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Research Article

Fungicides and Application Timing for Control of Early Leafspot, Southern Blight, and *Sclerotinia* Blight of Peanut

W. James Grichar[1] and Jason E. Woodward[2,3]

[1] *Texas A&M AgriLife Research and Extension Center, 10345 State Highway 44, Corpus Christi, TX 78406, USA*
[2] *Texas A&M AgriLife Research and Extension Center, 1102 East FM 1294, Lubbock, TX 79403, USA*
[3] *Department of Plant and Soil Science, Texas Tech University, 2500 Broadway, Lubbock, TX 79409, USA*

Correspondence should be addressed to W. James Grichar; w-grichar@tamu.edu

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Field studies were conducted in 2013 and 2014 in south Texas near Yoakum and from 2008 to 2011 in central Texas near Stephenville to evaluate various fungicides for foliar and soilborne disease control as well as peanut yield response under irrigation. Control of *Sclerotinia* blight caused by *Sclerotinia minor* Jagger with penthiopyrad at 1.78 L/ha was comparable to fluazinam or boscalid; however, the 1.2 L/ha dose of penthiopyrad did not provide consistent control. Peanut yield was reduced with the lower penthiopyrad dose when compared with boscalid, fluazinam, or the high dose of penthiopyrad. Control of early leaf spot, caused by *Cercospora arachidicola* S. Hori or southern blight, caused by *Sclerotium rolfsii* Sacc., with penthiopyrad in a systems approach was comparable with propiconazole, prothioconazole, or pyraclostrobin systems and resulted in disease control that was higher than the nontreated control. Peanut yield was also comparable with the penthiopyrad, propiconazole, prothioconazole, or pyraclostrobin systems and reflects the ability of the newer fungicides to control multiple diseases found in Texas peanut production.

1. Introduction

In the southwestern United States, the management of soilborne and foliar diseases found in peanut (*Arachis hypogaea* L.) requires the use of a wide range of fungicides [1–5]. In all the peanut production areas of the USA, chlorothalonil has been the most widely used fungicide for control of early leaf spot, caused by *Cercospora arachidicola* S. Hori; late leaf spot, caused by *Cercosporidium personatum* (Berk. & M.A. Curtis); and rust, caused by *Puccinia arachidis* Speg. for over 30 years [6–8]. Despite its widespread use across the peanut belt, chlorothalonil continues to provide effective control of foliar diseases [3, 8]. Also, chlorothalonil is a protectant with no "reach back" or curative activity [2, 3, 5, 8]. However, chlorothalonil has no activity against any diseases caused by soilborne pathogens such as southern blight, caused by *Sclerotium rolfsii* Sacc.; *Rhizoctonia* pod or limb rot, caused by *Rhizoctonia solani* Kühn; or *Sclerotinia* blight, caused by *Sclerotinia minor* Jagger [2, 5, 8–10].

Currently, the sterol biosynthesis inhibitors (SBI) tebuconazole and prothioconazole, which are in the triazolinthione class of fungicides [11], have shown activity against *C. arachidicola* and *C. personatum*, as well as *S. rolfsii* and *R. solani* [3, 12]. Prothioconazole has been used for the control of cereal diseases in Europe when applied alone or in combination with other fungicides [11]. In addition, the activity of this fungicide on foliar diseases is of special interest because populations of both leaf spot pathogens have displayed reduced sensitivity to tebuconazole and noticeable reductions in efficacy of that fungicide [12].

The quinone outside inhibitor (QoI) fungicides which include azoxystrobin and pyraclostrobin (FRAC, Group 11) have been registered for use in peanut in the USA for control of both foliar and soilborne diseases [2, 6, 9, 13, 14]. Depending on the fungicide, a calendar-based spray regime in the southeastern USA may result in as many as seven applications [6, 7] while in the southwest peanut growing region a maximum of five fungicide applications are generally made during the growing season depending on weather conditions [1, 2]. Chlorothalonil is used in combination with programs utilizing azoxystrobin, pyraclostrobin, or tebuconazole to minimize the risk of fungal pathogens developing resistance [7]. Currently, the fungicides fluazinam and boscalid are used to control *Sclerotinia* blight [4, 5, 15].

