# Germination of Ascospores and Infection of *Vitis* by *Uncinula necator*

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## ABSTRACT

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Ascospores of *Uncinula necator* were released from cleistothecia periodically from November (leaf fall) to May (bud break) by fracturing the ascocarp wall and allowing the spores to be discharged onto glass slides. Water potential of ascospore cytoplasm decreased continuously during this period. Ascospores did not germinate before November. Thereafter (until April), ascospores germinated only in the presence of free water but frequently burst in water, presumably because of high pressure potentials within the spore. After April, ascocarps dehisced naturally when wet, the released ascospores rarely burst in water, and ascospores germinated in water and at a vapor pressure deficit (VPD) of 1453 Pa at 25 C. However, germination at 25 C was reduced from 92 to 97% in water or 0 Pa VPD, to 40.4% at 600 Pa, 30.6% at 760 Pa, and 16.9% at 1453 Pa VPD. Similar effects of free water and humidity upon appressorium formation were recorded. The percentage of ascospores that germinated and formed appressoria increased as temperature was increased from 10 to 23 C. At 10, 15, 20, 22, and 25 C, ascospores infected 23, 57, 94, 92, and 89%, respectively, of inoculated in vitro plants of the *Vitis* interspecific hybrid cultivar Chancellor. Ascospores did not form appressoria or infect tissue culture plants at 5 C, or at 31 and 36 C. Ascospores incubated at 20–25 C germinated within 4 hr, formed lobate appressoria within 12 hr, and occasionally formed multiple germ tubes after 24 hr at 0 Pa VPD. *U. necator* is generally considered to be a xerophyte whose anamorph is adversely affected by free water. However, free water is required for ascocarp dehiscence and ascospore discharge and has no deleterious effect upon ascospore germination or infection of *Vitis* by ascospores.

*Additional keywords:* cleistothecium, grapevine, powdery mildew.

Cleistothecia of *Uncinula necator* (Schw.) Burr. were once widely believed to be relatively unimportant in the epidemiology of grape powdery mildew (1,12,16). The ascigerous state was relegated to a minor role in the epidemiology of grape powdery mildew for two reasons. First, in moderate climates the pathogen can overwinter successfully in the vegetative state as mycelium in dormant infected buds, and ascocarps are not necessary to insure perpetuation of the disease (16). For example, *U. necator* overwintered successfully for nearly 50 yr after its introduction to Europe before cleistothecia were found (17). Secondly, ascospores did not germinate (14) or infect (7,14,17) grapevines in several investigations. In 1895, Galloway (7) observed that ascospores of *U. necator* frequently burst in water. Spores that

did not burst eventually germinated, although attempts to reproduce the disease with ascosporic inoculum failed. Yossifovitch (17) attempted to repeat Galloway's experiments in 1923 but reported that ascospores did not germinate. In Romania, Toma (14) performed extensive inoculations, but ascospores did not germinate or infect grapevine buds or leaves.

The effects of environment on germination of and infection by conidia of *U. necator* are well understood. Thorough studies by Delp (2) demonstrated the harmful effects of free water on germination of conidia, the relative insensitivity of conidia to low humidity, and established cardinal temperatures for germination and infection that have not been altered significantly by subsequent research (8,14). We are not aware of similar studies of the effects of host or environmental factors upon ascospore germination or infection.

We have recently demonstrated that ascocarps are the principal source of primary inoculum in New York (9). Subsequent reports have indicated that cleistothecia are potential sources of primary inoculum in California (13) and Germany (3), where dormant infected buds are an additional source of spring infection (10,11). Cleistothecia may form on all infected tissues, but are then dispersed to the bark of the vine by rain during late summer (4). The ascocarps overwinter in bark crevices and discharge ascospores during spring rains between bud break and bloom of grapevines (4,6,9). Ascospores are wind-dispersed, and initial infections are generally found on the lower surfaces of leaves growing in close proximity to bark (9). In the present investigation, we have correlated the seasonal changes in germination of ascospores of *U. necator* with changes in water potential of ascospore cytoplasm; determined the effects of temperature, free water, and vapor pressure of water upon germination of ascospores and infection of *Vitis*; and commented upon possible reasons for difficulties encountered in some earlier studies of infection by ascospores.

