Insect Pollinivores of *Sorghum bicolor* and Plant Traits that Influence Visitation[1]

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Abstract Grasses such as sorghum, (*Sorghum bicolor* (L.) Moench), are rarely considered as an important food resource for pollinators. Here, we report insects collecting or consuming pollen of sorghum over the 8-wk flowering period of a mapping population in Tifton, GA. We also examine the response of insects to plant morphological traits and disease damage and the ability of bumble bees (*Bombus impatiens*) to cross-pollinate sorghum. The most numerous insect observed collecting/consuming sorghum pollen was the hover fly *Toxomerus politus* Say (i.e., the maize calligrapher) followed by honey bees (*Apis mellifera* L.), lined earwigs (*Doru taeniatum* Dorhn), lovebugs (*Plecia nearctica* Hardy), southern carpenter bees (*Xylocopa micans* Lepeletier), common eastern bumble bees (*Bombus impatiens* Cresson), exotic stripetails (*Allograpta exotica* Wiedemann), margined soldier beetles (*Chauliognathus marginatus* F.), a signal fly (*Rivellia* sp. Robineau-Desvoidy), and a dusky-winged hover fly (*Ocyptamus fuscipennis* Say). Maximum flowering occurred at Week 3, which coincided with the maximum number of maize calligraphers, bumble bees, and honey bees observed. A positive linear relationship was seen between the number of flowering plots and the number of maize calligraphers and honey bees. The probability of observing a bee on a panicle increased as plant height increased, whereas the probability of observing a bee or hover fly decreased as plant disease percentage increased. These results suggest that inflorescence abundance, plant height, and plant disease impact bee visitation in sorghum. Furthermore, field-captured bumble bees successfully fertilized sorghum in a greenhouse study, suggesting that bumblebees can pollinate sorghum flowers in field conditions.

Key Words grasses, pollinators, *Toxomerus politus*, honey bee, earwig

Insect pollination is essential for much of global food production (Larson et al. 2001, Rader et al. 2020). Pollen from crop and noncrop plants is packed with proteins, carbohydrates, lipids, vitamins, and minerals and is highly sought after by bees (Hymenoptera: Apoidea), flies (Diptera), and many other insects (Larson et al. 2001, Lundgren 2009, Thorp 2000). Bees use pollen to feed and raise larvae (Thorp 2000). In contrast to bees which often have specialized structures to collect pollen and transport it elsewhere, hover flies consume the pollen directly from the

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anthers or from their bodies during floral visitation (Holloway 1976, Thorp 2000). Pollinators, however, are under threat globally and suffer from resource deprivation, habitat decline, pesticide exposure, and infection by parasites and pathogens (Rhodes 2018). Recommendations to increase bee populations suggest utilizing nectar rich plants but rarely recommend wind pollinated plants such as grasses for habitat enhancement (Saunders 2018).

Despite being primarily wind pollinated, at least 51 genera of grasses have been documented to serve as pollen sources for bees and hover flies (Diptera: Syrphidae) (Saunders 2018). These grasses range from row crops such as corn (*Zea mays* L.) and rice (*Oryza sativa* L.) to turfgrasses such as centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.) and bahiagrass (*Paspalum notatum* Flugge) (Jones 2014, Joseph et al. 2020, Joseph and Hardin 2022, Liu et al. 2023, Moeller 1971). Grasses also serve as nesting resources for several species, such as bamboo-nesting carpenter bees (*Xylocopa* spp.), grass-nesting queen bumble bees (*Bombus* spp.), and leaf-cutting bees (Megachilidae) (Hurd 1978, MacIvor 2016, Mawdsley and Carter 2015, Svensson et al. 2000).

Sorghum in 2023 was planted on 2.9 million ha in the United States (USDA-NASS 2024) and is planted for grain, forage, and syrup production. Sorghum produces a large amount of pollen (up to 24 million pollen grains per panicle) and is primarily self-pollinated (Karper and Quinby 1947). However, outcrossing, excluding germplasm with cytoplasmic male sterility, can occur at 0.1–73% depending on the race grown, field conditions, crop maturity, spikelet position within the panicle, and method used of calculating outcrossing (Barnaud et al. 2008, Djè et al. 2004, Pedersen et al. 1998). Outcrossing in sudangrass (*Sorghum bicolor* ssp. *drummondii* [Nees ex Steud.] de Wet & Harlan) can be as much as 100% (Garber and Atwood 1945, Pedersen et al. 1998). In the sorghum literature, it is frequently stated that outcrossing can occur only by wind, and the role of insects is not considered (ex. Ellstrand and Foster 1983), even though numerous bees, beetles, and flies collect, consume, and transport the pollen. Bees of genera *Spatunomia*, *Patellapis, Lipotriches*, *Nomia*, *Lasioglossum*, *Bombus*, *Xylocopa*, and *Apis* have been identified collecting sorghum pollen (Harris-Shultz et al. 2022, Schmidt and Bothma 2005, Siede et al. 2021). Insects documented consuming sorghum pollen include pollen beetles (*Astylus atromaculatus* Blanchard), maize calligraphers, and lined earwigs (Harris-Shultz et al. 2022, Nunes-Silva et al. 2010, Schmidt and Bothma 2005). Bees enhanced seed yield and seed number of sorghum under unfavorable weather conditions for wind pollination (Siede et al. 2021). Sorghum pollen has been reported to contain 13–26% raw protein and a honey bee diet of only sorghum pollen is adequate for raising bee brood (Shen 1992, Siede et al. 2021, Standifer 1967).

In a previous study, we identified for the first time that bumble bees and southern carpenter bees were collecting sorghum pollen, and lined earwigs were consuming sorghum pollen in a subset of a sweet sorghum population consisting of 25 lines with 3 replications where insect data were collected over a 4-wk flowering period (September–October) (Harris-Shultz et al. 2022). In this study, we replicated the entire sweet sorghum population ($N = 124$ and the 2 parents), took insect data over the entire flowering period (8 wk), and measured a myriad of sorghum traits to understand which insects are utilizing sorghum pollen as a food source and if there are certain sorghum traits which increase insect abundance. In addition, we conducted an experiment to determine if cross-pollination of sorghum by bumble bees is possible.