A recently developed fungicide, penthiopyrad, is in the carboxamide group and is classified as a succinate dehydrogenase inhibitor (SDHI) that limits fungal growth by interfering with energy production in the mitochondrial electron transport group [16]. This mode of action is different from that of the SBI or Qol fungicides. In addition, cross-resistance between either the SBI or Qol fungicides and carboxamide fungicides does not appear to be likely [16–18]. Penthiopyrad was registered for use in peanut in the USA during the 2012 growing season [19].

Most of the irrigated peanuts in Texas are treated with fungicides approximately two to five times during the growing season to control leaf spot and soilborne diseases (author's personal observations). Fungicide applications are typically initiated 45 to 60 d after planting and subsequent applications follow a 21-to-28 d interval. Since little information is available on the use of penthiopyrad in peanuts, the objective of this study was to determine the effectiveness of various fungicides including penthiopyrad on foliar and soilborne diseases of peanut and peanut response to these fungicides under Texas growing conditions at several locations across the state. Of particular interest were comparisons of penthiopyrad with chlorothalonil for foliar disease control, comparison of penthiopyrad with fluazinam and boscalid for *Sclerotinia* blight control, and comparison of prothioconazole and tebuconazole combinations for southern blight control.

2. Materials and Methods

2.1. Field Experiments. Studies were conducted in two different peanut growing regions of Texas to determine disease control and peanut response to applications of penthiopyrad in comparison with other fungicides applied alone and in combination. Field studies at south Texas were conducted at the Texas AgriLife Research site near Yoakum (29.276°N, 97.123°W) while the central Texas studies were conducted at the Texas AgriLife Research and Extension Center near Stephenville (32.253°N, 98.191°W). Soil at Yoakum was Tremona loamy fine sand (thermic Aquic Arsenic Paleustalfs) with less than 1% organic matter and pH 7.0 to 7.2. This field site has been in continuous peanut for over forty years so there was a high concentration of soilborne and foliar disease inoculum. Soil at Stephenville was a Windthorst loamy sand (fine mixed thermic Udic Paleustalfs) with less than 1% organic matter and pH of 7.2 and has also been in extensive peanut production for the past fifty years.

2.2. Study Variables

2.2.1. South Texas. Studies in south Texas were conducted from 2013 to 2014 to determine early leaf spot and southern blight control by fungicides. Fungicides were applied with a CO_2-propellant backpack sprayer equipped with three D2–23 hollow-cone spray nozzles per row in 140 L of water/ha at a pressure of 504 kPa. The experimental design was a randomized complete block with four replications. All studies included a nontreated control. Each plot consisted of four rows spaced 97 cm apart and 6.3 m long. The varieties Georgia 09B [20] and McCloud [21] were planted on June 6, 2013, and June 5, 2014, at a seeding rate of 112 kg/ha. Fungicides were applied 60 days after planting (DAP), 80 DAP, 100 DAP, or 120 DAP or combinations of the above.

These studies included the following treatments: (1) nontreated control; (2) the premix of propiconazole (0.036 kg ai/L) plus chlorothalonil (0.479 kg ai/L) (TiltBravo 4.3SE®, Syngenta Crop Protection Inc., Greensboro, NC) at 1.75 L/ha applied 60 and 100 DAP plus azoxystrobin (0.249 kg ai/L) (Abound 2.08F®, Syngenta Crop Protection Inc.) at 0.88 L/ha and cyproconazole (0.099 kg ai/L) (Alto 100SL®, Syngenta Crop Protection Inc.) at 0.4 L/ha applied 80 and 120 DAP; (3) the premix of propiconazole plus chlorothalonil at 1.75 L/ha applied 60 DAP plus prothioconazole (0.144 kg ai/L) plus tebuconazole (0.288 kg ai/L) (Provost® 433SC, Bayer CropScience, Research Triangle Park, NC) at 0.59 L/ha applied 80, 100, and 120 DAP; (4) chlorothalonil (0.719 kg ai/L) (Bravo WeatherStik 6SC®, Syngenta Crop Protection Inc.) at 1.75 L/ha applied 60 DAP plus prothioconazole plus tebuconazole at 0.59 L/ha applied 80, 100, and 120 DAP; (5) the premix of propiconazole plus chlorothalonil at 1.75 L/ha applied 60 DAP plus penthiopyrad (0.2 kg ai/L) (Fontelis®, Dupont Crop Protection, Wilmington, DE) at 1.17 L/ha applied 80, 100, and 120 DAP; (6) pyraclostrobin (0.25 kg ai/L) (Headline® 2.09EC, BASF Corp., Research Triangle Park, NC) at 0.66 L/ha applied 60 DAP, and penthiopyrad at 1.17 L/ha applied 80, 100, and 120 DAP; (7) pyraclostrobin at 0.66 L/ha applied 60 DAP, and the premix of prothioconazole plus tebuconazole at 0.51 L/ha applied 80, 100, and 120 DAP; (8) pyraclostrobin at 0.66 L/ha applied 60 DAP plus flutolanil (0.455 kg ai/L) (Convoy®, Nichino America, Wilmington, DE) at 1.17 L/ha applied 80, 100, and 120 DAP; and (9) chlorothalonil alone at 1.75 L/ha applied 60, 80, 100, and 120 DAP.

