Efficacy of Seed Treatment Chemicals for Black Root Rot, Caused by *Thielaviopsis basicola*, on Cotton

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ABSTRACT

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The efficacy of triazole and host resistance-inducing seed treatment chemicals was examined for black root rot on cotton caused by *Thielaviopsis basicola* in both artificially and naturally infested soils with and without nematodes. In naturally infested soil, myclobutanil was effective in reducing root and hypocotyl discoloration over a wide range of soil population densities. Treatments containing high rates (42 g a.i./100 kg seed) of myclobutanil provided greater reductions in disease than low rates (21 g a.i./100 kg seed) in some experiments. Acibenzolar-S-methyl applied to the seed reduced black root rot or colonization by *T. basicola* on seedlings in artificially infested soils. Rates of acibenzolar-S-methyl did not differ in efficacy. In controlled studies, root colonization by *T. basicola* was significantly lower when seeds were treated with both myclobutanil and acibenzolar-S-methyl than with either chemical alone. In naturally infested soil under low (24 CFU/g soil) and high (154 CFU/g soil) populations of *T. basicola*, a combination of myclobutanil and acibenzolar-S-methyl at the high rate resulted in the lowest root discoloration and colonization. The nematocidal seed treatment abamectin improved the control of black root rot in the presence of *Meloidogyne incognita*. The semi-selective medium TB-CEN allowed the importance of *T. basicola* to be evaluated in the presence of other pathogens that contribute to the seedling disease complex on cotton by quantifying the isolation frequency and percent colonization of *T. basicola*.

Thielaviopsis basicola (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & Kendrick) was first reported on cotton as causing a collar rot of mature cotton plants (*Gossypium barbadense* L.) in late summer or early fall in Sacaton, AZ as early as 1922 (15,16). *T. basicola* was later found to cause a root rot on cotton seedlings of *G. barbadense* and *Gossypium hirsutum* L. (16,27). Currently, *T. basicola* is recognized as an economically important seedling pathogen of cotton throughout the world (1,11). The fungus has a host range of over 230 species and is a destructive pathogen on the roots of many crop and ornamental plants (26).

The fungus causes a cortical root rot of cotton seedlings starting as early as 10 to 14 days after planting (11,20). Gomaa (10) could not isolate *T. basicola* from cotton seedlings until the fifth day after planting and found that isolation increased dramatically between 5 and 10 days after planting. The fungus causes a dark-brown to black discoloration of the roots and hypocotyl, resulting in stunted, less vigorous seed-

lings and delayed flowering or maturity (1). Black root rot is most severe early in the growing season when soil temperatures are below 24°C and soil water content is high (25). As soil temperatures increase and the plants develop, the diseased cortical tissues slough off and secondary root growth occurs (19,20).

T. basicola may interact synergistically with *M. incognita* on cotton (29–31). A significant increase in seedling death and a decrease in plant growth and yield occurred in microplots infested with both pathogens compared to plants grown in microplots infested with either pathogen alone. In histological observations, infection of cotton roots by the root-knot nematode allowed *T. basicola* to colonize vascular tissues, which are not normally colonized in *G. hirsutum* in the absence of the nematode (30).

There are limited management options for the control of black root rot. Crop rotation can reduce *T. basicola* populations (12,23); however, extended rotations are rarely practiced by cotton producers. No resistant cultivars are available for black root rot on cotton (32,33). Seed treatment fungicides are nearly universally used on cotton for the control of various pathogens in the seedling disease complex. However, the extent of control of black root rot with these fungicide seed treatments is unknown. Myclobutanil (6) and triadimenol (4), two triazole fungicides, have been

shown to have some efficacy for the control of black root rot. The rates of these fungicides used on cotton, however, are directed at the control of *Rhizoctonia solani* and are generally not used at rates considered sufficient to provide significant control of *T. basicola*. Recently, acibenzolar-S-methyl (Bion), one of the systemic acquired resistance (SAR) chemical inducers, has been shown to reduce black root rot on cotton (21) and was registered recently for cotton in Australia.

The objective of this study was to evaluate the efficacy of seed treatment chemicals, including fungicides and host resistance inducers, individually and in combination for controlling black root rot on cotton. The efficacy of seed treatments was examined in both artificially and naturally infested soils with and without nematodes due to the synergistic interaction between *T. basicola* and *M. incognita*.

MATERIALS AND METHODS

Cottonseed, not treated and treated with appropriate pesticides, were supplied by D. H. Long (Syngenta Crop Protection, Greensboro, NC). The cotton cultivars DP 444 BG/RR or DP 555 BG/RR (Delta and Pineland, Scott, MS) were used in these studies. All seed also were treated with CaCO₃ (198.44 g/100 kg seed), polymer (Secure 28.34 g/100 kg seed, Becker Underwood, Ames, IA), and dye (Color Coat Blue 28.34 g/100 kg seed, Becker Underwood), standard components of a seed treatment combination on cotton to neutralize acid-delinted cottonseed and color the seed to indicate the presence of pesticides on the seed.

In addition to the seed treatments evaluated, seed were often treated with chemicals important in managing other seedling pathogens, early-season insects, or nematodes. The base seed treatment fungicides used in this study included fludioxonil (Maxim 4 FS), mefenoxam (Apron XL), and azoxystrobin (Dynasty). In addition to base seed treatment fungicides, custom seed treatment fungicides were azoxystrobin + fludioxonil + mefenoxam (Dynasty CST). The insecticide seed treatment was thiamethoxam (Cruiser). The nematocidal seed treatment was abamectin (Avicta). Aldicarb (Temik 840 g/ha; Bayer CropScience, Research Triangle Park, NC) was included as an in-furrow treatment for thrips and nematodes in the field study. Seed treatment fungicides examined for

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their efficacy against *T. basicola* were myclobutanil (Systhane) and acibenzolar-S-methyl (Bion).

T. basicola inoculum was prepared using the isolate 3N-25B recovered from cotton seedlings at the Delta Branch Station near Clarkdale, AR. Individual chlamydospores were prepared as described by Candole and Rothrock (8). Briefly, 6-week-old cultures of *T. basicola* grown on 10% carrot juice agar were flooded with sterile distilled water and decanted repeatedly to dislodge endoconidia. The chlamydospores along with the remaining endoconidia and hyphae were scraped from the surface of the cultures with a rubber policeman, suspended in sterile distilled water, and passed through a monofilament nylon fabric with 53- μ m openings (Tetko, Inc., Depew, NY). The suspension was then filtered through a monofilament nylon fabric with 20- μ m openings and was rinsed with sterile distilled water to remove endoconidia. Chlamydospore chains retained on the nylon fabric were scraped off with a spatula into a sterile 15-ml centrifuge tube containing about 50 mg of 212 to 300 μ m diameter glass microbeads. To digest chlamydospore chains, 12.5 units of chitinase from *Streptomyces griseus* (Sigma Chemical Co., St. Louis, MO) in 1 ml of buffer was added to the centrifuge tube with 12 ml of sterile distilled water. The suspension was placed on a shaker at 75 cycles per minute at 37°C for 24 h and then vortexed at high speed for 2 min. Chlamydospore chain separation was confirmed by microscopic examination, and the mixture was poured through nylon fabric with 20- μ m openings. After several washings with a minimal volume of sterile distilled water, the filtrate was collected and centrifuged. The chlamydospores were resuspended in sterile distilled water and centrifuged three times to remove the chitinase. Chlamydospores were resuspended in sterile distilled water and stored in a refrigerator at 2 to 5°C. Chlamydospore viability, percent germination, was determined before each experiment by plating spores on carrot juice agar.

