Initiation, Development, Dispersal, and Survival of Cleistothecia of *Uncinula necator* in New York Vineyards

David M. Gadoury and Roger C. Pearson

Research associate and associate professor, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456.

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ABSTRACT

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Cleistothecia of *Uncinula necator* began development as undifferentiated spherical groups of pseudoparenchymatous cells attached to the mildew colony by two parent hyphae of compatible isolates of the heterothallic fungus. As diameter of the ascocarp approached 30 μm, cells of the outer ascocarp wall produced anchorage hyphae that became intertwined in the surrounding mildew colony but did not anastomose or form appressoria. Anchorage hyphae were distinct from appendages, which appeared as the ascocarp grew to 75 μm in diameter, and which were directed upward and away from the mildew colony. Asci formed once the ascocarp had reached 75 μm in diameter, and, at 20 C, ascospores formed within 7 days of the first appearance of asci. The final event in development was the necrosis of the anchorage and parent hyphal connections to the mildew colony, followed by the formation of a basal concavity in the ascocarp wall. The mature concavo-convex ascocarps were dispersed in vineyards during rains and were deposited on the bark of the vine and on the soil surface. *Ampelomyces quisqualis*, a mycoparasite, had infested 23–61% of the mildewed leaves collected in September 1986 and 10–100% of the leaves collected in September 1987. In five of eight vineyards studied, leaf litter decomposition was complete by the time of bud break in 1986 and 1987. Of the cleistothecia that remained on leaves, canes, and berry cluster stems in spring, approximately 95–100% had died during winter without releasing ascospores. Only dead cleistothecia were recovered from the upper 1 cm of vineyard soils in spring. However, large numbers of cleistothecia were recovered from suspensions prepared from the exfoliating bark of several cultivars, and viability in populations of cleistothecia on bark ranged from approximately 50 to 90%. Temperature, day length, humidity, leaf age, and host resistance did not affect the initiation of cleistothecia, which appeared to require only the pairing of compatible mating types. However, the rate of growth and maturation of cleistothecia was affected by temperature and host resistance. No growth occurred at 4 or 32 C, whereas at 10 C cleistothecia increased in diameter but did not advance beyond the stage of early ascus development. Mature cleistothecia were produced on tissue culture plants and on detached leaves within 25–36 days of inoculation at 16–25 C. Cleistothecia grew and matured more rapidly on susceptible cultivars than on resistant cultivars. The date of first appearance of cleistothecia in vineyards and the date of first dispersal of cleistothecia were a function of the incidence and severity of powdery mildew. In the more severely diseased vineyards, cleistothecia formed and dispersed earlier and in greater numbers than in the less severely diseased vineyards. Dispersal of mature cleistothecia generally preceded parasitism of mildew colonies by *A. quisqualis*. Cleistothecia parasitized by *A. quisqualis* were common on leaves, but no parasitized cleistothecia were found on bark. Cleistothecia of *U. necator* appear to be adapted to dispersal to and overwintering on the bark of grapevines. Dispersal of cleistothecia from the tissues where they form to the bark of the vine places the cleistothecia immediately adjacent to emerging shoots in spring, insures that destruction of shed organs by detritivores does not result in a reduction in primary inoculum, and removes the dispersed cleistothecia from infection by *A. quisqualis*.

Additional keywords: grape powdery mildew, *Oidium tuckeri*.

Uncinula necator (Schw.) Burr., the grape powdery mildew fungus, repeatedly has been reported to survive winter as mycelium in dormant buds (7,8,25,28,36). However, the role of cleistothecia in overwintering of the pathogen in most viticultural regions is unclear (1,8,12,13,28,30,37). In 1987, we reported that cleistothecia and not mycelium in dormant buds was the principal form of overwintering in New York (24).

Considered as a whole, earlier studies of the cleistothecia of *U. necator* present a confusing and sometimes contradictory account of the effects of host and environmental factors on initiation, development, and survival of cleistothecia. For example, cleistothecia have been reported to form primarily or exclusively on senescent foliage (20) and on mid-cane leaves (30). Reports based on field studies or observations have suggested that initiation of ascocarps is triggered by severe powdery mildew (3,30), drought (4,9,12,22,31), cold (12), heat (12,31), or an environment generally unfavorable for the parasite (1,8). Host nutrition has been reported to be an important factor in initiation of cleistothecia, but both poor nutrition (12) and healthy growth (29) are reported as stimulatory for production of ascocarps. Host resistance also has been reported to affect initiation of cleistothecia (3,8,16). Finally, some reports have stated that cleistothecia die during winter (21,37) without releasing ascospores.

Our objective was to determine the effects of host and environmental factors on initiation, growth, dispersal, and survival of cleistothecia of *U. necator* in New York vineyards.

MATERIALS AND METHODS

Production of plantlets in culture. Several 2-mm nodal stem sections were cut from young greenhouse-grown plants of the *Vitis* interspecific hybrid cultivar Chancellor, surface-disinfested in 0.05% NaClO for 20–30 min, rinsed with sterile distilled water, and placed on a medium to promote shoot proliferation. Proliferation medium was prepared by adding 4.3 g of Murashige and Skoog salt mixture (Gibco Laboratories, Grand Island, NY), 30 g of sucrose, 7.5 g of agar, 1 mg of indolebutyric acid, 1 mg of benzylaminopurine, 10 mg of *myo*-inositol, 1 mg of thiamine, 1 mg of nicotinic acid, and 1 mg of pyridoxine to 1 L of distilled water. The medium was heated to 50 C and dispensed into baby food jars (30–40 ml/jar) with clear plastic lids (Magenta Corp., Chicago, IL). Jars and medium were sterilized in an autoclave for 15 min at 121 C. After 2–3 wk, individual shoots were transferred to 25- × 150-mm culture tubes containing a rooting and growth medium

prepared as above, but without benzylaminopurine. Within 3 wk, the plants grew to a height of 5–7 cm and could be inoculated with *U. necator*.

Mildew isolates. Single-spore isolates of *U. necator* from *Vitis vinifera* L. 'Pinot noir' and *Vitis labrusca* L. 'Ives,' hereafter referred to as PNG2 and IVF, respectively, were maintained on Chancellor tissue culture plants. In an earlier study (11), these isolates were compatible with each other and readily produced cleistothecia when paired. PNG2 and IVF were kept at 20 C and transferred every 3–6 wk to new tissue culture plants by removing a mildewed leaf and brushing it against the leaf of a plant to be inoculated.

Effects of temperature, day length, and humidity. Chancellor tissue culture plants were inoculated with PNG2 and IVF, one isolate on each side of a single expanded leaf, and were incubated at 20 C for 24 hr. Plants were examined 72 hr later with the aid of a stereomicroscope at 20× to determine the success of each inoculation. An inoculation was considered to be successful if germ tubes of conidia were two to three times the length of conidia and there was no necrosis of the inoculated area. Plants successfully inoculated with both isolates were incubated at 8, 10, 16, 20, 25, or 32 C. At 10, 15, 20, 30, and 45 days after inoculation, a leaf of five plants at each temperature was removed and examined under epiilluminescence microscopy. The diameter of the 10 largest cleistothecia on each leaf and the stage of development of these cleistothecia were recorded. Development was categorized as follows: 1) yellow, spherical, immature ascocarps; 2) brown, spherical, immature ascocarps; and 3) dark brown, concavo-convex, mature ascocarps (Fig. 1). The number of mature and immature ascocarps per square centimeter produced on five plants at each temperature was recorded 45 days after inoculation. Plants at 20 C also were subjected to the following photoperiods: 12 hr light/12 hr dark, 16 hr light/8 hr dark, 8 hr light/16 hr dark, 24 hr dark, and 24 hr light. These plants were examined 15 days after inoculation under a stereomicroscope at 20×, and the number of immature ascocarps produced per leaf on the leaves of five plants per treatment was recorded.

