Foliar- and Seed-Applied Insecticides for Management of *Melanaphis sorghi* (Hemiptera: Aphididae) in Alabama[1]

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J. Entomol. Sci. 59(2): 105–124 (April 2024)
DOI: 10.18474/JES23-38

Abstract Following the emergence of *Melanaphis sorghi* (Theobald) (Hemiptera: Aphididae) as a new pest of sorghum in the United States, research was conducted to identify tools and techniques successful at reducing populations and preventing economic losses in grain sorghum, *Sorghum bicolor* (L.) Moench. The objective of this study was to assess the efficacy of seed- and foliar-applied insecticide treatments for management of *M. sorghi*. Small plot experiments were replicated at two locations to evaluate residual activity of neonicotinoid seed treatments and foliar insecticides. Natural enemy presence was recorded in each of these trials to determine which predator and parasitoid species were using *M. sorghi* as prey. All seed treatments suppressed *M. sorghi* populations below a treatment threshold of 75 aphids per plant for 30% of plants for >6 weeks after planting. Foliar insecticides including flupyradifurone, sulfoxaflor, and thiamethoxam provided 3–4 weeks of population suppression, irrespective of *M. sorghi* pressure. Fifteen natural enemy species were identified in this study, and community structure varied temporally and geographically. In general, natural enemy species richness was correlated with aphid abundance. We identified the most efficacious insecticides available for management of *M. sorghi* and determined that they should be compatible with biological control and integrated pest management programs.

Key Words seed treatment, foliar insecticides application, management, sorghum, aphid

Melanaphis sorghi (Theobald) (Hemiptera: Aphididae) (previously reported as *Melanaphis sacchari* (Zehntner) in the United States from 2013 to 2018), is distributed worldwide and feeds on sugarcane, sorghum, corn, millet, and rice species (Singh et al. 2004; Nibouche et al. 2014). It is considered an economic pest of sorghum in approximately 30 countries across Africa, Asia, Australia, and Central and South America, and all sorghum production regions in Mexico (Bowling et al. 2016a, Lahiri et al. 2019, Singh et al. 2004). *Melanaphis sorghi* was first reported as a damaging pest of sorghum in Texas in late 2013 and rapidly spread across Louisiana, Mississippi, and Oklahoma (Bowling et al. 2016a, Kerns et al. 2015, Nibouche et al. 2018). By 2015, *M. sorghi* was reported in 17 states that account for 98% of sorghum production in the United States (Bowling et al. 2015, 2016b).

[1]Received 29 June 2023; accepted for publication 11 August 2023.
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The aphid directly damages plants by its feeding behavior, piercing plant tissues and siphoning phloem sap, causing desiccation, discoloration, and necrosis (Singh et al. 2004). *Melanaphis sorghi* indirectly damages plants by excreting honeydew on the lower leaves, which can promote black sooty mold growth (Narayana 1975). This activity reduces the photosynthetic capacity of the plant and may subsequently cause crop mortality (Armstrong et al. 2015). High densities of *M. sorghi* feeding on plants results in economic yield losses of 20–100% for farmers, and extensive honeydew on leaves can clog harvesting equipment (Bowling et al. 2016a, Kerns et al. 2015, Zapata et al. 2018). In 2015, it was reported that *M. sorghi* caused crop yield losses of between $62 and $432 per ha in sorghum in Texas alone (Bowling et al. 2016b). To mitigate the economic impacts of aphids, integrated pest management strategies are needed that reduce populations of *M. sorghi* (Etheridge et al. 2018, Haar et al. 2019).

Insecticide applications have been shown to be effective in suppressing the incidence of *M. sorghi* on sorghum (Lahiri et al. 2021). Foliar applications can occur anytime during the season when economically damaging populations are detected, whereas seed treatments provide early-season management that helps protect young crops early in the growing season. Insecticide seed treatments of imidacloprid, thiamethoxam, and clothianidin have been reported to suppress *M. sorghi* populations for 4–6 weeks post germination in Louisiana (Jones et al. 2015) and Texas (Etheridge et al. 2018, Knutson et al. 2016, Szczepaniec 2018). They can delay or eliminate the need for foliar sprays, especially when sorghum crops are planted later in the season (Bowling et al. 2016a) when dispersal peaks of *M. sorghi* have already passed (Lee et al. 2023). The most efficacious active ingredients for foliar- and seed-applied insecticides belong to the same class of chemicals, the neonicotinoids, and selection for insecticide resistance is a concern (Colares et al. 2017). Testing a variety of mode of actions may help identify products that could be used in rotation to help delay evolution of resistant populations.

Another important consideration for choosing an insecticide is its effects on natural enemies. In total, at least 19 species of natural enemies of *M. sorghi* have been identified in other studies conducted in North America (Brewer et al. 2017, 2022; Colares et al. 2015a,b; Hewlett et al. 2019; Maxson et al. 2019; Rodríguez-del-Bosque et al. 2018; Salas-Araiza et al. 2017). The insect families Coccinellidae, Chrysomelidae, Hemerobiidae, Anthocoridae, and Syrphidae all have members that function as predators (Zhang and Swinton 2012). Preserving natural enemy function may help suppress aphid populations in the landscape, reduce insecticide applications, and help delay development of insecticide resistance (Hewlett et al. 2019, Zhang and Swinton 2012). Previous studies have shown that flupyradifurone and sulfoxaflor suppress *M. sorghi* populations and are less toxic to predatory insects in Coleoptera, Hemiptera, and Neuroptera that function as natural enemies (Barbosa et al. 2017, Hakeem and Parajulee 2019) than alternative insecticides such as nicotinic acetylcholine receptor agonists (Colares et al. 2017).

When *M. sorghi* was first reported in Alabama in 2014, there was limited information on either the effectiveness of chemical control options for management in grain sorghum or the natural enemy communities present and their response to insecticides in the field. Regional variation has been reported in other management tactics such as host plant resistance (Pekarcik and Jacobson 2021) and biological control

(Brewer et al. 2022). Thus, this study investigated the efficacy of foliar- and seed-applied insecticides against *M. sorghi* in Alabama, each at two locations, as part of a regional effort. The objectives of this study were to (a) evaluate the efficacy window provided by a single application of foliar insecticides containing different active ingredients, (b) determine how long neonicotinoid seed treatments suppress *M. sorghi* populations below the action threshold following initial colonization, and (c) identify the natural enemy communities present in these studies and examine differences among insecticide treatments evaluated in the field. These results will provide Alabama sorghum farmers with the most effective preventative and curative chemical control options for *M. sorghi* that are the least impactful on natural enemy communities.

