Ascocarp Dehiscence and Ascospore Discharge in *Uncinula necator*

David M. Gadoury and Roger C. Pearson

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

Portions of this research were funded by grants from the New York Wine and Grape Foundation.

We are grateful for the technical assistance of Lori Pscheidt, Christine Senack, Diane Colasurdo, and for the assistance of T. Bourette and J. Alvernaz in preparing materials for electron microscopy.

Accepted for publication 29 September 1989 (submitted for electronic processing).

ABSTRACT

Gadoury, D. M., and Pearson, R. C. 1990. Ascocarp dehiscence and ascospore discharge in *Uncinula necator*. Phytopathology 80: 393-401.

Cleistothecia of *Uncinula necator* were hemispherical with a basal concavity unless in contact with free water. Upon wetting, the cleistothecia swelled to become spherical within 2 min. Water potential of ascospore cytoplasm decreased during maturation and overwintering, presumably resulting in increased pressure potential when intact cleistothecia were wet. Water potential of ascospore cytoplasm decreased from −670 KPa in September, 2 wk after detachment of cleistothecia from the mildew colony, to −2,990 KPa in May, 8 mo after leaf fall. The mass required to fracture the ascocarp wall also decreased during maturation, from approximately 3.0 g in autumn to 2.0 g in winter to 1.5 g in early spring. The most rapid decrease in the strength of the ascocarp wall occurred during a 4-wk period in early spring just before dehiscence in the field. This decrease occurred without a commensurate decrease in cytoplasmic water potential. Water potential of cytoplasm decreased when young ascocarps were incubated at 20 C, but not at 4 C. Flexing of the ascocarp wall during repeated cycles of wetting and drying at 4 C did not significantly weaken the ascocarp wall. Cleistothecia dehisced circumscissilely at the junction of the concave and convex surfaces of the outer ascocarp wall, near the site of a thin zone in the ascocarp wall, immediately beneath the equatorially attached appendages. Transmission electron micrographs of cleistothecia collected at various times during overwintering showed decreases in the quantity and changes in the distribution of cytoplasmic lipids as water potential decreased during maturation. Reducing sugars were not present at detectable levels in cleistothecia in autumn, but were detected in spring. Following ascocarp dehiscence, ascospores were discharged through a slitlike rupture of the apex of the ascus. In laboratory tests, the proportion of cleistothecia that dehisced after 4 hr was directly proportional to temperature between 10 and 32 C. Some cleistothecia dehisced within minutes of wetting, but the greatest proportion of cleistothecia dehisced within 4 hr after wetting. There was little change in the number of dehiscent ascocarps between 24 and 48 hr after wetting. Ascocarp dehiscence was nearly completely suppressed at 4 C. Initial dehiscence and ascospore release in vineyards coincided with bud burst of grapevines and continued for approximately 5 wk. Ascospores of *U. necator*, collected by a volumetric trap in a vineyard for two seasons, were trapped between bud burst and bloom of grapevines only during or immediately following rains of more than 2.5 mm. The anamorph of *U. necator* is widely regarded as a xerophytic plant pathogen, and rain generally is considered to be deleterious to development of epidemics of grape powdery mildew. However, rainfall appears to be a critical event in the release of ascospores and the initiation of powdery mildew epidemics in areas where cleistothecia are sources of primary inoculum.

Additional keywords: cleistocarp, *Oidium tuckeri*.

Although the Erysiphaceae comprise a group of major plant pathogens, fundamental knowledge of physiological and environmental factors affecting ascocarp dehiscence and ascospore discharge is incomplete. Upon close examination this is not surprising, because functional ascocarps have been described for relatively few powdery mildews (30). Cleistothecia of *Uncinula necator* (Schw.) Burr. only recently have been shown to be a significant source of primary inoculum for powdery mildew of grape (25). In New York, cleistothecia of *U. necator* form on infected tissues in mid- to late summer and are dispersed by rain to the bark of the vine, where they overwinter (8). In spring, cleistothecia discharge ascospores that infect the emergent leaves (25). Ascospore release appears to be restricted to a relatively brief period between bud burst and bloom of grapevine in New York (25). Most studies that have specifically dealt with ascocarp dehiscence and ascospore release in the Erysiphaceae have examined the effects of temperature and moisture on the occurrence of these events under laboratory conditions (3,4,18,22,28,35) or have described the unique form of ascocarp dehiscence in *Phyllactinia* (2). Although free water has been reported to induce ascocarp dehiscence and ascospore release (4,17,27,28,34–36), it is not clear whether the mechanism of spore release is related to imbibition, osmosis, or both. Reports of ascospore release at high relative humidities (22,31) would indicate that perhaps free water is unnecessary for dehiscence of cleistothecia. Martin et al (21) stated that the highly melanized cells of the ascocarp wall of *Sphaerotheca mors-uvae* were nearly impervious to aqueous fixatives, thus, the permeability of the ascocarp wall to water was questionable.

Cleistothecia of powdery mildews apparently can function in overwintering (29) and oversummering (31), but the factors that lead to the conservation of inoculum during the intercrop period and the release of inoculum as the host resumes growth are unknown. The conversion of glycogen to sugars in the cytoplasm, resulting in an increase in cytoplasmic pressure potential, has been reported as a general mechanism for spore discharge in the ascomycetes (11,12,32), and, in conjuction with a weakening of the ascocarp wall, specifically for *S. mors-uvae* (14). However, neither the strength of ascocarp walls nor cytoplasmic water potentials were measured in these studies. Further, the iodine stains that were used to identify glycogen (11,14,32) are nonspecific and stain a variety of cytoplasmic substances in addition to glycogen (24). Jackson and Gay (13) analyzed some of the chemical constituents of cell walls of mycelium and intact ascocarps of *S. mors-uvae*, identified bacteria and fungi that occasionally were found within the lumina of dead cells of the outer ascocarp wall, and speculated that microbial degradation of cell wall constituents might be a requisite for ascocarp dehiscence. However, in no investigation of cleistothecia of the Erysiphales have cytoplasmic water potential and strength of the ascocarp wall been measured directly. Unless it is demonstrated that these factors actually change during development, their role in ascocarp dehiscence and ascospore discharge will remain unknown. In this study, we describe some of the anatomical, cytological, and physiological changes that precede dehiscence of cleistothecia of *U. necator*, we report on cytoplasmic water potential and strength of the ascocarp wall during overwintering, and report the effects of

temperature and wetting upon the mechanism of ascocarp dehiscence and ascospore discharge under controlled conditions and in vineyards.

MATERIALS AND METHODS

Strength of the ascocarp wall. Cleistothecia were collected from the exfoliating bark of grapevines as described previously (25) at 2- to 4-wk intervals from September to May 1985–1986 and from August to April 1986–1987. The following technique was devised to measure the resistance of hydrated cleistothecia to crushing. Single cleistothecia were placed in a drop of distilled water containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, MO) on a glass microscope slide. A 1-cm^2 coverglass was placed over the cleistothecium and additional distilled water was added dropwise until the coverglass was submerged, centered over, and rested upon the cleistothecium. The slide was transferred to the pan of an analytical balance, the balance was tared, and the point of a fine scalpel attached to a micromanipulator was used to apply pressure to the coverglass directly over the ascocarp while the cleistothecium was observed through a stereomicroscope (70×) suspended over the balance. The weight at which the ascocarp wall fractured was recorded for each of 20–30 cleistothecia from each collection date.

