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Improved Management Efficacy of Late Leaf Spot on Peanut Through Combined Application of Prothioconazole with Fluxapyroxad and Pyraclostrobin

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Abstract: Late leaf spot, caused by *Nothopassalora personata*, is the most economically important fungal disease affecting peanut foliage in South Carolina and can result in combined management and yield loss costs of greater than 490 dollars/ha. Application of protectant fungicides is a critical part of effective integrated management under commercial production, and their strategic alternation and combination in management programs can provide enhanced control. Trials were conducted in Blackville, SC, from 2017 to 2019 to investigate whether combinations of prothioconazole with fluxapyroxad plus pyraclostrobin could provide more efficacious management of late leaf spot compared to either product alone. Two applications of 0.11 kg/ha prothioconazole with 0.05 kg/ha fluxapyroxad plus 0.1 kg/ha pyraclostrobin resulted in significantly ($p < 0.05$) less (24% to 42%) peanut canopy defoliation compared to the same number of applications of either product applied individually, with the combined application reflecting significant ($p < 0.0202$) synergism compared to component products as assessed through independent action methodology. An increased rate of fluxapyroxad plus pyraclostrobin application (0.1 and 0.2 kg/ha, respectively), with 0.16 kg/ha prothioconazole did not improve management relative to their combination at the examined lower rate ($p = 0.89$). Peanut yield was not adversely affected following combined applications. Cost-effectiveness of this combination depends on the actual disease intensity and yield potential of a given crop.

Keywords: *Arachis hypogaea*; *Nothopassalora personata*; *Cercosporidium personatum*; groundnut; fungicide

1. Introduction

Peanut (*Arachis hypogaea* L.) is an important agricultural crop both in the U.S. and worldwide, with recent U.S. annual production estimates valued over $1.22 billion per year [1]. Late leaf spot, caused by *Nothopassalora personata* (Berk. & M.A. Curtis) S.A. Khan & M. Kamal (syn. *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton), is among the most damaging biotic diseases of peanut and can contribute to yield losses of 70% in the absence of effective fungicide protection [2]. An ascomycete, *N. personata* overwinters on peanut residue in the soil; its conidia are dispersed during the following growing season by rain splash and wind, with epidemic progression being polycyclic [3]. An integrated approach is recommended to manage late leaf spot [3], with the application of protectant fungicides as one such pertinent tactic.

At least seven fungicide modes of action [4] are currently registered for management of late leaf spot on peanut in the U.S., with the majority of these having single-site activity [3]. Over the years, research has reported decreased field efficacy of several single-site mode of action fungicides including benomyl [5,6], tebuconazole [7] and prothioconazole [8], with further research having recently documented the regional occurrence of varying levels of *N. personata* fungicide resistance to azoxystrobin, prothioconazole, thiophanate-methyl and benzovindiflupyr in South Carolina [9].

Culbreath et al. [10] recently reported that combined applications of prothioconazole plus micronized sulfur resulted in significantly improved management of late leaf spot infections compared to either product alone. While the mechanistic details of this interaction were outside the scope of that study, it did illustrate how certain combinations can result in substantially improved disease control. Accordingly, the evaluation of further fungicide combinations represents an opportunity for improved disease management through the use of additional effective fungicide mixtures. The objective of this work was to examine combined applications of prothioconazole [10] with fluxapyroxad plus pyraclostrobin [11], for possible improved management of late leaf spot on peanut. Associated with this objective was the hypothesis that the simultaneous application of three single-site fungicide modes of action will be associated with greater management efficacy compared to fungicide applications with fewer modes of action among the compounds examined in this study. Were such a combination to significantly improve management of late leaf spot while remaining cost-effective, this would represent another tool that could be used in conjunction with other integrated approaches (e.g., cultivar resistance, planting date, crop rotation) to manage potential development of fungicide resistance to these fungicides in the field.