The effects of relative humidity on ascocarp initiation were studied with detached leaves of greenhouse-grown Chancellor plants. Leaves were inoculated with a spore suspension prepared by shaking two Chancellor tissue culture plants inoculated with PNG2 and IVF in water. The spore suspension was sprayed onto the leaves, the leaf surfaces were allowed to dry, and the leaves were incubated at 20 C for 21 days in double petri dishes as described by Pearson and Gadoury (24). Humidity either was maintained near 100% by placing wet filter paper within the upper plate, or the lids of the plates were removed and relative humidity remained at ambient levels, which fluctuated between 50–70% during the first experiment and 20–30% when the experiment was repeated. The number of cleistothecia per square centimeter was recorded on each of five leaves per treatment.

Effects of leaf age and the seasonal development of cleistothecia. Leaves from the 1st (basal), 15th, 20th, 30th, and 35th node of field-grown Chancellor vines were collected from a vineyard in Geneva, NY (vineyard 1), in late summer and were placed in double petri dishes. The leaves were inoculated with a spore suspension of PNG2 and IVF as above and incubated at 20 C. The percentage of leaves in each age class that bore immature ascocarps was recorded 10 days after inoculation. Three groups of 10 leaves from each node were examined. Periodically during the summers of 1986 and 1987, the severity and incidence of powdery mildew were assessed in vineyards of the *Vitis* interspecific hybrid cultivar Rosette in Geneva (vineyards 5, 6, and 7) and Naples (vineyards 8 and 9), NY. Mildewed leaves were collected at 7- to 14-day intervals from these vines at nodes 1, 15, 20, 25, and 30 and were examined for cleistothecia. The presence or absence of ascocarps of *U. necator* and the most advanced stage of cleistothecium development were recorded on 20–50 mildewed leaves per node.

Effects of host resistance on ascocarp development. Leaves were collected from vineyard 1 and from vines of *V. labrusca* 'Concord' and 'Delaware,' and the *Vitis* interspecific hybrid cultivar Cayuga White in vineyards hereafter referred to as 2, 3, and 4, respectively,

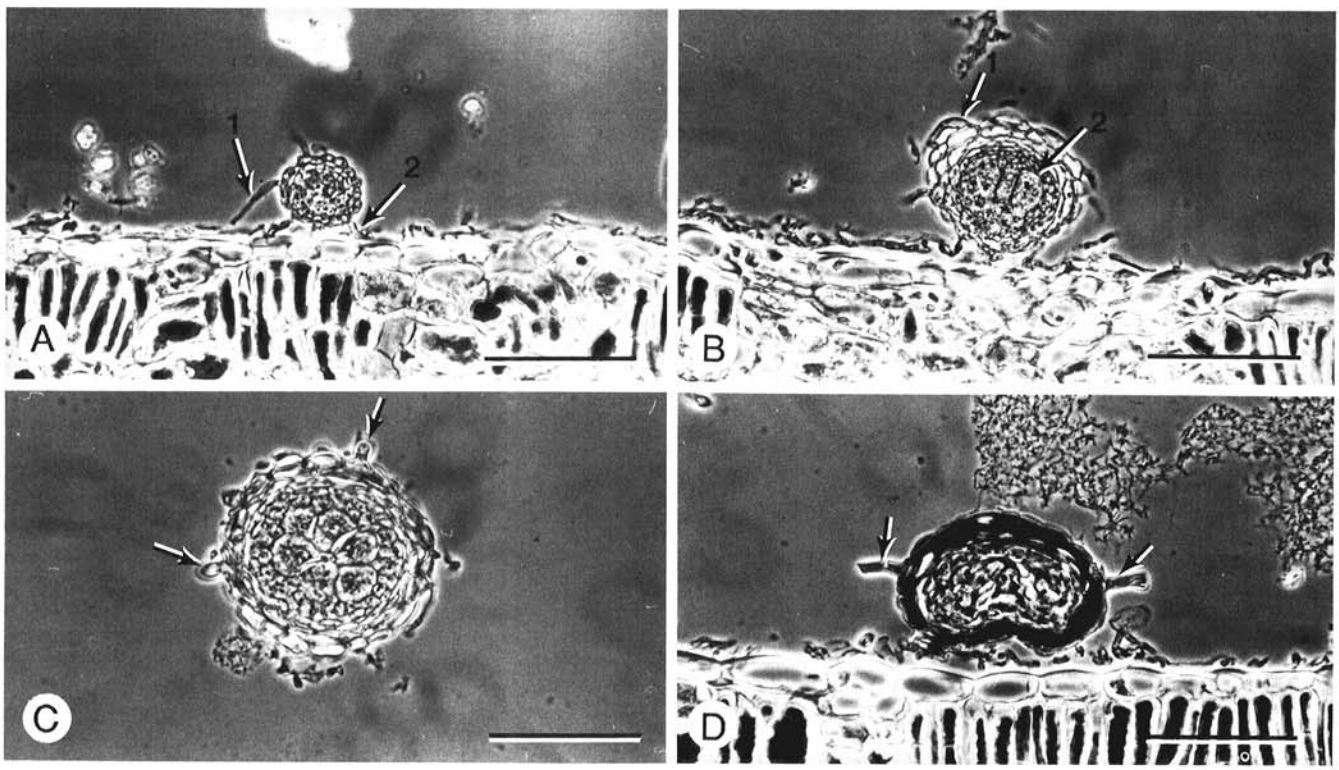

Fig. 1. Stages of development of cleistothecia of *Uncinula necator*. **A,** Hyaline, spherical ascocarp initial at approximately 10 days after inoculation at 20 C. Arrows indicate anchorage hypha (1) and parent hypha (2). **B,** Immature ascocarp at approximately 15 days after inoculation at 20 C, but before darkening of the outer ascocarp wall. Arrows indicate an anchorage hypha (1) and young ascus (2). **C,** Equatorial cross section of immature ascocarp showing arrangement of asci in centrum approximately 20 days after inoculation at 20 C. Cells of the outer wall have darkened, and an equatorial band of appendages (indicated by arrows) has begun to form. **D,** Mature concavo-convex ascocarp approximately 25 days after inoculation at 20 C. Functional connections to the mildew colony are severed and a basal concavity has formed. Bases of appendages are indicated by arrows. All scale bars indicate 70 μm.

in Geneva, NY. Leaves were placed in double petri dishes, inoculated with PNG2 and IVF as above, incubated at 20 C, and observed twice daily until sporulation occurred. Leaves were examined, and the diameter and stage of development of the 10 largest cleistothecia on five leaves per cultivar were recorded 20 and 30 days after inoculation. Similar studies were conducted with greenhouse-grown vines of Chancellor, Concord, Delaware, and *V. vinifera* 'White Riesling.' These plants were inoculated with PNG2 and IVF and were kept in the greenhouse at temperatures that varied from 20 to 28 C. At 20 and 30 days after inoculation, two leaves were collected from each of five plants, and the diameter of the 10 most advanced cleistothecia was recorded for each leaf.