2.2.2. Central Texas. Studies in central Texas were conducted from 2008 through 2011 in a field severely infested with *S. minor*. These studies included the fungicides boscalid (Endura® 70DG, BASF Corp.) at 701.0 g/ha and fluazinam (Omega 500F®, Syngenta Crop Protection, Inc.) at 1.78 L/ha in comparison with penthiopyrad at 1.22 and 1.78 L/ha. Each plot consisted of two rows spaced 91 cm apart and 7.9 m long. Fungicides were applied 70 DAP with a second application approximately 30 d later using a CO_2-propellant backpack sprayer equipped with two 8003 flat fan spray nozzles per row in 187 L of water/ha at a pressure of 241 kPa. The runner-type variety Flavor Runner 458 [22] was planted each year of the study at a seeding rate of approximately 15 seeds/m or 95 kg/ha.

2.3. Disease Evaluations. Peanut phytotoxicity ratings were taken 7 d after treatment at Yoakum. Peanut injury was visually estimated on a scale of 0 to 100 (0 indicating no leaf chlorosis or necrosis and 100 indicating completely killed peanut), relative to the nontreated control. Severity of leaf spot was rated in the two center rows using the Florida leaf spot scoring system where 1 = no leaf spot and 10 = plants

Table 1: Rainfall and irrigation for each year of the study.

Month	South Texas				Central Texas							
	Rainfall (R)		Irrigation (I)		Rainfall (R)				Irrigation (I)			
	2013	2014	2013	2014	2008	2009	2010	2011	2008	2009	2010	2011
	mm											
June	26.7	78.5	0.0	0.0	30.5	7.6	76.2	1.1	95.3	38.1	38.1	25.4
July	63.7	5.6	69.4	50.8	47.2	79.0	173.7	0.0	203.2	133.4	88.9	152.4
August	88.1	31.5	50.8	50.8	50.3	96.3	70.4	101.3	114.3	127.0	190.5	196.8
September	160.8	87.6	0.0	25.4	55.9	57.4	202.4	8.4	101.6	95.3	63.5	165.1
October	122.7	24.1	0.0	38.1	27.7	106.2	38.9	152.4	38.1	0.0	0.0	88.9
November	0.0	65.3	0.0	0.0	40.4	14.7	11.2	2.3	0.0	0.0	6.4	12.7
Total	462.0	292.6	120.2	165.1	252.0	361.2	572.8	265.5	552.5	393.7	387.4	641.4
Total $R + I$	582.2	457.7			804.5	754.9	960.2	806.6				

completely defoliated and dead because of leaf spot [6, 12]. Values of 1 through 4 on the scale reflect increasing incidence of leaflets with spots and occurrence of spots in lower versus upper canopy of the plots, whereas values 4 through 10 reflect increasing levels of defoliation [23]. The leaf spot rating was taken immediately prior to peanut digging.

Loci of southern stem blight were counted immediately after peanut plants were inverted, whereas loci of *Sclerotinia* blight were counted prior to peanuts being inverted. A locus represented 31 cm or less of linear row with one or more plants infected with *S. rolfsii* or *S. minor* [24].

2.4. Rainfall, Irrigation, and Weed Control. Rainfall and irrigation data was collected at each location (Table 1). Peanuts were dug approximately 140 d after planting at the south and central Texas locations.

All test areas were maintained weed-free with a preemergence tank-mix application of pendimethalin (Prowl H_2O®, BASF Corp.) at 1.06 kg/ha plus *S*-metolachlor (Dual Magnum® 7.62 L, Syngenta Crop Protection, Inc.) at 1.42 kg ai/ha. Overhead sprinkler irrigation was applied on a 1-to-2 wk schedule throughout the growing season as needed (Table 1).

