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TURFGRASS DISEASE AND INSECT PEST MANAGEMENT - SHORT COMMUNICATION

Diversity, composition, and freedom to choose drive the effects of St. Augustinegrass cultivar blends on an herbivorous insect

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Abstract

Warm-season turfgrasses are conventionally produced and maintained as cultivar monocultures, which leaves them less resilient to pest attack than more diverse plantings. Recent evidence has indicated that mixing St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze] cultivars can provide pest management benefits compared with cultivar monocultures. Research in other systems has shown that the effects of plant diversity on herbivores often depends on the number and identity of plant species present. Host plant diversity can affect herbivore fitness via post-consumptive physiological effects or before consumption by influencing herbivore behavior or plant defenses. To investigate the mechanisms by which St. Augustinegrass cultivar diversity and composition affect an insect herbivore, six cultivars were mixed to create two levels of cultivar diversity, and fall armyworm life history traits were tracked under no-choice diet mixing conditions and conditions where the larvae could forage among mixed cultivars. These experiments demonstrated that specific cultivar blends can reduce herbivore fitness and herbivory, and that the effects on fall armyworm fitness are likely driven by insect–plant interactions rather than postconsumptive physiological effects. The results will inform pest management strategies in warm-season turfgrass production and management, adding to the literature focused on the effects of intraspecific plant diversity.

1 | INTRODUCTION

Increasing plant species diversity is often proposed as a strategy to reduce crop susceptibility to pests (Andow, 1991; Barbosa et al., 2009), and mounting evidence suggests similar benefits can be obtained by increasing intraspecific diversity (Tooker & Frank, 2012). Most warm-season turfgrasses are produced as single cultivar monocultures. Recent evidence has indicated that mixing St. Augustinegrass [*Stenotaphrum secundatum* (Walter) Kuntze] cultivars enhances resilience to insect pests, weed invasion, and other biotic and abiotic stress factors compared with cultivar monocultures (Doherty et al., 2019; Pinkney et al., 2021; Whitman et al., 2022). Doherty et al. (2019) found that in the absence of pest-resistant St. Augustinegrass cultivars, mixing two or four cultivars reduced fall armyworm (*Spodoptera frugiperda* [J.E. Smith], Lepidoptera: Noctuidae) body size, colonization, and herbivory compared with cultivar monocultures. Others have observed similar effects of increasing crop cultivar diversity on herbivore fitness (Grettenberger & Tooker, 2016). The bottom-up effects of increasing cultivar diversity on insect herbivores have been attributed to reduced host selection efficiency (Barbosa et al., 2009; Root, 1973) and the increased

Abbreviations: M2, mixtures of two cultivars; M4, mixtures of four cultivars.

physiological cost of acclimating to a mixed diet (Wetzel & Thaler, 2016).

Although plant diversity alone can have pest management value, the effects of diversity often depend on plant community composition. For example, Grettenberger and Tooker (2016) found that the effect size of wheat (*Triticum aestivum* L.) cultivar diversity on aphid fitness depended on the focal host cultivar. Mixed plant diets can also influence herbivore fitness via pre- and postconsumptive effects. Several studies have shown that herbivores experience fitness effects when forced to consume a set regimen of mixed diets (Lefcheck et al., 2013; Schoonhoven & Meerman, 1978). However, negative effects of diet mixing may be diluted when herbivores can freely choose resources (Wetzel & Thaler, 2018). Additionally, neighboring plants may generate associational resistance or susceptibility by influencing herbivore behavior (Barbosa et al., 2009), or may inhibit herbivore success by increasing the vegetation's structural complexity (Randlkofer et al., 2010), neither of which can occur under no-choice conditions. Therefore, comparing an herbivore's response to mixed diets under limited-choice and no-choice conditions can reveal the mechanisms by which plant diversity influences herbivore fitness. Because the effects of plant diversity can produce contradicting results and be driven by different mechanisms, it is important to understand how turfgrass cultivar blends affect key insect pests when developing pest management tactics.

