Factors Affecting Chemical Control of Southern Blight of Peanut in Oklahoma

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ABSTRACT

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Resistance to pentachloronitrobenzene (PCNB) was not detected among 112 isolates of *Sclerotium rolfsii* collected from commercial peanut fields in Oklahoma and assayed for inhibition of radial growth in vitro. Values of PCNB concentration that provided 50% inhibition of growth (EC_{50}) were normally distributed about a mean of 2.86 ± 1.45 μg/ml. An isolate of *S. rolfsii* from apple, collected where PCNB had never been used, had a comparable EC_{50} of 3.29 ± 0.18 μg/ml. The recommended PCNB use pattern, one or two applications at 0.56 kg/ha in a wide (30-cm) band over the row, gave variable control of southern blight (23–90%) and peanut yield increases (102–915 kg/ha) in five field trials. In field trials comparing different combinations of PCNB rates and band widths, half rates (2.8 kg/ha) applied in a wide band resulted in no disease control in one trial where full rates (5.6 kg/ha) in a wide band and half rates in a narrow (10-cm) band were effective. None of the PCNB treatments were effective in a second trial. Increasing the concentration of PCNB at the plant crown with full rates in a narrow band did not improve disease control compared to full rates in a wide band. One (1.12 kg/ha) or two (0.84 kg/ha) banded sprays with flutolanil or three foliar sprays of tebuconazole (0.14–0.16 kg/ha) provided more consistent disease control than did PCNB. However, yield increases from the experimental fungicides were not always greater than those from PCNB nor were they as large as those reported in areas where yields are higher and both southern blight and Rhizoctonia limb rot are problems. EC_{50} values for isolates of *S. rolfsii* were determined for flutolanil (0.08 ± 0.04 μg/ml) and tebuconazole (0.06 ± 0.02 μg/ml) for references in resistance monitoring if these fungicides are registered in the future.

Southern blight or stem rot, caused by *Sclerotium rolfsii* Sacc., limits yield of peanut (*Arachis hypogaea* L.) in Oklahoma and other parts of the southern United States. The fungus frequently kills plants prior to harvest, and its effect on yield is proportional to disease incidence (4,18). *S. rolfsii* also causes pod rot, which may occur on plants without aboveground symptoms (2).

Control of southern blight can be achieved with the integration of cultural practices such as crop rotation, deep plowing, and nondirting cultivation (14). In Oklahoma, most of the peanut hectarage is irrigated, and a lack of profitable rotational crops frequently results in short (1-yr) rotations or continuous peanuts, which increase inoculum levels over time. Therefore, growers have relied on chemicals to augment cultural practices in problem fields. Pentachloronitrobenzene (PCNB) has been the most commonly used fungicide against southern blight for many years in Oklahoma and other states (5,16,17). A common pattern of PCNB use in other states has been one application of 11.2 kg/ha in a 30-cm band over the row when plants are actively pegging (growth stage R3 or R4) (3). However, disease control has been inconsistent (5,7–10,15,16,25).

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The recommended use pattern for PCNB in Oklahoma has been a split application of 5.6 kg/ha in a 30-cm band, once at pegging and again 3–4 wk later, if needed. Complaints from growers and crop advisors about poor disease control with PCNB have been numerous in recent years. The cost of PCNB has risen sharply, and it has sometimes been in short supply during this time. One possible cause of variable results with PCNB is the use of reduced application rates in an attempt to lower chemical costs. Also, to avoid vine damage during a second application, broadcast treatments are sometimes made, which further decrease PCNB concentration at the plant crown where infections occur. Csinos (10) reported that half rates of PCNB were as effective as full rates when applied in a narrow (10-cm) band. Resistance to PCNB in *S. rolfsii* is a suspected cause of variable disease control in Texas (24). Resistance to PCNB has been reported in *S. rolfsii* (1,26) and *Rhizoctonia solani* (13,20) but not in *Sclerotinia minor* (6).

The experimental fungicides tebuconazole and flutolanil provide better control of southern blight than PCNB (5,9,15) but are not registered for use on peanuts. Tebuconazole is a member of the ergosterol biosynthesis inhibitor (EBI) class of systemic fungicides to which there is potential for resistance development following intensive usage (12,13). Flutolanil is also a systemic fungicide that inhibits an enzyme involved with respiration in basidiomycete fungi (technical report, NOR-AM, Wilmington, DE), but the potential for resistance development to flutolanil is not known.

