Fusarium proliferatum as a Biocontrol Agent Against Grape Downy Mildew

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ABSTRACT

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Postinfection applications of microconidial suspensions of *Fusarium proliferatum* G6 reduced sporangial production of *Plasmopara viticola* on grape leaf disks by 97% and prevented resporulation. Microscopic examination of the hyphal interaction in vitro showed hyphae of *F. proliferatum* G6 coiled around and inside sporangiophores of *P. viticola*. In the field from 1992 to 1995, where substantial natural grape downy mildew developed, weekly applications of *F. proliferatum* G6 microconidia reduced disease development on leaves and fruit clusters of *Vitis* interspecific hybrid cultivars Chancellor and Lakemont. Severity of downy mildew on Chancellor clusters was reduced by 77% in 1992, 80% in 1993, and 53% in 1994 and was reduced on leaves by 71% in 1992. On Lakemont, disease severity on clusters was reduced by 99% in 1993, 94% in 1994, and 81% in 1995 and was reduced on leaves by 79% in 1992, 67% in 1994, and 60% in 1995. Fumonisin mycotoxins, which are produced by *F. proliferatum*, including *F. proliferatum* G6, were not found in grape berries or juice made from berries sprayed with microconidia in the field. Agar tests with fungicides showed sulfur, copper, and metalaxyl to be minimally detrimental to *F. proliferatum* G6. Biological control with *F. proliferatum* might be practical in conjunction with a management program in which sulfur and copper are used or in an antiresistance management program with metalaxyl, on moderately resistant cultivars, or in areas where downy mildew is not severe.

Additional keyword: oomycete.

During the late 1980s, there were several constraints on control of major grape diseases with fungicides in New York: captan and mancozeb use was (and still is) prohibited or severely restricted by grape processors, metalaxyl was not registered for use on grapevine and severe resistance to the product had occurred elsewhere (28), and copper fungicides were phytotoxic to important cultivars (14,33). This stimulated the initiation of a number of projects at the New York State Agricultural Experiment Station at Geneva on alternative means of controlling downy mildew (*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni in Sacc.) and other major fungal diseases of grapevine, especially powdery mildew. Investigations to establish alternative control for powdery mildew included the use of the mycoparasite *Ampelomyces quisqualis* (7,8), vapor-action treatment with triazole fungicides (29), dormant-season applications of calcium polysulfide (12), heat treatments of grapevines in the field (D. M. Gadoury, *unpublished data*), and seasonal exposure to ultraviolet light (13).

Fusarium proliferatum (T. Matsushima) Nirenberg (teleomorph *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura) was discovered overgrowing sporulating colonies of *P. viticola* in 1991 by R. C. Pearson. In this study, we report on the use of *F. proliferatum* to provide biological control of grape downy mildew. Our specific objectives were to determine (i) the effect of pre- and postinfection applications of *F. proliferatum* on *P. viticola*-infected leaf disks and leaves; (ii) the nature of the relationship between *F. proliferatum* and *P. viticola*; (iii) the effect of *F. proliferatum* applied in the field on development of grape downy mildew; (iv) whether fumonisins are produced by *F. proliferatum* when applied to grapes in the field; and (v) the sensitivity of *F. proliferatum* to fungicides used in viticulture. A preliminary report has been published (9).

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MATERIALS AND METHODS

Discovery and description of the biocontrol agent. *F. proliferatum* was isolated from atypical grape downy mildew lesions on *Vitis vinifera* L. 'Chardonnay' in Suffolk County, NY, during the summer of 1991. On potato dextrose agar (PDA) (Difco Laboratories, Detroit), *F. proliferatum* produced white fluffy colonies that were purple in reverse and reached a maximum colony diameter of 7.5 cm (standard error [SE] = 0.1 cm, $n = 3$) at 25°C after 8 days. Microconidia were clavate (7 to 11 × 2 to 3 μm) and were produced in false heads and chains on phialides and polyphialides. Macroconidia were falcate to subfalcate, three to five septate, and 40 to 60 × 2.5 to 3.5 μm. Identification to *Fusarium* species was made by P. Nelson (The Pennsylvania State University, University Park). The single-spore isolate G6 has been deposited under the Budapest Treaty with ATCC (American Type Culture Collection, Rockville, MD) and has the accession number 74149.

Effects of pre- and postinfection applications of *F. proliferatum* on *P. viticola*-infected leaf disks and leaves. Grape seedlings were produced from seeds of *V. vinifera* 'Riesling' as described previously (11). Germlings were planted in Jiffy-7 starter pellets (Jiffy Products of America, Batavia, IL) and placed in a growth chamber (Vesgro Environmental Chamber, model 206, Vestal Modern Design Corp., Vestal, NY) at 21°C with a 12-h photoperiod. After 2 to 3 weeks, seedlings were transferred to 15-cm-diameter pots containing Cornell potting mixture and were returned to the growth chamber. Seedlings

were fertilized at 3 weeks with slow-release fertilizer (Osmocote, 14–14–14).

P. viticola was isolated from leaves of *Vitis* interspecific hybrid cultivar Lakemont at Geneva, NY, in 1991. The pathogen was maintained, at weekly intervals, by spraying a suspension of sporangia washed from sporulating lesions onto leaves from the potted vines grown from Riesling seeds. Inoculated leaves were placed inside moist chambers in the growth chamber. Suspensions of *P. viticola* sporangia were prepared for infection of leaves and leaf disks by washing sporangia from sporulating lesions on leaves with distilled water in a nonchlorinated fluorocarbon aerosol sprayer (Preval, Precision Valve Corporation, Yonkers, NY). The collected suspension of sporangia was adjusted to 1×10^5 sporangia per ml with a hemacytometer. The suspension was kept at room temperature (22°C) for 20 to 30 min to allow the sporangia to release zoospores before application to leaf disks and leaves.

Suspensions of microconidia of *F. proliferatum* isolate G6 were prepared for application to leaf tissue pre- or postinfection with *P. viticola*. A microconidial suspension was prepared by scraping mycelium of *F. proliferatum* G6 from 2- to 3-week-old cultures grown on PDA at 20 to 25°C. A homogenized conidial suspension was obtained by vortex mixing (Thermolyne Maxi mix, Sybron Corp., Dubuque, IA) of the mycelium and conidia in distilled water. The suspension was filtered through a double layer of cheesecloth and adjusted to 1×10^6 microconidia per ml with a hemacytometer. Microscopic examinations revealed that suspensions consisted almost entirely of microconidia.

