Partial Control of Grape Powdery Mildew by the Mycoparasite *Ampelomyces quisqualis*

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ABSTRACT

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Ampelomyces quisqualis normally infects senescent colonies of *Uncinula necator* in late summer. Our objective was to introduce the mycoparasite at the start of an epidemic, and thereby reduce the rate of disease increase. Prior to establishing field trials, isolates of *A. quisqualis* were evaluated for pathogenicity, virulence, and host range in greenhouse and laboratory assays. Infection of powdery mildew colonies only occurred when plants were kept wet and resulted in sporulation of *A. quisqualis* within 10 days. Two isolates of *A. quisqualis* (G5 and G273) were evaluated for pathogenicity and virulence against 18 monoconidial isolates of *U. necator* on grape seedlings and showed little evidence of pathogenic specialization. Three isolates (G273, SF419, and SF423) were equally pathogenic to *Sphaerotheca fuliginea* on cucurbit, *S. macularis* on strawberry, and *U. necator* on grape seedlings. All *A. quisqualis* isolates appear to have a broad host range and cause significant damage to powdery mildew colonies. Pycnidia of *A. quisqualis* G273 were produced on cotton wicks saturated with malt extract agar or wheat bran malt extract agar. Wicks were suspended above grapevines of *Vitis vinifera* 'Riesling' and *Vitis* interspecific hybrid Aurore. Conidia were dispersed during rain to infect powdery mildew colonies while leaf surfaces were wet. Conidia were released for 3 months in 1990 from a single deployment of wicks. Higher numbers of conidia were released during the entire growing season in 1992 and 1993 due to replenishment of colonized wicks at monthly intervals. Wicks released conidia for 1 to 2 months in 1992 and 1993 before becoming depleted. Powdery mildew development was reduced on Riesling vines in 1990 following deployment of colonized wicks at 15 cm of shoot growth. Disease severity but not incidence was reduced on Aurore vines in 1992 under *A. quisqualis*–colonized wicks. High rainfall in 1992 provided ample opportunities for dispersal of inoculum of the mycoparasite and the wet conditions were conducive to parasitism. Disease development was late and much reduced in 1993, which was a drier season than 1992. Consequently, no differences were observed in *A. quisqualis*–treated and untreated plots in the same vineyard that year.

Ampelomyces quisqualis Ces. is a naturally occurring pycnidial hyperparasite of powdery mildew fungi. The mycoparasite is wholly internal within the mycelium, conidiophores, conidia, and ascocarps of several important species of the Erysiphaceae, including the powdery mildews of grapevine, *Uncinula necator* (Schwein.) Burrill (2,6,8,23); cucurbits, *Erysiphe cichoracearum* DC. and *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci (3,13, 16,17,21,24,25); apple, *Podosphaera leucotricha* (Ellis & Everh.) E. S. Salmon (1,17,18,23); and rose, *Sphaerotheca pannosa* (Wallr.:Fr.) Lév. (23,24,37). *Ampelomyces quisqualis* requires free water to infect mildew colonies (16,21,22). Infection can occur in 24 h at 20°C (30,35). After infection occurs, the mycoparasite ramifies throughout the host hyphae (5, 14), resulting in reduced growth, and eventually death, of the mildew colony. After 7 to 10 days, pycnidia of *A. quisqualis* form within the conidiophores of the powdery mildew fungus (30). When pycnidia are wet by rain, conidia of *A. quisqualis* are exuded in a cirrhus, which then dissolves and conidia are dispersed by rain splash (5,30,38).

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In nature, populations of *A. quisqualis* may lag several weeks behind populations of powdery mildew fungi in their development (8). The mycoparasite may not be observed until late in the growing season, long after the powdery mildews have formed the overwintering cleistothecia that ensure perpetuation of the disease. While this may have some evolutionary significance in that the mycoparasite allows its host to reproduce before killing it, the end result is that powdery mildew epidemics reach damaging levels on susceptible hosts before their growth is arrested by *A. quisqualis*. In the case of grape powdery mildew, foliar infection by *U. necator* is observed in mid-May in New York, or about 2 weeks after bud break. However, infection of mildew colonies by *A. quisqualis* has not been observed before August (8).

Many researchers have attempted to control powdery mildews by seeding mildewed crops with conidia of *A. quisqualis* (1,4,15,16,20,21,22,27,28,29,31,32,34,35, 36,37). The mycoparasite is usually applied in concentrated (10^6 per ml) conidial suspensions (1,4,15,28,34,35,36). However, the requirement of free water for infection by conidia has resulted in the greatest degree of disease suppression being confined to controlled environments and greenhouses (4,16,21,22,29,34). A reduction in incidence of powdery mildew on carrot from 43 to 13% was achieved by spraying field plants with conidial suspensions, but 7 weekly treatments of 10^6 conidia per ml were required to achieve this level of disease control (34).

The likelihood of successful infection of a mildew colony by *A. quisqualis* under field conditions could be maximized by introducing the inoculum as a spray immediately before or during rain events. However, the logistical problems attending this approach makes it impractical for commercial use. The unpredictable nature of rain events make pre-rain applications difficult. Applications during rain require a rapid response to the onset of rain, regardless of the hour, and travel through wet plantings with spray equipment. Thus, after many investigations of *A. quisqualis* as a biological control of powdery mildews, there are no examples of its use in commercial agriculture.

We have developed a means to place the mycoparasite in the field, where inoculum can be naturally dispersed onto crop plants by rain under ideal conditions for infection by the mycoparasite. In 1990, we devised a method to culture *A. quisqualis* on cotton wicks (12) and form pycnidia on the surface of the wick. These wicks were suspended in grapevine trellises to allow natural dispersal of the mycoparasite in rain. In this paper, we report on i) screening of *A. quisqualis* isolates for parasitism of several *U. necator* isolates, ii) screening of *A. quisqualis* isolates for parasitism of different powdery mildew species, iii) an evaluation of different wick substrates and growth media for colonization by *A. quisqualis*, iv) the efficacy of wick cultures against *U. necator* in grape vineyards, and v) the dispersal and viability of conidia from wick cultures.

MATERIALS AND METHODS

Screening *A. quisqualis* for pathogenicity and virulence against *U. necator* isolates. Grapevine seedlings were

produced from seed harvested from *Vitis vinifera* L. 'Riesling.' The seed were stratified for 6 months at 4°C, after which they were germinated in flats of Perlite at 20°C. Once cotyledons had emerged, seedlings were transplanted to Jiffy-7 peat pots contained within an 8-oz polystyrene cup. Seedlings were grown under natural light until two true leaves had formed, and were then inoculated with *U. necator*.

