Peer-reviewed version available at *Agronomy* **2020**, *10*, 571; doi:10.3390/agronomy10040571

Article

Bees Occurring in Corn Production Fields Treated with Atoxigenic *Aspergillus flavus* (Texas, USA)

Krishna B. Bhandari [1] *, Scott D. Longing [1], Charles P. West [1]

[1] Texas Tech University, Department of Plant and Soil Science, Lubbock, TX, USA; krishna.bhandari@ttu.edu (K.B.B); scott.longing@ttu.edu (S.D.L); chuck.west@ttu.edu (C.P.W)

* Correspondence: krishna.bhandari@ttu.edu

Abstract: A saprophytic soil fungus, *Aspergillus flavus*, produces aflatoxin (toxigenic strains) in the kernels of corn (*Zea mays* L.) and seeds of many other crops. Many strains of *A. flavus* do not produce toxigenic aflatoxin, and soil application of these atoxigenic strains is a suppressive control tactic to assist in controlling toxigenic conspecifics. Effects of atoxigenic *A. flavus* applications on honey bees (*Apis mellifera* L.) and other bees are unknown, and basic information on bee occurrences in corn fields treated with and without this biological pesticide is needed to inform integrated pest management in corn. Fields with atoxigenic *A. flavus* applications were compared to nearby control fields in three counties in corn production regions in eastern Texas. In each corn field, twenty bee bowl traps were deployed along four equal transects located between corn rows, with contents of the bowls (i.e. bees) retrieved after 24 hours. Eleven bee genera from four families were collected from corn fields, with only two honey bees collected and zero honey bees observed in transects. The sweat bee genus *Agapostemon* (primarily composed of the Texas-striped sweat bee *A. texanus*) was most abundant in corn fields (44% of the total number of bees collected) followed by long-horned bees (*Melissodes* spp., 24%). The southernmost county (i.e. San Patricio) produced over 80% of the total number of bees collected. Bee communities occurring in corn production fields with applications of atoxigenic *A. flavus* applications were not significantly different from nearby control fields. While little is known of bee resource use in corn production systems in Texas, the abundant yet variable bee communities across latitudes in this study suggests a need to investigate the influence of farming practices on bee resources in regional corn production systems.

Keywords: Aflatoxin treated corn; *Aspergillus flavus*; atoxigenic aflatoxin; bee community; biological pesticide; saprophytic soil fungus

1. Introduction

Aspergillus flavus is a common saprophytic soil fungus which produces toxigenic aflatoxin in the kernels of corn (*Zea mays* L.) [1], seeds of cotton (*Gossypium hirsutum* L.) [2], and seeds of many other crops both before and after harvest [3]. Toxigenic *A. flavus* causes ear rot in corn, one of the most important diseases, which diminishes grain quality and marketability, and livestock health if affected grain is consumed. Corn yields and profitability can be negatively impacted by toxigenic *A. flavus* by producing aflatoxin on corn before harvest and in storage [4,5], and therefore advancing practices for its control is necessary. A previous study reported that one of several species of *Aspergillus* causes stonebrood in honey bees (*Apis mellifera* L.) [6], and therefore applications of *A. flavus* should consider impacts to pollinator health.

It is expected that bees are minimally exposed to aflatoxin in corn fields, but evidence suggests bees visit corn during flowering [7] and therefore could be exposed to agrochemicals used in corn. Use of atoxigenic conspecific strains of *A. flavus* is the most widely used biocontrol method for reducing aflatoxin contamination in corn [8], in which toxigenic *A. flavus* strains were found to be altered and displaced by atoxigenic *A. flavus* strains [9]. Some registered microbial pesticides that

Peer-reviewed version available at Agronomy 2020, 10, 571; doi:10.3390/agronomy10040571

reduce toxigenic *A. flavus* populations are Aflaguard™ (Strain NRL 21882, Syngenta) and Ensure™ (Strain AF36; Arizona Cotton Research and Protection Council). In Texas, a new product (FourSure™) contains four atoxigenic strains of *A. flavus* which are expected to provide control of toxigenic *A. flavus* for several years following application [10,11]. It is recommended that FourSure™ be applied between the 7th leaf stage and tasseling to ensure *A. flavus* presence and its exposure to foraging insects at the time of flowering. Another bee resource that could be exposed to and affected by applications of *A. flavus* is soil nesting habitat for native bees, since approximately 75% of over 4000 species of wild bees in North America provision pollen in subsurface-soil brood chambers. However, how adults and immature stages of bees are affected by these pest control applications remains largely unknown.