Soil populations for artificially and naturally infested experiments were determined for *T. basicola* by the pour-plate technique using the semi-selective medium TB-CEN (28) amended with Penicillin G, 60 mg/liter (ICN Biomedicals Inc., Aurora, OH). Twenty-seven grams of soil was suspended in sufficient sterile 0.15% water agar to make 250 ml. The sample was shaken on a wrist action shaker for 20 min prior to assaying populations. One milliliter of soil suspension was pipetted into each petri plate (100 $\times$ 15 mm). Twenty-seven to thirty milliliters of molten medium (~45°C) was then poured into each plate, and plates were swirled to uniformly distribute the soil suspension. Ten plates were used for each soil sample. Plates were kept at room temperature (20 to

22°C) in the dark for 14 days prior to counting colonies growing on the medium. Counts were adjusted to colony forming units per gram of soil based on soil oven dry weight (odw).

The population of *M. incognita* used was collected from an infested field in Drew County, AR, and increased on tomato (*Lycopersicon esculentum* L. cv. Rutgers) in a greenhouse at the University of Arkansas Southwest Research and Extension Center at Hope, AR. Nematode eggs and juveniles were extracted from infected tomato roots using 0.5% NaOCl for 2 min as described by Hussey and Barker (13).

Soil samples from sites naturally infested with *T. basicola* were sent to the Arkansas Nematode Diagnostic Clinic Laboratory, University of Arkansas Southwest Research and Extension Center, to determine nematode populations. Nematodes were extracted from the samples using a semi-automatic elutriator (7) followed by centrifugal flotation (14), and nematodes were quantified with a stereoscope at $\times 40$ to 60 magnification.

Experiments in artificially infested soil. A Rilla silt loam soil (40% sand, 56% silt, and 4% clay) from a field known to be infested with *T. basicola* and *M. incognita* was utilized in this study. The soil was pasteurized for 0.5 h at 70°C prior to infestation.

A controlled-environment experiment was conducted to examine the efficacy of myclobutanil and acibenzolar-S-methyl alone and in combination for black root rot control. Four different seed treatment combinations, including no seed treatment, were used in this experiment (Table 1). The soil was infested with 60 viable chlamydospores of *T. basicola* per gram by diluting inoculum in 50 ml of deionized water and mixing the suspension into soil

(12,800 g of soil odw) for each tub (40 $\times$ 25 $\times$ 13 cm). Fourteen seed of the cotton cultivar DP 555 BG/RR were planted per treatment in a tub. The experiment had four replications and was conducted twice.

A separate controlled-environment experiment was conducted in the presence and absence of *T. basicola* and *M. incognita* in growth chambers. The soil in each tub (12,800 g odw) was infested with 100 viable chlamydospores of *T. basicola* per gram of soil, 16 eggs or juveniles of *M. incognita* per gram of soil, both pathogens, or not infested. Soil was infested with *M. incognita* after planting by applying inoculum to the surface of the soil in 250 cm³ of water using a watering container. Eleven different seed treatment combinations (subplots) were used in this study (Table 2). Eight seed of the cotton cultivar DP 444 BG/RR were planted per treatment in each tub. The experiment was a randomized complete block split-plot design with four replications, and the experiment was conducted twice.

For controlled-environment experiments, tubs were placed into growth chambers with a diurnal temperature regime of 15°C night and 24°C day, temperatures typical of the soil temperatures early in the growing season (22), with a 14-h photoperiod. The average light intensity for this study was 207 μ mol/m²/s, ranging from 130 to 288 μ mol/m²/s. Tubers were watered every other day. An additional set of experiments for the four-treatment study was conducted in the greenhouse. For this study, soil temperatures averaged 25°C day and 19°C night. The average light intensity at noon on a sunny day was 503 μ mol/m²/s, ranging from 441 to 630 μ mol/m²/s. Experiments were terminated 28 days after planting.

Plants were removed from the tubs, washed under running tap water to remove

Table 1. Efficacy of myclobutanil and acibenzolar-S-methyl seed treatments on black root rot in artificially infested soil in the growth chamber^v

Treatments	Rate (g a.i./100 kg seed)	Root discoloration (%) ^w	Hypocotyl lesions (%) ^x	Colonization (%) ^y	Isolation (%)
Nontreated	...	35.1 A ^z	68.9 A	87.3 A	100.0 A
Acibenzolar-S-methyl	1	12.5 B	37.3 B	49.0 B	91.7 AB
Myclobutanil	21	6.5 BC	11.5 C	27.4 C	82.3 B
Acibenzolar-S-methyl + myclobutanil	1 + 21	2.4 C	3.3 C	25.9 C	84.3 B
P value		<0.0001	<0.0001	<0.0001	0.0047

^v Experiments were conducted in a growth chamber with average temperatures of 24°C day and 15°C night and light intensity of 207 μ mol/m²/s.

^w Root discoloration was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system discolored. Analyses were conducted on mid-percentile values.

^x Hypocotyl discoloration was assessed on a 1 to 4 scale, where 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Percentages of plants having hypocotyl lesions, ratings of 3 or 4, were analyzed and presented.

^y Colonization was assessed by a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system having growth of *T. basicola* on the medium. Analyses were conducted on mid-percentile values.

^z Combined data from two experiments. Means in a column followed by a common letter are not significantly different, protected LSD ($P \leq 0.05$).

soil, blotted dry, and weighed. The numbers of nodes above the cotyledonary node and plant weights were recorded for all plants. Root discoloration was rated by using a scale of 0 to 10, in which 0 = 0%, 1 = 1 to 10%, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60%, 7 = 61 to 70%, 8 = 71 to 80%, 9 = 81 to 90%, and 10 = 91 to 100% of the root system discolored. Hypocotyl discoloration was rated on a scale of 1 to 4, in which 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Gall-ing associated with the root-knot nematode was evaluated for a root system on a scale of 1 to 6, with 1 = no galls, 2 = 1 to 2 galls, 3 = 3 to 10 galls, 4 = 11 to 30 galls, 5 = 31 to 99 galls, and 6 = galls $\geq$ 100.

