Effects of Ontogenic Resistance upon Establishment and Growth of *Uncinula necator* on Grape Berries

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ABSTRACT

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Grape berries become resistant to powdery mildew early in development and are nearly immune to infection within 4 weeks after bloom. In this study, ontogenic resistance did not reduce attachment, germination, or appressorium formation of *Uncinula necator* on 3- to 4-week-old berries of *Vitis vinifera* 'Chardonnay' or 3-week-old berries of *V. labruscana* 'Concord'. Pathogen ingress halted at the cuticle before formation of a penetration pore. As berries aged, hyphal elongation and colony growth slowed until finally no secondary hyphae formed on fully resistant berries. More appressoria formed per unit of hyphal length as berries aged, indicating that failure to penetrate older berries led to increased

attempts to penetrate resistant fruit. Additionally, hyphae within the colonies began to die as berries aged. Finally, the number of degree-hours between germination and sporulation of the colony (latent period) increased and sporophore density decreased with berry age at time of inoculation. Thus, ontogenic resistance both slows, and eventually halts disease development on grape berries, and limits the likelihood of spread by reducing absolute supply of conidia and delaying their formation. It furthermore has a consistent, stable, and predictable impact on grape powdery mildew and operates in a similar fashion and to a similar degree in both *V. labruscana* and *V. vinifera*, although at a slightly earlier phenological stage in *V. labruscana*.

Additional keywords: adult plant resistance, grape diseases, integrated pest management, *Oidium*.

Several earlier studies have reported that grape berries become less susceptible to powdery mildew as they mature (1–3,13). More recent studies by Gadoury and coworkers (4,5) and Stark-Urnau and Kast (12) have shown that ontogenic resistance to powdery mildew is strongly expressed at an earlier stage of berry development than was indicated in the previous reports. Berries of several cultivars of *Vitis vinifera* and *V. labruscana* are immune to infection within 3 to 5 weeks after bloom (4,5,12). However, exactly how pathogen establishment, growth, and reproduction are affected by ontogenic resistance, and the stage at which the pathogen is stopped on resistant berries, was not investigated. Earlier work focused upon the macroscopic symptoms of disease and signs of the pathogen, i.e., the macroscopic consequences of inoculating berries of different ages. None followed the effect of ontogenic resistance on the actual steps in pathogen development; attachment, germination, appressorium formation, penetration, growth of secondary hyphae, mortality, latent period, or sporulation as berries change from being highly susceptible to nearly immune. Our objective was to document the effects of ontogenic resistance on establishment of *Uncinula necator* on grape berries, colony development, and inoculum potential for subsequent disease cycles. These factors are critical components in predicting the risk of powdery mildew epidemics and subsequent economic losses. Furthermore, a more thorough understanding of where pathogen ingress is halted should aid subsequent biochemical and genetic studies of the mechanisms involved in ontogenic resistance. We included two grape species and three cultivars, *V. labruscana*

'Concord', *V. vinifera* 'Chardonnay', and *V. vinifera* 'Riesling', in our field and laboratory studies to broaden the applicability of the results.

MATERIALS AND METHODS

Vineyards and potted grapevines. Field-grown grapevines, as well as large potted vines, were used in studies of ontogenic resistance. Vineyard studies were conducted in single vine and half vine plots of 'Concord' and single vine plots of 'Chardonnay' vines established in 1974 and located in a 1-ha vineyard at the New York State Agricultural Experiment Station (NYSAES) at Geneva, New York. Additional vineyard studies were conducted in single cluster plots of 'Chardonnay' from 1998 to 2001, and on 'Riesling' in 2001 at the Commonwealth Scientific Industry Research Organization (CSIRO) in Adelaide, South Australia.

To provide fruit that were disease-free at the time of inoculation, vines at NYSAES and CSIRO were sprayed each year with a variety of locally systemic fungicides (e.g., myclobutanil and fenarimol) at 10- to 12-day intervals beginning at 7 to 15 cm shoot length to control powdery mildew. To provide berries that were not only disease-free, but also free of fungicide residues, we used a previously described procedure (4) in which fruit clusters scheduled for inoculation were bagged with clear polyethylene bags during any fungicide application immediately prior to inoculation and during all fungicide applications subsequent to the inoculation. Clusters were visually inspected to confirm the absence of powdery mildew immediately prior to inoculation.

'Chardonnay' and 'Concord' vines planted in 5-liter plastic containers filled with Cornell soil mix (compressed peat and vermiculite mixed 1:2) were vernalized at 4°C at NYSAES for at least 4 months. Vines were removed from cold storage at stag-

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gered intervals and placed in a screen house, leading to the simultaneous development of different phenological stages, and allowing berries of different ages to be evaluated for ontogenic resistance in the same environment. No later than 10 days prior to inoculation, JMS Stylet Oil (1%) (JMS Flower Farms Inc., Vero Beach, FL) was applied to foliage with a hand-held atomizer to eradicate any existing powdery mildew colonies.

