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Cleistothecia, the Source of Primary Inoculum for Grape Powdery Mildew in New York

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ABSTRACT


Vineyard surveys of more than 54,000 vines over a 3-yr period failed to provide evidence that Uncinula necator survived winter as mycelium in dormant infected buds. Ascospores of U. necator, but not conidia, were collected by a volumetric spore trap operated continuously in a vineyard for 40 days after bud burst. In spring, the first powdery mildew colonies were consistently found on leaves of shoots (7-30 cm) growing close to exfoliating bark on the head and trunk of the vine. Cleistothecia were found in spring on all plant parts infected during the previous growing season and also in leaf scars and in crevices of exfoliating bark. Most (79-97%) of the cleistothecia borne on leaves, canes, and cluster stems died during winter and spring without releasing ascospores, but 45-75% of the cleistothecia found in bark crevices were viable. When overwintered cleistothecia collected from vineyards at 7- to 14-day intervals were induced to discharge ascospores, about 75-100% of the ascospores were discharged between bud burst and bloom over a 4-yr period. Such ascospores, released from cleistothecia suspended over detached leaves or tissue culture plants, germinated, infected, and gave rise to typical powdery mildew colonies. Cleistothecia appear to be the principal means of overwintering of U. necator in New York vineyards.

Additional key words: ascocarps, cleistocarps, Oidium tuckeri, perithecia.

The source of primary inoculum for epidemics of grape powdery mildew in New York has never been determined. It has been assumed that the fungus Uncinula necator (Schw.) Burr. survives winter as mycelium in infected dormant buds, as has been demonstrated in California (18), Western Europe (4,13), and South Africa (22). In fact, survival of U. necator as mycelium in dormant buds has been accepted as the principal form of overwintering in most viticultural regions (5,26) despite the occurrence of cleistothecia in Germany (23,25), France (27), Romania (2), California (3), New York (17), and Australia (24).

The role of cleistothecia in the epidemiology of grape powdery mildew is unknown. Reddick and Gladwin (17) stated in 1915 that the method of hibernation of the fungus is not definitely known, but they assumed that the fungus overwintered as ascocarps in cleistothecia. Although germination of mature ascospores was observed as early as 1895 (11), repeated inoculations of grape with ascospores by Galloway in 1895 (11), Yossifovitch in 1923 (27), and Aurel in 1974 (2) failed to reproduce the disease. Because overwintering mycelium of U. necator in dormant buds has been shown to be a source of primary inoculum and ascospores have not, it has frequently been stated that ascocarps are unnecessary for successful overwintering and that the asexual stage is of minor or no importance in the disease cycle of grape powdery mildew (5,7,19,26). It is possible that U. necator overwinters exclusively as mycelium in buds when cleistothecia are absent. After introduction of U. necator to Europe in the mid-19th century, U. necator overwintered for nearly 50 yr before the first cleistothecia were found (5). However, U. necator also overwinters successfully in New York, where we have been unable to find any evidence of survival of mycelium in dormant buds. In this paper, we show that U. necator overwinters as cleistothecia in New York, that ascospores formed in these cleistothecia are functional inoculum, and that infection of dormant buds is not necessary for perennation of the disease.

MATERIALS AND METHODS

Vineyard surveys. Shoots that emerge from buds infected by U. necator are stunted, distorted, heavily coated with mycelium (5,18), and are referred to as flag shoots (27). Vineyard surveys to detect flag shoots or other evidence of infection by U. necator were conducted in 1983 on Long Island and in the Finger Lakes region of New York and in the Lake Erie region of New York and Pennsylvania. From 27 vineyards, 35,000 vines of several cultivars were examined, including Vitis vinifera L. ‘White Riesling,’ ‘Chardonnay,’ ‘Pinot noir,’ ‘Merlot,’ ‘Cabernet Sauvignon,’ and ‘Gewürztraminer’; V. labrusca L. ‘Concord’; Vitis interspecific hybrids Aurore and Rosette; and various unnamed seedlings. Vineyard surveys in 1984 were restricted to V. vinifera cultivars on Long Island, where winters are generally milder than in central and western New York and survival of infected buds would have been
more likely to occur. More than 18,000 vines in 26 vineyards were examined in 1984, including the following cultivars: Merlot, Pinot noir, Sauvignon blanc, and White Riesling. No surveys were made in 1985, but in 1986, 1,458 vines of the cultivar Rosette were examined in a single vineyard in the Finger Lakes region. All vineyards were surveyed during late May and early June, when emerging shoots were about 20–90 cm long.

