EFFICACY OF AZOXYSTROBIN FUNGICIDE AGAINST SORE SHIN OF SHADE TOBACCO CAUSED BY *RHIZOCTONIA SOLANI*

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Azoxystrobin fungicide was evaluated for efficacy against *Rhizoctonia solani*, causal agent of tobacco sore shin, in greenhouse and in vitro tests. Azoxystrobin significantly increased root weights and reduced disease ratings compared with untreated controls in 2004 and 2005, and also reduced the percent of the stem girdled in 2005 (not measured in 2004). Some phytotoxicity (observed as flecks) was visible on 1 or 2 leaves of Quadris-treated transplants about 1 week after treatment. Leaves that expanded after treatment did not show symptoms. Activity of azoxystrobin fungicide in vitro was not as effective as might be expected from field observations and greenhouse experiments. Dose–response experiments of *R. solani* mycelial growth rate on azoxystrobin-amended half-strength Potato dextrose agar measured mycelial growth inhibition of only 30% by 1,000 μg/ml ai. Salicylhydroxamic acid (SHAM), used to inhibit the alternative oxidase pathway in a number of fungi, was tested at 100 μg/ml SHAM in media to determine the efficacy of azoxystrobin against 2 isolates of *R. solani* in the absence of the alternative oxidase pathway. In the presence of SHAM, the inhibition of mycelial growth was over the LD_{50} for all concentrations tested, indicating that *R. solani* does use an alternative oxidation pathway in vitro. However, the observed inhibition decreased over time; inhibition averaged over all azoxystrobin concentrations was 63.3% after the first 24 hr, 52.0% after 48 hr and only 26.5% during the third–fourth day of exposure (P = 0.001). It appears that an additional mechanism of alternative oxidation may become active over time in *R. solani*. Azoxystrobin (Quadris) is registered for management of tobacco blue mold and should be valuable as a transplant band treatment to protect shade-grown tobacco plants from sore shin caused by *R. solani*.

Additional key words: alternative oxidase pathway, *Nicotiana tabacum*, Quadris, sore shin

INTRODUCTION

Sore shin, caused by *Rhizoctonia solani* Kuhn, is a common disease of tobacco, causing stunted, uneven growth of shade-grown cigar wrapper tobacco transplants in certain years, especially those with intermittent heavy rains (8). Management of tobacco sore shin in shade tobacco in the Connecticut River Valley has been primarily conducted by cultural practices consisting of cultivation and hoeing to break up soil surface crusts and hilling around plants to stimulate additional root growth. Plants may recover if environmental conditions are favorable for plant growth. However, if conditions remain conducive for disease, plants are often completely girdled, or simply damaged again in a different location further up the stem.

A number of fungicides have been evaluated for sore shin management (2), but none are currently registered for use in tobacco. Quadris fungicide (active ingredient azoxystrobin, Syngenta Crop Protection, Wilmington, DE) was registered for management of blue mold (*Peronospora tabacina*) with a Section 18 registration in Connecticut from 2003 to 2006 and a full Section 3 registration after 2006. Quadris has been shown to be effective against target spot of tobacco, a foliar leaf spot disease caused by *Thanetephorus cucurmeris* (teleomorph of *R. solani*; 5,12), and a number of other diseases caused by *R. solani* including crown and root rot of sugar beet (6,7) and sheath blight of rice (3). Connecticut Valley shade tobacco growers have observed that sore shin symptom development was reduced when tobacco plants were treated with Quadris for blue mold protection in the field 1–2 weeks after transplanting (LaMondia, unpublished).

The objectives of this research were to evaluate the activity of Quadris fungicide against *R. solani* in vitro and also in greenhouse pots when applied after transplanting at blue mold field application dosages.

MATERIALS AND METHODS

Greenhouse experiments were conducted in 2004 and 2005. Six-week-old Connecticut shade tobacco cultivar 8212 were transplanted to and grown in 400-cm³ pasteurized sandy field soil (Merrimac fine sandy loam Entic Haplorthod; 71.8% sand, 23.0% silt, 5.2% clay; pH 6.0, 3.0% organic matter) in 10-cm-diameter plastic pots. Plants were grown on heated propagation mats held at 18°C. Plants were inoculated with 4 0.5-cm-square plugs of half-strength potato dextrose agar (PDA) infested with *R. solani*, halfway between the stem and the edge of the pot in 4 holes 0.5 cm in diameter and 1 cm deep per pot. The *R. solani* isolate used was isolated in 2004 from shade tobacco in Windsor, CT, with typical sore shin symptoms, and maintained in culture. There were 17 and 11 replications of each treatment in 2004 and 2005, respectively. Five days (2004) or 1 day (2005) after inoculation, plants were treated with 0.022-ml Quadris fungicide (22.9% azoxystrobin, Syngenta Crop Protection, Greensboro, NC) per plant in 2.3 ml water per plant (equivalent to 0.585 L/ha or 8.0 oz/acre of formulated product in a 10-cm band over the row) or left untreated. Plants and soil were sprayed with a handheld mist sprayer. Two weeks after treatment, plants

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Table 1. The effect of Quadris fungicide on shade tobacco root and stem health and weight.

