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Surviving the Summer Dearth: Managed and Wild Bees Utilize Soybeans During a Resource-Poor Season

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ABSTRACT

Bee declines are being driven by a combination of factors, and some potential responses we can take include establishment of wildflower habitats among croplands and provision of late-season forage when natural resources are becoming scarce. We tested three trap types and three trap colors for monitoring pollinators in soybean plots during late summer of 2018 and 2019 in the Gulf Coast region of the United States. More bees were collected from blue or white bowl traps compared with yellow, and vane traps were the most effective type. Aside from honey bees, native bees such as bumble bees, sweat bees and long-horned bees were abundant. Particularly in years when late-season wild forage is less available, soybeans may provide a valuable resource for pollinators.

Additional index words: Pollinators; Bee Bowls; Vane Traps; Nutrition; Habitat

With widespread reports of declining entomofauna in recent years, new attention is being placed on biodiversity assessments before important ecosystem services are further jeopardized (Sanchez-Bayo and Wyckhuys 2019; Hallmann et al. 2017). Responsible for the pollination of 75% of the major food crops worldwide, bees are one of the most economically important insect groups experiencing decline, including honey bees and several North American bumble bees (Klein et al. 2007; Goulson et al. 2015; Cameron et al. 2011). Bee declines are being driven by several important and interacting stressors including habitat loss, exposure to pesticides, parasites and pathogens, dietary stresses and other factors associated with climate change (Goulson et al. 2015; Kammerer et al. 2021). For example, bee populations experiencing loss of habitat to agricultural intensification and urbanization become more susceptible to parasite and pathogen stress due to the reduced abundance and diversity of floral resources (DeGrandi-Hoffman et al. 2010; Dolezal and Toth 2018).

Honey bee (*Apis mellifera* L.) colony stress is maximized in winter when energy-depleting cold temperatures can cause bees to tightly cluster together and reduce sanitary activities such as grooming. Grooming is an important behavioral adaptation that can protect individual bees as well as the colony by limiting the introduction of contaminants and removing parasites such as Varroa mite (*Varroa destructor* L.; Land & Seeley 2004). While winter losses often are attributed to Varroa infestation, the relationship between dietary nutrition and colony health should not be underestimated. Bee colonies need to accumulate abundant and diverse stores of nectar and pollen in order to make it through the winter months, and the timing of floral supply may be as important as total nectar availability in limiting pollinator populations (Hendriksma and Shafir 2016; Timberlake et al. 2019). While the fall season can bring a variety of wildflower forage in southern Mississippi, there often is an extended dearth period in late summer where forage is lacking (Gratz 2019). Nutritional stress during the summer dearth can leave colonies unprepared for winter, constituting a primary mortality factor for winter losses (Spleen et al. 2013; Couvillon et al. 2014). Improving bees' nutritional resilience is therefore an important opportunity for reducing colony declines.

One way to obtain nutritional resilience in pollinator populations may be through the provision of forage crops. Soybeans are one of the most important agricultural crops in Mississippi, with numerous cultivars varying in flowering date. Some cultivars have an extended juvenile period, and manipulation of planting dates also can provide an opportunity for late-summer flowering. While soybean flowers normally self-pollinate, reducing their dependency on insect pollinators, bean yield can be enhanced 10-50% by honey bees (Gazzoni 2016). Soybean plants also have a unique extrafloral nectary that can facilitate excretion of excess waste and serve as an attractant for beneficial predators that protect a plant from herbivores, and also potentially as an attractant for pollinators (Horner et al. 2003; Roy et al. 2017). Soybean crops can be subjected to damage from a variety of insect pests including stink bugs and aphids, and therefore may need occasional inputs of insecticidal sprays, which can unintentionally impact bee populations (DiBartolomeis et al. 2019). However, by using insecticidal seed treatments or spraying in the early evening, growers may reduce the risk to pollinator populations (Stewart et al. 2014).