## MATERIALS AND METHODS

**Collection of cleistothecia.** Cleistothecia were harvested, as described previously (4), from the exfoliating bark of vines of the *Vitis* interspecific hybrid cultivars Aurore and Rougeon in each year of the study. In studies of seasonal changes in ascospore maturation and germination, the ascocarps were collected at 2- to 4-wk intervals from August through May. Ascocarps used as inoculum sources for studies of effects of environment upon germination and infection were collected shortly before bud break in April of each year, and were stored dry on filter paper disks at 4 C until needed. The latter studies were completed within 10 wk of the collection of the cleistothecia.

**Seasonal changes in germination of ascospores.** At 2- to 4-wk intervals, beginning in August of 1986 and 1987, twenty cleistothecia were placed upon each of several 2-cm disks of filter paper, and the paper was wet with distilled water. Because ascocarps in a previous study had not dehisced readily before spring (9), the ascocarp walls were fractured with fine forceps after 3–5 min of wetness while being observed at a magnification of 32×. Once the ascocarps on a disk were ruptured to induce spore release, the disk was immediately transferred to the lid of a 9-cm petri plate containing either a dry glass slide or a depression slide bearing a droplet of distilled water. Both the base and lid of the petri plate were lined with wet filter paper to provide a saturated atmosphere. Three plates per treatment were incubated in the dark at 20 C for 1 hr, the disks bearing the cleistothecia were removed, and the plates were incubated at 20 C for an additional 23 hr. Thus, all of the ascospores on a slide were discharged within the same 1-hr period, and all were incubated for 23–24 hr. A slide bearing discharged ascospores was also incubated as above at 20 C in a plate containing an aqueous slurry of NaCl, which, at 20 C, maintained a relative humidity (RH) of 76% and a vapor pressure deficit (VPD) of 600 Pa (15). Following incubation, the slides were mounted in lactophenol and were observed at 200–400× magnification. The number of ungerminated, germinated, and ruptured ascospores on each slide was recorded. At 2- to 6-wk intervals, the point of incipient plasmolysis of ascospore cytoplasm was determined for 30 ascospores as described by Gadoury and Pearson (6).

Cleistothecia collected in August, 1986, and in January and May, 1987, were prepared for transmission electron microscopy