The objectives of this study were to determine if St. Augustinegrass cultivar diversity and cultivar blend composition influenced fall armyworm fitness, and if the effects of cultivar diversity on fall armyworm fitness were driven by pre- or postconsumptive interactions between the insect and plants. This study tested the hypotheses that (a) increasing cultivar diversity would reduce herbivore fitness, (b) the effect of cultivar diversity would be driven by specific cultivars, and (c) when herbivores were given the opportunity to forage, the negative effects of more diverse diets would be reduced.

Core Ideas

- Increasing cultivar diversity negatively affects fall armyworm life history traits.
- The effects of cultivar blends are driven by the presence of specific cultivars.
- The negative effects of diversity are linked to preconsumptive insect–plant interactions.
- Given finite cultivar pools, there is pest management value in maximizing cultivar diversity.

2 | METHODS AND MATERIALS

2.1 | Study organisms and design

St. Augustinegrass is produced by vegetative propagation and planted in lawns as a single cultivar. This experiment used six commercially produced St. Augustinegrass cultivars: 'Bitterblue', 'NUF-76' (Captiva®), 'Variety Not Stated' (Classic™, hereafter referred to as Classic), 'SS-100' (Palmetto®), 'Floratam', and '615866' (Seville™). Cultivars were maintained in pots in a greenhouse in Gainesville, FL (29°39′5.58″ N, 82°20′34.12″ W), where they were watered as needed and fertilized once every 2 wk with MiracleGro (24–8–16 %N–P–K).

The fall armyworm is a key insect pest of turfgrasses throughout the United States (Vittum, 2020). Doherty et al. (2019) evaluated all six previously mentioned cultivars in monoculture and found that all were equally susceptible to fall armyworm. Fall armyworm larvae of the rice strain were reared at the USDA ARS, Gainesville, FL, at 25 °C under a 16:8 light/dark photoperiod in 4- by 4- by 1-cm cell trays (Model RT32W, Frontier Agricultural Science) on a multiple species diet (Southland Products). After the eggs hatched, neonates were stored in growth chambers (Percival Scientific) kept at 27 °C, 70% relative humidity, and a 14:10 light/dark photoperiod at the University of Florida (Gainesville, FL).

Two cultivar diversity treatments were created from the pool of six cultivars: mixtures of two cultivars (M2) and mixtures of four cultivars (M4). Both diversity treatments included each unique combination of cultivars, resulting in 15 cultivar blends of two and of four cultivars (Table 1).

2.2 | No-choice experiment

To isolate the effects of cultivar diversity and composition on fall armyworm fitness in the absence of pre-consumptive plant–herbivore interactions, a no-choice experiment with plant clippings placed in rearing trays was conducted. Larvae in the M2 treatment received fresh clippings from two alternating cultivars, one cultivar every 2 d. Larvae in the M4 treatment received one of four cultivars every 2 d, receiving all four assigned cultivars after 8 days. Larvae were given more clippings than they could consume, and leftover clippings were removed before adding more. Feeding continued until pupation, and insects remained in the rearing trays until eclosion. Larval weight 12 d after egg hatch, days to eclosion, and survival rate were recorded. Trays were kept in growth chambers at 27 °C, 70% relative humidity, and a 14:10 light/dark photoperiod. This experiment was repeated four times.

2.3 | Limited-choice experiment

Fall armyworm life history traits were also measured in potted (15 cm in diameter) microcosms where plants could

TABLE 1 St. Augustinegrass cultivar blends with two cultivar combinations and blends with four cultivar combinations. Each experimental replicate included one replicate of each cultivar combination

Mixes of two	Mixes of four
Bi + Nu	Bi + Ca + Cl + Fl
Bi + 61	Bi + Ca + Cl + 61
Bi + Fl	Bi + Ca + Fl + 61
Bi + Cl	Bi + Cl + Fl + Ss
Bi + Ss	Bi + Cl + Ss + 61
Nu + Ss	Bi + Ca + Cl + Ss
Nu + Fl	Bi + Fl + Ss + 61
Nu + 61	Bi + Nu + Ss + 61
Nu + Cl	Bi + Cl + Fl + 61
Cl + 61	Bi + Nu + Fl + Ss
Cl + Ss	Nu + Cl + Ss + 61
Cl + Fl	Nu + Cl + Fl + Ss
Fl + Ss	Nu + Cl + Fl + 61
Fl + 61	Nu + Fl + Ss + 61
Ss + 61	Cl + Fl + Ss + 61