This research was conducted to examine the factors affecting control of southern blight with PCNB in Oklahoma. The objectives were to determine whether resistance to PCNB exists in the local population of *S. rolfsii*, to assess the efficacy of application rates and methods in disease control, and to compare the disease and yield responses to treatments with PCNB, flutolanil, and tebuconazole. In addition, the sensitivity of several isolates of *S. rolfsii* to tebuconazole and flutolanil was determined.

MATERIALS AND METHODS

In vitro studies. Peanut stems with symptoms and signs of infection by *S. rolfsii* were collected from 26 fields in five Oklahoma counties in 1990. Most fields had a long history of peanut culture and PCNB usage. Sample size varied from one to 10 stems per field. Infected stems were incubated on moist paper towels in sealed chambers until sclerotia of *S. rolfsii* were observed. One sclerotium per stem was transferred to potato-dextrose agar (PDA) plates acidified to pH 5.0 with lactic acid. Plates were incubated at room temperature for 21 days, and sclerotia were harvested, air-dried, and stored in vials for later use.

Sensitivity to PCNB was determined for 112 isolates (one to nine per field) by measuring radial growth on amended PDA. Autoclaved PDA was cooled to approximately 50 C and amended with aqueous suspensions of Terraclor 75W (Uniroyal, Middleberry, CT) to achieve PCNB concentrations of 0, 0.1, 1, 5, 10, and 100 μg/ml. Amended plates were inoculated with 4-mm-diameter mycelial plugs taken from the periphery of 3-day-old colonies that were initiated from sclerotia in the storage vials. The assay was blocked over time because of limited incubator space and labor. Each of the three blocks consisted of one plate per isolate and PCNB concentration. The plates were randomized by isolate and incubated in darkness for 72 hr at 25 C. A reference isolate of *S. rolfsii*, obtained from an apple seedling that was collected at a site in Oklahoma where PCNB had never been used, was also assayed for sensitivity to PCNB.

The sensitivity of *S. rolfsii* to flutolanil, tebuconazole, and PCNB was compared using the methods described above for eight of the isolates selected at random. Aqueous suspensions of Moncut 70WDG (NOR-AM) and Folicur 3.6F (Miles, Kansas City, MO) were each added to PDA to obtain concentrations of 0, 0.001, 0.01, 0.1, 1, 10, and 100 μg/ml of flutolanil and tebuconazole, respectively. Cultures were randomized by isolate and fungicide on three incubator shelves (blocks).

The effective concentration for 50% inhibition of radial growth (EC_{50}) was calculated by regression analysis. Percent inhibition (y), determined by comparing radial growth on each amended plate to that on a nonamended plate, was regressed on the log of fungicide concentration by isolate and block. The mean EC_{50} value and its standard deviation were calculated for each isolate. Analysis of variance was used to test for block effects. The distribution of EC_{50} values to PCNB was compared for goodness of fit to a normal distribution by chi-square analysis.

Field studies. Five trials were conducted at three locations in Oklahoma during 1989–1991 to assess southern blight control with the recommended PCNB use pattern of 5.6 kg/ha applied in a 30-cm band. The performance of PCNB was compared to that of flutolanil and tebuconazole in four and two of the trials, respectively. Each site had been cropped to peanut the previous year and had a history of damage from *S. rolfsii*. The first site at Stillwater had a Port silt loam soil and was seeded with the cultivar Spanco on 11 May 1989, 15 May 1990, and 15 May 1991. The second site at Holdenville had a Bates fine sandy loam and was seeded with the cultivar Okrun on 5 June 1990. The third site at Perkins had a Vanoss silt loam and was seeded with the cultivar Spanco on 17 May 1991. Cultural and weed control practices were applied to the crops according to Oklahoma Cooperative Extension Service recommendations (23).