Bowl traps have become a standard method for monitoring bee populations, typically painted different colors and placed in both crop canopy and ground levels to attract a greater diversity of species (Droege et al. 2010). Vane traps meanwhile have been found to capture the greatest abundance and diversity of bees, and may become the standard method for future surveys (Acharya et al. 2022). In each case, bees can be attracted to the trap color and, when contacting the soapy water held within, will drown and be preserved for collection.

For these reasons, we describe here the abundance and diversity of bees collected from soybean plots in late summer over two years in the Gulf Coast region of the United States using three colors and two types of traps.

MATERIALS AND METHODS

To maximize the potential visitation of bees to soybean plants throughout the summer months (July – October), nine cultivars and two planting dates per year were selected, including both commercially standard and newly developed soybean cultivars (Table 1).

Table 1. Cultivars of soybeans planted over two dates each in both 2018 and 2019.

Soybean Cultivars	Source
CM422	USDA-ARS
Otoño	Rio Farms, Monte Alto, TX
49W3X™	AgVenture.Com
S12-1362	Univ. of Missouri
67W7X™	AgVenture.Com
Vernal	Rio Farms, Monte Alto, TX
54K4RR™	AgVenture.Com
38H4R™	AgVenture.Com
S14-10555	Univ. of Missouri

These cultivars were selected based on their trait of having an extended juvenile period, potentially providing floral resources longer into the season. Fields were prepared at the USDA-ARS research farm in McNeill, MS, with disking and herbicide applications [Prowl (BASF, Florham Park, NJ); 2 Pts/A] to remove grasses prior to seeding. Plots were seeded using a randomized complete block design, with two replicates of each of the nine cultivars planted over two dates, totaling 36 plots in each year. Plantings occurred approximately 3 weeks apart, on May 1^{st} and May 23^{rd}, 2018 and May 24^{th} and June 1^{st} of 2019. Each individual plot consisted of 6 rows of one soybean cultivar measuring 15m in length, with 0.75 m row spacing and 6 m fallow alleys between plots. The soybean field was located next to a research apiary (~300 m away), consisting of at least 16 colonies of queenright honey bees managed for Varroa mites with applications of Apistan (Vita Bee Health, Basingstoke, UK) or CheckMite (Bayer, Shawnee Mission, KS).

Bees were collected initially using canopy and ground bowl traps, with each canopy trap body made of 2.5 cm PVC piping and three elbow shelf brackets held together with six 0.6 cm sheet metal screws (Droege et al. 2010) (Fig. 1a). Rebar stakes measuring 1.3 cm diameter and 1.8 m in length were hammered at least 0.3 m deep into soil at the interior edge of soybean plots. A pan trap body was then mounted onto the rebar and held in place at soybean canopy height by an electrical cable-to-conduit connector (Halex, Harrison, OH) tightened onto the rebar. Three 96 mL plastic bowls (Solo Cup Co., Urbana, IL) painted white, fluorescent blue or fluorescent yellow (Krylon, Sherwin-Williams Co., Cleveland, OH) were then secured at the ends of brackets using Velcro tabs (Velcro USA, Inc., Manchester, NH).

