Activity of Tebuconazole on *Cercosporidium personatum*, a Foliar Pathogen of Peanut

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ABSTRACT

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The different modes of action of chlorothalonil and the ergosterol biosynthesis inhibiting (EBI) fungicides tebuconazole and diniconazole on *Cercosporidium personatum* were exhibited in in vitro studies. Chlorothalonil effectively prevented germination of conidia at rates as low as 0.1 μg/ml, whereas conidia exposed to high rates (100 μg/ml) of either EBI were able to use stored ergosterol and germinate. Diniconazole and tebuconazole effectively inhibited mycelial growth and sporulation at concentrations of 0.1 and 1.0 μg/ml, respectively. In the field, tebuconazole effectively controlled late leaf spot (*C. personatum*) of peanut (*Arachis hypogaea*) at the lowest rate tested (188 g/ha). Efficacy of tebuconazole was essentially equivalent to chlorothalonil (1,260 g/ha) as measured by visual disease ratings. The percentage of reflectance recorded by a multispectral radiometer indicated that plants treated with tebuconazole had more green leaf area than those treated with chlorothalonil. Significantly higher yields were also obtained with tebuconazole. Tebuconazole provided effective control of late leaf spot when applied full season (seven times) or alternated with chlorothalonil.

Peanut (*Arachis hypogaea* L.) is the most valuable crop grown in Georgia and is very important economically to other southeastern states in the United States. Successful peanut production requires the control of several often-damaging diseases that are endemic to the region. Every hectare of peanuts grown in Georgia is subject to loss from late leaf spot caused by *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton. Financial losses to growers have been estimated by extension service personnel to be \$43, \$31, and \$77 million (including control costs) in 1987, 1988, and 1989, respectively (S. S. Thompson, *personal communication*).

The estimates of financial loss include the cost of controlling late leaf spot, which averages about \$27 million annually in Georgia (S. S. Thompson, *personal communication*). All of this expense is for foliar fungicides, with chlorothalonil being used almost exclusively. As many as eight to nine applications may be used during the growing season, although efforts are being made to develop a late leaf spot forecasting system that could reduce spray frequency (9).

Chlorothalonil generally provides excellent control of leaf spot. However, with a heavy reliance on chemical control, it is dangerous to have only one effective product available. Other fungicides are being screened and some have shown potential for leaf spot control, particularly the ergosterol biosynthesis inhibitors (EBIs). Hancock and Weete (4) reported an ED_{50} of 0.15 μg/ml for growth inhibition of *C. personatum* by propiconazole. Csinos et al (3) documented excellent activity of diniconazole on *C. personatum* and soilborne pathogens of peanut in the field. Diniconazole also acts as a growth regulator (7), and, because of its high efficacy, became the standard fungicide for comparison of other experimentals in peanuts.

Tebuconazole (HWG 1608 = Folicur) is another EBI that has shown promise for controlling several peanut pathogens. It is not registered for use in the United States, but a registration package has been submitted and is currently being evaluated by the Environmental Protection Agency. In experimental plots, tebuconazole has provided effective control of peanut leaf spot when applied full season via chemigation or conventional ground sprays (2). There is concern that fungicide resistance will become a problem with this class of compounds (6) and they will not be recommended as full-season, stand-alone products. The availability of an effective protectant fungicide, such as chlorothalonil, offers the potential for a variety of resistance management strategies.

In vitro studies documenting the sensitivity of *C. personatum* to tebuconazole or diniconazole have not been published. Such information is useful for comparing the fungitoxicity of compounds and establishing a baseline of sensitivity should resistance occur in the future. Therefore, the objectives of this research were to document the in vitro sensitivity of *C. personatum* to tebuconazole, chlorothalonil, and diniconazole and to assess efficacy of various rates and use patterns of tebuconazole in combination with chlorothalonil for control of late leaf spot of peanut.

MATERIALS AND METHODS

In vitro studies. Chlorothalonil was supplied as Bravo 720, diniconazole as Spotless 25W, and tebuconazole as Folicur 1.2 EC. Aqueous stock suspensions of different fungicide concentrations were obtained by serial dilution in sterile deionized water. These were added to water agar (1.5%) after it was autoclaved at 121 C for 15 min and cooled to about 50 C. The amended agar was mixed thoroughly and poured into 10-cm-diameter petri plates. All three fungicides were evaluated at concentrations of 0, 0.01, 0.1, 1.0, 10, and 100 μg a.i./ml. These rates were chosen after a preliminary study evaluating the fungitoxicity of tebuconazole.