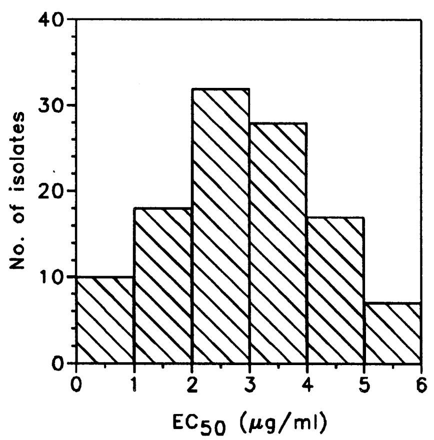

Fig. 1. Frequency distribution of EC_{50} values to PCNB determined for 112 isolates of *Sclerotium rolfsii* collected from peanut fields in Oklahoma. The mean EC_{50} was 2.86 ± 1.45 μg/ml.

All sites received sprinkler irrigation as needed to prevent moisture stress.

Treatments were applied to four-row plots that were arranged in four randomized complete blocks. Rows were 7.5 m long and spaced 0.9 m apart; all data were collected from the middle two rows. Terraclor 10G (Uniroyal) was applied at a rate of 5.6 kg/ha of PCNB in a 30-cm band with a granular applicator driven by a bicycle wheel. All sites received two applications except Stillwater in 1989, which received one. The first application was made at pegging (approximately 60 days after planting, growth stage R3-R4) and the second was made 21–30 days later. Foliar sprays of tebuconazole or flutolanil were applied with a wheelbarrow sprayer. Tebuconazole (Folicur 3.6F) was applied three times on a 14-day schedule as a full-canopy spray through three hollow-cone nozzles per row in 243 L/ha. The first application of tebuconazole was made on 30 July 1990 at Holdenville (0.16 kg/ha) and 6 July 1991 at Stillwater (0.14 kg/ha). Flutolanil (Moncut 50W) at 1.12 kg/ha was applied once at pegging in 243 L/ha either as a full-canopy spray at Holdenville in 1990 or as a 30-cm band through one flat-fan nozzle centered over the row at Stillwater in 1990 and at Perkins in 1991. Two full-canopy applications of flutolanil at 0.84 kg/ha were made 14 days apart at Stillwater in 1991 beginning at pegging. All plots at each site received four to six sprays of Bravo 720 at 1.26 kg/ha of chlorothalonil on a 14-day schedule to control early leaf spot.

Incidence of southern blight was determined prior to harvest by counting row segments with infected plants (18). Disease incidence (%) was calculated with the formula: ((number of 15-cm row segments with infected plants × 15)/row length) × 100. Plants were dug and dried in windrows for 2 days, then pods were mechanically removed from vines with a stationary thresher. Yields were determined after drying pods to approximately 10% moisture. Digging dates were 4 October at Stillwater in 1989, 26 September at Stillwater in 1990, 29 October at Holdenville in 1990, and 30 September at Stillwater and Perkins in 1991.

Different rates, timings, and band widths of PCNB application were compared in additional field trials at Calera in 1991 and 1992. The field had a Larton loamy fine sand and a history of continuous peanut culture and southern blight. The cultivars Florunner and Tamspan 90 were seeded on 11 May 1991 and 1 May 1992, respectively. Production practices, experimental design, irrigation, and equipment for treatments were the same as described above. Two applications of Terraclor 10G at 2.8 and 5.6 kg/ha of PCNB were made in both 30- and 10-cm bands at pegging and 30 days after pegging. These were compared with single applications of Terraclor 10G (11.2 kg/ha of PCNB) and Moncut 50W (1.1 kg/ha of flutolanil) at pegging in 30-cm bands. Early leaf spot was controlled in all plots with five (1991) and six (1992) applications of Bravo 720 at 1.26 kg/ha of chlorothalonil on a 14-day schedule. Disease incidence and yield were measured as described above except that incidence was evaluated after plots were dug in 1992. Digging dates were 6 November 1991 and 9 September 1992.

Simple correlation analysis was used to assess the contribution of disease incidence to variation in yield for each field test. Analysis of variance was performed on disease incidence and yield data by year and location. Treatment means were separated with the least significant difference (LSD) test.

