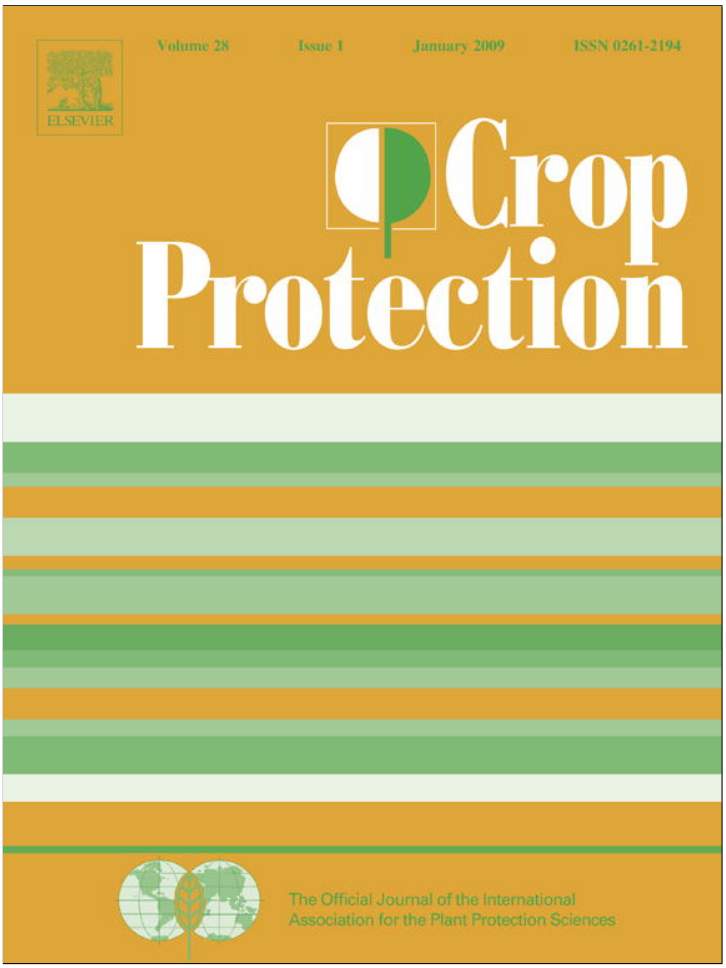

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Efficacy of fungicides and a systemic acquired resistance activator (acibenzolar-*S*-methyl) against tobacco blue mould

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ABSTRACT

Tobacco blue mould, caused by *Peronospora tabacina* Adam (*Peronospora hyoscyami* f. sp. *tabacina* Skalicky 1964) can be an economically devastating leaf spot disease in shade and broadleaf cigar wrapper tobacco (*Nicotiana tabacum* L.) types grown in Connecticut and Massachusetts. We investigated the effects of dimethomorph plus mancozeb and azoxystrobin fungicides as well as acibenzolar-*S*-methyl, a systemic acquired resistance inducer, on disease severity over 2 years in both shade-grown and broadleaf tobaccos. All fungicide and fungicide plus acibenzolar-*S*-methyl treatments applied were effective in reducing the number of blue mould lesions per plant. Treatments containing acibenzolar-*S*-methyl were the most effective, resulting in almost complete control. Substituting two or three applications of acibenzolar-*S*-methyl at label rates for dimethomorph plus mancozeb treatments in a spray program increased blue mould control over the same number of dimethomorph plus mancozeb applications by 28–94 percent. The effects of acibenzolar-*S*-methyl application on cured leaf quality were determined in commercial shade tobacco fields in 2000 and 2001. Leaves were cured, processed and commercially evaluated for quality in a blind test. Standard fungicide applications of dimethomorph plus mancozeb applied on a 14-d interval were compared to three acibenzolar-*S*-methyl treatments. Economic value was not different between treatments in 2000, but acibenzolar-*S*-methyl applied at 10-d intervals was associated with reduced value in 2001 when plants were more subject to drought and heat stress.