Inoculum of *C. personatum* consisted of conidia collected from late leaf spot lesions on Florunner peanut leaves. Leaves were surface-disinfested in 0.53% NaOCl, rinsed in tap water, and placed abaxial side up in a moist chamber to induce sporulation. Conidia were collected with a cyclone spore collector (ERI Machine Shop, Ames, IA) and stored dry at 5 C until needed. Conidia were then suspended in sterile, deionized water plus Tween 80 (two drops per 100 milliliters). The spore suspension contained about 1×10^4 conidia per milliliter, and 0.5 ml was deposited on the surface of each plate via pipet and distributed with a sterile glass rod. Five replicate plates per fungicide concentration were seeded. Plates were then incubated with a 12-hr photoperiod (45–75 $\mu E \cdot m^2 \cdot s$) at 23–26 C.

Seven days after seeding, spore germination was observed with a microscope (×400). Twenty-four spores per plate were selected at random and evaluated according to a rating scale where 0 = no germination; 1 = short and/or shriveled germ tube, little growth; 2 = moderate growth and well developed hyphae but no sporulation; and 3 = abundant growth and conidia present. Because most response variables did not fit a classic dosage-response pattern, data were grouped by ratings and evaluated for differences across concentrations of each fungicide by a likelihood ratio chi-square test ($P \leq 0.05$).

Field studies. Studies were conducted over a 4-yr period from 1985 to 1988.

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All tests were conducted near Tifton, GA, in fields of Tifton sandy loam soil with some history of peanut production. The fields were subsoiled, bedded, and tilled with a Rototiller. Florunner peanuts were planted in all studies in single rows 0.91 m apart. Seeding rates varied from 84 kg/ha in 1987 to 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 (5). 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 (Folicur 1.2 EC) was compared with the industry standard, chlorothalonil (Bravo 500 or 720). Sprays were applied 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 spray schedule with seven sprays per season.

Late leaf spot was rated visually at harvest by estimating both the percentage of necrotic leaf area with a standard area diagram and percentage of defoliation by counting the number of missing leaflets and the total number of nodes on six main stems randomly chosen from each plot. Several tests were also evaluated with a hand-held multispectral radiometer on the 750-nm wavelength (CROPSCAN, Inc., Fargo, ND). The percentage of reflectance of radiation is an indication of the amount of healthy green leaf area and has been shown to be highly correlated with both foliar disease ratings and peanut pod yields (10). 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 (11).

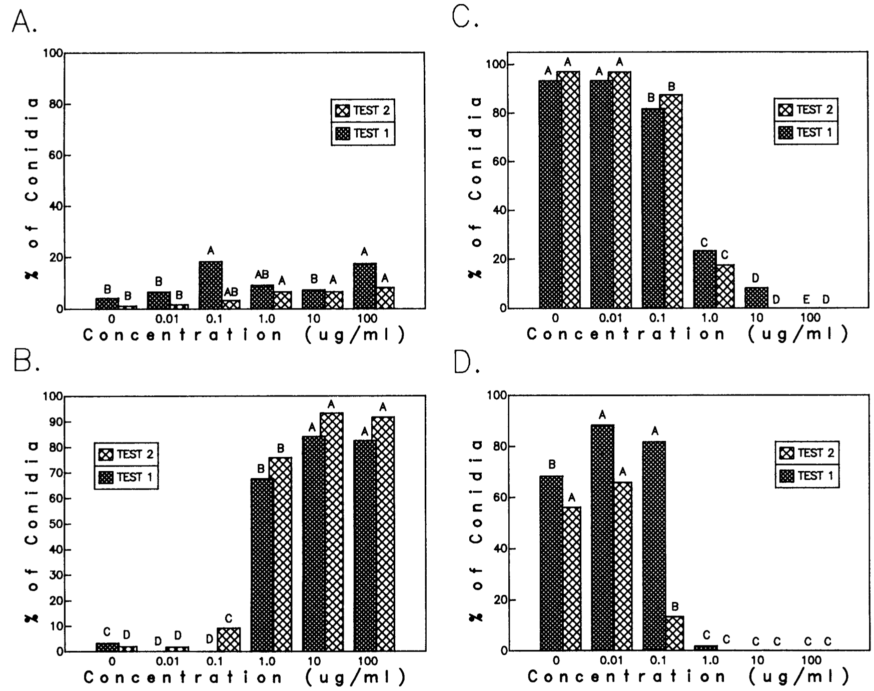

Fig. 1. Sensitivity of conidia from *Cercosporidium personatum* 7 days after seeding on tebuconazole-amended water agar, tests 1 and 2. **(A)** Percentage with no germination (rating class 0), **(B)** percentage with only a short or shriveled germ tube (rating class 1), **(C)** percentage with good mycelial growth, with or without sporulation (rating class 2 and 3), and **(D)** percentage with excellent mycelial growth and sporulation (rating class 3). Within each test, bars with the same letters in each figure are not significantly different according to a likelihood ratio chi-square test ($P \leq 0.05$).

