989 were 7, 14, and 12 C for the 19 April and 1 and 10 May inoculation dates, respectively. In 1990, the mean temperatures were 8, 13, and 18 C for the 24 hr after the 9, 19, and 24 April inoculations.

On 22 May 1989 (just after the flower petals dropped), 10 fruitlets that developed from the inoculated buds were collected and examined for sepal infections. In 1990, similar data were collected from 24 fruitlets collected on 16 May. Each of the five sepals per fruitlet was examined for macroscopic lesions,

Table 1. Overwintering conidia of *Venturia inaequalis* in McIntosh flower buds from western New York[u]

Phenophase	Date	Orchard	Conidia/bud[v]	SE	Percent germination[v]	SE	Viable conidia/bud[w]	SE
Dormant bud	27 March	A	0.4	0.1	*[x]	*	*	*
1-cm green[y]	28 April	A	1.9	0.6	74.0	5.3	1.4	0.5
Dormant bud	27 March	B	0.0	0.0	*	*	*	*
1-cm green	28 April	B	0.07	0.04	*	*	*	*
Dormant bud	22 and 27 March	C	25.1	16.4	21.8	12.4	5.7	2.0
Silver tip[z]	30 March and 4 April	C	3.1	1.8	40.9	8.7	1.4	1.1
1-cm green	28 April	C	141.5	121.5	88.8	1.6	117.0	99.4

[u] Buds were dissected, sonicated in water, and the number of viable and nonviable conidia were counted.
[v] Numbers represent the means and standard errors (SE) of three replicates of 100 buds each.
[w] Number of viable conidia determined by multiplying the total number of conidia by the proportion that germinated.
[x] Insufficient numbers of conidia observed.
[y] Stage of bud development when buds are swollen and budscales begin to open.
[z] Buds with approximately 1 cm of green tissue extending beyond the budscales.

excised from the fruitlet, and placed on a microscope slide to observe the presence of conidia. Ten fruitlets for each inoculation date, of about 1 cm diameter, were collected on 20 June 1989, and 24 fruitlets per treatment were collected on 11 June 1990 to determine the severity of scab on fruitlets. In 1989, conidia per fruitlet were determined by washing surfaces of fruitlets with a DeVilbiss Model 163 sprayer at 138 kPa. The final volume of wash water per fruitlet was adjusted to 1 ml, and the number of conidia was determined with a hemacytometer. In 1990, ratings were not conducted on the fruitlets because 95% of the uninoculated fruit had scab. In both years, uninoculated sepals and fruitlets were used as controls. The number of scab-infected cluster leaves that developed at the inoculation sites was recorded on 24 clusters per treatment on 16 May 1990. Analysis of variance using Minitab 6.0 (Minitab Inc., State College, PA) was used to evaluate differences between means for numbers of sepal, fruit, and foliage infections.

Meteorological analysis. Because conidia were detected within buds, temperature data at Geneva from October through April for 1987–1988, 1988–1989, and 1989–1990 were compared to the 82-yr averages (37) to search for atypical meteorological conditions that might have correlated with the detection of conidia.

RESULTS

Overwintering of conidia on infected leaves, fruit, and shoots. From October 1986 to May 1987, the mean number of conidia per square centimeter of scab lesion on leaves decreased from about 195 to 58 in the weather shelter and to 15 and eight on leaves in the tree and on the ground, respectively. The mean number of conidia per square centimeter on leaves was approximately 100 in October 1987 and decreased to approximately four the following May. The percentage of viable conidia decreased from approximately 35 and 28 in October 1986 and 1987, respectively, to zero by March. When pseudothecia were removed from leaves in May 1987, mature ascospores were observed only from leaves overwintered on the ground.

Two hundred ± 95 (standard error) conidia per square centimeter were initially detected in fruit lesions in October. That number increased to 640 ± 205 conidia per square centimeter in December then decreased steadily to near zero in March. Percent germination also increased from 12 ± 8% in October to 20 ± 6% in December, then decreased to 2% in March, and to zero percent in April.