After disease evaluation, roots were dis-infested in 0.5% NaOCl for 1.5 min, blot-

ted dry on a paper towel, and plated on the amended TB-CEN medium. Plates were kept at room temperature (20 to 22°C) in the dark. Roots were observed at 7 to 10 days on TB-CEN medium and rated using a 0 to 10 scale similar to the root discoloration scale for percent *T. basicola* colonization, the percentage of the root system with growth of *T. basicola* on the medium.

Experiments in naturally infested soil. Controlled-environment experiments were conducted to evaluate the efficacy of seed treatment chemicals to control the black root rot pathogen, *T. basicola*, in naturally infested soils. Field soils were selected that differed in soil populations of *T. basicola* and the presence of plant parasitic nematodes.

Three soils from different locations were used in this study: (i) soil infested with *T. basicola* (24 CFU/g soil) and *Rotylenchu-*

lus reniformis, reniform nematode, from a field near St. Charles, AR, (ii) soil infested with *T. basicola* (68 CFU/g soil) from a field near Trumann, AR, and (iii) soil infested with *T. basicola* (154 or 162 CFU/g soil) and *M. incognita* from a field near Portland, AR. Soil texture for each location was determined using the hydrometer particle-size method (3): (i) a silt loam soil (26.4% sand, 58% silt, 15.6% clay), (ii) a loamy sand soil (70.4% sand, 26% silt, 3.6% clay), and (iii) a sandy loam soil (58.4% sand, 40% silt, 1.6% clay), respectively. Soils were collected on 20 May 2007, 15 October 2007, and 15 October 2007 or 9 May 2008 for the St. Charles, Trumann, and Portland fields, respectively, and were re-frigerated at 2 to 5°C prior to use.

Six different seed treatment combina-tions were used (Table 3). Six seed of the

Table 2. Efficacy of seed treatment combinations on black root rot in soils artificially infested with *Thielaviopsis basicola*^a

Treatments ^v	Rate (g a.i./100 kg)	Root discoloration (%) ^w	Hypocotyl lesions (%) ^x	Colonization (%) ^y	Isolation (%)
Nontreated	...	53.0 A ^z	63.3 A	55.6 AB	94.3 A
Seed treatment		45.8 ABC	66.2 A	38.3 BCD	88.7 A
Myclobutanil	21	33.4 BCD	20.0 B	31.1 CD	90.0 A
Acibenzolar-S-methyl	0.3	40.5 BCD	50.0 AB	46.7 ABC	90.0 A
Acibenzolar-S-methyl	0.6	28.2 DE	39.1 AB	38.7 BCD	88.3 A
Acibenzolar-S-methyl	0.9	32.5 CD	37.5 AB	30.7 CD	87.5 A
Abamectin + myclobutanil	208.04 + 21	14.1 E	20.0 B	21.6 D	80.0 A
Abamectin + acibenzolar-S-methyl	208.04 + 0.3	47.1 ABC	58.1 A	45.0 BC	92.5 A
Abamectin + acibenzolar-S-methyl	208.04 + 0.6	48.6 ABC	50.6 AB	47.0 ABC	85.0 A
Abamectin + acibenzolar-S-methyl	208.04 + 0.9	34.1 BCD	50.0 AB	29.8 CD	78.9 A
Abamectin	208.04	48.9 AB	57.5 A	64.4 A	91.2 A
P value		<0.0001	0.0202	0.0005	0.7882

^a Experiments were conducted in a growth chamber with average temperatures of 24°C day and 15°C night and light intensity of 207 μ mol/m²/s.

^v All seed treated with azoxystrobin + fludioxonil + mefenoxam (Dynasty CST, 41.60 g a.i./100 kg seed), except nontreated.

^w Root discoloration was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system discolored. Analyses were conducted on mid-percentile values.

^x Hypocotyl discoloration was assessed on a 1 to 4 scale, where 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Percentages of plants having hypocotyl lesions, ratings of 3 or 4, were analyzed and presented.

^y Colonization was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system having growth of *T. basicola* on the medium. Analyses were conducted on mid-percentile values.

^z Combined data from two experiments. Means in a column followed by a common letter are not significantly different, protected LSD ($P \leq 0.05$).

Table 3. Efficacy of seed treatments for the control of black root rot in soil naturally infested with low *Thielaviopsis basicola* populations¹

Treatments	Rate (g a.i./100 kg)	Root discoloration (%) ^u		Hypocotyl lesions (%) ^v	Nodes	Colonization (%) ^w	Isolation (%)
		Exp. 1	Exp. 2				
Base seed treatment ^x		29.4 A ^y	38.1 A	22.9 A	2.1 A	60.3 A	91.6 A
Myclobutanil ^{x,z}	21	18.1 B	30.7 AB	11.1 BC	2.2 A	41.4 B	86.8 A
Myclobutanil + abamectin ^{x,z}	42 + 208.04	31.1 A	21.1 BCD	9.3 BC	2.3 A	51.1 AB	91.3 A
Myclobutanil + abamectin + acibenzolar-S-methyl ^{x,z}	21 + 208.04 + 0.6	16.2 BC	23.4 BC	13.6 AB	2.3 A	37.4 B	81.4 A
Myclobutanil + abamectin + acibenzolar-S-methyl ^{x,z}	21 + 208.04 + 1.0	22.1 AB	17.2 CD	7.4 BC	2.2 A	41.7 B	85.3 A
Myclobutanil + abamectin + acibenzolar-S-methyl ^{x,z}	42 + 208.04 + 1.0	7.9 C	11.1 D	0 C	2.2 A	13.7 C	58.7 B
P value		<0.0001	<0.0001	0.0055	0.6395	<0.0001	<0.0001

¹ Soil had a population of *T. basicola* of 24 CFU/g soil. Experiments were conducted in a growth chamber with average temperatures of 24°C day and 15°C night and light intensity of 207 μ mol/m²/s.

^u Root discoloration was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50, 6 = 51-60%, 7 = 61-70%, 8 = 71-80, 9 = 81-90%, and 10 = 91-100% of the root system discolored. Analyses were conducted on mid-percentile values.

^v Hypocotyl discoloration was assessed on a 1 to 4 scale, where 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Percentages of plants having hypocotyl lesions, ratings of 3 or 4, were analyzed and presented.

^w Colonization was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50, 6 = 51-60%, 7 = 61-70%, 8 = 71-80, 9 = 81-90%, and 10 = 91-100% of the root system having growth of *T. basicola* on the medium. Analyses were conducted on mid-percentile values.

^x Seed treated with thiamethoxam + mefenoxam + fludioxonil (471.56 + 7.5 + 2.5 g a.i./100 kg seed).