Seasonal pattern of ascospore release. During the autumns of 1982–1985, grape leaves bearing cleistothecia were collected from various cultivars of V. labrusca, V. vinifera, and from Vitis interspecific hybrids. In 1982, leaves were collected from Concord; in 1983, from Chardonnay and Pinot noir and from the Vitis interspecific hybrids Chancellor and Rosette; and in 1984 and 1985, from Rosette. The leaves were cut into 2.5-cm squares, placed in 35-μm polyester mesh bags and were either attached to the north or south face of a black, vertical board in an open field or hung in vines. At 7- to 14-day intervals from late March to late July of 1983–1985, leaf segments were retrieved from the field, dried on the lab bench overnight, and then soaked in distilled water for 1–6 hr. A single wet leaf segment was then placed on the inside surface of each of the lids of 10 glass petri dishes that contained glass slides. The petri dishes were incubated at room temperature for about 24 hr, then the glass slides were examined microscopically after application of the mountant lactophenol. The number of ascospores of B. necator observed on the slides was recorded.

At 7- to 14-day intervals beginning in mid-April 1986, Rosette leaf segments were retrieved from bagged samples overwintered in the vineyard. Cleistothecia were harvested by placing 50 leaf segments in 300 mL of water, shaking vigorously for 1–3 min, and sequentially pouring the resultant suspension through a 50- and 100-mesh Cobb sieve, respectively. The cleistothecia retained by the 100-mesh sieve were resuspended in 100 mL of water that was then divided equally between three 9-cm dishes of filter paper in glass funnels. The cleistothecia were rinsed with water to the centers of the dishes. The wet filter paper disks were placed in the lids of petri dishes containing glass slides. After about 24 hr of incubation at 20°C, the slides were examined microscopically and the number of ascospores of B. necator on the slides was recorded.

In 1986, a Burkard volumetric spore sampler was operated continuously for 40 days after bud burst in a research vineyard near the cultivar Chancellor at the N.Y. State Agricultural Experiment Station in Geneva. The trap sampled 10 L of air per minute. At weekly intervals, the tape was removed from the trap, dissected, mounted in lactophenol on glass slides, and examined hourly (2 mm) intervals at 200X. The number of spores identified as ascospores or conidia of B. necator was recorded. Weather instruments located 10 m from the trap provided hourly records of temperature, humidity, rainfall, and leaf wetness.

Inoculation of detached leaves. Throughout spring in 1983–1985, duplicate samples of leaf segments from collections described above were attached to the inside surface of lids of plastic double petri dishes (16), each containing a healthy, mildew-free leaf from greenhouse-grown grapevines. Mildew-free plants were maintained in the greenhouse by exposure to vapors of penconazole (Topas 10WP) incorporated into cheesecloth panels hung over the plants (20). The detached leaves were from V. labrusca 'Delaware' in 1983, from White Riesling in 1984, and from Chancellor in 1985. A total of 10–15 plates were prepared on each sampling date for each leaf collection. Ten plates containing only detached leaves served as controls. After 18–24 hr of incubation at room temperature, the leaf segments were removed from the lids of the double petri dishes, and the detached leaves were examined microscopically for colonies of B. necator after 5–7 days. The number of colonies on each leaf was recorded.

Scanning electron microscopy. Five to 7-day-old colonies of B. necator, as confirmed by inspection using epifluorescence light microscopy, were excised from the detached leaves used in the inoculation studies and were immediately fixed in 4% glutaraldehyde buffer with 0.05 M KPO₄ at pH 6.5 for 2 hr. The samples were rinsed six times in 0.05 M KPO₄ at 10-min intervals, rinsed twice in distilled water, and subsequently postfixed in 1% OsO₄ for 1.5–2 hr. The leaf pieces were then rinsed in distilled water six times at 10-min intervals, dehydrated in a seven-step acetone series, critical-point-dried, mounted, and sputter-coated with gold before examination and photography.

In April 1986, exfoliating bark collected from the upper trunk, head, and corms of unsprayed vines of the Vitis interspecific hybrid Rougeon was frozen at –15°C. Cleistothecia were later harvested from about 30 g of frozen dry bark in 300 mL of water, transferred as before to moist filter paper, and suspended over mildew-free Chancellor leaves in double petri dishes. Cleistothecia from this bark were also used to inoculate Chancellor tissue culture plants.