The impetus for this project was a need to determine if negative impacts to honey bees could occur in fields with applications of commercial atoxigenic *A. flavus*. In 2003, it was determined that atoxigenic *A. flavus* strain AF36 in cotton represented low risk to honey bees, yet a high-mortality event observed in a cotton field on the thirteenth day following application [9] emphasized a need for further investigating potential non-target effects. The objectives of this study were to sample bee communities occurring across corn production fields in Texas (USA), and to compare generic richness and relative abundances of bees in fields with and without applications of atoxigenic *A. flavus* (hereafter FourSure™). The conservation of wild, native bees in corn production systems and further research needs are discussed in relation to findings.

2. Materials and Methods

2.1 Description of Field Sites

The study was conducted in corn fields in three counties across a latitudinal range from northern to extreme southern Texas (Figure 1). The geographical extent of the study ranged from the Blackland Prairie and Cross Timbers ecoregions in the northern part to the Coastal Prairies in the extreme southern region of the state (Figure 1). Bee community sampling was performed in FourSure-treated and nearby control corn fields in San Patricio County (28°07′04.53″ N, 97°49′28.38″ W) near Sinton, TX, Ellis County (32°36′75.0″ N, 96°88′45.0 W) near Waxahachie, TX, and Grayson County (33°33′22.0″ N, 96°30′41.2″ W) near Sherman, TX, during May and June of 2019. Three treated and three nearby control fields were selected in San Patricio and Grayson counties, and five pairs of treatment-control were visited in Ellis county, TX for a total of 11 treated and 11 control fields.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 March 2020 doi:10.20944/preprints202003.0216.v1

Peer-reviewed version available at *Agronomy* **2020**, *10*, 571; doi:10.3390/agronomy10040571