1. Introduction

Tobacco blue mould, caused by *Peronospora tabacina* Adam (*Peronospora hyoscyami* f. sp. *tabacina* Skalicky 1964) is a periodically re-introduced pathogen in the Connecticut River Valley tobacco growing area (LaMondia and Aylor, 2001). The disease can be economically devastating to the cigar wrapper tobacco (*Nicotiana tabacum* L.) types grown in this region as they need to be completely free of blemishes of any kind to be marketed as natural leaf wrapper. Severe blue mould occurred in production fields in 1979 and 1980 (Aylor et al., 1982) and was re-introduced again in 1997. The pathogen has been present in Connecticut and Massachusetts and caused some level of disease each year from 1997 until present.

The lack of disease from 1981 until 1997 was associated with widespread use of the highly effective systemic fungicide metalaxyl. Reports of metalaxyl-resistant strains of the pathogen were made in Nicaragua in 1981 and 1982, in Mexico in 1984 (Wiglesworth et al., 1988) and in the United States in 1991. The widespread presence of metalaxyl-resistant *P. tabacina* has made management of tobacco blue mould difficult. This is especially true in cigar wrapper tobaccos where the tolerance to damage is very low, it is difficult to achieve good coverage of the crop canopy (LaMondia and Horvath, 2001) and environmental conditions are often very conducive for disease (Waggoner et al., 1959). Currently available fungicides such as mancozeb, dimethomorph and azoxystrobin result in acceptable control, but their protectant or limited systemic activity requires excellent coverage to minimize losses (LaMondia and Horvath, 2001).

The objective of this research was to determine the efficacy of fungicides and a systemic acquired resistance initiator for management of tobacco blue mould in Connecticut shade and broadleaf cigar wrapper tobacco types.

2. Materials and methods

Experiments were conducted in a cloth-covered shade tobacco tent (shade-grown tobacco cultivar 8212) or in field plots (broadleaf tobacco cultivar C9) in 2000 and 2001 at the Connecticut

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Agricultural Experiment Station Valley Laboratory Research Farm in Windsor, CT. Materials tested for efficacy against blue mould were: dimethomorph plus mancozeb [Acrobat MZ, BASF Corp., Research Triangle Park, NC]; azoxystrobin [Quadris SC, Syngenta Crop Protection, Inc., Greensboro, NC]; and acibenzolar-*S*-methyl [Actigard 50 WG, Syngenta Crop Protection, Inc., Greensboro, NC].

Shade-grown tobacco plots 5 by 5 m consisting of four planted rows were fertilized annually with cottonseed meal based 10-8-10 (approximately 168 kg ha^{-1}) before planting in early June. Mefenoxam [Ridomil Gold EC at 1.2 l ha^{-1}] and chlorpyriphos [Lorsban 4E at 7 l ha^{-1}] were lightly harrowed in at the same time prior to planting to control root rot and cutworms, respectively. Plots were planted with blue mould susceptible shade tobacco cultivar 8212 transplants in early June in four rows (15 plants per row 30 cm apart within rows with 39 cm between rows). Plots were side-dressed with approximately 168 kg ha^{-1} 10-8-10 and cultivated in mid-June for a total of 336 kg N ha^{-1}. Pendimethalin [Prowl 3.3E at 2.9 l ha^{-1}] was applied as a lay-by directed spray using a 8004E nozzle at 175 kPa in late June to control weeds. Plants were hand suckered and tied at the end of June and wrapped in early and mid July. Fungicide sprays were applied to the two inside plot rows using hand pump backpack sprayers (Solo, Newport News, VA) at 175–200 kPa in 225 l ha^{-1} (first spray date), 450 l ha^{-1} (second spray) and 900 l ha^{-1} (sprays three through six) to achieve coverage of spray rows. The two outside rows were unsprayed borders in each plot. Treatments were arranged in a randomized block design.

Field-grown broadleaf tobacco plots were fertilized and treated as above. Thirty-six (2000) and twenty-four (2001) two-row plots were planted in mid-June with blue mould-susceptible cultivar C9 broadleaf tobacco transplants in two rows (10 plants per row) per plot 1 m apart with plants 0.6 m apart within rows. Fungicide sprays were applied to extinction to the two inside plot rows using Solo backpack sprayers at 175–200 kPa in 225 l ha^{-1} (first spray date), 450 l ha^{-1} (second spray) and 900 l ha^{-1} (sprays three through six) to achieve coverage of spray rows. The two outside rows were unsprayed borders in each plot. All plots were bordered by a row of unsprayed tobacco. There were four replicate plots of each spray treatment for all experiments. Data were analyzed by the nonparametric Kruskal–Wallis One-Way Analysis of Variance on ranks and means were separated by the Kruskal–Wallis Multiple Comparison *Z*-test ($P \leq 0.05$).

