Overwinter Survival of *Podosphaera clandestina* in Eastern Washington

Gary G. Grove and Robin J. Boal

Assistant plant pathologist and research technologist, respectively, Washington State University Tree Fruit Research and Extension Center, 1100 N. Western Avenue, Wenatchee 98801.

Plant Pathology New Series no. 0062, Project 0795, Washington State University Agricultural Research Center, Pullman 99164.
We gratefully acknowledge the funding of this project by the Washington State Tree Fruit Research Commission. We thank Nancy Roberts for her excellent technical assistance.
Accepted for publication on 30 September 1990 (submitted for electronic processing).

ABSTRACT

Grove, G. G., and Boal, R. J. 1991. Overwinter survival of *Podosphaera clandestina* in eastern Washington. Phytopathology 81:385-391.

Podosphaera clandestina survived winter as cleistothecia on senescent cherry leaves on the orchard floor or trapped in tree crotches, as cleistothecia in partially decomposed leaves trapped in tree crotches, or as cleistothecia trapped in bark crevices. Ascocarp viability ranged from 55–90% in early February to 5–33% in mid-May. Inoculation of cherry seedlings with overwintered cleistothecia collected from early March through mid-May resulted in infection, with the largest number of mildew colonies resulting from inoculation with cleistothecia that were collected around bud burst. Ascospore release began 1 mo before bud burst and continued until after the bloom period. Trapping of ascospores and cleistothecia from the orchard air preceded the initial appearance of mildew colonies. Results of orchard surveys and experiments with dormant buds failed to provide evidence that *P. clandestina* survived winter as mycelium in dormant infected buds. Cleistothecia appear to be the principal source of primary inoculum for epidemics of sweet cherry powdery mildew in eastern Washington.

Epidemics of powdery mildew of sweet cherry (*Prunus avium* L.) have occurred in eastern Washington in recent years. The fungus that causes the disease in Washington fits the description of *Podosphaera clandestina* (Wallr.:Fr.) Lév. (1,21,24). In 1888, Galloway (10) provided the first report of the disease on cherry foliage in North America; in 1947, English (8) provided the first account of the disease on sweet cherry in Washington and the first report of fruit infection. Occurrences of the disease on sweet or tart (*Prunus cerasus* L.) cherries have been documented previously in several fruit-growing regions of the United States (5,10,12,15-17,20,26,28,29,32) and Canada (6).

In eastern Washington, foliar infections are first observed in late April to mid-May (G. G. Grove, *unpublished*), approximately 4–6 wk after bud break. Incidence and severity of foliar symptoms generally increase throughout the months of May and June.

© 1991 The American Phytopathological Society

Developing mildew colonies are a potential source of secondary inoculum for fruit infections that are hastened by rain showers during the latter stages of fruit development (8). In 1986 and 1987, rain showers occurred in mid-June, resulting in high incidences of fruit infection. Many Washington producers reported that entire crops were rejected for fresh-market sale because of fruit infection by *P. clandestina*. Foliar infection continues throughout the summer resulting in the infection of nearly all terminal shoots. As the mildew colonies age, numerous cleistothecia of the fungus are formed (8,10). Galloway (10) stated that cleistothecia carry the fungus over the winter, but failed to provide any evidence to support that contention. Sprague (27) contended, without evidence, that perennation occurred as mycelium in dormant, infected buds of sweet or wild cherry (*Prunus virginiana* L.), and that the initial appearance of mildew colonies 3–4 wk after bud burst was attributable to infection by spores that originated on infected wild cherries in close proximity to orchards. Other workers have speculated on the mode of overwintering (3,13,17,32), but the overwintering mode or propagule of the fungus has not been demonstrated conclusively.

The purposes of this study were to investigate the perennation of *P. clandestina* in dormant sweet cherry buds, to determine the significance of cleistothecia as a primary inoculum source in the epidemiology of sweet cherry powdery mildew, and to determine when primary infection occurs.

MATERIALS AND METHODS

Orchard descriptions. An 11.2-ha orchard composed of 40-yr-old sweet cherry (cv. Bing) trees located near Malaga, WA, was used throughout the study. During the 1989 growing season, a 0.8-ha block of 35-yr-old sweet cherry (cv. Bing) trees located near Orondo, WA, also was used for a portion of the study. Epidemics of cherry powdery mildew occurred at both sites in 1986, 1987, and 1988.

Ascospore viability assessment methods. In April 1987, senescent leaves were collected from both orchard floors and observed microscopically for the presence of cleistothecia. Ten leaves with abundant cleistothecia were chosen from each sample. Each leaf was hand-macerated, placed in a plastic centrifuge tube that contained 15 ml of sterile distilled water, and vortexed at high speed for 60 sec. Suspensions were then combined in a flask, mixed by shaking for 60 sec, and filtered in 15-ml aliquots over 10-cm-diameter filter paper disks. Cleistothecia were placed into several drops of europium chelate differential fluorescent stain (4,14,25) on glass slides and were gently crushed. Ascospore viability was assessed immediately at 160 and 400× by phase-contrast microscopy with incandescent light. A visual assessment method similar to that used by Pearson and Gadoury (22) was used for assessment of cleistothecial contents. Ascospores were categorized as viable if they were elliptical or subreniform in shape and contained light-colored cytoplasm composed of fine granules (Fig. 1A), vacuoles, or small, spherical lipid globules (Fig. 1B). Ascospores were considered degenerate if they were misshapen with dark-yellow or amber cytoplasm (Fig. 1C). After 10 min, ascospores were observed under epifluorescent light at 250×. A Zeiss microscope with a UV light source, dichroic excitation filters (Zeiss equipment 46 78 85, 46 78 88), and barrier filters (Zeiss equipment 47,53) was used to view fluorescing ascospores. Ascospores that fluoresced orange were considered viable, while those that fluoresced blue or blue-green were considered degenerate. In April 1990, senescent leaves were collected from the floor of the Malaga orchard and the cleistothecia from 10 leaves were harvested onto filter paper disks, as previously described but with slight modifications; washings from each leaf were filtered individually over single filter paper disks. Cleistothecia harvested from five leaves were placed in sterile distilled water on glass slides. Viability was determined with the visual assessment method that incorporates an ascocarp shape criterion (9). Cleistothecia from the second five-leaf subsample were placed into europium chelate and viability was assessed by the visual assessment method. After 10 min, viability was assessed by epifluoresence microscopy. Ascocarp shape was not used as a viability index in the latter method because the stain was dissolved in ethanol. When the visual assessment method and shape criteria were used, ascocarps were considered degenerate if they remained concavo-convex in water and contained misshapen, shrunken ascospores with dark-yellow cytoplasm (9). In a third study, cleistothecia were harvested from infected living