Inoculum production and inoculation. Inoculum for field and laboratory studies was produced on grapevine seedlings and detached grape leaves. Seeds from 'Chardonnay' and 'Riesling' grapes were stratified at 4°C for at least 12 weeks, planted in flats upon radicle emergence, and transferred to 4-cm-diameter pots after developing two true leaves. Seedlings were inoculated with conidia of *U. necator* after developing four true leaves and were used as a source of inoculum once colonies were 7 to 14 days old. Three to four seedlings covered with sporulating colonies were used to prepare a spore suspension in 25 to 30 ml of distilled water. The spore suspension was adjusted to a concentration of 10^5 conidia per ml and applied to fruit clusters with a hand-held atomizer as described by Gadoury et al. (4), or was applied to berries as individual 2- to 3- μ l droplets by a micropipette. Berries in certain experiments were inoculated by touching them with 4-cm-diameter leaf disks bearing sporulating mildew colonies. Such leaf disks were cut either from inoculated seedlings or from detached leaves and supported 7- to 10-day-old mildew colonies that bore three to five spores per conidiophore. Detached berries were also inoculated in a 1 m high, 0.17-m-diameter spore-settling tower by tapping three to four leaves or two to three whole seedlings over a wire-mesh screen at the top of the tower.

Attachment of conidia to the surface of berries of different ages. To determine if berry age might influence attachment or adhesion of conidia, 10 berries on each of two clusters from one potted 'Concord' and one potted 'Chardonnay' vine were inoculated in 2000 at NYSAES at the susceptible (at bloom and 7 days postbloom, respectively) or the nearly resistant stage (at 14 and 21 days postbloom, respectively) by touching an infected leaf disk to the berry surface. Initial density of conidia deposited on each berry was determined by counting the number of conidia per five $\times 35$ microscopic fields immediately after inoculation. The vines were incubated at 22 to 25°C and 30 to 50% relative humidity (RH); the final density of the conidial deposit was assessed after 24 h on 'Chardonnay' and after 72 h on 'Concord'. Reductions in the density of the conidial deposit between time of inoculation and 24 to 72 h later were calculated for younger versus older berries, and treatment means were compared using the Student's *t* distribution test. The experiment was repeated in 2001 with the following modifications: five susceptible (3 days old) and five resistant (24 days old) 'Concord' berries and five susceptible (4 days old) and five resistant (30 days old) 'Chardonnay' berries were detached from each of four clusters and placed in microtiter plates filled with 1% water agar. The berries were inoculated using the previously described settling tower. After 24-h incubation at 25°C and 70% RH, the berries were exposed to an air stream of 63 km/h for 1 s. Reductions in the density of the conidial deposit between time of inoculation and 24 h later were calculated for younger versus older berries, and treatment means were compared using the Student's *t* distribution test as described previously.

Germination of conidia, formation of appressoria, and formation of secondary hyphae on berries of different ages. In 1998 at NYSAES, 10 or more berries on each of five 'Chardonnay' clusters and five 'Concord' clusters on each of five vines per treatment were inoculated by placing a single 2- to 3- μ l droplet of spore suspension on each berry with a micropipette at each of the following times: 'Concord' berries were inoculated either at 7, 10, 14, or 21 days after bloom, whereas 'Chardonnay' berries were inoculated either at bloom, 7, 14, 17, or 28 days after bloom. Five berries per inoculated cluster were collected 1 day after inoculation. A single section from the surface of each inoculated berry

was prepared by slicing the berries tangentially under a dissecting microscope at $\times 32$, yielding a disk approximately 4 to 5 mm in diameter. These disks were stained with 0.01% Calcofluor M2R (Pylam Corp., Queenstown, NY) in distilled water for 1 min and viewed with a fluorescence microscope at $\times 400$ (excitation filter BP 450 to 490 nm, chromatic beam splitter FT 510 nm, and barrier filter LP 520 nm). The total number of propagules visible on each disk were counted (up to a maximum of 50 per disk) and the propagules were classified as (i) ungerminated conidium, (ii) bearing only a primary germ tube with or without a terminal appressorium, or (iii) bearing an appressorium and at least one secondary hypha. The experiment was repeated in 1999 at NYSAES with the following modifications: 'Concord' clusters were inoculated at 8 or 28 days after bloom, and 'Chardonnay' clusters were inoculated 10, 21, and 28 days after bloom by touching each inoculated berry with a leaf disk bearing a newly sporulating mildew colony. Berries were collected 3 days after each inoculation, and disks of berry tissue were prepared and examined as described previously. The experiment was repeated in 2000 at CSIRO with the following modifications: 'Chardonnay' clusters were inoculated by spraying them with an atomized spore suspension at 7, 17, and 25 days after bloom. Berries were collected 1 and 6 days after each inoculation date. Disks of epidermal tissue were cut from the berries as described previously, mounted in water on glass slides, and viewed unstained under brightfield microscopy at $\times 400$. In addition to the responses noted above, an ocular micrometer was used to measure the length of the longest secondary hyphal segment of 10 or more randomly selected propagules per disk for berries collected 6 days after inoculation. The experiment was repeated a final time in 2001 at NYSAES with the following modifications: 'Concord' berries were collected at 2 and 13 days after bloom, whereas 'Chardonnay' berries were collected at 5 and 28 days after bloom. The berries were placed immediately in microtiter plates filled with 1% water agar and inoculated in the spore-settling tower. Glass slides were placed beside each microtiter plate during inoculation and were incubated with the berries at 22°C and 75% RH. Conidium density on berries was estimated to be 12 to 14 conidia per mm^2 based on the density of the spore deposit on the slides. Disks were cut from the berries at 1 and 3 days after inoculation, mounted in water on glass slides, and viewed unstained under brightfield microscopy at $\times 400$. As before, the total number of propagules visible on each disk were counted (up to a maximum of 100 per disk) and the propagules were classified as (i) ungerminated conidium, (ii) bearing only a primary germ tube with or without a terminal appressorium, or (iii) bearing an appressorium and at least one secondary hypha. Treatment means were compared using the Student's *t* distribution test or were analyzed by regression of the power-transformed response against berry age (REGRESS, Minitab 10.1, Minitab Inc., State College, PA).

