Botanical and synthetic pesticides alter the flower visitation rates of pollinator bees in Neotropical melon fields[★]

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A R T I C L E   I N F O

A B S T R A C T

The ecological and economic contributions of pollinator bees to agricultural production have been threatened by the inappropriate and excessive use of pesticides. These pesticides are often applied in areas with ecological peculiarities (e.g., the Neotropical savannah-like region termed as Cerrado) that were not considered during the product development. Here, we conducted field experiments with melon (i.e., *Cucumis melo* L.) plants cultivated under Brazilian Cerrado conditions and evaluated the impacts of botanical (i.e., neem-based insecticide) and synthetic (i.e., the pyrethroid insecticide deltamethrin and the fungicides thiophanate-methyl and chlorothalonil) pesticides on the flower visitation rates of naturally occurring pollinator bees. Our results revealed that both honey bees (i.e., *Apis mellifera* L.) and non-*Apis* bees visited melon flowers and the intensity of bee visitation was moderately correlated with yield parameters (e.g., number of marketable fruits and fruit yield). Pesticide treatments differentially affected bee species. For instance, *Plebeia* sp. bees were not affected by any pesticide treatment, whereas both *A. mellifera* and *Halictus* sp. bees showed reduced visitation intensity after the application of deltamethrin or neem-based insecticides. Fungicide treatment alone did not influence the bee's visitation intensity. Deltamethrin-treated melon fields produced significantly lighter marketable fruits, and the melon yield was significantly lower in melon fields treated with the neem-based insecticide. Thus, our findings with such pollinator bees reinforce the idea that field applications of botanical pesticides may represent as risky as the applications of synthetic compounds, indicating that these alternative products should be submitted to risk assessments comparable to those required for synthetic products.

1. Introduction

The utilization of pesticides (e.g., herbicides, insecticides, and fungicides) has enabled farmers to increase high quality food production (Bourguet and Guillemaud, 2016; Cooper and Dobson, 2007; Lima et al., 2016; Skevas et al., 2013). However, the unintended effects of pesticides on the biodiversity of beneficial insects represents a global trend that is garnering much concern (Barbosa et al., 2015b; Cutler and Scott-Dupree, 2014; Cutler et al., 2014; Kessler et al., 2015; Muratet and Fontaine, 2015; Sánchez-Bayo and Tennekes, 2017; Tomé et al., 2017). The inappropriate application and overuse of these agricultural tools has been shown to harm many pollinators (Breeze et al., 2014; Fairbrother et al., 2014; Johnson, 2015; Kovács-Hostyánszki et al., 2017; Muratet and Fontaine, 2015), and such non-intended effects on naturally occurring pollinator bees can reduce the valuable contribution of pollination to agricultural production (Garibaldi et al., 2018; Garibaldi et al., 2013; Goulson et al., 2015; Klatt et al., 2014).

Declines in pollinator populations threatens global food production to such an extent that federal agencies from the USA and Europe have been attempting to establish strategies on pollinator health to prevent or recover such phenomenon (EFSA, 2014; Obama, 2014). Much attention was initially given to pesticide-mediated reductions in the number of honey bee pollinators (mainly *A. mellifera* in the USA and in some European

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countries). However, those initial concerns have been replaced by a broader concern in the decline of other pollinator bees (Del Sarto et al., 2014; Fürst et al., 2014; Lima et al., 2016; Melathopoulos et al., 2014; Potts et al., 2010; Rundlof et al., 2015; Tomé et al., 2017).

Although wild bee species exist in almost all terrestrial ecosystems, the vast majority of large-scale managed pollination and toxicological studies have used honey bee (i.e, *A. mellifera*) and some bumble bee species (e.g., *Bombus* sp.) in their investigations. The majority of studies assessing pesticide impacts on pollinators have focused on *A. mellifera* with fewer studies assessing the susceptibility of wild bee species to synthetic or botanical pesticides (Barbosa et al., 2015a; Del Sarto et al., 2014; Jacob et al., 2015; Stanley et al., 2015; Tomé et al., 2017; Valdovinos-Núñez et al., 2009). This may be due to the inaccurate but still prevalent consensus that *A. mellifera* is extremely sensitive when compared with other bee species (Arena and Sgolastra, 2014; Decourtye et al., 2013). Recent studies, however, describe sublethal effects or higher susceptibility in other bee species, caused by compounds generally considered to be safer products after risk assessments with honey bees (Arena and Sgolastra, 2014; Barbosa et al., 2015b; Bernardes et al., 2018; Del Sarto et al., 2014; Ollerton et al., 2014; Tomé et al., 2015; Tomé et al., 2017).

Exposure to synthetic insecticides (e.g., neurotoxic compounds such as the pyrethroids and neonicotinoids) or fungicides (e.g., ergosterol biosynthesis inhibitor (EBI) and non-EBI compounds) have been shown to alter the activities of cytochrome P450-mediated enzymes in pollinator bees (Iwasa et al., 2004; Sgolastra et al., 2016; Tomé et al., 2017), making these bees more susceptible to compound effects. Interestingly, botanical insecticides such as the ones obtained from neem (i.e., *Azadirachta indica*) plants can also harm pollinator bees by affecting these insects' development or reproduction. Such neem-based compounds have been shown to disrupt the neuroendocrine and reproductive systems of pollinator bees (Mordue (Luntz) and Blackwell, 1993; Mordue (Luntz) and Nisbet, 2000; Barbosa et al., 2015a,b, Bernardes et al., 2018).