Materials and Methods

Planting material, insect surveys, and plant measurements. A sweet sorghum (AN109 x No. 5 Gambela) F_5 mapping population, where each replicate consisted of 124 F_5 lines (1 plot of each) and 2 plots of each parent, was planted at Belflower Farm (31°30'23"N 83°33'22"W) in Tifton, GA on 9 June 2022 in a randomized complete block design (RCBD). There were 4 replicates of each entry, and plots were 4.3-m long with 1.8-m alleys on each end. Rows were 0.9-m apart. Surrounding the field was a border of 526 plots of commercial grain sorghum hybrid NK8416.

From 10 August to 28 September 2022, sorghum plots were evaluated for flowering and insect visitation. For each plot, the date at which 50% of the plot had flowered (half of the heads had anther shed halfway down the head) was recorded as the flowering date. Three panicles per plot were randomly observed for 3 min between 08:31 and 13:16, and all insects collecting or consuming sorghum pollen were recorded. Insects seen ingesting anthers were recorded as consuming pollen, whereas insects seen putting pollen on hind legs were recorded as collectors. Insects traveling from panicle-to-panicle were only recorded once and insect data were not recorded at times of heavy rain. There were 26 rating dates in total.

To examine the effects of sorghum traits on insect visitation, we measured length and width of the flag leaf, plant height at maturity (from the ground to the top of the panicle), and panicle length for 3 plants in each plot. We also examined the prevalence of plant pathogens. Bacterial stripe, caused by *Pseudomonas andropogonis* Smith, was rated using a 0–5 scale per plot where 0 = no visible symptoms, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = 76–100% of the leaf area exhibited symptoms (Akhtar 1984). Rough leaf spot, caused by *Ascochyta sorghi* Sacc., was rated on 3–9 August 2022 using the 1–4 scale of Zummo and Broadhead (1984). Overall disease damage was assessed from 30 August to 1 September 2022 and was the percentage of the plot that was impacted by any disease on any part of the plant.

Use of *Bombus impatiens* to determine if bumble bees can pollinate sorghum. Twenty pots of AN109 (pollen sterile) and 20 pots of BN109 (pollen fertile) were planted in a greenhouse on 7 June 2023 to align with sorghum planting in the field in Tifton, GA on 1 June 2023. AN109 and BN109 are isogenic except that one is sterile (cytoplasmic male-sterile A) and the other is fertile, respectively. Plants were thinned to 1 seedling per 5-gal pot and the soil was a mixture of 2 parts Promix BX Biofungicide potting mix (Premier Horticulture, Quakertown, PA) to 1 part mason sand. When panicle emergence began (24 July 2023), panicles were bagged immediately prior to anther shed. Crossing with *Bombus impatiens* or no bees began when each panicle had fully released anthers.

Bumble bees were the prevalent bee foraging for pollen in sorghum field plots planted in Tifton, GA in 2023 at Belflower Farm and, thus, were used for the crossing experiment. Bumble bees foraging on the pollen of 2 commercial grain sorghum hybrids (coded here as SW3/SW4) at approximately 08:30 were caught in 5-gal

Ziploc style plastic bags by rapidly moving the bag up as the bee was hovering to collect pollen. Bags were sealed and transported to the greenhouse. To allow transfer of the bee to the greenhouse grown sorghum panicles, bees in bags were cooled on ice for 5 min in a cooler. The bag containing the bee was placed over the panicle and the bag was stapled into place. Once the bumble bee began to move around in the bag, the bag was left on the sorghum panicle for 1 h 25 min. One plant at a time, the bee was released outside the greenhouse and a paper bag was placed over the panicle. For the control plants, the same procedure was used except no bees were present in the bag. Pots were placed in a RCBD with 4 replicates and allowed to set seed for 45 d. Seed was dried in a dryer at 38°C for 2 wk and then hand threshed. The number of seeds for each genotype x treatment x replicate were counted. Threshed seed was placed back in the dryer for 2 wk until planting. For the BN109 (fully fertile) heads, 18 seeds (1 seed per pot) were planted for each sample while for the AN109 (male-sterile) heads, every seed that was created was planted. AN109, BN109, and 2 hybrids coded SW3 and SW4 were used as controls to determine crossing.

DNA extraction and genotyping of AN109/BN109 derived seedlings. To determine whether each seedling from the bee crossing experiment (described above) resulted from an outcross or self-pollination, DNA was extracted by first placing plant tissue into a 2-ml microcentrifuge tube containing 4 Zn-plated BBs (Daisy Outdoor Products, Rogers, AR). Samples were then placed into liquid nitrogen and repeatedly ground using a vortexer until each sample became a fine, frozen powder. Lastly, DNA was purified from each sample using a GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific).

Simple sequence repeat (SSR) and repeat junction (RJ) markers were amplified from each sorghum DNA sample using a 10-μl reaction. The reaction contained 2 μl of 5x Colorless GoTaq Flexi buffer (Promega, Madison, WI), 1 μl of 25 mM $MgCl_2$, 0.8 μl of 2.5 mM dNTP mix, 1.8 μl of 1 μM M13 primer (M13-TGTAAAACGACGGCCAGT) 5' labeled with HEX, 0.5 μl of 1 μM forward primer containing a M13 tag on the 5' end of the primer sequence, 2 μl of 1 μM reverse primer, 0.04 μl of GoTaq Flexi DNA polymerase (Promega), 0.86 μl of water, and 1 μl of DNA (2.5 ng μl^{-1}). The SSR and RJ markers used, and their primer sequences are listed in Supplemental Table 1 (https://doi.org/10.15482/USDA.ADC/26381755.v2). Thermocycler conditions were initial denaturation at 94°C for 3 min, 39 cycles at 94°C 30 s, 50°C for 1 min, 72°C for 1 min 10 s, and a final elongation at 72°C for 40 min.