Penetration, formation of haustoria, and growth of secondary hyphae on berries of different ages. In 2001 at NYSAES, 10 berries were picked from each of three 'Chardonnay' clusters at 5 and 31 days after bloom and placed in microtiter plates filled with 1% water agar. Berries were inoculated in the spore-settling tower and incubated in the growth chamber for 24 h at 22°C and 75% RH. A single disk of tissue was cut from each berry as before, fixed in warm lactophenol/ethanol (1:2, vol/vol) for 5 to 10 min, and dehydrated in an ethanol series (50, 70, 95, 100, and 100% for 10 min each). The tissue was infiltrated with methyl salicylate (J. T. Baker, Phillipsburg, NJ) (50 and 100% in ethanol, 15 min each) and left in 100% methyl salicylate overnight. Sections remained on glass slides with the berry surface directed upward during the fixation and clearing process and mounted in methyl salicylate after the clearing was completed. Sections were viewed with a differential interference contrast microscope under an oil immersion lens at $\times 1,000$. The total number of propagules visible on each disk that had formed appressoria were counted (up

to a maximum of 100 per disk) and the appressoria were classified as (i) appressorium only, no penetration pore, or (ii) appressorium with visible penetration pore. Presence or absence of haustoria and secondary hyphae, and distance of the penetration pore from the nearest anticlinal wall, was also recorded for each germinated propagule. The study was repeated on 'Chardonnay' and *V. vinifera* 'Riesling' at CSIRO in 2001 with the following modifications: 'Chardonnay' berries were inoculated at 4 and 31 days after bloom, and 'Riesling' berries were inoculated at 2 and 30 days after bloom. The study was repeated a final time in the field using berries left attached to the vines at CSIRO in 2001. Clusters on 'Riesling' and 'Chardonnay' vines were inoculated with a spore suspension using an atomizer at their susceptible (2 and 4 days after bloom, respectively) and nearly resistant (30 and 31 days after bloom, respectively) stages. To moderate potentially inhibitory high temperatures and low humidity forecasted for the time of the second inoculation (30 to 31 days postbloom), small cages were placed around each cluster after inoculation to increase the RH and to provide shade and evaporative cooling to the inoculated cluster. These cages were prepared from pieces of aluminum window screen, 15 to 20 cm long, that were rolled to form a 10 to 15 cm long tube. The tube was held together by staples and placed around each cluster in the field about one half hour after inoculation, wrapped in four layers of moist paper towels, and left on the cluster for 8 h after inoculation. Berries were sampled 3 days after each inoculation, and disks of berry tissue were prepared, fixed, cleared, examined, and scored as described previously. Treatment means were compared using the Student's *t* distribution test or were analyzed by simple linear regression of the response against time after inoculation (REGRESS).

Frequency of appressorium formation and cell discoloration on berries of different ages. In 1999 at CSIRO, 35 'Chardonnay' clusters were inoculated with an atomized spore suspension at either 14 or 33 days after bloom. Five berries per cluster, five clusters in total were sampled at 1, 2, 3, 4, 5, 6, and 9 days after each inoculation. Berries were viewed under brightfield microscopy at $\times 400$, and the number of appressoria along the longest secondary hyphal strand in the colony, not including the appressorium formed by the germ tube, were recorded. The experiment was repeated in 2000 on 25 'Chardonnay' clusters at 7, 17, and 25 days after bloom. Berries collected after 6 days were prepared and examined as described previously. The number of penetration attempts, as determined by appressorium formation along the secondary hyphal segment, length of the hyphal segment, as well as the percentage of appressoria associated with discoloration of the subtending epidermal cells was recorded. Treatment means were compared using the Student's *t* distribution test.

