Activity of Tebuconazole on *Sclerotium rolfsii* and *Rhizoctonia solani*, Two Soilborne Pathogens of Peanut

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ABSTRACT

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Tebuconazole, an ergosterol biosynthesis inhibitor, had a high level of activity against *Sclerotium rolfsii* and *Rhizoctonia solani* in vitro. Mean ED_{50} values for inhibition of mycelial growth of two isolates each of *R. solani* anastomosis group 4 (AG-4) and *S. rolfsii* were 0.17 and 0.08 μg/ml, respectively, for tebuconazole, compared with 24.3 and 3.9 μg/ml, respectively, for the pentachloronitrobenzene (PCNB) standard. The ED_{50} values for the inhibition of sclerotia formation by *S. rolfsii* were 0.13 and 4.99 μg/ml for tebuconazole and PCNB, respectively. ED_{50} values for inhibition of sclerotial initials were 0.14 and 2.75 μg/ml for tebuconazole and PCNB, respectively. In the field, tebuconazole provided good control of both Rhizoctonia limb rot (*R. solani* AG-4) and southern stem rot (*S. rolfsii*) of peanut (*Arachis hypogaea*) when applied seven times as a foliar spray at rates of 188–250 g/ha. Control of both diseases was positively correlated with a range of rates between 125 and 280 g a.i./ha. Efficacy was maintained when tebuconazole was applied as a block of sprays either at the beginning or end of the season or in an alternating schedule with chlorothalonil. Pod yield for plants in treated plots was approximately 50% greater than in those treated with chlorothalonil, which controlled late leaf spot (*Cercosporidium personatum*), but had little impact on the soilborne pathogens.

Soilborne pathogens have been a problem for peanut (*Arachis hypogaea* L.) growers in the southeastern United States for many years. Because of the lack of economically viable, alternate crops, more hectares of peanuts are being planted each year. This has exacerbated the problem with soilborne pathogens because of the shorter rotations employed, especially in irrigated fields in which more disease problems tend to occur. The two most damaging soilborne pathogens of peanut in Georgia are *Sclerotium rolfsii* Sacc. and *Rhizoctonia solani* Kühn anastomosis group 4 (AG-4). From 1987 to 1989, the average annual losses to Georgia growers to southern stem rot (*S. rolfsii*) and limb rot (*R. solani*) have been estimated by the Georgia Cooperative Extension Service to be $37 and $26 million, respectively (S. S. Thompson, *personal communication*).

The literature concerning resistance of peanut genotypes to *S. rolfsii* was reviewed by Aycock (2). Although one of the earliest reports indicated the potential for near immunity to *S. rolfsii* (10), no cultivars with appreciable levels of resistance to the fungus are available. An exception to this is the recently released variety Southern Runner, which does have some resistance to *S. rolfsii* (1). However, Southern Runner has met with some objections from shellers and processors and accounts for only a very small percentage of the peanut crop. Therefore, the primary means of control in problem fields has been either cultural (rotation, non-dirting cultivation, etc.) or chemical, with pentachloronitrobenzene (PCNB) being the most commonly used product. Control of stem rot with PCNB can be erratic (13), and recent price increases have made PCNB less attractive to growers. Work by Csinos (7) demonstrated that half rates of the chemical provide control equivalent to that of the full rate, which has helped moderate the cost, but efficacy is still limited.

Unfortunately, even fewer control options exist for Rhizoctonia limb rot than for stem rot. *R. solani* is found throughout the world where peanuts are grown and will attack all plant parts (11). Limb rot, resulting from infections of the lower lateral limbs, was first reported in 1982 (14) and has been a major problem in Georgia. Rotation may be of limited value because the pathogen is known to be quite persistent in many soil types, especially where organic matter is added as crop debris (11). Development of crops resistant to *R. solani* historically has been difficult (4). Although differences in the susceptibility of peanut cultivars to limb rot have been documented (3), resistance among available runner cultivars is not known.

No currently labeled fungicides provide control of Rhizoctonia limb rot (3). Several experimental fungicides, particularly the ergosterol biosynthesis inhibitors (EBIs), have shown efficacy against both *R. solani* and *S. rolfsii*. This has been documented for diniconazole (8), and other EBIs, including tebuconazole (= HWG1608 = Folicur), have shown promise in preliminary work (6). The in vitro and in vivo activity of tebuconazole against *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton, causal agent of late leaf spot of peanut, has been demonstrated (5). Similar information is needed for the major soilborne pathogens.