Materials and Methods

Field sites and experimental design. Small plot replicated sorghum experiments were performed in 2015 to evaluate the efficacy of foliar- and seed-applied insecticides for suppressing *M. sorghi* populations. The foliar insecticide trial was conducted at the Brewton Agricultural Research Unit (Brewton) in Brewton, AL, and at the Wiregrass Research and Extension Center (Headland) in Headland, AL. The seed treatment trial was conducted at the Gulf Coast Research and Extension Center (Fairhope) in Fairhope, AL, and at the E.V. Smith Research and Extension Center's Plant Breeding Unit (Tallassee) in Tallassee, AL. All experiments were conducted using a randomized complete block design with four replicates per treatment. Research plots were four rows wide by 6.1 m long. Plots were seeded at a rate of approximately 148,263 seeds per ha, managed for weeds, and fertilized per commercial recommendations. One application of 1.462 L/ha Prevathon® (DuPont™, Wilmington, DE) was made on 23 July for whorl worms at Fairhope. No other insecticide applications for non-aphid pests were made at other locations. All experiments were performed under dryland conditions.

Foliar insecticide trials in Brewton and Headland used 84P80 (Pioneer®, Bushnell, IL), a known aphid-susceptible grain sorghum variety pretreated with Apron XL®, Maxim®, and Dynasty® (Syngenta® Crop Sciences, Greensboro, NC). Research plots were planted on 15 June in Headland and on 17 June in Brewton. The foliar insecticides and rates of formulated product evaluated in the studies include 946.4 ml/ha chlorpyrifos (Lorsban® Advanced, Dow® AgroSciences, Indianapolis, IN), 473.2 ml/ha dimethoate (Dimethoate 4EC, Cheminova, Research Triangle Park, NC), 473.2 ml/ha chlorpyrifos with 473.2 ml/ha dimethoate, 118.3 ml/ha flupyradifurone (Sivanto™ 200SL, Bayer© CropScience, Research Triangle Park, NC), 207.0 ml/ha flupyradifurone, 82.8 ml/ha β-cyfluthrin (Baythroid® XL, Bayer), 0.07 kg/ha sulfoxaflor (Transform® WG, Dow AgroSciences), 0.11 kg/ha sulfoxaflor, 0.07 kg/ha thiamethoxam (Centric® 40WG, Syngenta), and a nontreated control. Insecticide treatments were applied in Headland by using a backpack sprayer with TXV8 hollow cone nozzles at a rate of 140.3 L/ha and 45 psi (3.2 kg/cm^2) and in Brewton by using a John Deere 6000 sprayer with flat fan TeeJet 8004 nozzles at a rate of 187.1 L/ha and 30 psi (2.1 kg/cm^2).

Four neonicotinoid seed treatments were evaluated for reducing early-season infestations of *M. sorghi* in Fairhope and Tallassee by comparing them with a control that did not receive insecticide. All treatments used K73-J6 (Chromatin Inc., Chicago, IL), a known susceptible grain sorghum variety treated with Concep® III safener (Syngenta). Research plots were planted on 17 June at both locations. Rates of insecticide seed treatments (in kilograms of active ingredient [ai] of formulated product per 45 kg of seed) include 0.135 kg of thiamethoxam (Cruiser® 5FS, Syngenta), 0.113 kg of imidacloprid (Gaucho® 600, Bayer), 0.113 kg of clothianidin (NipsIt Inside®, Bayer), and 0.113 kg of clothianidin (Poncho® 600, Valent U.S.A, Walnut Creek, CA).

Data collection in foliar insecticide evaluations. Plots at each site were scouted for *M. sorghi* weekly after planting, and data collection began the first week infestations were detected. The total number of *M. sorghi* was counted weekly from an upper fully expanded leaf (highest leaf below flag leaf) and lower leaf (second from the bottom, or lowest green leaf) for 10 random plants in the interior rows for each plot. Foliar insecticide applications were made when aphid populations in the foliar insecticide trial reached a treatment threshold of 75 aphids per plant for 30% of all plants (Brewer et al. 2017). After insecticide application, aphids were counted at 5 and 10 d post treatment and then weekly until populations rebounded to treatment threshold; at least one plot for the nontreated control plot (and when time allowed, for treatments that surpassed treatment threshold) was assessed on each evaluation period. Once aphid populations for all foliar insecticide treatments rebounded to treatment threshold, all plots were sprayed with 0.105 kg/ha Transform WG and maintained below threshold through harvest. Plant growth stage was recorded weekly, and maturity was noted when 50% of plants from each plot had fully exerted panicles. Each plot was rated for injury using a 1–9 injury scale adapted from Webster et al. (1991), Burd et al. (2006), Armstrong et al. (2015): 1 = healthy, 2 = 1–5% injury and spotted, 3 = 5–20%, 4 = 21–35%, 5 = 36–50%, 6 = 51–65%, 7 = 66–80%, 8 = 81–95%, and 9 = 95–100% or dead. Ratings were made on the interior rows of each plot during weekly aphid counts. The last injury rating was conducted when aphid populations rebounded to the treatment threshold. The two interior rows were harvested for yield (tonnes per hectare) when grain moisture reached approximately 14%.

Data collection for seed treatment evaluations. Plots were scouted for *M. sorghi* weekly after planting. Once infested, the total number of *M. sorghi* was counted from 10 random whole plants in the exterior rows (one and four) of each plot by destructive sampling during the V1–V7 growth stages. Once plants reached the V8 growth stage, aphid counts were taken from a single leaf (either the fourth, fifth, or sixth whole leaf from the bottom of the plant) for 10 random plants per plot. Aphids were counted weekly until populations reached the treatment threshold of 75 aphids per plant for 30% of all plants, after which they were sprayed with 0.105 kg/ha Transform WG and maintained aphid free until harvest. Plots at Fairhope and Tallassee were sprayed 7 and 14 August, respectively.

Plant growth stage (i.e., number of fully developed true leaves) was recorded from 10 random plants per plot of the interior rows at 28 d post emergence. The interior rows of each plot were rated for injury using the 1–9 rating scale as described previously once all treatments reached treatment threshold. Plant maturity was noted when 50% of plants from each plot had fully exerted panicles. The two interior rows were harvested for yield (tonnes per hectare) when grain moisture reached approximately 14%.