Colony growth and viability of hyphae on berries of different ages. In 1998 at CSIRO, 30 'Chardonnay' clusters were inoculated with an atomized spore suspension at 15, 22, and 39 days after bloom. Five berries bearing powdery mildew colonies were

collected from each of five clusters for each inoculation date, 6 to 8 days after inoculation and at 4-day intervals thereafter. The percentage of berry surface colonized was estimated with a dissecting microscope at $\times 35$. Berry diameter was then measured to allow estimation of the absolute area colonized. From the berries inoculated at 15 and 22 days after bloom, a single disk of tissue was cut from each berry at 22 to 23 days after inoculation. The disks were submerged in a fluorescent vital stain (5 μ g of aqueous fluorescein diacetate per ml; Acros Organics, N.V., Fairlawn, NJ) for 5 min under constant agitation and were viewed with a fluorescence microscope at $\times 200$ (325 to 500 nm excitation and transmission filter above 530 nm). The percentage of the colony composed of viable (fluorescing) hyphae was estimated based on the size and density of the colony under brightfield compared with fluorescence views. The experiment was repeated at NYSAES in 1999 with the following modifications: 'Chardonnay' clusters were inoculated at 10, 21, or 28 days after bloom. Berries were collected at 4-day intervals from 11 to 31 days after inoculation. 'Concord' clusters were inoculated at 8 days after bloom, and beginning 6 days after inoculation and at 4-day intervals thereafter, berries were collected until 39 days after bloom. Once collected, disks were immediately cut from the berries and examined as described previously. Treatment means were compared using the Student's *t* distribution test or were analyzed by simple linear regression of the response against time after inoculation (REGRESS).

Latent period and sporophore density on berries of different ages. In 2000 at NYSAES, 35 'Concord' clusters were inoculated with an atomized spore suspension. Five infected berries per cluster from each of five clusters were collected at 6, 8, 10, 13, 17, 20, 23, and 27 days after the first inoculation date (bloom), 8 days after the second inoculation date (7 days postbloom), and 6, 7, 9, 10, 13, 17, 25, and 32 days after the third inoculation date (11 days postbloom). On each sampling date, the collected berries were viewed under a dissecting scope at $\times 35$ and checked for sporophore development and number of sporophores per $\times 35$ microscopic field. A data logger (CR10, Campbell Scientific Inc., Logan, UT) recorded hourly temperature in the vineyard over the course of the study.

The latent period of *U. necator* is known to vary with temperature (3), and temperature differed under field conditions in the above experiment as berries aged. It was therefore necessary to account for the variable effect of temperature on latent period in order to isolate and quantify the effect of ontogenic resistance on latent period. We used data reported in an earlier study by Delp (3), in which he described the duration of the latent period at various constant temperatures, to develop the following regression model: $Y = 385.97 \times T^{-1.311}$, where *Y* is latent period (days) and *T* is temperature (degree Celsius).

We initially expressed the duration of the latent periods observed in the above experiment in Celsius degree-hours (base =

TABLE 1. Effect of ontogenic resistance upon penetration of grape berries and formation of haustoria by *Uncinula necator*

Cultivar	Location ^a	Detached or attached ^b	% Appressoria with penetration pores ^c		% Appressoria with haustoria ^c	
			2- to 5-day-old berries	30- to 31-day-old berries	2- to 5-day-old berries	30- to 31-day-old berries
Chardonnay	NYSAES	Detached	97.3 (2.7)	0.0 (0)	49.2 (4.3)	0.0 (0)
		Detached	94.4 (2.1)	43.2 (5.8)	71.4 (11.5)	2.0 (1.5)
	CSIRO	Attached	100.0 (0)	10.7 (7.7)	100.0 (0)	0.0 (0)
Riesling	CSIRO	Detached	90.9 (2.7)	42.0 (8.1)	52.1 (13.1)	14.5 (6.6)
		Attached	91.7 (9.6)	0.0 (0)	83.3 (11.1)	0.0 (0)

^a Location where studies were performed. NYSAES is the New York State Agricultural Experiment Station at Geneva, NY. CSIRO is the Commonwealth Scientific Industry Research Organisation, Adelaide, South Australia, Australia.

^b Berries were either detached and placed on water agar before inoculation, or were inoculated and left attached to vines in the vineyard. Penetration was assessed 1 day after inoculation of detached berries or 3 days after inoculation of berries left in the vineyard.

^c Treatment means and standard errors, in parentheses, indicate the percentage of propagules associated with penetration pores or haustoria beneath appressoria (based on examination of 100 propagules per berry and 10 berries per treatment). Within rows, penetration and haustorium formation were significantly (*P* = 0.01) reduced on berries 30 to 31 days old according to the Student's *t* distribution test.

0°C) based on hourly temperatures recorded in the vineyard, which ranged from 9.9 to 30.1°C. The equation was then used to adjust heat unit accumulation of each hour based on temperature as follows. The latent period estimated by the equation for 30.1°C was 4.44 days. Degree-hour accumulations at all temperatures below 30.1°C were scaled according to the ratio of the estimated value of Y to 4.44. For example, a constant temperature of 25.9°C would yield an estimated latent period of $385.97 \times 25.9^\circ\text{C}^{-1.311} = 5.42$ days. Therefore, the adjustment factor for heat unit accumulation for an hour in which the mean temperature was 25.9°C was $4.44/5.42 = 0.819$. Unadjusted heat unit accumulation in the above example would be 25.9 degree-hours (base = 0°C). Adjusted heat unit accumulation would be $25.9 \times 0.819 = 21.2$ degree-hours. This process was repeated for each hour between the time of inoculation and the observation of sporulation; the adjusted values were summed and were used as the independent variable to estimate the isolated effect of berry age on latent period.