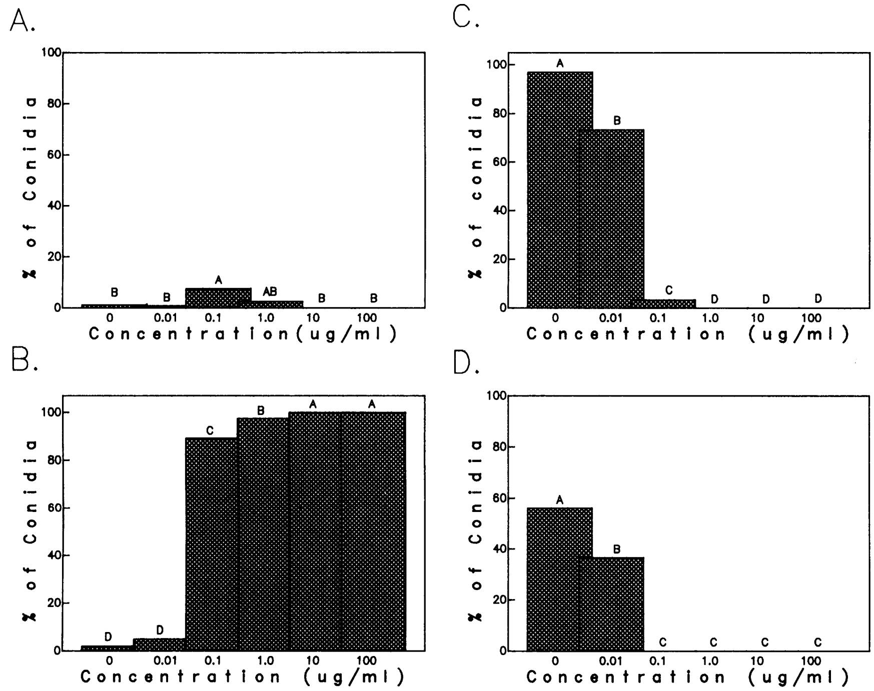

Fig. 2. Sensitivity of conidia from *Cercosporidium personatum* 7 days after seeding on diniconazole-amended water agar. **(A)** Percentage with no germination (rating class 0), **(B)** percentage with only a short or shriveled germ tube (rating class 1), **(C)** percentage with good mycelial growth, with or without sporulation (rating class 2 and 3), and **(D)** percentage with excellent mycelial growth and sporulation (rating class 3). Within each test, bars with same letters are not significantly different according to a likelihood ratio chi-square test ($P \leq 0.05$).

RESULTS

In vitro studies. The sensitivity of conidia of *C. personatum* to tebuconazole was similar in both trials, although sporulation was somewhat lower in test 2 than in test 1, even on the nonamended agar. Tebuconazole did not inhibit spore germination, even at the 100 μg/ml rate (Fig. 1A). However, when the concentration was 1.0 μg/ml or higher, the majority of conidia had only very short or shriveled germ tubes (Fig. 1B). The ability of germinated conidia to produce more extensive hyphal growth was greatly inhibited at 1.0 μg/ml and virtually eliminated at 10 μg/ml (Fig. 1C). Overall, conidial production responded similarly to mycelial growth (Fig. 1D).

Diniconazole also did not prevent conidia from germinating (Fig. 2A) but was more effective than tebuconazole in preventing mycelial growth (Fig. 2B,C). The parameter exhibiting the greatest difference in comparison with tebuconazole was sporulation (Fig. 2D). No conidia were formed on media amended with diniconazole at 0.1 μg/ml, whereas at least 1.0 μg/ml of tebuconazole was required to achieve the same effect (Fig. 1D).