Eighteen monoconidial isolates of powdery mildew described in a previous study (11) were used to evaluate the pathogenicity and virulence of two isolates of *A. quisqualis*. The powdery mildew isolates were named Delaware-F, Chardonnay-LI-2, Chardonnay-LI-3, *Vitis argentifolia* GV25, *V. champinii* GV25, *V. betulifolia* GV42, Pinot Noir-G-2, Bunchgrape-NCSU-1, Riesling-F, Elvira-F-1, Concord-F, Ives-F, Delaware-F-HG, Elvira-F-HG, Rosette-N-1, *V. solonis* GV21, *V. labrusca* SRI, and Rosette-N-11. Mildew isolates were grown as clonal isolates on tissue culture plants of the *Vitis* interspecific hybrid Chancellor in 75-mm culture tubes. Once cultures were sporulating, the mildewed plant was removed from the tube and shaken in 20 ml of distilled water containing 0.05% Tween 20 (a surfactant). Five droplets (20 µl, 10^5 conidia per ml) of the resultant conidial suspension were placed onto each of the two youngest expanded leaves on grape seedlings. After inoculation with *U. necator*, seedlings were incubated under artificial light for 7 days, until sporulating mildew colonies were present on the inoculated leaves.

Ampelomyces quisqualis isolates were originally obtained from parasitized mildew colonies on leaves of the *Vitis* interspecific hybrid cultivar Rosette in Geneva, N.Y., in the autumn of 1989. Two monoconidial isolates were arbitrarily selected for further study: G5 and G273. The isolates were grown on potato-dextrose agar (PDA) dishes at 20 to 25°C for 2 weeks prior to preparation of conidial suspensions. Approximately 5 ml of sterile distilled water was added to each dish, and the surface of the colony was rubbed with a transfer loop to wet the pycnidia. The dishes were allowed to stand for 15 min while conidia were released into the water within the dish. The conidial suspensions were enumerated with a hemacytometer, and were adjusted to 10^6 conidia per ml.

Distilled water was atomized onto seedlings immediately before inoculation of the mildew colonies with *A. quisqualis*. Each mildew colony was inoculated at its center with a single 10-µl drop of spore suspension. Control plants were inoculated with droplets of distilled water. The cups then were covered with translucent lids and were incubated at 25°C for 24 h. After this time, the lids were removed, the leaves were allowed to dry, and the seedlings were grown under artificial light for an additional 10 days. All treatments were replicated on 10 seedlings and the experiment was repeated.

Ten days after inoculation, mildew colonies were examined at 50× for evidence of infection by *A. quisqualis*. The condition of the mildew colony was examined and the percentage of the conidiophores that had collapsed was estimated by recording the number of erect conidiophores out of 100 conidiophores at the point of inoculation. Sporulation of *A. quisqualis* was also assessed as the number of pycnidia present in one 50× field of view at the point of inoculation, and was recorded as a numerical rating: 0 = no pycnidia; 1 = 1 to 10 pycnidia; 2 = 11 to 50 pycnidia; 3 = more than 50 pycnidia.

Virulence of G5 and G273 was evaluated as radial expansion of *A. quisqualis* in mildew colonies. Mildewed grape seedlings were inoculated with G5 and G273 as above, and were incubated for 10 days. The diameter of the collapsed area of the mildew colony surrounding the point of inoculation then was measured under a stereo microscope. Treatments were replicated on 10 seedlings and the experiment was repeated.

Pathogenicity of *A. quisqualis* to *U. necator* on wet and dry leaf surfaces. Protectant activity of conidial suspensions of *A. quisqualis* on wet and dry leaf surfaces also was evaluated. Grape seedlings were inoculated with G5, and G273 as above. The droplets of conidial suspension were allowed to dry for 24 h. One half of the plants were misted with distilled water, and the others were left dry. All of the plants then were inoculated with a 10-µl droplet of a conidial suspension of *U. necator* at the same location as the *A. quisqualis* conidia. This droplet dried upon the leaf surface within 10 min on the dry plants, but persisted for 24 h when misted plants were kept covered. The plants then were incubated for an additional 10 days, and then were examined for signs of powdery mildew and mycoparasitism. Treatments were replicated on 10 seedlings and the experiment was repeated.

Screening of *A. quisqualis* for pathogenicity and sporulation on other powdery mildew species. Three isolates of *A. quisqualis* were screened for pathogenicity and ability to sporulate on cucurbit powdery mildew (*Sphaerotheca fuliginea*), strawberry powdery mildew (*Sphaerotheca macularis* (Wallr.:Fr.) Lind) and *U. necator*.

Cucurbita pepo L. 'Seneca' zucchini were seeded directly into pots and grown in the greenhouse. After 2 weeks, when the true leaves were expanding, they were inoculated with *S. fuliginea* obtained from naturally infected *Cucumis sativus* L. in the greenhouse at the New York State Agricultural Experiment Station (NYSAES), Geneva, N.Y. Conidia were suspended in distilled water with Tween 20 (0.02%) adjusted to 10^4 per ml and immediately sprayed onto leaves to obtain a uniform heavy inoculation with powdery mildew. After 7 days, developing powdery mildew colonies were inoculated with *A. quisqualis* and held in the greenhouse for 10 days, the first 24 h of which the plants were in humidity chambers constructed in the greenhouse. Treatments consisting of three *A. quisqualis* isolates, an untreated and a distilled water check, were replicated on plants in five pots. The experiment was arranged in a completely random design on the greenhouse bench and was conducted four times.

Runners of strawberry (*Fragaria* × *ananassa* Duchesne) were transplanted from the field to flats and grown in the greenhouse. Plants of three cultivars were used. The cultivar Selva originated from a commercial farm near Red Creek, N.Y., and cvs. Allstar and Earliglow from NYSAES. Leaves were allowed to become naturally infected with *S. macularis* in the greenhouse before being detached and inoculated with *A. quisqualis* in the laboratory. Leaves were placed on wire mesh racks inside plastic crispers with the leaf petioles immersed in water. After inoculation crispers were sealed for 24 h and then uncovered for an additional 9 days. Crispers of leaves were placed under artificial light from fluorescent tubes on a 16-h light schedule. The five treatments of three *A. quisqualis* isolates and an untreated and a distilled water check were replicated on five leaves. The experiment was conducted two times with cv. Selva and once each with Allstar and Earliglow.