Natural enemies. The presence of natural enemy species was recorded for each plant sample on which aphids were counted. In the foliar insecticide trials, natural enemies were recorded in Headland on 30 July, and 6 and 13 August and in Brewton on 21, 26, and 29 July, and 4 and 10 August. In the seed treatment trial, natural enemy species were recorded in Tallassee on 21 and 27 July, and 3 and 12 August and in Fairhope on 22 and 28 July. The natural enemies identified included coccinellid larvae and adults (Coleoptera: Coccinellidae), syrphid larvae (Diptera: Syrphidae), lacewing larvae (Neuroptera: Chrysopidae and Hemerobiidae), minute pirate bug (*Orius insidiosus* Say, Hemiptera: Anthocoridae), and parasitoids as identified by mummies (parasitized aphids) (e.g., black or blue mummies [Hymenoptera: Aphelinidae] and tan mummies [Hymenoptera: Braconidae]). Mummies were transported to the lab, and the parasitoids were reared to verify identification. Relative abundances per plot were calculated separately for each natural enemy species by summing the number of plant samples per plot with an observation. We also calculated species richness (S) per plot for each location and date by summing the total number of species identified from the 10 plant samples.

Statistical analysis. Before statistical analyses, the number of aphid-days per sample (either whole plant or two-leaf sample) was calculated for each experiment and evaluation period following the equation developed by Ruppel (1983):

$$\text{Aphid–days} = (X_{i+1} - X_i)\left[(Y_i + Y_{i+1})/2\right]$$

in which X_i and X_{i+1} are two adjacent observation periods and Y_i and Y_{i+1} are the aphid densities corresponding to X_i and X_{i+1}. The aphid-days measurement is indicative of the severity of an insect attack, and it takes into consideration the number of surviving insects between time periods (Brewer et al. 2017, Kieckhefer et al. 1995, Ruppel 1983). Cumulative aphid-days were calculated by adding the number of aphid-days from each prior data collection period. To account for the transition between whole-plant samples and single-leaf samples in the seed treatment trials, X_i was designated as the final observation period using whole-plant samples, whereas X_{i+1} was the first observation period using single-leaf samples.

Data for the seed treatment and foliar insecticide were analyzed in separate one-way analyses of variance (ANOVAs) by location and evaluation date to compare the average number of aphid-days per sample, the average plant stand counts, plant growth stages, injury ratings, maturity ratings, and sorghum yields among treatments. Analyses were conducted using PROC GLIMMIX (SAS 9.4, SAS Institute 2013) with treatment as a main effect and block, residuals, and plant (only for aphid-days analyses) as random effects; block was included as a random effect because block was not a significant main effect in preliminary analyses. Mean comparisons were conducted using a simulated post hoc test with LS means at a $P \leq 0.05$ level.

Natural enemy species richness per plot was compared among treatments in separate analyses by experiment and location by using PROC GLIMMIX as previously described, but with treatment, date, and treatment $\times$ date interaction as main effects. We then used a nonparametric multivariate ANOVA (permutational multivariate ANOVA [PERMANOVA]) with the vegan package (Oksanen et al. 2022) in R 4.2.2 (R Core Team 2022) to compare the natural enemy community assemblages (from the relative abundance data per plot) among (a) the different sites for each experiment and (b) insecticide treatments over time at each site. For each analysis, we first created a dissimilarity matrix based on the relative abundance data for all species by using the vegdist function with a Bray–Curtis distribution. Each resulting matrix was square root transformed and input to the adonis2 function for PERMANOVA. To account for temporal differences in the natural enemy communities present in each experiment, we only included data from weeks when both sites were evaluated; the foliar insecticide analysis only included data from treatments evaluated at both sites during the weeks of 26 July and 2 August (i.e., control, flupyradifurone, sulfoxaflor, and thiamethoxam) and the seed treatment analysis included data from all treatments during the weeks of 19 and 26 July. To compare natural enemy communities among sites for each location, we designated site as the main effect and used the Bray–Curtis distribution model with 1,000 permutations. To compare natural enemy communities among insecticide treatments over time at each location, we designated treatment, date, and treatment × date interaction as main effects, and we used the Bray–Curtis distribution model with 1,000 permutations. Average aphid-days per plot was not included in the model because it did not significantly alter the output for any of the analyses. Multiple comparisons tests were conducted using the pairwise Adonis package (Martinez Arbizu 2020) in R (R Core Team 2022) with 1,000 permutations. For each site, we also conducted separate robust regression analyses (Chen 2002) with PROC ROBUSTREG (SAS Institute 2013) to assess the relationships between average number of aphids and natural enemy species per plot from all sampling periods.

Results

Foliar insecticide efficacy. Performance of foliar insecticide sprays for reducing populations of *M. sorghi* were consistent among locations despite differences in the severity of initial infestations (Tables 1, 2). Aphids were first detected 8 and 9 July (21 and 24 d after planting) in Brewton and Headland, respectively. Populations were above threshold at this time in Headland, and foliar sprays were applied the next day. At Brewton, populations were above threshold the following week on 14 July, and insecticide applications were made for all treatments on 16 July. The number of aphid-days per two-leaf sample significantly varied among treatments on all evaluation dates at both locations (Tables 1, 2).

The insecticides β-cyfluthrin, dimethoate, or chlorpyrifos and dimethoate (in combination) did not suppress aphid populations below threshold at either location (Tables 1, 2), and yields from these plots were not significantly different from those in the nontreated control plots (Table 3). Insecticide treatments of chlorpyrifos did not significantly suppress aphids at Headland and suppressed aphids below threshold for only 5 d at Brewton (Table 1). At both locations, applications of sulfoxaflor suppressed populations below threshold for approximately 2–3 weeks (Tables 1, 2), but the yields were only significantly higher than those of the control in Headland at the high application rate (Table 3). Flupyradifurone and thiamethoxam suppressed populations below threshold for >20 d (Tables 1, 2), and yields from these plots were significantly higher than those of all other treatments at both locations (Table 3). There were no significant differences in plant growth stage at either location (Table 3). In Headland, where plots were initially heavily infested and over threshold, injury ratings in plots sprayed with flupyradifurone, thiamethoxam, and the high rate of sulfoxaflor were significantly lower than those of the nontreated control plots (Table 3). Foliar insecticides did not affect maturation time at Brewton (Table 3), but at Headland (Table 3) plots receiving

Table 1. Mean (±SE) number of aphid-days per two-leaf sample among plots receiving different foliar insecticides in Brewton, AL. Foliar insecticides were applied on 16 July 2015. Summary statistics below each evaluation period are for the main effect of treatment. DAT = days after treatment application.

