Distribution and Retention of Cleistothecia of *Uncinula necator* on the Bark of Grapevines

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ABSTRACT

Cortesi, P., Gadoury, D. M., Seem, R. C., and Pearson, R. C. 1995. Distribution and retention of cleistothecia of *Uncinula necator* on the bark of grapevines. Plant Dis. 79:15-19.

Clesitothecia of *Uncinula necator* were dispersed by late summer and early autumn rain to the bark of grapevines. Rain-dispersed ascocarps accumulated rapidly on bark during a 10-wk period and were then retained on bark through subsequent rain events between leaf abscission and budbreak the folowing spring. The density of populations on bark was significantly correlated (r = 0.72–0.88) with catches of cleistothecia in filter-paper funnels attached to the trunk of grapevines. The density of populations on bark during winter was not correlated with several measures of disease incidence and severity from the previous growing season. We concluded that while incidence and severity may determine the potential population available for dispersal, rain events determine the actual efficiency of transfer from infected organs to the bark of the vine. The percentage of ascocarps that reacted positively with the fluorescent vital stain fluorescein diacetate ranged from 50 to 62% and did not change significantly during overwintering until cleistothecia began to dehisce in spring. Therefore, the density and viability of populations of cleistothecia on bark at the time of budbreak appear to have been determined at the time of leaf fall the previous autumn and were not modified by subsequent environmental conditions. The densest aggregations of cleistothecia occurred on the cordons of cordon-trained vines, with successively lower densities occurring on the bark of the upper and lower trunks. The pruning and training system of vines of *Vitis labrusca* cv. Concord did not affect the density of populations of cleistothecia on bark.

Additional keyword: oidium

There are two principal sources of primary inoculum for grape powdery mildew: mycelium overwintering in infected buds (10) and cleistothecia (8,9). The role of cleistothecia in the epidemiology of grape powdery mildew has been studied recently in New York vineyards, where they constitute the only source of primary inoculum (9). *Uncinula necator* (Schwein.) Burrill produces cleistothecia under vineyard conditions once the incidence and severity of disease have increased to allow colonies of compatible mating types to merge (2,6). The final event in development of the ascocarps is the necrosis of the hyphal connections to the mildew colony (2). Subsequent rain washes cleistothecia to the bark of the vine and to the vineyard soil (2). Ascocarps die during overwintering in or on soil in New York (2), but most of those on the bark survive winter (2) and release infectious ascospores during spring rains (4,5,8).

We have partially controlled epidemics of grape powdery mildew by eradicating a portion of the cleistothecia overwintering on the bark of the vine (7). Although the disease may often be substantially delayed, our current approach of using high-volume applications of calcium polysulfide is rarely cost-effective (7). We are currently investigating a variety of other means to reduce survival of cleistothecia on bark: fungicidal alternatives to calcium polysulfide, use of the mycoparasite *Ampelomyces quisqualis* Ces., and applications of heat to the trunk of the vine. The efficacy of these methods may be affected by temporal variations in the dispersal of cleistothecia to the bark, persistence of ascocarps on the bark, and the spatial distribution of ascocarps on the trunk of vines. Our objectives in this study were to quantify the dispersal of cleistothecia to the bark of the vine, their distribution on the vine, and their persistence through winter. A portion of this work was reported earlier (3).

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Accepted for publication 27 September 1994.

MATERIALS AND METHODS

Dispersal of cleistothecia during rain events. Funnels prepared from folded 9-cm disks of No. 1 filter paper were attached to the cordons and trunks of unsprayed grapevines at an experimental vineyard in Geneva, New York. Funnels were secured to the vines by pushpins inserted through the top edge of the funnel into the cordon or trunk of the vine. The vineyard was composed of six rows of vines of the *Vitis* interspecific hybrid cultivar Rosette, which were mid-wire cordon-trained and spur-pruned. Four vines were selected at random, and a total of 12 funnels were attached to each vine. Two funnels were attached to each of the two cordons, four funnels were attached to the upper trunk, and four were attached to the lower trunk. Each funnel was examined for cleistothecia at 20–30×, and the number of mature ascocarps was recorded. Funnels were installed on 25 July 1986, when immature ascocarps were detected first, and were replaced after each rain event until 7 October. The study was repeated in the same vineyard from 13 July to 14 October 1987. In both years of the study, 90% of the leaves had fallen from the vines by the date of the last sampling.