PCR fragments were diluted with 20 μl of molecular biology-grade water. To load PCR fragments onto the SeqStudio Genetic Analyzer (Thermo Fisher Scientific), each sample contained 0.5 μl of GeneScan 500 ROX dye size standard (Thermo Fisher Scientific), 8.5 μl of Hi-Di formamide (Thermo Fisher Scientific), and 1 μl of diluted PCR sample. Samples were denatured at 94°C for 5 min on a thermocycler, then loaded on the SeqStudio and run using default program "fragment analyses." GeneMapper software version 6 (Thermo Fisher Scientific) was used to analyze the fragment data.

Data analysis. Correlations were calculated in the CORR procedure of SAS v. 9.4 (SAS Institute Inc., Cary, NC). Most of the insect count data consisted of zeros and ones, so a regular ANOVA model was not adequate to analyze these data. Thus, a binary logistic model was run in SAS v. 9.4 PROC GLIMMIX to model the probability of observing an insect (hover flies, bees, and overall insects) on a sorghum panicle over time. (Data for the less common taxa were not analyzed statistically because most observations were zeros.) Data were grouped into 2 classes – insect (1 or more) versus no insect (0). Week of observation was included as a fixed class effect with replicate and entry as random effects. Week 8 had only 7 observations and, thus, had limited predictive power, so it was excluded from further analysis. Probabilities were calculated using the ILINK option in the LSMEANS statement. A similar binary model was run in SAS Proc Logistic to model the probability of observing an insect (bees, hover flies, or total insects) versus continuous independent variables, such as plant height and disease percentage. Week of observation was included in the model as a class variable. Probability curves were generated using the EFFECT-PLOT option.

To determine if genotype and treatment impacted seed number for the greenhouse crossing experiment, genotype, treatment, and genotype x treatment were treated as fixed effects and replicate was treated as a random effect in JMP (SAS Institute Inc., Cary, NC). The personality was standard least squares, the emphasis was effect screening, and the method was REML.

Results

Insects collecting/consuming sorghum pollen. By far the most found insect collecting or consuming sorghum pollen was the maize calligrapher hover fly, at 417 observed individuals. Following these were honey bees, lined earwigs, lovebugs, southern carpenter bees, common eastern bumble bees, exotic stripetails, margined soldier beetles, a signal fly, and a dusky-winged hover fly (Table 1, Fig. 1). For this study, the number of honey bees per plot ranged from 0–4, and 0 (380 plots), 1 (46 plots), 2 honey bees (11 plots) were the most frequent, whereas 3 (3 plots) and 4 (1 plot) were rarely observed, and only during Week 3 when bees were most abundant. Maize calligraphers, the most common hover fly species, ranged from 0–5 per plot, with 0 (194 plots), 1 (126 plots), or 2 (81 plots) were most frequently observed, whereas 3 (26 plots), 4 (9 plots) and 5 (3 plots) were less frequent.

Weather conditions and plant disease. The weather for the summer of 2022 was frequent rainfall followed by a drought starting from September 11 to the end of insect observations (September 28) (Supplemental Fig. 1, https://doi.org/10.15482/USDA.ADC/26381755.v2). The heavy rain caused a large amount of disease in the plots especially bacterial stripe and rough leaf spot, but zonate leaf spot (*Gloeocercospora sorghi* D. Bain & Edg.) and anthracnose (*Colletotrichum sublineola* Henn. ex Sacc. & Trotter) were also present. Percent disease damage had weak negative correlations with other plant characteristics including flag leaf length (FLL), flag leaf width (FLW), height, panicle length, and days to flower (Table 2).

Effect of week and number of flowering plots on insect abundance. The number of plots flowering increased each week until maximum flowering occurred at Week 3 (24–26 August 2022) with 107 plots and after Week 3 the number of plots flowering steadily decreased (Fig. 2). The number of maize calligraphers showed a very similar distribution to the number of plots flowering. The maximum number of maize calligraphers (152), bumble bees (7), and honey bees (47) were all seen at Week 3, which was the week when the most plots flowered. Lovebugs showed a slight peak (18 individuals) in Week 4.

Table 1. Insects observed consuming or collecting sorghum pollen at Belflower Farm (Tifton, GA) on a sorghum (N109A x No. 5 Gambela) F_5 mapping population from the start of flowering 10 August 2022 to the end of flowering on 28 September 2022.

Order: Family	Genus/Species	Common Name	No. Observed
Diptera: Syrphidae	*Toxomerus politus*	Maize calligrapher	417
Hymenoptera: Apidae	*Apis mellifera*	Honey bee	81
Dermaptera: Forficulidae	*Doru taeniatum*	Lined earwig	39
Diptera: Bibionidae	*Plecia nearctica*	Lovebugs	29
Hymenoptera: Apidae	*Xylocopa micans*	Southern carpenter bee	19
Hymenoptera: Apidae	*Bombus impatiens*	Common eastern bumble bee	15
Diptera: Syrphidae	*Allograpta exotica*	Exotic stripetail	6
Coleoptera: Cantharidae	*Chauliognathus marginatus*	Margined soldier beetle	2
Diptera: Platystomatidae	*Rivellia* sp.	Signal fly	1
Diptera: Syrphidae	*Ocyptamus fuscipennis*	Dusky-winged hover fly	1

There were some positive correlations between weekly insect counts and the number of plots flowering each week for maize calligraphers ($r = 0.92$, $P = 0.001$), total bees ($r = 0.77$, $P = 0.026$), and total insects ($r = 0.91$, $P = 0.002$; Table 3). Obviously observing more flowering plots would be expected to lead to higher total insect counts, so correlations were also adjusted for number of insects per plot. On a per-plot basis, the number of maize calligraphers per plot each week was still correlated with total number of plots flowering ($r = 0.80$, $P = 0.017$; Table 3). The weekly number of maize calligraphers per plot was also correlated with honey bees per plot ($r = 0.85$, $P = 0.007$), bumble bees per plot ($r = 0.83$, $P = 0.012$), and total bees per plot ($r = 0.91$, $P = 0.002$). Honey bees and bumble bees per plot were also correlated with each other ($r = 0.86$, $P = 0.006$; Table 3).