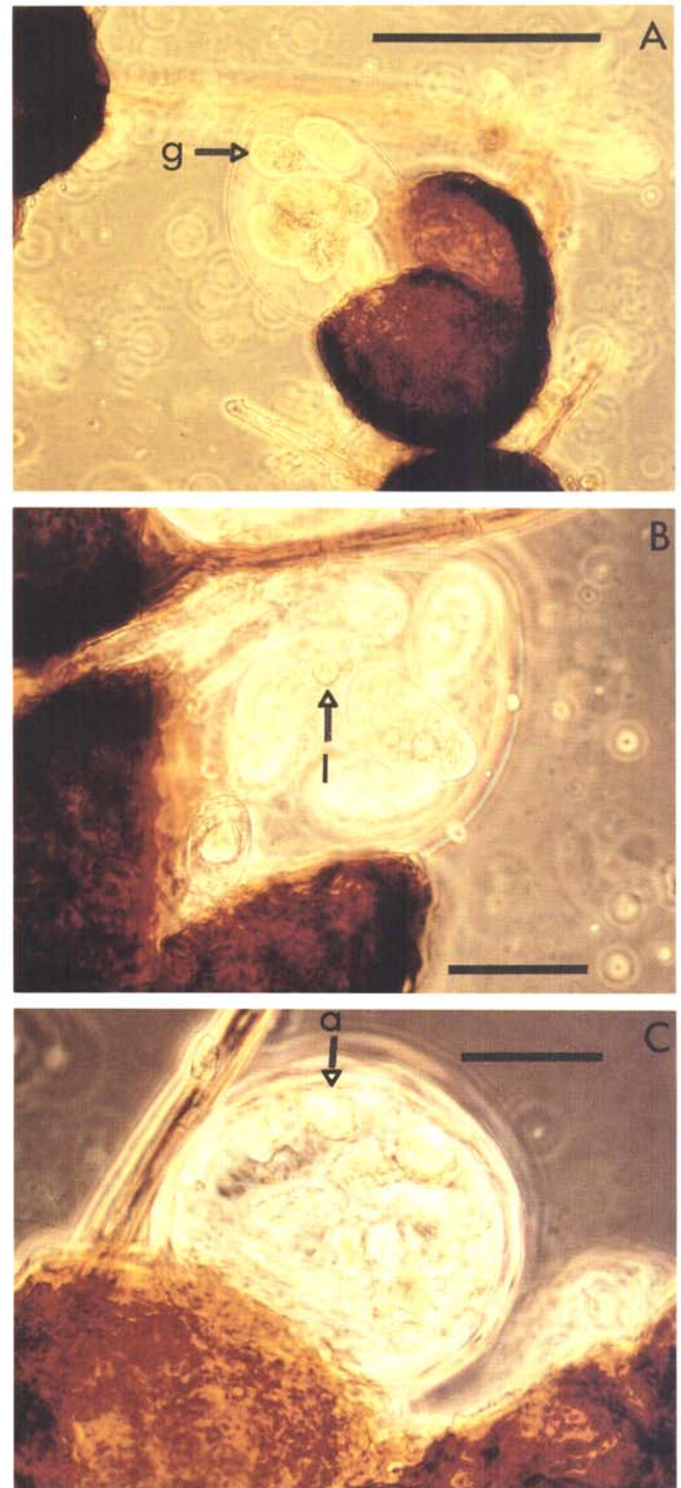

Fig. 1. Viable (**A,B**) and degenerate (**C**) cleistothecia of *Podosphaera clandestina* harvested from sweet cherry leaves. Cleistothecia with asci that contain light-colored granular (**A**) or vacuolate ascospores, or light-colored ascospores with small, lipid aggregations (**B**) were considered viable, while those with misshapen, dark-yellow ascospores (**C**) were considered abortive or degenerate. Bars (**A, B, C**) represent 75, 25, and 25 μm, respectively.

leaves collected from trees at the Orondo orchard in mid-July 1990. Leaves were examined at 40× for the presence of cleistothecia. Ten leaves bearing numerous cleistothecia were placed in 500 ml of distilled water and shaken vigorously for 1 min. Cleistothecia in the resultant suspension were harvested onto filter paper and transferred to europium chelate as was described. Cleistothecia were ruptured with pressure sufficient to cause the asci to protrude from the ascocarps, but insufficient for ascospore release from the asci. In preliminary vital-staining experiments with young nonoverwintered cleistothecia, many ascospores that had released from the asci were observed to burst soon after release (G. G. Grove, *unpublished*). Ascocarp viability was assessed immediately with the visual assessment method and, after 10 min, assessed with epifluoresence microscopy.