Water potential of ascospore cytoplasm. The water potential of ascospore cytoplasm was determined by two means. The point of incipient plasmolysis (1) in solutions of NaCl was determined in the first method. Cleistothecia were collected as before, and individual ascocarps were mounted in approximately 50 μl of saline solution on a glass slide, crushed, and the ascospores were observed under magnification (400×) for evidence of plasmolysis. The second method used was a measurement of cell distension (1) in solutions of NaCl. Five ascospores from each of 30 cleistothecia were examined. Slides were examined within 3 min of preparation to avoid effects due to concentration of the saline solutions as the mountant dried. Cleistothecia removed from leaves in September 1986, approximately 2 wk after the ascocarps had matured in a vineyard of the *Vitis* interspecific hybrid cultivar Rosette, and cleistothecia collected from the bark of the same vines 8 mo later, in May 1987, were crushed in distilled water and 0.2, 0.4, 0.6, 0.8, and 1.0 M NaCl. The water potentials generated by these solutions were 0; −670; −1,262; −1,838; −2,411; and −2,990 KPa (33). Cleistothecia collected from bark of Rosette vines on 21 August, 15 November, and 22 December 1986, and 10 February, 5 March, and 7 April 1987 were crushed in distilled water and in 0.5 and 1.0 M NaCl.

Effects of cyclical wetting upon strength of the ascocarp wall. Cleistothecia collected from the bark of grapevines in September 1987 were subjected to a wetting interval of 6 hr per 24-hr period at temperatures of 4 and 20 C. Wetting intervals were achieved by enclosing cleistothecia within 2-cm^2 envelopes of 170-mesh nylon screen that were attached to 5-cm arms on a clock cylinder. The cylinder rotated once every 24 hr, and was mounted horizontally above a pan of water. Depth of water in the pan was adjusted such that one-fourth of the arc circumscribed by an arm on the clock cylinder (or 6 hr) passed through the water in the pan. At 7- to 14-day intervals, the point of incipient plasmolysis of ascospore cytoplasm and the strength of the ascocarp wall was determined for 20–30 cleistothecia selected from three envelopes subjected to each temperature-wetting treatment.

The effects of cyclical wetting over longer periods were examined in a modification of the above experiment. Cleistothecia collected from the bark of grapevines on 5 October 1988 were placed on filter paper in petri plates, incubated at 4 and 20 C, and were wet every 3, 7, or 14 days. To wet the ascocarps, 2 ml of distilled water was added to each plate. After 12 hr, the filter paper was blotted and allowed to air dry for 1 hr at 4 or 20 C. Thus, treatments consisted of a 12-hr wetting at 3-, 7-, or 14-day intervals at 4 and 20 C. On 4 January and 6 April 1989, ascocarp wall strength and the point of incipient plasmolysis of ascospore cytoplasm were determined for 30 cleistothecia selected from three plates subjected to each temperature-wetting treatment.

Electron microscopy and cytochemistry. Intact ascocarps were impervious to Epon and Spurr's resins (Electron Microscopy Sciences, Fort Washington, PA), which did not penetrate beyond the first cell layer of the ascocarp wall, despite the use of graded infiltration series and infiltration times of up to 5 days. Therefore, cleistothecia collected in August and January were fixed in 4% glutaraldehyde buffered with 0.05 M KPO_4 at pH 6.5 for 2 hr. The ascocarps were rinsed six times in 0.05 M KPO_4 at 10-min intervals, and a small fracture was made in the ascocarp wall with the apparatus described in the above study of ascocarp wall strength. Cleistothecia then were postfixed in 1% OsO_4 in 0.05 M KPO_4 for 2 hr, rinsed six times in distilled water at 10-min intervals, dehydrated in an acetone series, embedded in Spurr's resin, and sectioned on an ultramicrotome. Ultrathin sections were observed under transmission electron microscopy, and thick sections were observed under light microscopy. Cleistothecia collected in May were placed on wet filter paper and were observed under a stereomicroscope (50×). As cleistothecia dehisced, they were immediately transferred to 4% glutaraldehyde in 0.05 M KPO_4 for fixation, and were subsequently rinsed, postfixed, embedded, and sectioned as above. Thus, collections were composed of cleistothecia in three stages of development: cleistothecia recently formed and less than 1 mo old (August collection); cleistothecia approximately 6 mo old (January collection); and overwintered, 10-mo-old cleistothecia at the stage of dehiscence (May collection).

Large amounts of a yellow, water-insoluble substance were exuded in water mounts of young cleistothecia that were crushed and observed under the light microscope. In previous reports, this substance was identified as a lipid by reaction with Sudan Black B (7,8). Distribution and quantity of lipids in cleistothecia were determined by extracting the fixed lipids from thin sections with sodium methoxide in methanol and benzene (5) and comparing the extracted sections with unextracted serial sections under the transmission electron microscope. Micrographs of extracted ascospores were electronically analyzed with a video camera (Newvicon Series 66, Dage-MTI, Inc., Michigan City, IN) linked to an image processing system (FD5000, Gould Electronics, San Jose, CA). This system allowed the identification of areas occupied by lipid bodies in micrographs of extracted ascospores and computed the cross-sectional area of ascospore cytoplasm that was composed of lipid. Micrographs of sections of 20 ascospores from five cleistothecia were analyzed from each collection date.

To test for the presence of glycogen, 20 cleistothecia from each collection were mounted in Lugol's iodine (24) on microscope slides. Cleistothecia were crushed to reveal intact asci with ascospores, examined at 400×, further crushed to release ascospores from the asci, and then were examined again at 400×. Presence of glycogen in embedded material was determined by

TABLE 1. Mass required to fracture the wall of cleistothecia of *Uncinula necator*

Year	Date	Mass (g)[a]
1985–1986	1 September	3.21
	14 April	2.17*
	29 April	2.08
	13 May	1.63*
1986–1987	20 August	2.94
	15 November	2.22*
	22 December	2.27
	10 February	2.23
	5 March	2.06*
	23 March	2.09
	7 April	1.89*
	28 April	1.55*

[a]*Indicates a significant decrease in mass from previous collection date ($P = 0.05$).

digesting the glycogen in thin sections with amylase (1,4-α-D-glucan glucanohydrolase, Sigma Chemical Co., St. Louis, MO). Thin sections on platinum grids were floated on a droplet of amylase (0.5 mg/ml) in 0.05 M KPO_4 buffer at pH 6.5 for 4 hr at 20 C. Sections then were floated on several changes of distilled water and were dried overnight in a dessicator. Amylase-treated and untreated serial sections of clesitothecia were compared by transmission electron microscopy. Micrographs of treated and untreated sections were analyzed electronically as above, and the cross-sectional area of ascospore and ascus cytoplasm that was composed of glycogen deposits was determined.

The cytoplasm of cleistothecia from the August and May collections was analyzed for the presence of reducing sugars by reaction with phenylhydrazine hydrochloride and sodium acetate in a modification of a technique described by Mangham (20). Five milligrams of cleistothecia was suspended in 10 μl of distilled water, crushed with a mortar and pestle, and 5μl of the resultant suspension was filtered and transferred to a glass microscope slide. Five microliters of 10% (w/v) phenylhydrazine hydrochloride in glycerol and 5 μl of 10% (w/v) sodium acetate in glycerol was added to the slide, the droplets were mixed, and the slide was heated over a boiling water bath for 30 min. A coverglass was placed over the droplet and the slides were stored at 25 C for 7 days. Slides were examined at 400× magnification for the presence of phenylosazone crystals. Three samples of cleistothecia were processed from each of the two collection dates. Statistical differences between treatment means in this experiment, and in preceding experiments, were determined by t tests at $P = 0.01$, 0.05, or 0.10.

Dehiscence of cleistothecia also was observed by light microscopy. Dehiscent cleistothecia were prepared for scanning electron microscopy as described by Pearson and Gadoury (25).

Effects of temperature on dehiscence of cleistothecia. Bark was collected from vines of the *Vitis* interspecific hybrid cultivar Rougeon on 16 days in May 1986. Cleistothecia harvested from each bark collection were transferred to filter paper in petri plates. Twenty cleistothecia were placed in each plate, the filter paper was wet with distilled water, and three plates were placed at each of the following temperatures: 4, 10, 16, 20, 25, and 32 C. At 2, 4, 24, and 48 hr after wetting, the number of dehiscent cleistothecia was recorded.