Refinement of a method to harvest cleistothecia from bark. The method used to harvest cleistothecia from bark was essentially the same as that used by Pearson and Gadoury (9) for collection of cleistothecia from overwintered leaves, but the method was further refined and the efficiency and reproducibility of the assessments were documented. A 10-g sample of dry bark was collected from each of four unsprayed vines of the *Vitis* interspecific hybrid cultivar Chancellor on 19 February 1992. Vines were established in 1978, spur-pruned, and trained to a mid-wire bilateral-cordon system. The bark was placed in a 2-L Erlenmeyer flask containing 500 ml of water. The flask was shaken vigorously for 3 min, and the resultant suspension was poured into a stack of nested Cobb sieves of 50, 80, 100, 120, 150, 170, and 250 mesh, corresponding to pore sizes of 300, 180, 150, 125, 106, 90, and 63 μm, respectively. Cleistothecia and bark debris collected on each sieve were resuspended in 25 ml of water, and four 5-ml aliquots of the suspension were transferred to four 9-cm filter-paper disks. The cleistothecia on the disks were counted at 64×. The bark remaining in the Erlenmeyer flask then was resupended in 500 ml of water and shaken an additional 60 sec. The suspension was poured into the nested sieves, and the cleistothecia were enumerated as above. This process was repeated for a total of nine rinses of each the four bark samples.

A total of 25 cleistothecia from each sieve in each repetition of the above assessment were transferred to glass microscope slides. A drop of water con-

taining 0.01% (w/v) of the fluorescent vital stain fluorescein diacetate and a coverglass were added to the slide, and the ascocarps were crushed to expose the asci to the stain. After 5 min, slides were examined at 80× under fluorescence microscopy as described by Widholm (11). Cleistothecia were considered viable if 50% of the visible ascospores displayed a bright green fluorescence.

Persistence of cleistothecia on bark. Four 20-g samples of bark were collected from vines chosen arbitrarily in the Chancellor vineyard at 10-day intervals from February to June 1992. Monthly collections were again made from September 1992 until March 1993, and bark was collected at 14-day intervals during April and May 1993. Cleistothecia were harvested in water as described above, using an agitation time of 3 min in the first washing and of 60 sec in three additional washings. The suspension was poured through nested 50- and 120-mesh sieves. The cleistothecia collected on a 120-mesh sieve were resuspended in 100 ml of water, four 5-ml aliquots of the suspension were poured on filter paper, and the ascocarps were counted as above. The viability of the cleistothecia was determined for each sample as previously described. Because the mature cleistothecia may dehisce when wet (4), the number of dehiscent ascocarps also was recorded.

Distribution of cleistothecia on cordons and trunks. A 10-g sample of bark was collected separately from the cordons, the upper half of the trunk, and the lower trunk of four randomly selected two-vine plots in the Chancellor vineyard on 30 March 1992. The experiment was repeated on 10 April 1992.

Bark was also collected on 8 May 1992 from vines of *V. labrusca* L. 'Concord' in Fredonia, New York. Rows of the vineyard were trained to a top-wire cordon system, which was either hand-pruned or hedged, or vines were trained to an umbrella Kniffen system and were hand-pruned. The bark was taken from the cordon, upper trunk, and lower trunk of cordon-trained vines or from the upper and lower trunk only for umbrella Kniffen-trained vines. Samples consisting of 7.5 g of dry bark were collected from four randomly selected four-vine plots of each pruning and training system.