Electron and light microscope studies of ascocarp development. Growth of cleistothecia was observed at daily intervals on Chancellor tissue culture plants and on detached leaves of various cultivars with the stereomicroscope and epiilluminescence microscopy. Cleistothecia in all stages of development were prepared for scanning electron microscopy as previously described (20). Cleistothecia also were fixed in 2% Formalin, dehydrated in a tertiary butyl alcohol series, infiltrated with paraffin, and serially sectioned at 10–12 μm for examination under the light microscope.

Dispersal of cleistothecia. Cleistothecia were trapped in filter paper funnels attached to the trunk of Rosette vines in Geneva, NY, and in Naples, NY, in 1986, and in vineyards 5 and 9 in 1987. The funnels were made from 9-cm disks of No. 1 paper, which were fastened to the vine with pushpins. Twelve funnels were attached to each of three vines per vineyard: four on the lower trunk, four on the upper trunk, and four on the arms of the vine. The funnels were changed after each rain or every 7 days and were examined at 20×. The number and stage of development of cleistothecia in each funnel were recorded.

Survival of cleistothecia. Viability of populations of cleistothecia on fallen leaves in vineyard 1 was monitored from November 1985 to April 1986. Cleistothecia were considered dead if they remained concavo-convex in water and if the ascospores contained darkened cytoplasm with numerous lipid droplets (24). Cleistothecia were harvested from a sample of 10–30 leaves with Cobb sieves as described previously (24) at 1- to 4-wk intervals during winter and spring. In April and May 1986, similar assessments of ascocarp viability were made with berry clusters collected from vineyard 1, canes from a vineyard of *V. vinifera* 'White Riesling' in Fredonia, NY (vineyard 10), and bark collected from vineyards 1, 10, a Concord vineyard in Fredonia, NY (vineyard 11), and vineyards of the *Vitis* interspecific hybrid cultivars Aurore (vineyard 12) and Rougeon (vineyard 13) in Geneva, NY. Ascocarp viability was assessed from three samples of 10 berry clusters, 1 m of cane, or 30 g of dry bark. Cleistothecia were harvested in water, collected, and examined as previously described (24). In November 1986 and April 1987, leaf, cane, berry cluster, and bark samples were collected as above from vineyards 7 and 9 and from a vineyard of *V. labrusca* 'Niagara' (vineyard 14) in Naples, NY. Three samples of the upper 1 cm of soil also were collected from vineyards 7, 9, and 14. Ascocarp viability in the leaf, cane, berry cluster, and bark samples was determined as before. Soil samples were allowed to dry. One hundred cubic centimeters of soil was suspended in 300 ml of water in a 1-L flask, and the flask was repeatedly shaken and then allowed to stand for 5 sec. The water containing the suspended cleistothecia was decanted and sieved. Viability of the ascocarps harvested from soil was determined as above.

At the time of bud break in 1986 and 1987, the proportion of the vineyard floor covered by leaf litter was measured (10) in vineyards 1, 2, 7, 9, 14, a vineyard of *V. vinifera* 'Chardonnay' in Hector, NY (vineyard 15), a Concord vineyard in Westfield, NY (vineyard 16), and a vineyard of the *Vitis* interspecific hybrid cultivar Seyval (vineyard 17) in Dresden, NY.

RESULTS

Effects of temperature, day length, and humidity. Ascocarp initials were found on Chancellor tissue culture plants 10 days after inoculation at 16, 20, and 25 C, and at 15 days after inoculation at 10 C. No hyphal growth occurred at 8 C; however, when the incubation temperature was increased to 20 C 45 days after inoculation, normal growth of the mildew colony resumed and immature ascocarps were formed within 10 days. No cleistothecia formed at 32 C, and the mildew colony died within 10 days of inoculation. Cleistothecia at 10 C increased in diameter but did not advance in development beyond the yellow sphere stage (Fig. 1). Growth resumed when the incubation temperature was raised from 10 to 20 C 45 days after inoculation. The mean number of days from inoculation until 50% or more of the cleistothecia in the sample were mature was 33 days at 16 C and 25 days at 20 and 25 C (Fig. 2). The equivalent degree-day accumulations required for production of mature cleistothecia at 16, 20, and 25 C were 528, 500, and 625 (base = 0 C). Temperature had no significant effect ($P = 0.01$) on the number of ascocarps produced. At 10, 16, 20, or 25 C, an average of 460, 725, 690, and 825 cleistothecia formed per square centimeter, respectively. In vineyard 8 in 1986, cleistothecia advanced from the hyaline sphere stage to maturity in 18 days. The mean temperature during this period was 19.5 C. The following year, cleistothecia in vineyard 8 advanced from the hyaline sphere stage to maturity in 17 days, when the mean temperature was 22 C.

Day length during the 15-day period following inoculation of tissue culture plants kept at 20 C had no significant effect ($P = 0.01$) on initiation or growth of cleistothecia, with the exception of the inoculated plants kept in continual darkness, which died after 12 days. None of the three humidity levels used (100, 50–70, and 20–30%) significantly affected the number of cleistothecia initiated during the 21-day period following inoculation of detached leaves kept at 20 C.

Effects of leaf age on the seasonal development of cleistothecia. Leaf age had no significant effect ($P = 0.01$) upon the number of ascocarps present 30 days after inoculation of Chancellor detached leaves. Nor was there any apparent effect of leaf age upon the occurrence of cleistothecia in two Rosette vineyards in 1986 or 1987. In vineyard 5 in 1986 and in vineyard 8 in 1987, leaves were infected by *U. necator* and cleistothecia were formed in the order that the leaves were formed (Fig. 3). In no case did infected leaves at any position on the shoot appear unsuitable for initiation or growth of ascocarps. By September of both years, 50–100% of the leaves at nodes 1, 15, 20, 25, and 30 bore cleistothecia in all vineyards examined (Table 1).

Cleistothecia first were found on June 25 in vineyard 9 in 1986 and on July 14 in vineyard 8 in 1987. The percentage of leaves at the first node that bore mature cleistothecia was directly proportional to disease incidence (Figs. 4 and 5). Cleistothecia consistently appeared later in vineyards with less disease. Shifts in time of disease progress curves were mirrored in a temporal shift of cumulative curves of ascocarp production (Figs. 4 and 5).

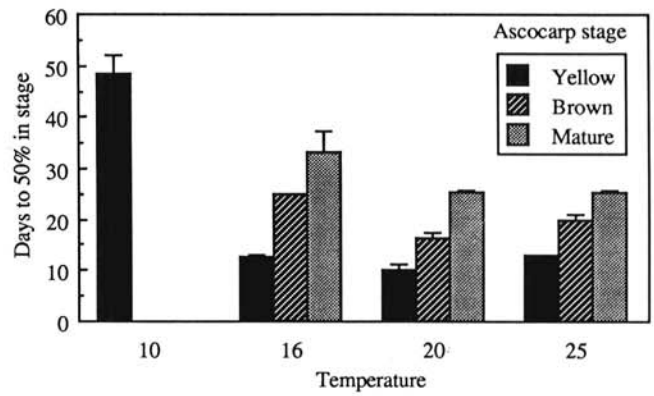

Fig. 2. Effects of temperature on the maturation of cleistothecia of *Uncinula necator* on tissue culture plants of the *Vitis* interspecific hybrid cultivar Chancellor. Developing cleistothecia were grouped into three categories in ascending order of maturity: 1) yellow, spherical, immature ascocarps, 2) brown, spherical, immature ascocarps, and 3) mature, concavo-convex ascocarps. The time from inoculation until 50% of the 50 most advanced ascocarps at each temperature had reached a certain maturity class is reported. Bars represent one standard error.