2. Materials and Methods

Field experiments were conducted at the Edisto Research and Education Center of Clemson University in Blackville, SC, from 2017 to 2019 in irrigated fields of Barnwell loamy sand. Fields were previously rotated to corn and cotton (2017 and 2018) or two years of cotton (2019) before planting peanut with conventional tillage. Planting dates were 17 May, 2017, 18 May, 2018 and 21 May, 2019. Cultivars planted were susceptible to late leaf spot [3] and included 'Georgia 13M' [12], 'TUFRunner 511' [13] and 'Bailey' [14] in 2017, 2018 and 2019, respectively. Plots were four single rows spaced 0.97 m apart and 12 m long, with blocks separated by 3 m fallow alleys.

Treatments were applied according to a randomized complete block design, with four replications per experiment. Examined treatments included a nontreated control, 0.11 kg/ha prothioconazole (plus tebuconazole at 0.22 kg/ha; Provost Opti, Bayer CropScience LP, St. Louis, MO, USA), fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha (Priaxor, BASF Corporation, Research Triangle Park, NC, USA), fluxapyroxad at 0.1 kg/ha plus pyraclostrobin at 0.2 kg/ha, prothioconazole at 0.11 kg/ha (plus tebuconazole at 0.22 kg/ha) plus fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha, prothioconazole at 0.16 kg/ha (Proline, Bayer CropScience LP, St. Louis, MO, USA), plus fluxapyroxad at 0.1 kg/ha plus pyraclostrobin at 0.2 kg/ha, and chlorothalonil at 1.26 kg/ha (Bravo Weather Stik, Adama Americas Inc., Aventura, FL, USA). Across experiments, treatments were consistent, with the exception of 2017, where fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha was absent. For the purposes of this study and based on previous research [7] and field performance in SC [3], late leaf spot management with tebuconazole within Provost Opti is considered negligible; as such, tebuconazole in the above treatments is listed in parentheses and is not further mentioned with regards to treatments involving Provost Opti. Fungicide treatments were applied using two DG8002 nozzles per row (48 cm spacing) at the rate of 140 l/ha at 345 kpa and initiated once late leaf spot leaflet incidence in the field reached approximately 10%. Fungicide treatments were applied twice per experiment and spaced approximately 14 days apart: 28 July and 11 Aug, 2017, 10 and 23 Aug, 2018 and 12 and 26 Aug, 2019. In 2018, due to increased overall late leaf spot infections, a cover spray of chlorothalonil at 1.26 kg/ha was applied over the entire field, including the nontreated control, 21 days after the second treatment application timing (13 Sep). Other than this exception, the experiments received no other fungicide applications, with standard extension recommendations followed for the remaining production practices (e.g., fertility, irrigation and weed management) [3]. The estimated cost per treatment was based on local prices in South Carolina in 2018.

The proportion of late leaf spot defoliation among total canopy leaflets (0% to 100%) was visually rated prior to harvest at 141, 136 and 129 days after planting (DAP) in 2017, 2018 and 2019, respectively. Plots were inverted 140 DAP in 2018 and 132 DAP in 2019. Pod yield was mechanically harvested using a Hobbs 2-row combine fitted with a load cell from two rows per plot 150 and 140 DAP in 2018 and 2019, respectively, and standardized to 10% moisture. Yield was not collected in 2017.

The GLIMMIX procedure of SAS 9.4 (SAS Institute, Cary, NC) was used to analyze data. Proportion defoliation was analyzed according to Equation (1),

$$y_{ij} = \theta + \tau_i + u_j + \varepsilon_{ij} \quad (1)$$

where y_{ij} is proportion defoliation corresponding to treatment i (τ_i, fixed effect) and experiment j (u_j, random effect), θ is an intercept, and ε_{ij} is the residual. The random effect of experiment × treatment was excluded from the defoliation analysis due to its inclusion having not improved fit of the model to the data based on Akaike's Information Criterion (AIC) [15]. A maximum likelihood estimation based on Laplace approximation was used to improve standard error estimation [15,16]. Yield data were modeled similar to Equation (1), substituting treatment i yield (kg/ha) per experiment j for y_{ij}, including a random effect term for replicate (experiment) (+ v_{ij}), and assuming a lognormal response distribution. As with Equation (1), the model for the yield data excluded the experiment × treatment random effect following lack of model improvement. Estimated treatment defoliation was separated following Fisher's protected least significant difference ($\alpha = 0.05$).