Some studies have demonstrated that crop productivity is improved by bee-mediated pollination (Garibaldi et al., 2013; Klatt et al., 2014; Motzke et al., 2015), whereas other investigations suggest that the benefits accrued from pollination are achieved only when plant protection (e.g., pest and disease management) practices are used (Lundin et al., 2013; Melathopoulos et al., 2014). Thus, plant protection practices should be set in regional contexts (Baldock et al., 2015; Boreux et al., 2013; Lundin et al., 2013; Melathopoulos et al., 2014; Motzke et al., 2015) to conserve the benefits provided by naturally occurring pollinator bees.

In this context, one of the world's most important agricultural frontiers and ecological hotspots is the Brazilian savannah-like biome (also termed as Brazilian Cerrado) (Maia et al., 2013; Mittermeier et al., 2004). This Cerrado area covers almost a quarter (i.e., 25%) of the Brazilian territory (Diniz-Filho et al., 2009; Ratter et al., 1997), but investigations on the naturally-occurring bee fauna (and pollination services) are scarce (Tschoeke et al., 2015) and crop management practices (e.g., cultivar types, soil and plant protection practices) have not been developed with this region in mind. This research gap is surprising because the introduction of commercial plants dependent on insect-mediated pollination in commercial fields, such as the melon *C. melo*, has increased rapidly during the last decade (IBGE; Tschoeke et al., 2015). Thus, we conducted field experiments in a typical Brazilian Cerrado area to evaluate which plant protection practices (e.g., synthetic or botanical pesticides) would minimize impacts on flower visitation rates by naturally occurring pollinator bees in melon fields.

2. Material and methods

2.1. Study site, cultivation and experimental design conditions

Excepting the fact that we planted melon seeds of the cultivar 'Casca de Carvalho Comprido' (Feltrin Sementes LTDA, Farroupilha, RS, Brazil) between July 2014 and September 2014, our study sites (i.e., melon fields), cultivation conditions and experimental design were similar to (with small modifications) those described elsewhere (Tschoeke et al., 2015). Briefly, we used a typical Brazilian "savannah-like" (i.e., Brazilian Cerrado) area (latitude 11°44′46.55″S, longitude 49°3′6.595″W, and altitude of 280 m) to cultivate melon fields, using a randomized block experimental design consisting of five treatments (pesticide regimens used to control insect pests or to control fungal plant diseases) and four study fields.

The pesticide regimes evaluated were the synthetic insecticide deltamethrin (application rate of 0.3 mL/L; Bayer Crop Science LTDA, São Paulo-SP, Brazil); the neem-based insecticide (application rate of 300 mL/L; Neenmax, Insetimax Indústria Química, Jardinópolis, SP, Brazil); the commercial mixture of the synthetic fungicides thiophanate-methyl (200 g a.i./kg) and chlorothalonil (200 g a.i./kg) (application rate of 2 g/L, Cerconil WP, Iharabras Indústrias Químicas S.A, Sorocaba, SP, Brazil); the simultaneous application of deltamethrin and fungicides (at the same application rate of each pesticide used in the sole applications); and tap water (control). Three pesticide applications were carried out during the flowering season, with seven days apart and always at the late afternoon (around 5:00 p.m.). Extensive efforts were made to avoid the influence of pesticide drifts, by using manual sprayers and physical barriers (plastic canvas with 1.5 m height) during each pesticide application.

2.2. Diversity and flower visitation rates of pollinator bees

In order to avoid the confusion between pollinator bees and mere floral visitors, we recorded only bee species that were clearly carrying pollen and interacting with the flower reproductive structures (i.e., anthers and stigmas). Specimens of these bees were captured, killed in a jar using ethyl acetate, prepared, and taxonomically identified at the Phytosanitary Laboratory (Federal University of Tocantins, Gurupi, TO, Brazil), where voucher specimens were deposited.

We conducted preliminary investigations (see Tschoeke et al., 2015 for more details), and observed that visits of pollinator bees to *C. melo* flowers were influenced by the time of day, with visit intensity peaks between 8:00 and 11:00 a.m., decreasing to zero by approximately 12:00 p.m. Only a few *A. mellifera* individuals were observed during the afternoon time (mostly after 4:00 p.m.). Thus, in this study, we only recorded the bee visitation frequency in the morning (from 07:00 a.m. – 12:00 p.m.). The 5th, 12th and 19th days of the flowering season were chosen for pesticide applications. Bee visitation frequencies were recorded on the mornings of application days as well as mornings following pesticide applications. These observations were always performed during the first 10 min of each anthesis hour, as described in other studies (Courcelles et al., 2013; Tschoeke et al., 2015; Wolf et al., 1999). We used one observer per experimental block.

2.3. Yield traits under pesticide treatments

As the bee frequency evaluation was recorded per experimental field study place, all the following yield parameters were evaluated for the same experimental unit. We evaluated not only fruit qualitative parameters (e.g., the total soluble solids [TSS] and the total

titratable acidity [direct titration in citric acid]), but also quantitative parameters such as the number of marketable fruits, the average fruit weight, and the estimated yield per hectare. The total soluble solid determinations were performed by using a portable refractometer (Master T 2312, Atago do Brasil LTDA, Ribeirão Preto, SP, Brazil), and the values were expressed in °Brix. The total titratable acidity values were obtained according the procedures described by the Instituto Adolf Lutz, 2008 and were expressed as percentages. The number of marketable fruits, average fruit weight, and estimated yield per hectare were calculated and subjected to correlation analysis with the number of bees that visited the experimental plots.