	Mean no. of aphid-days per two-leaf sample*						
Treatment (rate ha^{-1})	8 July check	14 July check	21 July 5 DAT	26 July 10 DAT	29 July 13 DAT	4 August 19 DAT	10 August 25 DAT
Control	63.5 ± 7.9ab	339.1 ± 71.6	2,698.6 ± 339.8a**	6,020.8 ± 354.0a**	4,486.2 ± 110.3a**	7,191.9 ± 416.6a**	4,643.1 ± 341.5a**
Sulfoxaflor (0.07 kg)	47.3 ± 7.9bc	299.0 ± 79.5	317.2 ± 324.4bc	19.3 ± 354.0e	83.1 ± 55.5b	1,559.1 ± 208.3b**	—†
Sulfoxaflor (0.11 kg)	45.6 ± 7.9bc	376.4 ± 73.1	305.4 ± 324.4bc	21.0 ± 354.0e	63.0 ± 55.5b	1,604.8 ± 208.3b**	2,888.3 ± 341.5b**
Flupyradifurone (118.3 ml)	77.1 ± 7.9ab	250.0 ± 77.5	466.1 ± 324.4bc	6.0 ± 354.0e	9.5 ± 55.5b	23.1 ± 208.3c	23.3 ± 176.4c
Flupyradifurone (207.0 ml)	24.2 ± 7.9c	129.6 ± 66.3	213.6 ± 324.4c	10.6 ± 354.0e	11.5 ± 55.5b	17.3 ± 208.3c	16.1 ± 176.4c
Thiamethoxam (0.07 kg)	92.1 ± 7.9a	353.1 ± 75.1	528.5 ± 324.4bc	8.6 ± 354.0e	26.0 ± 55.5b	159.5 ± 208.3c	521.1 ± 176.4c
Chlorpyrifos (946.4 ml)	53.6 ± 7.9bc	364.2 ± 75.1	575.6 ± 324.4bc	1,828.9 ± 354.0d**	—	—	—
Dimethoate (473.2 ml)	45.1 ± 7.9bc	318.6 ± 71.7	1,177.4 ± 324.4bc**	4,352.7 ± 354.0bc**	—	—	—
β-Cyfluthrin (82.8 ml)	55.3 ± 7.9bc	259.0 ± 79.2	1,569.1 ± 324.4ab**	2,992.8 ± 354.0cd**	—	—	—
Chlorpyrifos (473.2 ml) + dimethoate (473.2 ml)	92.6 ± 7.9a	206.9 ± 72.0	1,379.1 ± 324.4abc**	4,425.4 ± 354.0b**	—	—	—
df	9, 378	9, 114	9, 374	9, 378	5, 192	5, 192	4, 123
F value	7.68	1.67	6.65	43.83	245.85	179.08	218.50
P value	<0.0001	0.1040	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Column means followed by double asterisks (**) indicate when aphid populations for that insecticide treatment rebounded to the treatment threshold of 75 aphids per plant for 30% of plants. Column means followed by the same lowercase letter are not significantly different (LS means, $P \leq 0.05$).
† Aphids were not counted after populations rebounded to the treatment threshold, except in the control plots.

Table 2. Mean (±SE) number of aphid-days per two-leaf sample among plots receiving different foliar insecticides in Headland, AL. Foliar insecticides were applied on 10 July 2015. Summary statistics below each evaluation period are for the main effect of treatment. DAT = days after treatment application.

	Mean no. of aphid-days per two-leaf sample*						
Treatment (rate ha^{-1})	9 July check	15 July 5 DAT	20 July 10 DAT	24 July 14 DAT	30 July 20 DAT	6 August 27 DAT	13 August 34 DAT
Control	769.3 ± 117.0b	1,410.5 ± 339.3b**	7,403.4 ± 412.5a**	11,438.6 ± 634.9a**	20,800.5 ± 799.0a**	18,830.1 ± 1,026.3a**	14,414.0 ± 1,063.1a**
Sulfoxaflor (0.07 kg)	1,143.2 ± 198.2ab	778.0 ± 390.2b	656.2 ± 357.5bc	451.4 ± 634.9b	508.4 ± 402.3b	5,325.8 ± 560.9b**	7,917.6 ± 1,063.1b**
Sulfoxaflor (0.11 kg)	1,136.6 ± 198.8ab	1,135.3 ± 392.2b	542.4 ± 357.5bc	218.7 ± 639.9b	205.0 ± 402.3b	1,683.8 ± 560.9c**	—†
Flupyradifurone (118.3 ml)	1,630.5 ± 165.8a	744.6 ± 357.5b	163.0 ± 357.5c	58.0 ± 645.1b	65.2 ± 402.3b	158.5 ± 560.9c	977.8 ± 576.5cd**
Flupyradifurone (207.0 ml)	926.6 ± 198.8ab	676.8 ± 392.2b	210.4 ± 357.5c	124.4 ± 625.2b	38.9 ± 402.3b	113.3 ± 556.2c	198.5 ± 574.9d**
Thiamethoxam (0.07 kg)	1,280.0 ± 166.0ab	707.9 ± 357.3b	335.1 ± 357.5bc	244.1 ± 634.9b	123.8 ± 402.3b	841.9 ± 560.9c**	2,530.7 ± 576.5c**
Chlorpyrifos (946.4 ml)	1,037.7 ± 274.0ab	1,057.5 ± 477.1b**	1,802.9 ± 357.5b**	—	—	—	—
Dimethoate (473.2 ml)	1,206.8 ± 147.0ab	2,649.3 ± 357.5a**	—	—	—	—	—
β-Cyfluthrin (82.8 ml)	352.2 ± 274.0b	3,699.5 ± 477.3b**	—	—	—	—	—
Chlorpyrifos (473.2 ml) + dimethoate (473.2 ml)	1,024.7± 198.8ab	2,504.4 ± 392.2a**	—	—	—	—	—
df	9, 118	9, 208	6, 250	5, 218	5, 191	5, 191	4, 122
F value	4.18	7.84	31.25	96.09	103.68	140.18	166.97
P value	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Column means followed by double asterisks (**) indicate when aphid populations for that insecticide treatment exceeded the treatment threshold of 75 aphids per plant for 30% of plants following application. Column means followed by the same letter are not significantly different (LS means, $P \leq 0.05$).

† Aphids were not counted after populations rebounded to the treatment threshold, except in the control plots.