Seeds harvested from grapes cv. Riesling, which had been stratified for at least 6 months at 4°C, were germinated on wet paper toweling at 22°C. After 2 weeks, germinated seeds were transplanted to Jiffy-7 peat pots in 16-oz clear plastic cups. The cups were covered with a sheet of tissue held in place by a plastic band. Seedlings were grown under artificial light from fluorescent tubes on a 16-h light schedule for about 4 weeks until true leaves had formed. Leaves were inoculated with *U. necator* obtained from *Vitis vinifera* L. 'Chardonnay' in Dresden, N.Y., by transferring conidia by gently touching with an already infected leaf. After 7 days, powdery mildew colonies were inoculated with *A. quisqualis*, held in humidity chambers made by placing Saran wrap over the cups for 24 h, and then the tissue lids were replaced for an additional 9 days. The five treatments previously described were replicated on five seedlings and the experiment was repeated.

Comparison of pathogenicity of *A. quisqualis* isolates on the three powdery mildew species was made using the following three *A. quisqualis* isolates: G273 described previously; SF419 from pycnidia on *U. necator* colonies on *Vitis riparia* Michx. leaves in Albany, N.Y., in

autumn 1991; and SF423 from parasitized cleistothecia of *U. necator* on leaves of *Vitis* interspecific hybrid Chancellor in Geneva, N.Y., in autumn 1991. Each isolate was grown on wheat bran malt agar (WBMA) made from the strained extract of 100 g of wheat bran, 20 g of malt extract, 2 g DL-asparagine, 15 g of agar in 1 liter of distilled water, pH 6.5, (33) by spreading a 0.5-ml suspension of *A. quisqualis* conidia over the agar surface with a glass rod. After 2 to 3 weeks, conidia were harvested from these dishes by adding 10 ml of sterile distilled water to the dish and rubbing with a glass rod. Conidia were adjusted to 2×10^6 per ml and Tween 20 was added to 0.02%. Suspensions were sprayed onto powdery mildew–infected leaves of Seneca zucchini and grapevine seedlings and detached strawberry leaves using a nonchlorinated fluorocarbon aerosol sprayer (Preval, Precision Valve Corporation, Yonkers, N.Y.). After inoculation, plants or leaves were kept at 100% relative humidity for 24 h in the dark and then uncovered for an additional 9 days before pathogenicity and virulence of the *A. quisqualis* isolates were determined. Pathogenicity was determined by estimating the percent collapse of conidiophores and sporulation by counting the number of *A. quisqualis* pycnidia per cm^2 of powdery mildew colony. Ten 5-mm-diameter fields of view were examined for each replicated treatment. The upper leaf surfaces of zucchini and grapevine seedlings and the lower surfaces of strawberry leaves were examined.

Growth of *A. quisqualis* on wick substrates. Three wick substrates were examined for their ability to support the growth of *A. quisqualis*. Wick substrates consisted of twine made from cotton (30 ply, cotton/poly blend, King Cotton, John H. Graham & Co., Oradell, N.J.), jute (3 ply, King Cotton), or sisal (heavy duty, King Cotton, John H. Graham & Co., Elmwood Park, N.J.). Twine was washed in distilled water for 2 h, wrung out to remove most of the water, and single 1-m lengths of twine were placed in 113-g baby-food jars, and sterilized by autoclaving. Jars were inoculated with 8 ml of a suspension of *A. quisqualis* conidia (10^6 conidia per ml) in cooled (40°C) modified malt extract agar (MEA) (40 g of malt extract, 2 g of DL-asparagine, 1 g of agar per 1 liter of distilled water, pH 6.5) and incubated for 18 days in the dark at 24°C. Growth was measured by counting the number of pycnidia produced along two sides of a 10-cm segment of wick and by the number of conidia released after soaking the wick in jars for 1 h in 20 ml of distilled water with shaking. Conidia were counted with a hemacytometer. The experiment was replicated on five jars with three different *A. quisqualis* isolates; G273, SF419, and SF423 described previously.

An improved culture medium was assessed as a nutrient source for the culture of *A. quisqualis* G273 on cotton wicks. Baby-food jars containing cotton twine were inoculated as previously described with *A. quisqualis* conidia in modified MEA or WBMA (0.1% agar). Jars were incubated for 20 days at 24°C in darkness. Growth was measured by counting the number of conidia released from the wick as previously described. Treatments were replicated five times and the experiment was repeated.

Production of wick cultures for field trials. In 1990, cotton twine was used as a substrate for the growth of G273. One-meter-long lengths of twine were washed in distilled water, placed in baby-food jars, and amended with 10 ml of semisolid malt dextrose agar (15 g of malt extract, 15 g of dextrose, 5 g of agar per liter of distilled water). The jars containing the twine and medium were autoclaved, allowed to cool to 30°C, and then were inoculated with 1 ml of a conidal suspension of G273 containing 10^6 conidia per ml. The wick cultures were incubated at 25°C for 3 weeks, removed from the jars, and hung in the greenhouse overnight to dry before deployment in the vineyard.

In 1992 and 1993, *A. quisqualis* isolate G273 was grown on 1-m-lengths of cotton twine in baby-food jars. Twine was inoculated with 8 ml of a suspension containing 10^7 conidia per ml of *A. quisqualis* G273 in WBMA (0.1% agar). Wick cultures were incubated in the dark at 24°C for 3 weeks, at which time they were dried overnight in the greenhouse and stored at 4°C until needed.

Field trials with wick cultures. In 1990, a field trial was conducted in a 0.1 ha planting of cv. Riesling at Geneva, N.Y. The vines were 8 years old, hand-pruned, and umbrella-Kniffen trained. Treatments were assigned to three-vine panels and were replicated five times in a randomized complete block design. Treatments consisted of the deployment of a 1-m length of wick cultures of G273 as described above at the following times: (i) at 15 cm of shoot growth, (ii) at bloom, (iii) at 15 cm of shoot growth and again at bloom, (iv) untreated control. Wick cultures were wrapped around a wire suspended 30 cm above the top wire of the trellis and held in place with clothespins. Disease incidence on leaves was assessed as number of infected leaves per shoot from 10 shoots per vine and severity as the percentage of the leaf surface with powdery mildew. Severity of fruit infection was determined on 10 fruit clusters per vine.