2.4. Statistical analysis

Repeated-measures analysis of variance (ANOVA) was used to analyze the effect of pesticide treatment on the numbers of pollinator bees that visited melon flowers. The percent change observed in the number of bees after each pesticide application (i.e., on the mornings of the 6th, 13th and 20th days of flowering season) were used as replicated measurement (within sample variation) to avoid time pseudo-replication problems (Paine, 1996; von Ende, 1993). This analysis was conducted using procedures and statements previously described elsewhere, von Ende (1993). When necessary, post hoc Tukey's HSD tests ($\alpha = 0.05$) were used to compare treatment means. The GLM procedure with the PROFILE statement was used for this analysis (SAS Institute, 2008). In addition, we also established correlation analysis (PROC CORR; SAS Institute, 2008) between the number of bees of each bee species (as well as the bee abundance) and productivity traits (the number of fruits set, the number of marketable fruits, the average fruit weight, the total soluble solids and the total titratable acidity). We checked the assumptions of normality and homogeneity of variance by using the UNIVARIATE procedure (SAS Institute, 2008), but no data transformation was necessary.

3. Results

The average temperature and relative humidity on days when the visitation frequency was evaluated are presented in Supplementary Fig. 1A. In the morning, when greater bee visitation rates were observed, the temperature ranged from 21.3 °C to 33.9 °C, and the average relative humidity ranged from 36.3% to 80.8%. Furthermore, the monthly average temperature varied from 20.9 °C (minimum temperature) to 32.9 °C (maximum temperature), and the rainfall was primarily concentrated from January to May and

September to December, with an annual precipitation of 1428.6 mm (Supplementary Fig. 1B).

3.1. Diversity and flower visitation rates of pollinator bees

The honeybee *A. mellifera* L. and three wild bee species (*Halictus* sp., *Plebeia* sp., *Trigona spinipes* F.) were observed visiting both hermaphrodite and male flowers in all six days of evaluations. However, the number of *T. spinipes* visits was too small (Supplementary Table 1) for any statistical analysis. All bee species exhibited similar foraging behaviors and collected nectar and pollen. The repeated-measures ANOVA for the number of bees visiting *C. melo* flowers showed that the visitation rates of each bee species varied significantly with the anthesis hour, and was differentially affected by the pesticide treatments ($P < 0.05$; Tables 1–3). For *A. mellifera* and *Halictus* bees, significant effects were also observed for the interactions between the application of deltamethrin + fungicides and anthesis hour ($P < 0.05$; Tables 1 and 2).

The visitation rates of *Plebeia* bees were not affected by either botanically based or synthetic pesticides, but naturally decreased at the end of flowering period (Table 3, Fig. 1). Without application of pesticides (i.e control treatment), the visitation intensity of *A. mellifera* decreased significantly over the flowering period, and peaked between 8:00 and 11:00 a.m. (*Fig. 2A*). The visitation rates of *Halictus* bees in the absence of pesticide treatment differed from those observed for *A. mellifera*, being significantly (Table 2; Fig. 2B) higher after the middle (i.e., the second week) of the flowering period. For both *A. mellifera* and *Halictus* bees, significant reductions in visitations were observed in the field study places treated with deltamethrin (alone or mixed with fungicides) or neem-based insecticides (Figs. 3 and 4; Supplementary Figs. 2 and 3). In Figs. 3 and 4, we show only the changes in percentage (%) but the variations on the absolute number of bees' visitations are provided in Supplementary Figs. 2 and 3. For each anthesis hour, we calculated such changes in percentage by using the following mathematical analysis: $CP = \{[(NB_{after}/NB_{prior})*100]-100\}$, where CP = changes in percentage, NB_{after} = number of visiting bees at an x hour on the day immediately after the pesticide application, NB_{prior} = number of visiting bees at an x hour on the day immediately prior to the pesticide application.

The treatment with fungicides alone did not affect the visitation intensity of any pollinator bees (Tables 1–3).

3.2. Yield traits under pesticide treatments

Although the deltamethrin-treated field sites produced similar

Table 1
Repeated measures analysis of variance for the total number of *Apis mellifera* at *C. melo* flowers.

Sources of variation	Df	Control		deltamethrin		fungicide		deltamethrin + fungicide		neem	
		F	P	F	P	F	P	F	P	F	P
Between subjects											
Hour (H)	5	2.92	0.037*	6.71	0.0006*	8.42	<0.001*	20.6	<0.001*	6.58	0.0007*
Pesticide application (PA)	1	0.04	0.83	4.81	0.0362*	1.47	0.24	10.8	0.0026*	9.82	0.0039*
H x PA	5	0.78	0.55	1.55	0.21	0.25	0.91	3.07	0.0315*	1.20	0.33
Error	30	–	–	–	–	–	–	–	–	–	–
	df$_{den}$/df$_{num}$	Wilks' lambda	F P	Wilks' lambda	F P	Wilks' lambda	F P	Wilks' lambda	F P	Wilks' lambda	F P
Within subjects											
Time (T)	29/2	0.305	10.3 0.0004*	0.975	33.1 <0.001*	0.642	7.82 0.002*	0.429	19.3 <0.001*	0.647	7.65 0.0022*
T x H	58/8	0.647	1.81 0.09	0.546	2.56 0.0253*	0.819	0.74 0.66	0.646	1.77 0.10	0.573	2.24 0.0372*
T x PA	29/2	0.995	0.07 0.94	0.885	1.88 0.17	0.876	1.98 0.16	0.416	20.3 <0.001*	0.955	0.66 0.52
T x H x PA	58/8	0.507	2.93 0.0080*	0.631	1.87 0.08	0.656	1.64 0.14	0.502	2.98 0.0072*	0.793	0.86 0.57

* Significant at $P < 0.05$.