Effect of host resistance on ascocarp development. The rate of growth of cleistothecia, measured as the increase in volume of spherical ascocarps, was directly proportional to susceptibility of the host to powdery mildew, measured as the time between inoculation and sporulation (Table 2). Cleistothecia on detached leaves grew most rapidly on the cultivar Chancellor, grew at similar rates on Delaware and Concord, and grew most slowly on Cayuga White. On the potted vines, cleistothecia on Chancellor and White Riesling vines grew at similar rates but grew more rapidly than did cleistothecia on Concord and Delaware vines (Table 2). Significant differences in host susceptibility were reflected in significant ($P = 0.01$) differences in the rate of growth of cleistothecia (Table 2).

Electron and light microscope studies of ascocarp development. Under epiilluminescence microscopy, the cleistothecia appeared first as hyaline spheres approximately 10–20 μm in diameter. Cleistothecia increased in diameter from approximately 10 μm in diameter at 10 days after inoculation to approximately 70 μm in diameter at 20 days after inoculation at 20 C. As the ascocarp increased in diameter, the hyaline cleistothecia yellowed due to the intracellular accumulation of an unidentified lipid. This lipid was not stained by the general lipid stains Sudan III or Sudan IV but was stained darkly by Sudan Black B. Cells of the outer ascocarp wall darkened as the ascocarp approached a diameter of 100 μm (Fig. 1).

In cross section, ascocarp initials 20 μm in diameter (Fig. 1) appeared as undifferentiated spheres of pseudoparenchymatous tissue. Ascus initials appeared at 40 μm diameter and continued to expand as the cleistothecium enlarged. Ascospores formed when the ascocarp was approximately 70–80 μm in diameter. At maturity, the asci were wedge shaped and distributed in the centrum similar to the arrangement of carpels in an orange (Fig. 1). The wall of the mature ascocarp consisted of an outer cell layer one to two cells thick of thickened, dark-colored cells devoid of cytoplasm. Internal to this layer was a layer of thinner-walled pseudoparenchymatous tissue two to three cells in thickness (Fig. 1).

Cleistothecia were initiated when hyphal contact occurred between the two compatible mating types PNG2 and IVF. Ascocarp initials were produced within 48 hr of hyphal contact at 20 C. The ascocarp initial was attached to the mildew colony only through the two parent hyphae (Fig. 6). As the diameter of the ascocarp approached 20 μm, cells of the outer ascocarp wall produced hyphae (Fig. 6), hereafter referred to as anchorage hyphae, that radiated outward and became intertwined in the hyphae of the surrounding mildew colony but formed no anastomoses or appressoria, even though the anchorage hyphae were often several hundred microns long. Anchorage hyphae were distinct from the appendages of the cleistothecium, which appeared later in development, were more robust, and were

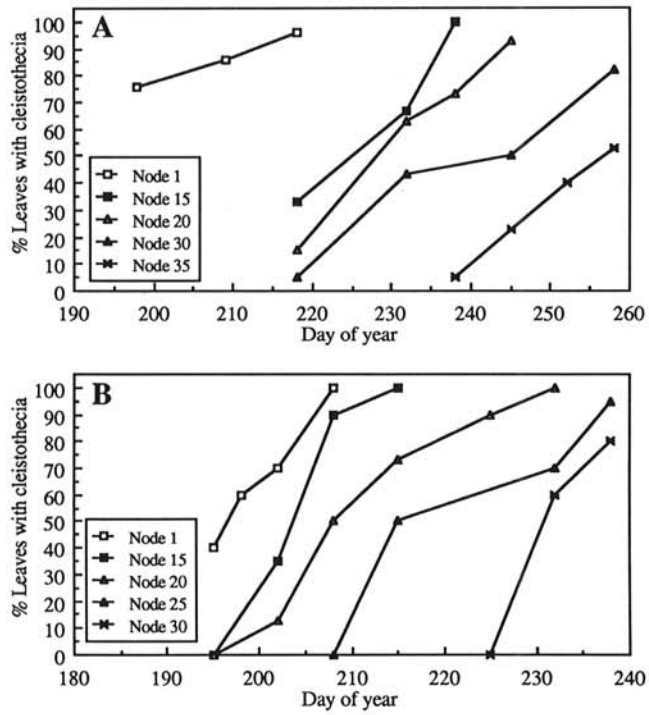

Fig. 3. Seasonal development of cleistothecia of *Uncinula necator* on leaves of various ages of the *Vitis* interspecific hybrid cultivar Rosette in vineyard 5 in 1986 **(A)** and vineyard 8 in 1987 **(B)**. Samples of mildewed leaves were collected from different positions on shoots (node 1 = basal node) throughout the growing season and were examined at 20× for the presence of cleistothecia.

TABLE 1. Percentages of leaves of various ages bearing cleistothecia of *Uncinula necator* in September 1986 and 1987

		Percent leaves bearing cleistothecia at node				
Year	Vineyard	1	15	20	25[a]	30
1986	5	96	100	93	···	82
	6	100	100	93	···	90
	7	100	93	93	···	97
	8	90	95	95	···	50
1987	5	100	95	100	100	95
	8	100	100	100	95	80
	15	100	100	100	100	100

[a]No data taken on node 25 in 1986.