The binary model revealed significant week of flowering effects for observation of hover flies (all species), total bees, and total insects. The probability of observing a hover fly was greater for Week 3 than any other week examined ($F = 5.97$; df $= 6, 301$; $P < 0.0001$) (Supplemental Fig. 2, https://doi.org/10.15482/USDA.ADC/26381755.v2). The probability of observing a bee was highest during Weeks 3–6 ($F = 3.59$; df $= 6, 301$; $P = 0.0019$) (Supplemental Fig. 3, https://doi.org/10.15482/USDA.ADC/26381755.v2). Similarly, because hover flies and bees made up the

Fig. 1. Insects, not previously documented utilizing sorghum pollen as a pollen food source. (A) Lovebugs (*Plecia nearctica*), (B) exotic stripetail (*Allograpta exotica*), (C) dusky-winged hover fly (*Ocyptamus fuscipennis*), (D) margined soldier beetle (*Chauliognathus marginatus*), and (E) signal fly (*Rivellia* sp.).

majority of all insects observed, the probability of observing an insect (all species) was highest at Weeks 3 and 6 ($F = 3.92$; df $= 6, 301$; $P = 0.0009$) (Supplemental Fig. 4, https://doi.org/10.15482/USDA.ADC/26381755.v2).

Effect of plant height and disease damage on the probability of observing insects. Plant height had a significant effect on the probability of observing honey bees and total bees. The binary model showed that there is increasing probability of observing a bee (all species) with increasing plant height (Wald $\chi^2 = 17.36$; df $=$ 1; $P < 0.0001$) as shown in Fig. 3A. A very similar probability curve was observed for honey bees (not shown), which made up the majority of the total bees

Table 2. Correlations (r) among sorghum morphological, phenological, and disease traits.

	Flag Leaf Length	Flag Leaf Width	Plant Height	Panicle Length	Days to Flower	Bacterial Stripe	Rough Leaf Spot
Flag leaf width	0.60***						
Plant height	0.13**	0.14**					
Panicle length	0.22***	0.12**	0.38***				
Days to Flower	0.11*	0.30***	0.40***	0.18***			
Bacterial stripe	−0.22***	−0.14**	−0.05	−0.24***	0.060		
Rough leaf spot	0.00	−0.04	0.23***	0.06	0.07	0.13*	
Plant disease %	−0.26***	−0.35***	−0.26***	−0.36***	−0.35***	0.26***	0.17***

*,**, and*** indicate significance at the 0.05, 0.01, and 0.001 levels, respectively.

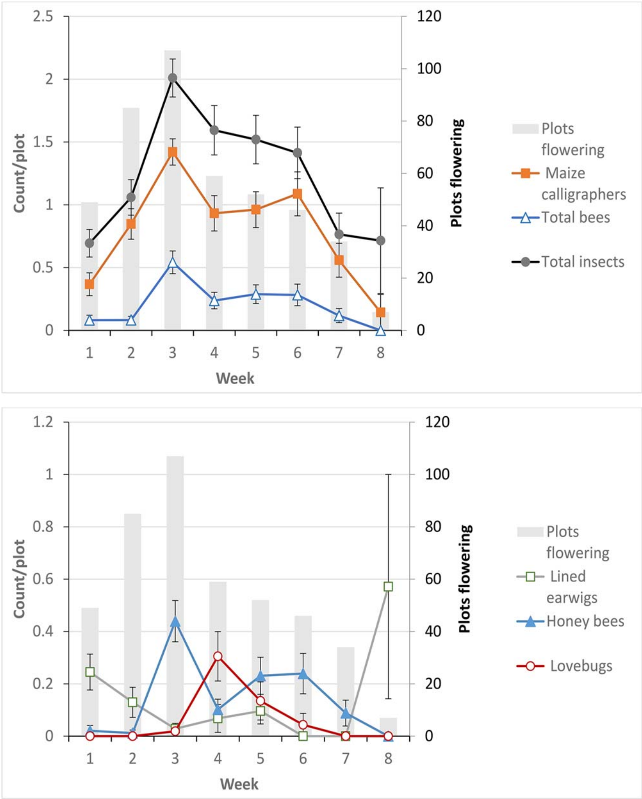

Fig. 2. Number of plots flowering (rated) and number of insects observed per plot collecting or consuming sorghum pollen each week (major taxa only) in 2022 at Tifton, GA. Error bars represent 1 standard error of the mean. Week 1 – August 10, 11, 12; Week 2 – August 13, 15, 17; Week 3 – August 24, 25, 26; Week 4 – Aug. 29, 30, 31, September 2; Week 5 – September 6, 8, 9; Week 6 – September 12, 13, 14, 15, 16; Week 7 – September 19, 21, 22; Week 8 – September 26, 28.

observed. Increasing plant height did not significantly increase the probability of observing a hover fly (Wald $\chi^2 = 2.37$; df = 1; $P = 0.124$), though there was a slight effect for total insects (Wald $\chi^2 = 4.84$; df = 1; $P = 0.028$; curves not shown). The probability of observing a bee also increased with increasing panicle length (Wald $\chi^2 = 6.32$; df = 1; $P = 0.012$; not shown), though this trait is correlated with plant height ($r = 0.38$). There was also a linear correlation of plant height with days to flowering ($r = 0.40$), with taller plants tending to flower later, as expected with sorghum, but days to flower did not affect the probability of observing a bee in the same manner (Wald $\chi^2 = 0.55$; df = 1; $P = 0.458$; week of observation is confounded with days to flowering, so it was not included in this model). There was a decreasing probability of observing a bee (all species) on a sorghum panicle as the percent plant disease increased as shown in Fig. 3B (Wald $\chi^2 = 5.14$; df = 1;

Table 3. Correlations (r) between number of plots flowering each week and weekly insect counts (total counts – top table and counts adjusted on a per plot basis – bottom table).