In 1992 and 1993, field trials were conducted in a 20-year-old commercial vineyard of the *Vitis* interspecific hybrid Aurore at Dresden, N.Y. Treatment with *A. quisqualis* consisted of suspending wick cultures of G273 about 30 cm above vines by attaching to nylon twine suspended between spikes nailed into the tops of the vineyard trellis posts. Two 1-m-length wick cultures were suspended above all vines in 36 vine plots (six rows by six vines). Control plots were untreated. Experimental plots were surrounded by untreated vines that were sprayed with fungicides using a hooded boom sprayer as part of the normal vineyard maintenance. The minimum distance between plot corners was approximately 15 m. All plots were replicated three times in 1992 and were completely random. In 1992, wick cultures were deployed at 10-cm shoot growth on 21 May and additional wick cultures were deployed over those already present on 18 June and 22 July. In 1993, plots were replicated four times in a randomized complete block design. Wick cultures were deployed at 3-cm shoot growth on 11 May and again at bloom on 14 June and on 13 July. Incidence of powdery mildew was assessed on 10 vines in each plot by examining 5 or 10 shoots per vine to determine the number of infected leaves per shoot and 30 infected leaves were examined to determine the severity of infection as the percentage of leaf surface infected. Incidence and severity of cluster infection was determined from 20 clusters per vine. Disease progress for each disease variable was additionally measured as the area under the disease progress curve (AUDPC) (26). This was calculated using the method of trapezoids, using days of the year as the x axis and either number of infected leaves per shoot, percent leaf surface infected, percent fruit clusters infected, or percent fruit surface infected as the y axis.

Dispersal and viability of conidia from wick cultures. In 1990, wick cultures of G273 were suspended as described above from a horizontal wire within a separate vineyard approximately 25 m from the Riesling vineyard. Three replicate funnel-traps were suspended beneath the wicks to catch rain splash and drips from the wicks. Each trap consisted of a 5-cm diameter glass funnel that emptied into a 30 × 200 mm glass culture tube. A small amount (0.1 g) of tribasic copper sulfate was placed within each tube to preserve the conidia. The tubes were changed after each rain event, and the total number of conidia of G273 in the rainwater suspension were enumerated with a hemacytometer. In 1992, funnel-traps were placed beneath wick cultures attached to trellis wires in plots in the Aurore vineyard. Conidia released from single, and later multiple deployed, wick cultures were collected after each rain and enumerated with a hemacytometer. Conidia were preserved with a small amount (0.1 g) of cupric acetate. Four replicate funnel-traps were randomly placed in the vineyard. As funnel-traps do not allow the viability of conidia to be determined, in 1992 and 1993, pieces of wick were collected to determine the percent germination of the

conidia. Regular samplings also enabled determination of the number of conidia on the wick cultures. A piece was removed every 1 to 2 weeks from each of five 1-m-long wick cultures randomly placed in the vineyard. Each batch of newly deployed wick cultures was sampled separately beginning a few days following deployment. Small pieces of wick (1 or 2 cm long) were soaked for 3 h in 1 ml of distilled water in microfuge tubes with regular vortexing and the conidia released were enumerated with a hemacytometer. A few drops of suspended conidia from each tube were spread on the surface of water agar in a petri dish, incubated at room temperature for 24 h, and, where sufficient conidia were present, the percent germination was determined in two counts of 50 conidia per dish. Conidia were considered germinated when the length of the germ tube exceeded the length of the conidium.

RESULTS

Screening of isolates of *A. quisqualis* for pathogenicity and virulence. Isolates G5 and G273 were able to infect and reproduce upon all of the powdery mildew isolates used in this study. Nearly complete collapse of the mildew colony was seen at the point of inoculation with both isolates. The percentage of collapsed conidiophores ranged from 79 to 100% on colonies inoculated with G5, and did not differ significantly among the various mildew isolates (Table 1). A slightly broader range in the percentage of collapsed conidiophores (66.4 to 99.8%) was recorded on colonies inoculated with G273 (Table 1), but only the *U. necator* isolate Delaware-F was significantly less affected than the other mildew isolates (Table 1).

In all cases, *A. quisqualis* produced pycnidia upon parasitized mildew colonies within 10 days after inoculation (Table 1). However, this sporulation of *A. quisqualis* was not always commensurate with the destruction of conidiophores. For example, when the mildew isolate Elvira-F-1 was inoculated with G273, the percentage of mildew colonies bearing 10 or more pycnidia of *A. quisqualis* was the lowest observed in our study (22%), although almost total collapse of hyphae and conidiophores of *U. necator* was observed (Table 1). Conversely, G273 sporulated profusely on the mildew isolate Delaware-F, even though the percentage of collapsed conidiophores (66.4%) was significantly lower than observed on other mildew isolates (Table 1).

The radial expansion of G5 in colonies of Delaware-F-HG and *V. argentifolia* GV25 differed significantly from that observed in 16 other mildew isolates (Table 1). No significant differences in radial expansion of G273 were observed following inoculation of colonies of 18 *U. necator* isolates (Table 1).

Pathogenicity of *A. quisqualis* to *U. necator* on wet and dry leaf surfaces. When plants were kept dry, neither G5 nor G273 were effective mycoparasites when applied as protectants (24 h prior to inoculation of grape seedlings with *U. necator*). All plants inoculated in this manner were infected by *U. necator*, no collapse of mildew colonies was observed, and no sporulation of *A. quisqualis* was observed. However, when plants were kept wet for 24 h following the application of the *U. necator* inoculum, substantial mycoparasitism occurred, and there was a marked (>50%) reduction in the percentage of plants infected by 17 of the 18 isolates of *U. necator*. The percentage of plants infected by Chardonnay-LI-3 was not reduced, but 100% of the mildew colonies that formed were infected by G5 and G273 when plants were kept wet following the application of *U. necator* inoculum. Among the other *U. necator* isolates, the percentage of mildew colonies infected ranged from 67 to 100% for G5, and ranged from 75 to 100% for all mildew isolates and G273.

