AQ10 Biofungicide Combined with Chemical Fungicides or AddQ Spray Adjuvant for Control of Cucurbit Powdery Mildew in Detached Leaf Culture

N. Shishkoff and M. T. McGrath, Department of Plant Pathology, Long Island Horticultural Research and Extension Center, Cornell University, 3059 Sound Avenue, Riverhead, NY 11901-1098

ABSTRACT

Shishkoff, N., and McGrath, M. T. 2002. AQ10 biofungicide combined with chemical fungicides or AddQ spray adjuvant for control of cucurbit powdery mildew in detached leaf culture. *Plant Dis.* 86:915-918.

The biofungicide AQ10, a pelleted formulation of conidia of *Ampelomyces quisqualis*, did not significantly reduce the size of colonies of the cucurbit powdery mildew (*Podosphaera xanthii*) in detached squash leaf culture but did reduce the amount of inoculum produced by each colony. No significant reduction in colonization of powdery mildew colonies by AQ10 was observed when it was sprayed in conjunction with the fungicides myclobutanil at 10 µg/ml or triadimefon at 100 µg/ml, suggesting that it is not sensitive to the fungicides at these concentrations. The spray adjuvant AddQ did not increase percent colonization by *A. quisqualis* but reduced the size of mildew colonies when used alone or with AQ10.

Additional keywords: biological control, *Sphaerotheca fuliginea*, *Sphaerotheca fusca*

Identifying biocontrol agents that can supplement conventional chemical fungicides is a valuable contribution to disease management, especially for a disease like powdery mildew of cucurbits, caused by *Podosphaera* (sect. *Sphaerotheca*) *xanthii* (Castagne) U. Braun & N. Shishkoff (previously known as *Sphaerotheca fusca* (Fr.) S. Blumer and *S. fuliginea* (Schlechtend.:Fr.) Pollacci). Fungicide applications are the principal management practice for this important disease (11). The biofungicide AQ10 (Ecogen Inc., Langhorne, PA) is a pelleted formulation of conidia of *Ampelomyces quisqualis* Ces. ex Schlechtend., a fungus that parasitizes powdery mildew colonies. AQ10 is intended for use as part of an integrated management program; therefore, information is needed on its compatibility with conventional chemical fungicides. In Norway, *A. quisqualis* was found to tolerate a number of fungicides. Good greenhouse cucumber yields were achieved when *A. quisqualis* was supplemented with one-third the normal rate of triforine (14,15). In Israel, *A. quisqualis* used alone significantly decreased powdery mildew severity and increased cucumber yields compared with unsprayed controls. When the hyperparasite was used in combination with pyrazophos at half the normal rate, yield was as good as using the

fungicide alone at full strength (16). In the United States, triadimefon has been used extensively to control powdery mildew. Myclobutanil, another ergosterol biosynthesis inhibiting fungicide, has been registered recently. It is imperative to use these fungicides with methods of control that have a different mode of action in order to manage resistance (10,13). Biological controls present one possibility.

A. quisqualis is now thought to be a polyphyletic species complex rather than a single species (5,7). It is found worldwide and infects nine genera of powdery mildews (6), but it does not usually control powdery mildew at natural concentrations. Therefore, it must be augmented by inundative sprays. AQ10 must be applied under climatic conditions allowing good germination of conidia of *A. quisqualis*, which means spraying when wetness will remain on leaves. The spray adjuvant AddQ (Ecogen) is reported to increase the hyperparasite's ability to colonize powdery mildew colonies by retarding the evaporation of water from the emulsion.

The objective of this study was to examine the hyperparasitic ability of AQ10 when used alone, combined with AddQ, or with the fungicides triadimefon and myclobutanil under controlled conditions. Quantification of powdery mildew colony size, degree of hyperparasitism, and conidium production was done with detached leaf culture within a controlled temperature and relative humidity.