Table 2
Repeated measures analysis of variance for the total number of *Halictus* sp. at *C. melo* flowers.

Sources of variation	df	control		deltamethrin (d)		fungicide(f)		deltamethrin + fungicide		neem	
		F	P	F	P	F	P	F	P	F	P
Between subjects											
Hour (H)	5	13.30	<0.0001*	7.28	0.0003*	7.58	0.0002*	20.58	<0.001*	11.05	<0.001*
Pesticide application (PA)	1	1.29	0.27	5.75	0.0329*	0.17	0.68	10.77	0.0002*	4.86	0.0353*
H x PA	5	1.71	0.17	1.14	0.36	1.19	0.33	3.06	0.0315*	1.67	0.18
Error	30	—	—	—	—	—	—	—	—	—	—

	df_{den}/df_{num}	Wilks' lambda	F	P	Wilks' lambda	F	P	Wilks' lambda	F	P	Wilks' lambda	F	P	Wilks' lambda	F	P
Within subjects																
Time (T)	29/2	0.251	43.4	<0.0001*	0.299	33.3	<0.001*	0.374	27.2	<0.001*	0.429	19.9	<0.001*	0.423	19.7	<0.001*
T x H	58/8	0.349	5.02	<0.0001*	0.422	3.91	0.0010*	0.329	5.38	<0.001*	0.646	1.77	0.10	0.402	4.17	0.0010*
T x PA	29/2	0.437	18.7	<0.0001*	0.400	21.7	<0.001*	0.816	3.27	0.0523	0.417	20.3	<0.001*	0.572	4.77	0.0161*
T x H x PA	58/8	0.370	4.67	0.0002*	0.530	2.70	0.0135*	0.683	1.52	0.16	0.502	2.98	0.0072*	0.796	0.88	0.54

* Significant at $P < 0.05$.

Table 3
Repeated measures analysis of variance for the total number of *Plebeia* sp. at *C. melo* flowers.

Sources of variation	df	control		deltamethrin (d)		fungicide(f)		deltamethrin + fungicide		neem	
		F	P	F	P	F	P	F	P	F	P
Between subjects											
Hour (H)	5	15.42	<0.0001*	13.97	<0.0001*	18.93	<0.0001*	14.52	<0.001*	12.61	<0.001*
Pesticide application (PA)	1	3.16	0.08	0.12	0.73	3.31	0.07	1.12	0.29	0.01	0.94
H x PA	5	0.24	0.91	1.41	0.25	2.47	0.06	0.31	0.87	0.39	0.81
Error	30	—	—	—	—	—	—	—	—	—	—

|	df_{den}/df_{num}	Wilks' lambda	F	P	Wilks' lambda	F	P	Wilks' lambda	F	P	Wilks' lambda	F	P	Wilks' lambda	F	P
Within subjects																
Time (T)	29/2	0.382	23.4	<0.0001*	0.207	55.5	<0.001*	0.332	29.1	<0.001*	0.443	18.2	<0.001*	0.341	28.0	<0.001*
T x H	58/8	0.583	2.24	0.0369*	0.435	3.74	0.0014*	0.460	3.43	0.0027*	0.524	2.76	0.0118*	0.520	2.81	0.0107*
T x PA	29/2	0.956	0.67	0.52	0.963	21.7	0.58	0.863	2.28	0.12	0.998	0.02	0.97	0.921	1.23	0.30
T x H x PA	58/8	0.600	2.11	0.0496*	0.840	2.70	0.72	0.680	1.54	0.16	0.502	0.90	0.52	0.730	1.24	0.29

* Significant at $P < 0.05$.
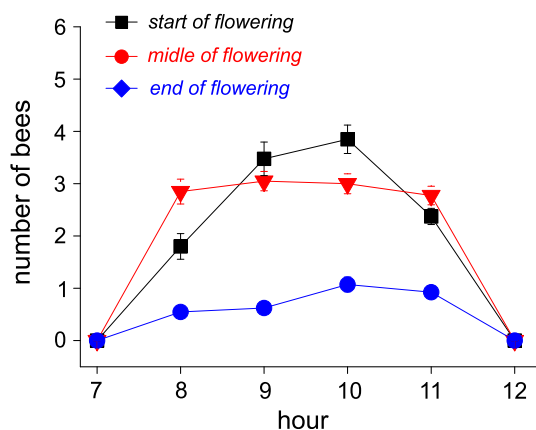
Fig. 1. Visitation rates of *Plebeia* sp. at *C. melo* flowers under Cerrado conditions. The number of *Plebeia* sp. was significantly affected only by the anthesis hour ($P < 0.05$; Table 3) and flowering season (time), and not by the pesticide applications ($P > 0.05$; Table 3). The average number of visits on the days before and after the pesticide applications is indicated by black (cumulative average for the 5th and 6th days of flowering), red (cumulative average for 12th and 13th days of flowering) and blue (cumulative average for 19th and 20th days of flowering) symbols. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 5A) quantities of marketable fruits compared to the control sites, these fruits were the lightest fruits produced among the field sites ($F = 18.0$, df = 4, $P < 0.0001$; Fig. 5B). Field sites treated with the neem-based insecticide produced fewer marketable fruits ($F = 3.9$, df = 4, $P = 0.023$; Fig. 5A), and resulted in the lowest fruit yield ($F = 3.9$, df = 4, $P = 0.023$; Fig. 5C). As shown in Fig. 5C, the pesticide treatment that resulted in the most satisfactory fruit yield was the joint application of deltamethrin and fungicides (i.e., thiophanate-methyl + chlorothalonil). No significant differences were observed for the qualitative parameters 'total of soluble solids' ($F = 1.5$, df = 4, $P = 0.240$) and 'total titratable acidity' ($F = 2.7$, df = 4, $P = 0.069$).