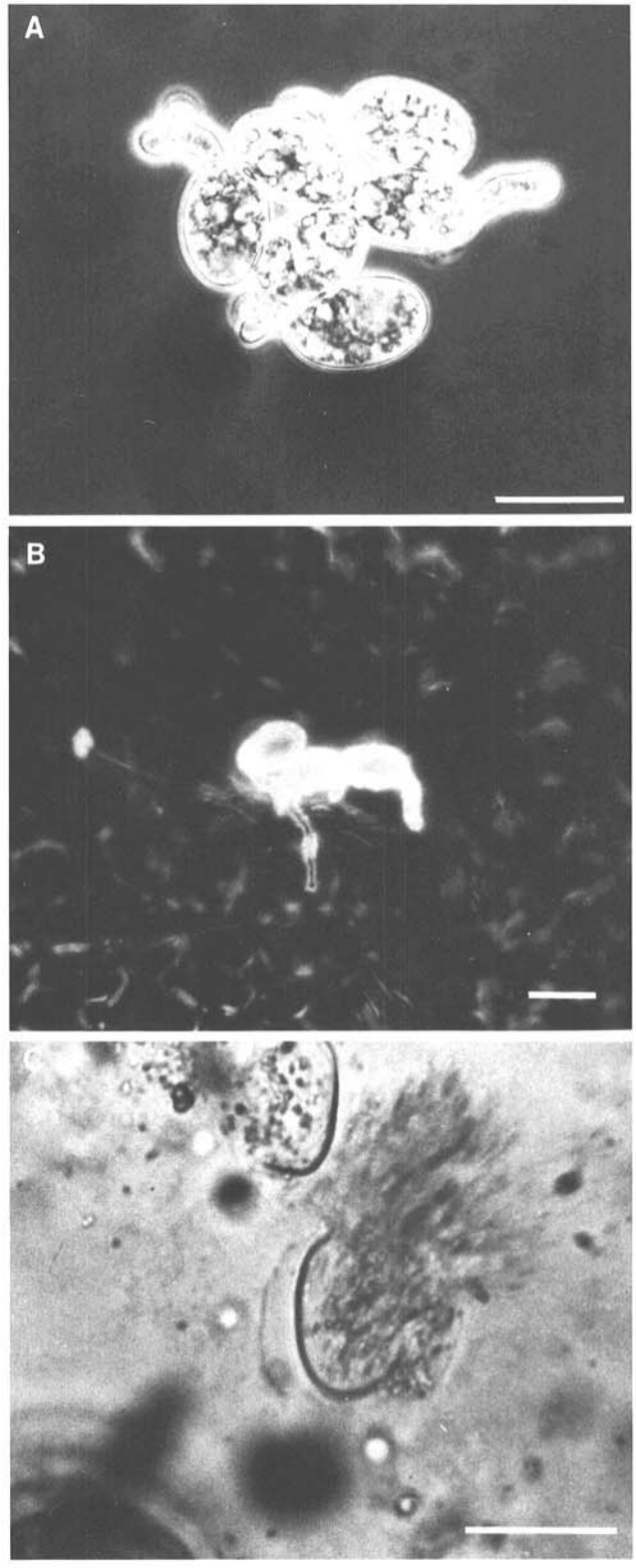


**Fig. 1.** Germination of ascospores of *Uncinula necator* on glass slides and on leaves of in vitro plants and bursting of ascospores in water. (**A**) Group of six ascospores germinating on glass slide after 24 hr at 0 VPD, 25 C. (**B**) Triad of germinated ascospores on leaf of Chancellor tissue culture plant after 16 hr at 20 C. (**C**) Ascospore bursting in water mount after harvest from a cleistothecium collected from the vineyard on 2 January 1986. Scale bars = 30 μm.

as previously described (6). The thickness of the ascospore cell wall was measured at four different points per spore at a magnification of 15,000×. Data on wall thickness were taken on four ascospores per cleistothecium and five cleistothecia per collection date.

**Effects of temperature, free water, and vapor pressure deficit on germination and appressorium formation.** In April through June of 1988 and 1989, intact cleistothecia on wet filter paper disks were suspended over dry glass slides or depression slides bearing droplets of distilled water as described above in order to collect naturally released ascospores. After 1 hr of wetting at 25 C, the disks bearing the ascocarps were removed from the plates. Depression slides containing the released ascospores were incubated as before for an additional 23 hr. Dry glass slides bearing ascospores were incubated for an additional 23 hr at 25 C in petri plates containing either wet filter paper or an aqueous slurry of one of the following salts: $(NH_4)_2\ SO_4$, NaCl, or $NA_2Cr_2O_7$, which maintained RH within the closed plates at 100, 81, 76, and 54%, respectively (15). At 25 C, VPD within the plates was 0, 600, 760, and 1453 Pa, respectively (15). Dry glass slides bearing ascospores collected as above were also incubated at 5, 10, 15, 20, 22, 25, 31, and 36 C, 0 VPD for 23 hr. Following incubation, the slides were mounted in lactophenol and the number of ungerminated, germinated, and appressorium-bearing ascospores on each slide was recorded. One petri plate containing 20 cleistothecia suspended over a single glass slide was incubated at each temperature or humidity, and the experiment was repeated twelve times in 1988 and three times in 1989.