2.1. 2000 Field experiment – shade tobacco

Dimethomorph plus mancozeb [Acrobat MZ] was applied to appropriate plots at the rate of 252 g a.i. ha^{-1} dimethomorph and 1.78 kg a.i. ha^{-1} mancozeb in 900 l ha^{-1}. Azoxystrobin was applied at a rate of 149 g a.i. ha^{-1} and was alternated with dimethomorph plus mancozeb. Acibenzolar-*S*-methyl was applied two or three times following dimethomorph plus mancozeb or azoxystrobin at 10 to 14-d intervals at rates ranging from 8.8 to 26.3 g a.i. ha^{-1} on the 2nd, 3rd, 4th, 5th or 6th spray dates.

Transplants were set into field plots on 5 June 2000 and treatments were applied on 7 July, 18 and 21 July, and 4, 8 and 18 August. The numbers of lesions per plant were counted on 21 and 31 August. Blue mould was first observed on the research farm on 7 August 2000. The numbers of blue mould lesions were counted on all leaves of all plants in the two spray rows. Leaves were not harvested or removed from the plants. Border row plants were not picked and severe disease levels typically occurred in borders of all plots.

2.2. 2000 Field experiment – broadleaf tobacco

Azoxystrobin was applied at 149 g a.i. ha^{-1} alone or alternated with dimethomorph (252 g a.i. ha^{-1}) plus mancozeb (1.7 kg a.i. ha^{-1}) at 14-d intervals. Acibenzolar-*S*-methyl was applied two to three times after azoxystrobin or dimethomorph plus mancozeb at rates of 8.8–26.3 g a.i. ha^{-1} at 10 or 14-d intervals. Sprays were applied on 7, 18, 21, and 28 July, and 4 and 8 August. Plants were topped on 10 August and were rated for phytotoxicity on 11 August on a scale of 0–3 where 0 = no leaf symptoms; 1 = few symptoms (less than 10 blemishes per leaf); 2 = moderate damage (11 to 100 blemishes per leaf) and 3 = severe damage (>100 blemishes per leaf). Blue mould lesions were counted at harvest on 30 August 2000 after stalk cutting plants.

2.3. 2001 Field experiment – shade tobacco

Dimethomorph plus mancozeb, dimethomorph alone (50%WP applied at 0.125 kg a.i. ha^{-1} – equivalent to the dimethomorph component of Acrobat MZ) and azoxystrobin alone were applied to appropriate plots on five occasions at 14-d intervals. Acibenzolar-*S*-methyl was applied two or three times following two applications of dimethomorph plus mancozeb or azoxystrobin at 14-d intervals at rates ranging from 8.8 to 17.5 g a.i. product on the 3rd, 4th, or 5th spray dates.

Transplants were set into field plots on 12 June 2001 and treatments were applied on 21 June, 6 and 20 July, and 3 and 16 August. The numbers of lesions per plant were counted on 4 and 17 September. Blue mould was first observed on the research farm on 23 August 2001. The numbers of blue mould lesions were counted on all leaves of all plants in the two spray rows. Leaves were not harvested or removed from the plants. Border row plants were not picked and severe disease occurred in borders of all plots.

2.4. 2001 Field experiment – broadleaf tobacco

Spray treatments were applied to the broadleaf plots at 14-d intervals on 21 June, 6 and 20 July, and 3 August. Dimethomorph (252 g a.i. ha^{-1}) plus mancozeb (1.7 kg a.i. ha^{-1}) was applied alone or acibenzolar-*S*-methyl was applied two to three times at rates of 8.8–26.3 g a.i. ha^{-1} at 14-d intervals after one application of dimethomorph plus mancozeb. Plants were topped on 13 August and blue mould lesions were counted at harvest on 10 September 2001 after stalk cutting plants.