^y Combined data from two experiments, except when presented separately. Means in a column followed by a common letter are not significantly different, protected LSD ($P \leq 0.05$).

^z Seed treated with azoxystrobin + fludioxonil + mefenoxam (Dynasty CST, 41.60 g a.i./100 kg seed).

cotton cultivar DP 444 BG/RR were planted in 10.5 cm diameter by 7.5 cm pots that contained approximately 450 g of soil (odw). Pots were arranged in a randomized complete block design (5 replications) and placed into growth chambers under the environmental conditions stated earlier. Pots were watered every other day. Experiments were terminated 28 days after planting. Plant growth and disease ratings were recorded as described previously. Experiments were conducted twice for each soil.

A field experiment was conducted to evaluate the efficacy of seed treatment chemicals in controlling the black root rot pathogen, *T. basicola*, and the root-knot nematode near Portland, AR in 2007. The field had a history of cotton production and severe damage from the root-knot nematode and seedling diseases, including black root rot. The field was treated with 28 liter/ha of 1,3-dichloropropene (Telone II, Dow AgroSciences, Indianapolis, IN) prior to planting. Eight different seed treatment combinations of the cotton cultivar DP 444 BG/RR were evaluated in this study (Table 4). The field was planted on 8 May 2007 at 3 seed/30 cm of row. Plot size was 6 rows by 30 m long with a 0.97-m row spacing. The experiment was a randomized complete block design with three replications. At 21 days after planting (DAP), 25 seedlings per plot were removed from arbitrary 30-cm sections of row, placed in reclosable plastic bags, placed on ice, returned to the laboratory, and refrigerated until processed. Seedlings were washed under running tap water to remove soil. The number of nodes and

plant weights were recorded for all plants. Root or hypocotyl discoloration and galling associated with the root-knot nematode was rated by using the scales described previously. Roots after disease evaluation were rinsed, disinfested, and plated on the amended TB-CEN medium as described previously. Percent isolation and colonization were recorded as described previously.

Statistics. Statistical analyses were done by the appropriate statistical design using the GLM procedure with SAS (SAS Institute Inc., Cary, NC). The artificially infested experiment examining treatment effect in the presence or absence of *T. basicola* and *M. incognita* was analyzed by infestation treatment (main plot). Root discoloration, colonization, and root galling were analyzed as mid-values for each category. Hypocotyl disease was analyzed as percentage of plants with lesions, a rating of three or four. Data from the multiple experiments were combined and analyzed by GLM with SAS when appropriate. Experiments were treated as random factors. Means were separated using Fisher's protected LSD at $P = 0.05$. Orthogonal comparisons were used to examine the efficacy of product or rate on disease for some experiments.

RESULTS

Experiments in artificially infested soil. In the growth chamber, root and hypocotyl ratings caused by *T. basicola* were significantly reduced by both myclobutanil and acibenzolar-S-methyl compared to the nontreated seed (Table 1). *T. basicola* colonization also was significantly reduced

by treatments containing either myclobutanil or acibenzolar-S-methyl or both compared to the nontreated seed. Isolation, the percentage of seedlings colonized by *T. basicola*, was lower in the treatments containing myclobutanil or myclobutanil with acibenzolar-S-methyl than in nontreated seed. In this study, myclobutanil alone or when combined with acibenzolar-S-methyl was more effective than acibenzolar-S-methyl alone in lowering hypocotyl ratings and root colonization. The number of nodes or plant fresh weight was not affected by the seed treatments in this study (data not shown).

In the greenhouse study using the four seed treatments, root discoloration was significantly reduced by all the treatments containing either myclobutanil or acibenzolar-S-methyl alone or in combination compared to the nontreated seed (Table 5). Hypocotyl lesions also were reduced in the treatments containing myclobutanil and myclobutanil with acibenzolar-S-methyl, but not acibenzolar-S-methyl alone, compared to the nontreated seed. Colonization of the root system by *T. basicola* was reduced by all treatments containing either myclobutanil or acibenzolar-S-methyl or a combination of both, relative to the nontreated seed treatment. Root discoloration was lower in the treatments containing myclobutanil or myclobutanil with acibenzolar-S-methyl than acibenzolar-S-methyl alone. Colonization was more significantly reduced by myclobutanil and acibenzolar-S-methyl together than by either chemical alone. There were no differences among treatments in percent isolation of *T. basicola*. The number of nodes and plant wet

Table 4. Efficacy of seed treatments for early-season diseases in the field^a

Treatments	Rate (g a.i./100 kg)	Root discoloration (%) ^r	Hypocotyl lesions (%) ^s	Root galling ^t	Colonization (%) ^u	Isolation (%)
Nontreated ^v		41.3 A ^w	44.7 A	25.7 A	27.8 A	88.0 A
Myclobutanil ^{x,y}	21	40.0 A	2.6 B	8.1 A	13.7 A	60.0 A
Myclobutanil + abamectin ^{x,y}	42 + 208.04	32.9 A	2.6 B	3.9 A	13.4 A	57.3 A
Myclobutanil + abamectin + acibenzolar-S-methyl ^{x,y}	21 + 208.04 + 0.6	37.3 A	4.0 B	6.3 A	15.9 A	57.3 A
Myclobutanil + abamectin + acibenzolar-S-methyl ^{x,y}	21 + 208.04 + 1.0	35.6 A	4.0 B	3.3 A	14.1 A	52.0 A
Myclobutanil + abamectin + acibenzolar-S-methyl ^{x,y}	42 + 208.04 + 0.6	38.7 A	6.0 B	4.0 A	11.4 A	62.6 A
Myclobutanil + abamectin + acibenzolar-S-methyl ^{x,y}	42 + 208.04 + 1.0	33.1 A	4.0 B	3.0 A	9.0 A	53.3 A
Myclobutanil + aldicarb ^{x,z}	21 + 840 g a.i./ha	37.9 A	2.6 B	0.33 A	10.0 A	52.0 A
<i>P</i> value		0.8863	0.0001	0.1038	0.1263	0.1608

^a Seedlings sampled 21 days after planting.

^r Root discoloration was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system discolored. Analyses were conducted on mid-percentile values.

^s Hypocotyl discoloration was assessed on a 1 to 4 scale, where 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Percentages of plants having hypocotyls lesions, ratings of 3 or 4, were analyzed and presented.

^t Galling was assessed on a 1 to 6 scale, where 1 = no galls, 2 = 1-2 galls, 3 = 3-10 galls, 4 = 11-30 galls, 5 = 31-100 galls, and 6 = galls ≥100. Analyses were conducted on mid-values.

^u Colonization was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system having growth of *T. basicola* on the medium. Analyses were conducted on mid-percentile values.