RESULTS

In vitro studies. PCNB inhibited the radial growth of each isolate of *S. rolfsii* at all concentrations tested. The mean EC_{50} value was 2.86 ± 1.45 μg/ml, with a minimum and maximum of 0.03 and 5.63 μg/ml, respectively, compared to the EC_{50} of 3.29 ± 0.18 μg/ml for the apple isolate. The frequency distribution of EC_{50} values was bell-shaped, and one standard deviation included 74% of the isolates (Fig. 1). Chi-square analysis indi-

Table 1. Sensitivity of eight isolates of *Sclerotium rolfsii* to the fungicides PCNB, flutolanil, and tebuconazole in PDA cultures

	EC_{50} (μg/ml)[b]		
Isolate[a]	PCNB	Flutolanil	Tebuconazole
24 Caddo	0.432 ± 0.140	0.074 ± 0.011	0.076 ± 0.007
30 Caddo	2.437 ± 0.396	0.082 ± 0.017	0.037 ± 0.007
44 Bryan	2.498 ± 1.277	0.067 ± 0.030	0.052 ± 0.002
66 Bryan	2.849 ± 0.356	0.060 ± 0.020	0.065 ± 0.010
80 Bryan	1.996 ± 0.098	0.073 ± 0.022	0.069 ± 0.022
82 Bryan	1.499 ± 0.434	0.042 ± 0.013	0.060 ± 0.025
100 Hughes	1.440 ± 0.631	0.105 ± 0.061	0.043 ± 0.025
115 Hughes	1.956 ± 0.872	0.147 ± 0.052	0.081 ± 0.033
Mean	1.888 ± 0.905	0.081 ± 0.041	0.060 ± 0.022

[a] From indicated county in Oklahoma.
[b] Effective concentration for 50% inhibition of radial growth determined by regression analysis of percent inhibition (y) vs. log (concentration). Values are means of three blocks per isolate ± the standard deviation.

cated that the observed distribution was not significantly different from a normal distribution ($\chi^2 = 0.65$, $P = 0.98$). Sectors of rapid growth indicative of PCNB resistance were not observed on any amended cultures during the incubation period.

Flutolanil and tebuconazole were more effective than PCNB in inhibiting radial growth of *S. rolfsii* (Table 1). Flutolanil and tebuconazole were 23 and 31 times, respectively, more fungitoxic than PCNB, according to comparisons of mean EC_{50} values. Sectoring was not observed on plates amended with any of the fungicides. The EC_{50} values to PCNB for the eight isolates in the two assays were within one standard deviation. Block effects were not significant at $P \leq 0.05$ in either assay.

Field studies. Incidence of southern blight in the controls ranged from 8.7 to 26.5% at the five sites where the recommended pattern of PCNB use was tested. Plot yields were negatively correlated with disease incidence ($P \leq 0.05$) at all locations except Holdenville in 1990. Correlation coefficients (r) ranged from −0.34 to −0.77. PCNB reduced disease incidence ($P \leq 0.05$) at three of the five locations (Table 2). Disease reduction averaged 54% over the five locations and ranged from 23% (not significant) at Holdenville in 1990 to 90% at Stillwater in 1990. Flutolanil and tebuconazole reduced disease incidence ($P \leq 0.05$) at all locations. The degree of disease reduction with these fungicides was generally better than that with PCNB (Table 2). Disease control averaged 82% with flutolanil and 70% with tebuconazole.

Yield of the PCNB treatments was more variable than disease incidence. PCNB increased yield ($P \leq 0.05$) at two of the five locations (Table 2). Yield increases with PCNB averaged 529 kg/ha over the five locations and ranged from 102 kg/ha at Stillwater in 1989, where only one application was made, (not significant) to 915 kg/ha at the same location in 1990. Flutolanil increased yield ($P \leq 0.05$) at three of four locations (Table 2). Yield increases from flutolanil averaged 677 kg/ha and ranged from 224 (not significant) to 1,007 kg/ha at Stillwater in 1989 and 1990, respectively. Yield response to flutolanil was significantly better than that with PCNB only at Perkins in 1991. Tebuconazole increased yield ($P \leq 0.05$) at one of two locations and was not significantly better than PCNB at either location (Table 2).

At Calera, where different PCNB rates and application methods were compared, disease incidence in the control exceeded 25% in both 1991 and 1992. Plot yields were negatively correlated ($P < 0.01$) with disease incidence in 1991 ($r = -0.71$) and 1992 ($r = -0.54$). In 1991, all PCNB treatments except the half rate (2.8 kg/ha) in a wide (30-cm) band reduced disease incidence by 54–70% and increased yield ($P \leq 0.05$) (Table 3). There were no significant differences in disease incidence or yield between split and single applications of full rates, between split applications of full rates on a wide band or half rates on a narrow (10-cm) band, or between split applications of full rates or half rates on a narrow band. Flutolanil reduced disease incidence (83%) and increased yield ($P < 0.05$) but was no better than any PCNB treatment except the split application of half rates in a wide band.