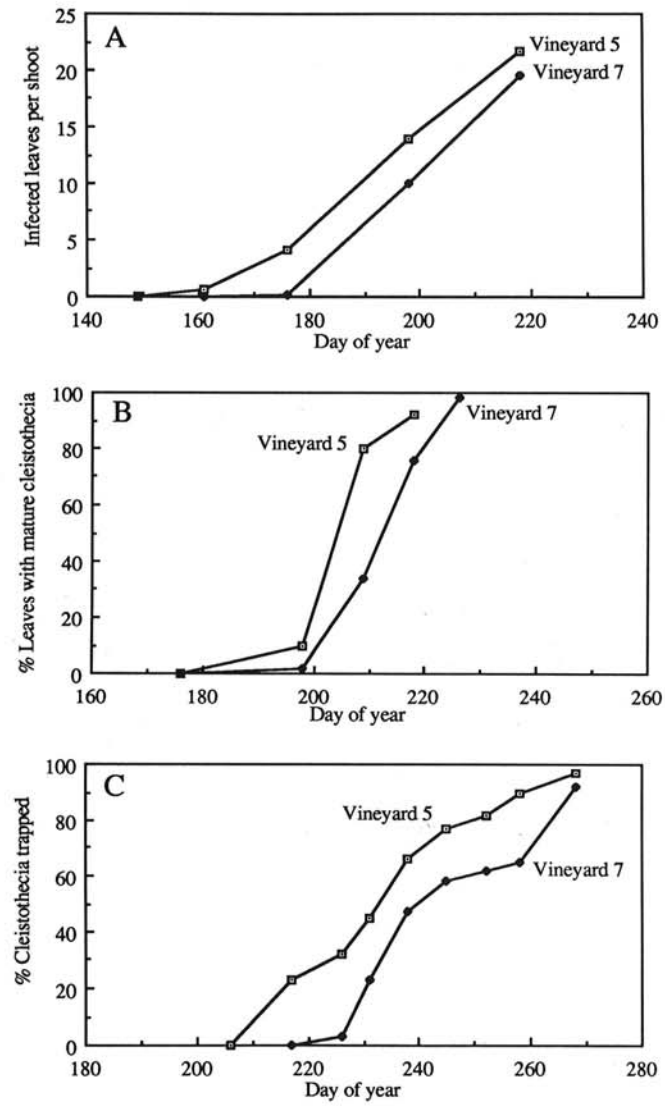

Fig. 4. Development of grape powdery mildew **(A)**, production of cleistothecia of *Uncinula necator* on mildewed leaves at node 1 **(B)**, and dispersal of cleistothecia **(C)** in 1986 in two adjacent vineyards of the *Vitis* interspecific hybrid cultivar Rosette.

directed up and away from the mildew colony (Fig. 6).

Dispersal of cleistothecia. The final event in the development of cleistothecia was the necrosis of the functional connections to the mildew colony. This was followed by a rapid loss of turgor and the formation of a basal concavity in the ascocarp wall (Fig. 1). This concavity formed within 5 min when hyphal connections to the mildew colony were broken by tearing the ascocarp free of the colony with forceps. In the vineyards, the presence of concavo-convex ascocarps coincided with the first dispersal of ascocarps during rain. The date of first ascocarp dispersal was a function of the incidence and severity of disease. Delayed disease resulted in delayed ascocarp formation and dispersal (Figs. 4 and 5).

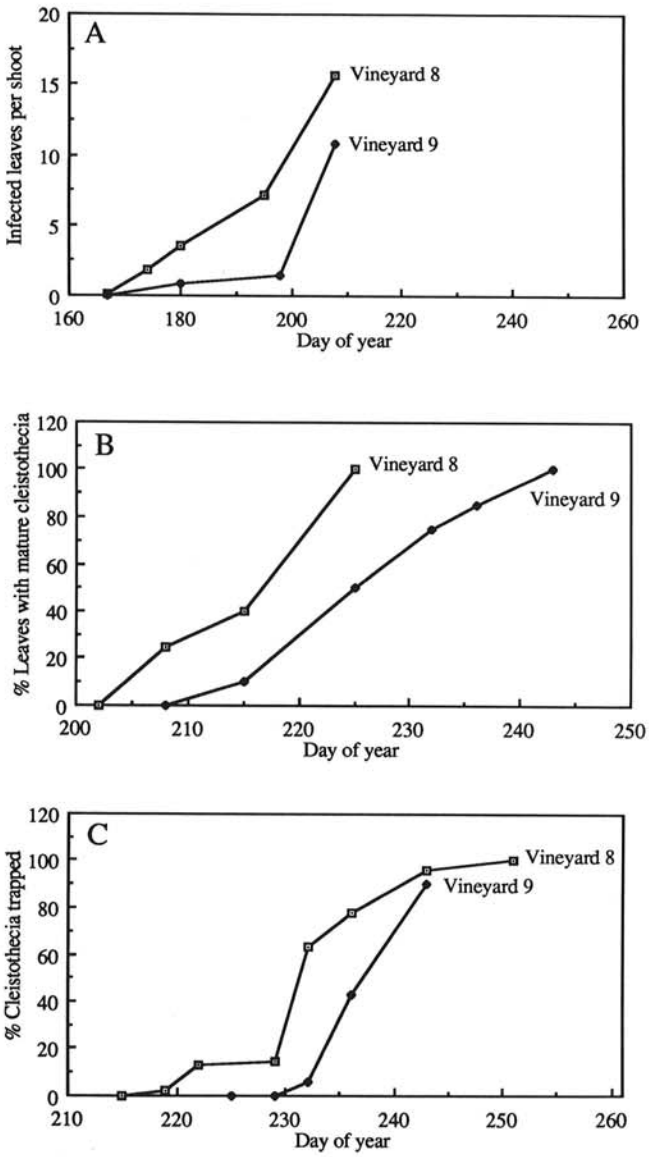

Fig. 5. Development of grape powdery mildew **(A)**, production of cleistothecia of *Uncinula necator* on mildewed leaves at node 1 **(B)**, and dispersal of cleistothecia **(C)** in 1987 in two adjacent vineyards of the *Vitis* interspecific hybrid cultivar Rosette.

Infection of mildew colonies by *A. quisqualis*. In September 1986, 54, 23, 38, 37, and 61% of the mildewed leaves collected from node 1 in vineyards 1, 2, 3, 5, and 12, respectively, bore colonies infected by *Ampelomyces quisqualis* Ces. In September 1987, 100% of the mildewed leaves collected from node 1 in vineyards 5 and 8 and 33% of the mildewed leaves collected from node 1 in vineyard 15 bore colonies infected by *A. quisqualis*. The incidence of mycoparasitism in autumn generally increased as age of the mildewed leaf increased. In September 1987, 100, 53, 53, 20, and 17% of the mildewed leaves at nodes 1, 15, 20, 25, and 30, respectively, were infected by *A. quisqualis* in vineyard 8. In vineyard 15 in September 1987, 33, 23, and 10% of the mildewed leaves at nodes 1, 15, and 30, respectively, were infected by *A. quisqualis*. Infection of mildew colonies by *A. quisqualis* generally lagged behind production of mature ascocarps and sometimes behind dispersal of cleistothecia (Fig. 7). Parasitized colonies could be recognized easily by the presence of pycnidia of *A. quisqualis* within the conidiophores of *U. necator* (Fig. 8). Cleistothecia attached to the mildew colony also were parasitized and exuded cirrhi of conidia of *A. quisqualis* when detached leaves bearing an infected mildew colony were incubated in double petri dishes (24) for 24–48 hr at 20 C.

Survival of cleistothecia in vineyards. Cleistothecia were recovered from all plant parts and from vineyard soils in April 1986 and 1987. The density of populations of cleistothecia on the

TABLE 2. Number of days from inoculation to sporulation of colonies and the rate of growth of cleistothecia of *Uncinula necator* on detached leaves and potted vines

	Detached leaves[y]		Potted vines[y]	
Cultivar	Time from inoculation to sporulation (days)	Rate of growth[z] (μm^3/days)	Time from inoculation to sporulation (days)	Rate of growth[z] (μm^3/days)
Chancellor	6.0 a	56,763 a	6.2 a	60,980 a
White Riesling	...	...	6.0 a	67,877 a
Delaware	7.2 b	31,802 b	7.4 b	36,944 b
Concord	7.2 b	32,452 b	7.6 b	32,380 b
Cayuga White	8.6 c	21,031 c	...	...

[y] Numbers within columns followed by the same letter do not differ significantly at $P = 0.01$ according to Duncan's multiple range test.

[z] Rate of growth was measured as the mean daily increase in volume of spherical ascocarps during the period between 20 and 30 days after inoculation.