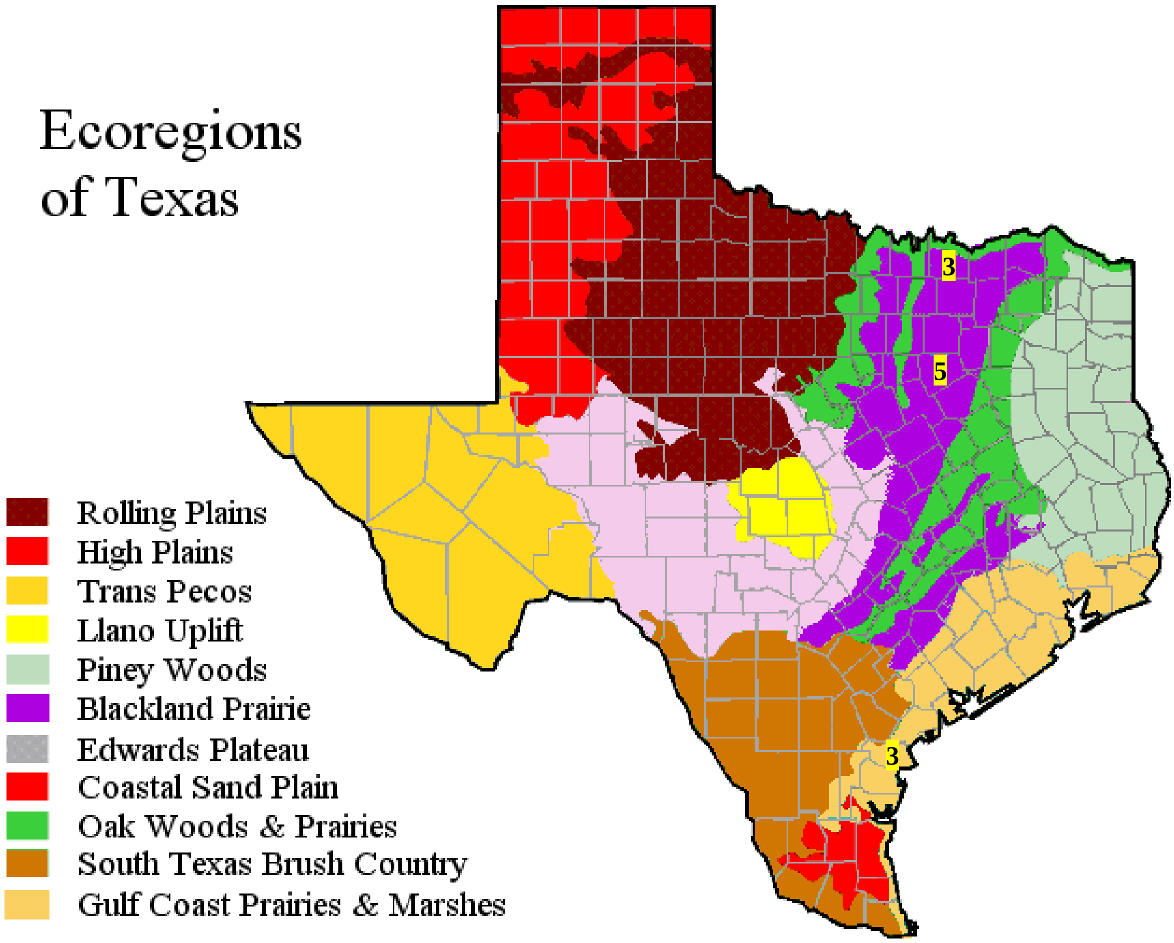

Figure 1. Ecoregions of Texas showing the counties sampled to collect bees in 2019. Number within a county is the number of pairs of control and treated fields sampled. (Source: http://kidsontheland.org/wpadmin/about-us/location/)

In San Patricio, corn was planted in February (14 and 21 February), and FourSure was applied on 22 April of 2019 (Table 1). In Ellis County, corn was planted on 8 March and 1 April 2019, and application of FourSure was performed on 19 May of 2019. Corn planting and FourSure application were performed on 22 March and 6 June 2019, respectively, in Grayson County. FourSure was applied at 11.3 kg ha^{-1} using an all-terrain vehicle-mounted spreader. Temperature and rainfall during the sampling period in each county are listed in Table 2. The temperature in San Patricio county was higher than in Ellis and Grayson counties during sampling. There was no rainfall in the week before sampling in San Patricio County, and thus the soil surfaces of corn fields were dry during the sampling. In contrast, three rain events of 0.3 mm, 33.4 mm, and 0.3 mm occurred on 5 June, 6 June, and 9 June, respectively before the sampling date (June 11) in Ellis County. The gravimetric water content of soil at 0-10 cm depth was determined by drying soil samples at 105°C for 48 h. The soil surfaces in Ellis County during the sampling were moist. In Grayson County, rain events of 14.5 mm, 16 mm, and 4.3 mm occurred on 16 June, 17 June, and 19 June, respectively, before the sampling date (20 June). Thus, the soil surfaces were wet during the sampling in Grayson County. During sampling in San Patricio county, there were storms moving through and occasional overcast skies and high wind speeds. The average wind speed for 24 h periods in San Patricio County was 5.1 m s^{-1}, while it was 4.3 and 9.3 in Ellis and Grayson Counties, respectively. The stages of the corn at sampling were silking (R1) and blister (R2). Corn at the time of sampling was late stage and mostly post-anthesis. Other studies in corn have shown bee abundance and diversity to be greatest during flowering [7,12].

Peer-reviewed version available at Agronomy 2020, 10, 571; doi:10.3390/agronomy10040571

Table 1. Dates of corn planting, FourSure application, and bee bowl setting in sampling sites in each county in 2019.

Variable	Treatment	County		
		San Patricio	Ellis	Grayson
Dates of corn planting	FourSure	February 14 & February 21	March 8 & April 01	March 22
	Control	February 14 & February 21	March 8 & April 01	March 22
Dates of FourSure	FourSure	April 15 & 22	May 19	June 6
	Control	April 15 & 22	May 19	June 6
Dates of bee bowl	FourSure	May 21	June 11	June 20
	Control	May 21	June 11	June 20

Table 2. Temperatures, rainfall, and degree-days data in each sampling site in 2019.

Variable			County		
			San Patricio	Ellis	Grayson
Air temperature (°C)†					
	Previous week of sampling	Maximum	31.3	29.8	30.9
		Minimum	23.8	20.5	20.7
		Average	27.6	25.2	25.8
	Sampling date	Maximum	34.4	26.1	31.4
		Minimum	26.7	16.1	21.1
		Average	30.6	21.1	26.3
Rainfall (mm)†		Total	-	34	35

† Temperatures are in a previous week of the sampling date and on the sampling dates within each county. Rainfall is the total rainfall in a previous week of the sampling date.