Ascospore discharge in vineyards. A Burkard volumetric spore sampler (Burkard Manufacturing Ltd., Rickmansworth, Hertsforshire, U.K.) was operated continuously in 1986 and 1987, beginning 2 wk before bud break and continuing until 4 wk after bloom. In 1986, the spore sampler was placed between the rows of a vineyard of the *Vitis* interspecific hybrid cultivar Chancellor trained to a mid-wire cordon system, and was surrounded by four bundles containing a total of 30 grapevine trunks. The trunks were collected the previous fall in a vineyard of *Vitis vinifera* 'Riesling', and it was estimated that each trunk bore several thousand cleistothecia of *U. necator* (D. M. Gadoury, *unpublished*). In 1987, the sampler was placed under the trellis in a hedged vineyard of the cultivar Rosette. Both Chancellor and Rosette are very susceptible to powdery mildew, were severely diseased in the season preceding the placement of the spore sampler, and, presumably, harbored large populations of overwintering cleistothecia. The trap was adjusted to sample 12 L/min. Removal, dissection, and examination of the trapping surface was as previously described (6). A modified hygro-thermograph (19) housed in a standard U.S. Weather Service instrument shelter, and a tipping-bucket rain gauge provided records of temperature, relative humidity, leaf wetness, and rainfall (duration, amount, and intensity) at each vineyard.

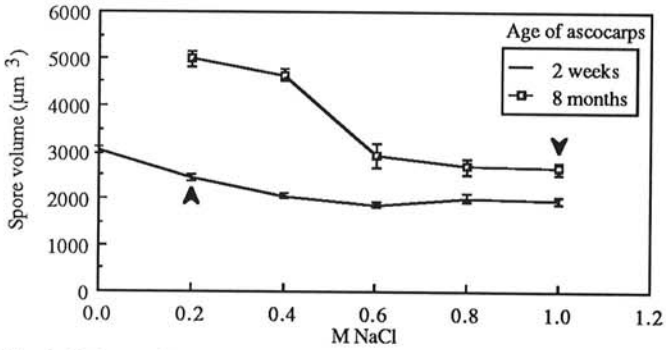

Fig. 1. Volume of ascospores of *Uncinula necator* in solutions of NaCl. Ascospore volume was computed from length and width measurements of ascospores, assuming a cylindrical shape. Arrows indicate the point of incipient plasmolysis of 50% of the ascospores sampled. Bars indicate one standard error of the mean.

RESULTS

Strength of the ascocarp wall. The strength of the ascocarp wall, measured as the mass required to fracture the wall, decreased significantly as cleistothecia aged (Table 1). The most rapid decrease in the strength of the ascocarp wall occurred during

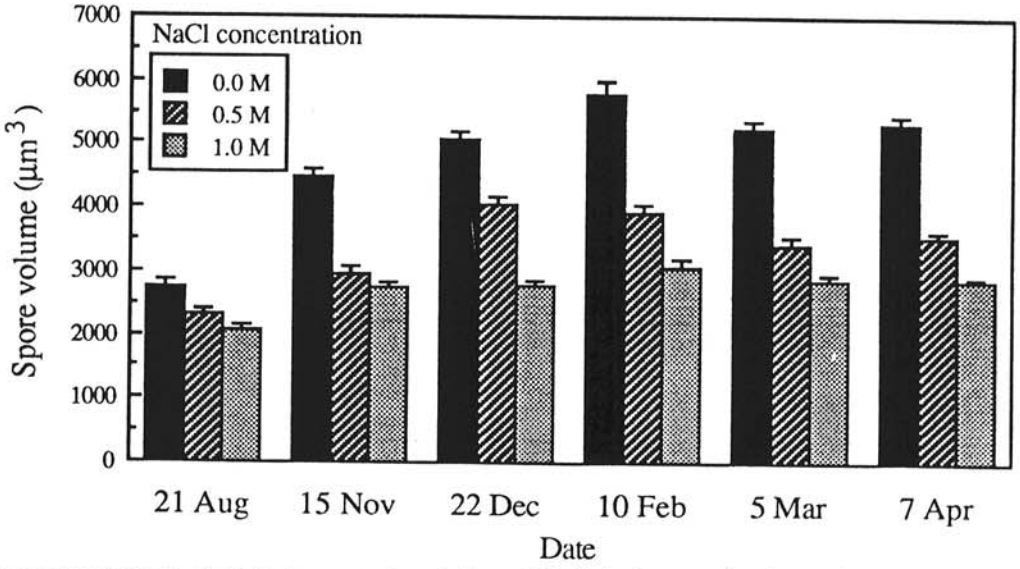

Fig. 2. Changes in ascospore volume of *Uncinula necator* in solutions of NaCl during overwintering during 1986–1987. Ascospore volume was computed from length and width measurements of ascospores, assuming a cylindrical shape. Lines above bars indicate one standard error of the mean.

a 4-wk period in early spring just before dehiscence in the field. Little change occurred between November and April (Table 1). In the cyclical wetting experiment of 1987, the mass required to fracture the ascocarp wall decreased significantly ($P = 0.05$) from 3.2 g at the start of the experiment to 1.9 g on day 28 at 20 C. During the same period, the force required to fracture the ascocarp wall of cleistothecia incubated at 4 C did not decrease significantly. In the cyclical wetting experiment of 1988–1989, the frequency of wetting had no significant ($P = 0.05$) effect on the mass required to fracture the ascocarp wall at 4 or 20 C. However, the strength of the ascocarp wall at 20 C declined significantly ($P = 0.05$) from 3.23 g on 5 October to 2.12 g on 4 January to 1.51 g on 4 April. During the same period, the strength of the ascocarp wall at 4 C decreased significantly ($P = 0.05$) from 3.07 g on 5 October to 2.58 g on 4 January, but did not decrease significantly thereafter.

Water potential of ascospore cytoplasm. The water potential of ascospore cytoplasm decreased as ascocarps aged, presumably resulting in increased pressure potential when older ascocarps were wet. Ascospores from cleistothecia that had been detached from mildew colonies for 2 wk were plasmolysed in 0.2 M NaCl (Fig. 1). Ascospores from cleistothecia harvested in May, 8 mo after leaf fall, were plasmolysed by 1.0 M NaCl, but not 0.8 M NaCl (Fig. 1). The proportion of the ascospores plasmolysed by 1.0 M NaCl in the May collection was not significantly ($P = 0.05$) affected by release from the ascus. Neither would intact asci swell in solutions that plasmolysed free ascospores, indicating that the water potential of the ascospore cytoplasm was equal to or less than the water potential of the epiplasm.

Ascospore volume, computed as the volume of a cylinder, was significantly ($P = 0.01$) affected by the molarity of mountants on glass slides (Figs. 1 and 2). This effect became progressively more pronounced as ascocarps aged from August to February (Fig. 2). Thereafter, there was a slight, but significant ($P = 0.05$), decrease in ascospore volume in distilled water and 0.5 M NaCl (Fig. 2). Ascospore volume in 1.0 M NaCl increased significantly between December and February, but not thereafter (Fig. 2). The minimum molarity of NaCl that resulted in plasmolysis of 50% of the ascospores was 0.2 M in September, 0.4 M in November, 0.6 M in February, 0.8 M in March and April, and 1.0 M in May.