	Plots flowering	Maize calligrapher	Earwigs	Bumble bees	Honey bees	Southern carpenter	Total bees
Total Counts							
Maize calligrapher	0.92**						
Earwigs	0.24	−0.06					
Bumble bees	0.66	0.86**	−0.41				
Honey bees	0.69	0.89**	−0.30	0.95***			
Southern carpenter	0.75*	0.54	0.60	0.23	0.17		
Total bees	0.77*	0.94***	−0.23	0.96***	0.99***	0.31	
Total insects	0.91**	0.99***	−0.07	0.90**	0.90**	0.55	0.95***
Insects/Plot							
Maize calligrapher	0.80*						
Earwigs	−0.57	−0.76*					
Bumble bees	0.46	0.83*	−0.68				
Honey bees	0.58	0.85**	−0.55	0.86**			
Southern carpenter	0.54	0.17	−0.13	−0.04	−0.19		
Total bees	0.69	0.91**	−0.62	0.90**	0.97***	0.02	
Total insects	0.69	0.93***	−0.56	0.88**	0.86**	0.20	0.93***

*,**, and*** indicate significance at the 0.05, 0.01, and 0.001 levels, respectively.

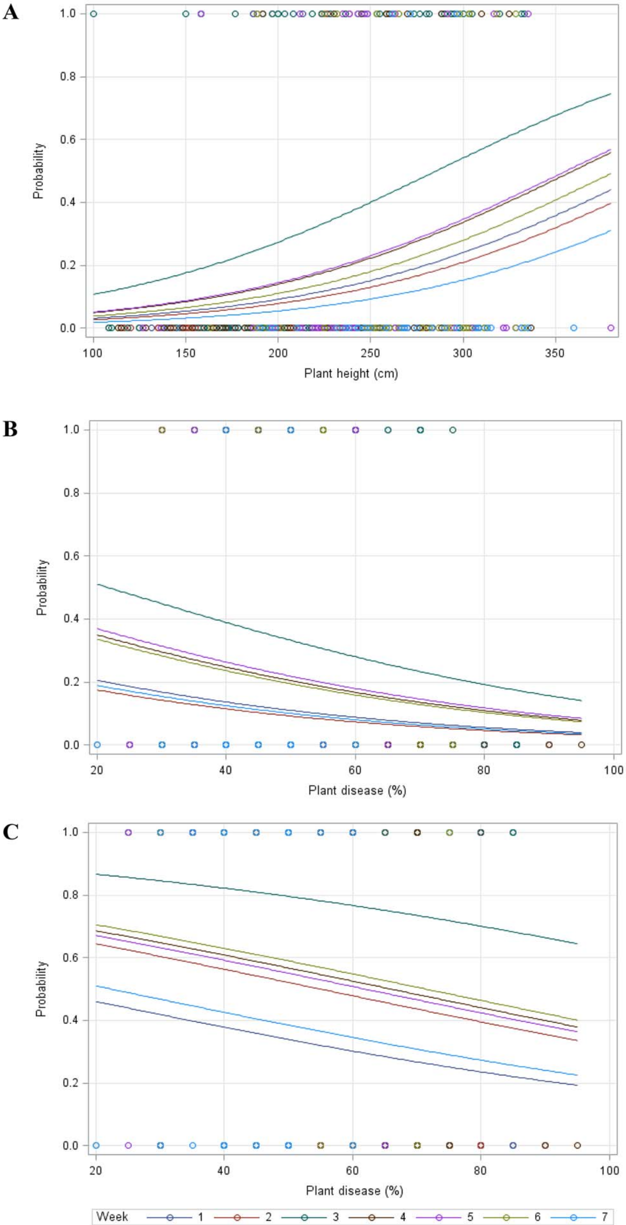

Fig. 3. Predicted probabilities of observing (A) a bee (any species) on a sorghum panicle versus plant height; (B) a bee (any species) on a sorghum panicle versus plant disease percentage; and (C) a hover fly (any species) on a sorghum panicle versus plant disease percentage, all by week of observation (Weeks 1–7, legend). Circles represent actual observations (1 = insect, 0 = no insect).

Table 4. Results of the *Bombus impatiens* sorghum crossing experiment. The # seed obtained, # crosses, and # self-pollinations are the sum of the 4 replicates.

Genotype	Treatment	# Seed Obtained	# Crosses	# Self-pollinations
AN109	*Bombus impatiens*	23	18	1
AN109	No bee	1	0	1
BN109	*Bombus impatiens*	1876	2	68
BN109	No bee	2075	0	72

$P = 0.023$). Again, the curve for honey bees was very similar, but the effect was only marginal (not shown). Hover flies also showed a decreasing probability of observation with increasing plant disease (Fig. 3C; Wald $\chi^2 = 3.99$; df = 1; $P = 0.046$). This effect was not significant for total insects (Wald $\chi^2 = 2.19$; df = 1; $P = 0.139$).

Can bumble bees cross-pollinate sorghum?. After bumble bee capture and placement in plastic bags on sorghum panicles, the bees lost their interest in foraging and spent their efforts flying to the top of the bag. During this process they would often rest on the top of the sorghum panicle and then pursue their escape efforts.

