Preliminary Studies of Signaling and Sporulation in *Uncinula necator*

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Introduction

Epidemics of grape powdery mildew are driven by the intense production of conidia by colonies of *Uncinula necator*. Whether descended from overwintered cleistothecia or mycelium within dormant buds, it is the continuous production of secondary inoculum that makes epidemics so destructive. Because neither high humidity nor free water is required for spore germination, every day is a potential infection period.

Control of grape powdery mildew has relied heavily on the use of fungicides. Beginning with Benlate in the 1980s and continuing through the sterol demethylation compounds in the 1990s and the strobilurin compounds in 2003, however, *U. necator* has successively developed resistance to most of the major classes used. There are few replacement materials presently near registration. Growers face a period of uncertain duration, during which there may be few highly effective options for chemical control.

Preliminary investigations by our research group indicate the presence of signaling processes related to the onset of sporulation in nascent mildew colonies and to the cessation of sporulation during formation of the ascigerous stage. This suggests an Achilles heel in the epidemic cycle and an attractive target for control of this and possibly other powdery mildews, should the switch be characterized and manipulated.

Morphogenesis in a Developing Colony

In mildew colonies comprised of a single mating type, colonies grow radially for 5-9 days before beginning to produce conidiophores. Thereafter, conidiophores rapidly form throughout the colony in less than 8 hours. This rapid and synchronous development of conidiophores suggests that conidiation may involve a molecular switch.

To illustrate the transmittance of a putative signal within the colony, detached Chardonnay leaves were inoculated with a conidial suspension of *U. necator*. Colony centers were excised four and five days after inoculation, leaving behind only the one-day old hyphae at the margin of the colony (Fig. 1).

In colonies where the excision was performed on day five, the remnant margin of the colony produced conidia on day 7, coincident with unaltered control colonies. Where the excision was performed on day four, however, sporulation was delayed until 10 days post-inoculation (Fig. 1). This indicates that any signal involved in conidiation was promulgated and transmitted throughout the colony sometime between day 4 and 5.

Removal of the colony center at day 4 had the effect of restarting a developmental clock. Conidial morphogenesis is therefore not a function of colony age, but depends rather on the dissemination of a signal that synchronously generates sporulation throughout the colony.

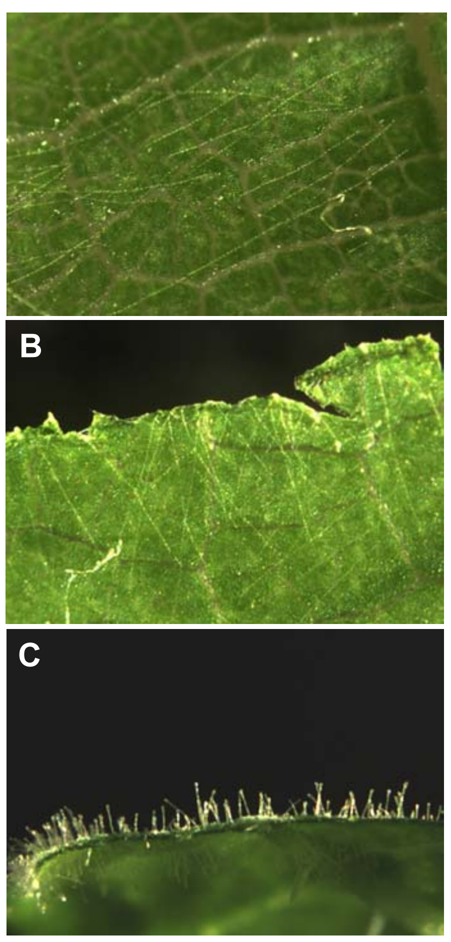

Figure 1. (A) Pre-sporulation growth at margin of 4-day old mildew colony (B) Continued radial growth of mildew colony without sporulation on day 7 following excision of colony center on day 4 (C) Sporulation at margin of mildew colonies on day 7 following excision of center of mildew colony on day 5. Colonies that were not surgically altered also sporulated on day 7.