In contrast to the two EBIs, chlorothalonil was extremely effective at preventing spore germination. Even at concentrations as low as 0.1 $\mu g/ml$, nearly 100% of the conidia did not form a germ tube (Fig. 3A). At 0.01 $\mu g/ml$ of chlorothalonil, mycelial growth was not affected, but there was some suppression of sporulation (Fig. 3C,D).

Field studies. Severe epidemics of late leaf spot developed in all eight tests evaluating full season sprays. The percentage of defoliation was consistently greater than 80% in untreated plots in each trial except for test B in 1988, when it was 59% (Table 1). Applications of chlorothalonil effectively slowed disease development with the final percentage of defoliation being only 20–30% in each test. Tebuconazole at 188, 210, or 250 g/ha applied full season (seven applications) gave leaf spot control equivalent to similar sprays of chlorothalonil at 1,260 g/ha (Table 2). This was reflected by ratings of the percentage of necrotic area as well as percentage of defoliation (Tables 1 and 2). All treatments increased pod yields, although the difference between the untreated plots and those treated with chlorothalonil was not always significant. Pod yields in plots treated with tebuconazole were consistently higher than in those treated with chlorothalonil, even though leaf spot control was essentially the same (Table 3).

Percent reflectance values, obtained from selected tests only, indicated that treatment with chlorothalonil (1,260 g/ha) resulted in large increases in the amount of green leaf area present compared with untreated plots (Table 4). Plots treated with tebuconazole seven times had reflectance values equal to or greater than the plots treated with chlorothalonil. Higher percentage of reflectance values usually indicate higher yields, as was the case in this study. Although the 800-nm wavelength was used in previous studies (10), two of these tests report reflectance of 750-nm wavelength radiation because of a mechanical problem with the radiometer. Reflectance of these two wavelengths of radiation is very similar and in 14 data sets on peanut was highly correlated ($r^2 = 0.95$, $P \leq 0.01$) (T. B. Brenneman, *unpublished*).

Various application strategies of tebuconazole and chlorothalonil demonstrated that there is flexibility in fungicide scheduling. Alternating sprays of tebuconazole (210 g/ha) with chlorothalonil (1,260 g/ha) provided leaf spot control similar to that obtained with a full-season regime of chlorothalonil (1,260 g/ha) (Table 5). This was true

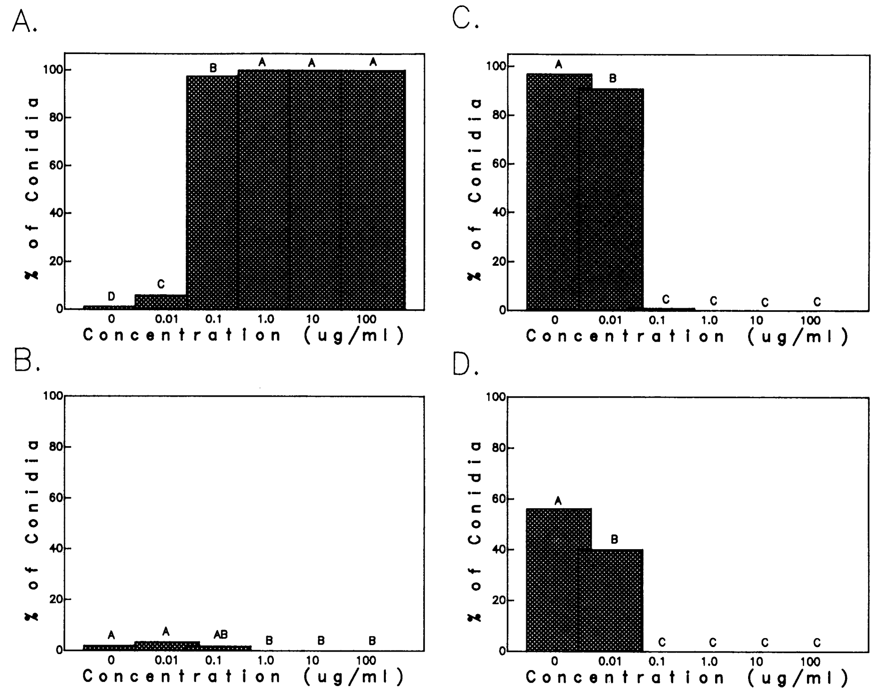

Fig. 3. Sensitivity of conidia from *Cercosporidium personatum* 7 days after seeding on chlorothalonil-amended water agar. **(A)** Percentage with no germination (rating class 0), **(B)** percentage with only a short or shriveled germ tube (rating class 1), **(C)** percentage with good mycelial growth, with or without sporulation (rating class 2 and 3), and **(D)** percentage with excellent mycelial growth and sporulation (rating class 3). Within each test, bars with same letters are not significantly different according to a likelihood ratio chi-square test ($P \leq 0.05$).