Ascospores were plasmolysed by 0.2 M NaCl on day 1 of the 1987 cyclical wetting experiment. When the experiment was terminated after 28 days, ascospores incubated at 20 C were not plasmolysed by less than 0.4 M NaCl. Ascospores from cleistothecia incubated at 4 C were still plasmolysed by 0.2 M NaCl on day 28. In the 1988–1989 cyclical wetting experiment, frequency of wetting had no significant ($P = 0.5$) effect on

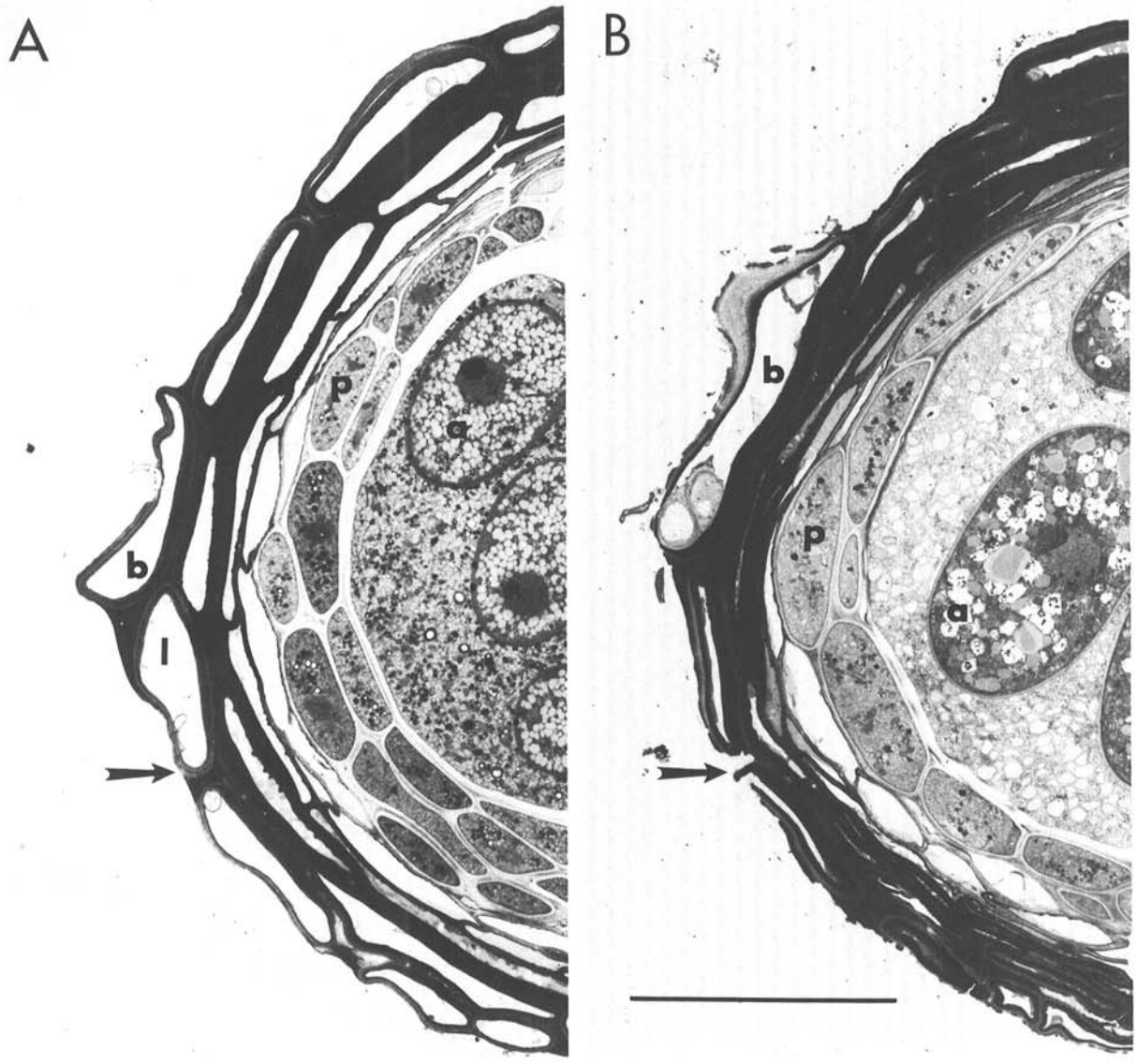

Fig. 3. Changes in cell layers of the ascocarp wall of *Uncinula necator* during overwintering. **A,** Cross section of cleistothecium collected in August. **B,** Cross section through cleistothecium collected in January. Arrows indicate zone immediately below point of attachment of appendages; a = ascospore, p = pseudoparenchyma, l = lumen of cell, b = basal cell of appendage. Bar = 25 μm.

cytoplasmic water potential at either 4 or 20 C. Homogeneity of error variances in the effects of wetting intervals supported pooling of data. At 4 C, at least 50% of the ascospores were plasmolysed by 0.2 M NaCl on 5 October, but this percentage was not plasmolysed by less than 0.4 M NaCl on 4 January. Water potential of ascospore cytoplasm did not increase at 4 C between 4 January and 6 April. At 20 C, the molarity of NaCl that resulted in plasmolysis of 50% of the ascospores increased from 0.2 M on 5 October, to 0.6 M on 4 January, to 0.8 M on 6 April.

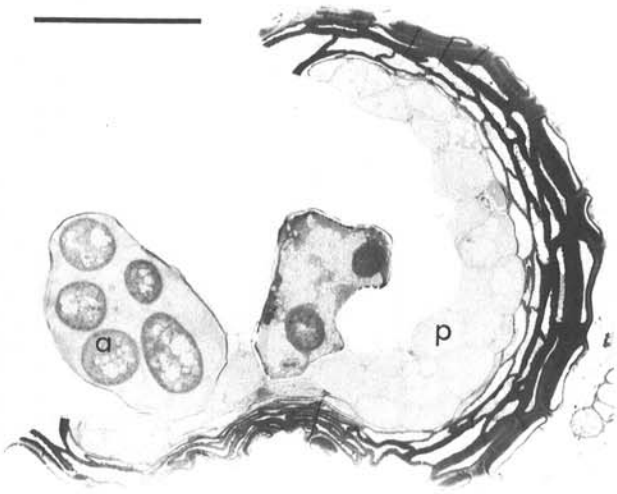

Fig. 4. Cross section through a dehiscent cleistothecium. a = ascospore, p = pseudoparenchyma. Bar = 25 μm.

Electron microscopy, light microscopy, and cytochemistry. The wall of the cleistothecium was composed of two distinct cell layers: an outer rind of two to three thick-walled dead cells, and an inner layer of one to three pseudoparenchymatous cells (Fig. 3A). Between August and January, the lumina of cells of the outer rind closed, resulting in a nearly solid structure of closely appressed cell walls (Fig. 3B). In general, the thinnest point in this outer cell layer of the ascocarp wall was slightly below the equatorially attached appendages. There was no reduction in the thickness of the pseudoparenchyma as cleistothecia aged (Figs. 3 and 4). Cells of the pseudoparenchyma were laterally flattened in intact cleistothecia, but became nearly isodiametric upon dehiscence (Fig. 4). Extraction by sodium methoxide (5) confirmed that lipid droplets were the predominant feature in the cytoplasm at all stages of development (Fig. 5A–D). However, the quantity and distribution of lipids in the cytoplasm of ascospores changed as cleistothecia aged. The mean percentage of the cross-sectional area of ascospores occupied by lipid was 71.9% in August (Fig. 5A), 33.1% in January (Fig. 5C), and 31.8% in May (Fig. 5D) (± 10.56, 3.48, and 1.92%, respectively, $P = 0.10$). The distribution of lipids changed from a uniform distribution of smaller droplets in ascospore cytoplasm in August (Fig. 5A) to a progressively more aggregated distribution of larger lipid droplets in January