2.5. 2000 and 2001 Commercial shade tobacco quality evaluations

The effects of acibenzolar-*S*-methyl application on cured leaf quality were determined in commercial shade tobacco fields in 2000 and 2001. Standard fungicide applications of dimethomorph plus mancozeb applied on a 14-d interval were compared to three acibenzolar-*S*-methyl treatments. All treatments were applied to 100 m^2 plots. In 2000, treatments started 6 weeks after transplanting and consisted of (1) two applications of 17.5 g a.i. ha^{-1} acibenzolar-*S*-methyl 10 d apart; (2) five applications of 17.5 g a.i. ha^{-1} acibenzolar-*S*-methyl at 14-d intervals; and (3) two applications of 26.3 g a.i. ha^{-1} acibenzolar-*S*-methyl 10 d apart. In 2001, treatments started 7 weeks after transplanting and consisted of (1) two applications of 8.8 g a.i. ha^{-1} acibenzolar-*S*-methyl 10 d apart; (2) four applications of 8.8 g a.i. ha^{-1} acibenzolar-*S*-methyl at 14-d intervals; and (3) two applications of 17.5 g a.i. ha^{-1} acibenzolar-*S*-methyl 10 d apart. Three leaves per week were harvested for 5 weeks in 2000 and 6 weeks in 2001, cured, processed and commercially evaluated for quality in a blind test. Weights were recorded and leaf value was determined by economic grade and expressed as a percent of the maximum grade and value possible. Data were analyzed by ANOVA and the Fishers LSD test ($P \leq 0.05$).

3. Results

3.1. 2000 Field experiment – shade tobacco

All treatments applied were effective in reducing blue mould symptoms compared to the non-treated control plots (Table 1). The most efficacious treatments were the acibenzolar-*S*-methyl applications with higher rates (26.3 g a.i. ha^{-1}) or the 17.5 g a.i. ha^{-1} sprays applied three times over the season. The combination of dimethomorph plus mancozeb or azoxystrobin followed by two acibenzolar-*S*-methyl sprays (17.5 g a.i. ha^{-1}) at 14-d intervals was also quite effective, resulting in better than 90% control compared to the non-treated plots. The application of three acibenzolar-*S*-methyl sprays after a single dimethomorph plus mancozeb application decreased blue mould lesions compared to four dimethomorph plus mancozeb applications by 91%. Dimethomorph plus mancozeb followed by two applications of acibenzolar-*S*-methyl 14 d apart was more effective than the same sprays applied at 10-d intervals.

3.2. 2000 Field experiment – broadleaf tobacco

All treatments applied were effective in reducing the number of blue mould lesions on harvested leaves and increasing marketable leaves harvested over the season compared to the non-treated control plots (Table 2). Treatments containing acibenzolar-*S*-methyl were the most effective. The application of three acibenzolar-*S*-methyl sprays after a single dimethomorph plus mancozeb application decreased blue mould lesions compared to four azoxystrobin or dimethomorph plus mancozeb applications by 80%. However, phytotoxicity as measured by the amount of flecking (small leaf blemishes) was associated with acibenzolar-*S*-methyl and azoxystrobin applications. Plants treated with acibenzolar-*S*-methyl exhibited small pinpoint brown fleck symptoms on leaves; while azoxystrobin treated plants had larger white fleck symptoms.

3.3. 2001 Field experiment – shade tobacco

All fungicide and fungicide plus acibenzolar-*S*-methyl treatments applied were effective in reducing the number of blue mould lesions per plant (Table 3). Acibenzolar-*S*-methyl after azoxystrobin treatments was more effective than acibenzolar-*S*-methyl after dimethomorph plus mancozeb. This may be due to the fact that five applications of azoxystrobin alone were more effective than five applications of dimethomorph plus mancozeb. Dimethomorph alone was as effective as dimethomorph plus mancozeb, indicating that activity against blue mould was primarily due to the dimethomorph component rather than the mancozeb component. The application of three acibenzolar-*S*-methyl sprays (17.5 g a.i. ha^{-1}) after two dimethomorph plus mancozeb applications decreased blue mould lesions by 28% compared to five dimethomorph plus mancozeb treatments and the application of three acibenzolar-*S*-methyl sprays (17.5 g a.i. ha^{-1}) after two azoxystrobin applications decreased blue mould lesions by 52% compared to five azoxystrobin applications.

Table 1
Effects of fungicide and acibenzolar-*S*-methyl (ASM) applications on blue mould development in shade tobacco (2000).