At Calera in 1992, none of the PCNB treatments reduced disease incidence or increased yields (Table 3). There were also no differences in disease incidence or yield among PCNB treatments. However, the flutolanil treatment reduced disease incidence by 73% and increased yield by 610 kg/ha ($P < 0.05$). Flutolanil reduced disease incidence compared to PCNB at full rate in a narrow band and half rate in a wide band. Flutolanil also increased yield over the half rate of PCNB applied in a wide band ($P < 0.05$).

DISCUSSION

Southern blight development in a given year and location varies with inoculum levels, crop management practices, and environmental conditions

Table 2. Efficacy of PCNB, flutolanil, and tebuconazole for control of southern blight of peanut in five field experiments during 1989–1991

Year/location[a]	Treatment	Rate (kg a.i./ha)	Application method[b]	Timing[c]	Disease incidence[d] (%)	Yield (kg/ha)
1989/Stillwater	Flutolanil 50W	1.12	30-cm band	P	0.7	3,275
	PCNB 10G	5.60	30-cm band	P	5.0	3,153
	Control	...	...	...	8.7	3,051
	LSD ($P \leq 0.05$)				3.1	643
1990/Stillwater	Flutolanil 50W	0.84	Foliar	P, P + 14	1.0	4,344
	Tebuconazole 3.6F	0.14	Foliar	14-day	4.2	3,947
	PCNB 10G	5.60	30-cm band	P, P + 21	1.0	4,252
	Control	...	...	...	15.5	3,337
	LSD ($P \leq 0.05$)				6.7	571
1990/Holdenville	Flutolanil 50W	1.12	Foliar	P	2.8	3,611
	Tebuconazole 3.6F	0.16	Foliar	14-day	3.1	3,407
	PCNB 10G	5.60	30-cm band	P, P + 30	7.2	3,763
	Control	...	...	...	9.4	3,051
	LSD ($P \leq 0.05$)				4.8	497
1991/Stillwater	PCNB 10G	5.60	30-cm band	P, P + 30	6.7	3,641
	Control	...	...	...	23.5	3,336
	LSD ($P \leq 0.05$)				11.1	723
1991/Perkins	Flutolanil 50W	1.12	30-cm band	P	6.0	2,828
	PCNB 10G	5.60	30-cm band	P, P + 30	15.0	2,523
	Control	...	...	...	26.5	1,912
	LSD ($P \leq 0.05$)				14.6	848

[a]Peanut cv. Okrun at Holdenville and cv. Spanco at Stillwater and Perkins.
[b]Foliar applications were directed sprays with three nozzles per row.
[c]P = pegging growth stage, P + number = number of days after pegging, 14-day = three applications made 14 days apart beginning at pegging.
[d]Determined just prior to harvest by counting the number of 15-cm row segments with infected plants. Incidence (%) was determined by calculating: ((number of row segments with infected plants × 15)/row length) × 100.

(21,22). Averaging fungicide performance over years and locations is thus useful in assessing disease control recommendations. PCNB performed somewhat better in our studies than in others when full rates applied in a wide band were compared. Disease was reduced by 36% and yield was increased by 575 kg/ha in the 18 field trials in the Southeast where the single application schedule was used (7–11,15,16,25). Disease control averaged 51% and yield was increased 661 kg/ha in the seven trials reported here with the split application schedule. Results from this study and another (7) show no difference in PCNB performance between the two schedules.

PCNB provided variable control of southern blight in this study, which is consistent with other reports from the Southeast where disease control ranged from 8 to 53% and yields were increased by 124–1,394 kg/ha (7–11,15,16,25). Results from the in vitro screening suggest that resistance to PCNB in *S. rolfsii* may not be responsible for the variable performance in Oklahoma. The mean sensitivity of peanut isolates was comparable to the reference isolate from apple, and the distribution of EC_{50} values was within the normal range of variability. EC_{50} values for the least sensitive isolates (approximately 5 μg/ml) were less than the 50 μg/ml reported by Sharma and Verma (19) for an isolate of *S. rolfsii* not believed to be resistant. The EC_{50} values of our isolates were also comparable to those reported for two peanut isolates in Georgia (5.89 and 1.84 μg/ml) (5). Where PCNB resistance in *S. rolfsii* has been reported, resistance was induced in vitro (1,26). PCNB resistance in *S. rolfsii* resulting from field applications of the fungicide has never been confirmed (12,13).