Senescent, infected leaves were collected from the floor of the Malaga orchard in April 1988. Leaves were examined at 40× for the presence of cleistothecia. Fifty leaves with abundant cleistothecia were selected from the sample, were air-dried, and then were comminuted (while dry) for 2 min at high speed in a Waring blender. Leaf powder was then transferred to a polyethylene bag and shaken vigorously by hand for 5 min. Five grams of leaf powder was then suspended in 125 ml of sterile distilled water and vortexed at high speed for 20 sec. The suspension was poured over nestled 0.047- and 0.120-mm sieves, and washed 5 min with tap water. Debris collected on the large sieve was discarded and debris on the smaller sieve was backwashed into a beaker with 1,500 ml of sterile distilled water. Four 30-ml aliquots were filtered over 7-cm-diameter filter paper disks. Each filter paper disk was attached to the inside of a glass petri plate bottom with a layer of silicon grease, remoistened with sterile distilled water from a surgeon's atomizer, and inverted over a single 30- × 15-cm glass slide in a petri plate top. Petri plates were sealed in a polyethylene bag, placed in an incubator in darkness, and incubated at 25 C for 24 hr. After incubation, the number of ascospores and the proportion of those germinating were recorded.

Seasonal ascospore viability. In 1988, senescent, infected leaves were collected from the orchard floor and tree crotches at the Malaga site from January through mid-May and were examined for cleistothecia. Cleistothecia were harvested onto filter paper, transferred to sterile distilled water on glass slides, crushed, and their viability determined by the visual assessment method as previously described. In October 1988, senescent leaves bearing numerous cleistothecia were collected from the orchard floors of the Malaga and Orondo orchards. Leaves from the Malaga orchard were stored over the winter on the orchard floor in a 1-m^3 wire mesh cage, while those from the Orondo orchard were stored in a similar fashion at the Tree Fruit Research and Extension Center (TFREC), Wenatchee, WA. Ten leaves were removed at random from the Malaga orchard cage in mid-February through mid-May and from the TFREC cage in early February through late April. In addition, 10 senescent leaves were collected from tree crotches at the Malaga site from late January through mid-May. Cleistothecia were examined and their viability determined as previously described. The proportion of cleistothecia with viable ascospores at each sampling date was subjected to analysis of variance with Minitab Data Analysis Software (Minitab Inc., State College, PA).

Detritus trapped in tree crotches at the Malaga site was sampled during the springs of 1988 and 1989. Samples from 10 trees were combined in a polyethylene bag, mixed by vigorous shaking for 60 sec, and then air-dried for 12 hr on a laboratory bench. Ten grams of the detritus was suspended in 300 ml of sterile distilled water, comminuted for 30 sec at high speed in a Waring blender, poured over nestled 0.047- and 0.120-mm sieves, and washed for 3 min with tap water. Debris on the larger sieve was discarded, while that on the smaller sieve was washed an additional 1 min with running tap water and then backwashed into a flask with 180 ml of sterile distilled water. Cleistothecia were collected by filtering 30-ml aliquots through separate 7-cm-diameter filter paper disks. Viability of cleistothecia was determined as described previously.

The main trunks of five trees were sampled for cleistothecia during the springs of 1988 and 1989. A roughly triangular open funnel was prepared from 0.025 mm of aluminum sheeting. A semicircular notch was cut in the broad end of the funnel, so that the aluminum would fit snugly against the trunk. The aluminum was then creased longitudinally to form a path for water flow. The notched end of the funnel was placed against the trunks, about 0.5 m above the soil surface, and the opposite end tilted downward at a 45° angle terminating immediately above nestled 0.120- and 0.047-mm sieves. The trunk area, 0–0.5 m above the point of contact with the funnel, was washed with 2 L of tap water. Debris collected on the smaller sieve was then washed for 2 min with tap water, backwashed into a flask with 250 ml of sterile distilled water, and filtered in 30-ml aliquots onto 7-cm-diameter filter paper disks. The viability of cleistothecia was determined by using the visual assessment method as previously described.

Seedling inoculations. Ascospore viability was verified by inoculating cherry seedlings (cv. Bing × Van). Seed was incubated in sterile, moist sand at 4 C in darkness for 5 mo. Stratified seed was germinated in a loam/sand/peat mixture (1:1:1, v:v:v) at 20 C in a 16-hr photoperiod. Ten-day-old seedlings with adherent cotyledons and one to three leaves were used in all inoculations. About 1 hr before inoculation, seedlings were planted in a 10-cm-layer of moist steam-disinfested vermiculite in chromatography jars (1988) or in 1.1-L Mason jars (1988 and 1989). Cleistothecia were collected in March, April, and May from leaves or crotch detritus and placed on 7-cm-diameter filter paper disks as described earlier. The disks, with the side bearing cleistothecia facing outward, were attached to jar covers that had been coated with a layer of silicone grease. Each cover was placed over a jar that contained several seedlings. Seedlings not exposed to cleistothecia served as a control. At least eight seedlings were inoculated at each sampling date. Seedlings were incubated 10 days at 20 C in a 16-hr photoperiod. The number of mildew colonies per leaf was determined by visual inspection. In addition, epidermal peels were taken from the seedling leaves by using the collodion epidermal peel technique (7,31). Collodion peels were mounted in lactophenol on glass slides and observed for ascospore germination.

Ascospore release. During the springs of 1988 and 1989, senescent leaves were periodically collected from the Malaga orchard floor. Leaves were air-dried for 24 hr on a laboratory bench and then soaked in sterile distilled water for 8 hr. Each leaf was secured to the inside of a glass petri plate bottom with a layer of silicone grease and then was inverted over a single Stickem-coated (Seabright Enterprises, Ltd., Emeryville, CA) glass slide in a petri plate top. Leaves were incubated for 16 hr at 20 C in darkness. Slides were then coated with lactophenol, covered with coverslips, and observed for ascospores. The numbers of ascospores and cleistothecia were recorded. The number of ascospores released per 100 cleistothecia was determined. Ratios for each sampling date were statistically analyzed as described.