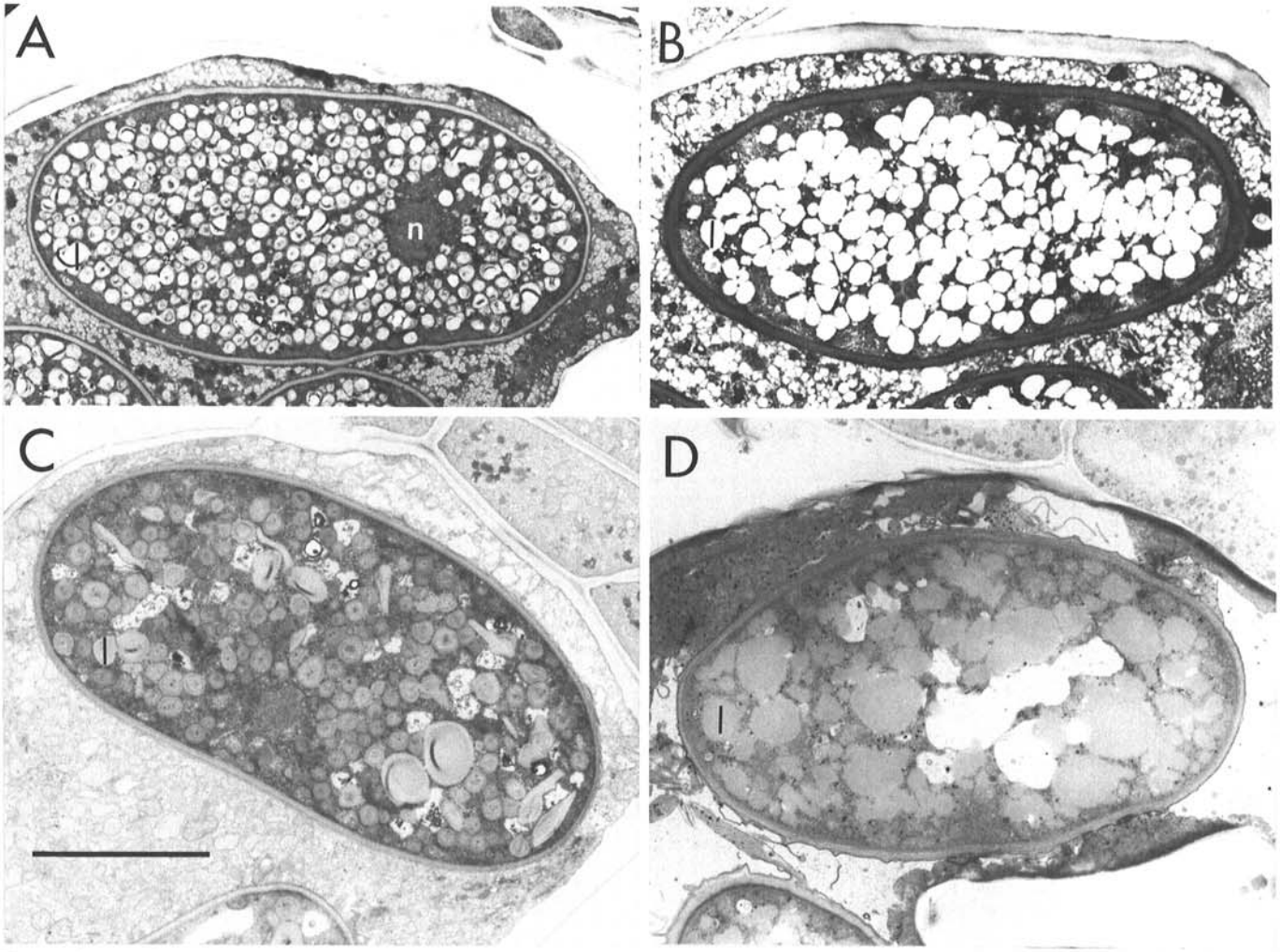

Fig. 5. Changes in distribution and quantity of cytoplasmic lipids in ascospores of *Uncinula necator* during overwintering. **A,** Ascospore from August collection before extraction of fixed lipids. **B,** Serial section of same ascospore after extraction of fixed lipids by sodium methoxide in methanol and benzene. **C,** Ascospore from January collection. **D,** Ascospore from May collection. l = lipid droplet, n = nucleus. Bar = 10 μm.

(Fig. 5C) and May (Fig. 5D). Phenylosazone crystals were absent from slides prepared from cleistothecia collected in August, but four to 17 crystals formed on each slide prepared from cleistothecia collected in May. The osazones were present as rosettelike groups of raphide crystals, similar to phenylosazones formed by the reaction of glucose described by Mangham (20). The epiplasm of asci of cleistothecia from the August collection was stained brownish-red by Lugol's iodine, indicating the possible presence of glycogen in the epiplasm. Free ascospores absorbed the stain, but no color change occurred. Neither the epiplasm of asci nor the freed ascospores from the January and May collections tested positively for glycogen. In the epiplasm of asci from cleistothecia of the August collection, numerous aggregates of glycogenlike particles (21) were observed in thin sections not treated with amylase. These aggregations of particles were partially or completely removed from serial sections by amylase, thus, they were assumed to be glycogen. The cross-sectional area of the epiplasm composed of glycogen particle aggregations was 2.20% ($\pm$ 0.162%, $P = 0.10$). No such aggregations of glycogen particles were observed in ascospore cytoplasm, or in cleistothecia from later collections.

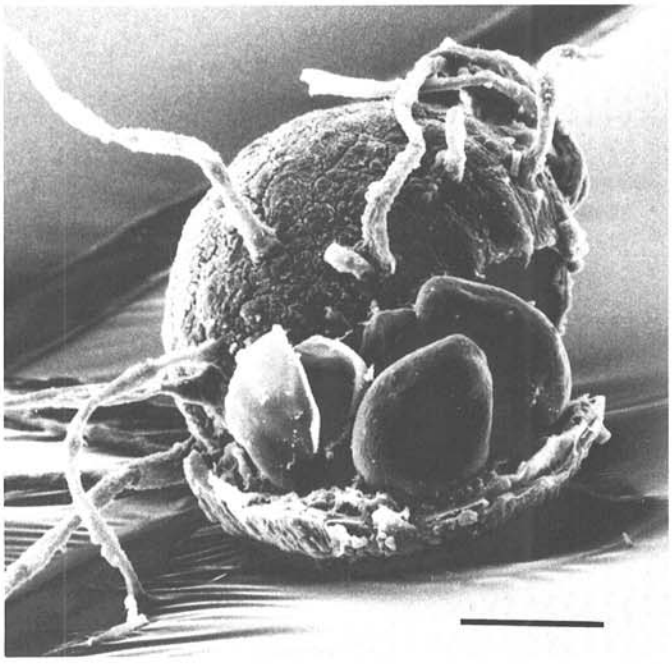

Fig. 6. Scanning electron micrograph of dehiscent cleistothecium of *Uncinula necator* from May collection. The ascocarp wall has ruptured circumscissilely immediately beneath the point of attachment of the equatorial appendages. One ascus has discharged spores through a slitlike rupture of the ascus tip. Bar = 30 μm.

Cleistothecia were hemispherical with a basal concavity when dry, but swelled to become nearly spherical within 2 min when wet. Dehiscence was circumscissile. The ascocarp wall broke below the point of attachment of the appendages, near the junction of the concave and convex surfaces of the ascocarp (Fig. 6). Swelling of the asci lifted the top portion of the ascocarp wall, which often remained attached at one point to the basal portion of the ascocarp wall, the point of attachment acting as a hinge. Ascospores from dehiscent cleistothecia in water mounts were forcibly discharged through a slitlike rupture in the ascus tip, which was also observed in scanning electron micrographs (Fig. 6). Ascocarps often closed after all asci had discharged, and showed little evidence of dehiscence under low power (20–50×) light microscopy.

Effects of temperature on dehiscence of cleistothecia. Temperatures between 10 and 32 C had little effect on the number of cleistothecia that dehisced after 24 hr of wetting. However, ascocarp dehiscence was nearly suppressed at 4 C (Fig. 7). As temperature was increased from 10 to 32 C, a progressively greater proportion of the cleistothecia that dehisced at each temperature had done so within 2–4 hr of wetting (Fig. 7). The proportion of dehiscent cleistothecia changed little between 24 and 48 hr at all temperatures (Fig. 7).