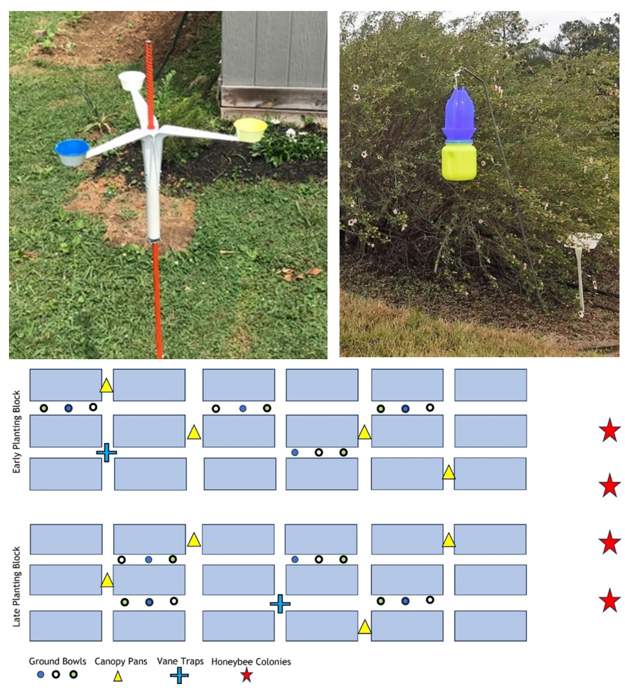

Figure 1. a-c. Bee collection methods used: a) pan trap body mounted onto rebar stake, with three colored bowls attached, b) vane trap affixed to Japanese beetle trap stand, and c) plot diagram.

Ground bowl traps were the same as those used on the canopy pan trap, but placed in a single file trap group on the ground in the fallow alleys between soybean plots, arranged randomly by color and spaced roughly 1 m apart. A total of 8 canopy pan traps and 24 ground bowls were used, divided evenly between early and late plantings totaling 4 individual canopy and ground trap groups within each of the early and late planting blocks. Traps were filled with a drowning solution of unscented laundry detergent in water (44 mL/3.8 L; Sun Products Corporation, Wilton, CT), in order to decrease water tension without lathering.

In 2019 we added blue vane traps (BanfieldBio Inc., Seattle, WA) to our methodology, hung from Japanese beetle trap stands (Tanglefoot, Grand Rapids, MI) at the interior edge of one randomly selected soybean plot in both the early and late plantation block (Fig. 1b-c). While pan and vane traps remained visible above the plant canopy and were left stationary on their rebar stakes, bowl traps were periodically moved next to actively flowering plots to accommodate mowing of grass between plots.

Once flowering began, traps were deployed and pollinators were collected daily by placing them into individually labelled plastic bags. Samples from the same color traps were pooled for each weekly sampling period and stored in a lab freezer until processing, with 12 weekly sample periods from July – October 2018 and 14 weekly sample periods from July – October 2019. Pollinators were identified to species, or as far as possible using taxonomic keys, but for the purposes of analysis were assigned to one of four groups: *A. mellifera, Bombus* spp., Halictidae, or other bees (Michener et al. 1994; Mitchell 1960; Mitchell 1962).

Two-way analysis of variance (ANOVA) was tested separately for 2018 and 2019 using the dependent variable of total bee capture per trap, and the independent variables of bee taxa, trap color and trap type, and their interactions (α = 0.05; Proc GLM, SAS). Trap groups composed of one each of blue, yellow and white bowls, placed either in the canopy or on the ground, were considered replicates. Tukey's HSD test was used to separate differences in mean captures of the bee taxa, trap colors and trap types. Trap capture data were log-transformed (x+1) for analysis, but untransformed data are presented.

RESULTS

Twenty-one taxa of bees were collected through two years of trapping in our soybean plots, including *A. mellifera*, two species of *Bombus*, at least eight species of Halictidae, and ten other bee species (Table 2). Weekly mean trap captures of *A. mellifera* and non-*Apis* bees followed similar trends from 2018 and 2019, with non-*Apis* bees peaking at 2.89 ($\pm$1.36) specimens during the week of 6 August 2018, and at 2.21 ($\pm$ 0.72) specimens during the week of 22 July 2019 (Fig. 2). In each year the non-*Apis* captures gradually declined as the season progressed into late summer. Conversely, *A. mellifera* captures climbed into the late summer period, with a peak of 3.67 ($\pm$ 1.15) specimens during the week of 10 September 2018 and 2.29 ($\pm$ 1.13) specimens during the week of 2 September 2019 (Fig. 3).