The objectives of this study were to document the in vitro sensitivity of *R. solani* and *S. rolfsii* to tebuconazole and PCNB and to assess the efficacy of various rates and use patterns of tebuconazole for control of peanut stem rot and Rhizoctonia limb rot. The efficacy of PCNB was not evaluated in the field because this has been thoroughly documented in numerous reports in *Fungicide and Nematicide Tests*, as well as in other publications (2,7,13).

MATERIALS AND METHODS

In vitro studies. Two isolates each of *S. rolfsii* (designated WM1 and WM3) and *R. solani* AG-4 (designated RS1 and RS6) obtained from peanut were used in this study. Materials tested were tebuconazole supplied as Folicur 1.2 EC and PCNB supplied as Terraclor 75W. Concentrations evaluated were 0, 0.01, 0.1, 1.0, 10, and 100 μg a.i./ml of medium. Stock suspensions of fungicides prepared with sterile deionized water were added to 1.5% water agar (Bacto agar, Difco Laboratories, Detroit, MI) after it was autoclaved at 121 C for 15 min and cooled to about 50 C. A dilution series was used to obtain the needed concentrations. The amended medium was mixed thoroughly and poured into 10-cm-diameter petri dishes. Inoculum for the study consisted of 5-mm plugs of agar and mycelium taken aseptically from actively growing cultures of each fungus isolate on potato-dextrose agar. Inverted plugs were placed onto the test medium at the edge of the dishes, which were then placed in plastic bags and incubated at 26 C in the dark. Radial growth was measured after 5 days, and numbers of sclerotia and sclerotial

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initials were counted 24 days after seeding.

Mycelial growth was compared with growth on nonamended medium. Percent inhibition was plotted as a function of the common logarithm of fungicide concentration. Linear regression was used to fit a line to the points and determine the dosage causing a 50% reduction in growth (ED_{50}). A similar analysis was used to evaluate the effect of the fungicides on production of sclerotia and sclerotial initials by *S. rolfsii*.

Field studies. Studies were conducted over a 2-yr period from 1987 to 1988. All tests were conducted near Tifton, GA, in fields of Tifton sandy loam soil. Each was planted to peanut the previous year, except for 1987 test A, which was preceded by grain sorghum in 1986 and peanut in 1985. The fields were subsoiled, bedded, and tilled. Florunner peanut was planted in all studies in single rows 0.91 m apart. Seeding rate varied from a low of 84 kg/ha in 1987 to a high of 134 kg/ha in 1988. Planting dates varied from the second week of April to the third week of May, and standard management practices of the Georgia Cooperative Extension Service were followed for everything except fungicide applications (9). Plots consisted of single beds (9.1 × 1.8 m) with two rows per bed. Two border rows separated each plot, and a randomized complete block design with four replications was used.

Tebuconazole was applied as Folicur 1.2 EC, and chlorothalonil was applied as Bravo 500 or Bravo 720. Plots were sprayed with a tractor-mounted, compressed air sprayer. Three D3-23 nozzles per row delivered 175 L/ha of spray at 414 kPa. Applications were made on a standard 14-day leaf spot spray schedule initiated about 7 wk after planting and terminated about 2 wk before harvest with seven total sprays applied per season of either tebuconazole, chlorothalonil, or an alternating program with both fungicides. Tebuconazole was evaluated at three potential use rates of 188, 210, and 250 g/ha.

A separate study was conducted to determine the disease control obtained with a wider range of dosages. Experimental parameters of rotational history, plot size, and application equipment, etc. were similar to those just described but the following rates of tebuconazole were evaluated: 125, 140, 156, 172, 188, 200, 210, 230, 250, 265, and 280 g/ha.

Both Rhizoctonia limb rot and stem rot were rated immediately after the

Table 1. In vitro inhibition of isolates of *Rhizoctonia solani* and *Sclerotium rolfsii* by tebuconazole and PCNB (percent inhibition [Y] as a function of $\log_{10}$ of concentration [X])

Parameter evaluated and treatment	Isolate	Linear regression	Correlation coefficient	ED_{50} (μg/ml)
Sclerotia				
S. rolfsii				
Tebuconazole	WM1	$Y = 45.5X + 90.9$	0.94	0.13
PCNB	WM1	$Y = 58.2X + 9.4$	0.90	4.99
Sclerotial initials				
S. rolfsii				
Tebuconazole	WM1	$Y = 43.5X + 87.0$	0.89	0.14
PCNB	WM1	$Y = 53.5X + 26.5$	0.79	2.75
Radial growth				
S. rolfsii				
Tebuconazole	WM1	$Y = 27.0X + 83.0$	0.95	0.06
	WM3	$Y = 27.9X + 79.4$	0.97	0.09
PCNB	WM1	$Y = 22.8X + 32.4$	0.77	5.89
	WM3	$Y = 18.6X + 45.1$	0.79	1.84
R. solani				
Tebuconazole	RS1	$Y = 23.3X + 66.2$	0.89	0.20
	RS6	$Y = 21.1X + 67.8$	0.87	0.14
PCNB	RS1	$Y = 25.3X + 16.2$	0.82	21.80
	RS6	$Y = 24.0X + 15.7$	0.85	26.80