Ascospore discharge in vineyards. The density of airborne ascospores of *U. necator* was relatively low in both years of the study and was near the threshold of detection of the spore sampler, thus, a detailed analysis of environmental effects on spore release was not attempted. Ascospores were trapped during eight rain-initiated wetting periods in 1986 (Fig. 8) and during four rain-initiated wetting periods in 1987 (Fig. 9). The minimum amount of rain during which ascospores were trapped was 2.5 mm on 22 May 1986 (Fig. 8), and ascospores were trapped whenever rainfall exceeded 2.5 mm. Ascospores were not trapped on 3 days when less than 2.5 mm of rain fell. Temperatures at the time that ascospores were trapped ranged from 6 to 24 C (Figs. 8 and 9). In 10 of 12 wet periods, ascospores were trapped within 6 hr of the start of rain, and in the remaining periods ascospores were trapped within 8 hr of the start of rain. With the exception of the wetting period beginning on 18 May 1986 (Fig. 8), no ascospores were trapped more than 12 hr after the start of rain, even when rain continued or leaves in the vineyard remained wet. All spore release periods occurred between the dates of bud

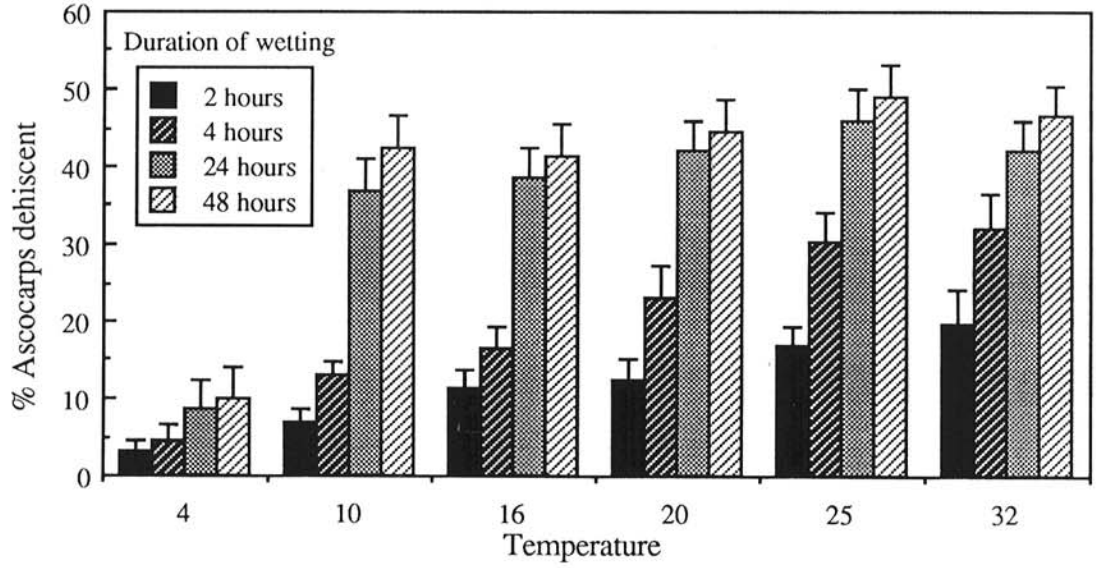

Fig. 7. Effects of temperature on dehiscence of cleistothecia of *Uncinula necator*. Lines above bars indicate one standard error of mean.

break (1 May 1986 and 28 April 1987) and bloom (10 June 1986 and 9 June 1987) of grape.

DISCUSSION

The mechanism of ascospore discharge. Ascocarp dehiscence and ascospore discharge in *U. necator* appear to be the result of two principal processes: a decrease in the strength of the ascocarp wall during overwintering and a simultaneous decrease in water potential, presumably resulting in increased pressure potential in wet, intact ascocarps. The decrease in strength of the ascocarp wall between August and January was associated with morphological changes in the outermost cells of the ascocarp wall, specifically, a lateral flattening of the cells, the closing of the lumina, and the formation of a circumscissile thin zone in the ascocarp wall immediately below the appendages. The signif-

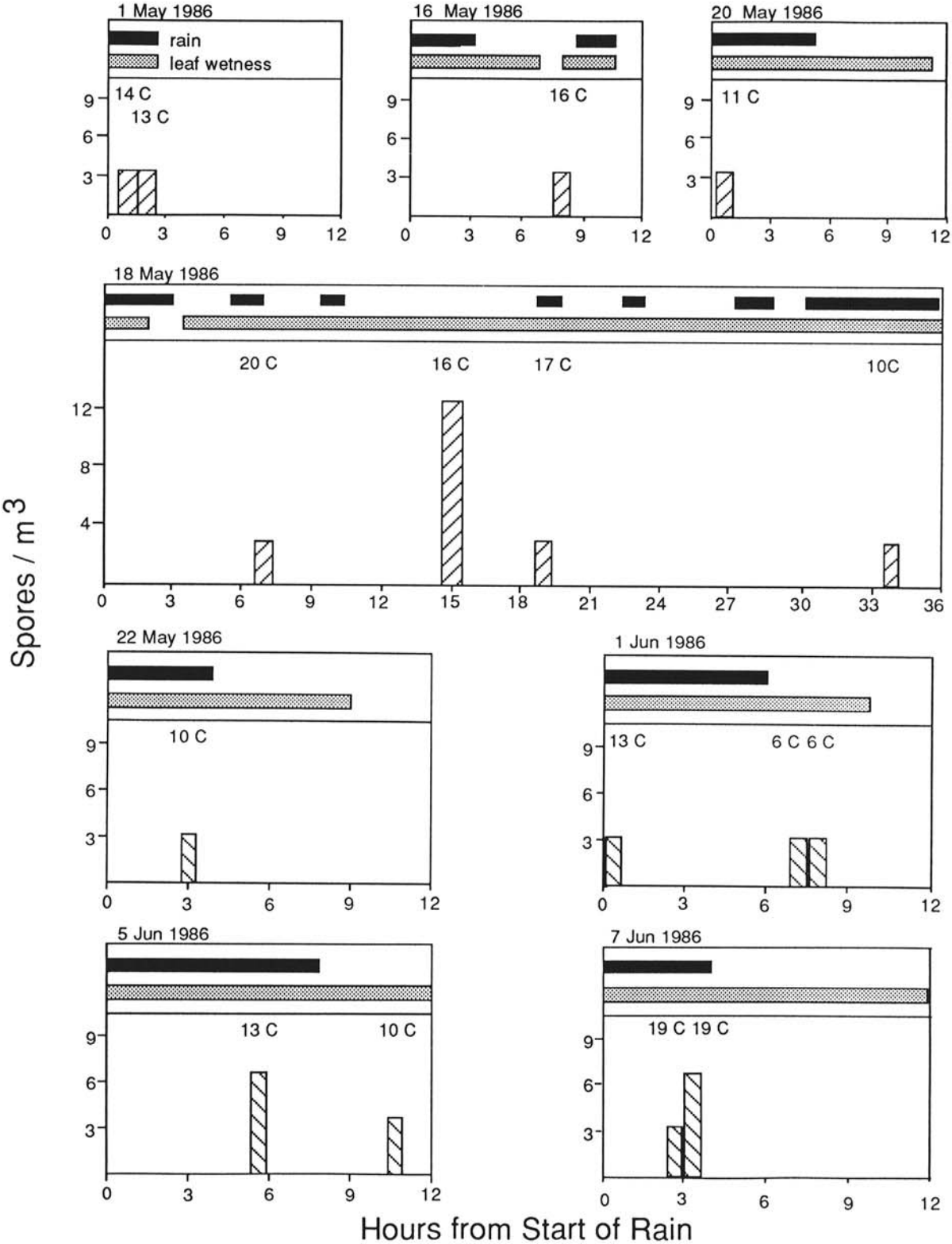

Fig. 8. Release of ascospores of *Uncinula necator* in vineyards during 1986. Ascospores were collected by a Burkard volumetric spore sampler operated continuously. Rainfall bar indicates hours during which more than 0.25 mm rain was recorded. Leaf wetness bar indicates hours during which leaves on vines were wet. Temperatures given are those recorded at the time that spores were trapped.

icance of the morphological change was seen as the consistent circumscissile rupture of the ascocarp wall at this point during dehiscence. When we subjected cleistothecia to repeated cycles of wetting and drying at 6 hr, and 3-, 7-, or 14-day intervals at 4 and 20 C, no decrease in the mass required to crush the ascocarps was observed unless the water potential of the cytoplasm decreased. The water potential of cytoplasm in ascospores of cleistothecia wet and dried at 4 C for 28 days did not decrease, nor did the mass required to rupture the wall decrease. At 20 C, cytoplasmic water potential decreased and the mass required to rupture the ascocarp wall decreased. The cycles of wetting and drying in the 1987 experiment took place over a 28-day period, and the cytoplasmic water potential at 20 C was less than one-half that observed in cleistothecia collected from vineyards in May. However, in the longer cyclical wetting experiment of 1988–1989, fatigue of the ascocarp wall due to flexing was not a determinant of the strength of the ascocarp wall.