Screening of *A. quisqualis* for patho-

Table 1. Percentage of collapsed conidiophores, production of pycnidia, and diameter of the collapsed area within colonies of *Uncinula necator* on grape seedling leaves inoculated with a conidial suspension of *Ampelomyces quisqualis*[w]

	Collapse of conidiophores within colony (%)		Colonies with ≥ 10 pycnidia of *A. quisqualis* (%)		Diameter of collapsed area of powdery mildew colony (mm)[x]	
***Uncinula* isolate**	**G5**	**G273**	**G5**	**G273**	**G5**	**G273**
Delaware-F	ND[y]	66.4 b	ND	90.0 a	2.99 a	3.90 a
Chardonnay-LI-2	100.0 a[z]	99.7 a	86.0 a	88.0 a	4.94 a	5.20 a
Chardonnay-LI-3	ND	94.4 a	ND	89.0 a	3.38 a	4.68 a
V. argentifolia GV25	ND	ND	ND	ND	1.82 b	5.50 a
V. champinii GV25	93.2 a	96.9 a	100.0 a	90.0 a	4.16 a	5.33 a
V. betulifolia GV42	93.0 a	93.6 a	100.0 a	95.0 a	4.55 a	3.90 a
Pinot Noir-G-2	ND	87.8 a	43.0 c	80.0 a	5.85 a	5.20 a
Bunchgrape-NCSU-1	90.1 a	82.7 a	70.0 b	100.0 a	4.42 a	3.25 a
Riesling-F	96.9 a	99.8 a	90.0 a	100.0 a	2.92 a	5.20 a
Elvira-F-1	99.1 a	99.7 a	67.0 b	22.0 c	6.15 a	3.90 a
Concord-F	99.3 a	99.8 a	100.0 a	90.0 a	5.85 a	4.55 a
Ives-F	ND	99.8 a	ND	89.0 a	2.93 a	4.42 a
Delaware-F-HG	79.0 a	96.9 a	56.0 bc	90.0 a	1.95 b	3.64 a
Elvira-F-HG	92.4 a	96.9 a	100.0 a	80.0 a	4.23 a	5.53 a
Rosette-N-1	92.4 a	94.4 a	89.0 a	89.0 a	5.80 a	2.29 a
V. solonis GV21	92.3 a	87.3 a	84.5 a	66.5 b	3.65 a	4.00 a
V. labrusca SRI	95.6 a	81.6 a	78.0 ab	72.0 b	4.42 a	3.25 a
Rosette-N-11	93.7 a	87.8 a	86.0 a	100.0 a	4.10 a	3.64 a
Control	3.1	4.7	0.0	0.0	ND	ND

[w] A 10-µl drop of *A. quisqualis* conidia (10^6 per ml) was applied to wetted leaves on grapevine seedlings bearing 7-day-old *U. necator* colonies. Leaves were kept wet at 25°C for 24 h and parasitism of the *U. necator* colonies was assessed after 10 days.

[x] The initial diameter of the inoculated area was approximately 1 mm.

[y] Not determined.

[z] Means within columns followed by the same letter do not differ significantly at $P = 0.05$ according to the Waller-Duncan *k*-ratio *t* test. Numbers are means of measurements from 10 seedlings and the experiment was repeated. Means of controls were excluded from the analysis.

Table 2. Percent collapse of conidiophores of three species of powdery mildew and production of *Ampelomyces quisqualis* pycnidia in powdery mildew colonies following parasitism by three isolates of *A. quisqualis*[x]

	Conidiophore collapse (%)			Pycnidia per cm²		
Powdery mildew[y]	**G273**	**SF419**	**SF423**	**G273**	**SF419**	**SF423**
U. necator	99.7 a[z]	95.8 a	99.8 a	1,270 a	1,790 a	1,640 a
S. macularis	93.7 a	97.7 a	98.0 a	56 b	26 b	53 b
S. fuliginea	39.4 b	28.4 b	44.6 b	420 b	180 b	490 b

[x] *A. quisqualis* conidia (2×10^6 per ml in 0.02% Tween 20) were sprayed until runoff onto 7-day-old powdery mildew colonies of *Uncinula necator* and *Sphaerotheca fuliginea* and older colonies of *S. macularis* on leaves. Leaves were kept wet for 24 h in the dark and then assessed for parasitism after an additional 9 days.

[y] Assays for parasitism were conducted on *U. necator* inoculated onto grapevine seedlings raised in plastic cups grown under artificial light, on *S. macularis* on detached strawberry leaves naturally infected in the greenhouse, and on *S. fuliginea* inoculated onto zucchini seedlings raised in the greenhouse.

[z] Numbers followed by the same letter within a column are not significantly different at $P = 0.05$ according to Bonferroni multiple comparisons. Numbers represent the mean of 10 5-mm-diameter fields of powdery mildew colony on five seedlings or detached leaves. Experiments were conducted twice for *U. necator* and four times each for *S. macularis* and *S. fuliginea*.

genicity and sporulation on other powdery mildew species. Collapse of powdery mildew colonies by *A. quisqualis* was almost complete for *U. necator* on grapevine seedlings (95.8 to 99.8%) and *S. macularis* on strawberry leaves (93.7 to 98.0%) when three isolates of the mycoparasite (G273, SF419, SF423) were assessed in the laboratory (Table 2). The collapse caused by these three isolates was significantly less ($P = 0.05$) on *S. fuliginea* on Seneca zucchini (28.4 to 44.6%) (Table 2). No collapse of powdery mildew occurred with either the distilled water or untreated check treatments. While a significant difference occurred among powdery mildew species, there was no significant difference among isolates of *A. quisqualis* (G273, SF419, and SF423) when examined by analysis of variance (ANOVA).

More pycnidia of *A. quisqualis* were produced on *U. necator* on grapevine seedlings (1270 to 1790 pycnidia per cm^2) than on the other powdery mildew species examined in laboratory or greenhouse assays (Table 2). In pairwise comparisons the number of pycnidia produced on *S. macularis* on strawberry leaves and *S. fuliginea* on Seneca zucchini differed at $P = 0.119$ to 0.157. As before, while there were significant differences in virulence by *A. quisqualis* on the different powdery mildew species, there were no significant differences among isolates of *A. quisqualis* in virulence when examined by ANOVA.

Growth of *A. quisqualis* on wick cultures. Cotton twine supported significantly better growth, as measured by production of pycnidia and release of conidia, for all three isolates (G273, SF419, SF423) of *A. quisqualis* (Table 3). Growth on jute was poor for all isolates and growth on sisal was intermediate for all except SF419, which also grew poorly on this twine.