Lesions on shoots from orchards A and C in July 1988 yielded an average of 191 ± 131 and 281 ± 90.8 conidia per lesion, and percent germination was 21 ± 7.2 and 47 ± 3.4, respectively. By October 1988, the number of conidia declined to 97.6 ± 29.7 and 9.8 ± 3.1 per lesion, respectively. Germination of these conidia was less than 7%. During April 1989, conidia were not observed in lesions from orchard C. However, 10 conidia were detected from one lesion in orchard A, of which five germinated. Shoot lesions initiated in 1989 yielded one viable conidium on 27 April 1990 from one lesion from orchard C, and no viable conidia from orchard A.

Viability of conidia associated with buds. The mean number of conidia per bud from samples collected in May 1988 ranged from 10.0 to 37.3; the number of viable conidia, as assessed by our cultural tests, ranged from 0 to 13.3 per bud. Viable conidia were detected in buds from all samples that were taken from orchards A and C in 1989 (Table 1). Only a few viable conidia were detected in orchard B from buds that had expanded in 1989 (Table 1), whereas none were detected in 1990. In 1989, the first infection period with ascospore discharge occurred on 29 April, which was 1 day after all samples in orchards A, B, and C were collected. Viable conidia were detected from each sample of McIntosh, Delicious, Rome Beauty, and RI Greening buds collected from orchard D (Table 2).

Distribution of conidia within buds. In 1989, an average of 0.02 ± 0.02 and 0.4 ± 0.3 viable conidia per bud were detected from the outer surface of intact buds and from the outer bud scales, which had been dissected from buds, whereas 2.1 ± 1.0 per bud were detected on the inner portions. In 1990, no viable conidia were detected from the outer surface of buds from orchards A and C. However, the outer bud scales that were dissected from buds yielded 34.3 ± 0.4 and 0.8 ± 0.4 viable conidia per bud, and 5.1 ± 0.4 and 7.1 ± 0.4 viable conidia per bud were detected from the inner bud portions.

Bud scale infections. Scab lesions were detected on the inner surfaces of 12 of 1,000 buds collected on 5 April 1990. Lesions were 1 to 2 mm in diameter and were observed on the adaxial surface of the second, third, or forth bud scale counting from the outside scale. Lesions were dark gray and developed only over the living portion of the bud scale. When infected bud scales were removed, hundreds of conidia remained on the immature leaf tissues adjacent to the

Table 2. Conidia of *Venturia inaequalis* isolated from flower buds of four apple cultivars collected from Wayne County, NY, in spring 1989

Cultivar / Factor	Sampling date: 7 April (silver tip)[t]	25 April (green tip)[u]	3 May (0.5-cm green)[v]	19 May (pink)[w]
McIntosh				
Conidia per bud[x]	18.5	37.3	20.7	1,054.0
SE	8.9	20.6	11.3	37.3
Percent germination[x]	77.8	59.1	87.8	87.9
SE	0.8	5.0	4.3	1.1
Viable conidia per bud[y]	14.3	29.3	19.8	927.5
SE	6.8	16.0	11.7	39.8
Rome Beauty				
Conidia per bud	5.3	0.6	77.4	32.6
SE	3.7	0.1	14.5	20.7
Percent germination	42.7	44.1	81.9	75.4
SE	21.1	6.1	2.0	4.4
Viable conidia per bud	1.5	0.3	63.9	24.9
SE	0.5	0.1	13.3	16.7
RI Greening				
Conidia per bud	*[z]	0.7	*	35.4
SE	*	0.1	*	14.3
Percent germination	*	44.4	*	41.8
SE	*	11.1	*	1.4
Viable conidia per bud	*	0.3	*	14.4
SE	*	0.3	*	5.3
Delicious				
Conidia per bud	*	0.8	*	2.6
SE	*	0.2	*	0.5
Percent germination	*	60.0	*	41.1
SE	*	11.7	*	5.5
Viable conidia per bud	*	0.5	*	1.1
SE	*	0.1	*	0.1

[t] Buds exposing silvery budscales.
[u] Buds exposing tips of green tissues.
[v] Buds with approximately 0.5 cm of green tissue extending beyond budscales.
[w] Blossoms separated from the cluster but before opening.
[x] Numbers represent means and standard error (SE) from three replicates of 100 flower buds.
[y] Number of viable conidia determined by multiplying the mean number of conidia per sample by the proportion germinated.
[z] Data not collected from these cultivars on this date.

lesion, and 76.8% of the conidia from the lesions germinated.