2.2. Bee Bowl Procedure

Pan traps (i.e. bee bowls) [13] were used to collect foraging bees. Bee bowls were set on 21 May in San Patricio County, 11 June in Ellis County, and 20 June in Grayson County (Table 1). Bee bowls were 104-mL plastic cups (New Horizons, Upper Marlboro, MD, USA) painted fluorescent yellow, blue or white on the inner surface. Five bee bowls, each positioned on 0.9-m elevated wooded stakes, were set in each transect of 20 m with 5-m distance between adjacent bowls (Figure 2). Each field replicate contained four transects with a total of 20 bee bowls established per field. The height of bee bowls was approximately 40% of the height of silking (R1) to blister (R2) stage corn. The extent of the total area in each field sampled in the four transects was less than one ha, and field sizes ranged from 24.3 to 72.8 ha. Two-thirds of each bee bowl was filled with a water and dish soap solution (approx. 5 to 10 drops of Dawn brand liquid soap per liter of water) to serve as a capturing and killing fluid. Bee bowls were left in the field for 24 h after which all bees from all bowls in each field replicate were collected and transferred into labeled glass vials containing 75% ethanol for

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 March 2020 doi:10.20944/preprints202003.0216.v1

Peer-reviewed version available at Agronomy 2020, 10, 571; doi:10.3390/agronomy10040571

preservation. Individual bees were identified to family and genus, and relative abundances of families and genera were compared between control and FourSure-treated fields (n = 11). In addition to bee bowl sampling, in each of the four transects per field, five-minute surveys were conducted after bee bowl establishment to record numbers and types of live foraging insects.

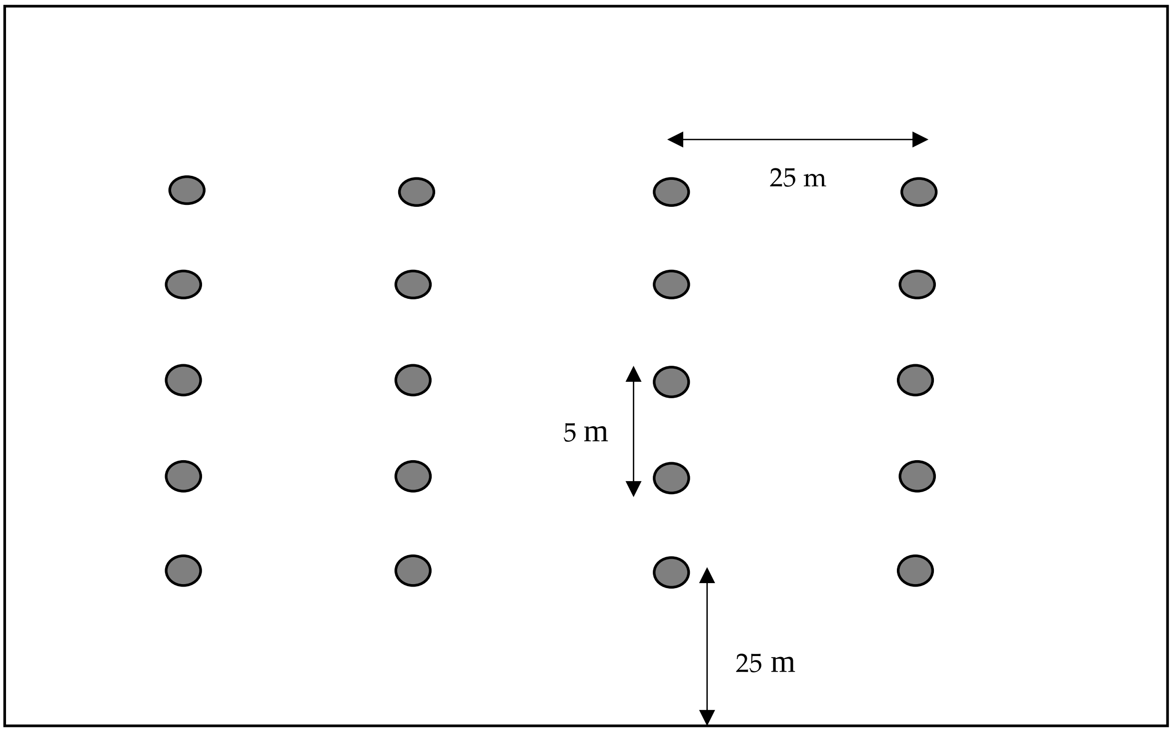

Figure 2. Diagram depicting the location within a corn field where bees were sampled; circles represent location of bee bowls on wooden stakes.