2.5. Data Collection. Peanut yields were obtained by digging each plot separately, air-drying in the field for 4 to 7 d, and harvesting pods from each plot with a combine. Weights were recorded after soil and trash were removed from plot samples and were adjusted to 10% moisture. Peanut grades were determined in south Texas but not from central Texas. Grade samples were determined by subjecting a 250 g pod sample using screens specified in USDA grading procedures [25].

2.6. Data Analysis. Data were subjected to ANOVA and analyzed using SAS PROC MIXED with locations and years designated as random effects in the model [26]. Treatment means were separated using Fisher's Protected LSD at $P < 0.05$. Since a treatment by year interaction was observed for all variables tested, means are presented individually.

3. Results and Discussion

3.1. Peanut Diseases

3.1.1. Early Leaf Spot. Early leaf spot incidence in 2013 was high due to late season (September and October) rainfall and/or irrigation (Table 1) resulting in high nighttime and early morning humidity and mild temperatures and ideal conditions for development of the early leaf spot fungus [3, 12]. All fungicides reduced the incidence of early leaf spot when compared with the nontreated control (Table 2). Propiconazole plus chlorothalonil applied 60 and 100 DAP followed by azoxystrobin plus cyproconazole applied 80 and 100 DAP resulted in the lowest early leaf spot development (3.2) while combinations which included penthiopyrad resulted in leaf spot control which ranged from 4.9 to 5.5 (based on the Florida scale). Pyraclostrobin followed by flutolanil and the nontreated control resulted in the highest levels of leaf spot. Chlorothalonil alone provided intermediate control. While pyraclostrobin may be the most effective fungicide for leaf spot [27], flutolanil is not active against leaf spots and thus may explain the poor leaf spot control [10].

Early leaf spot incidence in 2014 was not as great as in 2013 due to less rainfall and irrigation during the latter portion of the growing season (Table 1). Pyraclostrobin applied 60 DAP followed by prothioconazole plus tebuconazole applied 80, 100, and 120 DAP, propiconazole plus chlorothalonil applied 60 and 100 DAP followed by azoxystrobin plus cyproconazole applied 80 and 120 DAP, and propiconazole plus chlorothalonil applied 60 DAP followed by either prothioconazole plus tebuconazole or penthiopyrad alone applied 80, 100, and 120 DAP produced the lowest levels of early leaf spot while pyraclostrobin followed by flutolanil and the nontreated control produced the highest leaf spot levels (Table 2). Again, chlorothalonil was intermediate in early leaf

Table 2: Control of peanut diseases, yield, and grade when using foliar fungicides in South Texas.

Treatments[c]	L/ha	Appl[d]	Early leafspot[a] 2013	Early leafspot[a] 2014	Southern blight[b] 2013	Southern blight[b] 2014	Yield 2013	Yield 2014	Grade 2013	Grade 2014
			Florida scale[e]		% infection		kg/ha		% SMK + SS[f]	
Nontreated control	—	—	9.7	6.0	40.8	19.8	990	3440	67.9	65.4
Propiconazole + chlorothalonil	1.75	A C	3.2	1.7	9.2	3.5	4320	4250	70.0	71.3
Azoxystrobin	0.88	B D								
Cyproconazole	0.40	B D								
Propiconazole + chlorothalonil	1.75	A	4.1	1.8	9.8	10.3	4020	4060	69.5	70.3
Prothioconazole + tebuconazole	0.59	B C D								
Chlorothalonil	1.75	A	—	2.0	—	5.5	—	4690	—	71.4
Prothioconazole + tebuconazole	0.59	B C D								
Propiconazole + chlorothalonil	1.75	A	4.9	1.9	8.2	5.3	3900	4630	69.9	70.0
Penthiopyrad	1.17	B C D								
Pyraclostrobin	0.66	A	5.5	2.3	11.6	3.5	4000	4420	69.9	71.9
Penthiopyrad	1.17	B C D								
Pyraclostrobin	0.66	A	4.9	1.6	13.8	2.0	3900	4890	68.3	72.4
Prothioconazole + tebuconazole	0.51	B C D								
Pyraclostrobin	0.66	A	8.6	4.8	22.2	4.5	2130	4440	70.0	72.5
Flutolanil	1.17	B C D								
Chlorothalonil	1.75	A B C D	5.9	2.2	22.0	13.2	3490	3870	68.9	69.1
LSD (0.05)			0.7	0.4	7.8	7.5	450	630	NS	2.9