Preinfection application of F. proliferatum *G6 to leaf disks.* Preinfection applications of *F. proliferatum* G6 were applied to the undersurface of leaf disks (2 cm diameter) cut from grapevine seedling leaves grown in the growth chamber. The inverted leaf disks were arranged on moistened filter paper (Whatman No. 3, Maidstone, England) in glass petri dishes (9 cm diameter × 2 cm high) and sprayed until wet with a microconidial suspension of *F. proliferatum* G6 (1×10^6/ml) with a Preval sprayer. Five or six leaf disks were placed in each petri dish. A control treatment consisted of a distilled water spray. The droplets were allowed to dry, and the dishes were sealed with Parafilm and transferred to the growth chamber at 21°C and a 12-h photoperiod. After 24 h, five 10-µl drops of a suspension of *P. viticola* sporangia (1×10^5/ml) with released zoospores were placed on each leaf disk. After 20 h, the drops on the leaf disks were dried with absorbent paper tissues. The dishes were resealed and returned to the growth chamber. To assess the effect of *F. proliferatum* G6 on disease development, the production of *P. viticola* sporangia was quantified 7 days later. Sporangia were washed from each 2-cm-diameter leaf disk with 2 to 3 ml of distilled water with a Preval sprayer. The total number of sporangia per leaf disk was calculated by examining the suspension with a hemacytometer. The ability of *P. viticola* lesions to resporulate after the sporangia were washed off also was assessed. Resporulation was assessed by visual examination and quantified with a hemacytometer 24 to 48 h later. Treatments were replicated on five or six leaf disks, and each experiment was performed three times.

Postinfection application of F. proliferatum *G6 in drops to leaf disks.* Leaf disks for postinfection studies were inoculated first on the undersurface with five 10-µl drops of a suspension of *P. viticola* sporangia (1×10^5/ml) and released zoospores. After 20 h of incubation on moistened filter paper in glass petri dishes, the drops on the leaf disks were dried with absorbent paper tissues. The dishes, each containing four or five leaf disks, were resealed and returned to the growth chamber. Sporulation of *P. viticola* was first observed 4 days after inoculation. Five days after inoculation, each *P. viticola* lesion was inoculated with one 10-µl drop of a microconidial suspension of *F. proliferatum* G6 (1×10^6/ml). A control treatment consisted of drops of distilled water. After 20 h, the drops were dried with absorbent paper tissues, and the dishes were resealed and returned to the growth chamber. The production of *P. viticola* sporangia was quantified 7 days later, and an assessment of the ability of *P. viticola* lesions to resporulate was made after 4 days. Treatments were replicated on four or five leaf disks, and each experiment was performed seven times.

Postinfection application of F. proliferatum *G6 in a spray to leaf disks.* Similar postinfection applications of *F. proliferatum* G6 were made to leaf disks, as described above, except that a Preval sprayer was used to deposit microconidia (1×10^6/ml) onto already developed *P. viticola* lesions. A control treatment consisted of distilled water sprays. After air-drying, dishes were resealed, returned to the growth chamber, and the effect on production of *P. viticola* sporangia was quantified after 7 days. Treatments were replicated on four or five leaf disks, and each experiment was performed four times.

Postinfection application of F. proliferatum *G6 to seedlings.* Postinfection applications of *F. proliferatum* G6 were made to leaves on seedlings previously inoculated with *P. viticola*. The undersurfaces of the first three whole expanded leaves on seedlings with five to six leaves were inoculated with 15 to 20 10-µl drops of the *P. viticola* sporangia (1×10^5/ml) suspension containing released zoospores. Each plant was watered and sealed in a plastic bag sprayed with distilled water to produce moist conditions. Plants were transferred to the growth chamber (21°C, 12-h photoperiod) for incubation. After 20 h, the plastic bags were opened to dry the inoculation drops and plant surface. When dry, the plastic bags were resealed, and *P. viticola* sporulated 4 days after inoculation. Five days after inoculation with *P. viticola*, applications of *F. proliferatum* G6 were made by spraying a suspension of microconidia (1×10^6/ml) with a Preval sprayer onto the undersurface of leaves. Control treatments consisted of sprays with distilled water. The droplets of *F. proliferatum* G6 inoculum or distilled water were allowed to dry, and the plants were resealed in plastic bags and returned to the growth chamber. Moist chambers were in place for 3 of the next 7 days. At the end of 7 days, each lesion was examined visually, and the number of lesions per leaf on which *F. proliferatum* G6 was growing and *P. viticola* was no longer sporulating, indicating that growth of downy mildew had been stopped, were counted. Treatments were replicated on three plants, and each experiment was performed four times.

Microscopy of the in vitro interaction between *P. viticola* and *F. proliferatum*. The in vitro interaction between *F. proliferatum* G6 and *P. viticola* was examined at the level of the dissecting microscope, the scanning electron microscope (SEM), and the transmission electron microscope (TEM). The interaction was established postinfection on leaf disks inoculated with drops of *P. viticola* sporangia (1×10^5/ml) and zoospores, followed 24 h later by a spray of *F. proliferatum* G6 microconidia (1×10^6/ml). Leaf disks were incubated on moistened filter paper in glass petri dishes at 21°C for 4 to 6 days.

For SEM, samples were fixed by submersion in 1% OsO_4 overnight at 4°C and dehydrated through an ethanol series (20, 40, 60, 75, 95, and 100% at 30 min per step) followed by absolute ethanol overnight. After dehydration, the material was critical-point dried (ethanol/CO_2), mounted on specimen stubs, and sputter-coated with gold palladium. Samples were examined with a Hitachi S-530 SEM (Nissei Sangyo America, Ltd., Mountain View, CA) operated at 25 kV.

Segments (2 mm^2) of lesions were prepared for TEM by fixation in 3% glutaraldehyde buffered with 100 mM sodium phosphate buffer, pH 7.0, for 1.5 h. The segments were rinsed four times in buffer (15 min per rinse), followed by two rinses in distilled water (15 min per rinse). The samples were postfixed in 1% OsO_4 (aqueous) for 1 h, rinsed four times in distilled water, and dehydrated through an acetone series (20, 40, 60, 75, 95, and 100% at 30 min per step) followed by 100% acetone overnight. The specimens were embedded in an Epon-Araldite (Electron Microscopy Sciences, Fort Washington, PA) resin mixture, polymerized, and sectioned for electron microscopy. Thin sections were

stained for 15 min with 1% aqueous uranyl acetate and for 3 to 5 min with 0.5% lead citrate and examined with a JEOL (Tokyo) 100SX TEM operated at 100 kV.