2.3. Statistical Analyses

Data were analyzed by analysis of variance consisting of two treatments (i.e. control and FourSure-applied) and three replications (five replications in Ellis County) for within-site tests. Sites were also combined to test for main effects of treatment and treatment × site interactions on bee relative abundances using Proc Mixed in SAS 9.4 [14]. Treatments were set as fixed effects, and replicates and sites were set as random effects. LSMEANS procedure was used to compare means. Differences were considered significant at $P \leq 0.05$.

3. Results

Eleven bee genera among four families were collected: Apidae, Colletidae, Halictidae, and Megachilidae. A total of 245 bees were collected, and the total numbers of bees collected across control and FourSure-applied fields was not significantly different between treated and untreated fields. The Texas striped sweat bee (*Agapostemon texanus*) accounted for 44% of the total number of bees collected from three counties over the entire study period. Long-horned bee (*Melissodes* spp.) was the second most abundant bee in pan traps, accounting for 24% of the total number of bees collected, while the metallic sweat bee genera/subgenera *Lasioglossum* (*Dialictus* spp.), was the third-ranked bee in abundance (23%). These three bee taxa constituted 91% of the total number of bees collected. The small carpenter bee (*Ceratina* spp.), chimney bee (*Diadasia* spp.), and sweat bees in the genus *Halictus* were less common (Table 3). Only two honey bee and two green sweat bee (*Augochlorella* spp.) individuals were collected in bee bowls, while the long-horned bee (*Svastra* spp.), a masked bee (*Hylaeus* spp.), and a leafcutter bee (*Megachile* spp.) were collected as singletons. The dominance of Halictidae in our samples was expected considering the inherent sampling bias regarding this taxon and its typically high occurrence in pan traps/bee bowls [15,16]. Nonetheless,

Peer-reviewed version available at Agronomy 2020, 10, 571; doi:10.3390/agronomy10040571

relative occurrences and frequencies of bee taxa across fields provided robust data to investigate generalized community structures (e.g. relative abundances of bee genera) and differences among treatments.

Table 3. List of families of bees and total abundances recovered by bee bowl method summed over four transects per field replicate, three replicates (five in Ellis county), and two treatments in three counties (San Patricio, Ellis, and Grayson).

Family	Genus	Common name	Abundance count
Apidae	*Melissodes* spp.	long-horned bee	59
	Ceratina spp.	small carpenter bee	3
	Diadasia spp.	sunflower/chimney bee	3
	Apis mellifera	honey bee	2
	Svastra spp.	long-horned bee	1
Colletidae	*Hylaeus* spp.	masked bee	1
Halictidae	*Agapostemon texanus*	Texas striped sweat bee	110
	Lasioglossum (*Dialictus*) spp.	metallic sweat bee	57
	Halictus spp.	sweat bee	9
	Augochlorella spp.	green sweat bee	2
Megachilidae	*Megachile* spp.	leafcutter bee	1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 March 2020 doi:10.20944/preprints202003.0216.v1

Peer-reviewed version available at Agronomy 2020, 10, 571; doi:10.3390/agronomy10040571

Total number of bees was not significantly different ($P > 0.09$) between FourSure-treated and control fields in all counties (Table 4; Figure 3). Treatments were not significantly different ($P = 0.06$) in total number of bees when data from all three counties were combined. Although there were no significant treatment × site interactions ($P = 0.42$), there was a greater total number of bees collected in San Patricio County than those in Ellis and Grayson counties (Table 4).

Table 4. Total counts of bee genera compared between control and FourSure-treated fields, by county and by pooled county data.

Bees	Treatment	County			
		San Patricio	Ellis	Grayson	Mean
		(no. treatment^{-1})			
Total	FourSure	29.3	2.4	1.6	11.1
	Control	36.7	4.8	3.0	14.8
	Treatment effect	$P = 0.30$	$P = 0.09$	$P = 0.27$	$P = 0.06$
	Treatment x Site		$P = 0.42$		