Treatments[a] (ASM g a.i. ha^{-1})	Blue mould development (lesions per plant)	
	21 Aug	31 Aug
Non-treated control	175 a[b]	1328 a
(D + M) 4× at 14 d	13 b	196 c
AZ/D + M/AZ/D + M at 14 d	5 b	269 bc
AZ/ASM/AZ/ASM (17.5 g a.i. ha^{-1}) at 14 d	1 c	61 d
D + M 1×/ASM 2× (17.5 and 8.8 g a.i. ha^{-1}) at 14 d	9 b	116 c
D + M 1×/ASM 3× (26.3 g a.i. ha^{-1}) at 10 d	0 c	9 e
D + M 1×/ASM 2× (17.5 g a.i. ha^{-1}) at 10 d	9 b	293 b
D + M 1×/ASM 2× (17.5 g a.i. ha^{-1}) at 14 d	5 c	87 cd
D + M 1×/ASM 3× (17.5 g a.i. ha^{-1}) at 10 d	1 c	17 e
$P \leq$	0.0001	0.0001

[a] Applications of fungicide (consisted of dimethomorph plus mancozeb (D + M) at 252 g a.i. ha^{-1} dimethomorph and 1.78 kg a.i. ha^{-1} mancozeb alone or alternated with azoxystrobin (AZ) at 149 g a.i. ha^{-1}) and/or acibenzolar-*S*-methyl (ASM) at the listed rates of 17.5–26.3 g a.i. ha^{-1} at 10 or 14-d intervals. The first spray was applied on 7 July 2000.

[b] Numbers within a column followed by a common letter are not significantly different according to the Kruskal–Wallis Multiple Comparison *Z*-test ($P \leq 0.05$).

Table 2
Effects of fungicide and acibenzolar-*S*-methyl (ASM) applications on blue mould development and phytotoxicity in broadleaf tobacco (2000).

Treatments[b] (ASM g a.i. ha^{-1})	Blue mould development (lesions per plant)	Phytotoxicity rating[a]
	30 Aug	11 Aug
Non-treated control	74.7 a[c]	0.3 c
AZ/D + M/AZ/D + M at 14 d	0.5 bc	1.7 c
AZ 4× at 14 d	0.6 bc	2.2 b
AZ/ASM (17.5 g a.i. ha^{-1})/AZ/ASM at 14 d	0.3 bc	1.8 bc
D + M 1×/ASM (17.5 g a.i. ha^{-1}) 2× at 10 d	0.7 b	2.0 bc
D + M 1×/ASM (17.5 g a.i. ha^{-1}) 3× at 10 d	0.1 c	2.0 bc
D + M 1×/ASM (26.3 g a.i. ha^{-1}) 2× at 10 d	0.1 c	2.7 a
D + M 1×/ASM (17.5 g a.i. ha^{-1}) 2× at 14 d	0.3 bc	1.8 bc
D + M 1×/ASM (1× 17.5; 1× at 8.8 g a.i. ha^{-1}) at 14 d	0.1 c	1.8 bc
$P \leq$	0.0001	0.0001

[a] Phytotoxicity rating on a scale of 0–3 where 0 = no leaf symptoms; 1 = few symptoms (less than 10 blemishes per leaf); 2 = moderate damage (11–100 blemishes per leaf) and 3 = severe damage (>100 blemishes per leaf).

[b] Six applications of fungicide (consisted of dimethomorph plus mancozeb (D + M) at 252 g a.i. ha^{-1} dimethomorph and 1.78 kg a.i. ha^{-1} mancozeb alternated with azoxystrobin (AZ) at 149 g a.i. ha^{-1}) and/or acibenzolar-*S*-methyl (ASM) at the listed rates of 8.8–26.3 g a.i. ha^{-1} at 10 or 14-d intervals. The first spray was applied on 7 July 2000.

[c] Numbers within a column followed by a common letter are not significantly different according to the Kruskal–Wallis Multiple Comparison *Z*-test ($P \leq 0.05$).

Table 3
Effects of fungicide and acibenzolar-*S*-methyl (ASM) applications on blue mould development in shade tobacco (2001).