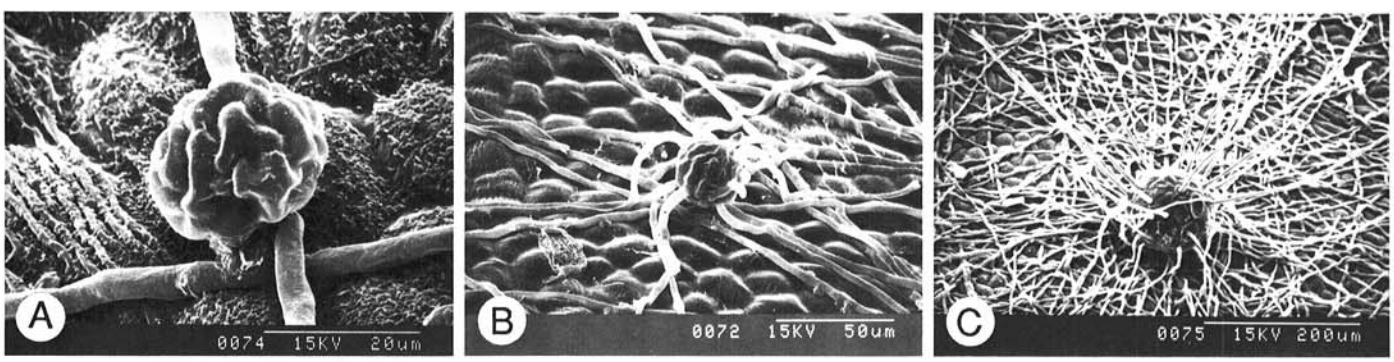

Fig. 6. Stages in development of cleistothecia of *Uncinula necator*. **A,** Ascocarp initial approximately 48 hr after contact of parent hyphae. **B,** Immature ascocarp producing anchorage hyphae. **C,** Nearly mature ascocarp. Appendages have formed but have not yet developed the uncinate tips that characterize the genus. The cleistothecium at this stage is still spherical, retains functional connections to the mildew colony through the parent hyphae, and has not yet developed a basal concavity.

surfaces of leaves collected from the vineyard floor ranged from 789 to 6,827 ascocarps/m^2. From 113 to 608 cleistothecia/m^2 were found on canes collected from the canopy. Population densities on exfoliating bark stripped from trunks ranged from 1,772 to 32,000 ascocarps/kg of bark. From 0.4 to 409 cleistothecia/rachis were recovered from berry cluster stems collected from vines or from the vineyard floor. Finally, from 9,200 to 69,000 cleistothecia/m^2 were recovered from the upper 1 cm of vineyard soils.

The percentage of viable ascocarps harvested from leaves from vineyard 1 declined steadily during winter. Approximately 70% of the cleistothecia were viable on 18 November 1985; however, viability declined to 60% on December 3, 51% on January 20, 46% on March 5, and 14% on March 15. By the time of bud break in April 1986, only 3% of the cleistothecia borne on leaves overwintered in vineyard 1 were viable. The remainder had died without releasing ascospores. A similar preponderance of dead ascocarps was found in spring collections from berry cluster stems in vineyard 1 and canes in vineyard 10, where 97 and 92%, respectively, of the cleistothecia harvested had died without releasing ascospores. Viable cleistothecia were abundant on exfoliating bark collected in April and May 1986 from vineyards 1, 10, 11, 12, and 13, where 51, 50, 45, 83, and 86%, respectively, of the cleistothecia examined were viable. No consistent differences were found among viability of populations of cleistothecia from leaves, canes, berry cluster stems, bark, or from the upper 1 cm of soil in vineyards 7, 9, and 14 in November 1986 (Fig. 9). However, by April 1987, only those populations of cleistothecia on bark contained viable ascocarps. The proportion of ascocarps on bark that was viable did not decrease significantly ($P = 0.05$) during winter (Fig. 9).

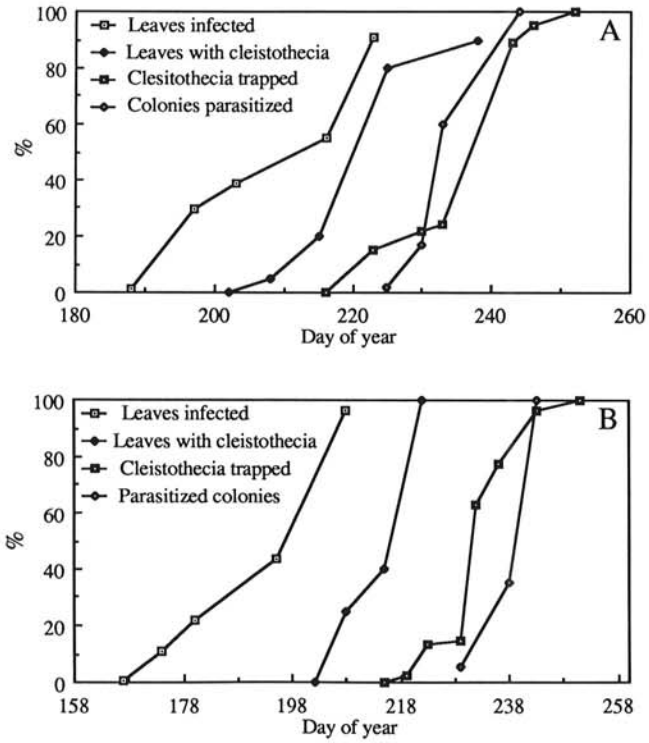

Fig. 7. Seasonal progression of grape powdery mildew, production of cleistothecia of *Uncinula necator*, dispersal of cleistothecia, and infection of mildew colonies by the mycoparasite *Ampelomyces quisqualis* in vineyards 5 **(A)** and 8 **(B)** in 1987.

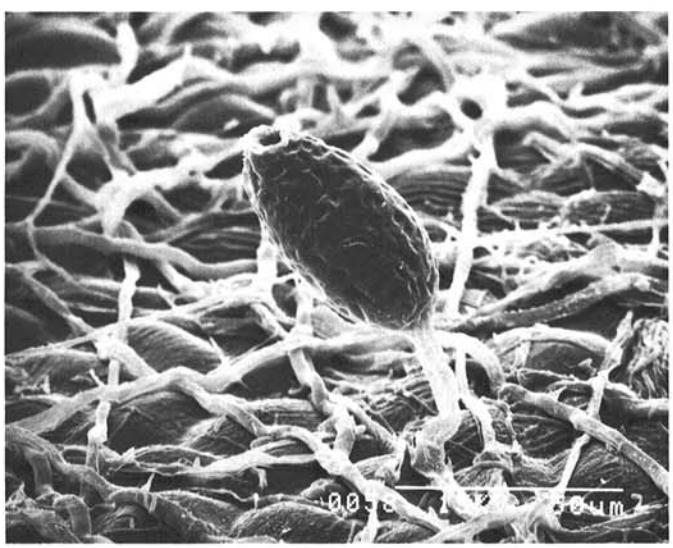

Fig. 8. Parasitism of *Uncinula necator* by *Ampelomyces quisqualis*. A pycnidium of *A. quisqualis* has formed within the conidiophore of *U. necator*.