Cleistothecia borne on bark from both the Chancellor and Concord vines were harvested and enumerated as described in the study on persistence of cleistothecia.

Direct and indirect estimates of inoculum dose. In August 1988, filter-paper funnels were attached to the trunks of grapevines in 14 New York vineyards. The vineyards consisted of seven plantings of *V. vinifera* L. 'Chardonnay' that were umbrella Kniffin-trained and hand-pruned, four plantings of the *Vitis* interspecific hybrid cultivar Seyval, one planting of the *Vitis* interspecific hybrid cultivar Rosette, and two plantings of Concord that were top-wire cordon-trained and hedge-pruned. At each vineyard, 10 vines were selected arbitrarily and two funnels were attached to the upper trunk of each vine. Funnels were installed prior to the initial detection of

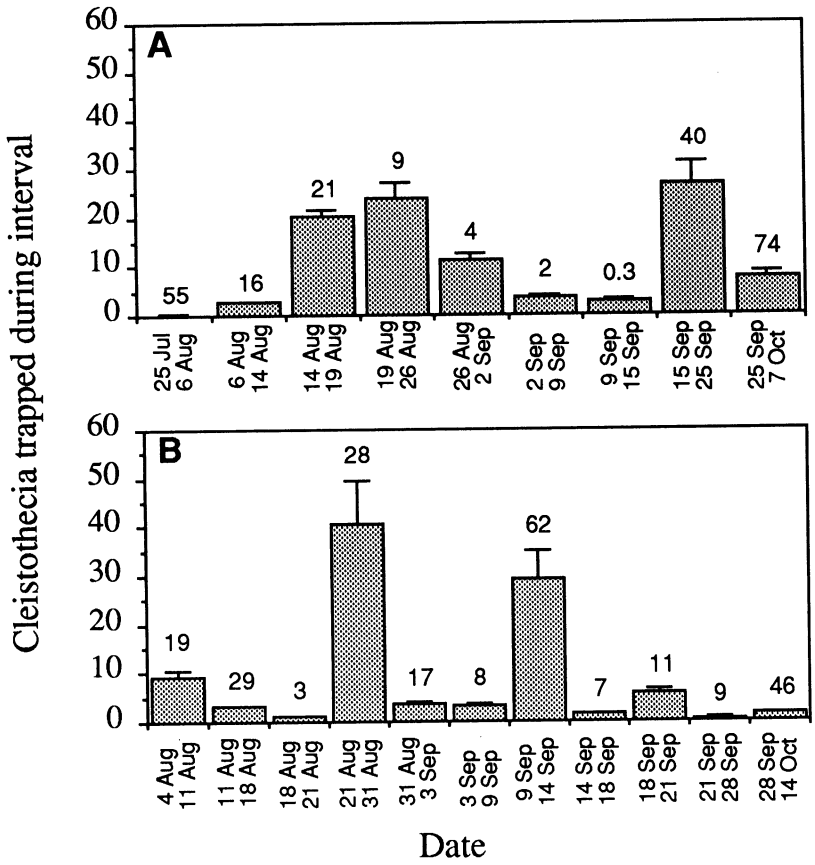

Fig. 1. Capture of rain-dispersed cleistothecia of *Uncinula necator* in funnels attached to Rosette grapevines in **(A)** 1986 and **(B)** 1987. Means of four replications of 12 funnels per vine on each sampling date. Bars indicate one standard error. Numbers above bars indicate millimeters of rain recorded in the vineyard during the time the funnels were present.

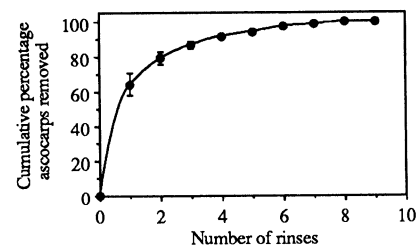

Fig. 2. Cumulative recovery of cleistothecia of *Uncinula necator* in Cobb sieves from sequential rinses of bark of grapevines.

