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

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ABSTRACT

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The development of grape powdery mildew (caused by *Uncinula necator*) was compared on leaves of various maturities of resistant and susceptible grapevine (*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.

Boubals (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 multilobed 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 resistant cultivars French Colombard and Rubired and the susceptible cultivar Thompson Seedless under controlled environmental conditions (growth chamber) and in the field. In one growth chamber experiment, leaves of cultivars Carignane, Chenin blanc, Zinfandel, and Grey Riesling were also used. All cultivars used were *V. vinifera* except Rubired which is *V. vinifera* × *V. rupestris* Scheele.

In all growth chamber experiments hardwood grape cuttings (10–20 cm long) having two or three buds were rooted in 10-cm-diameter pots in U.C. planting mix (2) and were grown in an isolated greenhouse with positive- pressure filtered air (so that the grapevines were mildew free) at approximately 25 C and 22,000 lux illumination until there were approximately 10 leaves per shoot (2–3 mo). The grapevines were inspected regularly to ensure they were mildew-free.

Conidial inoculum was taken from 8–13-day-old colonies growing on Thompson Seedless vines in a greenhouse. Approximately 20 cm^2 of the upper surface of the main lobe of a leaf was inoculated by lightly rubbing it with a detached infected leaf.

The growth chamber used was a Model 818, GCA (Precision Scientific Group, Chicago, IL 60647). The relative humidity in all growth chamber experiments was kept at approximately 50% by maintaining a free water reservoir in the growth chamber. Approximately 3,200 lux illumination was provided 12 hr each day.

The effect of leaf maturity on fungal development was determined by inoculating several leaves along the grapevine shoot, counting from the shoot tip. Grapevines selected for the growth chamber experiments had fully expanded leaves at the seventh node from the shoot tip and sometimes at the fifth node, while the leaves closer to the shoot tip were still growing.

Studies on powdery mildew development in the growth chamber. Fungal development on attached leaves was observed by removing inoculated plants from the growth chamber, carefully viewing colonies with a stage microscope with either side or bottom illumination, and then returning the plant to the growth chamber. The position of each colony on the leaf was mapped on paper and Polaroid photographs of the colonies were taken, thereby allowing the same colony to be located and viewed later.

In the first growth chamber experiment, grapevines of seven cultivars that, based on field observations, ranged from very susceptible to resistant (Carignane and Thompson Seedless, very susceptible; Chenin blanc and Zinfandel, moderately susceptible; French Colombard, moderately resistant; and Grey Riesling and Rubired, resistant) were inoculated with conidia and kept in the

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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-aceto-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 Coosmassie 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 papillalike 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 shrivelled 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 of 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 ($R^2 = 0.90$ untransformed, $R^2 = 0.92$ 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 1. Colony hyphal lengths of *Uncinula necator* on various-aged leaves of seven grapevine cultivars after 48 hr in a growth chamber at 20 C

Cultivar	Colony hyphal length (mm) on leaf number:[a] 1	3	5	Mean ($LSD_{.05} = 0.087$)
Thompson Seedless	0.585	0.473	0.368	0.475
Carignane	0.569	0.338	0.382	0.429
Chenin blanc	0.522	0.348	0.391	0.420
French Colombard	0.519	0.381	0.265	0.388
Zinfandel	0.394	0.321	0.334	0.349
Grey Riesling	0.418	0.321	0.225	0.321
Rubired	0.366	0.236	0.181	0.261
Mean ($LSD_{.05} = 0.068$)	0.482	0.345	0.306	

[a] Youngest leaf = 1 and the oldest leaf = 5, counting from the tip of the shoot.