[a]Early leafspot, *Cercospora arachidicola* S. Hori.
[b]Southern blight, *Sclerotium rolfsii* Sacc.
[c]In 2013, Treatment 3 included chlorothalonil only at C; Treatment 4 included chlorothalonil only at A.
[d]Application timing: A, 60 days after planting (DAP); B, 80 DAP; C, 100 DAP; 120 DAP.
[e]Leaf spot assessed using the Florida 1–10 scale where 1 = no disease and 10 = completely dead.
[f]SMK: sound mature kernels; SS: sound splits.

spot control. Although tebuconazole was effective against leaf spot in this study, leaf spot isolates with resistance to this fungicide have been reported [28–30].

Since chlorothalonil is a broad-spectrum protectant fungicide with no curative properties and no activity against soilborne diseases, it is most effective when applied prior to infection [31]. It can also be applied in alternating applications, alternating blocks of applications, or in application regime mixtures with other fungicides to prevent late season or secondary infections and to reduce the risk of developing resistance in *C. arachidicola* or *C. personatum* populations to systemic fungicides [32].

3.1.2. Southern Blight. As with early leaf spot, southern blight disease incidence was greater in 2013 than 2014 due to previously mentioned weather conditions (Table 1). Southern blight often is an issue due to moist conditions brought on by irrigation or rainfall [33, 34]. High soil moisture promotes infection and fungal mycelial spread between and within plants, especially in dense stands resulting from the use of high seeding rates such as used in these studies (approximately 16 seed/m) [35–37]. Southern blight infection is limited to basal stems, roots, pegs, and pods, and colonization of the tissues coincides with beginning peg and pod formation (R2 and R3) as defined by Boote [38] when peanut branches spread rapidly across the soil [37].

In 2013, propiconazole plus chlorothalonil applied 60 DAP followed by penthiopyrad applied 80, 100, and 120 DAP produced the lowest levels of southern blight (8.2%) while the nontreated control produced the highest level of disease incidence at almost 41% (Table 2). Propiconazole plus either azoxystrobin plus cyproconazole or prothioconazole plus tebuconazole also resulted in less than 10% southern blight infection. Prothioconazole is registered in a triazole mixture with tebuconazole to control leaf spots and southern blight [27]. Pyraclostrobin followed by flutolanil and chlorothalonil alone also provided poor southern blight control (22%). The lack of southern blight control with pyraclostrobin is thought to be due, at least partially, to high affinity of pyraclostrobin to leaf surface waxes and quick binding when applied to dry foliage [39]. Augusto et al. [40, 41] reported southern blight control and peanut yield were greatly improved when pyraclostrobin was applied at night when peanut leaves were folded and wet compared with day application when leaves were unfolded and dry. High rates (0.21 to 0.27 kg/ha) of pyraclostrobin may be necessary for effective control of southern blight [27].

In 2014, all fungicide treatments, with the exception of propiconazole plus chlorothalonil followed by prothioconazole plus tebuconazole or chlorothalonil alone, resulted in less than 6% southern blight disease incidence while the two above-mentioned treatments resulted in 10 to 13% disease

Table 3: Control of *Sclerotinia* blight with penthiopyrad in Erath County, Texas.

Treatments[a]	Rate/ha	Disease incidence 2008	2009	2010	2011	Yield 2008	2009	2010	2011
		(%)				(kg/ha)			
Nontreated control	—	49.0	52.3	44.8	42.8	4130	3120	3180	2500
Fluazinam	1.78 L	31.7	27.5	13.2	6.4	5130	3880	3800	4590
Boscalid	701.00 g	24.2	21.3	—	—	4830	4150	—	—
Penthiopyrad	1.22 L	36.0	20.6	43.2	26.8	4230	3400	3180	2720
Penthiopyrad	1.78 L	33.7	27.1	28.4	12.8	4650	3840	3490	4070
LSD (0.05)		17.4	24.2	14.6	13.8	660	440	320	510

[a]Initial application made approximately 70 days after planting with 2nd application 30 days later.

incidence (Table 2). The nontreated control resulted in almost 20% disease incidence.