The above experiment was repeated on cv. Chardonnay in 2000 at NYSAES, except that berries from each of the five clusters were collected at 4, 5, 6, 8, 11, 16, and 18 days after the first inoculation (7 days postbloom) and 8, 10, 15, 19, and 24 days after the second inoculation (14 days postbloom). Treatment means were compared using the Student's t distribution test.

RESULTS

Effects of berry age on prepenetration development of *U. necator*. Neither attachment nor germination of conidia, nor formation of appressoria, was consistently affected by age of berries. In 2000, loss of conidia at 3 days after inoculation of 14-day-old 'Concord' berries was not greater than the loss from 1-day-old berries (72.6%, SE = 7.0 versus 78.1%, SE = 5.0 per cm², respectively). In 2001, more conidia were lost from 24-day-old 'Concord' berries (48.4%, SE = 15.0%) exposed to an air stream after inoculation (SE = 15.0%) than from 3-day-old berries (17.8%, SE = 5.6%). In 2000, more conidia were detached from 21-day-old 'Chardonnay' berries (19.3%, SE = 1.0%) than from 7-day-old berries (10.6%, SE = 4.3%). However, the outcome was reversed in 2001 when the experiment was repeated and 'Chardonnay' berries were exposed to an air stream, i.e., 5.8% of the conidia were removed from 30-day-old 'Chardonnay' berries (SE = 1.5%), whereas 23.6% (SE = 2.1%) of the conidia were removed from 4-day-old berries.

Berry age had no significant effect on germination of conidia ($P = 0.05$). In none of the studies (vineyard or controlled environment) did more conidia germinate on younger berries than on older berries. Mean germination rates for conidia deposited on 'Concord' berries ranged from 32.8 to 38.0% in 1999 and from 62.5 to 87.1% in 2001, whereas mean germination rates on 'Chardonnay' berries ranged from 37.8 to 60.0% in 1999 and 77.7 to 82.2% in 2001. The mean temperature of the 24-h period following vineyard inoculations ranged from 19.9 to 25.2°C, within the optimal range for germination reported by Delp (3).

The percentage of germinated conidia that formed appressoria on 'Concord' and 'Chardonnay' berries was not affected by berry age ($P = 0.05$). The mean percentage of germinated conidia that formed appressoria ranged from 77.6 to 98.6% on 'Concord' berries and from 84.3 to 87.7% on 'Chardonnay' berries.

Effect of ontogenic resistance on penetration. In studies with detached berries at NYSAES in 2001, there was a significant decrease ($P = 0.05$, Student's t distribution test) in the number of penetration pores observed beneath appressoria formed on older 'Chardonnay' berries (Table 1). Similarly, at CSIRO in 2001, significantly more ($P = 0.05$, Student's t distribution test) penetration pores were formed beneath appressoria on detached 'Chardonnay' berries inoculated 4 days after bloom than on berries inoculated 31 days after bloom (Table 1). This difference was

observed within 24 h of inoculation (Table 1). The difference was still evident 3 days after inoculation, when we found that 100% of the appressoria were associated with a penetration pore on 4-day-old berries, but only 50% of the appressoria had penetration pores on 31-day-old berries (Table 1). Similarly, when 'Riesling' and 'Chardonnay' berries were left attached to incubate in the vineyard, the percentage of appressoria with penetration pores was significantly lower ($P = 0.05$) on resistant than on susceptible berries (Table 1).

The location of penetration pores on the epidermal cell surface was not random. Significantly more ($P = 0.05$) penetration pores were found at or near the anticlinal wall of the berry epidermal cells than near the center of the cell. On both 'Riesling' and 'Chardonnay' berries, 79.3% (SE = 7.1) and 77.3% (SE = 5.4) of the penetration pores were located at or close to the anticlinal wall of epidermal cells, respectively. Penetration pores near the middle of epidermal cells accounted for 20.7% (SE = 7.1) and 22.7% (SE = 5.4) of all observations on 'Riesling' and 'Chardonnay' berries, respectively.

Successful establishment of the pathogen, as evidenced by formation of secondary hyphae, was not related to location of the penetration pore with respect to the anticlinal walls of berry epidermal cells on either 'Riesling' or 'Chardonnay' berries. No differences were noted in the percentage of conidia forming secondary hyphae when observations were grouped by location of the penetration pore (i.e., near or not near an anticlinal wall). Of all penetrations at the anticlinal cell walls, 73.6% (SE = 8.8) resulted in formation of secondary hyphae, while the comparable value was 70.8% (SE = 13.0) for penetrations more than 3 µm from the nearest anticlinal wall.