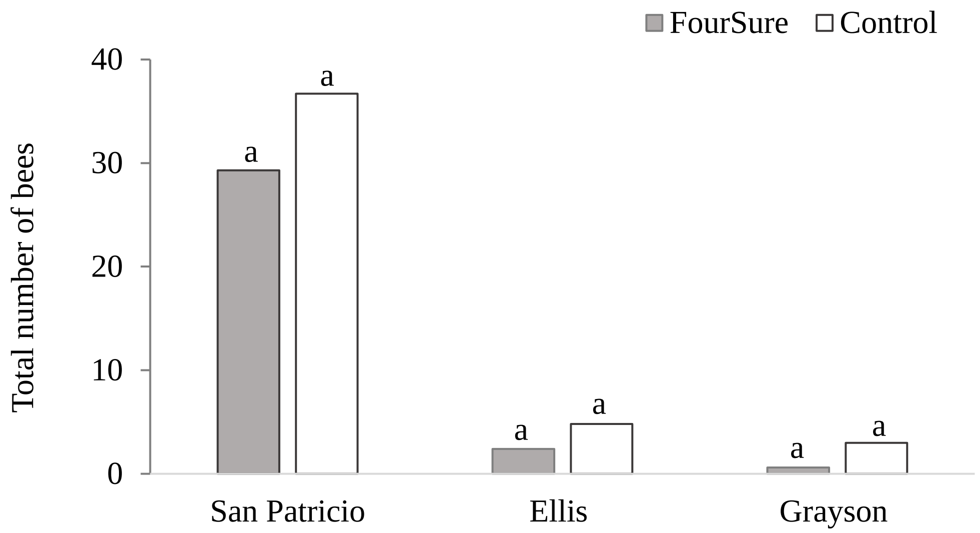

Figure 3. Total number of bees in FourSure-treated and control fields in three counties of Texas. Similar letters within a county indicate no significant treatment difference at a ≤ 0.05.

Because of low numbers of bees collected in Ellis and Grayson counties, an analysis of bee data from San Patricio County only was conducted using the three dominant bee taxa, i.e. Texas striped sweat bee long-horned bee, and metallic sweat bee Table 5). In San Patricio County, the differences in numbers of Texas striped sweat bees and long-horned bees between FourSure-treated and control fields were not significant ($P = 0.80$ and 0.63, respectively). Although the control fields had greater numbers of metallic sweat bees than did treated fields, the difference was not significant ($P = 0.30$).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 March 2020 doi:10.20944/preprints202003.0216.v1

Peer-reviewed version available at Agronomy 2020, 10, 571; doi:10.3390/agronomy10040571

Table 5. Statistics of ANOVA showing differences in the three dominant bee genera across control and FourSure treated fields in San Patricio County.

Treatment	*Agapostemon texanus*	*Melissodes* spp.	*Lasioglossum (Dialictus)* spp.
		(no. treatment^{-1})	
FourSure	16.0	7.3	4.7
Control	16.7	8.0	9.0
Treatment effect	*P* = 0.80	*P* = 0.63	*P* = 0.30

4. Discussion

This study documented honey bee and native bee communities occurring in both atoxigenic *Aspergillus flavus*-treated and nearby control corn fields across different corn production zones in Texas. While previous studies have reported honey bees foraging in corn [16,17], we found extremely few honey bees, which is similar to an earlier study [12] in which bee bowls were elevated at tassel height and few honey bees were recovered from traps. It was reported that height of bee bowl placement with the corn canopy may affect sampling accuracy of the pollinator community [15]. A previous study found a more abundant pollinator community in bee bowls deployed at tassel height than those deployed at ear height or ground height [12]. In a recent study in Texas pasturelands, honey bees were found to be the second most abundant after sweat bees of Halictidae family, using bee bowls on the soil surface [18]. Thus, it appears that the presence of extremely few honey bees in this study may not be due to bias associated with the height of the collection device (i.e., bee bowl).

Relatively high and unexpected abundances of wild native bees foraging in corn were counted in both FourSure-treated and nearby control fields in the current study. There were no differences in bee relative abundances between *A. flavus*-treated and control fields in each county, but greater bee abundances, particularly ground-nesting bees, were found in San Patricio County, and fields in this county generally contained lower soil moisture than those in the other sampled counties. Most native bees in Texas are ground-nesters and prefer well-drained ground habitat [19], and therefore soil conditions in corn could affect local uses by bees. Ground-nesting bees were more abundant in perennial grass pastures with low soil moisture compared to grass pastures with high soil moisture in the Texas High Plains [18]. The most abundant bee in our study was the Texas striped sweat bee followed by long-horned bee (Table 3). A sweat bee [*Lasioglossum* (*Dialictus*) spp.] is the next most abundant recovered in the current study. These results agree with a previous study [12] in which the most abundant bee species captured was [*Lasioglossum* (Dialictus) spp.] followed by *Melissodes* spp. in corn fields in Iowa.