Ascospore volume decreased in 0.5 M NaCl between 10 February and 7 April, which might indicate an increase in cytoplasmic water potential during this period. However, the minimum concentration of NaCl that caused plasmolysis of 50% of the ascospores increased from 0.8 M in March and April to 1.0 M in May. The decrease in ascospore volume in 0.5 M NaCl between February and April, therefore, was an indication of changes in the ascospore cell wall that restricted distension of the wall by an increased pressure potential. Evidence of this was reported in an earlier study (9) where the percentage of ascospores of *U. necator* that burst in distilled water decreased between late winter and early spring, despite decreasing cytoplasmic water potential.

Between autumn and early spring, the strength of the ascocarp wall may decrease in response to both decreasing water potential and morphological changes in the ascocarp wall. There was significant weakening of the ascocarp wall during April and May that occurred without a commensurate increase in the turgor pressure of the cytoplasm, and without a change in morphology of the ascocarp wall. However, the causes of the decrease in strength of the ascocarp wall may have occurred at the ultrastructural level and would not be seen as morphological changes.

Water potential provides the energy required for ascocarp dehiscence and ascospore discharge. The mean pressure potential of the cytoplasm of intact ascospores in water is equivalent to the absolute value of the chemical potential of a solution that causes 50% of the ascospores to plasmolyse (1). Thus, pressure potentials in wetted cleistothecia increased from 670 KPa in September to 2,990 KPa in May (33). The pressure potential is of sufficient magnitude to significantly affect identification of ascospores in certain mountants. Kapoor (15) reported the range of ascospore dimensions to be 15–25 × 10–14 μm, but did not specify the mountant used. When ascospores from cleistothecia collected in May 1987 were mounted in distilled water, we recorded dimensions of 18 × 11.6 to 36 × 18 μm. Although we measured water potential in ascospore cytoplasm, it is likely that the water potential of cytoplasm of the pseudoparenchyma of the ascocarp and the epiplasm of the ascus undergo similar changes, and, thus, contribute to ascocarp dehiscence. Pseudoparenchyma cells and asci were laterally flattened in intact ascocarps but swelled to become nearly isodiametric upon dehiscence, indicating a high pressure potential.

When ascocarps dehisced in distilled water, asci continued to swell, even after the ascospores were fully distended, indicating that water movement into the epiplasm was continuing. Although the water potential of the epiplasm was greater than or equal to that of the ascospore cytoplasm at the time of dehiscence, the relatively elastic wall of the ascus continued to be distended until discharge occurred, whereas distension of the ascospores eventually reached a maximum. Thus, a pressure potential due to water uptake by ascospores in an intact cleistothecium could contribute to ascocarp dehiscence. However, discharge of ascospores from the ascus following dehiscence could not be due to swelling of the ascospores alone.

Lipid droplets in the cytoplasm changed in quantity and distribution during overwintering. A decrease in total lipid and the aggregation of lipids into larger droplets occurred as ascocarps aged. A direct link between the decrease in lipid and the decrease in water potential has not been established, but one possible mechanism would be the conversion of lipids to a nonhydrophobic substance such as carbohydrates, resulting in decreased water potential in the cytoplasm. The formation of phenylosazone crystals indicated the presence of reducing sugars at a concentration above 1% (20) in the cytoplasm of cleistothecia collected in May, but not in August. It is possible that other nonhydrophobic substances accumulate in the cytoplasm as the ascocarps mature and contribute to decreased water potential of the cytoplasm. However, conversion of glycogen to sugars as a mechanism to provide the energy needed for forcible spore discharge, as has been proposed for other ascomycetes (11,12,14), is not consistent with our observations of changes in glycogen content and water potential in *U. necator*. Glycogen was observed in electron micrographs of the epiplasm of asci from cleistothecia collected in August, but not in later collections. At no time was glycogen observed in micrographs of ascospores or indicated by iodine staining in the cytoplasm of ascospores. Water potential decreased significantly in ascospore cytoplasm and, possibly, within the ascus throughout development, despite the absence of detectable glycogen.

Environmental effects on ascocarp dehiscence and ascospore discharge. Dehiscence of cleistothecia was significantly depressed at 4 C. Diehl and Heintz (4) also reported that ascospore discharge in *U. necator* was suppressed at 5 C. Temperatures of less than 8 C also have been reported to reduce ascospore release in *Erysiphe cichoracearum* (3), *Sphaerotheca humuli* (18), and *Erysiphe graminis* f. sp. *hordei* (22). In *U. necator*, the principal effect of increasing temperature from 4 to 32 C was that a larger proportion of the total ascospore release occurred in the first 4 hr of wetting. Regardless of temperature, ascospore release was nearly complete after 24 hr. Similar relationships between duration

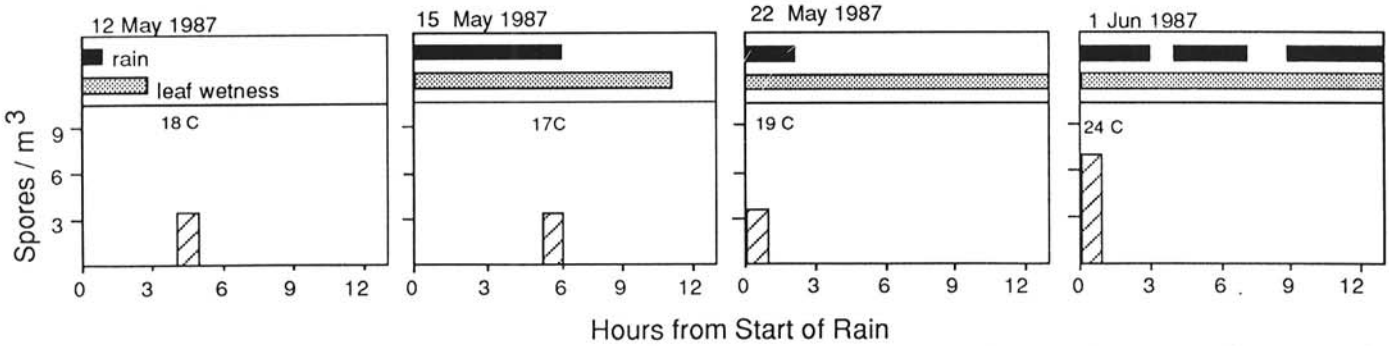

Fig. 9. Release of ascospores of *Uncinula necator* in vineyards during 1987. Ascospores were collected by a Burkard volumetric spore sampler operated continuously. Rainfall bar indicates hours during which more than 0.25 mm rain was recorded. Leaf wetness bar indicates hours during which leaves on vines were wet. Temperatures given are those recorded at the time that spores were trapped.

of wetting and ascospore discharge have been reported in *Erysiphe trina* (35) and *S. humuli* (17). Therefore, temperature will be significant in determining inoculum dose under two conditions: either when temperature is near the lower threshold for ascospore release, or when the duration of wetting is less than 4 hr.