Synergism of fungicide combination treatments was assessed following independent action methodology [17]. Differences of observed (E_{obs}) and anticipated estimated relative late leaf spot control (i.e., relative to the nontreated check) assuming independent fungicide component activity of the fungicide mixture (E_{mix}) were estimated using the *lsmeans* statement in the GLIMMIX procedure to produce a one-tailed test of significance (Fisher's protected least significant difference). E_{mix} was estimated following Equation (2),

$$E_{mix} = E_A + E_B - E_A \times E_B \quad (2)$$

where E_A and E_B are the respective relative control efficacies, 1—D_A/D_{NTC}, of fungicide A (prothioconazole at 0.11 kg/ha) and fungicide B (fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha), where D_A and $D_{NTC,}$ respectively, represent the estimated defoliation associated with fungicide A and the nontreated control from each replication and experiment. From the 2017 data where treatment with fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha was absent, data from the higher application rate of fluxapyroxad at 0.1 kg/ha plus pyraclostrobin at 0.2 kg/ha were substituted for E_B.

3. Results

Defoliation varied significantly among treatments ($p < 0.0001$; Table 1). Over the combined 2017 to 2019 data, all treatments resulted in significantly less defoliation compared to the nontreated control. Prothioconazole applied alone, and both rates of fluxapyroxad plus pyraclostrobin were not significantly different compared to the chlorothalonil standard; however, defoliation in peanut treated with fluxapyroxad plus pyraclostrobin was significantly less than that of prothioconazole-treated peanut. Both rates of combined application of prothioconazole plus fluxapyroxad plus pyraclostrobin were associated with significantly less defoliation compared to all other treatments. Compared to fluxapyroxad at 0.1 kg/ha plus pyraclostrobin at 0.2 kg/ha, combined application of prothioconazole at 0.11 kg/ha plus fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha resulted in significantly improved (19.7% less defoliation, $p = 0.005$) late leaf spot management while costing a nominal $6 more per hectare per application. Conversely, the mixture involving higher rates of the three active ingredients was associated with a treatment cost considerably greater than the three-way combination at the lower rate ($122 versus $83/ha per application; Table 1) while not resulting in significantly different late leaf spot control (20.3% compared to 19.3% defoliation, $p = 0.89$).

Table 1. Peanut canopy late leaf spot defoliation associated with fungicide treatments from field experiments conducted in Blackville, SC, from 2017 to 2019.

Treatment [1]	Application Rate (kg/ha)	Cost ($/ha) [2]	Late Leaf Spot Defoliation (%)				
			2017	2018 [3]	2019	Combined [4]	SE [5]
NTC	–	0	91.0	94.8	58.5	81.4 a	8.6
Chlor	1.26	20	55.3	71.0	28.3	51.5 bc	8.6
Proth	0.11	45	76.3	73.3	34.5	61.3 b	8.6
Flux plus pyr	0.05 plus 0.1	39	–	54.0	21.5	43.1 c	9.4
Flux plus pyr	0.1 plus 0.2	77	58.3	34.5	24.3	39.0 c	8.6
Proth plus flux plus pyr	0.11 plus 0.05 plus 0.1	83	23.0	21.3	13.8	19.3 d	8.6
Proth plus flux plus pyr	0.16 plus 0.1 plus 0.2	122	38.5	12.0	10.3	20.3 d	8.6

[1] NTC = nontreated control; chlor = chlorothalonil; proth = prothioconazole; flux = fluxapyroxad; pyr = pyraclostrobin. [2] Treatment cost is per application. [3] In 2018, due to increased overall late leaf spot infections, a cover spray of chlorothalonil at 1.26 kg/ha was applied over the entire field, including the nontreated control, 21 days after the second treatment application timing (13 September). [4] Estimates followed by the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Data analysis was performed on the pooled data. [5] SE = estimated standard error for the combined data.

When relative control was compared for individual fungicide treatments against the combined mixture, the resulting interaction was significant over the pooled data ($p = 0.0202$; Table 2). This indicated phenotypic synergism of the compounds (greater control than anticipated if independent). In 2019, when there was a lower overall amount of late leaf spot infections in the experiment compared to 2017 and 2018 (Table 1), the estimated difference between the observed and anticipated fungicide mixture relative control was not different from zero. However, when analyzed over the three-year dataset, the collective observed differences were statistically interpreted as phenotypic synergism.