Infection of mildew colonies on leaves by *A. quisqualis* was responsible for the death of some cleistothecia in early autumn, but the loss of viability during winter was not associated with mechanical damage to cleistothecia or with mycoparasitism. Microscopic examination of sectioned cleistothecia and crush mounts of whole ascocarps revealed that the cytoplasm of cleistothecia that had died during winter was darkened and contained numerous lipid droplets as previously described (24). No evidence of infection of cleistothecia by *A. quisqualis* was found among cleistothecia collected from bark.

Cleistothecia viewed with the scanning electron microscope often were found in bark crevices (Fig. 10). Appendages of the ascocarp, which were upright and bristlelike when first formed (Fig. 6), were closely appressed to the bark and appeared to anchor the cleistothecium to the bark (Fig. 10). When dry cleistothecia on bark were grasped by the appendages with fine forceps, bark pieces weighing up to 3 g could be lifted.

Leaf litter decomposition and removal was nearly complete by the time of bud break in 1986 and 1987. No measurable leaf litter remained at bud break in vineyards 5, 6, 7, 8, and 9 in 1986 or in vineyards 1, 5, 6, 7, 8, 9, and 15 in 1987. In vineyards 1, 2, and 12, the proportion of the vineyard floor covered by leaf litter was 0.06, 0.07, and 0.09, respectively, at the time of bud break in 1986. At the time of bud break in 1987, 3, 2, and 20% of the vineyard floor were covered by leaf litter in vineyards 2, 16, and 17, respectively.

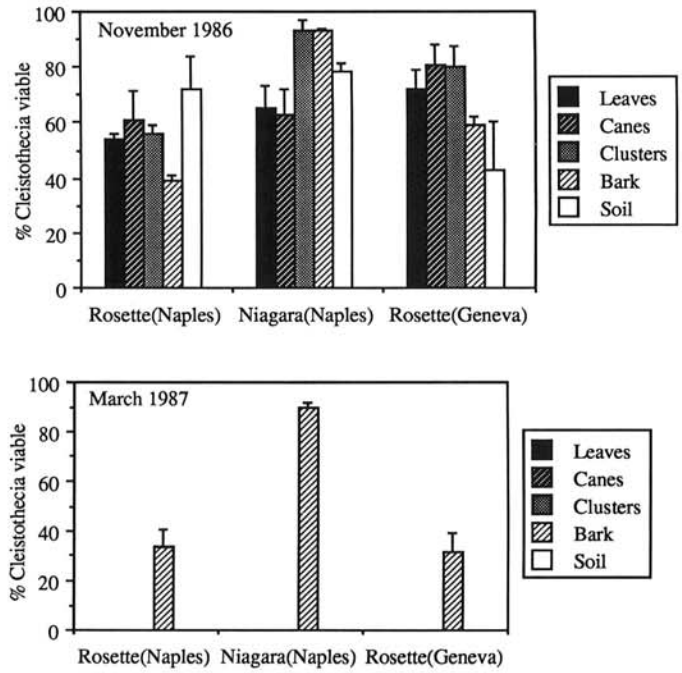

Fig. 9. Survival of cleistothecia over winter on leaves, canes, berry cluster stems, bark, and soil in vineyards 9 (Rosette, Naples), 14 (Niagara, Naples), and 7 (Rosette, Geneva). Bars represent one standard error.

Earthworms were major agents of leaf burial in all vineyards. In vineyards deviod of leaf litter in spring, up to 2,600 grape leaf petioles per meter of vineyard floor could be found protruding from earthworm burrows (Fig. 11).

DISCUSSION

U. necator is heterothallic (11,32). Our study was presumably the first study of ascocarp development of *U. necator* to use isolates of known mating type compatibility. Cleistothecia were initiated within 48 hr at 20 C when hyphal contact occurred between compatible isolates. None of the environmental or host factors examined in this study prevented the initiation of cleistothecia or reduced the numbers of cleistothecia initiated, unless the effect of the factor was so severe that either the host was killed or the growth of the pathogen was halted. The sole trigger for the initiation of cleistothecia appears to be hyphal contact between compatible isolates.

As disease incidence increases, so does the probability that compatible isolates will be paired on the same tissue. In our field studies, this relationship between pairing of compatible isolates and initiation of cleistothecia was exhibited as an effect of disease incidence on the time of appearance of cleistothecia in vineyards. Severe disease has been reported to be important in production of cleistothecia in vineyards (30). Thus, anything that directly or indirectly affected disease incidence could be seen as being stimulatory or inhibitory towards initiation of cleistothecia. We found that leaf age was directly proportional to the percentage of leaves within an age group that bore cleistothecia in the earlier phases of a mildew epidemic. Older leaves are exposed to inoculum for a longer period than younger leaves. This probably accounts for the report of cleistothecia on senescent foliage (20). However, we found that, given sufficient time for infection by compatible mating types, leaves of all ages supported production of cleistothecia. The relationship between host resistance and delayed or lowered ascocarp production in vineyards observed by Jovanovic (16) conceivably could have occurred through reduced disease incidence on resistant cultivars and delayed pairing of compatible mating types. It also is possible that correlations of ascocarp production in vineyards with seasonal factors such as high and low temperatures, drought (3,9,12,22,31), and succulent growth (29) were due to correlations of these factors with disease incidence. Cleistothecia can be expected to appear in vineyards whenever the incidence and duration of disease is sufficient to allow the pairing of compatible isolates.

Of the factors we studied, only temperature and host resistance directly affected the growth of cleistothecia once they were initiated. Cleistothecia failed to mature at 10 C, although they did increase in diameter at this temperature, indicating that low temperature inhibited processes essential to maturation but not processes related to diameter increase. The effect of host resistance on growth of cleistothecia was measured as the rate of volume increase. Although cleistothecia increased in volume more slowly on resistant cultivars, they did eventually mature on even the most resistant cultivars tested. It is important to distinguish between initiation of cleistothecia and subsequent growth. The rate of growth is dependent on temperature and host resistance. Initiation is dependent upon the pairing of compatible mating types. The time required for maturation on various cultivars was not examined in this study. However, on the susceptible cultivar Chancellor, cleistothecia matured between 500 and 625 degree days (base = 0 C) after inoculation when incubated at 16–25 C. We plan to use these values as minimum degree-day accumulations necessary for maturation of cleistothecia in commercial vineyards. If a vineyard is found to be relatively free of ascocarp initials on a certain date in late summer, we may be able to estimate whether cleistothecia could mature sufficiently to survive winter based on degree-day accumulation between the observation date and the date of leaf abscission or death.