Efficacy of *F. proliferatum* in vineyard trials against *P. viticola*. Microconidial suspensions of *F. proliferatum* G6 were prepared for field trials to evaluate biological control against *P. viticola*. PDA plates were inoculated with a single 5-mm-diameter plug of *F. proliferatum* G6 taken from an actively growing culture. After 2 to 3 weeks of growth at approximately 25°C, the mycelial mat with conidia was scraped from the surface of the agar and placed in distilled water. The suspension was shaken and filtered through a double layer of cheesecloth. The suspension was adjusted to 1×10^6 microconidia per ml in 0.02% Tween 20 (Sigma Chemical Company, St. Louis). Microscopic examination of the filtered suspension showed mostly microconidia. Viability of the inoculum was determined by assessing germination, after 24 h, of conidia applied to water agar at the time of application in the field. Germination averaged 93.6 ± 1.4% (n = 10) in 1993, 97.2 ± 0.9% (n = 12) in 1994, and 98.4 ± 0.6% (n = 10) in 1995.

Microconidial suspensions of *F. proliferatum* G6 were applied until runoff with a backpack sprayer (Solo, Inc., Newport News, VA) every 7 days, commencing at 15 cm of shoot growth. Applications were made from 22 May to 21 August 1992, from 30 May to 27 August 1993, from 3 June to 11 August 1994, and from 31 May to 9 August 1995. A fungicide treatment of mancozeb (Dithane M45 at 4.5 kg/ha) was applied with a CO_2 backpack sprayer in 1992, 1993, and 1994. Applications were made every 14 days from 22 May to 14 August 1992, from 30 May to 20 August 1993, and from 3 June to 11 August 1994. All treatments with microconidial suspensions and mancozeb were made in a volume of approximately 0.5 liters per vine (prebloom) and up to 1 liter per vine (postbloom). In 1995, the fungicide treatment consisted of metalaxyl/mancozeb (Ridomil MZ58 at 2.2 kg/ha) applied with an over-the-row hooded boom sprayer every 14 days from 1 June to 13 July. A check treatment consisted of vines receiving no treatments.

All treatments were applied to the downy mildew susceptible *Vitis* interspecific hybrid cultivar Chancellor in 1992, 1993, and 1994 and to the susceptible *Vitis* interspecific hybrid cultivar Lakemont in 1992, 1993, 1994, and 1995 at an experimental vineyard in Geneva. Treatments were applied to 2-vine plots of Chancellor in all years, to 2-vine plots of Lakemont in 1992 and 1993, and to 4-vine plots of Lakemont in 1994 and 1995. Plots were replicated five times in randomized complete block designs; there were four replicates of Lakemont in 1995. Chancellor vines were planted in 1979 and Lakemont vines in 1988. Downy mildew disease progress was monitored at intervals during the season by determining disease incidence and severity on clusters and leaves from two vines in each plot. Twenty-five clusters per vine were examined to determine disease incidence, and the percent surface area infected by downy mildew was estimated on all the examined clusters to determine disease severity. Ten leaves on each of five shoots per vine were similarly examined to determine the incidence and severity of leaf infections. Disease incidence (percent) and severity (percent area) were subsequently expressed as percentages and were transformed by arcsine square root (for homogeneity of variance) before analysis of variance (ANOVA), using Systat 5.2 (Systat, Inc., Evanston, IL). Comparison of means within treatments within years was done with Tukey's HSD. In 1992, powdery mildew was controlled by weekly applications of sulfur from 10 July to 19 August.

Production of fumonisins by *F. proliferatum*. Grape berries were collected to be analyzed for the presence of fumonisins. Berry clusters were collected randomly from vines treated with *F. proliferatum* G6, vines treated with mancozeb, and untreated vines of *Vitis* interspecific hybrid cultivar Delaware on 4 November 1992 and from cultivar Chancellor on 29 September 1993—6 weeks after the last application of *F. proliferatum* G6 in 1992 and 4 weeks after the last application in 1993. In 1992 and 1993 fresh berries were sent to P. Nelson, The Pennsylvania State University, University Park, to be analyzed for fumonisins and for isolation of *F. proliferatum* from berries with modified pentachloronitrobenzene medium (27). In 1993 juice was prepared from cultivar Delaware berries that had been stored frozen since 1992 and from the cultivar Chancellor berries collected in 1993. Juice was prepared by crushing berries and squeezing the juice through two layers of cheesecloth. Juice samples were sent to F. Ross, National Veterinary Services Laboratories, Ames, IA, to be analyzed for fumonisins by high-performance liquid chromatography/fluorescence (25).

Sensitivity of *F. proliferatum* to fungicides used in viticulture. Media were prepared, incorporating 1, 10, 100, or 1,000 µg of the active ingredient of fungicide per ml, with the commercial formulations. The fungicides were dissolved in acetone and added to PDA media at 55 to 60°C just prior to pouring into petri plates. For the assessment of hyphal growth, a 6-mm-diameter disk of *F. proliferatum* G6 from a 10-day-old colony was placed in the center of each of three replicate dishes. After 10 days of incubation at 20°C, the diameter of the colony was measured and expressed as the percent reduction in growth compared to the untreated control. Germination of *F. proliferatum* G6 was tested by spreading 0.2 ml of a suspension of 1×10^5 microconidia per ml over the surface of the agar with incorporated fungicides. Germination of 500 microconidia per treatment was assessed after 20 h of incubation at 20°C and expressed as the percent reduction in germination compared to the untreated control. The effects of the following fungicides were evaluated: benomyl (Benlate 50WP), captan (Captan 50WP), dinocap (Karathane WD), ferbam (Carbamate WDG), $CuSO_4$ (copper sulfate pentahydrate), COCS (copper oxychloride sulfate), iprodione (Rovral 50WP), mancozeb (Dithane M45), myclobutanil (Nova 40WP), sulfur (wettable sulfur), triadimefon (Bayleton 50DF), metalaxyl (Ridomil 2E), and fosetyl Al (Aliette).

TABLE 1. Effect of *Fusarium proliferatum* G6 on sporulation of *Plasmopara viticola* on leaf disks of grapevine seedlings of *Vitis vinifera* 'Riesling'

Treatment[v]	Application method[w]	% Reduction in sporangial production[x]	% Reduction in resporulation[y]	
			F. proliferatum G6	Distilled water
Preinfection	Spray	71 ± 12[z]	99 ± 2	1 ± 2
Postinfection	Drop	72 ± 3	99 ± 1	7 ± 1
Postinfection	Spray	97 ± 2	100 ± 0	0 ± 0

[v] *F. proliferatum* G6 was applied 24 h prior (preinfection) or 5 days after (postinfection) infection by *P. viticola*, which was applied in five 10-µl drops of sporangia (1×10^5/ml) with released zoospores to the undersurface of four to six leaf disks per treatment.