An even layer of *S. pomi* conidia were observed by SEM throughout the lesions (Fig. 1A). Annellophores typical of the genus *Spilocaea* covered the entire surface of the lesion, with up to 20 annellations on the tapering annellophores (Fig. 1B). At the margin of the lesions, ridges of raised cuticle were apparent with clusters of annellophores protruding through the crown of the ridge (Fig. 1C).

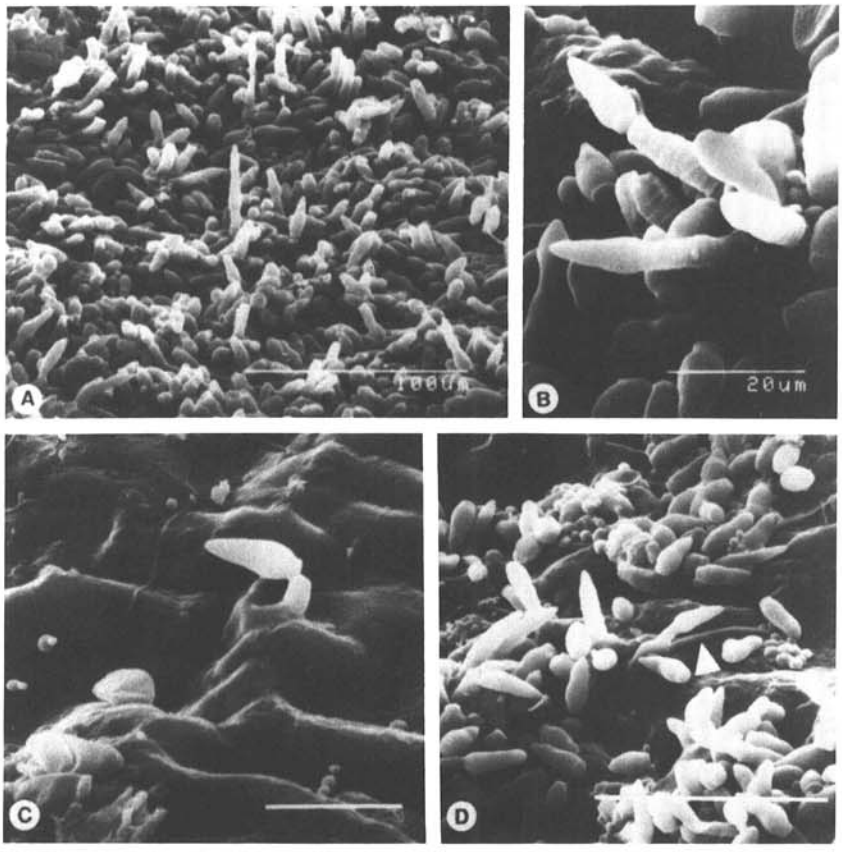

Fig. 1. Scanning electron micrographs of the adaxial surface of an apple bud scale infected with *Venturia inaequalis*. **(A)** Center of lesion showing the abundance of conidia with numerous annellophores. Scale bar = 100 μm. **(B)** Cluster of annellophores with mature conidia showing numerous annellations per conidiophore. Scale bar = 20 μm. **(C)** Margin of lesion showing swollen ridges with annellophores protruding through the cuticle. Scale bar = 20 μm. **(D)** Lesion showing hyphae growing along surface of the host (arrow), which is atypical compared with foliar infections. Scale bar = 50 μm.

Table 3. Apple scab infections on sepals, leaves, and fruitlets after inoculations with *Venturia inaequalis* at Geneva, NY, in spring 1989 and 1990

Inoculation stage[u]	Percent sepals with scab lesions[v] 1989	1990	Foliar infection[w] 1990	Percent fruit surface covered with scab[x] 1989	No. of conidia washed from fruit[x] (× 1,000) 1989
Green tip	46 a[y]	20 b	1.25 b	6.2 a	44.6 a
0.5- to 1-cm green	50 a	95 a	3.75 a	7.5 a	19.3 b
Tight cluster	*[z]	98 a	3.50 a	7.2 a	29.7 a
Uninoculated control	16 b	5 c	0.00 c	3.1 b	9.1 b

[u] The dates of inoculation for the respective phenological growth stages in 1989: 19 April for green tip, when green tissue just expanding beyond the budscales; 1 May for a late 1-cm green, when the first one to three leaves just separated from the bud, and 19 May for tight cluster, where the blossoms were visible, but not separated from the cluster. In 1990, green tip was on 9 April, 0.5-cm green was on 19 April, and tight cluster was on 24 April.