Table 2. Relative late leaf spot control efficacy observed and predicted for prothioconazole at 0.11 kg/ha plus fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha according to independent action from field experiments conducted in Blackville, SC, from 2017 to 2019.

Treatment	Relative Late Leaf Spot Control (%)				
	2017	2018 [3]	2019	Combined	(L, U) [4]
E_{obs}	74.9	77.6	76.2	76.3	(63.5, 89.0)
E_{mix} [1]	48.0	55.7	78.4	60.7	(48.0, 73.4)
p [2]	–	–	–	0.0202	–

[1] In 2017, data from treatment with fluxapyroxad at 0.1 kg/ha plus pyraclostrobin at 0.2 kg/ha was substituted for fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha, since the latter treatment was not conducted. [2] p values of one-tailed differences of relative late leaf spot control from observed (E_{obs}) fungicide mixtures versus predicted (E_{mix}) relative control assuming independent action according to Fisher's protected least significant difference ($\alpha = 0.05$). Data analysis was performed on the pooled data. [3] In 2018, due to increased overall late leaf spot infections, a cover spray of chlorothalonil at 1.26 kg/ha was applied over the entire field, including the nontreated control, 21 days after the second treatment application timing (13 September). [4] L and U respectively represent the lower and upper 95% confidence intervals of estimated relative late leaf spot control for the combined data.

Over the pooled data, pod yield significantly varied among treatments ($p = 0.0009$). While the chlorothalonil treatment was not significantly different compared to the nontreated control, all other treatments yielded significantly greater than the nontreated control. The yield following the combined application of prothioconazole plus fluxapyroxad plus pyraclostrobin was not significantly different compared to prothioconazole alone or fluxapyroxad plus pyraclostrobin (Table 3). Although treatment with prothioconazole at 0.11 kg/ha or fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha was not significantly different compared to the chlorothalonil standard, combined application of prothioconazole plus fluxapyroxad plus pyraclostrobin resulted in significantly greater yield compared to chlorothalonil. Taken together with the defoliation and relative control data, these results indicate that prothioconazole at 0.11 kg/ha applied with fluxapyroxad at 0.05 kg/ha plus pyraclostrobin at 0.1 kg/ha provided significantly improved control of late leaf spot versus either fungicide product applied alone, costs only marginally more than the higher labeled rate of fluxapyroxad plus pyraclostrobin and does not negatively impact pod yield.

Table 3. Peanut pod yield associated with fungicide treatments from field experiments conducted in Blackville, SC, from 2018 to 2019.

Treatment [1]	Application Rate (kg/ha)	Pod Yield (kg/ha)				
		Cost ($/ha) [2]	2018 [3]	2019	Combined [4]	SE [5]
NTC	–	0	2488	1804	2170 c	537
Chlor	1.26	20	3613	1771	2590 bc	641
Proth	0.11	45	4052	2265	3102 ab	767
Flux plus pyr	0.05 plus 0.1	39	4196	1871	2869 ab	710
Flux plus pyr	0.1 plus 0.2	77	4393	2313	3265 a	807
Proth plus flux plus pyr	0.11 plus 0.05 plus 0.1	83	5052	1970	3231 a	799
Proth plus flux plus pyr	0.16 plus 0.1 plus 0.2	122	4563	2501	3459 a	855

[1] NTC = nontreated control; chlor = chlorothalonil; proth = prothioconazole; flux = fluxapyroxad; pyr = pyraclostrobin. [2] Treatment cost is per application. [3] In 2018, due to increased overall late leaf spot infections, a cover spray of chlorothalonil at 1.26 kg/ha was applied over the entire field, including the nontreated control, 21 days after the second treatment application timing (13 September). [4] Estimates followed by the same letter are not significantly different according to Fisher's protected least significant difference ($\alpha = 0.05$). Data analysis was performed on the pooled data on the lognormal model scale, with means presented on the backtransformed data scale. [5] SE = estimated standard error for the combined data.