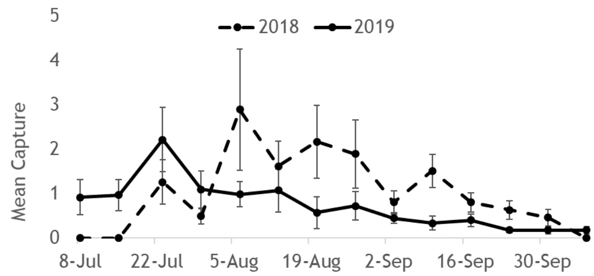

Figure 2. Weekly mean captures ($\pm$ SE) of non-*Apis* bees from experimental soybean plots using canopy and ground bowl traps in 2018 vs. bowl traps and vane traps in 2019.

Our statistical model was significant for both 2018 (F = 24.05; df = 15, 248; P < 0.0001) and 2019 (F = 32.25; df = 19, 372; P < 0.0001; Table 3), with descriptions of the interactions to follow. When comparing captures of the four groups of bees (*A. mellifera, Bombus* spp., Halictidae and all other bees), in 2018 we observed a significantly higher capture of Halictidae vs. the other groups (P < 0.0001), but no difference in captures among the two trap types (P = 0.306; Fig. 4).

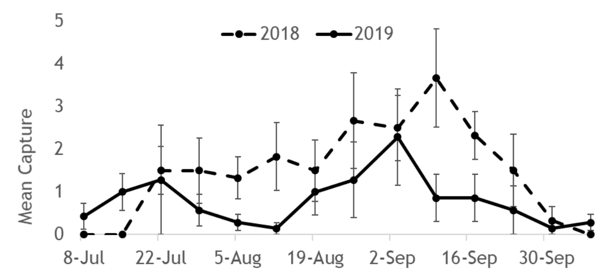

Figure 3. Weekly mean captures (+ SE) of *Apis mellifera* from experimental soybean plots using canopy and ground bowl traps in 2018 vs. bowl traps and vane traps in 2019.

In 2019 however, captures of all three non-*Apis* groups were significantly higher than the honey bees (P < 0.0001), and due to the introduction of the Vane trap, captures by trap type also were significantly different (P = 0.011; Fig. 5). Captures among the three trap colors also were significant in both 2018 (P < 0.0001) and 2019 (P < 0.0001), with more specimens collected from blue and white vs. yellow traps regardless of position in the canopy or on the ground.

Table 2. Bee taxa collected from three types of traps placed in a soybean plot from July – October in 2018 and 2019.

Taxa	Species	Ground Bowl		Canopy Bowl		Vane
		2018	**2019**	**2018**	**2019**	**2019**
Honey bees	*Apis mellifera*	**41**	**15**	**77**	**22**	**40**
Sweat bees	*Agapostemon splendens*	**16**	**2**	**10**	**3**	**3**
	Augochlora pura pura	**5**	**0**	**0**	**0**	**0**
	Augochlorella striata	**0**	**1**	**5**	**3**	**2**
	Augochloropsis metallica metallica	**4**	**0**	**1**	**1**	**0**
	Halictus ligatus	**23**	**4**	**21**	**23**	**34**
	Hemihalictus lustrans	**1**	**0**	**0**	**0**	**0**
	Lasioglossum spp.	**631**	**55**	**257**	**49**	**63**
	Sphecodes sp.	**2**	**0**	**0**	**0**	**0**
Bumble bees	*Bombus impatiens*	**19**	**13**	**16**	**15**	**270**
	Bombus pennsylvanicus	**24**	**4**	**43**	**3**	**69**
Other bees	*Calliopsis andreniformis*	**5**	**0**	**2**	**0**	**0**
	Megachile albitarsis	**1**	**0**	**1**	**0**	**0**
	Megachile inimica sayi	**2**	**0**	**0**	**0**	**1**
	Melissodes bimaculata	**0**	**2**	**5**	**11**	**104**
	Melissodes sp.	**16**	**8**	**63**	**32**	**147**
	Pseudopanurgus abdominalis tricolor	**1**	**0**	**0**	**0**	**0**
	Svastra aegis	**0**	**0**	**2**	**0**	**0**
	Xenoglossa strenua	**2**	**0**	**3**	**0**	**22**
	Xylocopa micans	**0**	**0**	**1**	**0**	**7**
	Xylocopa virginica	0	0	0	0	5
	Total specimens	**793**	**104**	**507**	**163**	**807**

Table 3. ANOVA output for trap captures of four bee taxa in soybean plots using two types of traps in three colors in 2018 and three types of traps in three colors in 2019.