Failure to apply a sufficient rate at the plant crown where infection occurs may contribute to variable disease control with PCNB. At Calera in 1991, where PCNB performed well, the full-rate applications in a wide (30-cm) band were effective but the half-rate application in a wide band was not. The latter treatment was chosen to simulate grower usage of reduced rates that have been common in recent years because of PCNB price increases. Broadcast applications of half rates would result in a further dilution of PCNB in the target area. Disease control with half rates was achieved with narrow (10-cm) band application, which agrees with previous reports (10,16) and provides an economic alternative to costly full-rate applications.

The experimental fungicide flutolanil provided more consistent control of southern blight than PCNB, as previously reported (9,10,15). Flutolanil reduced disease incidence by an average of 81% compared to 47% for PCNB in the six trials reported here where both

Table 3. Effects of application rate, timing, and band width of PCNB in comparison to flutolanil for control of southern blight of peanut at Calera, Oklahoma, in 1991 (cv. Florunner) and 1992 (cv. Tamspan 90)

Treatment	Rate (kg a.i./ha)	Application method	Timing[a]	Disease incidence[b] (%) 1991	1992	Yield (kg/ha) 1991	1992
PCNB 10G	5.6	30-cm band	P, P + 30	8.2	28.4	4,455	2,670
	2.8	10-cm band	P, P + 30	12.7	28.4	4,028	2,543
	11.2	30-cm band	P	11.2	24.7	4,475	2,619
	5.6	10-cm band	P, P + 30	12.5	37.8	3,947	2,695
	2.8	30-cm band	P, P + 30	24.0	36.2	3,112	2,390
Flutolanil 50W	1.1	30-cm band	P	4.5	9.1	4,109	3,026
Control	...	...	...	27.7	34.0	2,726	2,416
LSD ($P \leq 0.05$)				11.0	21.2	925	516

[a] P = pegging growth stage, P + 30 = 30 days after pegging.
[b] Determined just prior to harvest in 1991 and after plots were dug and inverted in 1992 by counting the number of 15-cm row segments with infected plants. Incidence (%) was determined by calculating: ((number of row segments with infected plants × 15)/row length) × 100.

fungicides were tested. However, the mean yield increase for flutolanil (783 kg/ha) was similar to that for PCNB (720 kg/ha). Disease control with flutolanil in this study compared favorably to the mean of 64% reported in Georgia (9,10) and to the mean of 61% in Alabama, where 1.1 kg/ha was applied twice (15). The mean yield increase from flutolanil in our trials with mainly Spanish cultivars was comparable to that reported in Alabama (618 kg/ha) but less than that in Georgia (1,844 kg/ha). Runner cultivars were used in the latter two studies. In addition to cultivar differences, the greater yield increases in Georgia may also be a result of higher overall yields and of control of Rhizoctonia limb rot in addition to southern blight. Limb rot is a minor disease in most years in Oklahoma, particularly on Spanish cultivars.

Tebuconazole also provided better disease control than PCNB (70% vs. 56%) at the two locations reported here where both chemicals were used. However, the yield increase from tebuconazole (483 kg/ha) was less than that for PCNB (813 kg/ha). The degree of southern blight control in our trials was nearly the same as that reported in Georgia (5) and Alabama (15), but yield increases from tebuconazole were greater in Georgia (1,725 kg/ha) and Alabama (849 kg/ha) than in our studies. In addition to differences between studies described above, a lower rate was used in our study.

Economic returns from chemical treatments for control of southern blight are largely impacted by fungicide price. The high cost of PCNB in recent years has reduced the profit margin resulting from its usage. Flutolanil and EBI fungicides such as tebuconazole offer distinct advantages over PCNB. Beside the more consistent control of southern blight, EBIs also control early and late leaf spots, while most EBIs and flutolanil also control Rhizoctonia limb rot. The economic advantage of EBIs and flutolanil over PCNB will depend on their cost. Furthermore, the development of resistance to EBI fungicides is an ongoing concern (12). Results from the in vitro studies could be used to monitor resistance development in Oklahoma should these fungicides be registered for use on peanut.

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