Assessment of ascospore viability. Throughout winter and spring of each year of the study, three samples of 10–30 cleistothecia were removed from leaves, cane, bark, or berry clusters, crushed on glass slides, and observed microscopically. The ascospores were categorized as containing immature spores with granular cytoplasm (Fig. 1A), mature spores with vacuolate cytoplasm (Fig. 1B), or degenerate spores containing dark cytoplasm with numerous lipid droplets (Fig. 1C). The percentage of ascospores containing ascospores in each category was recorded.

RESULTS

Vineyard surveys. No flag shoots were found in the vineyard surveys. When powdery mildew was found, it occurred as discrete colonies, generally on the abaxial surfaces of leaves on shoots 15–20 cm and often at the abaxial leaf margin. Most infected shoots were growing from the trunk or the head of the vine. For example, in 1983, 80% of the infected shoots found in the vineyard surveys originated from the trunk or the head of the vine, whereas only 20% of the infected shoots originated from canes. Steep

**Fig. 1.** Immature, mature, and degenerate ascospores of *Uncinula necator*. Cleistothecia were crushed on glass slides and ascospores categorized as containing A, immature spore with granular cytoplasm, B, germinating mature spore with vacuolate cytoplasm, or C, degenerate spores containing dark cytoplasm with numerous lipid droplets.
Seasonal pattern of ascospore release. Ascospores were released from cleistothecia on overwintered leaves beginning in mid to late April of each year and continued to be released for 6–10 wk. Initial spore release coincided approximately with bud burst. About 75% of the ascospores were discharged within 6 wk of bud burst, and 75–100% of the ascospores were released between bud burst and bloom (Fig. 2). Cleistothecia from leaves overwintered on the south face of the vertical board matured and released ascospores before similar samples overwintered on the north face of the board. However, with the exception of a shift in time of about 2 wk, the spore discharge curves were similar both to each other and to those shown in Figure 2.

Ascospores of U. necator were trapped on 11 of the 40 days that the spore trap was operated: 1, 16, 18, 19, 20, 22, 23, 33, 36, 37, and 38 days after bud burst. Each spore release occurred during rain or during a period of leaf wetness initiated by rain. Because the density of ascospores in the vineyard air was near the trapping threshold of the Burkard trap, a more detailed analysis of the results was not possible. Conidia of U. necator were not collected by the trap. The first powdery mildew colonies in this vineyard were found on 29 May 1986, 29 days after bud burst.

Powdery mildew colonies developed on detached leaves in numbers proportional to the number of ascospores observed on glass slides (Table 1). No mildew colonies developed on un inoculated control leaves. Dividing the number of colonies that developed on inoculated leaves by the number of ascospores released from duplicate leaf samples allowed the estimation of the infection efficiency of U. necator ascospores, which ranged from 3.1 to 8.8% under our test conditions using a variety of inoculum sources and host material (Table 1).

Scanning electron microscopy. Mildew colonies excised from inoculated detached leaves and tissue culture plants were found to originate from single spores (Fig. 3A) or groups of spores (Fig. 3B). Spore dimensions, as measured by epiluminescence microscopy before fixation and dehydration, were 25.7 ± 2.4 × 14.7 ± 1.5 μm; these are significantly (P = 0.05) smaller than those of conidia of U. necator (12). Based on these observations, the spores of origin in the center of these mildew colonies were identified as ascospores.

Development of the mildew colony was typical of that observed in conidial inoculations. Ascospores germinated and formed appressoria within 12 hr of inoculation at 20 C. Thereafter, there was a progressive growth and branching of the primary hyphae and formation of lobate appressoria at irregular intervals along the hyphal branches (Fig. 3B). Formation of conidiophores and sporation occurred within 6 days of inoculation on Chandler tissue culture plants.