[v] Ten fruitlets were collected from each treatment after petal fall on 22 May 1989 and 24 fruitlets were collected 16 May 1990. Each of the five sepals per blossom was viewed microscopically for the presence of *V. inaequalis* conidia.

[w] Mean numbers of leaves per cluster observed with at least one scab lesion on 16 May 1990.

[x] Ten fruit (approximately 7 mm in diameter) harvested per treatment on 20 June. Fruitlets were rated for the percentage of the fruit surface covered with apple scab lesions. Mean numbers of conidia removed from fruit surface as determined with a hemacytometer.

[y] Numbers followed by a common letter do not differ significantly at $P = 0.05$.

[z] Data not collected from sepals on this date.

Mycelium was also observed growing above the surface of the lesion (Fig. 1D).

Inoculation of flower buds in the spring. In 1989, significantly greater incidence of sepal infections ($P = 0.05$) resulted from conidial inoculations at green tip and 1-cm green (Table 3) compared with the uninoculated controls. The percentage of the fruit surface area covered with lesions after the 19 April and 1 and 10 May inoculations was also significantly greater ($P = 0.05$) compared with the uninoculated controls. In 1989, the first foliar infections were observed on 22 May simultaneously on inoculated and uninoculated cluster leaves. In 1990, significantly greater incidence ($P = 0.001$) of sepal infections occurred from inoculations made at green-tip, 1-cm green, and tight-cluster stages of growth compared with the uninoculated controls. In 1990, foliar infections were first observed on 9 May on the margins of the small, outside cluster leaves. Observations on 16 May (Table 3) revealed that lesions had developed on some foliage on all clusters that had been inoculated from the green-tip to tight-cluster phenological stages. Lesions were not observed on leaves from the uninoculated controls until 28 May.

Meteorological observations. The mean monthly temperatures from October through May during 1987–1988, 1988–1989, and 1989–1990 were similar to or below the 82-yr average (2,37). The greatest divergence was December 1989, when the mean temperature was 7.3 C lower than average, making it the coldest December on record. The second greatest divergence was January 1990, when the temperature was 4.6 C higher than average.

DISCUSSION

We have demonstrated that conidia of *V. inaequalis* overwinter internally in apple buds and may be an important source of inoculum in New York State. Conidia were detected in lesions on the inner surface of bud scales and in apparently healthy buds in the absence of evident lesions. In previous reports, conidia were detected in lesions on the outer surface of bud scales (10,16,27,33), on the outer surface of buds (26), and in air samples monitored with spore traps before the detection of scab lesions (15). We were unable to detect any viable conidia in lesions on leaves and fruit that had overwintered in the orchard. Conidia that overwinter in buds may cause the development of scab when infection periods develop before the maturity of ascospores (12). They also may be important in dry seasons when ascospore discharges are few but long dews occur at night.

Sepals are among the earliest green tissues to expand from buds (17,19), and they are thus vulnerable to infections at the green-tip phenological stage. They

remain attached to the developing fruit throughout the season; therefore, if they become infected, they could provide an important source of conidia for secondary infections (19). Our inoculations at early phenological stages of growth mimicked the availability of conidia from lesions on bud scales and demonstrated that sepals could become infected as early as green tip.

Because viable and nonviable conidia and germlings were detected on the adaxial surface of the outer four bud scales in August (2), it is possible that some of the conidia detected during the summer on the surfaces of the buds could become entrapped between the developing bud scales. Sufficient space occurs between bud scales for an average sized scab conidium (approximately 7 × 20 μm) to become entrapped in that space after movement with wind or rain water. By determining when buds become infected or infested, the timing of fungicide applications could be modified to reduce the overwintering of conidia within buds.