Spore-trapping studies. A Burkhard volumetric spore trap (Burkhard Mfg., Hertfordshire, UK) was placed in the Malaga orchard in March 1988 and 1989 and was operated continuously throughout both growing seasons. The trap was adjusted to sample 9.0 L of air per minute. Impaction tapes were retrieved at 7-day intervals and examined for ascospores and conidia of *P. clandestina*. Thermistors (Fenwall Electronics, Ashland, MA), printed-circuit leaf wetness sensors (Wong Labs, Cincinnati, OH), and relative humidity sensors (Phys-Chem Sensor, Phys-Chemical Research Co., New York, NY), connected to a CR-21 Datalogger (CR-21, Campbell Scientific, Logan, UT) located 2 m from the trap provided hourly records of environmental conditions. During the 1989 growing season, identical spore-trapping studies were also conducted in the Orondo orchard.

Perennation in dormant buds. In 1987 and 1988, 10 orchards were surveyed during late April and early May for evidence of overwintering in buds. Initially, orchards were surveyed for "flag" shoots similar to those of apple infected by *Podosphaera*

leucotricha (2,33). Floral parts of about 10 flowers per shoot and primary and secondary leaves (about 10 leaves per shoot) on 20 shoots of each of 25 trees at each location were examined with a hand lens for symptoms and signs of infection by *P. clandestina*. Each orchard was surveyed again at harvest for the presence of powdery mildew.

In late March 1987, 100 samples each of dormant, apparently healthy shoots, and dormant, blighted shoots were collected at random from throughout the Malaga orchard. Shoots were sectioned to 20-cm lengths, surface-disinfested for 30 sec in 0.525% NaOCl, rinsed twice with sterile distilled water, and individually placed in separate sterile-glass test tubes that contained 10 ml of sterile distilled water. Samples were covered with cheesecloth and incubated 3 wk at 20 C in a 16-hr photoperiod. Cheesecloth covers were kept moist with periodic applications of sterile distilled water. Emergent plant tissue was examined with a dissecting microscope for symptoms and signs of infection by *P. clandestina*. Representative young leaves, floral parts, and bud scales were removed from each respective bud with flame-disinfested forceps, cleared for 48 hr in acetic acid/ethanol (1:1, v:v) (30), mounted in lactophenol on glass slides, and observed at 160 and 400× for internal mycelium. This experiment was repeated with shoots collected in the same orchard in March 1988.

Overwintering of *P. clandestina* in dormant buds was examined under field conditions in 1987 and 1988. In March 1987, 100 stem apices each of dormant, apparently healthy shoots and of diseased dormant shoots with necrotic stem apices were tagged. Shoots were washed with tap water applied with a hand-sprayer. The upper 20 cm of 50 of each shoot type was covered with pieces of 5 μm of mesh Nitex cloth (Tekto, Inc., Ashland, MA) that were secured with an aluminum tag wire. The remaining shoots were left uncovered. Ten shoots were randomly harvested at bud burst, 20% bloom, full bloom, shuck fall, and full leaf. Floral parts and primary and secondary leaves were observed for symptoms and signs of powdery mildew. The experiment was repeated with slight modifications in 1988; shoots were sampled at full bloom and full leaf.

Dormant buds of nursery stock. In October 1987, 20 2-yr-old sweet cherry (cv. Bing) trees with symptoms of powdery mildew were obtained from a nursery near Quincy, WA. Trees were about 2.5-m tall, had served as untreated controls in an earlier fungicide trial, and had an average of 20 mildew colonies per leaf (11). Because disease pressure was high, the trees were selected for controlled-environment studies of perennation in dormant buds. Trees were totally stripped of senescent leaves and stored in a cold room at 4 C. Leaves were examined for the presence of cleistothecia, and those with cleistothecia were stored outside in a 2-m^2 wire mesh cage at TFREC.

In late February 1988, the trees were removed from storage, sprayed with tap water, and planted in a silt loam/sand/peat mixture (1:1:1, v:v:v). Four buds on each tree were individually covered with four layers of 5 μm of mesh Nitex cloth. Trees were placed in a 5.5-m^2 controlled-environment room, positioned in a circle around a Burkhard volumetric spore trap, and incubated for 28 days at 20 C in a 16-hr photoperiod. Relative humidity in the room was maintained between 75 and 90% with mist from a room humidifier. A CR-21 Datalogger provided hourly measurements of leaf wetness, temperature, and relative humidity. The spore trap was adjusted to sample 10 L of air per minute. Spore-trap impaction tapes were prepared for microscopic examination as previously described and were observed for propagules of *P. clandestina*. Leaves were examined daily for 23 days after bud break for symptoms and signs of powdery mildew.

RESULTS

Ascospore viability. A total of 56 and 49% of cleistothecia examined from Malaga and Orondo orchards, respectively, contained viable ascospores according to the visual assessment method. Of those that appeared viable in the first sample, the ascospores from all but two failed to fluoresce orange. Of the 55 cleistothecia determined as viable by the visual assessment method from the second orchard, 52 contained ascospores that fluoresced orange. Ascospores with light-colored granular cytoplasm, and those with light-colored cytoplasm that contained small, lipid globules fluoresced orange. Misshapen, dark-yellow ascospores, the ascus wall, and cleistothecia appendages fluoresced blue or blue-green. In the second study, viability in the subsample assessed according to ascocarp shape and visual assessment method ranged from 24.3 to 88.4%. Viability in the subsample assessed first by the visual assessment method and then by epifluorescent light ranged from 29.3 to 88.6%. Little difference was found when ascocarp viability was assessed by the visual assessment method, the visual assessment method and ascocarp shape, or the appearance of stained ascospores under epifluoresent light. In the third study, 64% of cleistothecia contained ascospores; ascospore cytoplasm appeared finely granular with the visual assessment method and appeared orange under epifluorescent light. Because there was little difference in viability assessments among the different methods tested, the visual assessment method was accepted as a reasonably accurate means of assessing ascospore viability.

