# Benomyl-Resistant Strains of *Uncinula necator* on Grapes

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**ABSTRACT**

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Benomyl sprays provided 97–100% control of grape powdery mildew caused by *Uncinula necator* in western New York during 1973-1976 but was much less effective in some vineyards in 1977 and 1978. Isolates of *U. necator* were collected in 1978 and 1979 and maintained on potted grapevines in biotrons or on detached grape leaves in plastic boxes. Resistance was determined by dipping leaves in a benomyl suspension and inoculating them with a spore suspension of the test isolate. After 7, 14, and 21 days, the amount of mildew on benomyl-treated leaves was not significantly different from that on untreated leaves inoculated with *U. necator* isolates from two western New York vineyards and one central New York vineyard. In a field trial in one of the western New York vineyards, four sprays of Benlate 50W at 1.12 kg/ha provided only 5 and 8% control of mildew on foliage and fruit clusters, respectively, in 1979. Field trials indicated that control of benomyl-resistant strains of *U. necator* with combinations of benomyl and one of several other fungicides, each at half the recommended rate, depended on the efficacy of the nonbenzimidazole component, but the half rate of benomyl alone was adequate to control benomyl-sensitive strains.

Additional key words: *Vitis* spp., fungicide resistance

Although Schroeder and Provvidenti's (14) 1969 report of resistance to benomyl in the cucurbit powdery mildew fungus *Sphaerotheca fuliginea* (Schlech.) Poll. was the first of many reports of fungi resistant to the benzimidazole fungicides (9), relatively few further cases of benomyl resistance in the powdery mildews have since been documented. Most reported cases have been in the genus *Erysiphe*, such as *E. cichoracearum* DC. on eggplant (5) and *E. graminis* DC. on bluegrass (16), or in the genus *Sphaerotheca*, including *S. fuliginea* on cucurbits (5,14) and *S. pannosa* (Wallr.) Lév. on rose (6,17). In addition, Iida (5) reported *S. humuli* (DC.) Burr. on strawberry as resistant to thiophanate-methyl in Japan. We report the first documented case of benomyl resistance in powdery mildew of grape (*Vitis labruscana* Bailey, *V. vinifera* L., and *V. vinifera* hybrids) caused by *Uncinula necator* (Schw.) Burr.

## MATERIALS AND METHODS

***U. necator* isolates.** Grape leaves bearing sporulating colonies of *U. necator* were collected from vineyards with a history of benomyl usage and decline in benomyl effectiveness. Conidia were rinsed from infected leaves with distilled water containing a surfactant (about 0.25 ml/L Triton X-100, Rohm and Haas Co., Philadelphia, PA 19105). The suspension was atomized onto young leaves of open-pollinated Delaware seedlings, Riesling cuttings, or detached, fully expanded Delaware leaves as described previously (1). The inoculated detached leaves were maintained by inserting the petiole through a hole in the lid of a plastic petri dish into distilled water, a technique used by H. S. Aldwinckle (*unpublished*). Isolates of *U. necator* on detached leaves were maintained in plastic boxes at 20–24 C under fluorescent lights (about 2,800 lux). Isolates on potted vines were maintained in biotrons at 25 C with 16 hr of fluorescent and incandescent lights (about 2,000 lux). Isolates were transferred monthly to new plants to maintain actively sporulating cultures. A known benomyl-sensitive isolate, used as a reference, was maintained in a separate biotron.

**Laboratory tests.** The foliage of potted Riesling vines (8–10 leaf stage) was dipped in a 600 $\mu$g/ml suspension of benomyl (prepared from Benlate 50W) and allowed to air dry. Four benomyl-treated and four untreated vines were inoculated by spraying (1) with a spore suspension ($1.8–3.0 \times 10^4$ conidia/ml) of the test isolate. Vines inoculated with the same isolate were incubated in the isolation of a biotron.