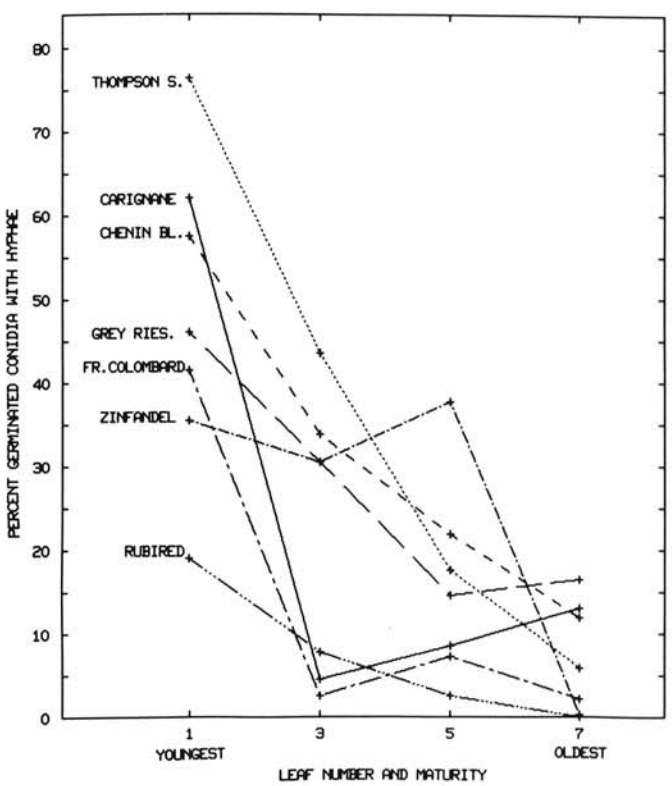

Fig. 1. Comparison of powdery mildew development on immature and mature leaves of seven grapevine cultivars (Thompson Seedless, Carignane, Chenin blanc, Grey Reisling, French Columbard, Zinfandel, and Rubired) 48 hr after inoculation in a growth chamber at 20.0 ±1.0 C.

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 untransformed, R^2 = 0.99 arcsine transformed) as leaf maturity increased.

There was no significant difference in colony 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 signficant ($P < 0.001$) effect of temperature on the length of hyphae. The largest 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 appressória were grouped close together (less than 10 μm apart). From a group of appressoria, at most only one normal-appearing haustorium was observed, although occasionally necrotic haustoria were present. The number of appressoria in the first and second group along the longest hypha was compared on the three cultivars after 72 hr at 20 C. On French Colombard, there was an average of 1.5 appressoria per group, 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 untransformed, R^2 = 0.85 arcsine transformed) 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 ($P < 0.005$) differences in hyphal development occurred on leaves of different ages. The mean colony hyphal length was 0.51 mm for the first leaf, 0.25 mm for the third leaf, and 0.28 mm for the fifth leaf ($LSD_{0.05}$ = 0.15). Hyphal length decreased linearly (R^2 = 0.64) as leaf maturity increased.

TABLE 2. Colony hyphal lengths of *Uncinula necator* on various-aged leaves of Thompson Seedless and French Colombard grapevines after 48 hr at three temperatures in a growth chamber

Temperature (C)	Colony hyphal length (mm) on leaf number:[a] 1	3	5	7	Mean ($LSD_{.05}$ = 0.141)
20	0.483	0.387	0.410	0.272	0.388
25	1.030	1.022	0.642	0.552	0.811
30	0.817	0.407	0.350	0.312	0.471

[a] Youngest leaf = 1 and the oldest leaf = 7, 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) Weltzien that formed hyphae on different sugarbeet cultivars (15). Fewer germinated conidia of *Erysiphe graminis* DC ex Merat f. sp. *hordei* formed hyphae on resistant barley cultivars than on a susceptible cultivar (20). Papillae 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 apple leaf waxes and some of their fractions had fungistatic properties against apple powdery mildew fungus, *Podosphaera leucotricha* (Ell. & Ev.) Salm. Peries (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. Goheen 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

TABLE 3. The percentage of germinated conidia of *Uncinula necator* that developed hyphae on immature and mature leaves of three grapevine cultivars in a vineyard at Davis, CA

Cultivar	Leaf maturity[a] 1	3	5	Mean of arcsine-transformed data ($LSD_{.05}$ = 13.0)
Susceptible				
Thompson Seedless	87.8	26.7	28.0	44.2
Resistant				
Rubired	76.4	8.0	1.5	28.1
French Colombard	51.3	10.5	3.5	25.2

[a] Youngest leaf = 1 and the oldest leaf = 5, counting from the tip of the shoot.