3.3. Correlations between the number of visiting bees and productivity traits

Our correlation analysis showed that the number of marketable fruits was significantly correlated with the number of *A. mellifera* ($r = 0.55$, $P = 0.013$), and with bee abundance ($r = 0.71$, $P < 0.0001$). The fruit yield was also significantly correlated with the number of *A. mellifera* ($r = 0.45$, $P = 0.047$), as well as bee abundance ($r = 0.58$, $P = 0.007$).

4. Discussion

Here, we recorded four naturally occurring bee species

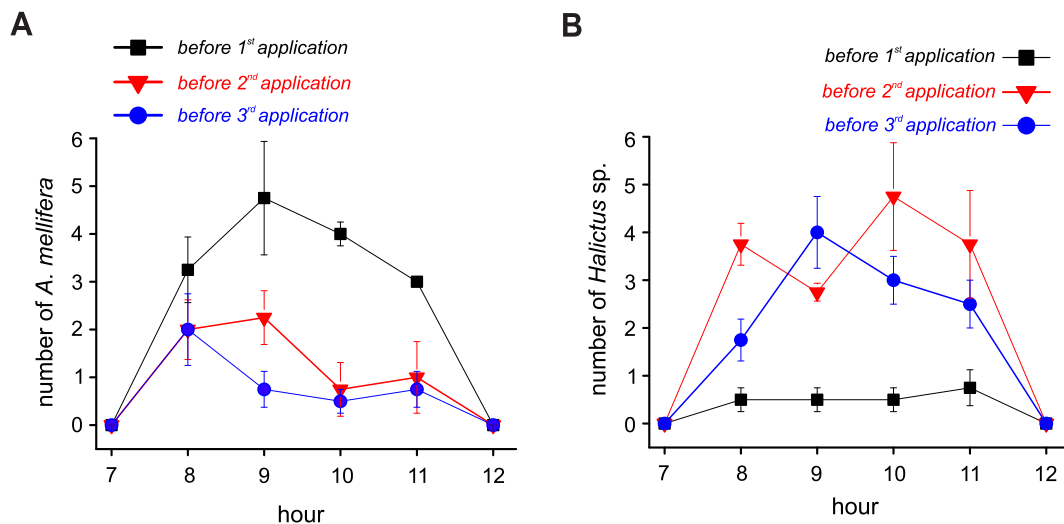## Flower visitation rates of *A. mellifera* and *Halictus* bees

Fig. 2. Visitation rates of *A. mellifera* and *Halictus* bees at *C. melo* flowers under Cerrado conditions, and in the absence of pesticide treatments. The number of visits by *A. melifera* (**A**) and *Halictus* bees (**B**) was affected by the anthesis hour and by the flowering period ($P < 0.05$, Tables 2–3). While *A. mellifera* visit rates decreased significantly over the flowering period, the visitation rates of *Halictus* bees were significantly (Table 2; Fig. 3B) higher after the middle (i.e., second week) of flowering period.

Pesticide-induced effects on flower visitation rates of *A. mellifera*
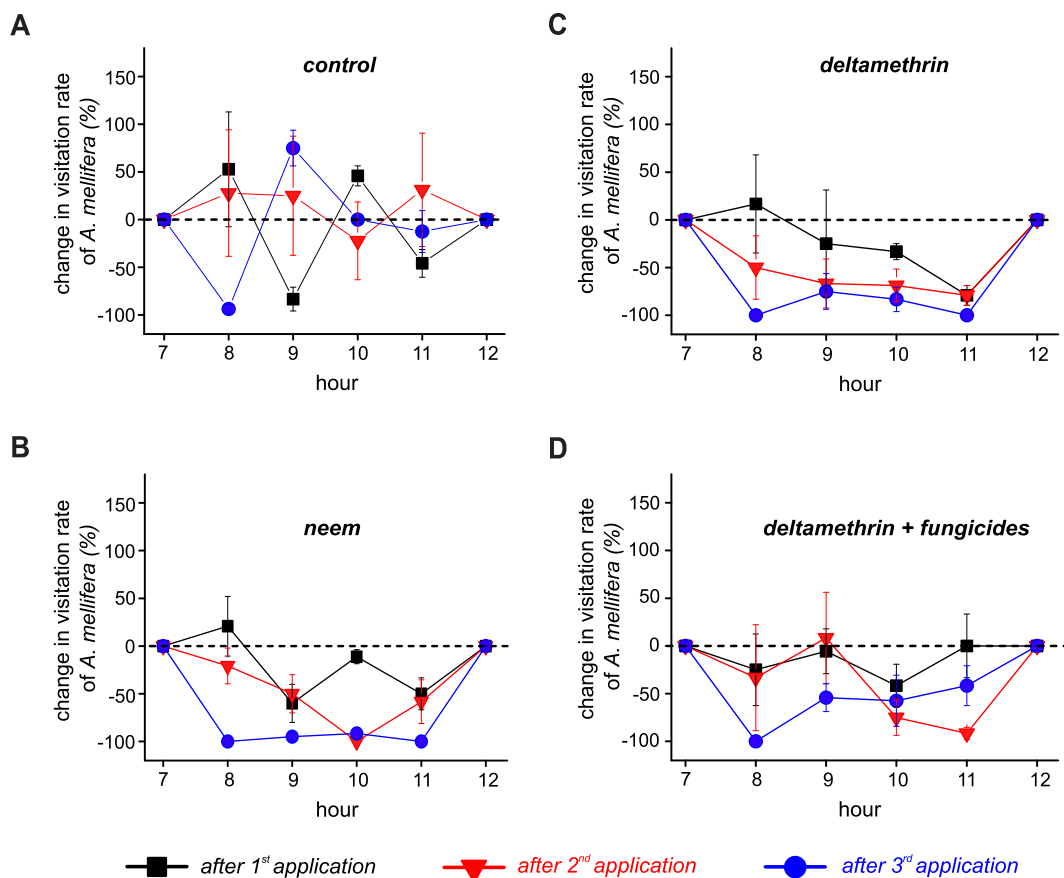
Fig. 3. Effects of pesticides on the visitation rates of *A. mellifera* at *C. melo* flowers under Cerrado conditions. **A.** Number of visits by *A. mellifera* was not affected by application of tap water ($P > 0.05$, Table 1). **B.** The application of neem-based insecticide (**B**), or deltamethrin alone (**C**) or mixed with fungicides (**D**) significantly reduced the visitation intensity of *A. mellifera* ($P < 0.05$, Table 1). The percent change in the number of visits on the day after the pesticide applications is indicated by black (after 1st application), red (after 2nd application) and blue (after 3rd application) symbols. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