Note. Cultivar abbreviations: Bi, 'Bitterblue'; Nu, 'NUF-76'; Cl, Classic; Fl, 'Floratam'; Ss, 'SS-100'; 61, 615866'.

interact, and larvae could choose among the available cultivars. The M2 treatments were created by planting four rooted cuttings of each cultivar. The M4 treatment was created by planting two rooted cuttings of each cultivar. After establishment, two second-instar larvae were placed in the pots and enclosed within a 0.95-L portion container with a mesh (Casa Collection Organza Fabric, Joann) top that was secured to the pot with Parafilm (Pechiney). Treatments were randomly arranged along benchtops and kept in a greenhouse at approximately 30 ˚C and 80% relative humidity. Eleven to twelve days after egg hatch, larval weight and the percentage of herbivory per pot were measured. Upon eclosion, development time and survival rate were also recorded. This experiment was repeated five times, but only larval weight and percent herbivory were collected from the fifth replicate because it did not continue beyond that point.

2.4 | Statistical analyses

One-way mixed-effect ANOVA was used to determine if the cultivar diversity level influenced fall armyworm life history traits in either experiment. Cultivar diversity was treated as the main effect, and experimental replicate was the random effect. Mixed-effect ANOVA was also used to identify cultivar-dependent effects of cultivar blends (pooling M2 and M4 data) in the no-choice and limited-choice experiments, treating the presence or absence of each of the six cultivars as the main effects and the experimental replicate as a random effect. All model residuals were normally distributed according to Wilcoxon tests and data visualization. All statistical analyses were carried out with JMP Pro version 15 (SAS). Models were considered significant at $P < .05$.

3 | RESULTS

3.1 | No-choice experiment

There was no effect of cultivar diversity level on fall armyworm weight, development rate, or survival during the no-choice experiment (Table 2). Cultivar blends that included Classic yielded larvae that were 13% smaller (284.8 ±8.7 mg) on average than larvae reared on blends that did not contain Classic (327.9 ±11.3 mg) ($F_{1,109} = 8.55$, $P = .004$; Table 2). There were no other cultivar-dependent effects on fall armyworm life history traits.

3.2 | Limited-choice experiment

Compared with the no-choice experiment, fall armyworm larvae in the limited-choice experiment developed into larger individuals, took longer to develop, and had lower survival (Table 2). There was a strong effect of cultivar diversity on fall armyworm life history traits. Larvae reared in M4 plantings developed more slowly (25 ± 1.3 d; $F_{1,97} = 8.61$, $P = .004$) and into smaller individuals (298.11 ± 17.7 mg; $F_{1,106} = 6.66$, $P = .01$) than those reared in the M2 plantings (24 ± 0.3 d and 353.2 ± 17.5 mg, respectively; Table 2). Larvae also caused less herbivory in M4 plantings (40 ± 2.3%) than in M2 plantings (47 ± 2.1%) ($F_{1,144} = 7.96$, $P = .006$).

There were several cultivar-dependent effects on fall armyworm larvae in the limited-choice experiment (Table 2). In blends containing 'Bitterblue', larval weight was 16% smaller (292.3 ± 18.5 mg; $F_{1,101} = 10.26$, $P = .002$) and survival was 20% lower (55.8 ± 4.6%; $F_{1,110} = 5.02$, $P = .03$) than larvae reared in blends without 'Bitterblue' (349.1 ± 16.9 mg and 70 ±4.3%, respectively). Cultivar blends containing Classic yielded fall armyworm larvae that developed nearly a full day slower (24.9 ± 0.4 days) than blends without this cultivar (24 ±0.3 d; $F_{1,92} = 6.42$, $P = .01$; Table 2). Finally, cultivar blends containing either 'Floratam' ($F_{1,139} = 8.23$, $P = .005$) or 'SS-100' ($F_{1,139} = 7.65$, $P = .006$) experienced less herbivory (40.5 ±2.2%) than other blends (47 ±2.3%).