Examination of lesions on the adaxial surface of bud scales from dormant buds with SEM revealed up to 20 annellations on many annellophores. One annellation corresponds to the production of one conidium (13), and in foliar lesions during the early summer, the holoblastic production of each conidium requires an average of 12 days (29). In 1990, the late winter and early spring temperatures in western New York were typical of the 82-yr average (37); temperatures were mostly −1.9 to 14 C, except for 5 days with high temperatures ranging from 20.0 to 27.8 C. Because such temperatures are not conducive for the production of the 20 successive sporulations observed by 5 April, we feel that lesions were actively sporulating during the previous summer or fall.

Our results indicated that the environment within apple buds is favorable for survival of the conidial state. Several other microorganisms are also known to survive within dormant apple buds. Greater populations of *Pseudomonas syringae* pv. *syringae* van Hall and *P. s.* pv. *papulans* (Rose) Dhanvantari were found on the inner bud scales of apple compared to the outer scales (5). Mycelium of *Podospherea leucotricha* (Ellis & Everh.) E. S. Salmon has been detected within apple buds (4,34), with colonization possible on the innermost bud scales (4). *Botryosphaeria obtusa* (Schwein.) Shoemaker, which causes black rot of apple, also overwinters in dormant buds (3). High numbers of epiphytic yeasts, filamentous fungi, and bacteria have been reported from the inner bud scales of deciduous fruit crops and ornamental trees (1,39).

Few or no conidia overwintered on the outer portions of dormant buds. Also, no conidia survived within scab lesions on overwintered leaves or fruit or on the exposed surfaces of shoots (2). Viable conidia were previously reported to overwinter in foliar lesions held in a weather shelter (17). In that study, infected leaves were placed in the shelter in July, when lesions would have had a high proportion of viable conidia and the leaves would have been green. No conidia survived in weather shelter in our study, possibly because the leaves were collected at leaf fall, when they were senescing, and when only 35% of the conidia in lesions were viable. Several factors may influence survival of conidia within foliar and fruit lesions, including senescence of the substrate, microclimate, increased competition from saprophytes, and parasitism by microorganisms. Conidia have been reported to overwinter on unwounded bark on shoots in Germany (18), but the cultivars and assays used were different from ours. Nonviable conidia have been detected on the surface of intact buds in South Africa (22). A study of the survival of conidia within foliar lesions showed that at low relative humidity (RH), only 2% of conidia germinated after 196 days and none germinated after at least 17 days at high RH followed by 33 days at low RH (21). These data suggest that conidia are unlikely to overwinter when exposed to fluctuating environmental conditions on the surface of apple tissues.

Apple scab infections on shoots previously have been reported in New York in the Hudson Valley in 1899 (35) and in Geneva in 1933 (11). They have also been reported in other states in the United States (7,8,20,30–32,38). Shoot infections were common in several orchards in NY in 1988 and 1989. These shoot lesions appeared to occur after long individual or excessive numbers of infection periods during the early summer (2). Reports of shoot scab predominate from regions with more moist climates such as the United Kingdom (25) and Ireland (27) and in the United States during wetter than average years (31,32). Only a few viable conidia were detected in overwintered shoot lesions. Therefore, we conclude that shoot lesions are not likely to provide a significant inoculum source in New York orchards.

The viable conidia detected in the overwintered buds were from orchards where moderate or high incidence of apple scab existed in the orchard during the previous season. Numbers of overwintering conidia vary from season to season, and detection is likely to be affected by sampling and detection methods. Three replicates of 100 buds were used to quantify overwintering conidia. However, because no conidia were detected in some of the replicates, a low proportion of buds may harbor a high proportion of the inoculum. This is supported by the occurrence of only 12 of 1,000 buds with macroscopic scab lesions. Successful overwintering may be affected by climatic conditions at certain periods during the growing season when buds are susceptible to infections. Apple buds infected with scab-conidia may also provide a means for pathogen dissemination in nursery trees.

Ascospores are typically detected in spore traps during the spring only when the ground surrounding the trap is seeded with scab-infected leaves, and the conidia detected overwintering in buds in this study were only from orchards that had substantial levels of scab the previous season. Further investigations concerning the relative significance of conidia in relation to ascospores as propagules for primary infections in commercial orchards with low levels of inoculum are, therefore, warranted.

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