The reasons for differences in abundances of wild bees between San Patricio and Ellis/Grayson are not known, but differences in weather conditions around the time of sampling (particularly rainfall) may be associated with patterns observed. There was no rainfall in San Patricio County, whereas three rain events occurred in Ellis and Grayson counties prior to sampling. Measurements of soil water contents (g g^{-1} soil) as described by (20, 21) indicated that soil water contents in San Patricio County (0.15) was lower compared to Ellis (0.25) and Grayson (0.24) counties.

Furthermore, while landscape context was not investigated here, larger areas of wild and uncultivated habitat in farmland could be influencing bee diversity and abundances [22], and this could have influenced the variability in bee abundances observed across latitudes. Although a functional relationship between bee abundance and corn plants is not clear, the observed diversity and abundances of bees suggests that the corn fields could be providing resources for native bees. Further study of bees in corn production systems in Texas are needed to better understand native bee resource use in corn fields in relation to weather variation and other local and landscape environmental factors, including those that could influence bee development in soil nests.

5. Conclusions

This study appears to be the first attempt to document bees occurring in corn fields in Texas. This survey of bees in corn was in part prompted by previous observations of dead honey bees in a cotton field following application of atoxigenic *Aspergillus flavus* (AF36 strain) to flowering-stage cotton in Arizona. We documented the honey bees and wild native bees in corn fields treated with atoxigenic *Aspergillus flavus*. The clearest result was that both FourSure-treated and control corn fields (particularly in San Patricio County) had fairly high and unexpected abundances of wild native bees foraging in corn. This suggests that atoxigenic FourSure had no negative effects on bee communities, yet toxicological studies and more field data are needed to elucidate potential negative impacts on bees as a result of its application. Among corn fields, only two honey bees were collected or observed during this study, which suggests a dearth of honey bees in corn production fields at this production stage. The reason for the greater abundances of bees in southerly San Patricio County is unknown, but differences in rainfall influencing soil moisture conditions during the sampling may have contributed to the observed variation. The potential benefits to pollinators in acquiring resources in corn (i.e. pollen and soils for nesting) and the use of corn by wild bees found in this study suggest a need to better understand non-target impacts to native fauna in corn production systems.

Author Contributions: Conceptualization, K.B.B., S.D.L., and C.P.W.; methodology, K.B.B. and S.D.L.; investigation, K.B.B., and S.D.L.; formal analysis, K.B.B.; writing—original draft preparation, K.B.B.; writing—review and editing, S.D.L. and C.P.W.; project administration, K.B.B.; funding acquisition, K.B.B. All the authors revised the manuscript.

Funding: This research was funded by Texas Corn Producers Board (TCPB) under 2019 Aflatoxin Research Program; https://texascorn.org.

Acknowledgments: We would like to thank cooperating growers Charles Ring and Jim Sugarek in San Patricio County, Kenneth Houdek and Scott Averhoff in Ellis County, and Bruce Wetzel and Mike Fallon in Grayson County that allowed us to sample on their corn fields and their field assistance. Field assistance by Shirish Acharya, Surendra Yadav, and Rahul Ukey is gratefully acknowledged. We also thank Aqeela Sehrish for her laboratory assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mehl, H.L.; Cotty, P.J. Variation in competitive ability among isolates of *Aspergillus flavus* from different vegetative compatibility groups during maize infection. *Phytopathology* 2010, 100, 150–159. doi: 10.1094/PHYTO-100-2-0150.
2. Cotty, P.J. Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the populations of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed. *Phytopathology*, 1994, 84, 1270-1277. doi: 10.1094/Phyto-84-1270.
3. Klich, M.A. Aspergillus flavus: The major producer of aflatoxin. *Mol Plant Pathol*. 2007, 8 (6), 713-22. doi: 10.1111/j.1364-3703.2007.00436.x.
4. Bacon, C.W.; Nelson, P.E. Fumonisin production in corn by toxigenic strains of *Fusarium moniliforme* and *Fusarium proliferatum*. *J. Food Prot*. 1994, 57, 514-521.
5. Payne, G.A. Aflatoxin in maize. *Crit. Rev. Plant Sci.* 1992, 10:423-440.
6. Nishie, K.; Cole, R.J.; Domer, J.W. Toxicity and neurophamacology of cyclopiazonic acid. *Food Chem. Toxicol.* 1985, 9, 831–839.
7. US EPA. Ecological Risk Assessment for Experimental Testing of the microbial pesticide end-use product, FourSure,.M (a.i.: Aspergillusflavus Strains TCI 6F, TC35C, TC38B and) to control aflatoxin-producing

(toxigenic *Aspergillus flavus*. 2016. EPA File Symbol 91I63-EUP-R, Tolerance Petition #5E8397; Decision No. 508471; Submission No. 972674; DP Barcode No: 4 I 9535; E-Sub #: 8226.