Table 2. Evaluation of full-season foliar tebuconazole sprays for control of two soilborne peanut pathogens, *Rhizoctonia solani* and *Sclerotium rolfsii*

		Rhizoctonia limb rot[w]				Stem rot[x]				Yield[y]			
		1987		1988		1987		1988		1987		1988	
Treatment	Rate (g/ha)	Test A	Test B	Test A	Test B	Test A	Test B	Test A	Test B	Test A	Test B	Test A	Test B
Untreated		13.8 a[z]	6.7 a	45.0 a	30.0 a	31.0 a	80.4 a	···	55.7 a	1.5 a	1.7 a	···	2.7 a
Chlorothalonil	1,260	6.0 b	4.2 bc	37.5 a	21.6 a	24.0 a	83.2 a	15.4 a	50.3 a	4.0 b	2.9 b	···	3.0 b
Tebuconazole	188	2.8 c	2.2 cd	15.0 b	···	2.0 b	26.8 c	7.1 a	···	6.0 c	4.6 c	···	···
Tebuconazole	210	2.5 c	2.7 cd	18.0 b	3.0 b	1.0 b	14.0 c	4.2 a	12.5 b	6.0 c	4.5 c	···	4.8 b
Tebuconazole	250	2.3 c	1.3 d	11.3 b	···	1.5 b	14.8 c	3.3 a	···	···	4.7 c	···	···

[w]Rhizoctonia limb rot expressed as percentage of symptomatic vines.
[x]Stem rot expressed as percentage of row with symptomatic plants.
[y]Yield expressed as t/ha.
[z]Means in columns with the same letter are not significantly different according to the Waller-Duncan k-ratio t test ($P < 0.05$).

Table 3. Evaluation of alternating or blocked sprays of tebuconazole for control of two soilborne peanut pathogens, *Rhizoctonia solani* and *Sclerotium rolfsii*

			1987 test A			1988 test A		1988 test B		
Treatment	Rate (g/ha)	Application[w]	Stem rot[x]	Limb rot[y]	Yield t/ha	Stem rot	Limb rot	Stem rot	Limb rot	Yield t/ha
Untreated			31.0 a[z]	13.8 a	1.5 a	···	45.0 a	55.7 a	30.0 a	2.3 b
Chlorothalonil	1,260	1–7	24.0 ab	6.0 b	4.0 b	15.4	37.5 a	50.3 a	21.6 b	2.5 b
Tebuconazole	210	1,3,5,7	9.0 bc	6.0 b	5.8 c	7.1	25.5 b	30.3 b	5.0 c	3.7 a
Tebuconazole	210	2,4,6	2.5 c	3.0 b	6.1 c	7.9	40.0 a	28.7 b	6.5 c	3.6 a

[w]Application refers to the application number in the standard 14-day leaf spot spray schedule. Chlorothalonil at 1,260 g/ha was applied when tebuconazole was not.
[x]*Sclerotium rolfsii*. Percentage of 30.5-cm sections of linear row per plot with at least one disease locus.
[y]Rhizoctonia limb rot measured as visual estimate of percentage of vines colonized by *Rhizoctonia solani* AG-4.
[z]Means with the same letter are not significantly different according to the Waller-Duncan k-ratio t test ($P < 0.05$).

plants were inverted in all tests. Limb rot ratings consisted of a visual estimate over the entire plot of the percentage of peanut vines that exhibited symptoms indicative of colonization by *R. solani*. Stem rot ratings were made by the method of Rodriguez-Kabana et al (12), which involves counting disease loci (infected area $\leq$ 30 cm) per plot. Peanuts were dug at physiological maturity and mechanically harvested after drying in the field. Yields were based on pod weight at 10% moisture (w/w). Data were analyzed by analysis of variance, and the Waller-Duncan *k*-ratio *t* test was used for means separation. Linear regression was used to examine the relationship between the amount of tebuconazole applied and the incidence or severity of stem rot and limb rot.