Table 1. Collection of cleistothecia of *Uncinula necator* from grapevine bark on nested Cobb sieves of various mesh sizes and the viability of cleistothecia captured at each sieve

Cobb sieve number	Mesh size (μm)	Cleistothecia collected (%)	Cumulative cleistothecia collected (%)	Cleistothecia viability (%)
50	300	6.0	6.0	78.7 (4.10)[z]
80	180	24.8	30.8	77.3 (1.97)
100	150	21.5	52.3	81.6 (3.48)
120	125	26.6	78.9	64.8 (3.62)
150	106	16.3	95.3	60.7 (3.95)
170	90	4.7	100.0	9.3 (3.51)
250	63	0.0	...	...

[z] Mean of four replicated samples of bark followed by the standard error of the mean in parentheses.

Table 2. Density of populations and viability of cleistothecia of *Uncinula necator* on bark from various locations on Chancellor grapevines

Location of bark	Cleistothecia/g	Viable cleistothecia (%)
Cordon	136.9 a[z]	66.4 a
Upper trunk	81.4 b	58.8 b
Lower trunk	60.1 c	46.1 c

[z] Means followed by different letters are significantly different according to the Waller-Duncan k-ratio t test ($P \leq 0.05$).

mature cleistothecia at each site and were removed once leaf fall exceeded 90%. The funnels were examined at 20–30× and the number of cleistothecia of *U. necator* was recorded. In March 1989, approximately 2 mo prior to budbreak, 10 vines were selected arbitrarily at the above sites and 10 g of bark was collected from the trunk of each vine. The bark was shaken in water and the cleistothecia in the resultant suspension were enumerated as described above.

Weekly to biweekly records of the foliar incidence and severity of powdery mildew (infected leaves per shoot and percentage of leaf surface infected) were available for each site for the period preceding the installation of the funnels. Data were analyzed by linear regression (1). The inoculum density at each site, expressed as the mean number of cleistothecia per kilogram of bark, was regressed against the following independent variables: 1) mean number of cleistothecia per funnel, 2) foliar disease incidence at bloom, 3) foliar disease severity at bloom, 4) foliar disease incidence at *véraison*, 5) foliar disease severity at *véraison*, 6) area under the foliar disease progress curve for incidence, and 7) area under the foliar disease progress curve for severity. The study was repeated in the autumn of 1989 and spring of 1990 in 18 vineyards, which included the 14 vineyards described above and four plantings of *V. vinifera* cv. Riesling that were umbrella Kniffen-trained and cane-pruned.

RESULTS

Dispersal of cleistothecia during rain events. Cleistothecia were captured in funnel traps during each rain event in both years of the study (Fig. 1). The cumulative catch per funnel was 4,858 in 1986 and 519 in 1987. Funnels on the cordons, upper trunk, and lower trunk caught equivalent ($P = 0.05$) numbers of ascocarps, both when individual rain events were analyzed separately and when an entire season's data were pooled. The percentage of cleistothecia collected in the funnel traps between collection dates varied greatly but was not related consistently to time after intial detection of cleistothecia, total rainfall, or occurrence of frost on grapevine foliage between collection dates (Fig. 1).

Refinement of a method to harvest cleistothecia from bark. The mean number of cleistothecia per 10-g bark sample collected from the Chancellor vineyard on 19 February 1992 was 2,702 ± 464 ($P = 0.05$). The removal of ascocarps in serial rinses was well fit by a quadratic function (Fig. 2). Over 60% of the ascocarps were removed in the first rinse, and 91% had been removed by the fourth rinse (Fig. 2). Nearly all cleistothecia passed through the 50-mesh sieve, while similar percentages (16–26%) were trapped in the 80-, 100-, 120-, and 150-mesh sieves (Table 1). Less than 5% of the cleistothecia passed through the 150-mesh sieve, and none passed through the 170-mesh sieve (Table 1). Fine bark debris and silt were retained in the 150-mesh sieve and to some degree in all smaller sieves. Viability of cleistothecia collected in the 50-, 80-, and 100-mesh sieves was significantly ($P = 0.05$, Student's t test, df = 3) higher than in the smaller mesh sieves (Table 1). Of the cleistothecia that passed the 150-mesh sieve, only 9.3% were viable (Table 1).

