Effects of Leaf Maturity and Cultivar Resistance on Development of the Powdery Mildew Fungus on Grapevines

M. A. Doster, and W. C. Schnathorst

Respectively, graduate research assistant, Department of Plant Pathology, University of California, Davis, and research plant pathologist, USDA, ARS, Department of Plant Pathology, University of California, Davis 95616.

This research was financially supported in part by the University of California Integrated Pest Management Program.

The authors thank Mary Ann Sall for assistance with this research.

Accepted for publication 1 November 1984 (submitted for electronic processing).

ABSTRACT


The development of grape powdery mildew (caused by Uncinula necator) was compared on leaves of various maturites of resistant and susceptible grapevines (Vitis vinifera) cultivars growing in a vineyard and in a growth chamber. As leaves matured, colony hyphal length and the percentage of germinated conidia that developed hyphae decreased on both the resistant and susceptible cultivars. In both growth chamber and field experiments, significantly more germinated conidia developed hyphae on young leaves of susceptible cultivars than on young leaves of resistant cultivars.

Temperatures of 20, 25, and 30°C did not significantly affect the number of germinated conidia that developed hyphae or interact with cultivar resistance to affect growth of hyphae. Hyphal growth was greatest at 25°C. Usually no signs of penetration or host response were associated with germinated conidia that did not develop beyond the formation of an appressorium, suggesting that mildew resistance may in part involve factors that inhibit or prevent penetration of epidermal cells.

Grape powdery mildew, caused by Uncinula necator (Schw.) Burr., is a major disease of grapevines (Vitis vinifera L.) in California. U. necator, like other powdery mildew fungi, penetrates the cuticle and cell wall, and establishes functional haustoria in the living epidermal cells of the host. In earlier studies on various powdery mildews, resistance was associated with prevention of penetration by pre-existing properties of the cuticle (13,16) or host response factors such as papillae (1,5). Resistance was also attributed to host cell osmotic pressure (18,23) or a hypersensitive host response (11,21) that interfered with the establishment and function of haustoria after penetration.

Bouba, (4), investigating the nature of resistance in the Vitaceae to U. necator, concluded that in some species, necrosis occurred around appressoria and haustoria, and in others, resistance possibly interfered with the nutrition of the fungus. Goheen and Schnathorst (9,10) showed that resistance in cultivars of V. vinifera may involve osmotic pressure of host cells.

U. necator develops best on young grape leaves and will not usually infect leaves over 2 mo old (17). The increased resistance of older leaves was observed with hosts of other powdery mildews (3,6,14,22,23). This type of resistance was associated with increased osmotic pressure (19,23) and a decrease of nutrients in host cells (22).

The normal sequence of development of U. necator on susceptible grape leaves is germination of the conidium, growth of a germ tube ending in a multiloculated appressorium, penetration, formation of a primary hypha from the end of the conidium opposite the germ tube, formation of other hyphae, and (eventually) conidiophores and conidia (7).

The objective of this study was to compare the development of U. necator on various-aged leaves of resistant and susceptible grape cultivars.

MATERIALS AND METHODS

Light microscopy was used to study development of U. necator on cleared detached leaves and on living attached leaves of plants of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

©1985 The American Phytopathological Society

318 PHYTOPATHOLOGY
growth chamber at 20 ± 1°C. Starting at the first node from the shoot tip having a leaf with the midvein length greater than 5 cm, the leaves at the first, third, fifth, and seventh nodes were inoculated. Two replications were inoculated at different times to give a total of four replications. Forty-eight hours after inoculation, the leaves were cut into rectangles 2 × 4 cm, fixed in formalin-acetic-alcohol, cleared in lactic acid, and stained in lactophenol cotton blue. The length of all hyphae from twenty germinated conidia with appressoria was measured on each leaf.

The second experiment was designed to investigate the effects of temperature and leaf maturity on fungal growth on leaves of the susceptible Thompson Seedless and resistant French Colombard cultivars. Three replications were used at 20.8 C±1.0 C, 25.6 C±1.0 C, and 29.5 C±1.0 C. Starting at the first node from the shoot tip having a leaf with the midvein length greater than 3 cm, the leaves at the first, third, fifth, and seventh nodes were inoculated. Forty-eight hours after inoculation, the leaves were treated as above.