**Effects of temperature on infection of *Vitis* by ascospores.** In April through June of 1988 and 1989, filter paper disks bearing 10 cleistothecia were wet with distilled water and were placed in the cap of a 12- × 75-mm culture tube containing a single tissue culture plant (4) of the *Vitis* interspecific hybrid cultivar Chancellor. After 1 hr at 25 C, the disk bearing the cleistothecia was removed, and five plants were incubated at 5, 10, 15, 20, 22, 25, 31, and 36 C with a photoperiod of 16 hr for 14 days. Plants were then examined at 20×, and the number of plants on which *U. necator* ascospores had formed colonies of more

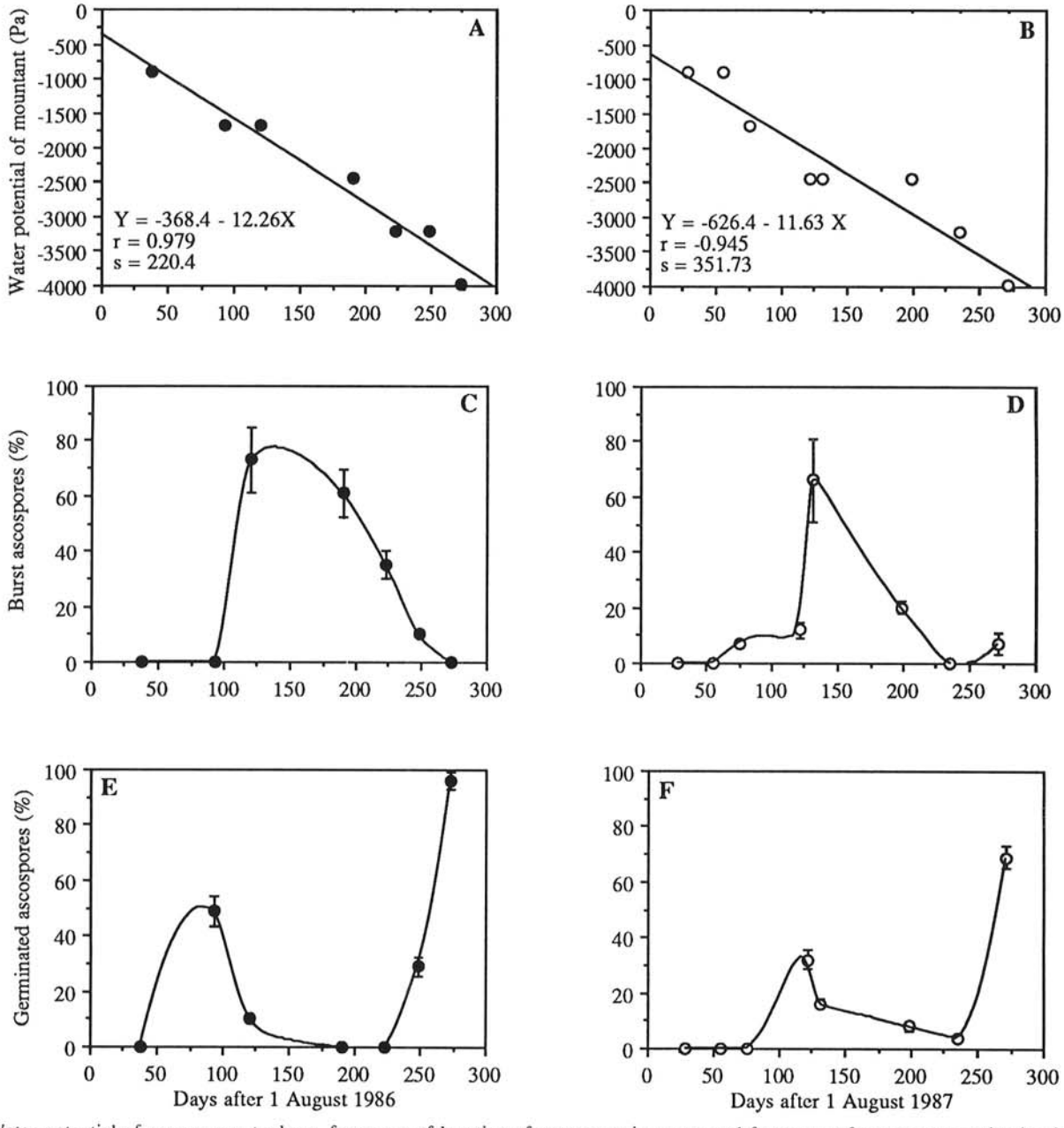


**Fig. 2.** Water potential of ascospore cytoplasm, frequency of bursting of ascospores in water, and frequency of ascospore germination in *Uncinula necator*. (**A**) Water potential of a solution of NaCl used as a mountant that caused incipient plasmolysis of 50% of the ascospores tested on glass slides in 1986-87 and (**B**) 1987-88. (**C**) Percentage of harvested ascospores that burst in water mounts after 24 hr at 25 C in 1986-87 and (**D**) 1987-88. (**E**) Percentage of ascospores that germinated in water mounts after 24 hr at 25 C in 1986-87 and (**F**) 1987-88.

than 0.1 mm was recorded. This study was repeated four times in 1988 and twice in 1989.

## RESULTS

**Seasonal changes in germination of ascospores.** In both years of the study, similar trends were seen in seasonal changes of the water potential of ascospore cytoplasm, bursting of ascospores in distilled water (Fig. 1), and germination of ascospores. The water potential of ascospore cytoplasm increased continuously during overwintering (Fig. 2A and B), presumably resulting in an increased pressure potential when ascospores were in distilled water. Ascospores were observed to burst in distilled water beginning in early November (Fig. 2C and D). The frequency of burst ascospores increased to a maximum in