For the number of seeds recovered in the crossing experiment, there were no treatment ($F = 0.20$; df = 1, 11; $P = 0.6668$) or genotype x treatment effects ($F = 0.30$; df = 1, 11; $P = 0.5918$), but significantly more seeds were obtained from BN109 than the male-sterile line AN109 (mean AN109 = 3, BN109 = 494) ($F = 96.29$; df = 1, 11; $P < 0.0001$). AN109 plants with no bees produced one seed (Table 4). To genotype the seedlings resulting from the crossing experiment, 14 markers were amplified to identify codominant markers that were polymorphic between A/BN109 and SW3/SW4 and 2 markers, Sg6rj2880 and RKNP342, were selected with the best amplification (Supplemental Table 1, https://doi.org/10.15482/USDA.ADC/26381755.v2). For the fully fertile genotype BN109, when bees or no bees were added, the seeds were all results of self-pollination, except for replicate 4 when bees were added which had 2 resulting seeds that were crosses from SW3/SW4 (3% outcrossing) (Table 4; Supplemental Table 1, https://doi.org/10.15482/USDA.ADC/26381755.v2). For the male-sterile line AN109, when no bees were added, only 1 seed was produced which was from self-pollination. In contrast, when bumblebees were added to AN109 inflorescences, the resulting seeds consisted of 18 crosses and 1 self (95% outcrossing). The self-pollinations in AN109 occurred because male sterility is not always complete, and a small amount of fertile pollen is sometimes produced.

Discussion

Insects collecting/consuming sorghum pollen. Here we add several new records of insects consuming sorghum pollen, including margined soldier beetles, exotic stripetails, lovebugs, signal flies, and dusky-winged hover flies (Fig. 1). Maize calligraphers, bees from genera *Spatunomia*, *Patellapis, Lipotriches*, *Nomia*, *Lasioglossum*, *Bombus*, *Xylocopa* and *Apis*, lined earwigs, and pollen beetles had previously been seen consuming or collecting sorghum pollen (Harris-Shultz et al. 2022, Schmidt and Bothma 2005, Siede et al. 2021). However, the most prevalent group of insects feeding on sorghum pollen were hover flies, namely the maize calligrapher (*Toxomerus politus*). Unlike many of its close relatives, larvae of this species specialize on Poaceae pollen (Nunes-Silva et al. 2010, Reemer and Rotheray 2009), so it may be presumed that many adults visiting these flowers were also ovipositing eggs. The effects of these insects on sorghum pollination are unknown, but at minimum they may create biotic winds which dislodges pollen into the air (Jones 2014) and onto nearby stigmas of the inflorescence or the inflorescences of neighboring plants. In addition to *T. politus,* we also observed *Allograpta exotica* and *Ocyptamus fuscipennis* 2 species of hover flies which have predacious larvae. *Allograpta exotica* larvae have been identified feeding on sorghum aphid (*Melanaphis sorghi* Theobald) infestations on sorghum (Fortoul-Diaz et al. 2020) and these 2 species may reduce aphid numbers as sorghum aphid and corn leaf aphid (*Rhopalosiphum maidis* Fitch) are frequently present on sorghum.

Margined soldier beetles and lined earwigs are also generalist predators/omnivores and are also likely to contribute biological control services. Margined soldier beetles have been previously seen on the flowering panicles of pearl millet (*Cenchrus americanus* [L.] Morrone) and were thought to be involved in cross pollination (Leuck and Burton 1966). This study confirms that numerous insects collect and consume sorghum pollen. Insecticide use in sorghum should therefore be used sparingly, preferably outside of the flowering period when possible, to protect pollinator health, supplement pollination and prevent pest resurgence.

Abundance of food source. The number of plots flowering per week was correlated with the number of maize calligraphers per plot. Furthermore, the probability of observing a bee or hover fly foraging on a sorghum panicle was greatest in Week 3 of the study when floral abundance was highest, suggesting that the decision to forage on sorghum may be due to plant inflorescence abundance. For honey bees, foraging efforts are determined by amount of stored pollen in the hive, the presence of brood, and their own genetics (Dreller et al. 1999, Page et al. 1995). The decision from which plants pollen should be collected is not determined by the protein content of the pollen but rather the odor of the pollen, the experience of the forager, and the abundance of plants in an area near the hive (Beekman et al. 2016, Klein et al. 2019, Liolios et al. 2015, Pernal and Currie 2001).

Bees showed a preference for taller sorghum plants. Within this field of sorghum of varying heights, the probability of observing bees was greater on taller plants than on shorter plants. Grain sorghum harvested with a combine is usually around 91–122 cm (3–4 feet tall) and sorghum at 1.8–3 m (6–10 feet tall) is used for syrup, forage, and bioenergy production. The preference of bees to flowering plants of taller heights has been seen in other species. In *Brassica rapa* L., *Cannabis sativa* L., and *Verbascum thapsus* L. bees preferred taller plants over shorter plants (Flicker et al. 2020, Lortie and Aarssen 1999, Zu and Schiestl 2017). One hypothesis for this is that it may be energetically favorable to forage at one height if the vertical distance between flowers exceeds the horizontal distances as well as the energy needed to navigate among stems and leaves (Gumbert and Kunze 1999, Levin and Kerster 1973). Alternatively, shorter flowering plants may be hidden within the canopy, whereas taller flowering plants may project out of the vegetation (Gumbert and Kunze 1999) and are simply easier to locate, particularly within mixed stands.

Bees and hover flies showed a preference for healthier plants. In this study, the probability of observing bees or hover flies on flowering sorghum panicles decreased with increasing severity of plant disease. Disease damage in sorghum, if severe, can prevent panicle emergence or cause male-sterility where no or reduced numbers of anthers are released. If there is a reduction in anther number, there is limited pollen for bees/hoverflies to gather/consume and, thus, these inflorescences are disregarded for more pollen-rich heads.

Bumble bees can cross-pollinate sorghum. This study shows for the first time that bumble bees can cross-pollinate sorghum and that the rate of cross-pollination was far higher in male-sterile lines than fertile lines (95% versus 3%). Field-grown sorghum would have multiple bee species (Table 1) quickly gathering pollen from the inflorescence with multiple visitors per inflorescence especially in the morning hours. In field conditions it is unlikely that bees would be attracted to male-sterile sorghum due to no/limited anther release. Yet, our results show that bumble bees can cross-pollinate fertile sorghum and this information is relevant when pollen control is needed as wind pollination is believed to occur within a few hundred meters, but bee pollination and foraging can extend up to 10 km (6.2 miles) (Beekman and Ratnieks 2000, Schmidt and Bothma 2005).