An average of 8.0, 3.6, 24.0, and 25.9% (mean = 15.4%) of the ascospores on four respective glass slides germinated. The cytoplasm of all ascospores contained small, spherical lipid globules. There were no apparent differences in the cytoplasm of germinated and nongerminated ascospores.

Viable cleistothecia were harvested from senescent leaves collected from the orchard floor and tree crotches, from detritus in tree crotches, and from trunk washings (Figs. 2 and 3). In general, the viability of cleistothecia harvested from senescent leaves ranged from 55–90% in early February to 5–10% in mid-May. Abundant cleistothecia were also present in detritus trapped in tree crotches; viability ranged from 55% in mid-February to 12% in mid-May. Sixteen percent of the cleistothecia retrieved from the combined washings of the main trunks washed in mid-May, 1988, contained viable ascospores; in 1989, viability declined from 58% in mid-March to 33.3% in mid-May.

Ascospore release. In 1988 and 1989, ascospore release was first detected in early March and continued until mid-May (Fig. 4). The highest ascospore per 100 cleistothecia ratios occurred at the end of March. The majority of ascospores were released between mid-March and mid-April.

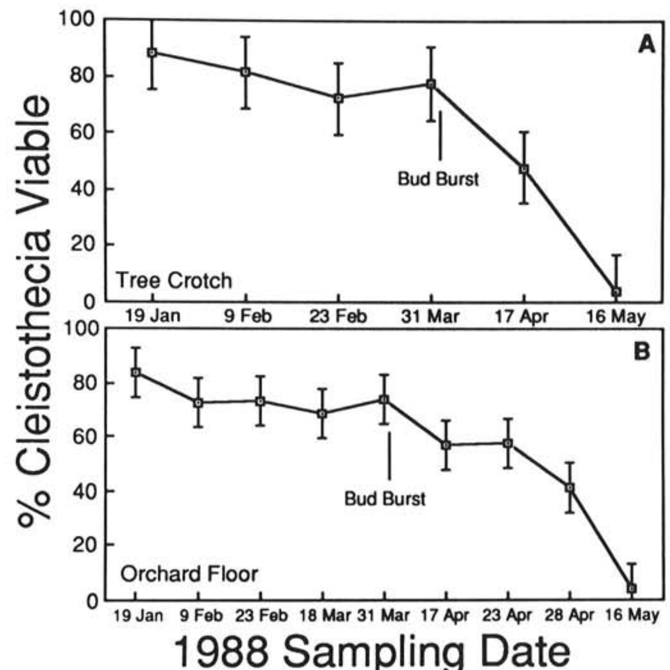

Fig. 2. Viability of cleistothecia of *Podosphaera clandestina* on leaves of sweet cherry overwintered in tree crotches (**A**) or on the orchard floor (**B**) in Malaga, WA, in 1988. Bars represent standard error of mean.

Spore-trapping studies. Thirty-one ascospores were trapped during late March–April 1988. Two ascospores were trapped during a rain period at bud burst on 30 March, while the rest were trapped 18–19 days later, during a 48-hr period of rain. Powdery mildew colonies were first observed on 28 April and were evident on leaves of several groups of trunk sprouts, or on leaves that originated from and were positioned close to scaffold branches or those positioned near tree crotches. Conidia were first trapped from the orchard air on 6 May. Cleistothecia that contained viable ascospores were trapped during April and again in late August.

In 1989, 23 and 37 ascospores were trapped at the Malaga and Orondo orchards, respectively. At the respective orchards, spores were trapped 0, 3, 6, 15–17 and 10, 14, 17–18 days after bud burst. Ascospores were trapped during periods of rain. The first powdery mildew colonies were found in the orchard on 28 April and 10 May in the Malaga and Orondo orchards, respectively. The distribution of primary mildew colonies was similar to that observed in 1988. Conidia of *P. clandestina* were first detected in early May in both orchards. Cleistothecia with viable ascospores were trapped in April and again in late August in both orchards.

Seedling inoculations. Seedlings inoculated with cleistothecia collected from leaves on the orchard floor, tree crotches, or isolated from crotch detritus developed symptoms and signs of powdery mildew (Table 1). In general, the number of mildew colonies per leaf increased from late winter to early spring, then gradually decreased. Powdery mildew did not develop on any uninoculated controls. Several germinating ascospores were observed on collodion peels of the surfaces of seedling leaves inoculated with overwintered cleistothecia. Although the collodion membrane severely limited microscopic resolution, the cytoplasm of germinated ascospores appeared to contain small, spherical lipid globules.