January, and then declined to near 0 at the time that ascocarps began to dehisce naturally in late April (Fig. 2C and D). In late August and early September, ascospores released from mechanically ruptured ascocarps would not germinate in water droplets or in saturated atmospheres (Fig. 2E and F). The percentage of ascospores that germinated increased in November, but then declined steadily, reaching 0 in late January and early February. Ascospore germination at 0 VPD again increased after early March (Fig. 2E and F). There was no significant ($P = 0.05$) change in the thickness of ascospore cell walls as cleistothecia aged. Ascospores did not germinate at 600 Pa VPD (76% RH at 20 C) before natural discharge of ascospores was observed in late April of either year of the study.

**Effects of free water, vapor pressure deficit, and temperature on germination and appressorium formation.** There was no significant difference in the percentage of ascospores that germinated or formed appressoria in droplets of distilled water or at 0 VPD at 25 C (Fig 3). However, both germination and appressorium formation were substantially reduced in less than saturated atmospheres. After 24 hr at VPD 600, 760, and 1453 Pa at 25 6, 40.4, 30.6, and 16.9% of the ascospores germinated, and 67.6, 54.0, and 31.2% of the germinated spores formed appressoria, respectively (Fig. 3). Ascospores at 0 VPD germinated after 24 hr at temperatures from 5 to 31 C and formed appressoria at 10–25 C (Fig. 4). The percentage of ascospores that germinated after 24 hr did not differ significantly at temperatures from 10 to 25 C but was reduced at 5 and 31 C (Fig. 4A). The percentage of germinated ascospores that had formed appressoria after 24 hr did not differ significantly at 20–25 C but was reduced at 15 and 10 C (Fig. 4B). Ascospores incubated at 20–25 C germinated within 4 hr, formed lobate appressoria within 12 hr, and occasionally formed multiple germ tubes after 24 hr at 0 VPD.