Distribution of cleistothecia on cordons and trunks. In the Chancellor vineyard, significantly more cleistothecia were found on bark from cordons than on bark from the upper trunk (Table 2), and bark from the upper trunk bore more ascocarps than bark from the lower trunk

Table 3. Density of populations of cleistothecia of *Uncinula necator* on bark from various locations on Concord grapevines under different pruning and training systems

Training system	Pruning system	Location of bark	Cleistothecia/g
Top wire cordon	Hand	Cordon	7.26 ± 1.47[z]
		Upper trunk	6.29 ± 1.53
		Lower trunk	4.69 ± 1.51
	Hedged	Cordon	6.62 ± 1.07
		Upper trunk	4.18 ± 1.16
		Lower trunk	3.52 ± 0.97
Umbrella Kniffen	Hand	Upper trunk	5.83 ± 1.05
		Lower trunk	6.96 ± 1.65

[z] Mean and 95% confidence interval of the number of cleistothecia per gram of bark.

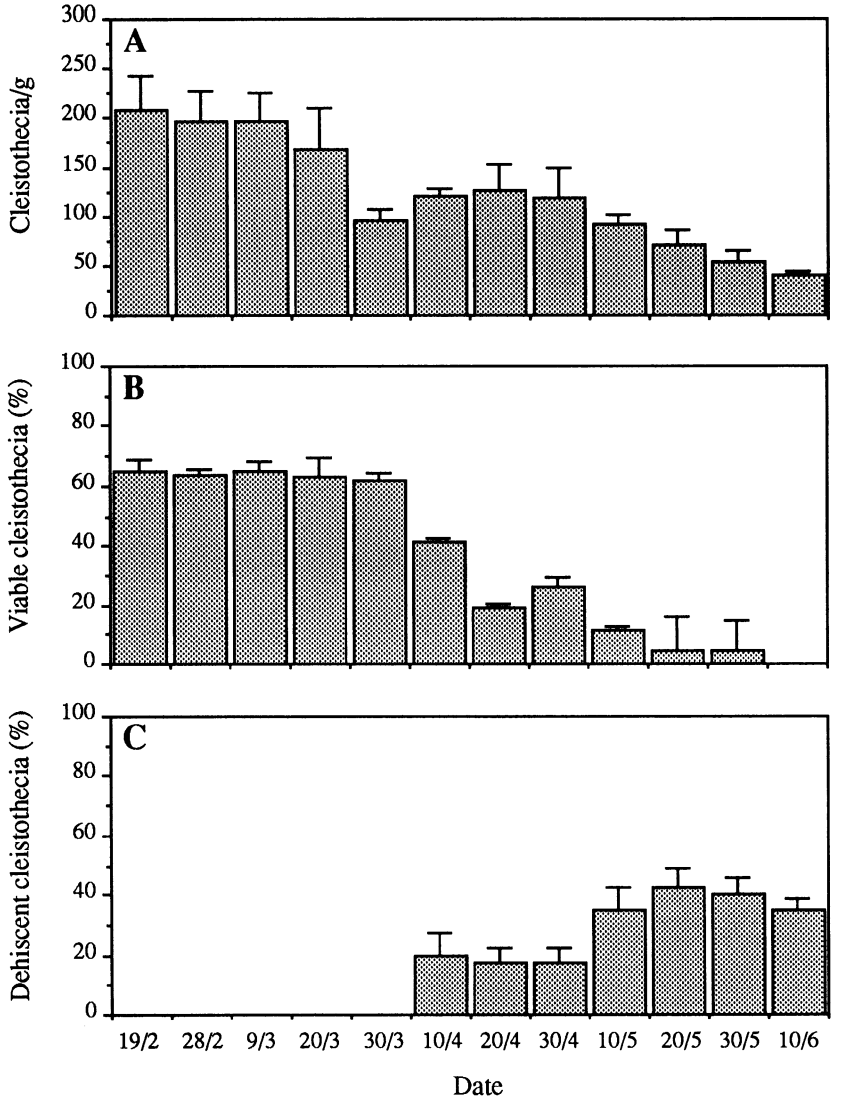

Fig. 3. (A) Density of overwintering populations of cleistothecia of *Uncinula necator* on bark of Chancellor grapevines in 1992, **(B)** percentage of the cleistothecia that were viable on the date of assessment, and **(C)** percentage of the viable cleistothecia that dehisced in suspension during the assessment.

(Table 2). A similar decrease in the percentage of viable cleistothecia was also observed among bark collected from cordons, the upper trunk, and the lower trunk (Table 2).

On top-wire cordon-trained Concord grapevines, bark from cordons of both hand-pruned and hedge-pruned vines bore denser populations of cleistothecia than bark collected from trunks (Table 3). Bark from the lower trunk of cordon-trained vines bore the fewest cleistothecia. However, no difference in the density of populations of cleistothecia was recorded between bark from the upper trunk and the lower trunk of umbrella Kniffen-trained vines (Table 3). Because of the differences in orientation between cordons and trunks (horizonatal vs. vertical), regression of density of cleistothecia against height aboveground was not attempted.