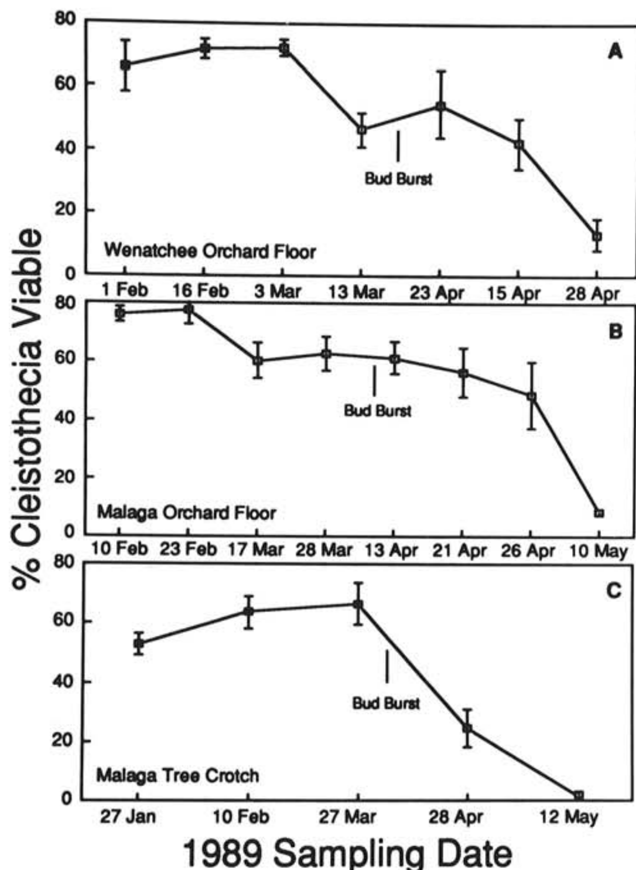

Fig. 3. Viability of cleistothecia of *Podosphaera clandestina* on leaves of sweet cherry overwintered on an orchard floor in Wenatchee (**A**) or Malaga (**B**), and in tree crotches in Malaga, WA (**C**), in 1989. Bars represent standard error mean.

Perennation in dormant buds. "Flag" shoots or other evidence of perennation of *P. clandestina* in dormant buds were not observed. The conidial state was not observed in any of the orchards until about 1 mo after bud burst (early May or late April 1987 and 1988, respectively). Primary colonies of *P. clandestina* were infrequent and generally observed on lower leaves of sucker shoots, on leaves from the main scaffold branches and positioned close to bark, or those positioned immediately above tree crotches. At harvest, foliar and fruit mildew was prevalent in all survey orchards in 1987. In 1988, foliar mildew was prevalent in all orchards. Fruit infections were not observed in 1988.

In laboratory experiments, bud swell failed to occur on twigs blighted by *P. clandestina* during the previous season. On shoots that appeared healthy when collected, symptoms and signs of infection were absent from unfolding leaves, floral parts, and bud scales. Microscopic examination of cleared plant tissues failed to reveal any evidence of infection by the fungus.

In orchard experiments, bud burst failed to occur on shoots with necrotic stem apices. Plant tissue covered with Nitex cloth remained free of powdery mildew. Uncovered shoots were free of mildew until 15 May 1987. Powdery mildew did not develop on shoots in 1988.

Dormant buds of nursery stock. Powdery mildew failed to develop on covered and uncovered leaves during the initial 28-day incubation period. However, several mildew colonies appeared on uncovered leaves 11 days after inoculation with overwintered cleistothecia. The first colonies to appear were located at the shoot apices near the inoculum source. Powdery mildew symptoms and signs were absent on all shoots covered with Nitex cloth. Propagules of *P. clandestina* were not trapped by the spore trap during the first 28 days of the experiment. Five, three, one, one, one, one, and one ascospores were trapped during days 1, 3, 4, 5, 8, 13, and 19, respectively, after introduction of cleistothecia into the controlled environment room. Conidia of *P. clandestina* were trapped on days 17–20.

DISCUSSION

Cleistothecia are the principal source of primary inoculum for epidemics of powdery mildew of sweet cherry in eastern

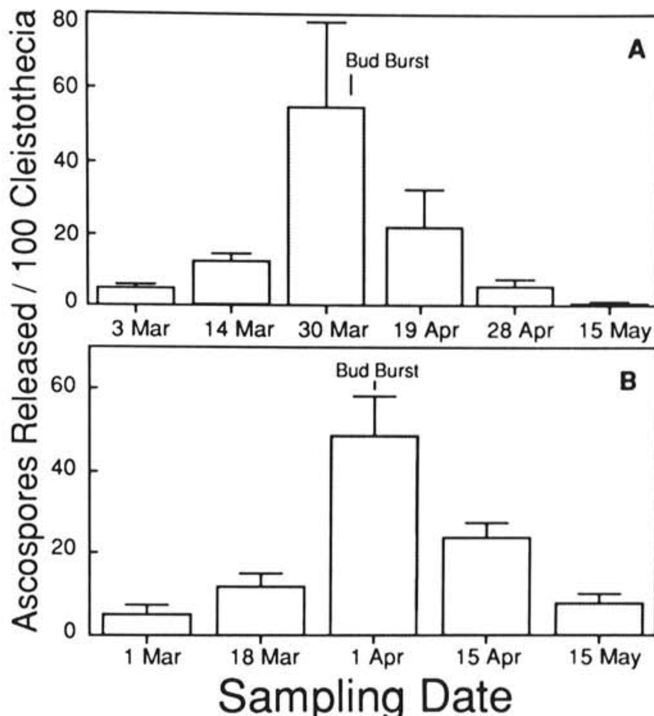

Fig. 4. Release of ascospores from cleistothecia of *Podosphaera clandestina* harvested from sweet cherry leaves overwintered on the orchard floor in Malaga, WA, in 1988 (**A**) and 1989 (**B**). Bars represent standard error of mean.

Washington. Numerous overwintered cleistothecia with nondegenerate ascospores were found in all survey orchards 1 wk before bud burst. This is in contrast with the findings of Khairi and Preece (18) who demonstrated that cleistothecia of *P. clandestina* are insignificant in the epidemiology of hawthorne (*Crataegus monogyna* Jaeq.) powdery mildew in England. Inspection of the same orchards several weeks later failed to provide any evidence of perennation of the fungus in infected buds. When powdery mildew appeared 4–5 wk after bud burst, the distribution of primary mildew colonies on tree foliage was similar to that reported for ascosporic infection of grapevines by *Uncinula necator* (22). Laboratory and field experiments with dormant shoots and experiments with nursery stock heavily infested with powdery mildew during the previous season failed to provide any evidence of perennation in infected dormant buds.