^v Seed treated with thiamethoxam (471.56 g a.i./100 kg seed).

^w Means in a column followed by a common letter are not significantly different, protected LSD, ($P \leq 0.05$).

^x Seed treated with azoxystrobin + fludioxonil + mefenoxam (Dynasty CST, 41.60 g a.i./100 kg seed).

^y Seed treated with thiamethoxam + mefenoxam + fludioxonil (471.56 + 7.5 + 2.5 g a.i./100 kg seed).

^z Seed treated with mefenoxam + fludioxonil (7.5 + 2.5 g a.i./100 kg seed).

weights were not affected by any seed treatment (data not shown).

In soils artificially infested with *T. basicola* or *T. basicola* and *M. incognita*, azoxystrobin + fludioxonil + mefenoxam or abamectin had no effect on root or hypocotyl symptoms or colonization by *T. basicola* compared to nontreated seed (Tables 2 and 6). In the absence of *M. incognita*, root discoloration caused by *T. basicola* was reduced by the treatments containing myclobutanil or acibenzolar-S-methyl compared to the nontreated seed, except for the low and intermediate rates of acibenzolar-S-methyl in combination with abamectin (Table 2). Root discoloration also was reduced for myclobutanil with abamectin compared to abamectin

alone, and root discoloration was reduced for the intermediate rate of acibenzolar-S-methyl compared to the seed treatment. All treatments containing myclobutanil significantly reduced the percentage of plants with hypocotyl lesions compared to the nontreated seed or the seed treatment with or without abamectin. *T. basicola* colonization was significantly reduced by myclobutanil and the high rates of acibenzolar-S-methyl compared to the nontreated seed. Colonization also was significantly reduced in the treatments containing myclobutanil with abamectin and the low and high rates of acibenzolar-S-methyl with abamectin compared to the abamectin control. Isolation of *T. basicola* was high and did not differ among treatments for

this study. In the presence of *T. basicola*, number of nodes and plant fresh weight were not affected by any of the seed treatments (data not shown).

In the presence of both *M. incognita* and *T. basicola*, only myclobutanil with abamectin significantly reduced root discoloration compared to the nontreated seed or abamectin (Table 6). Myclobutanil and the intermediate rate of acibenzolar-S-methyl without abamectin reduced root disease compared to the seed treatment azoxystrobin + fludioxonil + mefenoxam. Root discoloration was not affected by different rates of acibenzolar-S-methyl. The percentage of plants with hypocotyl lesions did not differ among treatments. Colonization of *T. basicola* in the presence of *M. incognita* was significantly reduced by the combination of myclobutanil and abamectin compared to myclobutanil alone. The treatments containing the intermediate rate of acibenzolar-S-methyl or low rate of acibenzolar-S-methyl with abamectin also reduced colonization compared to the nontreated seed or either the custom fungicide or abamectin seed treatments. The isolation of *T. basicola* was high in this study, and no differences were observed among treatments compared to the nontreated seed. In the presence of *T. basicola*, galling by *M. incognita* was more reduced by some treatments containing abamectin than by comparable treatments without abamectin. The myclobutanil treatment had greater gall ratings than almost all other treatments. According to orthogonal contrasts, all treatments with abamectin had reduced galling compared to comparable treatments without abamectin ($P = 0.000022$). The number of nodes and plant wet weight were not affected by the seed treatments (data not shown).

Table 5. Efficacy of myclobutanil and acibenzolar-S-methyl seed treatments on black root rot in artificially infested soils in the greenhouse^v

Treatments	Rate (g a.i./100 kg seed)	Root discoloration (%) ^w	Hypocotyl lesions (%) ^x	Colonization (%) ^y	Isolation (%)
Nontreated	...	38.8 A ^z	50.6 A	82.1 A	95.8 A
Acibenzolar-S-methyl	1	23.5 B	30.0 AB	45.8 B	82.1 A
Myclobutanil	21	6.0 C	9.5 B	38.6 B	86.2 A
Acibenzolar-S-methyl + myclobutanil	1 + 21	3.9 C	2.7 B	22.2 C	55.5 A
P value		<0.0001	0.0147	<0.0001	0.0602

^v Experiments were conducted in a greenhouse with average temperatures of 25°C day and 19°C night and light intensity of 503 $\mu\text{mol}/\text{m}^2/\text{s}$.

^w Root discoloration was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system discolored. Analyses were conducted on mid-percentile values.

^x Hypocotyl discoloration was assessed on a 1 to 4 scale, where 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Percentages of plants having hypocotyl lesions, ratings of 3 or 4, were analyzed and presented.

^y Colonization was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system having growth of *T. basicola* on the medium. Analyses were conducted on mid-percentile values.

^z Combined data from two experiments. Means in a column followed by a common letter are not significantly different, protected LSD ($P \leq 0.05$).

Table 6. Efficacy of seed treatments on black root rot in soils artificially infested with *Thielaviopsis basicola* and *Meloidogyne incognita*^t

Treatments ^u	Rate (g a.i./100 kg)	Root discoloration (%) ^v	Hypocotyl lesions (%) ^w	Colonization (%) ^x	Isolation (%)	Galling ^y
Nontreated	...	51.1 AB ^z	64.3 A	83.3 A	96.6 A	5.3 BC
Seed treatment		55.9 A	72.5 A	89.6 A	97.5 A	4.8 BCD
Myclobutanil	21	38.8 BC	34.2 A	83.7 A	99.9 A	10.5 A
Acibenzolar-S-methyl	0.3	46.2 AB	50.0 A	85.2 A	100.0 A	7.4 AB
Acibenzolar-S-methyl	0.6	39.6 BC	42.8 A	61.1 BC	86.6 A	3.9 BCD
Acibenzolar-S-methyl	0.9	48.8 AB	35.0 A	76.3 ABC	97.5 A	5.9 C
Abamectin + myclobutanil	208.04 + 21	27.8 C	32.5 A	61.5 BC	100.0 A	0.9 D
Abamectin + acibenzolar-S-methyl	208.04 + 0.3	42.5 B	50.0 A	57.9 C	97.5 A	2.2 CD
Abamectin + acibenzolar-S-methyl	208.04 + 0.6	43.3 AB	48.7 A	76.9 AB	100.0 A	2.5 CD
Abamectin + acibenzolar-S-methyl	208.04 + 0.9	41.0 B	42.5 A	76.7 AB	97.9 A	1.9 CD
Abamectin	208.04	51.6 AB	60.3 A	82.8 A	99.9 A	1.0 D
P value		0.0062	0.1173	0.0065	0.4775	0.0005

^t Experiments were conducted in a growth chamber with average temperatures of 24°C day and 15°C night and light intensity of 207 $\mu\text{mol}/\text{m}^2/\text{s}$.