Persistence of cleistothecia on bark. Once entrapped on the bark of the vine, cleistothecia persisted through winter. In 1992, no significant change in density of populations occurred until 30 March, shortly before a decline in viability and an increase in the percentage of dehiscent ascocarps was observed (Fig. 3). Thereafter, the number of cleistothecia recovered from bark samples and the viability of recovered cleistothecia steadily declined, as the percentage of dehiscent cleistothecia rose to over 40% (Fig. 3). Dehiscence of cleistothecia in bark suspensions on 10 April 1992 preceded budbreak of Chancellor grapevines in Geneva by 4 wk. Depletion of the supply of nondehiscent viable cleistothecia on 10 June 1992 preceded bloom of Chancellor grapevines by 8 days.

In 1992, the density of the population of cleistothecia increased between 4 September and 1 October (Fig. 4). Leaf fall was complete by the time of the third assessment on 3 November, and no subsequent increases were observed. A decrease in ascocarp density was recorded on 5 February 1993, but assessments made on 4 March and 5 April did not differ significantly from previous fall and winter assessments (Fig. 4). A consistent reduction in ascocarp numbers and in the percentage of viable ascocarps was observed after cleistothecia began to dehisce on 5 April (Fig. 4). Dehiscence of cleistothecia in bark suspensions on 5 April 1993 preceded budbreak of Chancellor grapevines in Geneva by 4 wk. By 25 May, when shoots on Chancellor vines were approximately 15 cm long, the percentage of viable cleistothecia had declined to less than 10%.

Direct and indirect estimates of inoculum dose. The number of cleistothecia trapped in funnels was the only independent variable that was significantly ($P = 0.05$) correlated with the density of populations of cleistothecia on grapevine bark in both years of the study (Fig. 5). There was a significant linear relationship between the $\log_{10}$ of the number of cleistothecia per kilogram of bark and the mean number of cleistothecia captured per funnel (Fig. 5). Both slope and intercept terms differed significantly between the 2 yr of the study, but in neither year was the regression significantly affected when data from umbrella Kniffen-trained vines was analyzed separately from the top-wire cordon-trained vines.