Table 1. Percentage defoliation of peanut after full-season, foliar sprays of tebuconazole and chlorothalonil on a 14-day schedule (seven applications) for control of late leaf spot (*Cercosporidium personatum*)

	Rate	1985	1986		1987		1988	
Treatment	**(g/ha)**	**Two tests**	**Test A**	**Test B**	**Test A**	**Test B**	**Test A**	**Test B**
Untreated		86.7 a[y]	85.7 a	82.9 a	92.2 a	83.0 a	98.1 a	59.1 a
Chlorothalonil	1,260	25.3 b	24.4 b	21.0 b	24.3 c	23.4 b	30.4 b	29.2 b
Tebuconazole	188	22.9 b	22.7 b	NT	29.8 b	28.0 b	31.0 b	NT
Tebuconazole	210	NT[z]	22.1 b	20.2 b	26.0 bc	26.3 b	27.6 b	27.9 b
Tebuconazole	250	23.2 b	NT	20.4 b	26.1 bc	27.9 b	28.8 b	NT

[y] Means in columns with the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).
[z] Treatment not included in this test or parameter was not evaluated.

Table 2. Percentage of necrotic leaf area of peanut after full-season, foliar sprays of tebuconazole and chlorothalonil on a 14-day schedule (seven applications) for control of late leaf spot (*Cercosporidium personatum*)

	Rate	1985	1986		1987		1988	
Treatment	**(g/ha)**	**Two tests**	**Test A**	**Test B**	**Test A**	**Test B**	**Test A**	**Test B**
Untreated		18.4 a[y]	15.8 a	14.8 a	16.5 a	19.2 a	18.5 a	18.7 a
Chlorothalonil	1,260	3.3 b	2.8 b	3.3 b	4.5 b	3.0 c	7.8 b	2.0 b
Tebuconazole	188	3.6 b	3.0 b	NT	3.3 c	5.6 b	4.5 c	NT
Tebuconazole	210	NT[z]	3.0 b	3.3 b	4.0 bc	3.6 c	6.5 b	2.0 b
Tebuconazole	250	3.3 b	NT	3.5 b	3.3 c	3.4 c	3.4 c	NT

[y] Means in columns with the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).
[z] Treatment not included in this test or parameter was not evaluated.

Table 3. Pod yield[w] of peanut after full-season, foliar sprays of tebuconazole and chlorothalonil on a 14-day schedule (seven applications) for control of late leafspot (*Cercosporidium personatum*)

Treatment	Rate (g/ha)	1985 Two tests	1986 Test A	1986 Test B	1987 Test A	1987 Test B	1988 Test A	1988 Test B
Untreated		2.8 a[x]	3.5[y]	4.6 a	1.5 a	1.7 a	NT	2.7 a
Chlorothalonil	1,260	7.0 b	4.7	5.2 a	4.0 b	2.9 b	NT	3.0 a
Tebuconazole	188	7.5 b	5.5	NT	6.0 c	4.6 c	NT	NT
Tebuconazole	210	NT[z]	5.1	6.2 b	6.0 c	4.5 c	NT	4.8 b
Tebuconazole	250	7.6 b	NT	6.2 b	NT	4.7 c	NT	NT

[w] Metric tons per hectare.
[x] Means in columns with the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).
[y] Only average yields per treatment were available.
[z] Treatment not included in this test or parameter was not evaluated.

when tebuconazole was applied on sprays 1, 3, 5, and 7 or on sprays 2, 4, and 6.

Reflectance from crop canopies treated with alternating spray regimes of tebuconazole and chlorothalonil were equal to or greater than reflectance from plots treated with only chlorothalonil (Table 4).

DISCUSSION

Tebuconazole inhibited growth and development of *C. personatum* at relatively low concentrations. Chlorothalonil was even more effective, but differences in the mode of action of these compounds must be considered. Chlorothalonil is a protectant-type material that is active only on the surface of the plant. It is a multisite inhibitor that disrupts numerous functions of fungal cells by binding to enzyme thiol groups (13). The EBIs, such as tebuconazole, are systemic and are thought to disrupt the synthesis of ergosterol by fungi. Ergosterol is essential to maintenance of fungal membrane structure, and without it the physical stability of membranes, as well as the activity of membrane-bound enzymes, can be affected adversely (1). It has been demonstrated previously that germinating spores may not be affected by EBIs (12) and, therefore, this parameter may not be appropriate for evaluating these fungicides.