^u All seed treated with azoxystrobin + fludioxonil + mefenoxam (Dynasty CST, 41.60 g a.i./100 kg seed), except nontreated.

^v Root discoloration was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system discolored. Analyses were conducted on mid-percentile values.

^w Hypocotyl discoloration was assessed on a 1 to 4 scale, where 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Percentages of plants having hypocotyl lesions, ratings of 3 or 4, were analyzed and presented.

^x Colonization was assessed by use of 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system having growth of *T. basicola* on the medium. Analyses were conducted on mid-percentile values.

^y Galling was assessed by a 1 to 6 scale, where 1 = no galls, 2 = 1-2 galls, 3 = 3-10 galls, 4 = 11-30 galls, 5 = 31-100 galls, and 6 = galls ≥ 100 . Analyses were conducted on mid-values.

^z Combined data from two experiments. Means in a column followed by a common letter are not significantly different, protected LSD ($P \leq 0.05$).

According to orthogonal contrasts, treatments containing myclobutanil significantly decreased root discoloration caused by *T. basicola* both in the presence and in the absence of *M. incognita*, compared to treatments without these chemicals. In the presence of only *T. basicola*, treatments containing myclobutanil had an estimated mean difference of 23.6 from the comparable treatments without myclobutanil ($P = 0.0001$). In the presence of both pathogens, treatments containing myclobutanil had an estimated mean difference of 20.1 from the comparable treatments without myclobutanil ($P = 0.0031$). In the presence of only *T. basicola*, treatments containing acibenzolar-*S*-methyl did not reduce root discoloration. However, in the presence of both pathogens, root discoloration was reduced for treatments containing acibenzolar-*S*-methyl compared to comparable treatments without acibenzolar-*S*-methyl ($P = 0.0279$). However, no differences were found for different rates of acibenzolar-*S*-methyl for root discoloration according to orthogonal contrasts.

Results from orthogonal contrasts also indicated that treatments containing myclobutanil reduced hypocotyl lesions caused by *T. basicola* in the presence or in the absence of *M. incognita*, compared to treatments without myclobutanil. In the presence of only *T. basicola*, treatments containing myclobutanil had an estimated mean difference of 41.8 from the comparable treatments without myclobutanil ($P = 0.00028$). In the presence of both pathogens, treatments containing myclobutanil had an estimated mean difference of 32.8 from the comparable treatments without myclobutanil ($P = 0.0030$). In the presence of only *T. basicola*, treatments containing acibenzolar-*S*-methyl did not affect the development of hypocotyl lesions. How-

ever, in the presence of both pathogens, treatments containing acibenzolar-*S*-methyl reduced hypocotyl lesions compared to comparable treatments without acibenzolar-*S*-methyl ($P = 0.0151$). Different rates of acibenzolar-*S*-methyl did not differ for hypocotyl lesions according to orthogonal contrasts.

In the absence of *M. incognita*, treatments containing myclobutanil reduced colonization of the seedlings by *T. basicola* compared to comparable treatments without myclobutanil ($P = 0.0009$). However, in the presence of both pathogens, treatments containing myclobutanil did not significantly reduce colonization from the comparable treatments without myclobutanil ($P = 0.0587$). In the presence of only *T. basicola*, treatments containing acibenzolar-*S*-methyl did not reduce colonization ($P = 0.0529$), but in the presence of both pathogens, treatments containing acibenzolar-*S*-methyl did reduce colonization from the comparable treatments without acibenzolar-*S*-methyl ($P = 0.0084$).

In the experiments not infested with either pathogen, no galling or seedling disease was observed, indicating the pasteurization treatment was effective in eliminating the soilborne pathogens present. In noninfested soil, number of nodes and plant wet weight were not affected by the seed treatments, indicating that there was no phytotoxicity (data not shown). In the presence of only *M. incognita*, treatments containing abamectin significantly reduced root galling, 16.5 galls per root system, compared to treatments without abamectin, using orthogonal contrasts ($P = 0.000020$).

Naturally infested soil experiments. In naturally infested soil from near St. Charles with a low *T. basicola* population (24 CFU/g soil) and in the presence of

reniform nematode (32,000/500 cm³ soil), there was a treatment by experiment interaction for root discoloration, and experiments are presented separately (Table 3). Myclobutanil reduced root discoloration in experiment 1 at the low rate and in experiment 2 at the high rate compared to the base seed treatment. When myclobutanil was combined with acibenzolar-*S*-methyl, root discoloration was consistently reduced, except for the low rate of myclobutanil with the high rate of acibenzolar-*S*-methyl in experiment 1. Myclobutanil, when used at high rate in combination with the high rate of acibenzolar-*S*-methyl, provided better control of root discoloration than myclobutanil at the low rate with or without acibenzolar-*S*-methyl for some treatments. Hypocotyl ratings also were reduced by the treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl compared to the base seed treatment, except for the treatment containing the low rates of myclobutanil and acibenzolar-*S*-methyl. No differences were detected in number of nodes. A significant reduction in colonization was provided by all the treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl, except for the treatment containing the high rate of myclobutanil with abamectin. Myclobutanil, when used at the high rate in combination with the high rate of acibenzolar-*S*-methyl, provided the greatest reduction in colonization of *T. basicola* compared to all other treatments. Percent isolation of *T. basicola* also was reduced by the high rate of myclobutanil in combination with the high rate of acibenzolar-*S*-methyl.

In naturally infested soil collected in the fall from near Trumann with an intermediate *T. basicola* population (68 CFU/g soil), root and hypocotyl ratings were signifi-

Table 7. Efficacy of seed treatments for the control of black root rot in soil naturally infested with intermediate *Thielaviopsis basicola* populations^t

Treatments	Rate (g a.i./100 kg)	Root discoloration (%) ^u	Hypocotyl lesions (%) ^v	Nodes	Colonization (%) ^w	Isolation (%)
Base seed treatment ^x		75.1 A ^y	92.5 A	1.7 B	95.5 A	100 A
Myclobutanil ^{x,z}	21	51.4 B	44.1 B	1.9 AB	81.6 BC	100 A
Myclobutanil + abamectin ^{x,z}	42 + 208.04	50.7 B	48.5 B	2.0 A	88.0 AB	100 A
Myclobutanil + abamectin + acibenzolar- <i>S</i> -methyl ^{x,z}	21 + 208.04 + 0.6	49.7 B	45.1 B	2.0 A	88.1 AB	100 A
Myclobutanil + abamectin + acibenzolar- <i>S</i> -methyl ^{x,z}	21 + 208.04 + 1.0	49.4 B	67.3 B	2.0 A	76.3 C	100 A
Myclobutanil + abamectin + acibenzolar- <i>S</i> -methyl ^{x,z}	42 + 208.04 + 1.0	41.7 B	49.5 B	1.8 AB	75.0 C	100 A
<i>P</i> value		0.0004	0.0019	0.0359	<0.0001	

^t Soil had a population of *T. basicola* of 68 CFU/g soil. Experiments were conducted in a growth chamber with average temperatures of 24°C day and 15°C night and light intensity of 207 μmol/m²/s.