DISCUSSION

The use of Cobb sieves was an efficient method to collect cleistothecia from bark suspensions when the bark sample were rinsed three or four times (Fig. 2). We recommend the use of a pair of Cobb sieves of mesh size 50 subtended by either a 120- or 150-mesh sieve for the collection and enumeration of cleistothecia of *U. necator* from leaf and bark samples. If

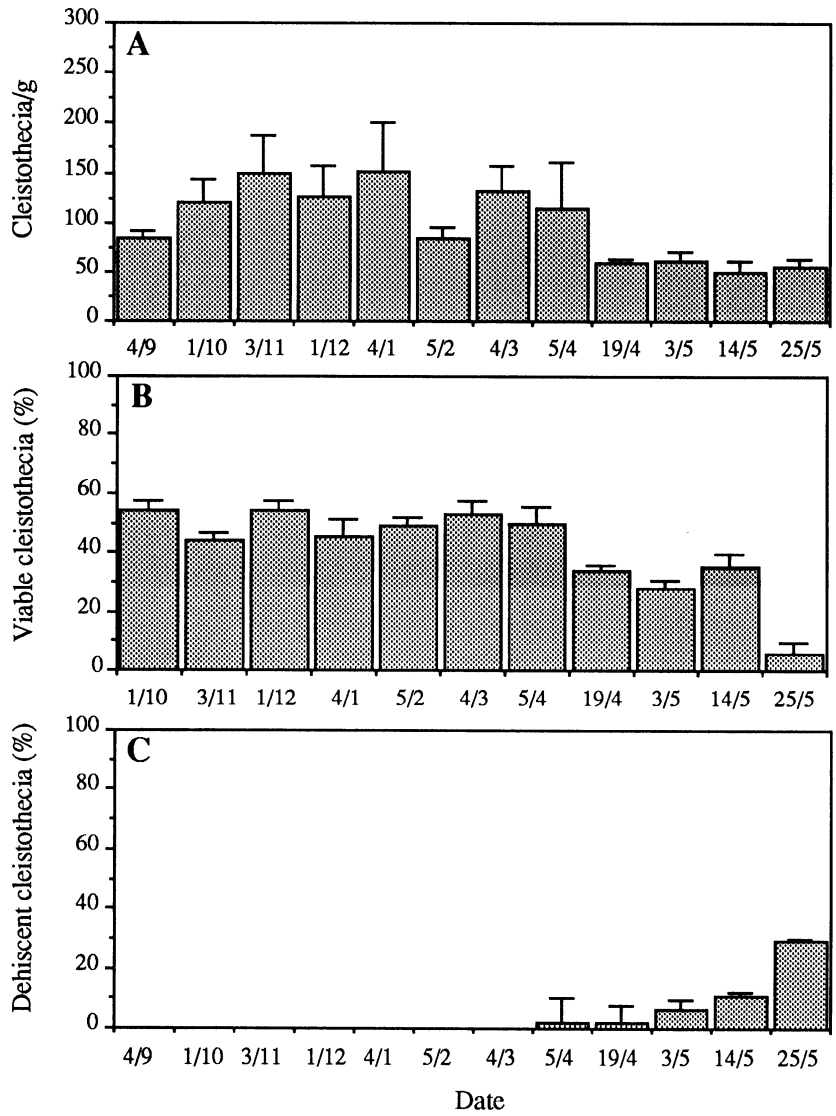

Fig. 4. (A) Density of overwintering populations of cleistothecia of *Uncinula necator* on bark of Chancellor grapevines from autumn 1992 to spring 1993, **(B)** percentage of the cleistothecia that were viable on the date of assessment, and **(C)** percentage of the viable cleistothecia that dehisced in suspension during the assessment.