[w] 1×10^6 microconidia of *F. proliferatum* G6 per ml was applied as a spray suspension until leaves were wet or in one 10-µl drop to *P. viticola* lesions.

[x] *P. viticola* sporangia were washed off each leaf disk after 7 days and were counted with a hemacytometer.

[y] Percentage of *P. viticola* lesions on leaf disks resporulating after removal of sporangia for counting.

[z] Numbers are means of n experiments ± 1 SE, where n = 3 (preinfection, spray), n = 7 (postinfection, drop), and n = 4 (postinfection, spray).

RESULTS

Effects of pre- and postinfection applications of *F. proliferatum* on *P. viticola*-infected leaf disks and leaves. Microconidia of *F. proliferatum* G6 sprayed onto grapevine seedling leaf disks 24-h preinfection reduced the number of sporangia of *P. viticola* produced by 71% (Table 1) compared to control-inoculated leaf disks. On these same leaf disks, resporulation of *P. viticola* was prevented in 99% (Table 1) of the lesions treated with *F. proliferatum* G6 after removal of sporangia by washing. Only 1% of untreated lesions failed to resporulate after sporangia were washed from the leaf disks (Table 1). Postinfection application of *F. pro-*

liferatum G6 microconidia by droplets to 5-day-old sporulating lesions of *P. viticola* on leaf disks reduced production of sporangia by 72% (Table 1) 7 days after treatment compared to lesions treated with distilled water. After these sporangia were washed off the leaf disks, resporulation was prevented in 99% of the lesions treated with *F. proliferatum* G6 and in only 7% of the lesions treated with distilled water (Table 1). When microconidial suspensions of *F. proliferatum* G6 were sprayed onto 5-day-old sporulating lesions of *P. viticola* on leaf disks, production of sporangia was reduced by 97% (Table 1) 7 days later. After these sporangia were washed off the leaf disks, resporulation was prevented in 100% of lesions treated with *F. proliferatum* G6, whereas all other lesions resporulated (Table 1). *F. proliferatum* G6 sprayed 5 days postinfection onto whole leaves of seedling grapevines in the growth chamber prevented further development of *P. viticola* in 91% (SE = 5%, $n = 4$) of the treated lesions compared to only 2% (SE = 2%, $n = 4$) of the lesions that failed to continue growth when treated with distilled water.

Microscopy of the in vitro interaction between *P. viticola* and *F. proliferatum*. *P. viticola* lesions sprayed with *F. proliferatum* G6 microconidia showed extensive growth of *F. proliferatum* G6 mycelium over the surface of colonies when examined after 6 days (Fig. 1A). Closer examination with SEM showed growth of *F. proliferatum* G6 over sporangia and sporangiophores of *P. viticola* and production of phialides and microconidia (Fig. 1B). *F. proliferatum* G6 hyphae were observed coiled around sporangiophores of *P. viticola* (Fig. 1C), and when observed by TEM 4 days after inoculation, hyphae also were observed inside sporangiophores of *P. viticola* (Fig. 1D).

Efficacy of *F. proliferatum* in vineyard trials against *P. viticola*. Late-season assessments of the incidence and severity of downy mildew in cultivar Chancellor grapevines are shown in Table 2 for 1992, 1993, and 1994. Chancellor grapevines were not examined in 1995. Treatments within years were examined by separate ANOVAs, and the means were compared by Tukey's HSD ($P = 0.05$). Treatment with *F. proliferatum* G6 reduced the incidence of downy mildew (percent infected) on clusters from 43.3 to 14.0% (68% reduction, $P = 0.066$) in 1993, and severity (percent area infected) was reduced from 27.4 to 6.4% (77% reduction, $P = 0.058$) in 1992 and from 9.2 to 1.8% (80% reduction, $P = 0.01$) in 1993. On leaves, severity (percent area infected) was reduced from 3.1 to 0.9% (71% reduction, $P = 0.042$) in 1992. Severity of downy mildew (percent area infected) on vines treated with *F. proliferatum* G6 was not different from the amount of disease that developed on mancozeb-treated vines on clusters in 1993 ($P = 0.103$) and on leaves in 1992 ($P = 0.839$).

For the remaining disease variables and in other years, the amount of downy mildew that developed on *F. proliferatum* G6-