^u Root discoloration was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system discolored. Analyses were conducted on mid-percentile values.

^v Hypocotyl discoloration was assessed on a 1 to 4 scale, where 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Percentages of plants having hypocotyl lesions, ratings of 3 or 4, was analyzed and presented.

^w Colonization was assessed by a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system having growth of *T. basicola* on the medium. Analyses were conducted on mid-percentile values.

^x Seed treated with thiamethoxam + mefenoxam + fludioxonil (471.56 + 7.5 + 2.5 g a.i./100 kg seed).

^y Combined data from two experiments. Means in a column followed by a common letter are not significantly different, protected LSD ($P \leq 0.05$).

^z Seed treated with azoxystrobin + fludioxonil + mefenoxam (Dynasty CST, 41.60 g a.i./100 kg seed).

cantly reduced by all treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl compared to the base seed treatment (Table 7). Plant development (number of nodes) was improved with the treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl compared to the seed treatment, except for the treatments containing the low rate of myclobutanil and high rate of myclobutanil with acibenzolar-*S*-methyl. Colonization of *T. basicola* was significantly reduced by the treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl compared to the base seed treatment (thiamethoxam + mefenoxam + fludioxonil), except for the treatments containing myclobutanil at the high rate and myclobutanil with acibenzolar-*S*-methyl at the low rates. Percent isolation of *T. basicola* was 100% for all treatments in this study.

For the naturally infested soil collected near Portland with a high *T. basicola* population (154 or 162 CFU/g soil) in the presence of the root-knot nematode (625 or 415/500 cm³ soil), root discoloration was significantly reduced by all treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl, compared to the base seed treatment (Table 8). The higher rate of myclobutanil with acibenzolar-*S*-methyl provided better control for root discoloration, except for the high rate of myclobutanil alone. Similarly, hypocotyl ratings were significantly decreased by the treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl, compared to the base seed treatment, except for the treatment containing low rate of myclobutanil with the high rate of acibenzolar-*S*-methyl. No differences in the number of nodes were detected among treatments. Experiment 1 had greater mean

root and hypocotyl ratings than experiment 2 ($P = 0.05$). There was an experiment by treatment interaction for colonization. For experiment 1, colonization of *T. basicola* was significantly reduced by the high rate of myclobutanil in combination with high rate of acibenzolar-*S*-methyl relative to other treatments, except for the treatment with myclobutanil with acibenzolar-*S*-methyl (low rates). Myclobutanil at the high rate also reduced colonization compared to the control. In experiment 2, no treatments differed from the base seed treatment for colonization. Percent isolation of *T. basicola* was 98% or greater for all treatments in this experiment.

In the field study near Portland in 2007, root discoloration was not affected by seed treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl compared to no fungicide seed treatment (Table 4). However, hypocotyl ratings were significantly reduced by all treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl compared to no fungicide seed treatment. Number of nodes was not affected by any of these treatments (data not shown). Percent colonization and isolation of *T. basicola* did not differ among treatments. However, using orthogonal contrasts treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl significantly reduced percent colonization compared to no fungicide seed treatment ($P = 0.0050$). Similarly, treatments containing myclobutanil or myclobutanil with acibenzolar-*S*-methyl had an estimated mean difference for percent isolation of 31.6 from no fungicide seed treatment ($P = 0.0051$). There were no differences among treatments containing either abamectin or aldicarb for root galling caused by *M. incognita*. Or-

thogonal contrast indicated that treatments containing either abamectin or aldicarb reduced root galling from the no fungicide seed treatment and fungicide seed treatment without nematicide ($P = 0.0094$).

DISCUSSION

Myclobutanil was effective in reducing root discoloration in experiments using artificially infested soil, and was more effective in experiments where soil populations were 60 CFU/g soil than 100 CFU/g soil. In experiments using naturally infested soils, myclobutanil was effective in reducing root discoloration at soil populations ranging from 24 to 162 CFU/g soil. Hypocotyl lesions also were reduced over a similar range of *T. basicola* populations. The high rate (42 g a.i./100 kg seed) of myclobutanil on the seed provided greater reduction in disease than the low rate (21 g a.i./100 kg seed) in some experiments. Few differences in plant development were found in these experiments, which were terminated after 28 days. However, plant development had reached only one or two nodes under these environmental conditions, making differences in plant growth difficult to detect. Myclobutanil (6) and triadimenol (4) are triazole fungicides that have been previously shown to have some efficacy for the control of black root rot. Butler et al. (6) demonstrated in the greenhouse, with soils artificially infested with *T. basicola*, that rates of myclobutanil as low as 20 g a.i./100 kg seed significantly reduced root necrosis even at high rates of inoculum (300 to 400 CFU/g soil). Similarly, seed treatments containing triadimenol with thiram and metalaxyl greatly reduced root necrosis, primarily attributed to *T. basicola*, from 77 to 47% in controlled-environment experiments using

Table 8. Efficacy of seed treatments for the control of black root rot in soil naturally infested with high *Thielaviopsis basicola* populations¹

Treatments	Rate (g a.i./100 kg)	Root discoloration (%) ^v	Hypocotyl lesions (%) ^w	Nodes	Colonization (%) ^u		Isolation (%)
					Exp. 1	Exp. 2	
Base seed treatment ^x		88.4 A ^y	93.3 A	0.9 A	95.9 A	54.3 AB	100 A
Myclobutanil ^{x,z}	21	67.2 BC	44.0 B	1.0 A	92.6 AB	33.8 B	100 A
Myclobutanil + abamectin ^{x,z}	42 + 208.04	59.9 CD	40.3 B	0.9 A	85.9 BC	40.5 B	98 A
Myclobutanil + abamectin + acibenzolar- <i>S</i> -methyl ^{x,z}	21 + 208.04 + 0.6	66.1 BC	50.7 B	1.0 A	83.6 CD	41.3 B	98 A
Myclobutanil + abamectin + acibenzolar- <i>S</i> -methyl ^{x,z}	21 + 208.04 + 1.0	72.5 B	58.5 AB	0.9 A	88.2 ABC	70.9 A	100 A
Myclobutanil + abamectin + acibenzolar- <i>S</i> -methyl ^{x,z}	42 + 208.04 + 1.0	51.8 D	40.1 B	0.9 A	76.7 D	43.9 B	99 A
<i>P</i> value		<0.0001	<0.0001	0.76	<0.0001	0.02	0.66

¹ Soil had a population of *T. basicola* of 154 or 162 CFU/g soil. Experiments were conducted in a growth chamber with average temperatures of 24°C day and 15°C night and light intensity of 207 μmol/m²/s.