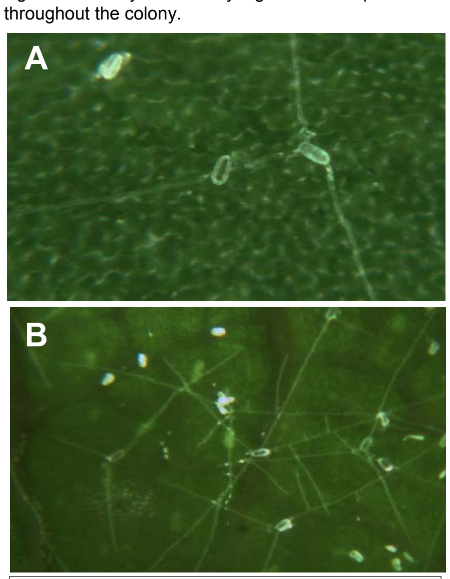

Figure 2. Colony growth after 48 hours of colonies deriving from (A) single conidia and (B) multiple conidia.

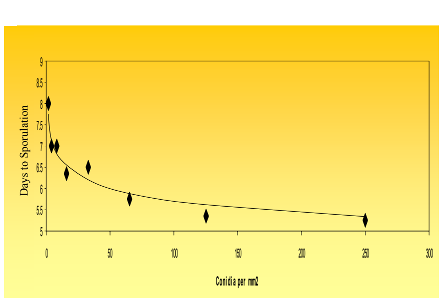

Figure 3. Density-dependent latent period of *Uncinula necator* at 26 C. Conidia were applied in 10 µl droplets containing up to 250 germinable conidia, covering an area of 1 mm^2 on detached leaves of *V. vinifera* 'Chardonnay'. The lowest density/inoculation was 2 germinable conidia per mm^2.

A Density Dependent Latent Period

Previous studies of the latent period in *U. necator* have involved depositing relatively large numbers of conidia by various means upon relatively small areas of host tissue. In nature, inoculum more commonly arrives at the infection court as a single conidium or ascospore (Fig. 2). We used a dilution series of a conidial suspensions to illustrate the effect of inoculum density upon the latent period.

The density of inoculation had a clear influence on days to sporulation. Most of the treatment effect was observed in the range of 1 to 10 conidia per mm^2 (Fig. 3), illustrating how rapidly one might exceed a natural inoculum density during experiments and thereby underestimate the latent period most likely to be observed in a natural epidemic..

The above is also relevant to signaling and conidiation and further indicates that conidiation is not entirely dependent upon colony age. Induction of conidiation may be influenced by the density of the mycelium in the colony, where a developing colony must reach a certain biomass before either the signal is turned on or is produced in sufficient quantity to initiate sporogenesis.

Cessation of Conidiation at the Switch to Sexual Reproduction

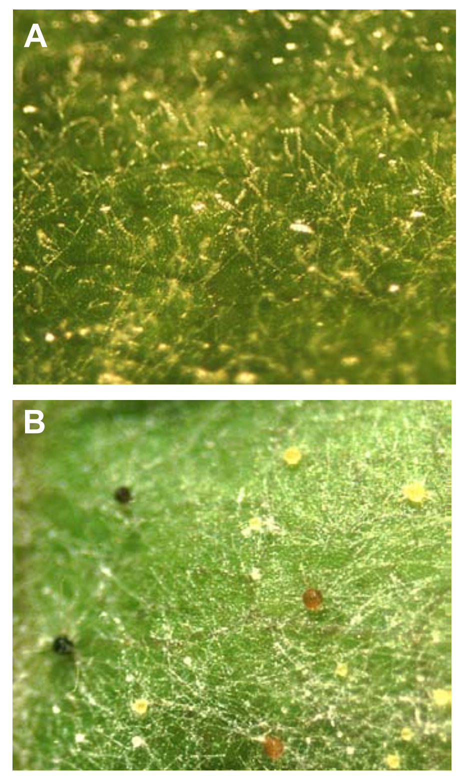

Figure 4. (A) Abundant sporulation of mildew colony of single mating type (B) Absence of sporulation within mildew colony composed of two compatible mating types accompanied by production of cleistothecia.