**Effects of temperature upon infection of *Vitis* by ascospores.** No infection was observed on plants incubated at 5, 31, or 36 C (Fig. 5). The mean and standard error of the percentage of plants that were colonized by ascospores was 23.3% ± 1.96% and 56.6% ± 6.35% at 10 and 15 C, respectively (Fig. 5). At 20–25 C, 89–94% of the plants were infected, and there was no significant ($P = 0.05$) effect of temperature upon incidence of infection (Fig. 5).

## DISCUSSION

The seasonal trends observed in germination and bursting of ascospores appears to be related to maturation of the ascospores during overwintering, decreasing water potential of ascospore cytoplasm, and changes in the strength of the ascospore wall. Ascospores released from mechanically fractured ascocarps in early autumn were able to germinate in distilled water, but did not germinate in less than saturated atmospheres. As the water potential of ascospore cytoplasm decreased during overwintering, an increasing proportion of mechanically released ascospores burst in water. The bursting of ascospores eventually resulted

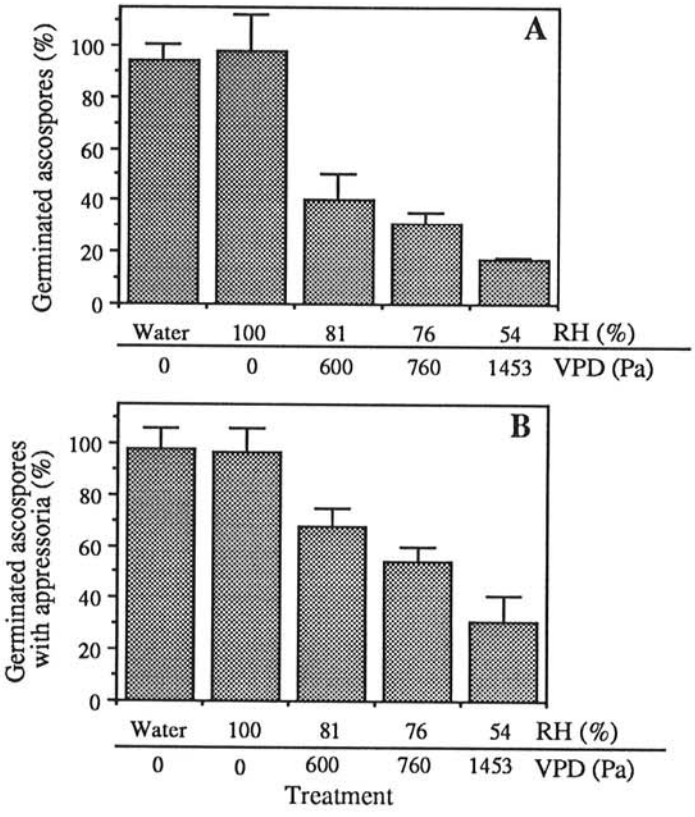


**Fig. 3.** (**A**) Germination of ascospores of *Uncinula necator* on glass slides after 24 hr at 25 C in water and at various humidities. Ascospores were naturally released from cleistothecia collected from vineyards in April of 1988 and 1989. (**B**) Percentage of the ascospores released above that formed appressoria.