Assessment of ascospore maturity and viability. Cleistothecia were not only found on all infected plant parts but were also found in large numbers in bark crevices and leaf scars, presumably having been brought by rain (Fig. 4). Cleistothecia on bark were found singly and in clumps. Bark collected from un sprayed Chancellor vines in March and Rosette vines in April 1984 bore an average of 7.1 ± 3.8 and 7.0 ± 2.4 cleistothecia per square centimeter, respectively (P = 0.10). Bark collected from un sprayed Aurore and Rougeon vines in May 1986 bore an average of 51 ± 11.4 and 18 ± 7.4 cleistothecia per gram of dry bark, respectively (P = 0.10). Only a small percentage of the cleistothecia collected from overwintered leaves during 1983–1985 ever produced mature ascospores. From 79 to 85% of the ascospores on leaves died by spring (Table 2) and contained only degenerate ascospores with large lipid inclusions (Fig. 1C). A similar preponderance of dead ascospores was found in limited examinations of canes and berry cluster stems during spring of 1986. Nearly 97% of the ascospores on berry cluster stems collected from Chancellor vines on 28 April 1984 had died without releasing ascospores. heavily infected canes collected from Vitis interspecific hybrid Seyval on 23 April had abundant cleistothecia, but 92% of these cleistothecia had died during winter. Whole mounts, crush mounts, and sections of the ascospores showed no evidence of mechanical damage or mycoparasitism. Conversely, a relatively high percentage of cleistothecia collected from bark were viable. Viability of cleistothecia on bark collected from the cultivars Chancellor,
White Riesling, Seyval, Rosette, Aurore, Rougeon, and Concord in late April and early May 1986 averaged 49, 50, 64, 75, 46, 52, and 45%, respectively.

Periodic assessment of the condition of the contents of cleistothecia on leaves collected from the ground in a Chancellor vineyard showed an initial rapid decrease in the proportion of viable ascospores between early October 1985 (leaf fall) and January 1986 (Fig. 5). No further loss occurred under snow cover during winter, but the degeneration increased at the end of March until 94% of the ascospores had died without releasing ascospores by budbreak in 1986.

**DISCUSSION**

We have demonstrated that cleistothecia of *U. nectar* survive winter in New York and release ascospores the following spring and that these ascospores can infect grape and function as primary inoculum. Although overwintering of mycelium in dormant infected buds has been demonstrated in three viticultural regions (4,13,18,22), this form of overwintering has not been demonstrated in New York in any previous study, nor were we able to find evidence of survival of dormant infected buds in our study. Flag shoots were not found in repeated vineyard surveys. Ascospores, not conidia, of *U. nectar* were detected in vineyard air at the time of primary infection. Finally, the distribution of initial infections in vineyards was not of the type to be expected from intense point-sources of inoculum such as flag shoots. Thus, cleistothecia are the only proven source of primary inoculum in New York. It is possible that buds of certain cultivars may become infected in New York. However, as is the case in apple buds infected by *Podosphaera leucotricha* (Ell. & Ev.) Salm., infection of the buds may preclude their survival of low winter temperatures in New York (6).

The fact that cleistothecia survive winter in New York and produce functional inoculum in spring raises the question of whether these ascospores might also be an additional source of inoculum in other viticultural areas where they are produced (2,3,23-25,27). Degeneration of cleistothecia on leaves, canes, and cluster stems may be one reason for the failure of past studies (2,11,27) to demonstrate the ability of ascospores to infect leaves. Unless large numbers of cleistothecia were used, the small proportion of viable ascospores coupled with an inoculum efficiency of 3-9% would render most inoculations ineffective. The probability of successful inoculation would be reduced even further if attempted at a time of year other than the approximate 6-wk period of spore release.

Seasonal maturation and release of ascospores coincided with availability of host tissue. Ascospore discharge could not be induced before April in repeated tests; however, ascospore maturation was accelerated when leaves were exposed on the south face of a black, vertical board. Therefore, temperature may be one environmental factor that determines the rate of ascospore maturation and the time of ascospore release.

**TABLE 1. Discharge of ascospores of *Uncinula nectar* over glass slides and detached leaves**

<table>
<thead>
<tr>
<th>Year</th>
<th>Cultivar</th>
<th>Host</th>
<th>Total ascospores released on slides</th>
<th>Total no. colonies on leaves</th>
<th>Efficiency of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>Concord</td>
<td>Delaware</td>
<td>478</td>
<td>42</td>
<td>8.8</td>
</tr>
<tr>
<td>1984</td>
<td>Rosette</td>
<td>White Riesling</td>
<td>3,256</td>
<td>161</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Chancellor</td>
<td></td>
<td>1,276</td>
<td>79</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>Pinot noir</td>
<td></td>
<td>619</td>
<td>29</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Chardonnay</td>
<td></td>
<td>280</td>
<td>11</td>
<td>3.9</td>
</tr>
<tr>
<td>1985</td>
<td>Rosette</td>
<td>Chancellor</td>
<td>358</td>
<td>11</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*Overwintered leaf segments bearing cleistothecia were wet for 1-6 hr and then placed in the lids of petri dishes containing glass slides or detached leaves for 18-24 hr.*