Fig. 1. Development of secondary hyphae of *Uncinula necator* on **A**, *Vitis labruscana* 'Concord' and **B**, *V. vinifera* 'Chardonnay' berries at different developmental stages in 1998 at NYSAES. Berries at different developmental stages were inoculated with an aqueous suspension of conidia and sampled after 1 day. The percentage of appressoria-bearing conidia that had progressed to form secondary hyphae was recorded.

Effect of ontogenic resistance on development of haustoria.

Significantly more ($P = 0.05$) appressoria were associated with a haustorium on younger 'Chardonnay' berries at NYSAES than on older berries (Table 1). A similar reduction in the number of haustoria

formed on older berries was observed on detached 'Chardonnay' berries in studies at CSIRO (Table 1). Repetition of the CSIRO study using attached berries in the vineyard produced similar results. After 3 days, there were significantly ($P = 0.05$) more appressoria associated with haustoria on younger 'Chardonnay' and 'Riesling' berries than on older berries (Table 1).

Effect of ontogenic resistance on the initiation and growth of secondary hyphae. The percentage of germinated conidia that formed secondary hyphae was inversely proportional to berry age at the time of inoculation for 'Concord' and 'Chardonnay' at NYSAES in 1998 (Fig. 1). No secondary hyphae formed when berries of either cultivar were inoculated 13 to 31 days after bloom (Fig. 2). Length of secondary hyphae increased linearly over time on both younger and older 'Chardonnay' berries (Fig. 3). The slope of an equation describing the increase of hyphal length over time was significantly higher ($P = 0.02$) for 7-day-old berries than for 25-day-old berries (Fig. 3).

Effect of ontogenic resistance on growth in colony area. Absolute and relative colony area increased over time on 'Chardonnay' berries inoculated in 1998 either at 15, 22, or 30 days after bloom (Fig. 4), but the slopes of equations describing rates of colony growth decreased significantly ($P = 0.05$) as berry age at inoculation increased (Fig. 4). In 1999, significant increases in colony area ($P = 0.05$) were observed during the first 2 weeks of colony development after 'Chardonnay' and 'Concord' berries were inoculated at 8 to 10 days of age (Fig. 5). No significant ($P = 0.05$) change in colony area was recorded after colonies were 15 days old, at which time the berries were 23 to 25 days old (Fig. 5).

Effect of ontogenic resistance on the frequency of penetration sites along the hyphal strand. Ontogenic resistance was associated with increased penetration attempts, as indicated by an

Fig. 2. The percentage of appressoria-bearing conidia that had progressed to form secondary hyphae of *Uncinula necator* on **A**, *Vitis labruscana* 'Concord' and **B**, *V. vinifera* 'Chardonnay'. In 1999, 'Concord' and 'Chardonnay' berries in the vineyard at NYSAES were inoculated by touching mildewed leaf disks to berries that were then examined microscopically 3 days later. In 2001, detached 'Concord' berries at NYSAES, and detached 'Chardonnay' berries at CSIRO, were inoculated in a spore-settling tower and examined microscopically 1 day (NYSAES) or 3 days (CSIRO) later. Bars indicate one standard error of the mean. Means or standard errors not visible approached zero.

Fig. 3. Early hyphal growth of *Uncinula necator* on berries of *Vitis vinifera* 'Chardonnay' inoculated 7 or 25 days after bloom. Berries were collected from the vineyard at 1-day intervals after inoculation and were examined at $\times 400$ magnification.

Fig. 4. **A**, Relative and **B**, absolute area colonized by *Uncinula necator* on *Vitis vinifera* 'Chardonnay' berries at CSIRO in 1998. Berries inoculated at different developmental stages were collected from the vineyard at 4-day intervals. Relative area was estimated as the percentage of berry surface colonized at $\times 35$ magnification. Berry diameter was measured to calculate berry surface area and the absolute area colonized.

increase in number of appressoria per unit of hyphal length. In 1999 at CSIRO, the number of appressoria per hyphae increased with berry age within 9 days on 'Chardonnay' clusters inoculated at either 14 or 28 days after bloom. When the number of appressoria along a hyphal strand was regressed against time after inoculation, the rate of increase in the number of appressoria per hyphal strand was greater ($P = 0.05$) for berries inoculated 28 days after bloom ($y = 1.556x - 2.954$, $r^2 = 0.93\%$, $SE = 0.31$) than for berries inoculated 14 days after bloom ($y = 0.691x - 0.961$, $r^2 = 0.94\%$, $SE = 0.57$). The study of appressorium frequency was repeated at CSIRO in 2000 and included quantitative measurements of hyphal length. Within 6 days of inoculation, the number of appressoria per millimeter of hyphae was significantly ($P = 0.05$) higher on more resistant berries (9.0 appressoria per mm, $SE = 0.25$) than on more susceptible berries (5.3 appressoria per mm, $SE = 0.51$).