Treatment	2004		2005		
	Disease Rating[a]	Root Weight (g)	Disease Rating	Root Weight (g)	% Girdled
Untreated control	1.41	0.33	1.05	0.73	45.5
Quadris[b]	0.53	0.83	0.00	1.05	1.8
P =	0.0005	0.002	0.0003	0.016	0.0003

[a] Root disease rating: 0 = healthy; 1 = discolored; 2 = collapsed. The experiment was conducted as a randomized group design and there were 17 and 11 replications of each treatment in 2004 and 2005, respectively. Data were analyzed by analysis of variance.
[b] 0.585 L/ha or 8.0 oz/acre of formulated product in a 10-cm band over the row.

were rated for disease (0 = healthy; 1 = discolored; 2 = collapsed), and roots were washed free of soil and weighed. The percent girdling of the stem just below the soil line was determined in 2005.

To evaluate the effects of azoxystrobin on mycelial growth, azoxystrobin fungicide was added to autoclaved, cooled (45°C), half-strength potato dextrose agar (½PDA) to result in 500, 250, 50, 5, 0.5, and 0 ppm (µg ai/ml). In additional experiments, salicylhydroxamic acid (SHAM; Fisher Scientific, Suwannee, GA) was added to 9-cm petri dishes at 100 µg/ml to inhibit the alternative oxidase pathway (10). For sensitivity evaluations in the presence of SHAM, SHAM was dissolved in methanol:acetone (1:1 [vol/vol]) (M), and ½PDA was amended to final concentrations of 100 µg/ml, including additional control plates of SHAM alone or just the methanol:acetone solvent alone not amended with azoxystrobin. Agar plugs were removed from the edge of actively growing ½PDA cultures and placed at 1 edge of control, fungicide-amended, or fungicide plus SHAM–amended plates. Two *R. solani* isolates were tested. Both were isolated in 2004 from shade tobacco in Windsor, CT, with typical sore shin symptoms and maintained in culture. Plates were held at ambient temperature in the laboratory. Colony diameter was measured for up to 10 days and the radial growth rate per day determined. There were 4 replicate plates each for the 2 *R. solani* isolates, and the experiment was conducted 4 times, twice on ½PDA amended with azoxystrobin fungicide alone and twice in experiments including SHAM. All data were analyzed by analysis of variance.

RESULTS AND DISCUSSION

Azoxystrobin fungicide (formulated as Quadris) significantly increased root weights (P = 0.002 and

Table 2. The effect of azoxystrobin (Quadris fungicide) on radial growth of *Rhizoctonia solani* in vitro.[a]

Azoxystrobin (µg ai/ml)	Radial Growth (cm)[b]	Growth Reduction (%)
0	0.83	–
50	0.65	22
500	0.65	22
1,000	0.58	30

[a] Azoxystrobin fungicide was added to autoclaved, cooled (45°C) half-strength potato dextrose agar to result in 1,000, 500, 50, and 0 ppm (µg ai/ml).
[b] Radial growth on media measured over 4 days. There were 4 replicate plates for each of 2 *R. solani* isolates, and the experiment was conducted twice. Data were analyzed by analysis of variance.

0.02) and reduced disease ratings (P = 0.0005 and 0.0003) compared with untreated controls in 2004 and 2005, respectively, and also reduced the percent of the stem girdled in 2005 (P = 0.0003). Percent stem girdling was not measured in 2004 (Table 1). Some phytotoxicity was observed as fleck symptoms (less than 1 mm in diameter) on 1 or 2 leaves of Quadris-treated transplants (of about 6 total leaves) about 1 week after treatment. Leaves that expanded after treatment did not show symptoms.