Another factor that makes spore germination alone to be of limited value in evaluating EBI fungicides is that these compounds function primarily inside the host plant and can serve in a curative fashion for existing infections (12). In fact, greenhouse studies with tebuconazole on peanut have shown that when acting systemically, it does not inhibit germination of spores of *C. personatum* on the leaf surface but rather serves to prevent colonization once a germ tube has entered the leaf (8). However, the rating system used in the in vitro portion of this study allows the differentiation between simple germination of conidia and their continued growth and development into a potentially infective entity.

These in vitro data indicate that tebuconazole should be an effective fungicide for controlling late leaf spot, and field studies verified this efficacy. Pod yields in plots treated with tebuconazole were consistently higher than in plots treated with chlorothalonil, probably because of the additional control of soilborne pathogens such as *Rhizoctonia solani* Kühn and *Sclerotium rolfsii* Sacc. Rates required for disease control are considerably less than for the current standard, chlorothalonil, but are somewhat higher than effective rates of diniconazole (3). This difference may be a reflection of the relative sensitivity of the pathogen to the two EBI fungicides as determined in vitro in this study.

There appears to be flexibility in scheduling tebuconazole sprays in conjunction with chlorothalonil applications. Multiple years of research indicate that in alternating spray programs, leaf spot control with tebuconazole tends to be superior when it is applied on sprays 1, 3, 5, and 7 as compared with sprays 2, 4, and 6 (A. P. Murphy, *unpublished*).

Table 4. Evaluation of radiation reflectance from peanut canopies treated with tebuconazole on a 14-day schedule (seven applications) or alternating with chlorothalonil

Treatment	Rate (g/ha)	Application no.[w]	Reflectance (%), 750 nm 1987 Test A[x]	1988 Test A	1988 Test B
Untreated			16.5 d[y]	10.5 d	25.7 c
Chlorothalonil	1,260	1–7	46.7 b	41.6 b	39.7 b
Tebuconazole	188	1–7	50.1 a	42.9 b	NT[z]
Tebuconazole	210	1–7	50.5 a	45.1 a	47.8 a
Tebuconazole	250	1–7	51.1 a	45.0 a	NT
Tebuconazole	210	1,3,5,7	43.5 c	44.3 a	46.4 a
Tebuconazole	210	2,4,6	50.6 a	39.5 c	46.2 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] 800 nm used instead of 750 nm.
[y] Means with the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).
[z] Treatment not included in this test.

Table 5. Peanut late leaf spot disease ratings and pod yields evaluating alternating spray schedules of tebuconazole and chlorothalonil

Treatment	Rate (g/ha)	Application no.[w]	1987 Test A Defoliation (%)	1987 Test A NA[x] (%)	1987 Test A Yield[y] (t/ha)	1988 Test A Defoliation (%)	1988 Test A NA[x] (%)	1989 Test B Defoliation (%)	1989 Test B NA[x] (%)	1989 Test B Yield[y] (t/ha)
Untreated			92.2 a[z]	16.5 a	1.5 c	98.1 a	18.5 a	59.1 a	18.7 a	2.3 b
Chlorothalonil	1,260	1–7	24.3 c	4.5 c	4.0 b	30.4 c	7.8 b	29.2 b	2.0 b	2.5 b
Tebuconazole	210	1,3,5,7	35.6 b	9.0 b	5.8 a	30.1 c	8.3 b	25.7 b	2.3 b	3.7 a
Tebuconazole	210	2,4,6	24.9 c	4.0 c	6.1 a	38.3 b	9.0 b	29.1 b	1.7 b	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] Percentage of necrotic leaf area.
[y] Metric tons per hectare.
[z] Means with the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t* test ($P \leq 0.05$).

This may be attributable to the curative type activity of this compound, which serves to abort existing infections (12). Such activity would most likely manifest itself at the beginning of the season when some infections have occurred before the first spray is applied.

Ultimate use patterns will be determined by resistance management philosophy, price structure, and other marketing considerations. Currently, there is still no consensus among the scientific community as to whether alternating or block sprays of EBIs with protectant fungicides such as chlorothalonil offer the most effective resistance management strategy (6). Regardless of the ultimate use pattern, tebuconazole should be a valuable tool for management of late leaf spot of peanut.

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