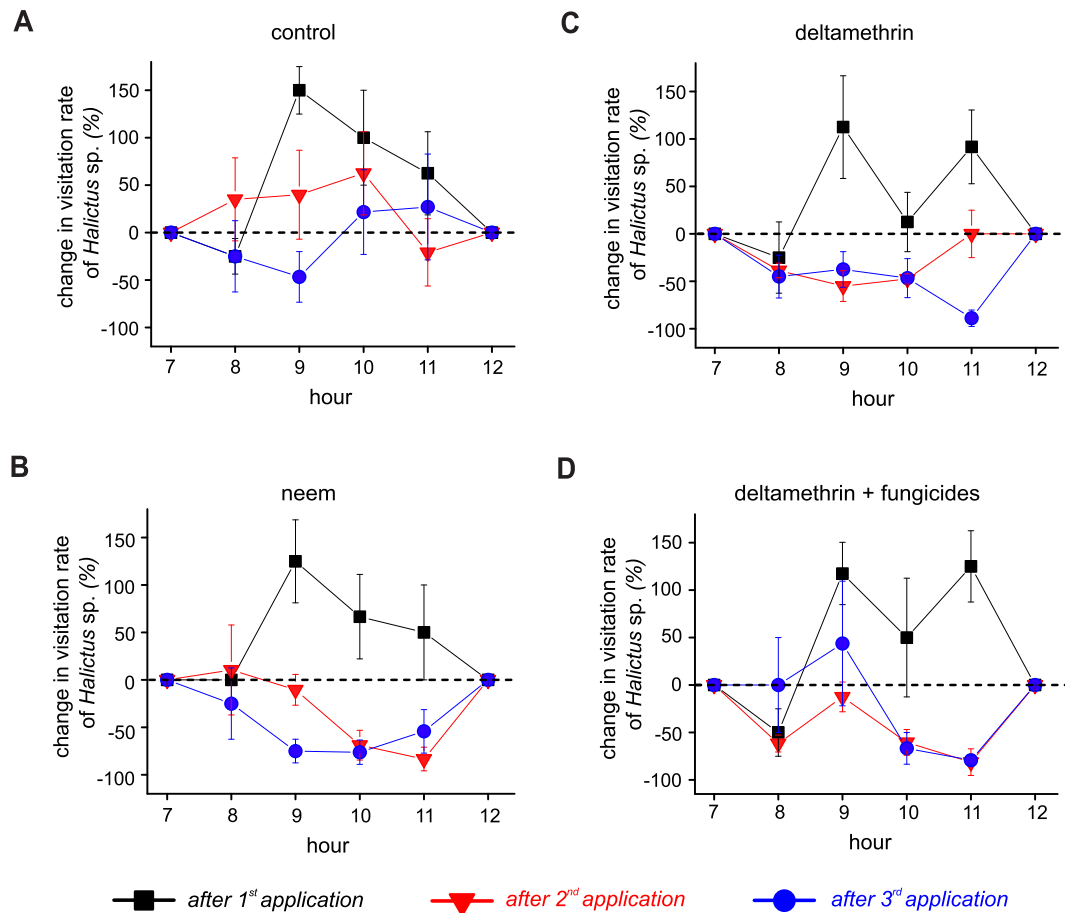### Pesticide-induced effects on flower visitation rates of Halictus bees