Delaware leaves were removed from vines grown in the field or greenhouse, soaked in running tap water for 10 min, rinsed in distilled water, and air dried. Some leaves were dipped in a 100 $\mu$g/ml suspension of benomyl, and the petioles inserted into distilled water in plastic petri dishes. Equal numbers of benomyl-treated and untreated leaves were inoculated with a spore suspension of the test isolate. All treatments were replicated six times. Detached leaves inserted into petri dishes were placed in plastic boxes to keep each *U. necator* culture in isolation. The boxes were then placed under 14 hr of cool-white fluorescent light (2,800 lux) per day at 20–24 C.

Barratt-Horsfall ratings of infected leaf area for all leaves on potted vines and each detached leaf were recorded daily and converted to percent leaf area infected with Elanco conversion tables (13).

**Field trials.** Experiments were conducted in a Delaware vineyard at Geneva, NY, where control of powdery mildew with benomyl had previously been satisfactory, and in several vineyards at Fredonia and Westfield, NY, where either benomyl-resistant isolates had been identified or control of powdery mildew with benomyl had not been adequate. In the Geneva trial, fungicides were applied at 935 L/ha with a five-nozzle vertical boom sprayer on 27 June, 11 July, 24 July, and 17 August 1979. Treatments were arranged in a randomized complete block design with four replications.

Trials at Fredonia were conducted in a Delaware vineyard (Trial A) and a Concord vineyard (Trial B), both at the New York State Agricultural Experiment Station Vineyard Laboratory. The Delaware vines in Trial A were trained to the Geneva Double Curtain and were sprayed with a vertical boom sprayer at 2,432 L/ha on 29 June, 12 July, 23 July, and 21 August 1979. Treatments were applied to plots of four rows of nine vines each and arranged in a randomized complete block design replicated four times. The Concord vines in Trial B were sprayed with an over-the-trellis hooded boom sprayer at 2,339 L/ha on 29 June and 12 July and at 2,806 L/ha on 17 August 1979. In a third trial conducted at Westfield in a commercial Concord vineyard (Trial C), treatments were applied with an air-blast sprayer at 234 L/ha on 2 July, 26 July, and 12 August 1979.

The incidence and severity of *U. necator* infection were evaluated on foliage and fruit clusters. We estimated the percentage of the exposed surface area (canopy) of individual vines that was infected or rated the vine canopy by the Barratt-Horsfall system and converted to percent area infected (Trial A). *U. necator* infection of fruit clusters was reported as

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a percentage of clusters infected, based on 30–60 clusters per plot.

We used the following fungicides in the field studies: 1) benomyl (Benlate 50WP, E. I. du Pont de Nemours & Co., Wilmington, DE 19898); 2) dinocap (Karathane 19.5WP, Rohm and Haas Co., Philadelphia, PA 19105); 3) folpet (Folpet 50WP, Stauffer Chemical Co., Westport, CT 06880); and 4) triadimefon (Bayleton 50WP, Mobay Chemical Corp., Kansas City, MO 64120).

**Table 1.** Resistance of *Uncinula necator* isolates from New York vineyards to benomyl

| Isolate number | Source | Percent leaf area infected after:[a] 7 days Check | 7 days Benomyl | 14 days Check | 14 days Benomyl | 21 days Check | 21 days Benomyl |
|---|---|---|---|---|---|---|---|
| | | | **Potted vines**[b] | | | | |
| 202 | Fredonia | 1.8 | 0.3 NS[c] | 8.7 | 2.4 NS | 11.8 | 5.3 NS |
| 203 | Fredonia | 1.4 | 0.7 NS | 6.6 | 4.2 NS | 14.6 | 9.4 NS |
| 204 | Fredonia | 10.9 | 2.5* | 17.4 | 7.9 NS | 25.7 | 15.4 NS |
| 235 | Geneva (greenhouse) | 4.1 | 0[d] | 12.4 | 0[d] | 13.2 | 0.4* |
| | | | **Detached leaves**[e] | | | | |
| 251 | Westfield | 3.5 | 5.6 NS | 9.0 | 11.3 NS | 14.8 | 18.8 NS |
| 252 | Westfield | 3.3 | 4.2 NS | 10.2 | 7.0 NS | 18.8 | 14.1 NS |
| 254 | Geneva | 3.3 | 4.7 NS | 14.1 | 14.1 NS | 26.2 | 23.4 NS |