Incorporation of an extract of wheat bran into the culture media significantly improved the growth of G273. The number of conidia released from cotton wicks was increased from $0.45 \pm 0.06 \times 10^6$ per cm in MEA to $6.15 \pm 0.32 \times 10^6$ per cm in WBMA ($P < 0.0001$, separate variance Student's t test, $n = 10$).

Field trials with wick cultures. In 1990, all treatments significantly but equivalently reduced the number of mildewed leaves per shoot (Table 4). The severity of infection on mildewed leaves was reduced below the level observed on control vines by single deployments of wick cultures at 15 cm of shoot growth and at bloom, but the greatest reduction occurred when cultures were deployed at 15 cm of shoot growth and again at bloom (Table 4). Fruit infection was significantly reduced when cultures were deployed at 15 cm of shoot growth, but not when the initial deployment was delayed until bloom (Table 4). Retreatment at bloom of vines treated at 15 cm of shoot did not result in any additional control of powdery mildew on fruit compared with vines treated only at 15 cm of shoot growth (Table 4).

In 1992, treatment with *A. quisqualis* wick cultures significantly reduced the severity (percent surface infected) but not incidence of both leaf and fruit infections on Aurore vines (Table 5). The severity of *U. necator* infections under treatment with *A. quisqualis* wick cultures was reduced throughout the season in 1992 compared with the progress of disease severity on leaves and fruit clusters of untreated vines (Fig. 1). In 1993, the amount of powdery mildew that developed in the same vineyard was much reduced compared with the previous season (Table 5). Consequently, treatment with *A. quisqualis* exhibited no effect on either the incidence or severity of leaf and fruit infections by powdery mildew. ANOVA of AUDPC values for the incidence and severity of leaf and fruit infections showed a significant effect of year of experiment on each of the four measures of disease but only significant treatment effects for measures of disease severity (percent surface infected) (Table 5).

Dispersal and viability of conidia from wick cultures. *A. quisqualis* conidia were detected during rain events for over 3 months in 1990 and 1992 (Fig. 2). Conidia were trapped in a rain event immediately after the deployment of the wick cultures in 1990, and in every rain event thereafter until the experiment was terminated. In 1992, conidia were released from wick cultures throughout the experiment with peaks of release occurring on 20 June, following the second deployment of the wick cultures on 18 June, and on 22 July following the third deployment the same day. Many more conidia were released in 1992 than in 1990; while a maximum of 1,566,000 conidia were trapped on 20 June immediately following deployment of the second wick cultures in 1992, the maximum number of conidia trapped in 1990 was only 91,000 conidia which occurred on 30 May following deployment of the wicks. The viability of conidia re-

Table 3. Growth of *Ampelomyces quisqualis* in modified malt extract agar (MEA) (40 g of malt extract, 2 g of DL-asparagine, 1 g of agar per 1 liter of distilled water, pH 6.5) on three wick culture substrates after 18 days at 24°C

		Growth	
Isolate[v]	Substrate[w]	Pycnidia per cm[x]	Conidia × 10^6 per cm[y]
G273	cotton	95 a[z]	0.52 a
	sisal	33 b	0.26 b
	jute	10 c	0.03 c
SF419	cotton	140 a	0.60 a
	sisal	3 b	0.02 b
	jute	0 b	0.03 b
SF423	cotton	199 a	0.38 a
	sisal	63 b	0.19 b
	jute	6 c	0.16 b

[v] Conidia of *A. quisqualis* (10^6 per ml) were suspended in 8 ml of 4% MEA with 0.2% DL-asparagine.
[w] Lengths of twine (1 m) were washed for 2 h in distilled water, placed in baby-food jars, and sterilized.
[x] Pycnidia were counted along a 10-cm length of twine.
[y] Conidia were released into 20 ml of distilled water added to jars containing wick cultures and counted with a hemacytometer.
[z] Numbers were transformed to their square roots prior to analysis. The means were averaged over five replicates and detransformed to the tabulated values. Numbers for each isolate within columns followed by the same letter are not significantly different at $P = 0.05$ according to Bonferroni multiple comparisons.

Table 4. Partial suppression of grape powdery mildew (*Uncinula necator*) on *Vitis vinifera* 'Riesling' at Geneva, N.Y., in 1990 by *Ampelomyces quisqualis* G273 grown on cotton wick cultures suspended within the trellis

Treatment[w]	Infected leaves per shoot[x]	Leaf surface infected (%)[x]	Fruit cluster surface infected (%)[y]
Control	10.2 a[z]	19.1 a	38.2 a
15 cm shoots	7.5 b	14.4 b	12.4 b
15 cm + bloom	5.2 b	6.6 c	15.7 b
Bloom	4.4 b	12.7 b	34.9 a

[w] *A. quisqualis* G273 was grown on cotton twine soaked in semisolid malt extract agar, which was then dried and suspended 30 cm above the grapevine trellis when shoots were 15 cm long and again at bloom, or at bloom only.
[x] Disease was assessed on 10 shoots per vine. The number of infected leaves per shoot and the percentage of the leaf surface infected on mildewed leaves was recorded on 10 August 1990.
[y] Disease was assessed on 10 fruit clusters per vine on 23 August 1990.
[z] Means within columns followed by the same letter do not differ significantly at $P = 0.05$ according to the Waller-Duncan k-ratio t test.

leased from wick pieces, although variable, did not decrease with time following deployment. In 1992, germination ranged from 42.2 to 83.0 % (mean 66.2 ± 4.2%, n = 10), and in 1993 from 48.4 to 93.2% (mean 77.2 ± 3.1%, n = 19). The number of conidia available for dispersal decreased with time following deployment (Fig. 3). In all cases conidia were depleted to negligible numbers remaining on the wicks in 1 to 2 months. In 1992, wick cultures deployed at all three times had greater than 600,000 conidia per cm at deployment. In 1993, the first deployment was poorly colonized, yielding only 146,000 conidia per cm at deployment. Later deployed wick cultures yielded over 900,000 conidia per cm at deployment.

DISCUSSION

We found no convincing evidence of widespread resistance of isolates of *U. necator* to *A. quisqualis*, nor does there appear to be substantial pathogenic specialization within *A. quisqualis* toward isolates of *Uncinula*. Virulence of G5, when measured as the radial spread through an infected mildew colony, differed significantly in only 2 of 18 isolates of *Uncinula*, and no differences were observed in G273. Both G5 and G273 appear to have a broad host range, cause significant damage to powdery mildew colonies, and sporulate upon parasitized colonies within 10 days. Reproduction of *A. quisqualis*, measured as the quantity of pycnidia produced on a parasitized mildew colony, does appear to occasionally be reduced on certain mildew isolates, but such mildew isolates are still severely damaged by *A. quisqualis*.