Effect of ontogenic resistance on latent period and sporophore density. The latent period increased significantly ($P = 0.05$) with berry age at time of inoculation. Adjusted degree-hour accumulation during the latent period of 'Concord' was 1,986 degree-hours ($SE = 0$) on susceptible versus 2,931 degree-hours ($SE = 227$) on more resistant berries. The latent period on resistant 'Chardonnay' berries was nearly double that of more susceptible berries: 2,472 degree-hours, $SE = 0$ versus 1,337 degree-hours, $SE = 146$, respectively.

The slope coefficient of a linear regression fit to sporophore density over time for 'Concord' berries indicated a significant ($P = 0.05$) increase over a period of 25 days for berries inoculated at bloom (Fig. 6A). However, the same analysis applied to 'Concord' berries inoculated 11 days after bloom, yielding an equation

Fig. 5. Area colonized by *Uncinula necator* on **A**, *Vitis vinifera* 'Chardonnay' berries and **B**, *V. labruscana* 'Concord' berries at CSIRO in 1999. Fruit were inoculated at **A**, 10 or **B**, 8 days after bloom. Berries were collected from the vineyard 11 days later and at 3- to 5-day intervals thereafter. The proportion of berry surface colonized was estimated at $\times 35$ magnification, and the area colonized was computed based on berry diameter. Bars indicate one standard error of the mean.

with a slope not significantly ($P = 0.05$) different from 0 (Fig. 6A). Sporophore density increased linearly over time on 'Chardonnay' berries inoculated at both 7 or 17 days after bloom, but increased significantly faster ($P = 0.05$) on the younger berries (Fig. 6B).

Effect of ontogenic resistance on mycelium viability. When 'Chardonnay' berries were inoculated 15 or 22 days after bloom at CSIRO, and then examined 3 weeks later, hyphal viability was significantly ($P = 0.01$) higher on berries inoculated 15 days after bloom (38.7%, $SE = 4.4$) than on berries inoculated 22 days after bloom (5.8%, $SE = 9.9$). The mean temperature of the 3-week periods following the above inoculations were 24 and 27°C, respectively, whereas the maximum temperatures recorded were 34 and 38°C, respectively: below temperatures that might cause hyphal mortality (3). The percentage of viable hyphae within mildew colonies declined significantly ($P = 0.05$) over time when 'Concord' and 'Chardonnay' berries were inoculated 8 to 10 days after bloom at NYSAES (Fig. 7). There were significantly more ($P = 0.05$) viable hyphae in 19-day-old colonies on 'Chardonnay' berries inoculated at 10 days after bloom (28.5%, $SE = 9.0$) than on berries inoculated 21 days after bloom (4.2%, $SE = 1.9$).

DISCUSSION

Ontogenic resistance of grape berries affects powdery mildew by reducing the proportion of propagules that become established; thus, by inhibiting the growth of those propagules that do establish, and through its effects on latent period and sporophore density, it reduces the potential for secondary cycles of disease. Although conidial attachment, germination, and appressorium formation were not affected by berry age, successful penetration of 'Chardonnay' and 'Riesling' berries, as well as formation of

Fig. 6. Effects of berry age at time of inoculation on change in sporophore density in colonies of *Uncinula necator* on berries in vineyards of **A**, *Vitis labruscana* 'Concord' and **B**, *V. vinifera* 'Chardonnay' at NYSAES in 2000.

haustoria, often declined to either nil or trace levels once ontogenic resistance was fully expressed in older berries. Hyphal development was severely inhibited by ontogenic resistance in 'Chardonnay', 'Riesling', and 'Concord' berries. *U. necator* appears to be halted by ontogenic resistance in many cases before it forms visible penetration pores. Hence, the mechanisms of ontogenic resistance may operate at, or very near, the interface between the appressorium and the berry cuticle. Once an appressorium is fully established an as-yet-unidentified host response or constitutive barrier halts further ingress of the pathogen. The frequent absence of a visible penetration pore on resistant berries could indicate either the lack of a penetration attempt or the rapid incapacitation of the pathogen. However, Heintz (7) showed that *U. necator* was able to penetrate artificial membranes. Failure of *U. necator* to form a penetration pore therefore seems unlikely to be related to the lack of a host-mediated signal required for further pathogen ingress. A penetration-inhibiting compound might be present within the cuticle of berries expressing ontogenic resistance or might be rapidly synthesized and exported to the cuticle in the earliest stages of spore attachment and germination. No such compound has yet been isolated from grape berry skins. Hence, although we now have a much more comprehensive view of the impact of ontogenic resistance on discrete stages of pathogen development, and have resolved the precise point at which the pathogen is halted during the infection process, the mechanisms of resistance that operate in older berries remain unknown.

Older detached berries in studies at CSIRO in 2001 still showed a degree of susceptibility, measured as successful penetration by the pathogen, while lack of penetration on similarly aged detached berries at NYSAES in 2001 was nearly absolute. This difference is possibly due to greater temporal heterogeneity in flowering of grape clusters in warmer climatic conditions (6), such as those prevailing in South Australia. Increased temporal heterogeneity in flowers would yield a population of berries with a more diverse phenological structure within clusters. Thus, samples taken 30 days after bloom could have included a small, but significant, number of berries in which ontogenic resistance was not yet fully expressed. It is noteworthy that 'Chardonnay' and 'Riesling' berries left attached to the vine in parallel studies at CSIRO showed a substantially lower frequency of penetration than the detached berries at 30 to 31 days after bloom (Table 1). However, design of the experiment did not allow direct statistical comparisons of the response on detached versus attached fruit.