In the laboratory, cleistothecia dehisced only when wet and, in the vineyard, ascospores were trapped only during or immediately following rain. Given that the mechanism of ascospore discharge involves a force generated by water potentials, a film of water on the ascocarp would be required for this mechanism to work. Free water was essential for ascospore release in *S. humuli* (17), *E. trina* (35), and in earlier studies of ascospore release in *U. necator* (4,7,10,25,36). Mur (23) reported that ultraviolet (UV) light stimulated ascocarp dehiscence in *U. necator*. However, the ascocarps used were collected in late autumn, a time when ascocarps are physiologically immature and do not release ascospores in New York (8,25), and were frozen at −27 C for 2 yr before exposure to UV light. When Diehl and Heintz (4) collected ascocarps of *U. necator* in spring and exposed them to UV light, an exposure of 2 hr had no effect and an exposure of 5 hr reduced ascocarp dehiscence. The role of UV light in ascospore release in *U. necator* thus appears uncertain, and the most important factors remain free water and temperature. In the vineyard, ascospores were trapped whenever rainfall exceeded 2.5 mm between the host phenophases of bud burst and bloom. The failure to detect ascospores on three occasions when rainfall was between 0.3 and 2.5 mm could be due to two causes. First, the airborne ascospore dose may have been below the threshold of detection of the spore sampler during the relatively brief rains. Secondly, rainfall of less than 2.5 mm may not sufficiently wet the cleistothecia on their normal substrate, which is the crevices of the exfoliating bark of grapevines (8).

The anamorph of *U. necator* is widely regarded as xerophytic and rain is considered deleterious to the development of powdery mildew (34). However, we have shown that rainfall is an essential event in release of the primary inoculum in areas where this inoculum comes entirely or in part from cleistothecia. Further, environmental conditions resulting in release of ascosporic inoculum for grape powdery mildew appear to be rainfall in excess of 2.5 mm and temperatures of more than 4 C between the phenophases of bud break and bloom. Current studies to identify the environmental conditions conducive to infection by ascospores (9) may allow the use of postinfection applications of fungicides and, possibly, the integration of the timing of fungicide applications for powdery mildew and grape diseases such as black rot and downy mildew, both of which are favored by and initiate infections following rain. In future work, we plan to examine more closely the relationship between conversion of lipids in the cytoplasm, ascocarp dehiscence, and the coincident timing of ascospore release with host phenology. We believe that *U. necator* may serve as a model of ascospore release for many members of the Erysiphaceae, because many of the environmental influences upon ascospore release (3,17,18,22,35), the abundance of lipids in ascospore cytoplasm at certain stages of development (14,16,21,26,35), and the possibly low water potentials of ascospore cytoplasm (14,27) observed in our study have been reported in other genera of the Erysiphaceae.

LITERATURE CITED

1. Bidwell, R. G. S. 1974. Plant Physiology. Macmillan Publishing Co., New York. 643 pp.
2. Cullum, F. J., and Webster, J. 1977. Cleistocarp dehiscence in *Phyllactinia*. Trans. Br. Mycol. Soc. 68:316-320.
3. Cutter, E. C., and Wheeler, B. E. J. 1968. Effect of temperature on ascospore discharge from cleistocarps of *Erysiphe cichoracearum* on *Arctium lappa*. Trans. Br. Mycol. Soc. 51:791-795.
4. Diehl, H. J., and Heintz, C. 1987. Studies on the generative reproduction of grapevine powdery mildew (*Uncinula necator* Berk.). Vitis 26:114-122.
5. Eurenius, L., and Jarskar, R. 1970. A simple method to demonstrate lipids in Epon-embedded ultrathin sections. Stain Technol. 45:129-132.
6. Gadoury, D. M., and MacHardy, W. E. 1983. A 7-day recording volumetric spore trap. Phytopathology 73:1526-1531.
7. Gadoury, D. M., and Pearson, R. C. 1986. A mechanism for ascospore discharge in *Uncinula necator*. (Abstr.) Phytopathology 76:1145.
8. Gadoury, D. M., and Pearson, R. C. 1988. Initiation, development, dispersal, and survival of cleistothecia of *Uncinula necator* in New York vineyards. Phytopathology 78:1413-1421.
9. Gadoury, D. M., and Pearson, R. C. 1988. Germination of ascospores and infection of *Vitis* by *Uncinula necator*. (Abstr.) Phytopathology 78:1538.
10. Galloway, B. T. 1895. Observations on the development of *Uncinula spiralis*. Bot. Gaz. 20:486-493.
11. Ingold, C. T. 1933. Spore discharge in the ascomycetes. New Phytol. 32:175-196.
12. Ingold, C. T. 1965. Spore Liberation. Clarendon Press, Oxford. 210 pp.
13. Jackson, G. V. H., and Gay, J. L. 1976. Perennation of *Sphaerotheca mors-uvae* as cleistothecia with particular reference to microbial activity. Trans. Br. Mycol. Soc. 66:463-471.
14. Jackson, G. V. H., and Wheeler, B. E. J. 1974. Perennation of *Sphaerotheca mors-uvae* as cleistocarps. Trans. Br. Mycol. Soc. 62:73-87.
15. Kapoor, J. N. 1967. *Uncinula necator*. C. M. I. Descriptions of Pathogenic Fungi and Bacteria, No. 160. 2 pp.
16. Khairi, S. M., and Preece, T. F. 1978. Hawthorne powdery mildew: Occurrence, survival and ascospore productivity of *Podosphaera clandestina* cleistothecia in England. Trans. Br. Mycol. Soc. 71:289-293.
17. Liyanage, A. S. 1973. Studies on resistance and overwintering in hop powdery mildew (*Sphaerotheca humuli*). Ph.D. thesis. University of London. 304 pp.
18. Liyanage, A. S., and Royle, D. J. 1976. Overwintering of *Sphaerotheca humuli*, the cause of hop powdery mildew. Ann. Appl. Biol. 83:381-394.
19. MacHardy, W. E., and Sondej, J. 1981. Weather-monitoring instrumentation for plant disease management programs and epidemiological studies. N. H. Agric. Exp. Stn. Bull. 519. 40 pp.
20. Mangham, B. A. 1911. On the detection of maltose in the tissues of certain angiosperms. New Phytol. 10:160-166.
21. Martin, M., Gay, J. L., and Jackson, G. V. H. 1976. Electron microscopic study of developing and mature cleistothecia of *Sphaerotheca mors-uvae*. Trans. Br. Mycol. Soc. 66:473-487.
22. Moseman, J. G., and Powers, H. R. 1957. Function and longevity of cleistothecia of *Erysiphe graminis* f. sp. *hordei*. Phytopathology 47:53-56.
23. Mur, G. 1985. Sur la maitrise du developpement in vitro des champignons parasites de la vigne a l'aide de rayons ultra-violets. Prog. Agric. Vitic. 102:363-365.
24. Pearse, A. G. E. 1960. Histochemistry, Theoretical and Applied. Little, Brown, and Co., Boston. 998 pp.
25. Pearson, R. C., and Gadoury, D. M. 1987. Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. Phytopathology 77:1509-1514.
26. Preece, T. F. 1965. Early stages of American gooseberry mildew. Plant Pathol. 14:83-87.
27. Salmon, E. S. 1903. Infection-powers of ascospores in Erysiphaceae. J. Bot. 41:159-165, 204-212.
28. Schnathorst, W. C. 1959. Heterothallism in the lettuce strain of *Erysiphe cichoracearum*. Mycologia 51:708-711.
29. Schnathorst, W. C. 1965. Environmental relationships in the powdery mildews. Annu. Rev. Phytopathol. 3:343-366.
30. Smith, C. G., and Wheeler, B. E. J. 1969. The overwintering of cleistocarps of *Erysiphe polygoni* on different hosts. Trans. Br. Mycol. Soc. 52:437-445.
31. Turner, D. M. 1956. Studies on cereal mildew in Britain. Trans. Br. Mycol. Soc. 39:495-506.
32. Walker, L. B., and Andersen, E. N. 1925. Relation of glycogen to spore-ejection. Mycologia 17:154-159.
33. Weast, R. C., ed. 1968. Handbook of Chemistry and Physics. Chemical Rubber Co., Cleveland.
34. Yarwood, C. E. 1957. Powdery mildews. Bot. Rev. 23:235-301.
35. Yarwood, C. E., and Gardner, M. W. 1972. Ascospore discharge by *Erysiphe trina*. Mycologia 64:799-805.
36. Yossifovitch, M. 1923. Contribution a l'etude de l'Oidium de la Vigne et de son traitement. Ph.D. thesis. Univ. Toulouse. 176 pp.