Relatively high proportions of cleistothecia contained viable ascospores. During both years of the study, however, viability declined rapidly after bloom. Unlike *Uncinula necator* on senescent grape leaves (9,22), large numbers of cleistothecia survived the winter on senescent leaves from the ground or from those trapped in tree crotches. Overwintered cleistothecia were also isolated from tree crotch detritus and bark fissures as late as May during both years of the study. Although cleistothecia of *P. clandestina* survived in various locations within the orchard, the winters in which this study was conducted were both about 5.0 cm below normal in precipitation, and about 2 C warmer than average. Perhaps survival in protected positions such as tree crotches and bark fissures would be more important under different meteorological conditions.

TABLE 1. Ascosporic infection of sweet cherry seedlings after inoculation with overwintered cleistothecia of *Podosphaera clandestina*

Collection date	Source[a]	Seedlings infected (%)	Total number of mildew colonies[b]	Mean number of colonies per leaf[c]
1988				
03-08	OF	45.5	63	3.3
03-18	OF	70.0	76	4.5
04-07	OF	92.3	91	7.0
04-17	OF	80.0	42	4.2
04-23	OF	44.4	20	2.2
05-06	OF	11.1	2	0.2
03-08	TC	64.3	47	3.4
03-18	TC	92.3	41	3.2
04-07	TC	100.0	31	6.2
04-17	TC	100.0	29	4.1
04-23	TC	70.0	22	2.2
05-06	TC	20.0	14	1.4
05-16	TC	10.0	12	1.2
04-17	CD	87.5	10	0.6
05-06	CD	12.5	6	0.8
05-16	CD	11.1	5	0.6
1989				
02-23	OF	16.6	12	0.3
03-17	OF	60.0	36	1.4
03-23	OF	85.7	132	3.2
03-30	OF	85.7	102	2.4
04-13	OF	80.0	57	3.9
04-28	OF	17.6	45	0.7
05-05	OF	20.0	21	0.7
02-14	TC	22.0	6	0.2
02-22	TC	25.0	9	0.4
03-13	TC	60.0	18	0.4
03-23	TC	75.0	81	1.4
04-13	TC	33.0	12	0.5
04-28	TC	33.0	21	0.7

[a]Seedlings were inoculated with overwintered cleistothecia collected periodically from the orchard floor (OF), from tree crotches (TC), or crotch detritus (CD) in a Malaga, WA, sweet cherry orchard, winters and springs of 1988 and 1989.

[b]Total number of mildew colonies present on seedlings at that sampling date.

[c]Total number of mildew colonies divided by the total number of leaves exposed to cleistothecia.

As confirmed by seedling inoculations, functional primary inoculum was present in the orchard from mid-February through mid-May. This corresponded to a host phenological period of 4–6 wk before bud burst to 4–5 wk after the initial appearance of host tissue. Colony per leaf ratios were highest between late-March and mid-April, corresponding to a host phenological period from immediately before bud burst to the bloom period. However, quantitative conclusions about infection efficiency (9,22) were not determined because the total number of cleistothecia used in the inoculations was not determined nor were the seedlings genetically uniform.

Ascospores of *P. clandestina* were trapped from the air of one orchard in 1988 and from two orchards in 1989. In two orchards, propagules were first trapped at bud burst and were trapped intermittently for several weeks, while in the third orchard ascospores were trapped only during the bloom period. Relatively few ascospores were trapped from the orchard air. The low recovery was probably attributable to the airborne inoculum dose that was below the 10 spores per cubic meter air-trapping threshold of the volumetric spore trap. Ascospores were trapped during periods of rain and trapped in the highest numbers during the bloom period. The trapping of ascospores preceded the appearance of primary mildew colonies. The first conidia of *P. clandestina* were trapped 5–8 days after the first appearance of mildew colonies. The chronological sequence of ascospore trapping, appearance of primary mildew, and the subsequent trapping of conidia further implicate ascospores as the principal primary inoculum form.

Cleistothecia recovered from bark surfaces and bark fissures originated from infected leaves. The deposition of cleistothecia away from points of initiation and development has been previously reported (9,19,21,34) and their dispersal attributed to wind (19) or water (9,19,34). Cleistothecia of *P. clandestina* were trapped from the orchard air in April and again in August. Those trapped during the primary infection period had probably developed on mildew colonies during the previous growing season and were wind-dispersed from senescent leaves or redistributed from bark or crotch detritus. Because the ascocarps trapped during August contained viable ascospores, they probably formed on mildew colonies earlier in the growing season. We have also observed viable overwintered cleistothecia on young, healthy leaves immediately after an air-blast fungicide application during April (*unpublished*). This raises interesting questions about wind as a dispersal and deposition agent of cleistothecia as well as epidemiological questions about ascospore release and deposition. The chances of ascospores reaching susceptible host tissue would be much higher were the spores released in close proximity to the leaf surface as opposed to being released at the orchard floor and wind-dispersed to host tissue.

The presence of primary inoculum in the orchard air before the initial calendar application of sulfur raises questions regarding the efficacy of earlier fungicide applications. The initial fungicide application in eastern Washington has typically been made at the onset of secondary inoculum production. In years when disease pressure is high, this fails to provide adequate control of the disease. Perhaps an earlier application of sulfur or other fungicides during the primary infection periods would provide better control of the disease by preventing primary infection and thus possibly delaying the onset of epidemics.

A functional sexual stage of *P. clandestina* has profound implications for future management strategies of cherry mildew. The increased potential for genetic variation (23) is of significance to breeders who develop mildew-resistant cultivars or to the producers who use benzimidazole or ergosterol-biosynthesis inhibiting fungicides. Management practices to delay the formation of fungicide-resistant strains of *P. clandestina* should be encouraged.