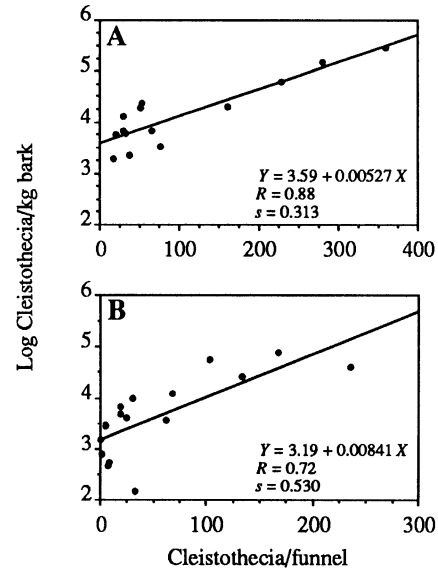

Fig. 5. Relationship between the $\log_{10}$ of the number of cleistothecia of *Uncinula necator* surviving winter on the bark of grapevines and the number of cleistothecia trapped during dispersal in autumn by funnels attached to the grapevine trunks in **(A)** 1989 and **(B)** 1990.

selective collection and removal of viable cleistothecia from suspensions is a primary objective, then the use of the 120-mesh sieve presents the advantage of retaining less silt and fine particulate matter, which makes the identification of ascocarps less tedious. The principal advantage of the 150-mesh sieve is that it retains nearly all cleistothecia, whereas approximately 20% pass through the 120-mesh sieve and are lost (Table 1). If parasitism of cleistothecia by *A. quisqualis* is of interest, we recommend the use of the 150-mesh sieve to retain parasitized immature ascocarps.

Mature cleistothecia of *U. necator* readily disperse in rain from infected tissues. Necrosis of anchorage hyphae on individual ascocarps was observed in earlier studies (2) and appears to be a prerequisite to the detachment of a cleistothecium during rain. Variations in the number of cleistothecia in the above stage at the time of a rain event may account for the observed lack of correlation between accumulated rain and numbers of ascocarps trapped (Fig. 1). Once removed from infected tissue by rain, cleistothecia were collected in equal numbers by traps on the cordons, upper trunk, and lower trunk. However, bark on cordons consistently retained a higher density of ascocarps than bark on the trunk, irrespective of pruning and training systems, perhaps because of the horizontal orientation of the cordons, as compared with the vertical trunks. The proximity of cleistothecia on the cordons to emerging shoots and developing fruit may be significant if it results in a greater probability of ascospores being intercepted by plant tissue.

While densities of cleistothecia on trunks were lower than those observed on cordons, the greater surface area of the trunk may more than offset the observed differences in density. Because cordons are commonly less than one-half the diameter and length of the trunk, a greater number of cleistothecia per hectare may reside on the trunk of the vine, even though the density is higher on the cordons. The differences in density were generally on the order of two- to threefold and were not so great that either source of inoculum should be ignored. Therefore, cleistothecia on both the cordons and trunk should be targets of any eradicant treatments to destroy overwintering ascocarps (7).

Estimation of the density of populations of clesitothecia on grapevine bark made at any time between leaf fall and budbreak should provide an equivalent measure of potential inoculum dose. Density of populations on bark did not change substantially during overwintering, despite frequent and heavy rains. Decreases in density were related to the dehiscence of ascocarps as they matured near budbreak, and dehiscent ascocarps were continually removed from samples, resulting in an apparent decline in numbers of cleistothecia in late spring. We found no evidence of substantial loss of ascocarps from bark during overwintering due to rain, other than the aforementioned decline due to dehiscence of cleistothecia during rain.

Trap catches provide the best indirect measure of potential inoculum dose. No measure of disease from the previous season was as highly correlated with numbers of ascocarps on bark during overwintering. This may reflect an effect of rainfall timing, amount, and intensity upon the efficiency of transfer to and deposition on bark. Disease incidence, severity, and duration could all be expected to influence the numbers of ascocarps formed on diseased tissues based on earlier studies (2). However, rain events may differ in the efficiency with which they transfer ascocarps to bark of grapevines, thereby accounting for the high correlation of trap catches (which measure dispersed numbers) and the low correlation of various measures of disease (which measure only potential for dispersal) with density of ascocarps on grapevine bark.

ACKNOWLEDGMENTS

We thank the New York Wine and Grape Foundation and the New York State IPM Program for their support of this research. The first author was supported by a fellowship from the National Research Council of Italy.

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