Year	Response variable	Factor	Num DF	Den DF	F Value	Pr > F
2018	No. Bees Captured	Trap	1	248	1.05	0.306
		Color	2	248	48.96	<0.0001
		Taxa	3	248	71.63	<0.0001
		Trap*Taxa	3	248	7.94	<0.0001
		Color*Taxa	6	248	3.84	0.001
2019	No. Bees Captured	Trap	1	372	6.47	0.011
		Color	2	372	24.67	<0.0001
		Taxa	3	372	15.55	<0.0001
		Trap*Taxa	3	372	1.3	0.273
		Color*Taxa	6	372	3.56	0.002

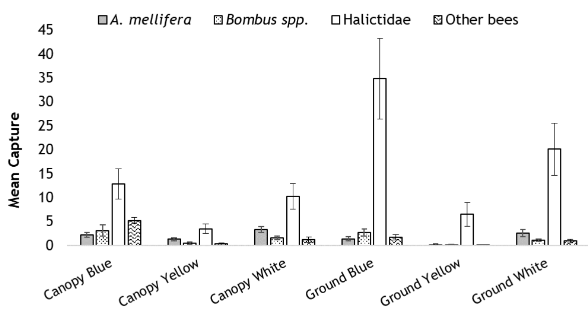

Figure 4. Mean captures (+ SE) of *Apis mellifera*, *Bombus* spp., Halictidae and other bees from three colors of bowl traps placed in the canopy and on the ground in 2018.

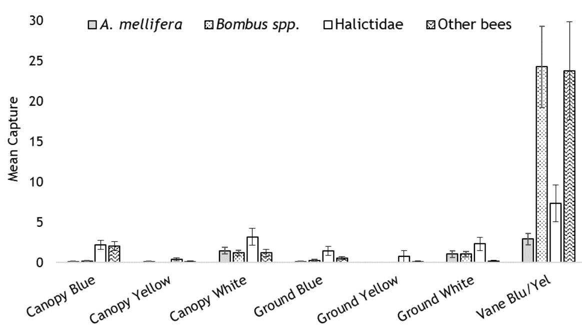

Figure 5. Mean captures (+ SE) of *Apis mellifera*, *Bombus* spp., Halictidae and other bees from three colors of bowl traps placed in the canopy and on the ground, and canopy vane traps, in 2019.

DISCUSSION

Throughout the collecting season from both years, honey bees were readily trapped in soybean plots with initially lower captures starting in July and hitting peak captures in early September, before again dropping off to the conclusion of the studies. In 2019 however, honey bee captures were lower even when including those collected from the new vane traps. Late summer rainfall was higher in 2019 than in 2018, leading to an unexpectedly large bloom of goldenrod (*Solidago* sp.) in the surrounding fields and meadows. This melliferous late-summer wildflower is visited predominately by honey bees, while bumble bees are less common, and it is likely that our late-season honey bee foragers were preferentially visiting goldenrod instead of soybeans (Frakas and Zajacz 2007; Gross and Warner 1983). Even so, our two years of data support previous observations that *A. mellifera* will actively forage in soybeans during the late summer when other resources are less available (Gazzoni 2016; Lin et al. 2022).

In contrast with honey bee captures, bumble bee captures saw a large increase from 2018 to 2019, primarily due to the inclusion of the vane traps which captured over 90% of *Bombus* specimens. It is possible that the newly available resource of a large soybean plantation at this research farm in 2018 allowed for the delayed response of a larger bumble bee population in the following year, but vane traps are known to be highly attractive to bumble bees and may even impact local populations when sampling too aggressively, making it harder to compare the populations across the two years (Gibbs et al. 2017). Halictidae also were readily captured in each year, especially those from the most species-rich of all bee genera, *Lasioglossum*.