[a] Values based on Barratt-Horsfall ratings of all leaves on potted vines and each detached leaf; values converted to percentages using Elanco conversion tables.
[b] Potted Riesling vines, inverted, dipped in a 600 μg/ml suspension of benomyl or untreated (check), and inoculated with the test isolate of *U. necator* (four replications).
[c] Student's *t* test for paired comparisons of check and benomyl treatments: NS = no significant difference, * = significant difference at $P \leqslant 0.05$.
[d] Student's *t* test for paired comparisons not possible when values are zero.
[e] Leaves detached from field-grown Delaware, dipped in a 100 μg/ml suspension of benomyl or untreated (check), and inoculated with the test isolate of *U. necator* (six replications).

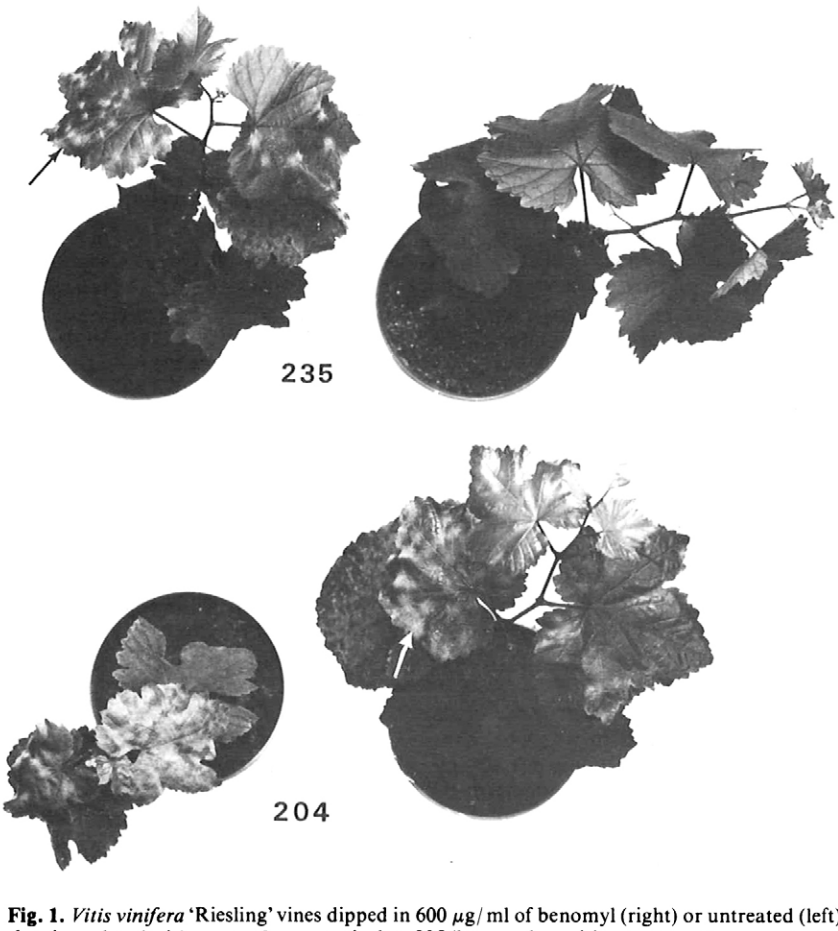


**Fig. 1.** *Vitis vinifera* 'Riesling' vines dipped in 600 μg/ml of benomyl (right) or untreated (left) and then inoculated with *Uncinula necator* isolate 235 (benomyl-sensitive, top) or *U. necator* isolate 204 (benomyl-resistant, bottom). Note powdery mildew symptoms indicated by arrows.