In a third experiment, the effect of the host cultivar on the formation of secondary appressoria (those formed after the first or primary appressorium) was investigated. Young leaves of comparable age on vines of Thompson Seedless, French Colombard, and Rubired were inoculated. The vines were placed in the growth chamber at 20.3 ± 1.0 C. After 72 hr, 2 × 4 cm rectangles were cut from the infected leaf, fixed and cleared in ethanol-acetic acid (1:1, v/v) overnight, and then stained with acid fuchsin. For each of the five replications, 20 fungal colonies per cultivar were examined. The number of appressoria formed were counted on the longest hypha from the infecting conidium.

Studies on powdery mildew development in the vineyard. In a vineyard near Davis, CA, leaves on vines of susceptible Thompson Seedless and resistant Rubired and French Colombard cultivars were inoculated. Two shoots per cultivar were inoculated on 10 May and two others 15 May 1983. There was no rain during this period. Starting at the first node from the cane tip having a leaf with the midvein length greater than 5 cm, the leaves at the first, third, and fifth nodes were inoculated. Seventy-two hours after inoculation, the leaves were cut into rectangles 2 × 4 cm, cleared and fixed in ethanol-acetic acid (1:1, v/v), and then stained in Coomassie Brilliant Blue according to the method of Wolf (24). The length of hyphae developing from 25 germinated conidia with appressoria was measured on each leaf that was evaluated.

All experiments were analyzed as a split-plot design by using analysis of variance. The different levels of leaf maturity were considered to be subplots because several leaves were inoculated on each shoot. Percentages were arcsine-transformed for the analysis. The trend of the variable as leaf maturity increased was investigated.

RESULTS

Growth chamber experiments. Forty-eight hours after inoculation, all germinated conidia had formed lobed appressoria. Frequently, some conidia on leaves were observed to germinate and form appressoria, but did not develop hyphae, while others nearby germinated and formed hyphae. After the leaf was cleared and stained, and germinated conidia that did not develop hyphae were removed while viewing with the light microscope, usually no signs of penetration of the host cell wall or host response were visible. Occasionally, golden coloration of the host cell or a papilliform structure was observed in the host cell.

Fifteen germinated conidia, that after 48 hr at 23 C had formed appressoria but no hyphae on young leaves of the Thompson Seedless and French Colombard cultivars, were examined on living leaves periodically for 8 days. These conidia appeared shrunken and no host response was apparent. After 8 days, none of the fifteen conidia had developed hyphae, even though other conidia on the same leaves had formed sporulating colonies. This indicates that germinated conidia that had not formed hyphae after 48-hr incubation had ceased development.

The percentage of germinated conidia that developed hyphae on leaves in the growth chamber was significantly different among the seven cultivars tested (P < 0.001) and among leaves of various ages (P < 0.0001) (Fig. 1). Significantly (P < 0.01) more conidia developed hyphae on leaves of susceptible Thompson Seedless than on leaves of resistant French Colombard and Rubired. Less than 17% of the germinated conidia formed hyphae on the oldest leaf (leaf 7) for all cultivars tested. The differences in frequency of hyphal development from germinated conidia among cultivars were greatest on the youngest leaf with values ranging from 19.2% for Rubired to 76.4% for Thompson Seedless. In general, the percentage of conidia that developed hyphae decreased linearly (R² = 0.90 untransformed, R² = 0.92 arcsine transformed) as leaf maturity (leaf number) increased.

Significant differences (P < 0.01) in colony hyphal lengths (including only those germinated conidia that developed hyphae) among the cultivars were detected (Table 1). Fungal colonies were significantly larger on Thompson Seedless than on French Colombard or Rubired. The smallest colonies occurred on Rubired. The ranking of the cultivars relative to extent of hyphal development (Table 1) is very similar to that in Fig. 1 which

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Colony hyphal length (mm) on leaf number: 1 3 5</th>
<th>Mean (LSD₀.₀₅ = 0.087)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thompson Seedless</td>
<td>0.585 0.473 0.368</td>
<td>0.475</td>
</tr>
<tr>
<td>Carignane</td>
<td>0.569 0.338 0.382</td>
<td>0.429</td>
</tr>
<tr>
<td>Chenin blanc</td>
<td>0.522 0.348 0.391</td>
<td>0.420</td>
</tr>
<tr>
<td>French Colombard</td>
<td>0.519 0.381 0.265</td>
<td>0.388</td>
</tr>
<tr>
<td>Zinfandel</td>
<td>0.394 0.321 0.334</td>
<td>0.349</td>
</tr>
<tr>
<td>Grey Riesling</td>
<td>0.418 0.321 0.225</td>
<td>0.321</td>
</tr>
<tr>
<td>Rubired</td>
<td>0.366 0.236 0.181</td>
<td>0.261</td>
</tr>
</tbody>
</table>

*Youngest leaf = 1 and the oldest leaf = 5, counting from the tip of the shoot.
corresponds to their ranking for mildew susceptibility. In general, fewer germinated conidia formed hyphae on resistant cultivars and the colonies were smaller. Colony size decreased linearly \((R^2 = 0.91)\) as leaf maturity (leaf number) increased.