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. necator* 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. necator* 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. necator* failed to form a functional haustorium from an appressorium, a group of secondary appressoria were formed nearby. Perhaps a host factor causing *U. necator* 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 corresponded 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

1. Aist, J. R., and Israel, H. W. 1977. Papilla formation: Timing and significance during penetration of barley coleoptiles by *Erysiphe graminis* hordei. Phytopathology 67:455-461.
2. Baker, K. F. 1957. The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. 23. 332 pp.
3. Berwith, C. E. 1936. Apple powdery mildew. Phytopathology 26:1071-1073.
4. Boublas, D. 1961. Étude des causes de la résistance des vitacées à l'oidium de la vigne, *Uncinula necator* (Schw.) Burr., et de leur mode de transmission héréditaire. Ann. Amelior. Plantes 11:401-500.
5. Carver, T., and Carr, A. 1977. Race non-specific resistance of oats to primary infection by mildew. Ann. Appl. Biol. 86:29-36.
6. Cimanowski, J., and Millikan, D. F. 1970. Resistance of apple to powdery mildew and its relationship to osmotic activity of the cell sap. Phytopathology 60:1848-1849.
7. Delp, C. J. 1954. Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology 44:615-626.
8. Doster, M. A., and Schnathorst, W. C. 1984. Comparative susceptibility of various grapevine cultivars to the grape powdery mildew fungus, *Uncinula necator*. Am. J. Enol. Vitic. (In press).
9. Goheen, A. C., and Schnathorst, W. C. 1961. Resistance to powdery mildew in leafroll-affected grapevines. Plant Dis. Rep. 45:641-643.
10. Goheen, A., and Schnathorst, W. 1963. Resistance to powdery mildew in grapevines related to osmotic value and associated factors. (Abstr.) Phytopathology 53:1139.
11. Hyde, P., and Colhoun, J. 1975. Mechanisms of resistance of wheat to *Erysiphe graminis* f. sp. *tritici*. Phytopathol. Z. 82:185-206.
12. Jhooty, J., and McKeen, W. 1965. Studies on powdery mildew of strawberry caused by *Sphaerotheca macularis*. Phytopathology 55:281-285.
13. Martin, J., Batt, R., and Burchill, R. 1957. Fungistatic properties of apple leaf wax. Nature (Lond.) 180:796-797.
14. Mence, M. J., and Hildebrandt, A. C. 1966. Resistance to powdery mildew in rose. Ann. Appl. Biol. 58:309-320.
15. Mukhopadhyay, A., and Russell, G. 1979. Development of *Erysiphe betae* on leaves of four sugar beet varieties. Phytopathol. Z. 96:15-20.
16. Peries, O. S. 1962. Studies on strawberry mildew, caused by *Sphaerotheca macularis* (Wallr ex. Fries) Jaczewski II. Host-parasite relationships on foliage of strawberry varieties. Ann. Appl. Biol. 50:225-233.
17. Sall, M. A., and Teviotdale, B. L. 1981. Powdery mildew. Pages 46-50 in: Grape Pest Management. D. Flaherty, F. Jensen, A. Kasimatis, H. Kido, and W. Moller, eds. Agricultural Sciences Publications, University of California, Berkeley.
18. Schnathorst, W. C. 1959. Resistance in lettuce to powdery mildew related to osmotic value. Phytopathology 49:562-571.
19. Schnathorst, W. C., and Weinhold, A. R. 1957. An osmotic mechanism for resistance to powdery mildew in lettuce and peach. (Abstr.) Phytopathology 47:533.
20. Stanbridge, B., Gay, J. L., and Wood, R. K. S. 1971. Gross and fine structural changes in *Erysiphe graminis* and barley before and during infection. Pages 367-379 in: Ecology of Leaf Surface Micro-organisms. T. F. Preece and C. H. Dickinson, eds. Academic Press, London and New York.
21. Stavely, J. R., and Hanson, E. W. 1966. Some basic differences in the reactions of resistant and susceptible *Trifolium pratense* to *Erysiphe polygoni*. Phytopathology 56:957-962.
22. Temmen, K. H., Gruppe, W., and Schlosser, E. 1980. Investigations on the resistance of plants to powdery mildew III. Basis for horizontal resistance to *Ribes* cultivars to *Sphaerotheca mors-uvae*. Z. Pflanzenkr. Pflanzenschutz 87:129-136.
23. Weinhold, A. R., and English, H. 1964. Significance of morphological barriers and osmotic pressure in resistance of mature peach leaves to powdery mildew. Phytopathology 54:1409-1414.
24. Wolf, G., and Fric, F. 1981. A rapid staining method for *Erysiphe graminis* f. sp. *hordei* in and on whole barley leaves with a protein-specific dye. Phytopathology 71:596-598.