4 | DISCUSSION

Greater cultivar diversity negatively affected fall armyworm life history traits, but the magnitude of these effects depended

TABLE 2 Effects of cultivar diversity and cultivar composition on fall armyworm larval weight, development rate, survival, and herbivory in the limited-choice and no-choice experiments. Mean (± SEM) values are provided for each dependent variable of the limited-choice and no-choice assays

Factor	Larval weight	Development rate	Survival	Herbivory
Limited-choice	323.21 ± 12.72 mg	24.46 ± 0.25 d	63.0 ± 3.21%	43.53 ± 1.58%
Cultivar diversity	M4 15% smaller than M2*	M4 3% (24 h) slower than M2**	NS	M4 7% less than M2**
Cultivar composition				
Bi	16% smaller**	NS	20% lower*	NS
Nu	NS	NS	NS	NS
Cl	NS	3% slower*	NS	NS
Fl	NS	NS	NS	7% less**
Ss	NS	NS	NS	7% less**
61	NS	NS	NS	NS
No-choice	306.19 ± 7.37 mg	22.10 ± 0.15 d	100 ± 0%	–
Cultivar diversity	NS	NS	NS	–
Cultivar composition				
Bi	NS	NS	NS	–
Nu	NS	NS	NS	–
Cl	13% smaller**	NS	NS	–
Fl	NS	NS	NS	–
Ss	NS	NS	NS	–
61	NS	NS	NS	–

Note. Cultivar abbreviations: Bi, 'Bitterblue'; Nu, 'NUF-76'; Cl, Classic; Fl, 'Floratam'; Ss, 'SS-100'; 61, 615866'. NS indicates $P > .05$; –, data not collected.
*Signifiant at $P < .05$.**Significant at $P < .01$.

on cultivar composition and the opportunity for plants and herbivores to interact. Contrary to previous research (Wetzel & Thaler, 2018), fall armyworm larvae suffered greater negative fitness consequences when they could forage, and plants could interact than under no-choice diet conditions. This suggests that the effects of mixing St. Augustinegrass cultivars on fall armyworm are caused by factors associated with insect–plant interactions rather than postconsumptive physiological effects (Wetzel & Thaler, 2016). There was 100% survival for all individuals forced to consume mixed diets in both the M2 and M4 treatments and no effect of increasing diet diversity. In contrast, there was lower survival and an effect of increasing diversity in the limited-choice experiment.

Previous work found that fall armyworm larvae preferentially colonized cultivar monocultures over cultivar blends (Doherty et al., 2019), suggesting that larvae exhibit host selection behavior against more diverse plantings. The presence of Classic reduced larval weight, but only in the no-choice experiment, suggesting that larvae avoided this effect in situ. In the limited-choice study, larvae may have spent more time foraging in M4 plantings, reducing their efficiency and fitness. St. Augustinegrass cultivars also vary in architecture and morphology, creating a denser vegetation canopy when mixed (Pinkney et al., 2021), which may further interfere with larval foraging.

The observed effect of increased diversity in the limited-choice study could also be explained by a sampling effect (Loreau, 1998), where M4 plantings were more likely than M2 to contain a detrimental cultivar. The effects and effect sizes of M4 are similar to the observed cultivar-dependent effects. There were several cultivar-dependent effects under limited-choice conditions only, the most pronounced being the presence of 'Bitterblue', where larval weight and survival were reduced by 16 and 20%, respectively. Plantings that contained 'Floratam' or 'SS-100' had reduced herbivory, which is a driver of insecticide use in turfgrass lawns. Given a finite pool of cultivars, when plants have the opportunity to interact with their neighbors and herbivores, there is value to mixing four cultivars rather than two. Ultimately, this work aims to reduce pests, pesticide use, and associated non-target effects in a ubiquitous feature of urbanized landscapes.

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