In the experiment investigating the effect of temperature on fungal development, there was no significant difference in the percentage of germinated conidia that developed hyphae at 20, 25, and 30 C. However, there was a significant \((P < 0.05)\) difference among the cultivars in the percentage of germinated conidia that developed hyphae. On Thompson Seedless, 71.7% of the germinated conidia developed hyphae on leaf 1 compared to 5.0% on leaf 7 while on French Colombard the percentages were 35.8% on leaf 1 and 7.2% on leaf 7. These data were similar to those obtained in the experiment comparing seven cultivars (Fig. 1), except for French Colombard, for which the percentage of conidia that formed hyphae on leaf three was higher (29.5%) in the temperature experiment. The percentage of conidia that developed hyphae decreased linearly \((R^2 = 0.99, R^2 = 0.99)\) as leaf maturity increased.

There was no significant difference in hyphal length between susceptible Thompson Seedless and resistant French Colombard when infected plants were maintained at three different temperatures. Interactions between cultivars and leaf maturity, and between cultivars and temperature in regard to hyphal growth were not significant. Therefore, the data for the two cultivars were averaged (Table 2). There was a significant \((P < 0.01)\) effect of temperature on the length of hyphae. The highest colonies were observed at 25 C. Colony size decreased linearly \((R^2 = 0.98)\) as leaf maturity increased. The interaction between leaf maturity and temperature on hyphal growth was not significant. A decrease in colony size with increasing leaf maturity was observed at all three temperatures, 20, 25, and 30 C.

Frequently, two or more secondary apressoria were grouped close together (less than 10 mm apart). From a group of apressoria, at most only one normal appearing haustorium was observed, although occasionally necrotic haustoria were present. The number of apressoria in the first and second group along the longest hypha was compared on the three cultivars at 72 hr at 20 C. On French Colombard, there was an average of 1.5 apressoria per leaf, significantly \((P < 0.01)\) more than on Rubired (1.3) or Thompson Seedless (1.2).

**Field experiment.** The percentage of germinated conidia that produced hyphae differed significantly \((P < 0.05)\) among three cultivars in a vineyard at Davis, CA (Table 3). More germinated conidia formed hyphae on susceptible Thompson Seedless than on resistant French Colombard with the largest difference on the youngest leaves. The percentage of germinated conidia with hyphae decreased linearly \((R^2 = 0.81, R^2 = 0.85)\) as leaf maturity increased. The interaction between cultivar and leaf maturity was not significant. The percentages of germinated conidia with hyphae on Thompson Seedless and French Colombard in the vineyard (Table 3) were similar to those obtained in the growth chamber (Fig. 1).

There were no significant differences in the length of hyphae on three cultivars in the vineyard, although the total length of hyphae was greater on leaves of Thompson Seedless than on those of French Colombard and Rubired (for the first leaf of these cultivars the means were 0.64 mm, 0.38 mm, and 0.50 mm, respectively). The standard deviation was large \((0.22)\). Significant \((P < 0.005)\)

<table>
<thead>
<tr>
<th>Temperature (C)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>Mean (LSD(_{0.05} = 0.141))</th>
<th>Leaf maturity*</th>
<th>Mean of arcsine-transformed data (LSD(_{0.05} = 13.0))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.483</td>
<td>0.387</td>
<td>0.410</td>
<td>0.272</td>
<td>0.388</td>
<td>20.0</td>
<td>44.2</td>
</tr>
<tr>
<td>25</td>
<td>0.1030</td>
<td>0.1022</td>
<td>0.642</td>
<td>0.352</td>
<td>0.321</td>
<td>8.0</td>
<td>98.1</td>
</tr>
<tr>
<td>30</td>
<td>0.817</td>
<td>0.407</td>
<td>0.407</td>
<td>0.350</td>
<td>0.312</td>
<td>1.5</td>
<td>25.2</td>
</tr>
</tbody>
</table>

*Youngest leaf = 1 and the oldest leaf = 5, counting from the tip of the shoot.