## RESULTS

**Laboratory tests.** Three *U. necator* isolates from Fredonia and one isolate from the greenhouse at Geneva were tested for resistance to benomyl on potted vines. For two of the Fredonia isolates, the amounts of *U. necator* growth on untreated leaves and on leaves treated with benomyl (600 μg/ml) were not significantly different ($P \leqslant 0.05$) 7, 14, and 21 days after treatment and inoculation (Table 1). The third Fredonia isolate (no. 204) showed significantly less *U. necator* growth on benomyl-treated leaves after 7 days but not after 14 or 21 days. No *U. necator* had grown on benomyl-treated leaves inoculated with the Geneva greenhouse isolate (no. 235) after 7 and 14 days, and only slight growth occurred after 21 days (Fig. 1). Although leaf area infected was significantly different in the untreated and benomyl-treated leaves of plants inoculated with isolate 204, the percentage of leaves infected after 7 days was much higher with the benomyl-resistant isolate 204 than with the benomyl-sensitive greenhouse isolate 235 (Fig. 2).

Two isolates from a commercial Concord vineyard in Westfield and an isolate from a *V. vinifera* cultivar planting at the New York State Agricultural Experiment Station at Geneva, where 10 sprays of Benlate 50W at 1.12 kg/ha failed to control mildew in 1979, were tested for benomyl resistance on detached Delaware leaves. The three isolates grew equally well on benomyl-treated and untreated leaves (Table 1).

**Field trials.** The field trial at Geneva, where control of mildew with benomyl had been satisfactory during the two previous years, showed that 0.56 kg/ha (half the recommended rate) provided 95% control of powdery mildew on

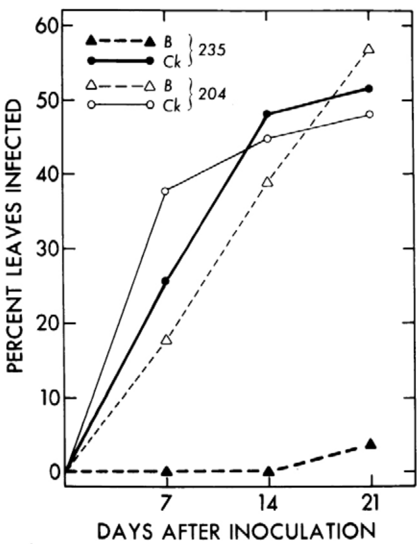


**Fig. 2.** Percent grape leaves infected with *Uncinula necator* 7, 14, and 21 days after inoculation of untreated (Ck) and benomyl-treated (B) leaves with benomyl-sensitive isolate 235 or benomyl-resistant isolate 204.

foliage and 99% control on clusters (Table 2). Benomyl at half the recommended rate combined with half rates of either dinocap or folpet did not improve control. Application of dinocap or folpet at half the recommended rate without benomyl provided only 54 and 47% control, respectively, of mildew on the foliage.

Field trials at Fredonia and Westfield confirmed the results of the laboratory studies indicating the prevalence of benomyl-resistant strains. In Trial A, benomyl provided only 5% control of canopy mildew and 8% control of mildew on clusters, whereas a relatively low rate of dinocap (1.4 kg/ha) provided 87 and 45% control of mildew on the canopies and clusters, respectively (Table 2). Data from a Concord vineyard (Trial B) showed 30% control of mildew on the canopy with benomyl, but no control of mildew on cluster stems. Disease development on the canopies of vines treated with benomyl plus folpet at half the recommended rates was not significantly different from the untreated check, and control of mildew on cluster stems was only 52%. Triadimefon at the low rate of 0.07 kg/ha (a.i.) provided 90% control of mildew on foliage, but only 27% control on cluster stems.

In Trial C, disease development in benomyl-treated blocks was not significantly different from that in untreated blocks. Furthermore, dinocap-treated blocks did not differ statistically in disease development from those where a reduced rate of dinocap was combined with benomyl at half the recommended rate.

A comparison of results with benomyl sprays over the past few years indicated a gradual buildup of resistance. Field trials in a Concord vineyard at Fredonia showed 97–100% control of mildew on cluster stems with three seasonal applications of Benlate 50W (1.12 kg/ha) from 1973 through 1976. Control of *U. necator* in this vineyard dropped to 61 and 69% in 1977 and 1978, respectively, and to 12% in 1979.