This study found that maize calligraphers, honey bees, lined earwigs, lovebugs, southern carpenter bees, common eastern bumble bees, exotic stripetails, margined soldier beetles, a signal fly, and a dusky-winged hover fly utilized sorghum pollen as a food source. These insects, especially bees, are important pollinators of a variety of crop and non-crop plant species, while soldier beetles, earwigs, and hover flies may play additional roles as biological control agents of sorghum-feeding aphids. Maize calligraphers, bumble bees, and honey bees were most abundant at peak floral density, and bees were more likely to collect sorghum pollen from taller and less-diseased plots. Hover flies also appeared to show a preference for healthier plants. Our results show that cross-pollination of sorghum by bumblebees is possible, but further research is needed to determine how often it occurs in the field and if it improves yield during unfavorable weather conditions. Nonetheless, pollination by bees may need to be considered by growers when planting sorghum with enhanced traits or when planting in isolated nurseries to maintain genetic purity. It is unknown if the pollen of different types of sorghum (grain, forage, sweet, or biomass) impacts insect diversity or abundance.

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References Cited

Akhtar, M.A. 1984. Bacterial stripe and bacterial leaf blight of sorghum: effect of temperature and relative humidity on disease development, tolerance among sorghum hybrids, and overwinter survival of the incitant bacteria. MS Thesis. Kansas State Univ., Manhattan.

Barnaud, A., G. Trigueros, D. McKey and H.I. Joly. 2008. High outcrossing rates in fields with mixed sorghum landraces: how are landraces maintained? Heredity (Edinb.) 101(5): 445–452. https://doi.org/10.1038/hdy.2008.77

Beekman, M., K. Preece and T.M. Schaerf. 2016. Dancing for their supper: Do honey bees adjust their recruitment dance in response to the protein content of pollen? Insect. Soc. 63: 117–126. https://doi.org/10.1007/s00040-015-0443-1

Beekman, M. and F.L.W. Ratnieks. 2000. Long-range foraging by the honey-bee, *Apis mellifera* L. Funct. Ecol. 14: 490–496. https://doi.org/10.1046/j.1365-2435.2000.00443.x

Djè, Y., M. Heuertz, M. Ater, C. Lefebvre and X. Vekemans. 2004. *In situ* estimation of outcrossing rate in sorghum landraces using microsatellite markers. Euphytica 138: 205–212.

Dreller, C., R. Page Jr. and M. Fondrk. 1999. Regulation of pollen foraging in honey bee colonies: effects of young brood, stored pollen, and empty space. Behav. Ecol. Sociobiol. 45: 227–233. https://doi.org/10.1007/s002650050557

Ellstrand, N.C. and K.W. Foster. 1983. Impact of population structure on the apparent outcrossing rate of grain sorghum (*Sorghum bicolor*). Theor. Appl. Genet. 66(3–4): 323–327.

Flicker, N.R., K. Poveda and H. Grab. 2020. The bee community of *Cannabis sativa* and corresponding effects of landscape composition. Environ. Entomol. 49(1): 197–202. https://doi.org/10.1093/ee/nvz141

Fortoul-Diaz, J.V., A. Huerta-de la Peña, J.R. Lomeli-Flores, J.H. Hernández-Salgado and A. Pérez-Magaña. 2020. Population fluctuation of *Melanaphis sacchari* (Zehntner) and identification of their predators in sorghum with traditional management in Puebla, Mexico. Southwest. Entomol. 45(2): 553–562.

Garber, R.J. and S.S. Atwood. 1945. Natural crossing in Sudan grass. J. Am. Soc. Agron. 37: 365–369.

Gumbert, A. and J. Kunze. 1999. Inflorescence height affects visitation behavior of bees-case study of an aquatic plant community in Bolivia. Biotropica 31(3): 466–477.

Harris-Shultz, K.R., J.S. Armstrong, M. Caballero, W.W. Hoback and J.E. Knoll. 2022. Insect feeding on *Sorghum bicolor* pollen and Hymenoptera attraction to aphid-produced honeydew. Insects 13: 1152. https://doi.org/10.3390/insects13121152

Holloway B.A. 1976. Pollen-feeding in hover-flies (Diptera: Syrphidae). New Zealand J. Zool. 3: 339–350.

Hurd, P.D. Jr. 1978. Bamboo-nesting carpenter bees (Genus *Xylocopa* Latreille) of the Subgenus *Stenoxylocopa* Hurd and Moure (Hymenoptera: Anthophoridae). J. Kansas Entomol. Soc. 51: 746–764.

Jones, T.M. 2014. Why is the lawn buzzing? Biodivers. Data J. 2: e1101. doi: 10.3897/BDJ.2.e1101

Joseph, S.V. and C.B. Hardin. 2022. Bees forage on bahiagrass spikelets. Florida Entomol. 105: 95–98.

Joseph, S., K. Harris-Shultz and D. Jespersen. 2020. Evidence of pollinators foraging on centipedegrass inflorescences. Insects 11(11): 795. doi: 10.3390/insects11110795

Karper, R.E. and J.R. Quinby. 1947. Sorghum—Its production, utilization and breeding. Econ. Bot. 1: 355–371. https://doi.org/10.1007/BF02858895

Klein, S., C. Pasquaretta, X.J. He, C. Perry, E. Sovik, J.-M. Devaud, A.B. Barron and M. Lihoreau. 2019. Honey bees increase their foraging performance and frequency of pollen trips through experience. Sci. Rep. 9: 6778. https://doi.org/10.1038/s41598-019-42677-x

Larson, B., P. Kevan and D. Inouye. 2001. Flies and flowers: Taxonomic diversity of anthophiles and pollinators. Can. Entomol. 133(4): 439–465. doi: 10.4039/Ent133439-4

Leuck, D.B. and G.W. Burton. 1966. Pollination of pearl millet by insects. J. Econ. Entomol. 59: 1308–1309.