Certain features of the dispersal and survival of cleistothecia of *U. necator* in New York vineyards may explain why some previous studies have not recognized the role of ascocarps in the epidemiology of grape powdery mildew. Early in ascocarp development, anchorage hyphae secure the developing ascocarp to the mildew colony. These anchorage hyphae are not unique to *U. necator* and have been reported to form in other *Uncinula* species and in the genus *Microsphaera* (35). Anchorage hyphae of *U. necator* appear to function only in attachment to the mildew

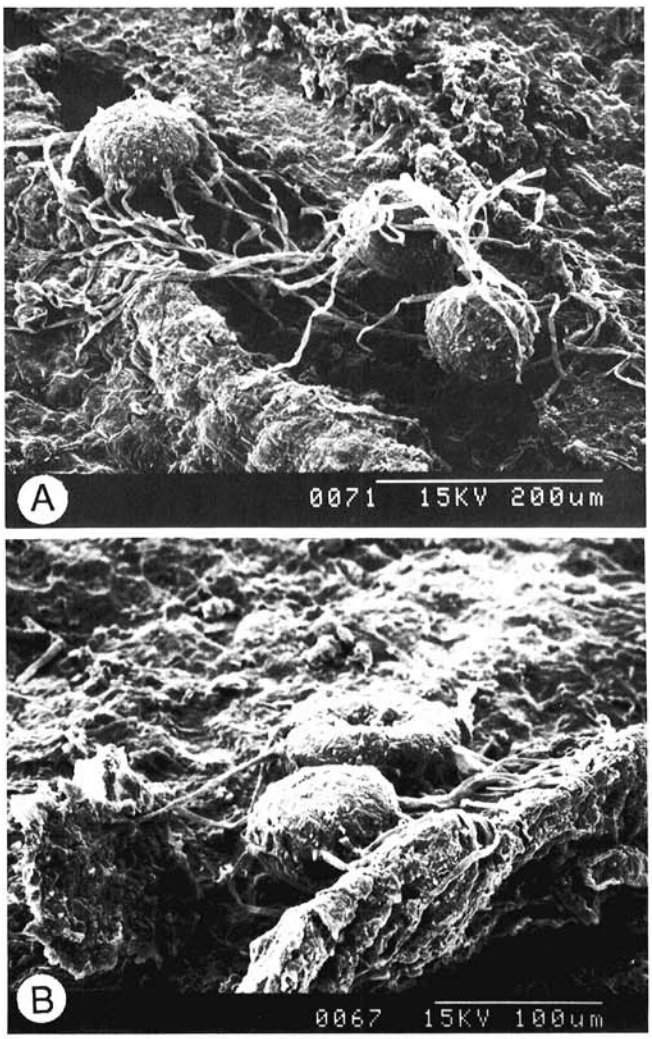

Fig. 10. Cleistothecia of *Uncinula necator* in crevices of exfoliating bark of grape vines. **A,** Group of three ascocarps suspended over fissure. **B,** Two ascocarps adjacent to ridge in bark. The basal concavity of the inverted ascocarp in the background is visible.

Fig. 11. Burial of grape leaves by earthworms. Leaves are drawn into burrows leaving only the protruding petioles.

colony. Although the hyphae were sometimes several hundred microns long, no anastomoses or appressoria were seen in more than 100 cleistothecia examined in the scanning electron microscope study. The final event in the morphological development of cleistothecia is the necrosis and abscission of hyphal connections to the cleistothecia and the formation of a basal concavity in the ascocarp wall. The concavo-convex ascocarps were dispersed during rain to the bark of the vine and to the surface of the vineyard soil. Neger (23) speculated that the formation of the concavity itself resulted in the breaking of all hyphal connections to the mildew colony, but later it was shown that concavo-convex ascocarps would remain firmly attached to mildew colonies unless they were wet (38). Dispersal of cleistothecia of *U. necator* in rain was first described by Yossifovitch (38), and a similar type of detachment and dispersal of cleistothecia has been reported for *Pleochaeta polychaeta* (19). A connection between dispersal of cleistothecia of *U. necator* and survival has been established in our study. The majority of cleistothecia that remain attached to the tissues on which they are produced are apparently physiologically immature and unable to survive winter. However, a small number of viable cleistothecia can be found on leaves in spring. In an earlier study (24), we successfully inoculated grapevines using cleistothecia from overwintered leaves, but the leaves were packed in layers in mesh bags that may have reduced the removal of mature ascocarps by rain. Diehl and Heintz (9) obtained similar results when cleistothecia used for inoculations of grape leaf disks were harvested from leaves that were collected in vineyards and then sheltered from rain in the laboratory. We found that survival of populations of cleistothecia on bark consistently and significantly exceeded that of populations from leaves, canes, berry cluster stems, or soil. In fact, only those cleistothecia on bark survived the winter of 1986-1987 in vineyards 5, 8, and 14.

Cleistothecia of *U. necator* appear to be adapted to dispersal to secondary substrates. Anchorage hyphae effectively hold the developing ascocarps to the mildew colony on the original substrate. Immature ascocarps, either yellow spherical or earlier immature stages, seldom were found in the funnels used to trap dispersed cleistothecia. The appendages of cleistothecia appear to fasten dispersed ascocarps to secondary substrates such as bark. In most previous studies in which ascospores were used to inoculate grapevines (2,13,24,38), inoculum was collected from leaves overwintered in the vineyard. Thus, failure of some previous studies to reproduce the disease from ascospore inoculations (2,13,38), the reported degeneration of cleistothecia during overwintering (21,37), and the resultant unclear role of cleistothecia in the epidemiology of grape powdery mildew (1,8,12,28,30,37) may all be related to the dispersal of cleistothecia by rain as they mature and the retention of morphologically mature, but physiologically immature, ascocarps on parasitized tissues.

Dispersal of mature cleistothecia is a significant event in the epidemiology of grape powdery mildew. Detachment and dispersal of cleistothecia from mildew colonies remove ascocarps from infection by *A. quisqualis*, a mycoparasite that was widespread among mildewed leaves in September 1986 and 1987. Dispersal to the bark of the vine also insures that destruction of shed organs, such as leaves, will not result in a reduction of primary inoculum. Leaf removal was complete in five of eight vineyard sites in 1987. The survival of mature cleistothecia on leaves is unlikely when the substrate itself is buried, as occurred when earthworms buried leaves in vineyards. No viable cleistothecia were recovered from soil in spring. Dispersal of cleistothecia to bark also places the primary inoculum immediately adjacent to emerging shoots in spring, the significance of which is highlighted by our finding in an earlier study (24) that early-season infections were most common on the basal leaves of shoots growing in close proximity to the bark of the trunk.

Earlier literature presented indirect evidence that overwintering cleistothecia on bark may be a source of inoculum for grape powdery mildew in viticultural areas other than New York. Bioletti (5) reported in 1907 that some California grape growers achieved partial control of powdery mildew by removing the exfoliating bark from vines, presumably also removing any attached ascocarps. Smith (33) reported that some California growers were using this technique, in conjunction with washing dormant vines with a copper sulfate solution, to control powdery mildew as early as 1879. In more recent literature, Boelema (6) recommended a dormant application of lime sulfur as an effective method to control powdery mildew in South Africa. Suit (34) was able to reduce the incidence of powdery mildew in New York by applying dinitro-oxy-creosol to dormant vines. We have found that lime sulfur, copper salts, and certain dinitro compounds are highly toxic to cleistothecia, can kill cleistothecia on bark when applied as dormant over-the-trellis sprays, and can substantially delay powdery mildew epidemics in New York vineyards (Gadoury and Pearson, *unpublished*). Presumably, disease reduction reported above (5,6,33,34) was due to the eradication of a significant portion of overwintering inoculum.

Dispersal of cleistothecia of *U. necator* from parasitized tissues to bark in *Vitis* may serve as a model for the survival of cleistothecia of powdery mildews of other deciduous perennial hosts. Many of the phenomena reported for *U. necator*, namely, the degeneration of ascocarps during winter (14,15,17,18,26,27) and the failure of ascospore inoculations to reproduce the disease (18,37), have been reported for powdery mildews of other deciduous perennials such as apple, oak, hawthorne, gooseberry, and rose.

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endodermis. After a diffusion period of 2 hr, the dye entered the