Fig. 7. Change over time in percentage of viable hyphae within colonies of *Uncinula necator* on berries of *Vitis labruscana* 'Concord' and of *V. vinifera* 'Chardonnay' at NYSAES in 1999. 'Concord' and 'Chardonnay' berries were inoculated at 8 and 10 days after bloom, respectively. Viability of hyphae was assessed at 4- to 5-day intervals using fluorescence microscopy and the fluorescent vital stain fluorescein diacetate at $\times 200$ magnification.

Approximately 80% of all penetration pores in 'Chardonnay' berries were found at, or near, anticlinal cell walls. However, our study did not show a correlation between penetration at anticlinal cell walls and successful establishment of a haustorium in underlying or neighboring cells. Recognition of a topographical signal is common in rust uredospores to locate stomata of the host cells (9), but has not been reported for powdery mildews, which penetrate directly. Nonetheless, it is intriguing that germlings of *U. necator* appear, to some degree, to preferentially form appressoria at the anticlinal walls of berry epidermal cells. A study of barley powdery mildew showed that *Blumeria graminis* differentiated between host and artificial surfaces and responded differently to them (10). The findings in that study lead us to speculate that *U. necator* might also be capable of sensing differences in the berry surface, such as anticlinal cell walls. How this recognition operates in grape powdery mildew and what benefit it might confer upon the fungus is not clear.

Increase in hyphal length and colony area slowed significantly as 'Chardonnay' and 'Concord' berries expressed ontogenic resistance and the colony matured. Likewise, hyphal death occurred to a greater degree in colonies on older berries. Age of the colony could itself be an impeding factor in colony expansion and survival and could therefore confound the effect of ontogenic resistance on colony growth and hyphal mortality. The potential life span of a hyphal segment and its associated haustoria is not known. If hyphal viability is intrinsically time-limited, then death of hyphae within the colony is a natural aging process independent of ontogenic resistance, and colonies of the same or similar ages should show a comparable percentage of viable hyphae and comparable rates of expansion. However, when we compared the growth of same-aged colonies on berries inoculated at different stages of development, the inhibitory effect of ontogenic resistance on colony growth and hyphal mortality was independent of colony age and directly proportional to age of the fruit.

Hyphal growth depends on the presence of functional haustoria to maintain a sufficient nutrient supply from the host. Initiation of new colonies on resistant berries is limited by prevention of penetration. New haustoria needed for further colony growth would likewise be reduced by the failure of penetration along the hyphal strand as berries age and increasingly express ontogenic resistance. More appressoria were formed along the hyphal strand on berries inoculated at later, more resistant stages, than on berries inoculated earlier. Nonetheless, the rate of hyphal growth was reduced. As suggested by Heintz and Blauch (8), increase in penetration sites or appressoria per unit of hyphal length does not necessarily lead to an increase in penetration success. The higher number of appressoria along a slower growing hyphal strand suggests an attempt to compensate for failed penetration attempts in the colony rather than an increase in successful penetration.

Latent period increased significantly in direct proportion to expression of ontogenic resistance in 'Concord' and 'Chardonnay' berries, while sporophore density decreased with berry age at time of inoculation. Ability to form sporophores early and in high density depends on the availability of nutrients for pathogen growth. Collectively, the foregoing results suggest that the pathogen becomes increasingly less efficient in accessing nutrients as berries age, forms fewer propagules, and thus requires more time to reproduce.

We have quantified many of the effects of ontogenic resistance on the increase of the pathogen biomass and disease severity, e.g., colony expansion rates, hyphal mortality, sporulation density, and latent period. Although not the subject of the present study, these rate determinations and fitness components could be integrated and may be of particular interest and use in simulation modeling of grape powdery mildew. This study may also serve as a model for studies of ontogenic resistance to other pathogens in grape berries and to powdery mildew in the leaves of grapevine, both of

which have been reported (11) but not investigated to the extent of this study.

Ontogenic resistance affected disease development at many levels. It both slowed, and eventually halted disease development on grape berries, and limited the likelihood of spread by reducing the absolute supply of conidia and delaying their formation. In several vineyard and laboratory studies, ontogenic resistance was shown to have a consistent, stable, and predictable impact on grape powdery mildew. It operated in a similar fashion and to a similar degree in both *V. labruscana* and *V. vinifera*, although at a slightly earlier phenological stage in *V. labruscana*. Elsewhere (5), we have demonstrated that grape berries will remain mildew-free at harvest if protected from infection until ontogenic resistance is fully expressed, and have shown how our present results could be used to maximize the efficiency of fungicide applications and reduce selection pressure for fungicide resistance among pathogen populations exposed to at-risk compounds.

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