Treatments[a] (ASM g a.i. ha^{-1})	Blue mould development (lesions per plant)	
	4 Sep	17 Sep
Non-treated control	56.0 a[b]	1300 a
D + M (5×)	9.6 b	181 b
AZ (5×)	1.4 b	85 cd
D (5×)	1.2 b	172 bc
D + M (2×)/ASM (2× at 17.5 g a.i. ha^{-1})	0.5 b	206 b
D + M (2×)/ASM (3× at 17.5 g a.i. ha^{-1})	0.5 b	130 bc
D + M (2×)/ASM (3× at 8.8 g a.i. ha^{-1})	0.4 b	159 bc
AZ (2×)/ASM (2× at 17.5 g a.i. ha^{-1})	0.8 b	138 bc
AZ (2×)/ASM (3× at 17.5 g a.i. ha^{-1})	0.1 b	41 d
AZ (2×)/ASM (3× at 8.8 g a.i. ha^{-1})	0.2 b	92 cd
$P \leq$	0.0001	0.0001

[a] Fungicide applied at 14-d intervals (dimethomorph alone or dimethomorph plus mancozeb (D + M) at 252 g a.i. ha^{-1} dimethomorph and 1.78 kg a.i. ha^{-1} mancozeb; azoxystrobin (AZ) at 149 g a.i. ha^{-1}) and acibenzolar-*S*-methyl (ASM) at the listed rates of 8.8–17.5 g a.i. ha^{-1} at 14-d intervals. The first spray was applied on 21 June 2001.

[b] Numbers within a column followed by a common letter are not significantly different according to the Kruskal–Wallis Multiple Comparison *Z*-test ($P \leq 0.05$).

3.4. 2001 Field experiment – broadleaf tobacco

All treatments applied were effective in reducing the number of blue mould lesions on broadleaf tobacco compared to the non-treated control plots (Table 4). Treatments containing acibenzolar-*S*-methyl were the most effective. The application of three acibenzolar-*S*-methyl sprays (17.5 g a.i. ha^{-1}) after one dimethomorph plus mancozeb application decreased blue mould lesions by 94% compared to four dimethomorph plus mancozeb applications.

3.5. 2000 and 2001 Commercial shade tobacco quality evaluations

There were no significant differences in cured leaf weight between treatments in either year (Table 5). Economic value was not different between treatments in 2000, but acibenzolar-*S*-methyl applied at 10-d intervals was associated with reduced value in 2001. Leaves ripened sooner in the field and cured leaf colour was uneven with green along the veins. Plants were more subject to drought and heat stress in 2001 than in 2000. Temperatures were above 32 °C on 1 d each in July and August 2000 and rainfall was 58 mm above average and 8 mm below average in July and August 2000, respectively. Temperatures exceeded 32 °C on 4 d in July and 8 d in August 2001 and rainfall was 56 mm below and 2 mm above average in July and August 2001, respectively.

4. Discussion

Blue mould can be economically devastating to Connecticut cigar wrapper tobaccos where the tolerance to damage is very low. A single lesion per leaf can reduce marketable values by 50%. Currently, there are no commercially acceptable cultivars with resistance to *P. tabacina* available and the climate and culture of shade tobacco are very conducive for disease. Connecticut shade tobacco is a long-season crop (about 100 d) grown at high plant densities of over 27,000 plants ha^{-1} each at a height of 3 m under cloth in a shade-covered tent with each plant individually tied to a wire running over each row. This makes spray coverage extremely difficult and expensive (LaMondia et al., 2006). Broadleaf tobacco is a shorter-season crop (about 70 d to harvest) field-grown at lower densities of about 16,000 plants ha^{-1}.

Our results demonstrate that currently labelled fungicides are effective against tobacco blue mould, especially in broadleaf tobacco, but spray coverage and timing are important (LaMondia and Horvath, 2001). Dimethomorph (Acrobat now sold as Forum) and azoxystrobin (Quadris) are locally translaminar systemic fungicides. Acibenzolar-*S*-methyl (Actigard) is the first synthetic systemic acquired resistance (SAR) chemical developed and labelled for use in the U.S. (Walters et al., 2005). SAR is a systemic defence response caused by the activation of plant genes by biological factors, salicylic acid or pathogenesis-related proteins (Kessmann et al., 1996; Mandel et al., 2008). The current label use rate for acibenzolar-*S*-methyl for blue mould management in tobacco is 17.5 g a.i. ha^{-1}. The results of the present study were achieved under severe inoculum pressure as adjacent unsprayed border rows had plants with more than 1300 lesions per plant on 20–22 leaves. Under these conditions, the application of Acrobat MZ, azoxystrobin or alternate applications of both fungicides resulted in 80–90% control of blue mould in shade tobacco. The addition of acibenzolar-*S*-methyl to a spray program resulted in nearly complete control.