RESULTS

In vitro studies. Tebuconazole inhibited mycelial growth of *R. solani* more than PCNB did as reflected by ED_{50} values, which were more than 100-fold higher for PCNB than tebuconazole (Table 1). This was true for both isolates of *R. solani*. The same trend was evident for *S. rolfsii*, where ED_{50} values for isolate WM1 and WM3 were approximately 98- and 20-fold higher, respectively, for PCNB than for tebuconazole (Table 1).

Mycelial growth of *S. rolfsii* was more sensitive to both fungicides than was that of *R. solani* (Table 1). When looking at the mean ED_{50}'s for both isolates of each fungus, it is apparent that values are approximately 50% lower for *S. rolfsii* than for *R. solani* with regard to sensitivity to tebuconazole (0.08 and 0.17 μg/ml, respectively). However, for PCNB, mean ED_{50} values for *R. solani* were nearly seven-fold higher than for tebuconazole (24.30 and 3.87 μg/ml, respectively) (Table 1).

S. rolfsii isolate WM3 formed very few sclerotia or sclerotial initials, even on nonamended agar. Isolate WM1 formed a mean of 6.6 sclerotia and 4.6 sclerotial initials per culture dish (*data not shown*). Tebuconazole completely inhibited production of sclerotia and initials at 1.0 μg/ml, whereas it took 100 μg/ml of PCNB to have the same effect. The ED_{50} values for sclerotial formation were 0.13 and 4.99 μg/ml and for initials were 0.14 and 2.75 μg/ml for tebuconazole and PCNB, respectively. Linear regressions for each variable and fungicide are given in Table 1.

Field studies. Rhizoctonia limb rot was light to moderate in 1987 and severe in 1988. This is reflective of the environmental conditions, particularly heavier rainfall, that caused limb rot to be more severe in 1988 (3). Full season sprays of tebuconazole provided good control of limb rot in 1987 at 188, 210, and 250 g a.i./ha (Table 2). In 1988 (test A), these same rates inhibited limb rot development by 67, 60, and 75%, respectively. Foliar sprays of chlorothalonil gave some suppression of limb rot in 1987 when disease severity was low but did not provide significant reduction in 1988.

Severe stem rot developed in both years of the study. Chlorothalonil had no effect on disease development, whereas tebuconazole was highly effective when applied as full-season foliar sprays (Table 2). Disease incidence was usually reduced 70–90% with tebuconazole application compared with the untreated or chlorothalonil-treated plants. There were no significant differences in efficacy among the three rates of tebuconazole evaluated.

Late leaf spot control with chlorothalonil generally increased yields compared with plots receiving no fungicide (5). Plots treated with tebuconazole yielded 1.8- to 4.0-fold higher than the untreated plots and were consistently about 1.5-fold higher than those treated with chlorothalonil alone (Table 2). There were no differences in yields with different rates of tebuconazole.

When applied in alternation with chlorothalonil, tebuconazole usually provided control of soilborne pathogens of peanut (Table 3). Reductions in stem rot incidence with tebuconazole ranged from 40 to 90% compared with plots sprayed with only chlorothalonil. Reductions in Rhizoctonia limb rot were less consistent. In 1988, test B, four sprays of tebuconazole at 210 g/ha reduced limb rot 77% compared with plots sprayed with only chlorothalonil. However, with greater disease severity in test A, limb rot control was not as good and the plants treated with three applications of tebuconazole (210 g/ha) had no less disease than the nontreated or plants treated with chlorothalonil. Chlorothalonil provided some suppression of Rhizoctonia limb rot but did not influence the incidence of stem rot.

In the full-season dosage-response test, a linear relationship was found to exist between rates of tebuconazole from 125 to 280 g/ha and the levels of both stem rot and limb rot. These relationships are illustrated in Figure 1. The correlation coefficients of the two lines were 0.78 and 0.90 for stem rot incidence and limb rot severity, respectively. Although not presented graphically, there was also a linear relationship between rate of tebuconazole (X) and severity of peanut leaf spot (Y) ($Y = 44.07 - 0.07X$, $r^2 = 0.88$).