Prior studies showed significantly greater bee captures from pans in the crop canopy in comparison with bowls on the ground, but our captures by trap type were not significant for 2018, with an even distribution of honey bees, bumble bees and other bees among the canopy and ground traps (Tuell and Isaacs 2009). It is possible that the variable trap orientation, single file vs. around a trap a stand, could impact captures, so future efforts using the same bowl arrangement for canopy and ground trapping will facilitate more accurate comparison. Captures by trap color varied significantly in 2018, with more captures from blue and white traps as compared with yellow, whether in the canopy or on the ground. Exceptions to these trends were the Halictidae, collected in higher numbers from each trap type and color, especially the blue ground bowls.

In 2019, captures again varied significantly for trap color with a similar preference for blue and white bowls, supporting prior findings of bee forager preference for blue traps (Leong and Thorp 1999; Stephen and Rao 2005). But trap type also was significant in 2019 due to the inclusion of the blue vane traps, which had greater mean capture of honey bees (2.8), Halictidae (7.3) and especially of bumble bees (24.2) in comparison with canopy or ground bowl traps. The inclusion of the vane traps was similarly impactful for the other species of wild bees (23.7). In particular, the Eucerine *Melissodes* spp. were extremely abundant in the vane traps as opposed to the other traps in 2019.

Previously assigned to the family Anthophoridae, the *Melissodes* are solitary bees that remain relatively understudied, but they are known to nest in large aggregations and feed widely on both wildflowers and soybeans throughout the U.S. (Cardel and Koptur 2010; Rust et al. 1980). It is possible that the *Melissodes* had a response to the soybean plots similar to that of the bumble bees, with a much larger population developing in the second research season in response to the provision of this large new resource in the previous year. But another likely explanation is the strong response to blue vane traps exhibited by some Eucerine longhorn bees (Gibbs et al. 2017). This exposes a limitation of passive sampling, whereby insects attracted to the trap but not necessarily the crop may be collected and misrepresented as crop pollinators. There also is the possibility that the large difference in trap size of bowl vs. vane traps would invalidate comparison of these trap types. Future efforts on this project will include active sampling with sweep nets to better support conclusions (Portman et al. 2020).

Interestingly, *Melissodes* have been shown to promote greater yields for self-fertile crops such as cotton (Esquivel et al. 2020). Like cotton, soybeans are considered autogamous, with floral structure allowing infrequent crosses of only 2%, due to the location of the pistil underneath the stamen anthers (Gazzoni 2016). However, this 2% can represent important overall yields, and some studies have shown yield increases from 10-50% for soybean crops visited by wild bees and honey bees, including greater weight and numbers of seeds per pod (Monasterolo et al. 2015). Aside from the benefits to soybean growers, beekeepers also can gain advantages by placing colonies in proximity to soybean fields, including prolific honey crops (Hoag 1981; Pellett 1976).

If we are to preserve the essential ecosystem services of pollinators, we need to understand the full range of stressors on bee populations as well as the complex interactions between habitat loss, dietary stress and parasites/pathogens. Particularly in late summer months when natural forage can be less abundant, bee colonies experiencing food scarcity and nutritional stress will have reduced fat stores and be more subject to winter loss (Dolezal et al. 2019). Establishing diverse and consistently-flowering wildflower habitats within an agricultural landscape may be the best way to increase nutritional variety and health of wild and managed bees alike (Neumuller et al. 2021; Dolezal and Toth 2018; Hendriksma and Shafir 2016; Sidhu and Joshi 2016). But this research shows that soybean crops may be able to promote pollinator health by providing forage during the resource-poor late summer dearth period.

Next steps for this research will include a comparison of foraging habits among the various cultivars using direct sampling methods rather than passive trapping, examination of pollen diversity from adjacent honey bee colonies using pollen traps, and analysis of sex ratios of bees captured within soybean plots.

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