MATERIALS AND METHODS

Isolates of *Podosphaera xanthii*. In experiments combining the application of AQ10 and fungicides, it was necessary to

use an isolate of *P. xanthii* with decreased sensitivity to the fungicides used. (If the host isolate was sensitive, the sensitivity of AQ10 to the fungicide could not be measured.) The isolate used (4at, collected in 1990 from an experimental field plot of pumpkin [*Cucurbita pepo* L.] in Riverhead, NY) was able to tolerate myclobutanil at 20 µg/ml and triadimefon at 50 µg/ml. Another isolate (LI3-S17-3, collected in 1994 from a experimental field plot of pumpkin in Riverhead, NY) that was able to tolerate myclobutanil at 2 µg/ml and triadimefon at 50 µg/ml was used for other experiments. Isolates were maintained on detached leaves or on cotyledon disks on water agar.

Detached leaf culture. Detached leaf culture allowed incubation of the pathogen and mycoparasite under controlled environmental conditions where quantifiable measurements of growth were made. It was done largely as described previously (9), using leaf chambers fashioned from pairs of 9-cm-diameter petri dishes. Leaves that had just reached the stage of full expansion were taken from 4- to 7-week-old yellow straightneck squash (*C. pepo* L. var. *melopepo* Alef.) 'Seneca Prolific' raised from seed in a controlled environment chamber set at 23 to 25°C with a 14-h photoperiod. After the leaf lamina had been trimmed to fit inside the upper portion of the leaf chamber (one petri dish), the petiole was inserted through a hole in the bottom of the chamber into soil mix contained in the second petri dish. Leaves in leaf chambers were then placed in an incubator set at 24°C with a 16-h photoperiod, where the detached leaves could survive for 3 to 4 weeks. In these studies, after the detached leaves had begun to root (3 to 4 days), the petri dish lid serving as the cover for the upper chamber containing the leaf lamina was replaced with an inverted clear plastic drinking cup to create a larger volume of air around the leaf (Fig. 1). Because the ability of hyperparasites to colonize powdery mildew colonies is strongly affected by relative humidity, such leaf chambers were used in order to provide a uniform relative humidity of approximately 80%, which was demonstrated in pilot studies by daily measurements taken over 2-week intervals using a humidity probe.

Treatments and materials tested. Treatments were applied to runoff using a DeVilbiss Model 152 atomizer (DeVilbiss Health Care, Inc., Somerset, PA) attached

Corresponding author: N. Shishkoff
E-mail: ns44@Cornell.edu

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to a compressed air source and operated at 138 kPa. Materials applied were AQ10 (*A. quisqualis*) and AddQ (an adjuvant, Eco-gen), myclobutanil (active ingredient, Rohm and Hass Company, Philadelphia, PA), triadimefon (technical grade Bayleton 50DF, Bayer Corporation, Kansas City, MO), and the inert ingredients used for formulation of Bayleton (as a control for the Bayleton treatment).

Inoculation with powdery mildew. Detached leaves were inoculated with powdery mildew by placing a small number of conidia (15 to 20, as visible in the dissecting microscope) of *Podosphaera xanthii* at six sites using a small camel's hair paintbrush. Inoculation was done before or after treatment, depending on the experiment. After inoculation, leaves in leaf chambers were incubated in growth chambers at 24°C with a 12-h photoperiod.

Measurement of percent infection by hyperparasitic and conidium production of the powdery mildew. Infected colonies of powdery mildew appear tan from the fruiting structures of *A. quisqualis*, and the morphological abnormalities of infected powdery mildew can be clearly seen at $\times 40$ magnification. Clearing and staining of infected colonies showed that *A. quisqualis* mycelium did not extend into symptomless areas (N. Shishkoff, unpublished). Colony diameter was measured with a centimeter rule approximately 2 weeks after inoculation when control colonies were >1 cm in diameter and infection by *A. quisqualis* was easily measurable. Colony diameter was measured along a radial axis from the leaf center through the center of the colony. Diameters of the six colonies were averaged for each leaf. Percent infection of the powdery mildew colony by AQ10 was measured approximately 2 weeks after

inoculation. This was done by estimating the proportion of "tan" area in each colony and averaging over the six colonies of powdery mildew per leaf. Spore density for each colony of powdery mildew was measured on a 1 to 4 scale to quantify the effect of the hyperparasite on conidium production. In this rating system, 1 was a colony with no sporulation at all, 2 showed sparse sporulation, 3 was moderately dense, and 4 showed dense sporulation. An uncolonized colony of powdery mildew typically rates 3 or 4 on this scale.