Dimethomorph and azoxystrobin have activity against a number of oomycete pathogens and are widely used against late blight of potato, downy mildew of cucurbits (Cohen et al., 1995) and *Phytophthora* spp. on many crops (Matheron and Porchas, 2000). Both fungicides have specific modes of action and are at risk of fungicide resistance in plant pathogens (Ishii et al., 2001; Stein and Kirk, 2004). As a result, mancozeb has been used as a mixing partner for dimethomorph, and azoxystrobin use requires a resistance management strategy such as tank mixing or alternating with fungicides that have different modes of action.

Results of these experiments were similar to commercial production fields in that blue mould incidence and severity is typically much greater for shade tobacco than broadleaf tobacco types due no doubt to the environment of the shade tent and the length of time that the crop is in the field. In 2000, we observed that acibenzolar-*S*-methyl applications at the same rates were more efficacious in shade when applied at 14-d intervals rather than 10-d intervals, but there were no such differences for broadleaf tobacco, likely due to the factors mentioned above.

In contrast to our results, acibenzolar-*S*-methyl at rates of 17.5 g a.i. ha^{-1} or lower did not result in blue mould management sufficient for cigar wrapper quality in Cuba (Perez et al., 2003). This may be due to the fact that the blue mould pathogen was reported to be present in the Cuban experimental fields at or prior to application of the first acibenzolar-*S*-methyl treatment 7 d after transplanting. In our experiments, the first application of acibenzolar-*S*-methyl was applied prior to the first observation of disease on the research farm. Acibenzolar-*S*-methyl, as an SAR inducer, requires a period of several days to accumulate pathogenesis-related proteins throughout the plant prior to initiation of

Table 4
Effects of fungicide and acibenzolar-*S*-methyl (ASM) applications on blue mould development in broadleaf tobacco (2001).

Treatments[a] (ASM g a.i. ha^{-1})	Blue mould development (lesions per plant)
	10 Sep
Non-treated control	24.1 a[b]
D + M (4×)	8.4 b
D + M (1×)/ASM (2× at 17.5 g a.i. ha^{-1})	0.5 d
D + M (1×)/ASM (3× at 17.5 g a.i. ha^{-1})	0.5 d
D + M (1×)/ASM (2× at 8.8 g a.i. ha^{-1})	2.2 c
D + M (1×)/ASM (3× at 8.8 g a.i. ha^{-1})	1.2 d
$P\leq$	0.0001

[a] Fungicide applied at 14-d intervals (dimethomorph plus mancozeb (D + M) at 252 g a.i. ha^{-1} dimethomorph and 1.78 kg a.i. ha^{-1} mancozeb) and acibenzolar-*S*-methyl (ASM) at the listed rates of 8.8–17.5 g a.i. ha^{-1} at 14-d intervals. The first spray was applied on 21 June 2001.

[b] Numbers within a column followed by a common letter are not significantly different according to the Kruskal–Wallis Multiple Comparison *Z*-test ($P \leq 0.05$).

Table 5
Effects of fungicide and acibenzolar-*S*-methyl (ASM) applications on cured tobacco leaf quality under commercial shade-grown conditions (2000 and 2001).

Treatments (ASM g a.i. ha^{-1} and timing)	Value[a]	Weight (kg)
2000		
D + M (14-d intervals×)	78.2	40.6
D + M/ASM (2× at 17.5 g a.i. ha^{-1} 10-d intervals)	75.4	39.2
D + M/ASM (5× at 17.5 g a.i. ha^{-1} 14-d intervals)	75.2	44.6
D + M/ASM (2× at 26.3 g a.i. ha^{-1} 10-d intervals)	78.9	41.9
$P\leq$	ns	ns
2001		
D + M (14-d intervals×)	73.5 a[b]	40.3
D + M/ASM (2× at 8.8 g a.i. ha^{-1} 10-d intervals)	58.0 b	43.3
D + M/ASM (4× at 8.8 g a.i. ha^{-1} 14-d intervals)	68.2 a	39.6
D + M/ASM (2× at 17.5 g a.i. ha^{-1} 10-d intervals)	50.7 b	45.0
$P\leq$	0.0005	ns

[a] Economic leaf value was determined by quality grade and value and expressed as a percent of the maximum value possible.