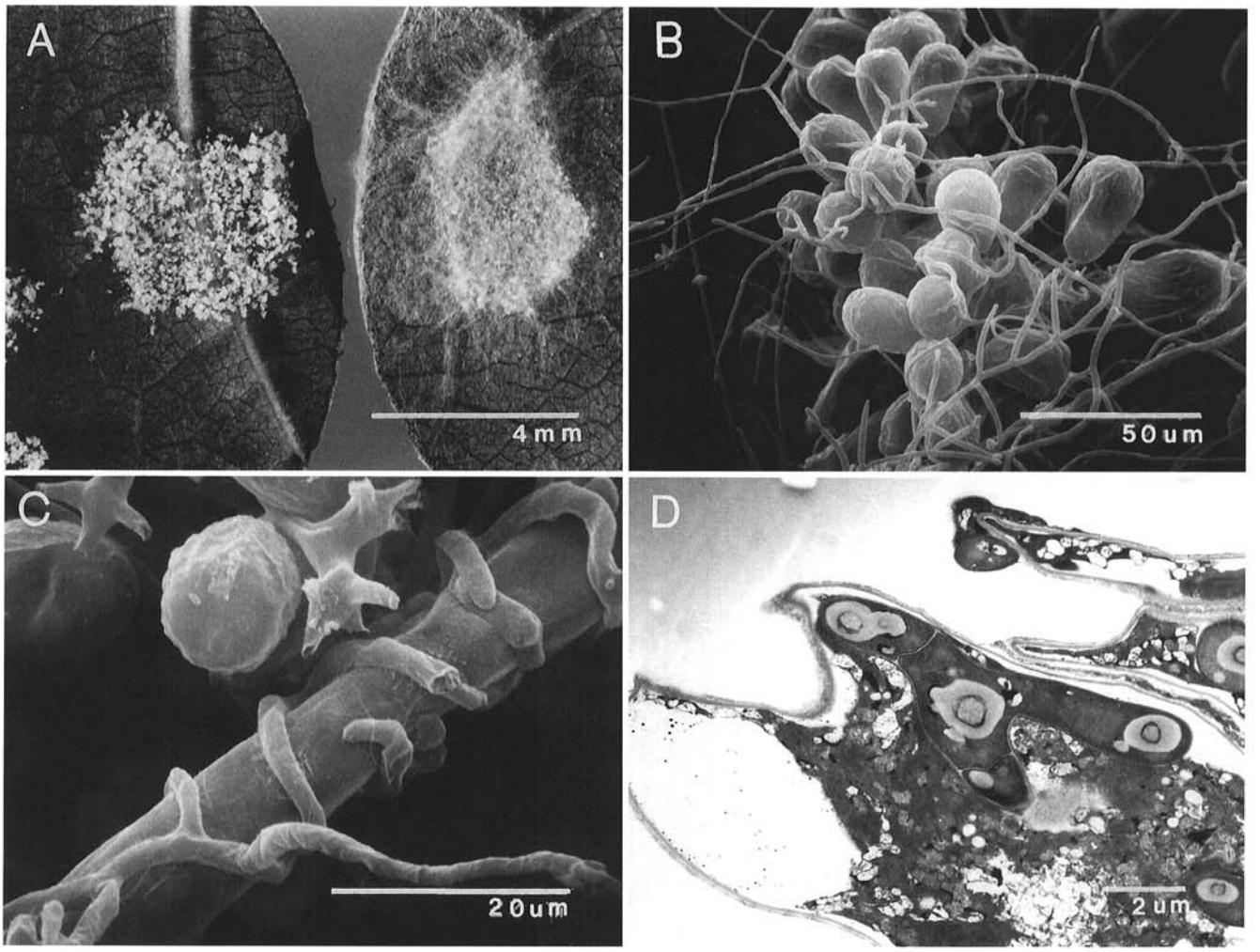

Fig. 1. In vitro hyphal interactions between *Fusarium proliferatum* and *Plasmopara viticola*. **A,** Lesions of *P. viticola* on the undersurface of 2-cm-diameter leaf disks of seedlings from *Vitis vinifera* 'Riesling' grapevines 11 days after inoculation with a 10 µl-drop of sporangia (1×10^5/ml) with released zoospores and incubation in a moist chamber. The lesion on the right was sprayed with microconidia of *F. proliferatum* G6 (1×10^6/ml) after 5 days. The lesion on the left was sprayed with distilled water after 5 days. **B,** Surface of *P. viticola* lesion viewed by scanning electron microscopy (SEM) showing small-diameter hyphae of *F. proliferatum* G6 growing over sporangia and sporangiophores of *P. viticola*. **C,** Hyphae of *Fusarium proliferatum* G6 coiled around a sporangiophore of *P. viticola* in a colonized *P. viticola* lesion, viewed by SEM. **D,** Transmission electron microscopy of septate hyphae of *F. proliferatum* G6 inside a sporangiophore of *P. viticola* 4 days after inoculating the *P. viticola* lesion with microconidia of *F. proliferatum* G6.

treated vines was intermediate between fungicide-treated and untreated vines (Table 2). ANOVA (Table 2) showed that the amount of downy mildew that developed on Chancellor clusters and leaves differed across years. More downy mildew occurred on clusters in 1992 than in 1993 or 1994. The only year in which substantial downy mildew developed on untreated leaves (percent infected = 29.1%, percent area infected = 3.1%) was 1992. The amount of downy mildew that developed on untreated leaves in 1993 was only 0.6 and <0.1% for percent infected and percent area infected, respectively, and in 1994 was 2.9 and 0.1%, respectively.

The incidence and severity of downy mildew in cultivar Lakemont grapevines in 1992, 1993, 1994, and 1995 are shown in Table 3 along with comparisons of means by Tukey's HSD (P = 0.05) of treatments within years. Incidence of downy mildew (percent infected) on clusters was reduced from 35.2 to 5.8% (84% reduction, $P < 0.001$) in 1993 and from 22.7 to 8.9% (61% reduction, $P = 0.017$) in 1995, and severity (percent area infected) was reduced from 10.1 to 0.8% (99% reduction, $P < 0.001$) in 1993, from 3.2 to 0.2% (94% reduction, $P = 0.071$) in 1994, and from 4.1 to 0.8% (81% reduction, $P = 0.019$) in 1995. On leaves, incidence (percent infected) was reduced from 68.7 to 37.0% (46% reduction, $P = 0.001$) in 1992 and from 40.8 to 20.8% (49% reduction, $P = 0.04$) in 1994, and severity (percent area infected) was reduced from 15.6 to 3.3% (79% reduction, $P = 0.001$) in 1992 and from 7.1 to 2.4% (67% reduction, $P = 0.016$) in 1994. Severity of downy mildew (percent area infected) on vines treated with *F. proliferatum* G6 was not different from the amount of disease that developed on mancozeb-treated vines on leaves in 1992 ($P = 0.185$).

In other years, the amount of downy mildew that developed on *F. proliferatum* G6-treated vines was intermediate between fungicide-treated and untreated vines. ANOVA showed that year did not affect the incidence (percent infected) of downy mildew on clusters ($P = 0.838$), but severity (percent area infected) on clusters differed across years at $P = 0.041$. Incidence of downy mildew on Lakemont leaves differed across years ($P < 0.001$), with more occurring in 1992 and 1995 than in 1994, whereas severity was greater in 1992 ($P < 0.001$) than in 1994 or 1995. Very little downy mildew developed on untreated Lakemont leaves in 1993 with incidence only 0.7% and severity only <0.1%.

Production of fumonisins by *F. proliferatum*. Fumonisins were not detected (<0.5 µg/ml) in berries sprayed with *F. proliferatum* G6 or in juice prepared from treated berries in either 1992 or 1993. In 1993 several isolates of *F. proliferatum* were obtained from the surface of berries treated with *F. proliferatum* isolate G6, from berries treated with mancozeb, and from untreated cultivar Chancellor berries.

Sensitivity of *F. proliferatum* to fungicides used in viticulture. The percent reduction in radial growth of *F. proliferatum* G6 on PDA media containing fungicides is shown in Table 4. Benomyl completely inhibited radial growth of *F. proliferatum* G6 at all concentrations of fungicide tested. Myclobutanil, incorporated at 100 and 1,000 µg of fungicide per ml into agar media, was the only other fungicide that completely inhibited radial growth of *F. proliferatum* G6. These concentrations are at or above the usual field rate of 90 to 150 µg/ml for myclobutanil. At lower concentrations (1 and 100 µg/ml) radial growth was substantially reduced (71.6 to 87.3%) by myclobutanil. Triadimefon reduced radial growth by 58.9 to 85.3% across the concentrations tested. Captan only reduced growth at 100 and 1,000 µg/ml (53.3 and 60.9%, respectively) and had no effect at lower concentrations. Iprodione only reduced growth substantially at concentrations above 10 µg/ml (59.9 to 80.7%). Ferbam, COCS, and fosetyl Al only reduced growth substantially (62.5, 74.6, and 61.4%, respectively) at 1,000 µg/ml and had little or no effect at lower concentrations. Dinocap had only a moderate effect on radial growth across all the concentrations of fungicide tested (40.6 to 48.2%), whereas mancozeb had only a moderate effect (40.6%) at 1,000 µg/ml and little effect at lower concentrations. Sulfur, $CuSO_4$, and metalaxyl had no or only a minor effect on radial growth of *F. proliferatum* G6 across all of the concentrations tested.