Free water appears to be beneficial, if not an absolute necessity, for infection of *U. necator* by *A. quisqualis*. Although, under vineyard conditions, some infection might be recorded at high humidities, this would probably be due to condensation of water on plant surfaces during minor temperature fluctuations, rather than germination of conidia at high humidities. Free water also appears to be needed for secondary spread of *A. quisqualis*. No exudation of conidia from pycnidia was observed on dry plant surfaces during the course of this investigation.

The broad host range of *A. quisqualis* also includes other powdery mildew species. Three isolates of *A. quisqualis* (G273, SF419, and SF423) did not differ in their ability to parasitize grape (*U. necator*), strawberry (*S. macularis*), or cucurbit powdery mildew (*S. fuliginea*). However, each species of powdery mildew reacted differently to parasitism in our assay. Compared with other powdery mildews, fewer conidiophores of *S. fuliginea* collapsed following parasitism and sporulation of *A. quisqualis* was only moderate on this powdery mildew. This was probably due to the profuse and rapid sporulation of *S. fuliginea* in the greenhouse. *Sphaerotheca macularis*, while heavily parasitized as measured by the percentage of conidiophores that collapsed, only supported low numbers of *A. quisqualis* pycnidia. This was due to the very sparse colonies produced by this species in our assay. The host range of *A. quisqualis* is very much broader than the three species examined here. Over 64 species in the genera *Brasiliomyces*, *Erysiphe*, *Leveillula*, *Microsphaera*, *Phyllactinia*, *Podosphaera*, *Sphaerotheca*, *Uncinula*, and the anamorphic genera *Oidium* and *Oidiopsis* have been reported as hosts of *A. quisqualis*. These reports have come from 256 species of host plants representing 172 genera in 59 families from 28 countries (S. P. Falk, *unpublished*).

Cotton twine was superior to sisal or jute twine for the growth of *A. quisqualis*. Cotton twine is made from a 50:50 blend of polyester and waste and raw cotton (Berk Carter, King Cotton Product Representative, *personal communication*) that is almost pure cellulose. Sisal twine is made from the leaves of *Agave sisalana* and jute twine from the stems of *Corchorus capsularis*. It would seem that compounds, perhaps tannins, toxic to *A. quisqualis* may be present in these plants and persist in the manufacturing process of the twine. These compounds, while desirable in making these twines more durable, may be responsible for inhibiting the growth of *A. quisqualis*.

Previous research by Sztejnberg (31) determined that improved growth of *A. quisqualis* on agar media could be accomplished by adding DL-asparagine to MEA (4% malt extract) and adjusting pH to 6.5. Subsequently, wheat bran extract was found to further enhance spore production on solid media (33). When growth of five isolates of *A. quisqualis* was compared on four agar media, significantly

Table 5. Area under disease progress curve (AUDPC)[u] for disease incidence and severity on leaves and fruit clusters of *Vitis* interspecific hybrid Aurore treated with the mycoparasite *Ampelomyces quisqualis* on cotton wick cultures or untreated at Dresden, N.Y., in 1992 and 1993

		AUDPC			
		Leaves		Fruit clusters[x]	
Year	Treatment	Number infected per shoot[v]	Percent surface infected[w]	Percent infected	Percent surface infected
1992	*A. quisqualis*	346	1,198	3,889	1,020
	Untreated	461	2,784	4,655	3,077
	P^y =	0.213	0.002	0.286	0.045
1993	*A. quisqualis*	72	452	582	94
	Untreated	50	416	365	60
	P^y =	0.454	0.755	0.588	0.619
Analysis of variance (both years)					
Year	P^z =	≤0.001	≤0.001	≤0.001	≤0.001
Treatment	P^z =	0.225	≤0.001	0.421	0.003
Year × Treatment	P^z =	0.085	≤0.001	0.163	0.002

[u] AUDPC (26) was calculated using the method of trapezoids with day of year as the x axis and disease variable as the y axis.
[v] Leaves were examined on 5 or 10 shoots per vine on 10 vines in each of three replicate 36-vine plots in 1992 and four replicate 36-vine plots in 1993.
[w] Percent leaf surface infected from 30 leaves per plot.
[x] Twenty fruit clusters were examined on 10 vines in each of three replicate 36-vine plots in 1992 and four replicate 36-vine plots in 1993.
[y] Probability of significant effect of treatment within each column for each year determined by separate variance Student's t tests.
[z] Probability of F value for analysis of variance with 1 df.

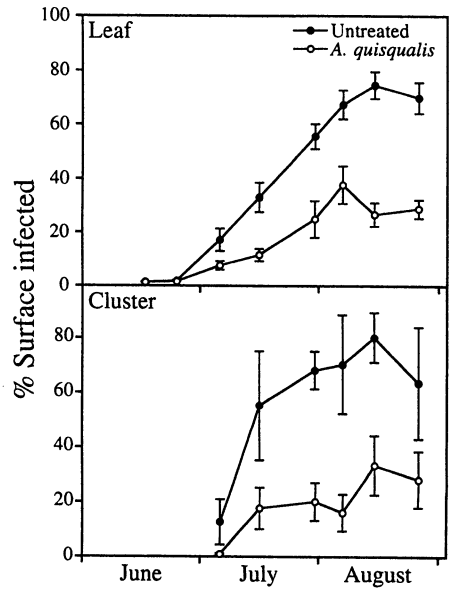

Fig. 1. Development of grape powdery mildew (*Uncinula necator*) on vines of the *Vitis* interspecific hybrid Aurore treated with the mycoparasite *Ampelomyces quisqualis* on cotton wick cultures or untreated at Dresden, N.Y., in 1992. Wick cultures were deployed, two per vine, on 21 May, 18 June, and 22 July. Plots, consisting of 36 vines, were replicated three times in a completely random design. Severity of leaf infection was determined from 30 infected leaves per plot and severity of cluster infection was determined from 20 clusters per vine. Error bars represent 1 standard error of the mean (n = 3).

more pycnidia were produced on WBMA compared with agar media amended with oat bran extract, PDA, or MEA and significantly more conidia were produced on WBMA compared with MEA (S. P. Falk, *unpublished*). Similarly, we have demonstrated improved production of conidia by addition of wheat bran extract to our semi-solid medium used for the growth of *A. quisqualis* G273 on cotton twine.