4. Discussion

The results reported in this study document the improved management efficacy of late leaf spot when prothioconazole is concurrently applied with fluxapyroxad plus pyraclostrobin relative to control associated with either product alone. This finding supports the hypothesis of improved late leaf spot control in the presence of multiple fungicide modes of action. The application of effective fungicide mixtures of multiple modes of action, in addition to the alternation of fungicide modes of action within a growing season, has been recommended as a key means of delaying or preventing the development of fungicide resistance [18–20]. Under the production conditions of the experiments reported in the present study, the lower rate of the examined three-way mixture provided significantly improved late leaf spot control that did not negatively impact yield. At the same time, pod yield associated with the three-way mixture was not significantly different from either (less expensive) product applied alone (not considering chlorothalonil). This raises uncertainty regarding the cost-effectiveness of the more expensive three-way mixture and reinforces the importance of information-driven fungicide management decisions.

It can be anticipated that in the absence of excessive leaf spot infections, utilization of more effective fungicide applications that cost more might not translate into an economic benefit (risk theory aside) during the year of their application and could, in some cases, negatively affect overall production budgets. Peanut pod yield has been reported to be highly significantly influenced by leaf spot defoliation [2]. Taken in the context of the meta-analysis conducted by Anco et al. [2], equivalent defoliation reductions reported in the current study (from 40% to 20% defoliation) of the lower-rate three-way fungicide mixture compared to fluxapyroxad at 0.1 kg/ha plus pyraclostrobin at 0.2 kg/ha would be anticipated on average to translate into economic yield savings of $21 to $45/ha, after accounting for product application costs for runner and Virginia market type peanut cultivars, respectively, assuming 4480 kg/ha yield potential and peanut contract prices of $470/1000 kg (runner market type) or $500/1000 kg (Virginia market type). These economic returns more than pay for the cost of the fungicide material inputs, but they are contingent on actual disease levels in the field. Nevertheless, while effective, the lower-rate three-way combined fungicide application reported herein is still costly and is not recommended for economical management under all production conditions and yield potentials.

It is not uncommon for conditions in the Virginia–Carolina region, however, to include periods of ample rainfall near harvest that delay field access, consequently resulting in increased defoliation from leaf spot infections and subsequent yield loss [21]. Accordingly, the benefit of applying a fungicide treatment with improved efficacy (e.g., the three-way mixture reported herein) includes additional disease-management insurance against situations that might delay field access or crop inversion and harvest. In the presence of conducive conditions (optimal infection near 20 °C with relative humidity >93% for >12 h or continual leaf wetness for 10 h) [22], it would take on average approximately 7 days for defoliation to progress from 20% to 40% [2]. Along these lines, while the actual benefits received from more effective fungicide treatment-related insurance varies across individual production conditions, under the conditions present in the current study, the application of prothioconazole plus fluxapyroxad plus pyraclostrobin appeared to provide about a weeks' worth of flexibility in terms of defoliation at harvest levels.

While this study demonstrated significant phenotypic synergism between prothioconazole and fluxapyroxad plus pyraclostrobin, it did not determine the mechanistic interactions responsible for this phenotype. Future studies could examine if the improved combined efficacy is associated with fungicide binding site activity, active ingredient metabolism or other factors. Currently, this remains an area for exploration that could shed additional light on further potentially synergistic fungicide combinations. Further research is similarly warranted to determine if the examined three-way combination that is synergistically effective in the present study is analogously synergistic across other crops and pathosystems.

Culbreath et al. [10] reported significant improvement of late leaf spot management when prothioconazole was applied in a mixture with micronized sulfur. In that study, reduced control of late leaf spot relative to previous studies [23] was reported, with end-of-season defoliation control attributed to prothioconazole (plus tebuconazole) not significantly different from the nontreated control in two locations in Georgia. Fungal cross-resistance to demethylation-inhibiting (DMI) fungicides has been reported to be common [24,25]. However, Culbreath et al. [23] have also reported prothioconazole to exhibit superior efficacy relative to other DMI fungicides, despite several of those other DMI fungicides being associated with reduced efficacy. The results of these collective studies, with those of the present work, are optimistic in the sense that while specific fungicides may show reduced efficacy over time, mixtures with additional fungicides represent a means of recapturing management efficacy while corroborating the recommendations limiting fungicide resistance development [18,19].

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