Fig. 4. Effects of pesticides on the visitation rates of *Halictus* sp. at *C. melo* flowers under Cerrado conditions. **A.** Number of visits by *Halictus* sp. was not affected by application of tap water ($P > 0.05$, Table 2). **B.** The application of neem-based insecticide (**B**), and deltamethrin alone (**C**) or mixed with fungicides (**D**) significantly reduced the visitation intensity of *Halictus* sp. ($P < 0.05$, Table 2). The percent change in the number of visits on the day after the pesticide applications are indicated by black (after 1st application), red (after 2nd application) and blue (after 3rd application) symbols. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(*A. mellifera*, *Halictus* sp., *Plebeia* sp., and *T. spinipes*) contributing to the pollination of melon fields cultivated under Neotropical savannah-like conditions (i.e., Brazilian Cerrado). We demonstrated variations in visitation patterns among honey bees (*A. mellifera*) and non-*Apis* bees (e.g., *Halictus* sp. and *Plebeia* sp. Bees) in field sites where no pesticide was applied. However, the application of synthetic (e.g. the pyrethroid deltamethrin) and botanical (e.g. neem-based) insecticides similarly affected the flower-visiting rates of these bees. Furthermore, sole applications of synthetic fungicides (a commercial mixture of thiophanate-methyl and chlorothalonil) did not affect these bee's visitation rates.

Variation in visitation patterns among *A. mellifera*, *Halictus* sp., and *Plebeia* bees might be related to the ecology of these pollinators. Regarding the food resources, *A. mellifera* is considered a generalist/opportunist capable of exploiting a variety of food sources over long distances from the colony (Beekman and Ratnieks, 2000), selecting only those resources with the greatest resource availability or with greatest energy gain (Seeley, 1985). In this respect, *Halictus* bees exhibit significant ecological differences, having small bodies (limiting their flight capacity), forming colonies with small numbers of individuals, and the colonies must be located in the vicinity of food sources and nest-building materials

(Michener, 2007; Wray et al., 2014; Zurbuchen et al., 2010).

It is not thoroughly understood how pesticide application influences bee-mediated pollination, and recent investigations have generated conflicting results (Lundin et al., 2013; Melathopoulos et al., 2014; Motzke et al., 2015). Whilst pollination was found to mitigate yield gaps more than pesticide use in tropical smallholder gardens (Motzke et al., 2015), other investigations suggest that the benefits accrued from pollination are linked to plant protection (e.g., pest and disease management) practices (Lundin et al., 2013; Melathopoulos et al., 2014).

Our productivity results suggest that the situation is even more complex, and demonstrate that sustainable recommendations for pollinator management need to be tailored to regional contexts. Here, effective pest control using a pesticide that is considered not very toxic to bees (i.e., a neem-based insecticide) not only significantly reduced the visitation intensities of *A. mellifera* and *Halictus* sp., but also resulted in the lowest productivity. This result reinforces the argument that botanical insecticides should not be exempted from risk assessment analysis (Barbosa et al., 2015a; Decourtye et al., 2013; EFSA, 2012; Lima et al., 2016; Sánchez-Bayo and Tennekes, 2017), and stresses the importance of conducting complementary assays (e.g., field analysis) for botanically based
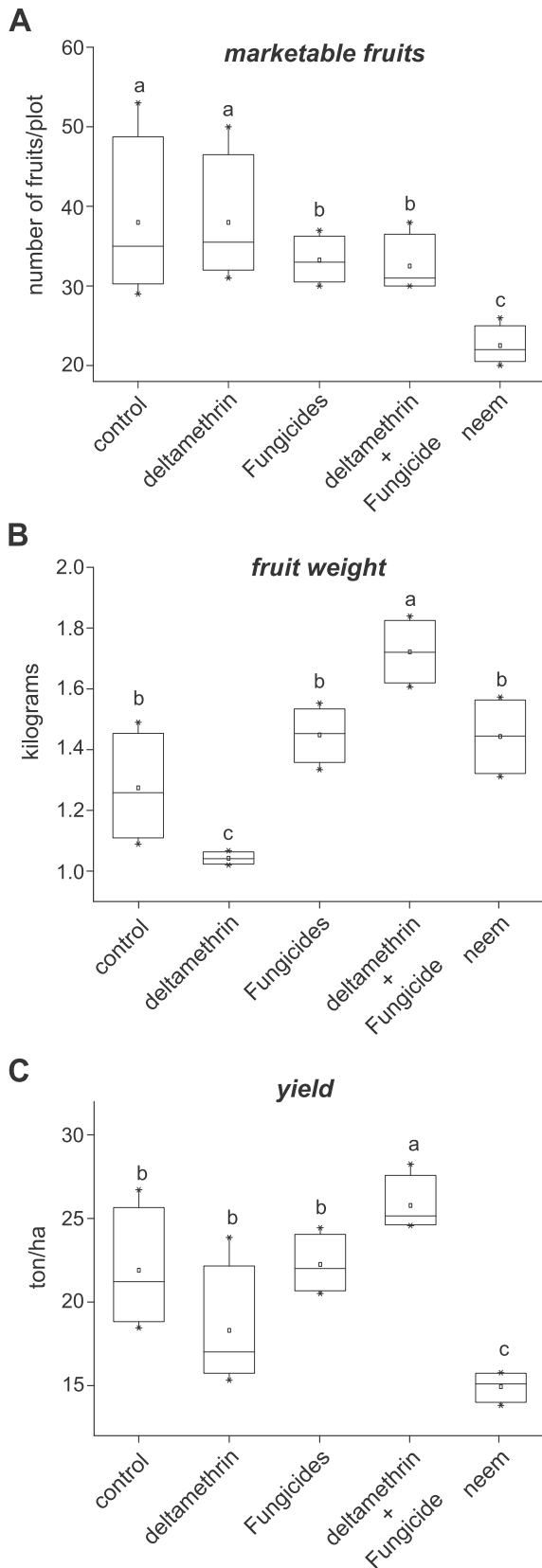
Fig. 5. Evaluation of the number of marketable fruits per plant (**A**), average fruit weight (**B**), and productivity (**C**) of *C. melo* planted under *Cerrado* conditions. Box plots indicate the range of data (lower and upper quartiles and extreme values), median, and average (symbols). Box plots with the same letter indicate that there were no significant differences among the pesticide treatments using Tukey's HSD test (*P* < 0.05).