Table 3. Plant growth characteristics including yield, maturation rate when 50% of plants per plot had their panicles exerted, 1–9 injury rating, and growth stage of sorghum plots receiving different foliar insecticide applications in Brewton, AL (A) and Headland, AL (B). Summary statistics below each evaluation period are for the main effect of treatment.

	Brewton, AL			
Treatment (rate ha^{-1})	Yield (tonnes/ha) 22 October	Maturation rate (days after plant)	Injury rating (1–9) 10 August	Growth stage 19 August
Control	0.3 ± 0.3c*	78.0 ± 8.8	8.0 ± 1.4a	12.0 ± 0.1
Sulfoxaflor (0.07 kg)	1.3 ± 0.3bc	80.0 ± 4.5	3.5 ± 0.9ab	13.5 ± 0.1
Sulfoxaflor (0.11 kg)	1.3 ± 0.3c	80.0 ± 4.5	3.3 ±0.9 ab	13.3 ± 0.1
Flupyradifurone (118.3 ml)	4.7 ± 0.3a	78.0 ± 4.4	2.0 ± 0.7b	14.0 ± 0.1
Flupyradifurone (207.0 ml)	4.1 ± 0.3a	78.0 ± 4.4	2.5 ± 0.8ab	13.8 ± 0.1
Thiamethoxam (0.07 kg)	4.1 ± 0.3a	78.0 ± 4.4	2.8 ± 0.8ab	13.8 ± 0.1
Chlorpyrifos (946.4 ml)	0.5 ± 0.3c	78.0 ± 8.8	7.5 ± 1.4ab	13.8 ± 0.1
Dimethoate (473.2 ml)	0.04 ± 0.3c	NA	7.5 ± 1.4ab	11.3 ± 0.2
β-Cyfluthrin (82.8 ml)	0.2 ± 0.3c	NA	7.8 ± 1.4ab	13.3 ± 0.1
Chlorpyrifos (473.2 ml) + dimethoate (473.2 ml)	0.7 ± 0.3c	86.0 ± 6.6	8.0 ± 1.4a	13.3 ± 0.1
df	9, 27	9, 27	9, 27	9, 27
F value	49.62	0.21	4.81	0.22
P value	<0.0001	0.9765	0.0007	0.9888

Table 3. Continued.

	Headland, AL			
Treatment (rate ha^{-1})	**Yield (tonnes/ha) 20 October**	**Maturation rate (days after plant)**	**Injury rating (1–9) 13 August**	**Growth stage 19 August**
Control	0.3 ± 1.1c	88.0 ± 4.7a	7.5 ± 1.4a	13.0 ± 0.1
Sulfoxaflor (0.07 kg)	1.3 ± 1.1bc	62.0 ± 4.0b	5.3 ± 1.1ab	13.3 ± 0.1
Sulfoxaflor (0.11 kg)	3.5 ± 1.1b	59.0 ± 3.8b	4.3 ± 1.0bc	13.0 ± 0.1
Flupyradifurone (118.3 ml)	8.2 ± 1.1a	59.0 ± 3.8b	2.3 ± 0.8c	13.3 ± 0.1
Flupyradifurone (207.0 ml)	8.3 ± 1.1a	59.0 ± 3.8b	2.3 ± 0.8c	13.0 ± 0.1
Thiamethoxam (0.07 kg)	7.0 ± 1.1a	59.0 ± 3.8b	4.0 ± 1.0bc	13.0 ± 0.1
Chlorpyrifos (946.4 ml)	0.5 ± 1.1c	109.0 ± 5.2a	7.3 ± 1.3a	13.3 ± 0.1
Dimethoate (473.2 ml)	0.4 ± 1.1c	109.0 ± 5.2a	7.3 ± 1.3a	13.3 ± 0.1
β-Cyfluthrin (82.8 ml)	0.4 ± 1.1c	109.0 ± 5.2a	7.0 ± 1.3a	13.0 ± 0.1
Chlorpyrifos (473.2 ml) + dimethoate (473.2 ml)	0.3 ± 1.1c	109.0 ± 5.2a	7.0 ± 1.4a	12.3 ± 0.1
df	9, 25	9, 27	9, 27	9, 27
F value	51.32	28.56	15.64	0.02
P value	<0.0001	<0.0001	0.023	1

* Column means followed by the same letter are not significantly different (LS means, $P \leq 0.05$). NA = not available because aphid infestations were so severe 50% maturity was not reached.

Table 4. Mean number of aphid-days accumulated per plant and leaf samples among plots receiving different insecticide seed treatments in Fairhope, AL. Summary statistics below each evaluation period are for the main effect of treatment.

	Mean no. of aphid-days*		
	per plant		per leaf
Treatment	14 July	22 July	28 July
Control	198.8 ± 17.3a**	364.5 ± 24.3a**	1,907.6 ± 178.4a**
Cruiser 5FS	30.4 ± 17.3b	67.8 ± 24.3b	106.3 ± 178.4b
Gaucho 600	83.0 ± 17.3b	140.9 ± 24.3b	472.9 ± 178.4b
NipsIt Inside	64.1 ± 17.3b	97.7 ± 24.3b	131.7 ± 178.4b
Poncho 600	46.8 ± 17.36b	59.4 ± 24.3b	56.7 ± 178.4b
df	4, 183	4, 183	4, 183
F value	15.86	27.61	19.69
P value	<0.0001	<0.0001	<0.0001

* Column means followed by a double asterisk (**) indicate when aphid populations for that insecticide treatment exceeded the treatment threshold of 75 aphids per plant for 30% of plants following application. Column means followed by the same letter are not significantly different (LS means, $P \leq 0.05$).

applications of flupyradifurone, thiamethoxam, and sulfoxaflor matured in 59–62 d, whereas plants in plots receiving applications of chlorpyrifos, dimethoate, β-cyfluthrin, or no insecticide matured in ≥88 d.