^u Colonization was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system having growth of *T. basicola* on the medium. Analyses were conducted on mid-percentile values.

^v Root discoloration was assessed on a 0 to 10 scale, where 0 = 0%, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100% of the root system discolored. Analyses were conducted on mid-percentile values.

^w Hypocotyl discoloration was assessed on a 1 to 4 scale, where 1 = healthy, 2 = slight discoloration, 3 = lesions, and 4 = girdling lesions. Percentages of plants that had hypocotyl lesions, ratings of 3 or 4, were analyzed and presented.

^x Seed treated with thiamethoxam + mefenoxam + fludioxonil (471.56 + 7.5 + 2.5 g a.i./100 kg seed).

^y Combined data from two experiments, except when presented separately. Means in a column followed by a common letter are not significantly different, protected LSD ($P \leq 0.05$).

^z Seed treated with azoxystrobin + fludioxonil + mefenoxam (Dynasty CST, 41.60 g a.i./100 kg seed).

naturally infested soil (34). The other fungicides used in these studies, azoxystrobin, fludioxonil, and mefenoxam, did not show any efficacy against *T. basicola*.

Acibenzolar-S-methyl was effective in soil artificially infested with *T. basicola* using 60 or 100 CFU/g soil, except for the artificially infested experiment conducted in the absence of *M. incognita* at 100 CFU/g soil. Rates of acibenzolar-S-methyl did not differ in efficacy. In a previous study, cottonseed soaked in solutions containing 25 or 50 µg of acibenzolar-S-methyl/ml distilled water for 12 h reduced the severity of black root rot in container and field experiments (21). Mondal et al. (21) reported that reductions in taproot discoloration for these acibenzolar-S-methyl treatments were generally 20 to 30%. In experiments examining acibenzolar-S-methyl alone, reductions in discoloration of the root system ranged from 39 to 64% using soil populations of 60 chlamydospores/g soil and from 12 to 38% using soil populations of 100 chlamydospores/g soil, giving similar results to Mondal et al. (21). Allen (2) reported acibenzolar-S-methyl seed treatment, in the absence of sterol-inhibiting fungicides, appeared to be more effective under low disease pressure than under high disease pressure for field evaluations. In naturally infested experiments, acibenzolar-S-methyl was not evaluated separately, but as part of a seed treatment combination with myclobutanil to manage the seedling disease complex. In these studies, acibenzolar-S-methyl was shown to be effective over a range of soil populations of *T. basicola* in the presence or absence of nematodes. Based on the level and consistency of black root rot control, the use of triazole fungicides like myclobutanil is a more important component of a seed treatment formulation, but acibenzolar-S-methyl would enhance the level of control as part of the seed treatment mixture. In Australia, acibenzolar-S-methyl is being used alone for black root rot control in the seed treatment formulation.

Seedling diseases on cotton are caused by a number of pathogens contributing to the seedling disease complex, making it difficult to determine the importance of individual pathogens or the efficacy of different fungicides against specific pathogens in the field. Colonization by *T. basicola* was significantly reduced by myclobutanil or acibenzolar-S-methyl, indicating the reductions in seedling disease were due to reductions in *T. basicola* in naturally infested soils and not other pathogens in the seedling disease complex. In the field experiment, treatments containing either myclobutanil or myclobutanil with acibenzolar-S-methyl reduced the percent isolation of *T. basicola* by 36% compared to treatments without these chemicals. Thus, isolation of *T. basicola* on the semi-selective medium TB-CEN

allowed the evaluation of the contribution of black root rot to seedling disease.

An additive effect for myclobutanil and acibenzolar-S-methyl was observed in both artificially and naturally infested soils for some experiments. In artificially infested experiments in the greenhouse study using four treatments, root colonization was lower in the treatment containing both myclobutanil and acibenzolar-S-methyl than with either chemical alone. When acibenzolar-S-methyl was combined with myclobutanil at the high rate (1 g a.i./100 kg seed) in naturally infested soils, significant reductions in colonization were observed over myclobutanil alone at low (24 CFU/g soil) and high (154 CFU/g) populations of *T. basicola* in some experiments. Similarly, an additive effect for survival of pepper plants from *Phytophthora* root and crown rot, caused by *Phytophthora capsici*, was reported under some environments when acibenzolar-S-methyl was used with mefenoxam (18). Additive effects from the use of acibenzolar-S-methyl with fungicides also have been reported for several foliar diseases including with trifloxystrobin for white rust on spinach (17), fenpropidin for powdery mildew on wheat (24), and a fungicide program using copper oxychloride, mancozeb, and iprodione with or without azoxystrobin for scab on passionfruit (35). Application of the SAR chemical inducer BABA (DL-3-aminobutyric acid) also has been reported to have additive or synergistic effects with fungicides for the control of late blight on potato and tomato (5), downy mildew on cucumber (5) and tobacco (9), and powdery mildew on wheat, barley, and cucumber (9). However, in this study the acibenzolar-S-methyl was applied to the seed instead of the foliage or stems.

T. basicola interacts synergistically with *M. incognita*, the root-knot nematode, on cotton (29–31). In microplot studies, root necrosis was greater in the presence of *M. incognita* than in the absence *M. incognita* (31). In our studies, colonization of root systems by *T. basicola* in the presence of nematodes was higher than colonization of roots infected by *T. basicola* alone. Colonization of *T. basicola* in the presence of *M. incognita* was lower in the treatment containing myclobutanil with abamectin than myclobutanil alone, suggesting that root-knot nematode plays a role in black root rot development. Even though little galling or nematode damage was observed on seedlings after 28 days, research has shown that early nematode infection plays an important role in colonization of the vascular tissue by *T. basicola* (30). These results emphasize the importance of management of early-season nematode damage on cotton to limit seedling disease losses.

In this study, the efficacy of triazole fungicides and SAR chemical inducers was documented over a range of *T. basicola* populations by using artificially and

naturally infested soils. Myclobutanil and acibenzolar-S-methyl appear promising for the control of black root rot. The efficacy for myclobutanil was demonstrated at rates used currently by seed companies. When myclobutanil was used at higher rates of application to the seed, it was more effective. Previous research measured only root discoloration and hypocotyl discoloration for disease evaluations. In the field or in naturally infested soils, root or hypocotyl discoloration may be caused by other pathogens or abiotic factors, complicating the interpretation of the importance of individual factors or efficacy of products against individual pathogens. Other indicators of black root rot severity, including colonization and incidence, were important in assessing black root rot as a result of the numerous pathogens in the seedling disease complex, giving a true indication of what portion of root or hypocotyl discoloration is associated with *T. basicola*.

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