Experiments performed. The first experiment examined development of powdery mildew isolate 4at on squash leaves treated with AQ10 and myclobutanil. The detached leaves were sprayed with water alone, AQ10 alone (at 0.05 mg/ml formulated product), myclobutanil alone (at 10.8 μ g/ml active ingredient), or AQ10 followed by myclobutanil. After spraying, leaves were allowed to dry and then inoculated at six sites with the mildew. The diameter of colonies of the powdery mildew was measured when control colonies were >1 cm in diameter (approximately 2 weeks). Percent colonization by the hyperparasite was also measured. The experiment was done two times with four replicate leaf chambers per treatment.

Experiment two examined the development of powdery mildew on squash leaves treated with AQ10 and triadimefon. Detached leaves were sprayed with triadimefon alone (at 100 μ g/ml active ingredient), AQ10 alone (0.05 mg/ml), AQ10 immediately followed by triadimefon, or with a blank formulation for Bayleton (the inert ingredients at 100 μ g/ml). After spraying, leaves were allowed to dry and then inoculated at six sites with the mildew. The diameter of colonies of powdery mildew was measured when water-sprayed control colonies were >1 cm in diameter (approximately 2 weeks). Percent colonization by the hyperparasite was also measured. The experiment was performed three times

with four to five replicate leaf chambers per treatment.

Experiment three examined the development of powdery mildew on squash leaves treated with AQ10 and AddQ. Because AddQ is thought to aid germination of spores by retarding evaporation of water, the procedure of the previous two experiments (in which leaves were sprayed, allowed to dry, and then inoculated with the mildew) could not be followed. Instead, leaves were inoculated at six sites with the fungus 1 day prior to treatment. Inoculated leaves were sprayed with AQ10 alone (at 0.05 mg/ml), with AddQ alone (at 0.3%), with AQ10 + AddQ, or with water. Leaf chambers were closed immediately after spraying in order that evaporation of water from the treated leaves occurred under as uniform conditions as possible. Percent colonization by the hyperparasite and inoculum quality were measured for each colony. The experiment was run twice with four to five replicate leaf chambers per treatment.

Statistical analysis. For each leaf, an average diameter of colonies of powdery mildew, average percent colonization by the hyperparasite, and (in experiments testing AddQ) average conidium production were determined. Data were analyzed using the analysis of variance (ANOVA) procedure of Minitab 8.21. Experiments were run as one-way ANOVAs in order to compare treatment effects on diameter and conidium production using Tukey's multiple comparison. Experiments were also analyzed as two-way ANOVAs (treatment a = fungicide application, treatment b = biocontrol application) in order to look for interactions caused by dual application of pesticide and biocontrol. Data were tested for random and normal distributions using plots of residuals versus predicted values and the "correlate" and "nscores" procedures. Data were log-transformed if necessary, using a square function. Analysis of variance could not be used to analyze per-

Fig. 1. Chamber for detached leaf culture consisting of two petri dishes, the lid of the top dish replaced with a clear plastic drinking glass. The trimmed leaf is placed in the top dish with the petiole pushed through hole A into soil mix in the bottom dish. Hole B allows the soil mix to be moistened.

Table 1. Characteristics of powdery mildew colonies developing on leaves sprayed with AQ10, the fungicide myclobutanil, or both immediately before inoculation

Treatment	Mildew colony diameter (cm) ^x		% Area of mildew colonized ^y	
	Trial 1	Trial 2	Trial 1	Trial 2
Control	1.82 a	1.11 a	0	0
AQ10	1.61 a	1.21 a	71.3 a	35.0 a
Myclobutanil	0.60 b	0.54 b	0	0
AQ10 + myclobutanil	0.44 b	0.59 b	47.5 a	8.8 a
<i>P</i> value for interaction of AQ10 and fungicide ^z	0.88	0.75	...	...
<i>P</i> value ^z	<0.0001	<0.0001	0.51	0.30

^x Colony diameter was analyzed by one-way analysis of variance; treatments followed by the same letter did not differ significantly by Tukey's test.

^y Percent colonization of powdery mildew colonies by the AQ10 biocontrol agent (*Ampelomyces quisqualis*) was compared in plants inoculated with AQ10 alone and with AQ10 plus myclobutanil using a *t* test (percent infection of colony by the biocontrol agent). Treatments followed by the same letter did not differ significantly.