Seed treatment residual activity. *Melanaphis sorghi* was first observed on 7 and 14 July (20 and 27 d post planting) at Tallassee and Fairhope, respectively (Tables 4, 5). At Fairhope, populations in nontreated control plots were above treatment threshold when first detected, whereas all other plots had significantly fewer aphid-days per plant sample and remained below treatment threshold through 28 July (Table 4). Because of inclement weather, aphid counts were not made on 4 August at Fairhope, although all plots including control and treatments were quickly scouted and determined to be well above threshold. Initial populations at Tallassee were below treatment threshold, but control plots had significantly more aphid-days per plant than plots receiving a seed treatment. Populations in control plots exceeded the treatment threshold on subsequent evaluation dates, whereas all other plots remained below threshold through 3 August (Table 5). On 12 August, Cruiser 5FS was the only treatment to remain below treatment threshold and had significantly fewer aphid-days per plant than Gaucho 600 and the control. Final plant growth stage and final injury ratings were not significantly different at either location (Table 6). Maturation time was similar among treatments at Tallassee (Table 6), but plots at Fairhope treated with Cruiser 5FS, NipsIt Inside, or Poncho 600 reached maturity nearly 2 weeks sooner than the control (Table 6). Sorghum yield data were unavailable for Fairhope plots because of undetected sorghum midge damage. At Tallassee, yields were significantly higher in plots receiving a seed treatment and produced approximately 3–4 tonnes/ha

Table 5. Mean number of aphid-days accumulated per plant and leaf samples among plots receiving different insecticide seed treatments in Tallassee, AL. Summary statistics below each evaluation period are for the main effect of treatment.

	Mean no. of aphid-days*					
	per plant		per leaf			
Treatment	7 July	13 July	21 July	27 July	3 August	12 August
Control	470.0 ± 25.6a**	3,715.0 ± 313.5a**	7,244.9 ± 506.6a**	8,094.8 ± 463.7a**	8,714.7 ± 543.7a**	7,537.7 ± 643.5a**
Cruiser 5FS	95.3 ± 25.6c	199.8 ± 256.5b	114.1 ± 506.6b	47.8 ± 463.7b	39.4 ± 543.7b	468.9 ± 643.5c
Gaucho 600	208.5 ± 25.6b	488.0 ± 249.6b	521.4 ± 506.6b	622.1 ± 463.7b	1,181.2 ± 543.7b	2,937.6 ± 643.5b**
NipsIt Inside	75.4 ± 25.6c	337.5 ± 300.7b	201.4 ± 506.6b	84.1 ± 463.7b	411.7 ± 543.7b	1,334.0 ± 643.5bc**
Poncho 600	94.4 ± 25.6c	266.4 ± 313.8b	151.7 ± 506.6b	62.3 ± 463.7b	32.1 ± 543.7b	937.8 ± 643.5bc**
df	4, 183	4, 98	4, 183	4, 183	4, 183	4, 183
F value	69.59	28.18	73.59	86.34	67.19	28.65
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Column means followed by a double asterisk (**) indicate when aphid populations for that insecticide treatment exceeded the treatment threshold of 75 aphids per plant for 30% of plants following application. Column means followed by the same letter are not significantly different (LS means, $P \leq 0.05$).

Table 6. Plant growth characteristics including yield, maturation rate when 50% of plants had their panicles exerted, 1–9 injury rating, and growth stage of sorghum plots receiving different insecticide seed treatments in Fairhope, AL (A) and Tallassee, AL (B). Summary statistics below each evaluation period are for the main effect of treatment.

	Fairhope, AL			
Treatment	**Yield (tonnes/ha) NA**	**Maturation rate (days after plant) NA**	**Injury rating (1–9) 4 August**	**Growth stage 11 August**
Control	NA	72.0 ± 4.1a*	6.8 ± 1.3	11.9 ± 0.1
Cruiser 5FS	NA	55.0 ± 3.5b	4.5 ± 1.1	12.1 ± 0.1
Gaucho 600	NA	67.0 ± 3.9ab	5.3 ± 1.1	12.2 ± 0.1
NipsIt Inside	NA	55.0 ± 3.5b	5.3 ± 1.1	12.0 ± 0.1
Poncho 600	NA	55.0 ± 3.5b	4.5 ± 1.1	11.8 ± 0.1
df		4, 12	4, 12	4, 12
F value		5.91	0.4	0.01
P value		0.0073	0.8047	0.9996
	Tallassee, AL			
Treatment	**12 October**	**NA**	**19 August**	**19 August**
Control	1.2 ± 0.2b	71 ± 4.2	6.5 ± 1.3	16.0 ± 0.1
Cruiser 5FS	5.4 ± 0.2a	63 ± 4.0	2.3 ± 0.8	16.0 ± 0.1
Gaucho 600	4.5 ± 0.2a	63 ± 4.0	3.3 ± 0.9	16.0 ± 0.1
NipsIt Inside	5.2 ± 0.2a	63 ± 4.0	3.3 ± 1.0	16.3 ± 0.1
Poncho 600	5.4 ± 0.2a	63 ± 4.0	3.5 ± 0.9	15.8 ± 0.1
df	4, 12	4, 12	4, 12	4, 12
F value	67.31	0.79	2.11	0.01
P value	<0.0001	0.5527	0.1426	0.9999

* Column means followed by the same letter are not significantly different (LS means, $P \leq 0.05$). NA =.

more yield than that of control plants (Table 6). Yields did not vary among plots receiving treatments with different active ingredients.

Natural enemies. Natural enemies were present on 185 (15.4%) of 1,200 plant samples in the seed treatment trial and 303 (16.1%) of 1,880 plant samples in the foliar insecticide trial. Throughout these studies, 15 natural enemy species were recorded either predating or parasitizing (i.e., presence of mummies) *M. sorghi* in Alabama and included 6 species of coccinellids [*Coccinella septempunctata* (L.), *Coleomegilla maculata* DeGeer, *Diomus terminatus* Say, *Harmonia axyridis* Pallas, *Hippodamia convergens* Guerin-Meneville, and *Scymnus* sp.], 4 syrphid species (*Allograpta obliqua* Say, *Pseudodorus clavatus* F., *Syrphus* sp., and *Toxomerus geminatus* Say), 2 lacewing species (*Cereaochrysa* sp. and *Hemerobius* sp.), the

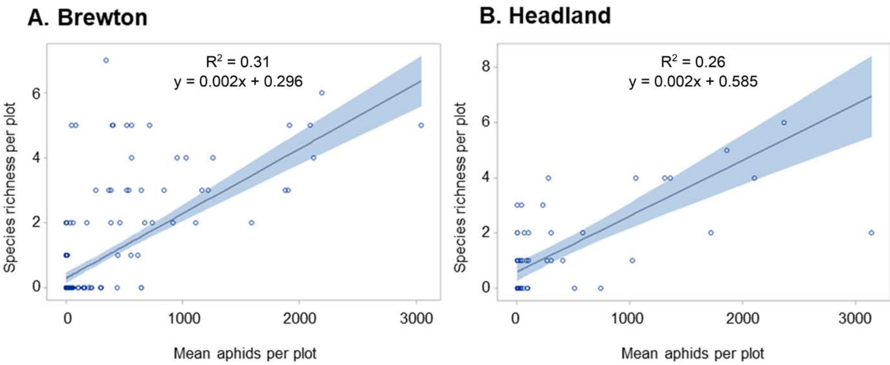

Fig. 1. Robust regression analysis between species richness and aphid density per plot in the foliar insecticide trials conducted in Brewton, AL (A), and Headland, AL (B), 2015. The shaded area outside of trendlines represents the 95% confidence interval.