Levin, D.A. and H.W. Kerster. 1973. Assortative pollination for stature in *Lythrum salicaria*. Evolution 27: 144–152.

Liolios, V., C. Tananaki, M. Dimou, D. Kanelis, G. Goras, E. Karazafiris and A. Thrasyvoulou. 2015. Ranking pollen from bee plants according to their protein contribution to honey bees. J. Apic. Res. 54(5): 582–592. doi: 10.1080/00218839.2016.1173353

Liu, F., G. Zhang, C. Zhang, W. Zhou, X. Xu, Q. Shou, F. Yuan, Q. Li, H. Huang, J. Hu, W. Jiang, J. Qin, W. Ye and P. Dai. 2023. Pesticide exposure and forage shortage in rice cropping system prevents honey bee colony establishment. Environ. Res. 219: 115097.

Lortie, C.J. and L.W. Aarssen. 1999. The advantage of being tall: Higher flowers receive more pollen in *Verbascum thapsus* L. (Scrophulariaceae). Écoscience 6(1): 68–71. doi: 10.1080/11956860.1999.11952207

Lundgren, J.G. 2009. The pollen feeders, Pp. 87–116. *In* Relationships of Natural Enemies and Non-Prey Foods. Progress in Biological Control, vol 7. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-9235-0_6

MacIvor, J.S. 2016. DNA barcoding to identify leaf preference of leafcutting bees. R. Soc. Open Sci. 3: 150623.

Mawdsley, J.R. and C.M. Carter. 2015. Urban populations of a declining bumble bee species, *Bombus pensylvanicus* (De Geer) (Hymenoptera: Apidae), in the District of Columbia, USA. Md. Entomol. 6(3): 22–29.

Moeller, F.E. 1971. Effect of pollen availability on poisoning of honey bees by carbaryl applied to sweet corn. J. Econ. Entomol. 64: 1314–1315.

Nunes-Silva, P., G.D. Cordeiro, D. Obregon, J.F.L. Neto, F.C. Thompson, B.F. Viana, B.M. Freitas and P.G. Kevan. 2010. Pollenivory in larval and adult flower flies: Pollen availability and visitation rate by *Toxomerus politus* Say (Diptera: Syrphidae) on sorghum *Sorghum bicolor* (L.) Moench (Poaceae). Stud. Dipterol. 17: 177–185.

Page, R.E. Jr, K.D. Waddington, G.J. Hunt and M.K. Fondrk. 1995. Genetic determinants of honey bee foraging behavior. Anim. Behav. 50: 1617–1625.

Pedersen, J.F., J.J. Toy and B. Johnson. 1998. Natural outcrossing of sorghum and sudangrass in the Central Great Plains. Crop Sci. 38: 937–939. https://doi.org/10.2135/cropsci1998.0011183X003800040009x

Pernal, S.F. and R.W. Currie. 2001. The influence of pollen quality on foraging behavior in honey bees (*Apis mellifera* L.). Behav. Ecol. Sociobiol. 51: 53–68.

Rader, R., S.A. Cunningham, B.G. Howlett and D.W. Inouye. 2020. Non-bee insects as visitors and pollinators of crops: Biology, ecology, and management. Annu. Rev. Entomol. 65: 391–407.

Reemer, M. and G.E. Rotheray. 2009. Pollen feeding larvae in the presumed predatory syrphine genus *Toxomerus* Macquart (Diptera, Syrphidae). J. Nat. Hist. 43(15–16): 939–949. https://doi.org/10.1080/00222930802610576

Rhodes, C.J. 2018. Pollinator decline-an ecological calamity in the making? Sci. Progr. 101(2): 121–160. https://doi.org/10.3184/003685018X15202512854527

Saunders, M.E. 2018. Insect pollinators collect pollen from wind-pollinated plants: implications for pollination ecology and sustainable agriculture. Insect Conserv. Divers. 11: 13–31. https://doi.org/10.1111/icad.12243

Schmidt, M. and G. Bothma. 2005. Indications of bee pollination in sorghum and its implications in transgenic biosafety. I.S.M.N. 46: 72–75.

Shen, L. 1992. Nutritional and chemical properties of sorghum, rapeseed, and sunflower pollens. MS Thesis. Univ. Arizona, Tucson.

Siede, R., B. Eickhoff, C. Freyer, S. Windpassinger and R. Büchler. 2021. The bioenergy crop *Sorghum bicolor* is a relevant pollen source for honey bees (*Apis mellifera*). Glob. Change Biol. Bioenergy 13: 1149–1161. https://doi.org/10.1111/gcbb.12835

Standifer, L.N. 1967. A comparison of the protein quality of pollens for growth-stimulation of the hypopharyngeal glands and longevity of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). Insectes Sociaux 14: 415–425.

Svensson, B., J. Lagerlof and B.G. Svensson. 2000. Habitat preferences of nest-seeking bumble bees (Hymenoptera: Apidae) in an agricultural landscape. Agric. Ecosyst. Environ. 77: 247–255.

Thorp, R.W. 2000. The collection of pollen by bees. Plant Syst. Evol. 222: 211–223.

USDA-NASS. 2024. Sorghum Acres United States. (https://www.nass.usda.gov/Charts_and_Maps/graphics/sorgac.pdf) (last accessed 3 September 2024).

Zu, P. and F.P. Schiestl. 2017. The effects of becoming taller: direct and pleiotropic effects of artificial selection on plant height in *Brassica rapa*. Plant J. 89: 1009–1019. https://doi.org/10.1111/tpj.13440

Zummo, N. and D.M. Broadhead. 1984. Sources of resistance to rough leaf spot disease in sweet sorghum. Plant Dis. 68: 1048–1049.