8. Jensen, A.B.; Aronstein, K.; Flores, J.M.; Vojvodic, S.; Palacio, M.A.; Spivak, M. Standard methods for fungal brood disease research. *J. Apic. Res.* 2013, 52 (1), 1-39. doi: 10.3896/IBRA.1.52.1.13
9. US EPA. Environmental hazard assessment for the microbial pesticide, Aspergillus flavus AF36 for conditional registration in Arizona and EUP Extension in Texas; 2003. EPA Reg. No. 071693-R; Barcode Nos. D288777; D288782; D286708
10. Bayman, P., Cotty, P.J. Vegetative compatibility and genetic variation in the Aspergillus flavus population of a single field. *Can. J. Bot.* 1991, 69, 1707-1711.
11. Ehrlich, K.C. Non-aflatoxigenic Aspergillus flavus to prevent aflatoxin contamination in crops: advantages and limitations. *Front. Micro*. 2014. doi: 10.3389/fmicb.2014.00050
12. Wheelock, M.J.; O'Neal, M.E. Insect pollinators in Iowa cornfields: community identification and trapping method analysis. *PLoS ONE* 2016, 11, 7, 1-13. doi: 10.1371/journal.pone.0143479
13. Droege, S.; Engler, J.D.; Sellers, E.; O'Brien, L.E. National protocol framework for the inventory and monitoring of bees. U.S. Fish and Wildlife Service, U.S. Department of the Interior. 2016. Available at the National Wildlife Refuge System Libraries. Accessed on 4 March 2020
14. Littell, R.C.; Milliken, G.A.; Stroup, W.W.; Wilfinger, R.D.; Schabenberger, O. SAS for mixed models. 2006, 2nd ed. SAS Inst., Cary, NC.
15. Tuell, J.K.; Isaacs, R. Elevated pan traps to monitor bees in flowering crop canopies. *Entomol. Exp. Appl.* 2009, 131 (1) 93–98. doi: 10.1111/j.1570-7458.2009.00826.x
16. Krupke, C.H.; Hunt, G.J.; Eitzer, B.D.; Andino, G.; Given, K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS ONE* 2012, 7, (1), 1-8. doi: 10.1371/ journal.pone.0029268
17. Keller, R.; Fluri, P.; Imdorf, A. Pollen nutrition and colony development in honey bees:part:1 Bee World 2005, 86 (1), 3–10. doi: 10.1080/0005772X.2005.11099641
18. Bhandari, K.B.; West, C.P.; Longing, S.D.; Brown, C.P.; Green, P.E.; Barkowsky, E. Pollinator abundance in semi-arid pastures as affected by forage species. *Crop Sci.* 2018, 58, 6, 2665-2671. doi: 10.2135/cropsci2018.06.0393.
19. Warriner, M.; Hutchins B. Management recommendations for native insect pollinators in Texas. Texas Parks and Wildlife Department. 2016. Available at the Texas State Publications Clearinghouse and/or Texas Depository Libraries. https://tpwd.texas.gov/publications/pwdpubs/media/pwd_bk_w7000_1813.pdf.
20. Bhandari, K.B.; West, C.P.; Acosta-Martinez, V.; Cotton, J.; Cano, A. Soil health indicators as affected by diverse forage species and mixtures in semi-arid pastures. *Appl. Soil Ecol*. 2018, 132, 179-186. doi:10.1016/j.apsoil.2018.09.002
21. Bhandari, K.B.; West, C.P.; Acosta-Martinez, V. Assessing the role of interseeding alfalfa into grass on improving pasture soil health in semi-arid Texas High Plains. *Appl. Soil Ecol*. 2019, 147, 103399. doi:10.1016/j.apsoil.2019.103399
22. Evans, E.; Smart, M.; Cariveau, D.; Spivak, M. Wild, native bees and managed honey bees benefit from similar agricultural land uses. *Agric. Ecosyst. Environ.* 2018, 268, 162–170. doi: 10.1016/j.agee.2018.09.014.