minute pirate bug, and 2 parasitoid species (*Aphelinus nigritus* Howard [identified by black-blue mummies; see Maxson et al. 2019]) and *Lysiphlebus testaceipes* Cresson [tan mummies]). Parasitoids (mummies) were the most frequently encountered natural enemy and were observed on 9.1% of the total 3,080 plants samples evaluated for this objective. Coccinellid larvae and adults (5.5% of plant samples) were the second most prevalent natural enemy group, followed by syrphid larvae (4.1% of plant samples), lacewing larvae (1.5% of plant samples), and minute pirate bug (0.1% of plant samples), which was the least common. Fourteen species were identified at Tallassee, followed by 13 species at Headland, 12 species at Brewton, and 5 species at Fairhope. All species were found at two or more locations, except for *P. clavatus* and *T. germinates*, which were only recorded at Brewton and Tallassee, respectively.

In the foliar insecticide trial, observed natural enemy communities were significantly different at Brewton and Headland ($F = 3.66$; df $= 1, 40$; $P = 0.0110$). At Brewton, foliar insecticides did not significantly influence the natural enemy community ($F = 1.17$; df $= 5, 17$; $P = 0.3020$), or species richness per plot ($F = 0.67$; df $= 9, 60$; $P = 0.7332$). Instead, the observed communities changed over time ($F = 3.66$; df $= 1, 17$; $P = 0.0050$), and the number of species per plot was positively correlated with aphid density (Fig. 1A; $R^2 = 0.31$, $\chi^2 = 201.75$, $P < 0.0001$). The relationship between species richness and aphid density varied among treatments ($F = 6.77$; df $= 9, 135$; $P < 0.0001$). Similar trends were observed at Headland, and foliar insecticides did not influence the natural enemy community assemblage ($F = 1.44$; df $= 5, 22$; $P = 0.1500$) or species richness per plot ($F = 2.14$; df $= 5,26$; $P = 0.0919$). The natural enemy community also changed over time ($F = 6.33$; df $= 1, 22$; $P = 0.0030$), and species richness was positively correlated with aphid density (Fig. 1B; $R^2 = 0.26$, $\chi^2 = 60.46$, $P < 0.0001$).

In the seed treatment trial, natural enemy communities were distinct at Fairhope and Tallassee ($F = 5.59$; df $= 1, 30$; $P = 0.0010$). At Fairhope, natural enemy communities varied among seed treatments ($F = 5.81$; df $= 3, 12$; $P = 0.0080$), but were

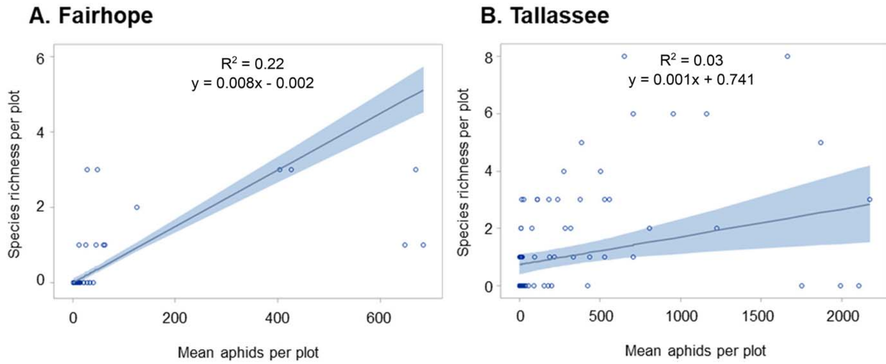

Fig. 2. Robust regression analysis between species richness and aphid density per plot in the seed treatment trials conducted in Fairhope, AL (A), and Tallassee, AL (B), 2015. The shaded area outside of trendlines represents the 95% confidence interval.

similar over time ($F = 2.75$; df = 1, 12; $P = 0.1079$). Species richness was not influenced by seed treatment ($F = 1.29$; df = 1, 30; $P = 0.2959$) or time ($F = 0.00$; df = 1, 30; $P = 0.9898$). Instead, species richness was positively correlated with aphid density (Fig. 2A; $R^2 = 0.22$, $\chi^2 = 249.01$, $P < 0.0001$). Zero natural enemies were recorded from the Poncho treatment, which had the fewest aphids, whereas four species were recorded from the control, which had the most aphids. At Tallassee, seed treatments also influenced average species richness ($F = 5.58$; df = 4, 60; $P = 0.0007$), but not the community assemblages ($F = 1.07$; df = 4, 43; $P = 0.4066$). Community assemblages changed over time ($F = 3.14$; df = 3, 43; $P = 0.0010$) and species richness per plot was correlated with aphid density (Fig. 2B; $R^2 = 0.03$, $\chi^2 = 7.90$, $P = 0.0049$).

Discussion

The results of these studies corroborate previous reports on the efficacy of chemical control options for management of *M. sorghi* on sorghum in Alabama and indicate that natural enemy communities respond to seed treatments and aphid density, but not foliar insecticides in the field. All seed treatments suppressed early-season infestations up to 47 d post planting regardless of active ingredient, which is consistent with other studies that reported efficacy from 3 weeks to 1 mo (Ahrens et al. 2013, Bowling et al. 2016b, Etheridge et al. 2018, Jones et al. 2015, Knutson et al. 2016, Lahiri et al. 2021, Szczepaniec 2018). Several foliar insecticides, including flupyradifurone, sulfoxaflor, and thiamethoxam, provided 20–30 d of population suppression in these experiments, irrespective of high *M. sorghi* pressure at application, which was also similar to findings of other studies (Buntin and Roberts 2016, Larsen et al. 2016, Studebaker and Jackson 2017, VanWeelden et al. 2016, Zarrabi et al. 2017). Only sulfoxaflor, flupyradifurone, and chlorpyriphos are available for commercial use as foliar sprays in sorghum. There is a cause for concern that *M. sorghi* will develop resistance because the three most successful foliar insecticides and all seed treatments are classified by the Insecticide Resistance Action Committee as Group 4 and have similar modes of action.