Under vineyard conditions, sexual reproduction by *U.necator* begins as populations have increased to a level where the pairing of compatible mating types occurs naturally on the host. On severely infected vines, this may occur as early as mid-summer. We have documented that initiation of cleistothecia is coincident with a near-total cessation of conidiation (Fig. 3). This does not appear to be the consequece of a source-sink process, where resources for asexual sporulation are reallocated to the developing cleistothecia. Production of new conidia ceases within 48 hours of contact between compatible mating types, prior to an increase in biomass due to formation of ascocarp initials. During the first few days following contact of the compatible mating type there appears to be rather a net decrease in the rate of biomass production due to the cessation of asexual sporulation prior to a gain due to cleistothecial biomass. In addition, cessation of conidiation is not limited to areas of the colony immediately adjacent to sites of ascocarp initiation. This process, like the sequence at the initiation of conidiation, appears to be the result of a biochemical signal that is transmitted throughout the colony which either functions to directly shut down sporulation or switches off a signal which maintains production of conidia.

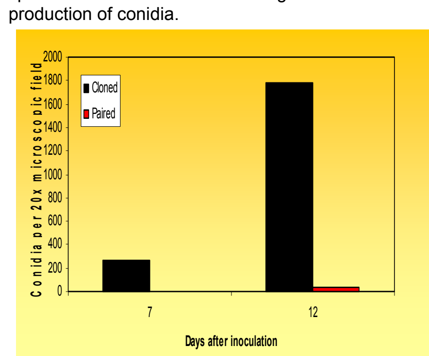

Figure 5. Differential production of conidia 7 and 12 days after inoculation of grapevine seedlings with two sexually compatible isolations of *U. necator* that were grown separately (clone) or paired on individual leaves.

The effect of this switch from asexual to sexual reproduction is significant both in terms of total sporulation and effect on disease progress. Vines which are inoculated with a compatible pair of isolates show near-zero production of conidia when compared with those inoculated with a single clone (Fig. 5).

Studies of disease progress in vineyards before and after initiation of cleistothecia have also shown a marked effect on epidemics. A typical example from a Chardonnay vineyard is shown in Figure 6. Disease incidence increased rapidly and linearly until cleistothecia were initiated. The slope of subsequent disease progress was not significantly different from zero (P = 0.05). The switch to sexual reproduction with its resultant cessation of asexual propagation therefore has a significant effect on disease progress in the field. It is also interesting ecological example of a survival strategy, where asexual reproduction and spread is shut down coincident with the impending need to survive the intercrop period through formation of overwintering structures by the pathogen.

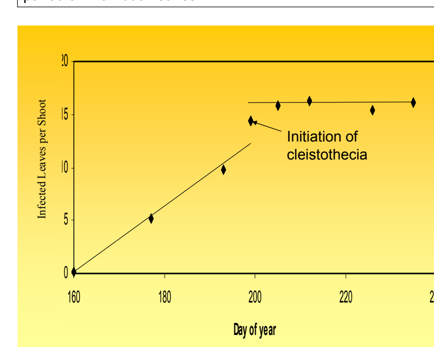

Figure 6. "Broken stick" regression of foliar disease progress in a powdery mildew epidemic in a commercial Chardonnay vineyard before and after initiation of cleistothecia on day 205.

Conclusions

♦ Preliminary studies suggest that sporogenesis in nascent colonies of *Uncinula necator* is induced by a molecular signal generated near the colony center sometime between 4 and 5 days after inoculation

♦ An analogous signal causes conidiation to cease when compatible mating types are paired on the same host tissue.

♦The duration of the latent period, as it operates under field conditions, will be substantially overestimated in experiments where inoculum dose is high.

Future Work

♦ Characterization of the biochemical inducers
- ♦ Bioactivity-guided sub-fractionation
  - ♦ Further subfractionation by High Pressure Liquid Chromatography
    - ♦ Bioassay and mass spectroscopy of putative inducers

♦ Genetic Analysis
- ♦ Design of primers using sporulation associated candidate genes from model Ascomycetes and other powdery mildews and identification of homologues in *U. necator*.
- ♦ Identification of sporulation-associated genes by Differential Subtraction Chain

♦Ecological Studies
- ♦ Effects of hierarchical levels of pairing of compatible mating types upon epidemic progress.