Germination of *F. proliferatum* G6 microconidia (Table 4) was greatly reduced (>75%) by mancozeb at 10, 100, and 1,000 µg/ml

TABLE 2. Effect of fungicide and *Fusarium proliferatum* G6 treatments on incidence and severity of grape downy mildew (*Plasmopara viticola*) on *Vitis* interspecific hybrid cultivar Chancellor grapevines at Geneva, NY

Year[s]	Treatment[t]	Cluster infection		Leaf infection	
		%[u]	% Area[v]	%	% Area
1992	Untreated	84.8 a[w]	27.4 a	29.1 a	3.1 a
	Mancozeb[x]	30.1 b	2.9 b	10.3 a	0.6 b
	F. proliferatum G6[y]	55.1 ab	6.4 ab	12.4 a	0.9 b
1993	Untreated	43.3 a	9.2 a	0.6 a	<0.1 a
	Mancozeb	0.9 b	0.1 b	0.0 a	0.0 a
	F. proliferatum G6	14.0 ab	1.8 b	<0.1 a	<0.1 a
1994	Untreated	37.4 a	5.3 a	2.9 a	0.1 a
	Mancozeb	2.6 b	0.2 b	0.7 a	<0.1 a
	F. proliferatum G6	31.5 a	2.5 ab	0.8 a	<0.1 a
Analysis of variance[z]					
Year		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Treatment		$P < 0.001$	$P < 0.001$	$P = 0.004$	$P = 0.001$
Year × treatment		$P = 0.784$	$P = 0.343$	$P = 0.389$	$P = 0.012$

[s] Data shown are from disease assessments conducted on 20 August 1992, 18 August 1993, and 29 July 1994.
[t] Treatments were applied to 2-vine plots and replicated five times in a randomized complete block design.
[u] Incidence (percent infected) was determined from 25 clusters per vine and 10 leaves from each of 5 shoots per vine.
[v] Severity (percent area infected) was estimated from each of 25 clusters per vine and 10 leaves from 5 shoots per vine.
[w] Data were transformed using arcsine $\sqrt{p}$, where p is a proportion, prior to statistical analysis, and the means were backtransformed to percents when reported in the table. Means within columns within years with different letters had probability levels <0.05 when analyzed by Tukey's HSD means comparisons after an analysis of variance (ANOVA).
[x] Mancozeb (Dithane M45 at 4.5 kg/ha) was applied at 14-day intervals in 1992 on 22 May; 5 and 19 June; 2, 16, and 30 July; and 14 August; in 1993 on 30 May; 11 and 25 June; 8 and 22 July; and 8 and 20 August; and in 1994 on 3, 16, and 30 June; 14 and 28 July; and 11 August.
[y] *F. proliferatum* G6 (1×10^6 microconidia per ml in 0.02% Tween 20) was applied at 7-day intervals in 1992 on 22 and 29 May; 5, 12, 19, and 26 June; 2, 10, 16, 24, and 30 July; and 7, 14, and 21 August; in 1993 on 30 May; 4, 11, 17, and 25 June; 1, 8, 16, 22, and 30 July; and 8, 13, 20, and 27 August; and in 1994 on 3, 9, 16, 23, and 30 June; 7, 14, 21, and 28 July; and 4 and 11 August.
[z] Probability of F value for analysis of variance.

in PDA media, by myclobutanil, benomyl, captan, dinocap, and ferbam at 100 and 1,000 µg/ml, and by iprodione at 1,000 µg/ml. Triadimefon and fosetyl Al across all of the concentrations tested, metalaxyl at 10, 100, and 1,000 µg/ml, and all other fungicides, unless specifically mentioned, reduced germination only moderately (35 to 65%). Myclobutanil and metalaxyl at 1 µg/ml and captan at 1 and 10 µg/ml had little effect (<25%) on germination of microconidia. Sulfur, $CuSO_4$, and COCS had little effect (<25%) on germination across all the concentrations tested; COCS at 1,000 µg/ml reduced germination by 31.9%.

DISCUSSION

F. proliferatum G6 was effective in reducing sporulation and further growth of *P. viticola* on leaf disks and leaves of grapevine seedlings in a growth chamber. Pre- and postinfection applications

TABLE 3. Effect of fungicide and *Fusarium proliferatum* G6 treatments on incidence and severity of grape downy mildew (*Plasmopara viticola*) on *Vitis* interspecific hybrid cultivar Lakemont grapevines at Geneva, NY

Year[r]	Treatment[s]	Cluster infection		Leaf infection	
		%[t]	% Area[u]	%	% Area
1992	Untreated	n.d.[v]	n.d.	68.7 a[w]	15.6 a
	Mancozeb[x]	n.d.	n.d.	14.5 c	1.0 b
	F. proliferatum G6[y]	n.d.	n.d.	37.0 b	3.3 b
1993	Untreated	35.2 a	10.1 a	0.7 a	<0.1 a
	Mancozeb	0.0 c	0.0 c	0.0 a	0.0 a
	F. proliferatum G6	5.8 b	0.8 b	0.7 a	<0.1 a
1994	Untreated	23.2 a	3.2 a	40.8 a	7.1 a
	Mancozeb	<0.1 b	0.0 b	<0.1 c	0.0 c
	F. proliferatum G6	9.9 a	0.2 ab	20.8 b	2.4 b
1995	Untreated	22.7 a	4.1 a	71.5 a	6.4 a
	Metalaxyl/mancozeb	0.0 c	0.0 b	10.7 b	0.3 b
	F. proliferatum G6	8.9 b	0.8 b	39.1 ab	2.6 ab
Analysis of variance[z]					
Year		$P = 0.838$	$P = 0.041$	$P < 0.001$	$P < 0.001$
Treatment		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Year × Treatment		$P = 0.267$	$P = 0.117$	$P < 0.001$	$P < 0.001$

[r] Data shown are from disease assessments conducted on 24 August 1992 and 25 August 1993, on clusters on 29 July 1994, and on leaves on 22 August 1994 and 16 August 1995.

[s] Treatments were applied to 2-vine plots in 1992 and 1993 and 4-vine plots in 1994 and 1995. Plots were replicated five times in a randomized complete block design; there were four replicates of Lakemont in 1995. Data were collected from 2 vines per plot.

[t] Incidence (percent infected) was determined from 25 clusters per vine and 10 leaves from each of 5 shoots per vine.