In 1990, introduction of the mycoparasite early in the growing season was more conducive to disease control than were repeated introductions. The early dispersal and establishment of the mycoparasite onto the first-formed mildew colonies was the most important factor in the degree of suppression of powdery mildew on leaves and fruit. Our aim in expanded field trials in 1992 and 1993, then, was to introduce the mycoparasite early in the season to impact subsequent disease development. We were able to do this in 1992 but not 1993. Our success in 1992 may have been due to above average rainfall that year, which created conditions conducive to dispersal of *A. quisqualis* inoculum from wick cultures and parasitism of developing powdery mildew colonies. Rainfall amounts for the months of May, June, July, August, and September in 1992 were 100, 62, 214, 77, and 119 mm respectively in the vineyard at Dresden. In 1993, the respective rainfall amounts were 35, 82, 36, 56, and 92 mm (D. M. Gadoury, *unpublished*). The respective 30-year averages at the nearby NYSAES were 76, 93, 75, 80, and 82 mm (T. DeYulio, *unpublished*). In addition to the drier conditions in 1993, disease did not develop until late in the season in 1993, compared with 1992, and was subsequently less severe.

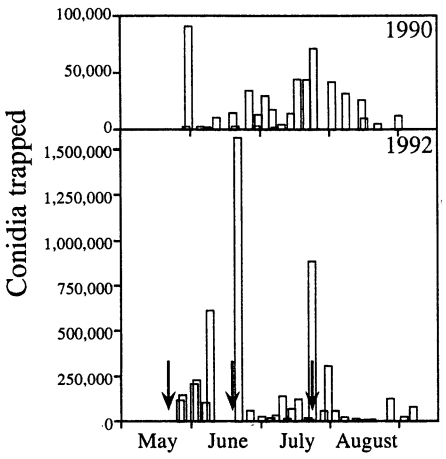

Fig. 2. Trapping of conidia of *Ampelomyces quisqualis* G273 growing on cotton wick cultures in 1990 and 1992. Conidia were dispersed in rain-splash and drip to glass funnels beneath the wicks and collected after each rain event. In 1992, arrows indicate time of deployment of wick cultures on 21 May, 18 June, and 22 July. Numbers are means of the total number of conidia counted in three replicate funnel-traps in 1990 and four in 1992.

Consequently, it was not possible to observe an impact on disease development in the low levels of disease that did appear.

Release of conidia and subsequent trapping in funnels is influenced by the number of conidia available for release as well as the amount of rainfall. Peaks of release immediately following deployment of wick cultures included conidia that had not been exposed previously to rain. Lesser peaks that occurred once the wick cultures had been in the field for some time followed periods of high rainfall. Examination of the funnel-trap data, then, may not show the depletion of conidia from the wick cultures over time. Data showing the number of conidia that could be removed from pieces of wick collected periodically from the field clearly shows this depletion within a period of 1 to 2 months. However, low numbers of conidia continued to be detected for 3 months. In 1990, the presence of conidia in the funnel-traps over a 3-month period may represent detection of these low numbers of conidia.

A major limitation in the use of *A. quisqualis* to control powdery mildew on most crops is the requirement of free water by the mycoparasite for infection. Our use of long-lived cultures deployed above the crop canopy appears to address this limitation. The biological control agent survives on wick cultures, and is available to release conidia during rain, under precisely the conditions that will lead to maximum parasitism of *U. necator*. Of course, this approach presupposes an adequate supply of rain or irrigation to result in dispersal of the mycoparasite coincident with infection periods by *U. necator*. In areas where cleistothecia are sources of primary inoculum for grape powdery mildew, release of ascospores of *U. necator* requires rain (9,10). However, after the initial cycle of primary infection, secondary infection can progress without rain (19). Not surprisingly then, we obtained our best disease control during 1990 and 1992, years of abundant rainfall, and comparatively poor disease control during a dry year, 1993.

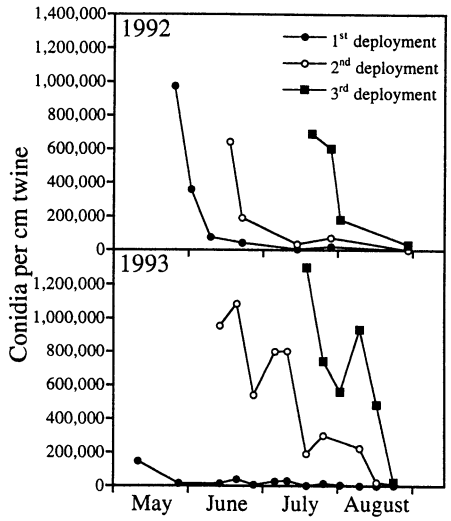

Fig. 3. Recovery of conidia (per cm wick) of *Ampelomyces quisqualis* G273 washed from pieces of wick culture in a laboratory assay following deployment over vines of *Vitis* interspecific hybrid Aurore at Dresden, N.Y., in 1992 and 1993. Five replicate samples of wick (1 or 2 cm long) were soaked for 3 h in 1 ml of distilled water, vortexed regularly, and the resulting conidial suspension counted with a hemacytometer.

Given the low tolerance for powdery mildew on high-quality wine grapes, the high susceptibility of these cultivars to powdery mildew, and the best level of disease control that we achieved in our trials, it is unlikely that *A. quisqualis* alone will provide adequate control of powdery mildew on this crop. However, the observed tolerance of G273 of many of the fungicides used in viticulture (S. P. Falk, *unpublished*), the significant reduction of disease that was observed often due to biological control, and the resultant reduction of overwintering inoculum both through suppression of disease and direct parasitism of cleistothecia (6,7) should not be regarded as unimportant. Less than optimal disease control methods are often required in viticulture. For example, resistance to the sterol demethylation–inhibiting fungicides triadimefon and myclobutanil has limited their use in some vineyards. Similarly, factors such as cultivar sensitivity to certain fungicides (phytotoxicity) and prohibitions imposed by grape processors can severely limit the available options for chemical control of grape powdery mildew. In this regard, the mycoparasite *A. quisqualis* and the strategies that we have developed for its use may be useful additions for the control of this disease.

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