**Table 2.** Control of benomyl-sensitive and benomyl-resistant strains of *Uncinula necator* in the field

| Treatment | Dosage (formulated) (kg/ha) | Canopy surface area infected[w,x] (%) | Clusters infected[w] (%) |
|---|---|---|---|
| Benomyl-sensitive strains[y] | | | |
| Untreated | | 76 a | 100 a |
| Benomyl 50W | 1.12 | 4 c | 1 d |
| Benomyl 50W | 0.56 | 5 c | 4 d |
| Dinocap 19.5W | 2.24 | 8 c | 4 d |
| Dinocap 19.5W | 1.12 | 35 b | 38 c |
| Benomyl 50W + dinocap 19.5W | 0.56 + 1.12 | 5 c | 2 d |
| Folpet 50W | 4.48 | 34 b | 76 b |
| Folpet 50W | 2.24 | 40 b | 90 a |
| Benomyl 50W + folpet 50W | 0.56 + 2.24 | 8 c | 4 d |
| Benomyl-resistant strains[z] | | | |
| Trial A: | | | |
| Untreated | | 82 a | 97 a |
| Benomyl 50W | 1.12 | 78 a | 89 a |
| Dinocap 19.5W | 1.40 | 11 b | 53 b |
| Trial B: | | | |
| Untreated | | 61 a | 64 ab |
| Benomyl 50W | 1.12 | 43 c | 86 a |
| Folpet 50W | 4.48 | 48 bc | 64 ab |
| Benomyl 50W + folpet 50W | 0.56 + 2.24 | 55 ab | 31 bc |
| Triadimefon 50W | 0.14 | 6 d | 47 bc |
| Trial C: | | | |
| Untreated | | 54 a | 98 a |
| Benomyl 50W | 1.12 | 49 a | 100 a |
| Dinocap 19.5W | 1.40 | 15 b | 76 b |
| Benomyl 50W + dinocap 19.5W | 0.56 + 0.84 | 21 b | 75 b |

[w] Values within each grouping followed by the same letter do not differ significantly (Waller-Duncan's exact Bayesian K-ratio LSD rule, $P \leq 0.05$).
[x] Values in Trial A based on Barratt-Horsfall ratings of vine canopy surface area converted to percent with Elanco conversion tables.
[y] Trial on Delaware grape at Geneva, NY.
[z] Trial A on Delaware grape at Fredonia, NY; Trials B and C on Concord grape at Fredonia and Westfield, NY, respectively.

## DISCUSSION

The short list of powdery mildew species resistant to benomyl or other benzimidazole compounds (9) presumably reflects their limited use against this group of fungi. Most powdery mildews are controlled with inexpensive sulfur applications (3). Unfortunately, sulfur is phytotoxic to Concord (65% of the grape acreage in New York) and some other American and French-hybrid grape cultivars grown in the eastern United States (2). Benomyl has become a popular fungicide in New York because it provides excellent control of *U. necator* and is not phytotoxic. Growers recognize benomyl's added benefit of controlling oxidant stipple (8), an air pollution problem that is widespread in grape-growing areas of the Great Lakes region (7).

The development of benomyl resistance in *U. necator* presents considerable problems in controlling powdery mildew because alternative fungicides are either much less effective—such as folpet—or are phytotoxic—such as sulfur, copper and lime (15), and dinocap (11,12). Furthermore, the use of copper and lime precludes the use of many insecticides as tank mixes and requires the grower to use more toxic compounds, such as parathion (4).

Our field studies indicated that where benomyl-sensitive strains of *U. necator* predominated, control of powdery mildew by the reduced-rate fungicide-combination approach could be attributed solely to the benomyl component. Where benomyl-resistant strains were present, disease control with combinations of benomyl and another fungicide, each at reduced rates, depended on the efficacy of the nonbenzimidazole component. If the nonbenzimidazole component of the fungicide mix has low efficacy, even full-rate combinations may not control resistant strains. For example, combinations of benomyl and captan, each at full recommended rates, failed recently to control benomyl-resistant strains of *Botrytis cinerea* in New York vineyards (10). Unfortunately, strategies for preventing the buildup of fungicide-resistant strains of plant pathogens remain speculative.

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