*Denotes source of inoculum and host material. Inoculum consisted of cleistothecia borne on overwintered leaves of the specified cultivar. The host was that cultivar used as the detached leaf in the double petri dish.*

*Data are yearly totals of tests performed at 10- to 14-day intervals throughout spring and early summer. There were 10 glass slides and 10 detached leaves exposed per treatment during each discharge and inoculation test.*

**Fig. 3.** Colonization of Chancellor leaf by *Uncinula nectar* ascospores. **A,** Germination and appressorium (arrow) formation from single ascospore 12 hr after inoculation at 20 C. **B,** Mildew colony originating from two ascospores 72 hr after inoculation at 20 C, showing formation of lobate appressoria (arrow).
TABLE 2. Maturity and viability of cleistothecia of *Uncinula necator* on grape leaves

<table>
<thead>
<tr>
<th>Year</th>
<th>Cultivar/</th>
<th>No. ascocarps</th>
<th>Percent ascocarps with mature spores</th>
<th>Percent ascocarps with immature spores</th>
<th>Percent ascocarps with degenerate spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>Concord</td>
<td>560</td>
<td>8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1984</td>
<td>Rosette</td>
<td>321</td>
<td>2.1</td>
<td>14.9</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Chancellor</td>
<td>1,189</td>
<td>0.8</td>
<td>16.2</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Chardonnay</td>
<td>458</td>
<td>5.7</td>
<td>14.3</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Pinot noir</td>
<td>634</td>
<td>4.1</td>
<td>16.9</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Rosette</td>
<td>531</td>
<td>1.5</td>
<td>13.5</td>
<td>85</td>
</tr>
</tbody>
</table>

*Assessments were performed at 2-wk intervals throughout spring. Cultivar on which cleistothecia were borne. No data collected.*

The discovery of relatively large numbers of viable ascospores residing in bark crevices raises some interesting questions regarding the suitability of various plant parts as overwintering substrates or the suitability of bark in retaining those ascospores most likely to survive winter. It has been shown in earlier studies that cleistothecia are readily removed from the mildew colony by rain and are redeposited elsewhere by runoff (1,27). It is possible that mature ascospores are more readily detached and dispersed (16) and that the death of cleistothecia on leaves, canes, and cluster stems observed over winter is actually the decomposition of the immature ascospores remaining on these organs after dispersal of the mature cleistothecia by rain. In any case, the occurrence of large numbers of viable cleistothecia on exfoliating bark from the head and trunk of the vine probably accounts for our consistent observation that most of the mildew colonies found in the vineyard surveys developed on leaves of young shoots emerging from the head and trunk of the vine and growing close to bark.

The significance of functional ascospores in *U. necator* reaches beyond the original question of how this fungus overwinters in New York vineyards. A functional sexual stage creates significant opportunity for variation in the fungus, specifically the development of pathogenic races and development of tolerance of highly selective fungicides such as benomyl (14) and members of the sterol biosynthesis inhibitor group. Pathogenic races have not been reported in *U. necator* but are common in several other powdery mildews. For example, Powers and Moseman (15) reported wide variation in pathogenicity from a collection of isolates derived from single ascospores of *Erysiphe graminis* f. sp. *graminii* and f. sp. *hordei*. They reported as many as three pathogenic races occurring in a single cleistothecium. Furthermore, identification of cleistothecia as the main source of
primary inoculum in New York has suggested new strategies for improving control of grape powdery mildew. In addition to the advantage of being able to identify the first infection periods in spring, the subject of current research, we have used dormant, over-the-trellis applications of lime sulfur in spring to destroy the overwintering cleistothecia on the bark and significantly delay the development of powdery mildew epidemics (R. C. Pearson and D. M. Gadoiry, unpublished). Currently, grape powdery mildew is controlled by repeated fungicide sprays timed according to grape phenology, with little or no regard to production of primary inoculum by the causal fungus (5,7). An approach similar to that proposed for management of apple scab, where inoculum dose, inoculum reduction, and the infection rate are used to forecast the onset of epidemics and initiate spray programs (8,9), may be feasible in management of grape powdery mildew.

**LITERATURE CITED**