LITERATURE CITED

1. Braun, U. 1987. A monograph of the Erysiphales. Beih. Nova Hedwigia 89:1-700.
2. Burchill, R. T. 1958. Observations on the mode of perennation of

apple mildew. Rep. Agric. Hortic. Res. Stn. Bristol 1957:114-123.
3. Burchill, R. T. 1978. Powdery mildews of tree crops. Pages 473-493 in: The Powdery Mildews. D. M. Spencer, ed. Academic Press, New York.
4. Caesar, A. J., and Pearson, R. C. 1983. Environmental factors affecting survival of ascospores of *Sclerotinia sclerotiorum*. Phytopathology 73:1024-1030.
5. Callan, N. W., and Miller, J. B. 1989. Preventive and curative applications of Rubigan fungicide for the control of powdery mildew on sour cherry, 1988. Fungic. Nematic. Tests 44:55.
6. Creelman, D. W. 1967. Diseases of fruit crops. B. Stone fruits. Can. Plant Dis. Surv. 47:55-56.
7. Delp, C. J. 1954. Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology 44:615-626.
8. English, W. H. 1947. Powdery mildew of cherry fruit in Washington. Phytopathology 37:421-424.
9. Gadoury, D. M., and Pearson, R. C. 1988. Initiation, development, dispersal, and survival of cleistothecia of *Uncinula necator* in New York vineyards. Phytopathology 78:1413-1421.
10. Galloway, B. T. 1889. The powdery mildew of cherry. US Agric. Comm. Rep. 1888:352-357.
11. Grove, G. G., and Covey, R. P. 1988. Evaluation of fungicides for control of cherry powdery mildew in Washington nursery stock, 1987. Fungic. Nematic. Tests 43:58.
12. Hein, W. H. 1908. Two prevalent cherry diseases: Powdery mildew. Nebr. Insect Plant Dis. Bur., Bot. Div. Circ. 2:1-2.
13. Hesler, L. R., and Whetzel, H. R. 1917. Manual of Fruit Diseases. The Macmillan Company, New York. 443 pp.
14. Hubbard, J. P., Harmon, G. E., and Eckensode, C. J. 1982. Interaction of a biological control agent, *Chaetomium globosum*, with seed coat microflora. Can. J. Microbiol. 28:431-437.
15. Jeffers, S. N. 1989. Evaluation of fungicides for management of sour cherry diseases in Wisconsin, 1989. Fungic. Nematic. Tests 44:56.
16. Jeffers, S. N. 1989. Evaluation of fungicides for management of sour cherry diseases in Wisconsin, 1988. Fungic. Nematic. Tests 44:57.
17. Jones, A. L., and Sutton, T. B. 1984. Diseases of tree fruits. Mich. State Univ. Coop. Ext. Serv. Bull., North Centr. Reg. Ext. Publ. No. 45. 40 pp.
18. Khairi, S. M., and Preece. T. F. 1975. *Podosphaera clandestina*. Descriptions of Pathogenic Fungi and Bacteria No. 187 in: Commonw. Mycol. Institute, Kew, Surrey, England.
19. Kimbrough, J. W. 1963. The development of *Pleochaeta polychaeta* (Erysiphaceae). Mycologia 55:608-618.
20. Manji, B. T., Ogawa, J. M., Tomich, T. P., and Coyier, D. L. 1977. Powdery mildew of sweet cherry and its control. (Abstr.) Proc. Am. Phytopathol. Soc. 4:93.
21. Parmlee, J. A. 1977. The fungi of Ontario. II. Erysiphaceae (mildews). Can. J. Bot. 55:1940-1983.
22. Pearson, R. C., and Gadoury, D. M. 1987. Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. Phytopathology 77:1509-1514.
23. Powers, H. R., Jr., and Moseman, J. G. 1957. Pathogenic variability within cleistothecia of *Erysiphe graminis*. Phytopathology 47:136-138.
24. Salmon, E. S. 1900. A monograph of the Erysiphaceae. Mem. Torrey Bot. Club 9:1-292.
25. Schaff, W. L., Jr., Dyer, D. L., and Mori, K. 1969. Fluorescent europium chelate stain. J. Bacteriol. 98:246-248.
26. Smith, C. A., Burr, T. J., and Seem, R. C. 1988. Evaluation of fungicides for control of brown rot and powdery mildew, 1987. Fungic. Nematic. Tests 43:54.
27. Sprague, R. 1957. Cause and control of powdery mildew of cherry. Proc. Wash. State Hortic. Assoc. 53:45.
28. Stewart, V. B. 1915. Some important leaf diseases of nursery stock. NY Agric. Exp. Stn., Cornell, Bull. 258:171-268.
29. Thomson, S. V., and Buhler, R. D. 1988. Effect of fungicides on control of powdery mildew and fruit quality of sour cherry, 1987. Fungic. Nematic. Tests 43:55.
30. Weinhold, A. R. 1961. The orchard development of peach powdery mildew. Phytopathology 51:478-481.
31. Weinhold, A. R. 1961. Temperature and moisture requirements for germination of conidia of *Sphaerotheca pannosa* from peach. Phytopathology 51:699-703.
32. Wilson, E. E., and Ogawa, J. M. 1979. Fungal, bacterial, and certain non-parasitic diseases of deciduous tree fruit in California. Div. Agric. Sci., Univ. Calif. Press, Berkeley. 190 pp.
33. Woodward, R. C. 1927. Studies on *Podosphaera leucotrica* (Ell. and Ev.) Salm. Trans. Br. Mycol. Soc. 12:173.
34. Yossifovitch, M. 1923. Contributions a l'etude de l'odium de la vigne et de son traitement. Ph.D. thesis. University of Toulouse, France. 172 pp.