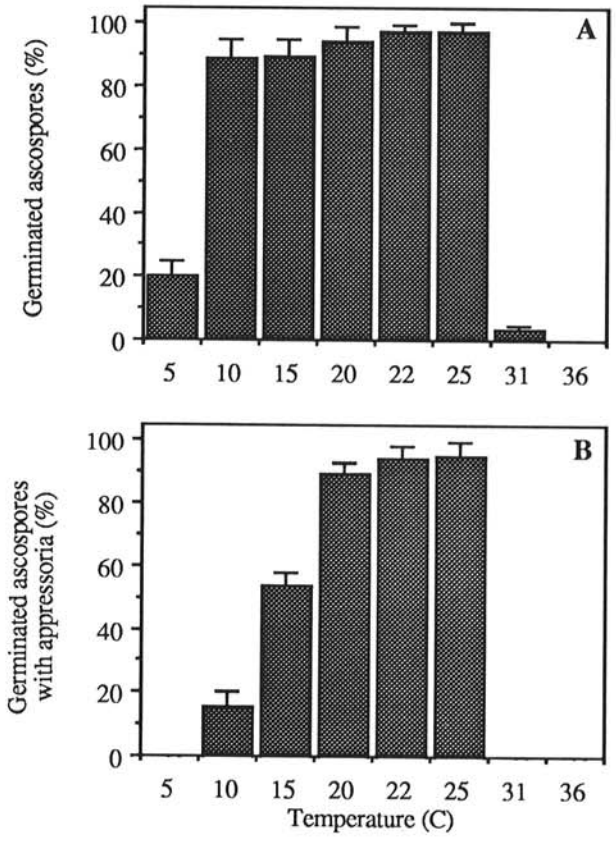


**Fig. 4.** (**A**) Germination of ascospores of *Uncinula necator* on glass slides after 24 hr at 5 to 36 C and 0 VPD. Ascospores were naturally released from cleistothecia collected from vineyards in April of 1988 and 1989. (**B**) Percentage of the ascospores released above that formed appressoria.

in a complete absence of germinated spores in midwinter of each year. However, in later months, the frequency of germination increased and the bursting of ascospores in water became less common. The decreasing incidence of burst ascospores in spring continued, despite a continual decrease in the water potential of ascospore cytoplasm. In a previous study (6), we reported that ascospores of *U. necator* swelled in distilled water mounts between August and February, but that ascospore dimensions did not increase significantly in distilled water mounts between February and April, again, despite a continual decrease in the water potential of ascospore cytoplasm. Our measurements of the cell walls of ascospores indicated that the decrease in the frequency of burst ascospores in spring is not related to increased thickness of the wall, and it may be, instead, a result of qualitative changes in the ascospore cell wall. The inability of ascospores to germinate in water immediately after formation, or in the absence of water before spring, does not appear to be related to the strength of the ascospore wall or to the water potential of ascospore cytoplasm. These events are probably associated with physiological processes of maturation not examined in our study.

Ascospores of *U. necator*, like conidia, can germinate and form appressoria in less than saturated atmospheres. However, the tolerance of ascospores to dry conditions is far less than that reported for conidia. Germination of ascospores was markedly reduced at VPD between 600 and 1453 Pa. In contrast, conidia of *U. necator* are relatively insensitive to VPD at temperatures below 25 C (2). Germination of conidia and infection of grapevines has been reported at 30 C and VPD of 4133 Pa (2). Unlike the conidia of *U. necator* (2) and several other Erysiphaceae (16), ascospore germination is favored by saturated atmospheres and the presence of free water. Although the anamorph of *U. necator* is generally considered a xerophytic plant pathogen (16), rainfall and leaf wetness are critical to release of ascospores from cleistothecia (6) and favor infection by ascospores.

Ascospores germinated equally well after 24 hr at temperatures of 10–25 C, but germination was reduced to less than 30% at 5 C and less than 5% at 31 C. Germinated ascospores failed to form appressoria at 5 and 31 C, and no infection was observed at these temperatures. The minimum temperature for germination and infection by ascospores and the reduction of infection by ascospores at temperatures of 10 and 15 C are similar to results reported by Delp (2) for the conidial state. However, Delp (2) reported that 40% of conidia germinated at 31 C, with a maximum germination temperature of 33 C. The minimum and maximum temperatures for infection by ascospores correspond to those reported for growth and maturation of cleistothecia of *U. necator* (4). Discounting the effects of humidity, the response of ascospores to temperature may be similar to that of conidia, with the possible exception of a greater sensitivity of ascospores to extremely high temperatures.