insecticides. Indeed, neem-based pesticides have been considered as excellent candidates for controlling honey bee pests (González-Gómez et al., 2012; Isman, 2006; Melathopoulos et al., 2000), but investigations characterizing the field effects of these botanical pesticides on non-*Apis* pollinator bees are still very scarce (Bernardes et al., 2018).

It is worth noting that *Plebeia* sp. visitation rate was not affected by the application of any pesticides. Such findings mean that this bee species can have higher exposure to these compounds than bee species that were supposedly repelled. In the long term, such exposures to these pesticides would be detrimental to *Plebeia* bees. For example, if we consider the neem-based compounds, which have been shown to disrupt the neuroendocrine (e.g., altering the functions of endocrine glands such as prothoracic gland and corpora allatum) and reproductive (e.g., preventing oogenesis and vitellogenesis by inhibiting cell division and protein synthesis) systems of other pollinator bees (Mordue (Luntz) and Blackwell, 1993; Mordue (Luntz) and Nisbet, 2000; Barbosa et al., 2015a,b, Bernardes et al., 2018), the long term exposure of *Plebeia* bees to these compounds can lead to reduction on he health of these colonies, which may imply in reduction in their pollination services.

Pollinator bees that can sense and avoid exposures to chemical compounds (i.e., repelled) generally reduce toxic exposure and damages to their colonies, whereas those that are not repelled are affected by the toxicity of the chemicals (Kessler et al., 2015). Despite the present investigation was not designed to evaluate the risk of the chemicals on the bees, but to evaluate the performance of the crop as pollinated by bees under several chemical treatments, it interestingly to note that at the end of flowering, there were fewer visitations by *Apis* bees, which could bee correlated to the fact that generally are less open flowers available at the end of flowering or to the continue exposure to pesticides. The findings described in the present investigation are not sufficient to disatangle such factors as the number of visitations by *Apis* bees was reduced in study fields treated with pesticides (i.e., neem-based compounds and deltamethrin alone or combined with fungicides) but also naturally decline along the season in the study fields with no pesticide application. However, for the visitation by *Halictus* bees, the reductions on visitations mediated by pesticide applications were more evident as a significant increase of visiting bees were recorded for the study field that did not receive pesticide applications.

Considering that the sole application of deltamethrin (which also reduced the visitation rates of *A. mellifera* and *Halictus* sp.) resulted in effective pest control and adequate productivity level, we cannot rule out the possibility that the neem-based insecticide might have strong repellent effects on these bee pollinators or affected some physiological processes of the melon plants (e.g., phytotoxic actions). For instance, neem plant products had shown phytotoxic effects for weeds and crops (Paul and Sharma, 2002; Xuan et al., 2004). The similar susceptibility of *Halictus* bees and *A. mellifera* to deltamethrin- and neem-based insecticide emphasizes the limited value of extrapolating the results of toxicity bioassays that compare *A. mellifera* to wild pollinator species. Other studies have documented sublethal insecticide effects on other bee species, even though these compounds were considered safe in a risk assessment with honey bees (Barbosa et al., 2015a; Bernardes et al., 2018; Decourtye et al., 2013; Del Sarto et al., 2014; Tomé et al., 2015; Tomé et al., 2017). The differential life histories (e.g., sociality, body size, target-site sensitivity, and capacity for detoxification by enhanced metabolism) of these pollinator bees might also help to explain these different pesticide susceptibilities (Brittain and Potts, 2011; Decourtye et al., 2013; Poquet et al., 2015).

Consistent with results of previous studies (Lundin et al., 2013; Melathopoulos et al., 2014), the highest melon productivities in this

investigation were achieved in study fields treated with both pest and plant disease control agents. This demonstrates that appropriate use of such agricultural practices potentiates the pollination services provided by the naturally-occurring pollinator bees. To achieve this, however, the chosen form of pesticide management needs to facilitate the maintenance or increase the diversity and abundance of the pollinator bees that can tolerate these agricultural practices. Further multi-year field investigations will help to capture eventual fluctuations between unintended pesticide effects and pollinator bees' abundance and richness. Collectively, the present investigation reinforces the contribution of *A. mellifera*, *Halictus* sp.*, Plebeia* sp., and *T. spinipes* to the pollination of melon plants cultivated in a novel Neotropical commercial area (i.e., the Brazilian Cerrado), but also identifies specific forms of pesticide management that optimize melon production in one of the world's most important agricultural frontiers, the Brazilian Cerrado.

Author contribution statement

PHT, EEO, and GRS conceived and designed the research. PHT, MSD and MCACS-T conducted the experiments. GRS, RAS, PHT and EEO contributed new reagents and/or analytical tools. EEO, RAS and PHT analyzed the data. EEO and PHT prepared all the figures. EEO, GRS and PHT wrote the manuscript. All authors read, corrected and approved the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were considered in the present investigation.

Informed consent

The six authors of this manuscript accepted that the paper is submitted for publication in the *Environmental Pollution*, and report that this paper has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.04.133.

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