[b] Numbers within a column followed by a common letter are not significantly different according to ANOVA and the Fishers LSD test ($P \leq 0.05$).

the resistance response (Vallad and Goodman, 2004). Mandel et al. (2008) correlated the induction of resistance in tobacco to *Tomato spotted wilt virus* with the induction of pathogenesis-related proteins by acibenzolar-*S*-methyl over a 5–6 d period. The lack of significant activity in Cuba at the same acibenzolar-*S*-methyl rates used successfully in Connecticut may be due to infection by the pathogen prior to the initiation of resistance.

Systemic acquired resistance would be desirable in an integrated management program for control of blue mould in Connecticut wrapper tobaccos. Acibenzolar-*S*-methyl was effective against blue mould in our experiments and experiments reported by other researchers (Cole, 1999; Perez et al., 2003). In the current experiments we report that adding acibenzolar-*S*-methyl to a spray program reduced lesions per plant by up to 99% compared to the non-treated control. Substituting two or three applications of acibenzolar-*S*-methyl at label rates (17.5 g a.i. ha^{-1}) for dimethomorph plus mancozeb treatments in a spray program decreased blue mould lesions compared with the paired fungicide-only treatments by 28 to 94 percent. In addition, systemic activity is very desirable, especially as spray coverage may be difficult to achieve in shade tobacco (LaMondia and Horvath, 2001). The mode of action of SAR inducers is indirect and does not exert selection pressure on pathogens to develop insensitivity as may the currently used single-mode of action fungicides dimethomorph and azoxystrobin (Vallad and Goodman, 2004). Currently, mancozeb is used as a protectant mixing partner to help manage fungicide resistance, but mancozeb is relatively ineffective against blue mould and can leave residues which can affect marketability. The induction of SAR by acibenzolar-*S*-methyl may be a more effective and desirable resistance management alternative. Finally, the use of SAR elicitors, which have no direct antimicrobial activity and low toxicity, is environmentally benign in comparison to pesticide alternatives (Vallad and Goodman, 2004).

However, induced resistance may carry some cost to the plant (Vallad and Goodman, 2004; Walters et al., 2005). In previous research, we observed flecking and off-colour cured leaves on shade tobacco at rates of 35 g a.i. ha^{-1} acibenzolar-*S*-methyl (70 g ha^{-1} Actigard, 2× label rates) (LaMondia, 2002). Commercial evaluation of shade tobacco treated with 17.5 g a.i. ha^{-1} acibenzolar-*S*-methyl over 2 years resulted in no detrimental effects or some reduced quality when acibenzolar-*S*-methyl was applied to drought-stressed plants. We have not observed any negative impacts on cured leaves at rates lower than 17.5 g a.i. ha^{-1}. These results are consistent with those of Csinos et al. (2001), who observed that stunting was associated with physiological stress rather than simply the rate of acibenzolar-*S*-methyl applied to plants. Our results differ from Perez et al. (2003) who found that acibenzolar-*S*-methyl affected plant height and yellowing, but not cured leaf quality. There may be different responses of different tobacco types, however. In these experiments, we observed flecking damage to broadleaf tobacco but no such damage to shade tobacco applied at the same time.

Blue mould can be successfully managed by dimethomorph and mancozeb in broadleaf tobacco, and by azoxystrobin, dimethomorph and mancozeb in shade tobacco. Disease control can be significantly improved by incorporating acibenzolar-*S*-methyl into the spray program. However, the application of acibenzolar-*S*-methyl at 10-d intervals to heat or drought-stressed tobacco significantly reduced leaf quality. Avoidance of applications during stress periods or the application of lower rates at longer intervals may be necessary to avoid quality losses. In our experiments, one-half label rates at 14-d intervals prior to pathogen infection resulted in increased efficacy over fungicides alone and reduced the risk of stress-associated loss of leaf quality. The effects of further rate reductions on efficacy and phytotoxicity remain to be determined.

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