**DISCUSSION**

In the present study, germinated conidia consistently produced hyphae less frequently on young leaves of resistant French Colombard than on those of susceptible Thompson Seedless. Similarly, cultivar resistance to powdery mildew in strawberry was correlated with the percentage of germinated conidia that failed to penetrate the cuticle and epidermal cell wall of leaves (16). A significant difference was found in the percentage of germinated conidia of *Erysiphe betae* (Vanha) Wiltzien that formed hyphae on different sugarbeet cultivars (15). Fewer germinated conidia of *Erysiphe graminis* DC ex Merat f. sp. hordii formed hyphae on resistant barley cultivars than on a susceptible cultivar (20). Papilae were usually (but not always) associated with germinated conidia of *E. graminis* on barley that produced no hyphae (1). In the present study, the lack of formation of hyphae on French Colombard could not be attributed to papillae as they were only rarely seen. With some cultivars (eg, Rubired), resistance could be partially explained by a host hypersensitive response, but the difference in susceptibility between Thompson Seedless and French Colombard was not associated with a visible host response (unpublished).

Cuticle thickness of other hosts has been suggested as a physical barrier to other powdery mildew fungi (12,14). Martin et al (13) found that leaf abaxial waxes and some of their fractions had fungistatic properties against apple powdery mildew fungus, *Podosphaera leucotricha* (Ell. & Ev.) Salm. Peres (16) stated that there was insufficient evidence on the importance of chemical and physical properties of the cuticle to draw a conclusion on their contribution to strawberry powdery mildew resistance. Schnathorst (18) showed that the osmotic value of epidermal cells of lettuce leaves was associated with differences in cultivar resistance to *Erysiphe cichoracearum* DC ex Merat while the cuticular and epidermal wall thickness was not. Gehenn and Schnathorst (9,10) presented evidence suggesting the involvement of an osmotic mechanism in the resistance of grape cultivars to grape powdery mildew. In the present study, when germinated conidia of *U. necator* did not produce hyphae on resistant French Colombard leaves, no haustoria or other signs of penetration were observed. This indicates that some factor in the cuticle or epidermal cell wall besides cell osmotic value may be involved in the resistance of grapevine cultivars.

In the present study, gradients in mildew development were observed on shoots; smaller colonies developed on older leaves (Tables 1 and 2) and a reduced number of colonies developed from germinated conidia (Table 3 and Fig. 1). The resistance to powdery mildew of mature apple leaves was associated with higher osmotic values of the epidermal cell (6). Mature peach leaf resistance to powdery mildew was also associated with increased osmotic pressure (19,23) and could be reversed by withholding light (23).

Unlike in the present study, in which the germinated conidia did not develop hyphae, rudimentary haustoria were observed in resistant
peach leaves (23). Temmen et al (22) suggested that the epidermal cells of mature gooseberry leaves had less cytoplasm, hence, less nutrients were available for the gooseberry powdery mildew fungus, and the leaves were more resistant. Either of these hypotheses might explain the slower growth of U. nectarator on mature grape leaves, but not the increase of germinated conidia which apparently failed to penetrate the host cell. There may be another factor, possibly some property of the cuticle, that inhibits penetration. A factor that inhibits penetration could slow colony development because powdery mildew fungi depend on repeated penetrations and secondary haustorial formation to sustain colony development. Although on both young French Colombard leaves and mature leaves of all cultivars only a few germinated conidia seemed to have penetrated the host cell, it has not been determined if the resistance factors involved are the same. It has been observed that late in the season U. nectarator develops profusely again on presenescent leaves of certain cultivars, thus exhibiting a distinct gradient in development (W. C. Schnathorst and A. C. Goheen, unpublished).

It appeared that where U. nectarator failed to form a functional haustorium from an appressorium, a group of secondary appressoria were formed nearby. Perhaps a host factor causing U. nectarator to form more appressoria per group on French Colombard than on Thompson Seedless was the same as that causing fewer germinated conidia to form hyphae on French Colombard than on Thompson Seedless.

The ranking of cultivars according to the percentage of germinated conidia that developed hyphae and the extent of hyphal growth on them correspond closely to their relative field susceptibility as determined by Doster and Schnathorst (8). For all cultivars tested, the decreasing susceptibility of leaves as they mature complicates comparisons of cultivar susceptibility. To obtain meaningful results on cultivar susceptibility we suggest that inoculations and observations on mildew development should be confined to comparable young leaves.

LITERATURE CITED