Activity in vitro was not as effective as might be expected from field observations and greenhouse experiments. On ½PDA with different concentrations of azoxystrobin, mycelial growth was only inhibited by 30% by 1,000 µg/ml ai (Table 2). Strobilurin fungicides inhibit mitochondrial respiration by binding to the Q_0 site of cytochrome b, blocking electron transfer and disrupting the production of ATP (1). A number of fungi, including *Venturia inequalis* and *Septoria tritici*, have been reported to be more sensitive to strobilurin fungicides in vivo than in vitro because of an alternative oxidase pathway that functions in vitro, but not in strobilurin fungicide–treated plant tissues (11,14). Although not all fungi utilize the alternative oxidative pathway (10), our results indicate that *R. solani* does utilize this alternative pathway in vitro. Host-plant antioxidants such as flavones may interfere with the induction of the alternative pathway in vivo (9). SHAM has been used to inhibit the alternative oxidase pathway (10,13). We tested the effects of 100 µg/ml SHAM incorporated into media on the efficacy of azoxystrobin against 2 isolates of *R. solani* (Table 3). The control plates used to evaluate the effect of SHAM were ½PDA with SHAM dissolved in methanol:acetone (1:1 [vol/vol]). The methanol–acetone solvent control resulted in a 15.3% reduction in growth at the concentration used compared to ½PDA alone. The addition of methanol–acetone solvent plus SHAM further inhibited mycelial growth by 40.1% in the absence of fungicide. *Rhizoctonia solani* growth in media amended with azoxystrobin fungicide was significantly inhibited in the presence of SHAM (P = 0.0001), even at the 5–µg/ml ai rate. This effect of SHAM on inhibition of fungal mycelial growth demonstrated that *R. solani* is among the fungi that use the alternative oxidase pathway in vitro. However, our results were intriguing in that *R. solani* inhibition by azoxystrobin in the presence of SHAM became less over time (Table 3). Jin et al. (4) investigated the relationship between azoxystrobin and the alternative oxidase pathway by measuring the rate of mycelial respiration for 4 fungi, including *R. solani*. They observed that azoxystrobin

Table 3. The effect of azoxystrobin (Quadris fungicide) in the presence of SHAM on radial growth of *Rhizoctonia solani* in vitro.

Azoxystrobin (μg ai/ml)[a]	Radial Growth (cm)[b]	(%) Growth Reduction[c]			
		2 Days	4 Days	8 Days	10 Days
0	1.37				
0 + Methanol	1.16				
0 + SHAM	0.81				
5	0.13	100	83.9	71.3	62.2
50	0.13	95	84.6	74.1	58.2
500	0.04	100	95.4	73.4	75.8
1,000	0.07	100	91.0	77.7	66.9

[a] Azoxystrobin fungicide was added to autoclaved, cooled (45°C) half-strength potato dextrose agar to result in 1,000, 500, 50, 5, and 0 ppm (μg ai/ml). SHAM was added at 100 μg/ml dissolved in methanol:acetone (1:1 [vol/vol] final concentration 0.1%), including control plates not amended with azoxystrobin.
[b] Radial growth per day on media measured over 2–10 days. There were 4 replicate plates for each of 2 *R. solani* isolates, and the experiment was conducted twice. Data were analyzed by analysis of variance.
[c] The effect of time (days) on percent growth reduction in comparison to SHAM plus solvent was significantly different ($P = 0.0001$).

fungicides in the presence of SHAM inhibited respiration and the alternate oxidase pathway, but that the observed inhibition decreased with time. Our results also demonstrated that the inhibition of mycelial growth decreased over time; inhibition averaged over all azoxystrobin concentrations was 98.8% after the first 2 days, 88.7% after 4 days, 74.1% after 8 days, and 65.8% after the 10th day of exposure ($P = 0.001$). Our results confirm those of Jin et al. (4), and indicate that an additional, less effective mechanism of alternative oxidation may become active over time in *R. solani*.

Rhizoctonia solani periodically causes substantial losses due to sore shin in shade and broadleaf tobacco in the Connecticut River Valley production area. The application of azoxystrobin to sugar beet resulted in reduced Rhizoctonia crown and root rot caused by *R. solani* AG 2-2. The mechanism was not determined but was thought to be either a direct effect on *R. solani* inoculum in soil, protection of susceptible plant tissues, or a combination of the 2 (7). We evaluated azoxystrobin fungicide for activity in vivo and in vitro and determined that Quadris may be valuable as a transplant band treatment to protect shade plants from sore shin caused by *R. solani*. Our results indicate that azoxystrobin fungicide may have significant direct impact on fungal inoculum in soil, as well as efficacy in combination with plant tissues. Our results also suggest that *R. solani* may utilize an alternate oxidase pathway in addition to the SHAM inhibited alternate oxidase pathway, for which further research is warranted.

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