^z Colony diameter was also analyzed by two-way analysis of variance for fungicide and AQ10 in order to look for an interaction of the variables. In each case, the *P* value for the model was significant at $P < 0.01$. *P* values for the interaction of variables that were >0.05 indicate the interaction was not significant.

cent colonization of powdery mildew colonies by the biocontrol agent (uninoculated treatments contributed too many “0” values); so colonization was analyzed by paired *t* tests.

RESULTS

Experiment one: growth of colonies of powdery mildew treated with AQ10 and myclobutanil. In experiments quantifying powdery mildew development on squash leaves sprayed with AQ10, myclobutanil, or both, colonies sprayed with myclobutanil were significantly smaller than those not sprayed with fungicide. AQ10 had no effect on colony size (Table 1). No interaction between treatments (fungal or chemical) was seen in either trial. Infection of powdery mildew colonies by *A. quisqualis* was successful in both treatments where AQ10 was used; however, the incidence of infection was variable, with no significant difference in percent colonization between colonies sprayed with AQ10 alone and in colonies sprayed with fungicide plus AQ10.

Experiment two: growth of colonies treated with AQ10 and triadimefon. In experiments quantifying powdery mildew development on squash leaves sprayed with AQ10, triadimefon, or both, AQ10 alone had no significant effect on colony diameter (Table 2). Triadimefon alone significantly reduced colony diameter in two of the three trials. The reduction in diameter caused by dual treatment with triadimefon and AQ10 varied; in one trial, no significant reduction was seen, in one it caused the greatest reduction, and in one it produced an intermediate reduction. The percent colonization of the powdery mildew fungus by *A. quisqualis* was not significantly reduced by triadimefon.

Experiment three: growth of colonies treated with AQ10 and AddQ. In experiments quantifying powdery mildew development on squash leaves sprayed with AQ10, AddQ, or both, AddQ alone significantly reduced colony diameter and AQ10 alone did not (Table 3). AQ10 and AddQ together reduced colony diameter as much as AddQ alone in one experiment but not as well as AddQ alone in the other. Colonization of the powdery mildew fungus by *A. quisqualis* was not significantly affected by AddQ under the conditions of the experiments. Inoculum quality of the mildew colony was highest in controls and was significantly reduced by the presence of the biocontrol agent.

DISCUSSION

Infection by *A. quisqualis* did not significantly reduce the size of colonies of powdery mildew in detached leaf culture but was observed to reduce the amount of inoculum produced by each colony. The inoculum that was produced appeared uninfected by the hyperparasite and if used as inoculum, gave rise to healthy colonies

(unpublished data). The percent infection of colonies of powdery mildew by the hyperparasite varied widely, perhaps due to small differences in humidity levels. The reduction in inoculum of powdery mildew also varied; some colonies had practically no viable conidia, while in others sporulation was only slightly inhibited by the hyperparasite. Based on these results, at best only moderate control of cucurbit powdery mildew would be predicted to occur under field conditions. Thus it was not surprising that AQ10 was not as effective as conventional chemical fungicides or other biocompatible fungicides (12).

AQ10 was compatible with conventional chemical fungicides. No significant reduction in colonization of colonies of powdery mildew was observed when AQ10 was

sprayed in conjunction with the fungicides myclobutanil at 10 µg/ml or triadimefon at 100 µg/ml. Therefore, AQ10 could be used as part of an integrated management program. Hofstein et al. (3) state that in order to achieve cost-effective, consistent disease control, AQ10 has to be applied before the incidence of powdery mildew had reached high levels.