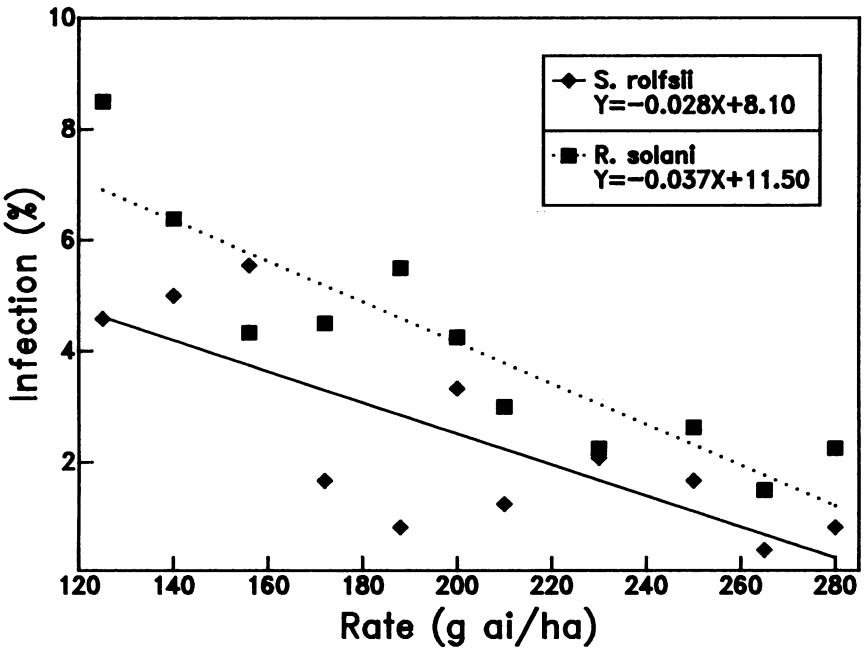

Fig. 1. Linear regressions relating the percentage of rows infected with stem rot (*Sclerotium rolfsii*) or the percentage of vines infected with limb rot (*Rhizoctonia solani* AG-4) to the amount of tebuconazole applied (seven applications) to Florunner peanuts.

DISCUSSION

Tebuconazole exhibited activity in vitro against *S. rolfsii* and *R. solani* that was superior to the currently used fungicide, PCNB. Although not compared directly in the field, PCNB has been evaluated in numerous trials and its efficacy is well documented (2,7,14). From 1987 to 1989, a total of 18 tests were conducted in Tifton evaluating the control of *S. rolfsii* and *R. solani* on Florunner peanut with PCNB. PCNB was applied at 5.6 kg a.i./ha in a narrow band (10–15 cm) over the row. In these trials, PCNB had no activity on limb rot; ratings of percent infected vines were 16 and 15% for the plots treated with PCNB and untreated plots, respectively. Disease incidence of southern stem rot was reduced about 20% from a mean of 27 to 22 infection sites per plot. Pod yields

were increased from 2,833 kg/ha in the untreated to 3,425 kg/ha in the plots treated with PCNB (A. S. Csinos and T. B. Brenneman, *unpublished*).

The in vitro sensitivity data help explain some of the efficacy trends observed in the field. Certainly, the basic difference in ED_{50} values for both fungi is significant, but the relative sensitivity of each fungus to the two fungicides is significant as well. When working with mean ED_{50} values for both isolates evaluated, the ED_{50} for *R. solani* is 143 times higher for PCNB than for tebuconazole (24.30 and 0.17 μg/ml, respectively). However, for *S. rolfsii*, that same ratio is only 48 (3.87 and 0.08 μg/ml for PCNB and tebuconazole, respectively). On this basis, tebuconazole should provide comparatively better control of limb rot than would PCNB, and this appears to be the case in the field (T. B. Brenneman, *unpublished*).

Tebuconazole offers flexibility concerning application timing for controlling both soilborne and foliar pathogens (5). No significant differences in control of stem rot were observed in any of the treatment regimes with tebuconazole. With Rhizoctonia limb rot, there was an indication that using tebuconazole for applications 1, 3, 5, and 7 was superior to using it for applications 2, 4, and 6. This may be attributable to the one additional application of tebuconazole in the first spray regime. It is also possible that applications 1, 3, 5, and 7 happened to correspond to favorable periods for infection by *R. solani* during that particular year. Studies with other fungicides have demonstrated the importance of early- to mid-season applications for control of Rhizoctonia limb rot (3).

Although no differences in efficacy were detected among the three standard use rates of tebuconazole evaluated (188, 210, and 250 g/ha), there was a definite rate response in the field at doses ranging from 125 to 280 g a.i./ha with both soilborne diseases. With most EBI fungicides, higher rates are needed to control soilborne pathogens than to control foliar pathogens such as *C. personatum*, and further testing under more diverse conditions may show tebuconazole to be no exception.

Tebuconazole offers an improved level of control of soilborne peanut pathogens. It can also be applied with conventional hydraulic sprayers, thus eliminating the need for specialized granular applicators and the extra trips over the field currently needed to apply PCNB. In addition, it effectively controls late leaf spot (5) and can substitute for chlorothalonil sprays, therefore making it more cost efficient. Registration of this product would greatly expand the options for peanut disease management.

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