3.1.3. Sclerotinia Blight. In 2008, disease incidence was lowest with fluazinam or boscalid while both rates of penthiopyrad provided intermediate control compared to the nontreated control (Table 3). In 2009, all fungicide treatments reduced the incidence of *Sclerotinia* blight compared with the nontreated control. In 2010, fluazinam and penthiopyrad at 1.78 L/ha reduced disease incidence compared to the nontreated control, while the 1.22 L/ha rate of penthiopyrad did not. In 2011, all fungicide treatments reduced Sclerotinia blight disease incidence compared with the nontreated control. Again, fluazinam and the high rate of penthiopyrad controlled *Sclerotinia* blight better than the low rate of penthiopyrad.

Fluazinam has provided good to excellent disease control depending on the rate applied [4, 5, 15, 42, 43]. Smith et al. [44] reported in field studies that the application of boscalid or fluazinam provided the best control of *Sclerotinia* blight and subsequent peanut yield increase. They suggested that disease advisories or intensive scouting should be used to determine when epidemics initiate so that a fungicide can be applied prior to infection, whereas Woodward and Russell [4] found applying fungicides on a calendar basis provided the most consistent level of control.

3.2. Peanut Yield

3.2.1. South Texas. In 2013, the use of a fungicide resulted in increased yield with all fungicide treatments (Table 2). However, pyraclostrobin followed by flutolanil resulted in the lowest yield among fungicide treatments while chlorothalonil alone produced lower yields than treatments that included propiconazole plus chlorothalonil followed by either azoxystrobin plus cyproconazole or prothioconazole plus tebuconazole, or pyraclostrobin followed by penthiopyrad. In 2014, the use of foliar fungicides improved yields over the nontreated control with the exception of propiconazole plus chlorothalonil followed by prothioconazole plus tebuconazole or chlorothalonil alone.

3.2.2. Central Texas. In 2008, all fungicides, with the exception of penthiopyrad at 1.22 L/ha, improved yield over the nontreated control with fluazinam producing the highest yield, while in 2009 only fluazinam and boscalid improved yield over the nontreated control (Table 3). Boscalid was not evaluated in 2010; however, fluazinam improved yield over the nontreated control while in 2011 peanut yields were improved with all fungicide treatments with the exception of the low rate of penthiopyrad.

3.3. Peanut Grade. In 2013, no differences in grade were noted between the nontreated control and any fungicide treatment (Table 2). In 2014, all fungicide treatments improved grade over the nontreated control; also, pyraclostrobin followed by prothioconazole plus tebuconazole and pyraclostrobin followed by flutolanil improved peanut grade over chlorothalonil alone.

4. Conclusion

Peanut is susceptible to numerous foliar and soilborne diseases; thus, fungicides are intensely used in most production areas in the USA. Several fungicides are registered for use in peanut; however, regimes comprised of multiple modes of action are recommended based on target diseases. In addition, sequential applications are required to provide season-long control. Results from these studies confirm previous reports that premixes of SBI and QoI fungicides provide superior control of foliar diseases compared to chlorothalonil alone [3, 12]. Furthermore, combinations of these fungicides are effective in the management of southern blight [1, 3, 7, 12, 32]. Penthiopyrad has been shown to possess excellent activity towards early and late leaf spot and southern blight in the southeastern USA [45] and results from these studies support those findings.

Damage caused by *Sclerotinia* blight can be severe [5] and management can be challenging, as fungicides registered for use against the disease are limited. Boscalid and fluazinam provided excellent control of *Sclerotinia* blight in these studies, which is consistent with previous findings [4, 5, 42–44]. Applications of higher rates of penthiopyrad provided intermediate control of Sclerotinia blight when compared to maximum label rates of boscalid and fluazinam. In addition to yield increases, the application of fungicides has improved quality, thus increasing overall value of peanuts [44, 46]. Responses in peanut quality (expressed as % SMK + SS) were not assessed in trials evaluating the efficacy of fungicides towards *Sclerotinia* blight as planting was delayed to increase pressure for disease development later in the season. Additional studies are needed to examine the influence of fungicide applications on peanut quality so that a more comprehensive economic analysis can be conducted.

Information regarding the performance of penthiopyrad for disease control in the southwestern US is lacking. These results provide a basis of comparison of penthiopyrad to other fungicides commonly used in the region. Penthiopyrad represents a new broad-spectrum active ingredient that producers can use in developing management strategies for various diseases. Furthermore, penthiopyrad provides another mode of action separate from the SBI and QoI fungicides and therefore should help prevent the development of fungicide resistance.

Competing Interests

The authors declare that there are no conflicts of interest regarding publication of this manuscript.

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