AddQ did not increase percent colonization by *A. quisqualis* in our experiments. However, when Marchetti and D'Aulerio (8) tested Ultra Fine Oil (refined mineral oil) and Ultra Fine Oil + AQ10 on powdery mildew-infected crape myrtle, good control was achieved with AQ10 + Ultra Fine Oil. Ultra Fine Oil on its own was least effective. In greenhouse experiments, Elad et al. (1) evaluated the ability of AQ10 to

Table 2. Characteristics of powdery mildew colonies developing on leaves sprayed with Bayleton Blank (inert formulation for triadimefon, used as the control treatment), AQ10, the fungicide triadimefon, or both AQ10 and triadimefon immediately before inoculation

Treatment	Mildew colony diameter (cm) ^x			% Area of mildew colonized ^y		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Control	1.45 ab	1.61 a	1.54 a	0	0	0
AQ10	1.54 ab	1.45 ab	1.53 a	12.5 a	53.0 a	52.3 a
Triadimefon	0.97 a	0.66 c	1.14 b	0	0	0
AQ10 + triadimefon	0.34 c	1.14 b	1.26 ab	14.0 a	46.9 a	14.4 a
<i>P</i> value for interaction of AQ10 and fungicide ^z	0.01	0.006	0.95	...	...	...
<i>P</i> value	<0.0001	<0.0001	<0.003	0.91	0.80	0.06

^x Colony diameter was analyzed by one-way analysis of variance; treatments followed by the same letter did not differ significantly by Tukey's test.

^y Percent colonization of powdery mildew colonies by the AQ10 biocontrol agent was compared in plants inoculated with AQ10 alone and with AQ10 plus myclobutanil using a *t* test (percent infection of colony by the biocontrol agent). Treatments followed by the same letter did not differ significantly.

^z Colony diameter was also analyzed by two-way analysis of variance for fungicide and AQ10 in order to look for an interaction of the variables. In each case, the *P* value for the model was significant at *P* < 0.01. *P* values for the interaction of variables that were >0.05 indicate the interaction was not significant.

Table 3. Characteristics of powdery mildew colonies developing on leaves sprayed with AQ10, the spray adjuvant AddQ, or both AQ10 and AddQ 1 day after inoculation. Controls were sprayed with water

Treatment	Mildew colony diameter (cm) ^w		% Area of mildew colonized ^x		Conidium production ^y	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Control	1.13 a	1.27 a	0	0	3.20 a	3.76 a
AddQ alone	0.71 b	0.48 b	0	0	2.65 ab	3.36 a
AQ10+water	1.18 a	1.19 ac	96.0 a	79.5 a	1.87 c	2.44 b
AQ10+AddQ	0.75 b	1.00 c	96.6 a	73.0 a	2.00 bc	2.42 b
<i>P</i> value for interaction of AQ10 and fungicide ^z	0.27	0.001	...	...	...	...
<i>P</i> value	<0.0001	<0.0001	0.77	0.57	<0.0001	<0.0001

^w Colony diameter was analyzed by one-way analysis of variance; treatments followed by the same letter did not differ significantly by Tukey's test.

^x Percent colonization of powdery mildew colonies by the AQ10 biocontrol agent was compared in plants inoculated with AQ10 alone and with AQ10 plus myclobutanil using a *t* test (percent infection of colony by the biocontrol agent). Treatments followed by the same letter did not differ significantly.

^y Conidium production was rated on a 1 to 4 scale, where 1 was a colony with no sporulation at all, 2 showed sparse sporulation, 3 was moderately dense, and 4 showed dense sporulation. Paired *t* tests showed a significant reduction in conidium production due to treatment with AQ10, but not from treatment with AddQ.

^z Colony diameter was also analyzed by two-way analysis of variance for fungicide and AQ10 in order to look for an interaction of the variables. In each case, the *P* value for the model was significant at *P* < 0.01. *P* values for the interaction of variables that were >0.05 indicate the interaction was not significant.

control *P. xanthii* on greenhouse cucumbers and found AddQ improved efficacy. In our experiments, the leaf chambers were designed to provide optimum conditions for *A. quisqualis*, and under less optimum conditions, the AddQ might have increased germination. On the other hand, powdery mildews of cucurbits and grape under field conditions were controlled equally effectively with AddQ alone and with AQ10 plus AddQ (12,17). This suggests that any increase in effectiveness was due to AddQ directly.

In most greenhouse experiments where *A. quisqualis* significantly controlled powdery mildew, relative humidity appeared to be high due to manipulation of greenhouse environment (4,15,16). However, raising the humidity in commercial greenhouses could encourage other pathogens such as *Botrytis*. In field situations, manipulation of humidity is impossible. When pycnidia of *A. quisqualis* were suspended above grapevines, conidia were dispersed during rain, and in years with high rainfall the wet conditions were conducive to parasitism (2).

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