Foliar applications of organophosphates and pyrethroids did not reduce populations below treatment threshold following application or significantly alter natural enemy communities. These products require higher application rates and are known to harm off-target organisms, whereas flupyradifurone, sulfoxaflor, and thiamethoxam are regarded to be compatible with the biological control agents evaluated (Barbosa et al. 2017; Bowling et al. 2016b; Colares et al. 2015a, 2017; Davis et al. 2019; Hakeem and Parajulee 2019; Szczepaniec 2018). In this study, we determined that neonicotinoid seed treatments, but not foliar-applied insecticides, influenced the natural enemy communities observed, perhaps because of the mode of action of neonicotinoid seed treatments and/or lack of aphid prey in plots receiving seed treatments. Seed applied insecticides are translocated throughout developing plant tissues and directly ingested by feeding aphids, suppressing populations as soon as they colonize the plant. Foliar applications might miss the aphids which are initially concentrated to the abaxial surface of lower leaves (Yang et al. 2021), providing natural enemies with increased prey availability. In addition, the control plots in the foliar insecticide trial, which remained above threshold while the other plots were sprayed, aided in the recruitment and retention of natural enemies while populations in the treated plots rebounded.

Fifteen of the 22 predators and parasitoids reported to feed on *M. sorghi* in grain sorghum fields in Alabama, Kansas, Louisiana, and Texas (Bowling et al. 2016a, Pekarcik and Jacobson 2021) were observed in this study, but there were differences in the communities and number of species recorded among locations. Because of the limited number of samples, sampling dates, and instances of some natural enemies, it was not possible to assess the natural enemy communities before foliar insecticide application or after seed treatment efficacy waned. However, preliminary analyses of natural enemies from dates and trials with higher sample sizes and instances (e.g., Colares et al. 2015a,b, 2017; Szczepaniec 2018) suggest that although coccinellid larvae and adults, lacewing larvae, and parasitoid mummies were observed in all plots of the insecticide seed treatment experiments, they were observed more frequently in the nontreated plots; syrphid larvae were only observed in the nontreated plots. In the foliar insecticide trials, syrphid larvae, coccinellids, and mummies were observed in plots after applications of foliar sprays and were more frequently observed as populations increased after application. Colares et al. (2017) showed that topical applications and residual activity of flupyradifurone and sulfoxaflor caused mortality to coccinellid larvae and adults, whereas Davis et al. (2019) observed reductions in natural enemy populations following field applications of chlorpyrifos, α-cypermethrin, or λ-cyhalothrin. We did not directly evaluate the efficacy of insecticides to natural enemy species, although this study indicates that the use of neonicotinoid seed treatments and foliar insecticides in July for *M. sorghi* does not suppress subsequent colonization of and oviposition by natural enemy adults (data not shown).

None of the insecticides evaluated in these studies suppressed *M. sorghi* populations below treatment threshold for the duration of this study after a single application. It possible that farmers will need to reapply products once populations rebound to the treatment threshold or following new colonization events. Insecticide application is variable and typically responsive to pest pressure. Zapata et al (2018) noted that farmers applied an average of 0.85 insecticide applications in 2015 as *M. sorghi* populations were smaller relative to 2014, when an average of 1.65 applications was made. Future work is needed to understand how additional applications of foliar insecticides—for example, overspraying plots with sulfoxaflor in this study after populations rebounded to the treatment threshold—influence natural enemies.

In the event that additional insecticide applications are needed, the elimination of prey could cause natural enemies to disperse elsewhere. In this study, natural enemy communities became more abundant and diverse over time, and they were positively correlated with aphid abundance in all trials, which is consistent with findings of other studies (Brewer et al. 2017, Szczepaniec 2018). However, natural enemies appeared after the aphids arrived, and their slower generation times were unable to keep up with *M. sorghi* population growth. Weather conditions of the southeastern United States also may have contributed to low natural enemy activity (Brewer et al. 2022) or lower aphid populations (Zapata et al. 2018) relative to other sorghum production regions (Brewer et al. 2022). All experiments were conducted under dryland conditions, and plots at Brewton and Headland received little rainfall ($<$10.7 cm). Brewer et al. (2022) determined that precipitation was positively correlated with *L. testaceipes* parasitism and coccinellid adult and lacewing and syrphid activity, whereas high temperatures decreased lady beetle adult activity. Zapata et al. (2018) observed a reduction in insecticidal sprays due to less *M. sorghi* pressure during lower average temperatures and increased precipitation.

The results of this study provide additional information about the regional efficacy of foliar- and seed-applied insecticides for management of *M. sorghi* and new data on the natural enemy community assemblages observed in Alabama. The chemical control options evaluated in this study, including seed treatments and foliar insecticides for early-season suppression and curative control, respectively, provided control of *M. sorghi* that was consistent with findings of studies conducted in other production regions in the United States (Ahrens et al. 2013, Bowling et al. 2016b, Etheridge et al. 2018, Jones et al. 2015, Knutson et al. 2016, Lahiri et al. 2021, Szczepaniec 2018). These tools are important components of an integrated pest management program for *M. sorghi* on sorghum in Alabama, because initial infestations and recolonization events are difficult to predict and populations increase exponentially. The integration of other control tactics such as host plant resistance (Pekarcik and Jacobson 2021) and biological control (Lahiri et al. 2021) show promise to suppress aphid population growth, reduce insecticide applications, and promote natural enemies. Recruitment and retention of these species early in the growing season are critical to suppress *M. sorghi* and prevent populations from growing exponentially and to reduce the need for rescue treatments as was necessitated in these studies. Future studies should investigate combinations of these tactics in Alabama to establish an integrated pest management plan for farmers that promotes the recruitment and retention of natural enemies, suppresses *M. sorghi* population growth, and ultimately increases economic threshold levels.

Acknowledgments

The authors thank the E.V. Smith Plant Breeding Research Unit, Brewton Agricultural Research Station, Gulf Coast Research and Extension Center, Prattville Agricultural Research Station, and Wiregrass

Research and Extension Center for assisting with planting, maintenance, and harvest of field plots. We thank David Kerns, Ian Ingram, Emily Ingram, Dylan Bartlett, Bruno Sausa-Viera, Rafael De Almeida Leite, and Charles Ray for assistance with this project. Funding for this study was provided by the United Sorghum Checkoff Program, Alabama Wheat and Feed Grain Producers, Alabama Farmers Federation, and the Alabama Agricultural Experiment Station. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. USDA is an equal opportunity provider and employer.

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