[u] Severity (percent area infected) was estimated from each of 25 clusters per vine and 10 leaves from 5 shoots per vine.

[v] Not determined.

[w] Data were transformed using arcsine $\sqrt{p}$, where p is a proportion, prior to statistical analysis, and the means were backtransformed to percents when reported in the table. Means within columns within years with different letters had probability levels <0.05 when analyzed by Tukey's HSD means comparisons after an analysis of variance (ANOVA).

[x] Mancozeb (Dithane M45 at 4.5 kg/ha) was applied at 14-day intervals (discussed in Table 2). Metalaxyl/mancozeb (Ridomil MZ58 at 2.2 kg/ha) was applied at 14-day intervals in 1995 on 1, 14, and 29 June and 13 July.

[y] *F. proliferatum* G6 (1×10^6 microconidia per ml in 0.02% Tween 20) was applied at 7-day intervals (discussed in Table 2) and in 1995 on 31 May; 7, 14, 21, and 28 June; 5, 12, 19, and 26 July; and 2 and 9 August.

[z] Probability of F value for ANOVA.

TABLE 4. Effect of fungicide on radial growth and germination of microconidia of *Fusarium proliferatum* G6 on potato dextrose agar (PDA) amended with 1, 10, 100, or 1,000 µg of active ingredient concentration of fungicide per ml

Fungicide[w]	Field rate[x] (µg/ml)	% Inhibition of radial growth[y]				% Reduction of germination[z]			
		1 µg/ml	10 µg/ml	100 µg/ml	1,000 µg/ml	1 µg/ml	10 µg/ml	100 µg/ml	1,000 µg/ml
Triadimefon (Bayleton)	112–150	58.9 ± 1.1	73.1 ± 0.6	84.3 ± 0.6	85.3 ± 0.6	48.6	52.1	59.5	64.5
Myclobutanil (Nova)	90–150	71.6 ± 0.6	87.3 ± 0.6	100.0	100.0	22.2	54.0	94.1	96.1
Benomyl (Benlate)	599–899	100.0	100.0	100.0	100.0	51.2	63.0	88.5	98.7
Captan (Captan)	1,198–2,397	0.0	0.0	53.3 ± 0.6	60.9 ± 0.6	0.7	5.7	91.3	98.4
Dinocap (Karathane)	467	41.1 ± 0.6	40.6	42.7 ± 0.6	48.2	42.7	44.7	80.7	90.9
Iprodione (Rovral)	899–1,198	7.7 ± 3.1	59.9 ± 0.6	64.5 ± 1.2	80.7 ± 2.5	29.6	41.1	42.8	77.5
Ferbam (Carbamate)	2,732–3,643	2.1 ± 2.7	8.7 ± 1.9	16.3 ± 1.1	62.5 ± 0.6	56.5	60.0	97.6	100.0
Sulfur	2,205–5,512	11.0 ± 0.8	11.7 ± 1.1	11.2 ± 0.6	10.2	1.5	8.3	10.0	10.9
$CuSO_4$	2373	0.0	0.0	0.0	21.9 ± 0.6	3.8	5.0	5.5	11.1
COCS	1,198	0.0	0.0	0.0	74.6 ± 1.2	15.3	20.2	23.9	31.9
Metalaxyl (Ridomil)	627	4.6 ± 0.6	4.6 ± 0.6	4.1	14.3 ± 2.7	21.4	35.7	41.5	42.3
Fosetyl Al (Aliette)	1,917–4,793	1.1 ± 1.1	2.1 ± 0.6	2.6	61.4 ± 5.0	43.7	45.4	50.4	58.4
Mancozeb (Dithane)	2,876–3,835	3.6 ± 2.7	6.6 ± 1.2	11.7 ± 1.1	40.6 ± 7.5	58.4	89.7	99.0	100.0

[w] Fungicides were prepared to the indicated active ingredient concentration and dissolved in acetone before adding to PDA media at 55 to 60°C. COCS = copper oxychloride sulfate.

[x] Field rate concentration of active ingredients of fungicides, assuming a spray volume of 935 liters/ha and using 0.21 to 0.28 kg of triadimefon per ha; 0.21 to 0.35 kg of myclobutanil per ha; 1.12 to 1.68 kg of benomyl per ha; 2.24 to 4.48 kg of captan per ha; 2.24 kg of dinocap per ha; 1.68 to 2.24 kg of iprodione per ha; 3.36 to 4.48 kg of ferbam per ha; 2.24 to 5.60 kg of sulfur per ha; 2.24 kg of $CuSO_4$ per ha; 2.24 kg of COCS per ha; 2.33 liters of metalaxyl per ha; 2.24 to 5.60 kg of fosetyl Al per ha; and 3.36 to 4.48 kg of mancozeb per ha.

[y] Numbers are the means of 3 replicates ± 1 SE of colony diameters measured after 10 days of growth at 20°C.

[z] Each number was determined based on examination of 500 microconidia.

were both effective, but posttreatment applications in the form of spray applications of microconidia of *F. proliferatum* G6 were the most effective. *F. proliferatum* G6 also prevented resporulation of *P. viticola* when sporangia were removed from lesions by washing, which simulated natural conditions under which sporangia dry-up between periods of leaf wetness. Microscopic examination of the hyphal interaction showed that *F. proliferatum* grew on *P. viticola* sporangia and sporangiophores and coiled around and grew inside the sporangiophores.

Biological control of grape downy mildew has been attempted previously. Culture filtrates of *F. gibbosum*, isolated from leaves and berries of grapevine inhibited development of the disease when sprayed onto detached leaves prior to inoculation with *P. viticola* zoospores (2). *Erwinia herbicola* inhibited infection, sporangia germination, and zoospore behavior of *P. viticola* (34); a *Trichothecium* species inhibited sporangial germination (1); and microorganisms in compost extracts suppressed development of *P. viticola* in the field (35). Biological control of a plant disease with *F. proliferatum* has been reported once previously when an isolate (M-685) protected gladiolus corms from *F. oxysporum* f. sp. *gladioli* (22). In that study, M-685 was called *F. moniliforme* 'subglutinans' (since 1983, *F. subglutinans* [27]), but M-685 recently has been identified as *F. proliferatum* (36). The nature of the biocontrol interaction between *F. proliferatum* and *F. oxysporum* f. sp. *gladioli* was not determined.

Field applications of *F. proliferatum* G6 reduced development of downy mildew on clusters and leaves of cultivar Chancellor and Lakemont grapevines during all years of our study. However, this reduction was substantial only during some years for some disease variables. The only clear pattern is that *F. proliferatum* G6 had a greater impact on reducing severity than on incidence of downy mildew.