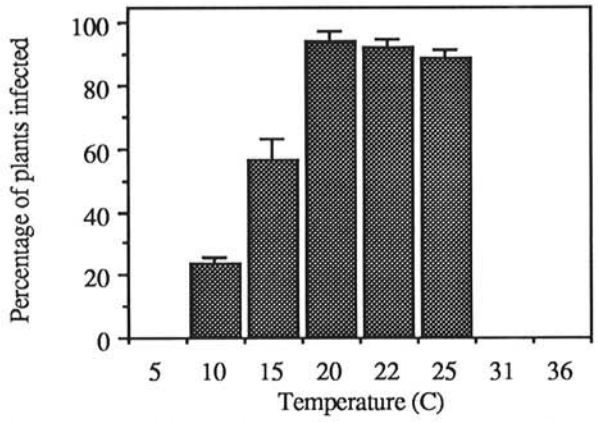


**Fig. 5.** Percentage of plants infected by ascospores of *Uncinula necator* at various temperatures. Tissue culture plants of the *Vitis* interspecific hybrid cultivar Chancellor were inoculated with ascospores released from overwintered cleistothecia in 1988 and 1989. Plants were examined 14 days after inoculation for mildew colonies.

The ability of conidia of powdery mildews to germinate without free water has been attributed to an endogenous water supply contained within the conspicuously large vacuoles of conidia (16). Such vacuoles are common in conidia of *U. necator*, but we have not observed similar water-containing vacuoles in ascospores at any stage preceeding ascospore discharge (6) or during germination. The cytoplasm of ascospores is uniformly granular and surrounds numerous large lipid droplets (6). Despite the apparent absence of an endogenous water supply analogous to that reported for conidia, ascospores of *U. necator* germinate on dry surfaces at 54% RH, albeit to a lesser degree than do conidia.

The changes that we observed in ascospore germination during overwintering are interesting laboratory phenomena, but with no counterpart under field conditions. Ascospores that have not overwintered are rarely released from cleistothecia under natural conditions in New York (6,9). The variable response of ascospores to free water and humidity and the bursting of ascospores in water occurred in our study when physiologically (and possibly morphologically) immature ascospores were artificially released from cleistothecia. These responses, however, are significant in light of previous attempts to reproduce grape powdery mildew from ascosporic inoculum. We reported earlier that survival of cleistothecia on substrates other than the bark of the vine was relatively low (4), and that there may be a period of several months between production of morphologically mature ascocarps and dehiscence under field conditions (6). The low proportion of viable cleistothecia from leaves may in part explain the negative results obtained by Galloway (7), Yossifovitch (17), and Toma (14) when ascocarps harvested from leaves were used as a source of inoculum. It is also possible that the lack of natural dehiscence in immature ascocarps (6,9), the requirement of immature ascospores for free water for germination, and the bursting of immature ascospores in water exacerbated the difficulty of achieving successful infection.

Considering that the release of ascospores follows rain, the fact that ascospores are more sensitive to VPD than conidia may often be inconsequential. We have observed that ascospores establish mildew colonies with secondary branching of hyphae in as little as 12 hr on grape leaf tissue at favorable temperatures (Gadoury and Pearson, *unpublished*). Several hours of leaf wetness and high humidity can follow rain. For example, during the months of May and June in Geneva, NY for the years 1986, 1987, and 1989, rain in excess of 2.5 mm fell on 43 days. Of the total 43 rain events, 12 were followed by 4–8 hr of leaf wetness, 11 were followed by 9–12 hr of leaf wetness, 8 were followed by 13–16 hr leaf wetness, and 12 were followed by 17 or more hr of leaf wetness (Gadoury and Pearson, *unpublished*). In an earlier report (6), we suggested that rain events greater than 2.5 mm and temperatures above 4 C between the phenophases of bud break and bloom of grape could serve as criteria to identify periods of ascospore release. We can now suggest that infection by the released ascospores is likely to follow release unless VPD increases above 1453 Pa or temperatures are less than 10 C or greater than 31 C. The use of these criteria to schedule postinfection fungicide applications for the combined control of powdery mildew and black rot of grape has been briefly described in a previous report (5).

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