Our control treatment in the field left vines untreated. We based this decision on the results of in vitro assays in which treatment of *P. viticola* lesions with water, Tween 20 (0.02%), or no treatment always developed substantially more and similar numbers of sporangia compared to treatment with *F. proliferatum* G6 microconidia (A. Sztejnberg, *unpublished data*). We are not aware of any studies showing an adverse effect of Tween 20 on downy mildew. Tween 20 has been shown to control cucurbit powdery mildew in vitro (*Sphaerothecia fuliginea*) (32) and reduce development of gray mold (*Botrytis cinerea*) in rose flowers at 10°C (5) but also has been shown not to inhibit spore germination nor mycelial growth of *Pyricularia oryzae* at 0.1% (18).

The amount of downy mildew that developed during our study varied from year to year. More downy mildew developed on cultivar Chancellor vines in 1992 than in any other year. This difference is most likely due to the more favorable conditions for infection by *P. viticola* that existed in 1992, because 263 mm of rain fell during July compared to the 30-year mean of 75 mm. This also was the only year in which substantial downy mildew developed on Chancellor leaves. Chancellor leaves are moderately resistant to downy mildew (10), but the high rainfall and more favorable conditions for infection in 1992 promoted higher disease levels on all cultivars. Downy mildew developed on Chancellor clusters in 1993 and 1994 even though it did not develop substantially on leaves during those years. Downy mildew development on cultivar Lakemont leaves also was impacted more by rainfall than mildew was on clusters. Very little mildew developed on leaves in 1993, when rainfall was below normal during June, average during July, and below normal during August. Foliar downy mildew occurred very late in the season in 1994 and 1995, increasing very rapidly during the last 3 weeks prior to the last disease assessment. In 1994 rainfall was slightly above average during June, below average during July, and above average during August, whereas in 1995 rainfall was much below average during June, above average during July, and below average during August.

Fumonisins are a class of carcinogenic mycotoxins produced by *F. proliferatum* and other related *Fusarium* species (26). Even though *F. proliferatum* G6 is a good producer of fumonisins when cultured on corn (P. E. Nelson, *personal communication*), it did not produce fumonisins in our study when applied to grape berries. It also appears that *F. proliferatum* occurs widely on grape berries, because it was possible to isolate different isolates of the fungus from fungicide-treated and untreated berries. No fumonisins occurred in these berries even though the *F. proliferatum* isolates were potent producers of fumonisins when cultured on autoclaved corn kernels (P. E. Nelson, *personal communication*). These isolates were different from G6 because they produced levels of fumonisins different from G6 and from each other.

F. proliferatum possibly could provide biological control of downy mildew in a vineyard where fungicides are applied to control other diseases. In agar tests, the least detrimental fungicides to *F. proliferatum* were sulfur, copper fungicides (especially $CuSO_4$), and metalaxyl. A management program that uses sulfur and copper fungicides might be compatible with the biocontrol agent. Similarly, management programs using metalaxyl also might be compatible with the biocontrol agent. Metalaxyl is a highly specific fungicide active against oomycetes and very effective for control of grape downy mildew. However, development of resistance to metalaxyl occurs readily and has been reported for *P. viticola* (20). Although metalaxyl alone is not registered for use on grapevines in the United States, metalaxyl/mancozeb and metalaxyl/copper formulations are. Use of the combination formulation aids in reducing development of resistance. *F. proliferatum* could have similar value in an antiresistance management program if used in combination with metalaxyl. At present, *F. proliferatum* might be of value in an antiresistance management program on other crops where metalaxyl is registered, because *F. proliferatum* has been shown in preliminary results to act against several other oomycetes (A. Sztejnberg, *unpublished data*).

We believe that *F. proliferatum* G6 is broadly antagonistic against oomycetes. Growth of *F. proliferatum* G6 on oomycete-infected lesions, coiling around sporangiophores, and reduced spread of lesions have been observed in detached leaves treated with microconidial suspensions of the antagonist for *Bremia lactucae* on *Arctotis* sp., *B. lactucae* on lettuce, *Peronospora farinosa* on *Chenopodium murale*, *Pseudoperonospora cubensis* on cucumber, and *Peronospora destructor* on onion, although coiling was not observed around *P. destructor* sporangiophores (A. Sztejnberg, *unpublished data*).

Additionally, *F. proliferatum* G6 has been observed by electron microscopy to coil around and penetrate sporangiophores of *Phytophthora infestans* and by light microscopy to coil around sporangiophores and sporangia of *Peronospora parasitica* on cabbage. Other isolates of *F. proliferatum* also are antagonistic. Three isolates were as effective as G6 in leaf-disk assays against *P. viticola* (A. Sztejnberg, *unpublished data*), and one of these isolates was as effective as G6 in a 1-year field trial (S. P. Falk, *unpublished data*). Antagonism has been found between only two other foliage diseases caused by oomycetes and different species of *Fusarium*. *F. equiseti*, an antagonist of *P. infestans*, inhibited development of the disease in vitro and when conidia or culture filtrates were applied to potato plants (17). Antagonism between *Sclerospora graminicola*, the cause of downy mildew of pearl millet, and *F. semitectum* involved penetration of oospores (30). This antagonism, which made the oospores nonviable, could be reestablished by spraying conidia onto detached diseased green ears of pearl millet and in the field. Whether *F. proliferatum* penetrates oospores of *P. viticola*, making them nonviable, is unknown.

F. proliferatum has been reported as a pathogen of asparagus (6,31), citrus (23), container-grown conifer seedlings (15), dracaena (37), maize stalks and ears (19,21), rice (4), and wheat seed (3). *F. proliferatum* can cause decay of banana fruit (16) and gladiolus corms (36) and has been examined for its potential as a

biocontrol agent of jointed cactus (24). In our studies, *F. proliferatum* G6 and other isolates were never observed in leaf-disk assays to spread to healthy leaf tissue from antagonist-treated lesions or from leaf tissue wounded with a hot needle (A. Sztejnberg, *unpublished data*). We also have not observed any pathogenicity of our isolates to grape leaves or berries when applied in the field or to detached leaves of *Arctotis* sp., lettuce, *Chenopodium murale*, cucumber, onion, potato, or cabbage sprayed with microconidial suspensions (A. Sztejnberg, *unpublished data*).

We were able to reduce the development of grape downy mildew in the field with weekly applications of *F. proliferatum* microconidia. Continued development would be needed to create a commercial formulation of *F. proliferatum* that could be used practically. This would likely be in conjunction with other control methods, such as in a management program with sulfur or copper, with metalaxyl as an antiresistance strategy, with moderately resistant cultivars, or in areas where downy mildew is not severe.

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