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RESEARCH ARTICLE

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Do agrochemicals used during soybean flowering affect the visits of *Apis mellifera* L.?

Guillermina A. Fagúndez[1], Diego C. Blettler[1], Carlos G. Krumrick[2], Mariana A. Bertos[1] and Cecilia G. Trujillo[1]

[1] *National Council of Scientific and Technical Research, Center of Scientific Research and Technology Transfer to Production (CONICET-CICyTTP). Autonomous University of Entre Ríos, Faculty of Science and Technology (UADER-FCyT). Laboratory of Modern Palynology. Dr. Materi y España, E3105BWA Diamante, Entre Ríos, Argentina.* [2] *Agricultural engineer, Private consultant. Entre Ríos. Argentina.*

Abstract

In the Pampa region of Argentina, most beehives are situated near to soybean [*Glycine max* (L.) Merr.] crop and honey bees (*Apis mellifera* L.) use its floral resources. Soybean is often sprayed with pesticides but very little is known about their repellent action against bees. This study evaluates the visit of honey bees to crop after the application of agrochemicals aiming to check for repellency of them and estimate the possible impact on crop pollination**.** For this, six treatments were used (glyphosate + cypermethrin; glyphosate; cypermethrin; lambda-cyhalothrin; methoxyfenocide; *Bacillus thuringiensis*) and developed on plots of 625 m^2, located in Oro Verde (Argentina), applying two sprays during the crop flowering. The bees were captured using entomological net every 4 days in three different times from the day after the first spraying and up the end of crop flowering. The results showed very little or no repellent action of pesticides on *A. mellifera*, noting that it foraged on soybean flowers regardless of the temporal proximity and the type of product used in sprays. Possible causes are discussed and the need for larger studies is evident in field conditions related to pesticides repellency and mixtures. Also, further evaluation of the effects of the different chemical formulations available on the market and used regionally where the subspecies *A. mellifera* can be found. Simultaneously some management practices that could help minimize the risk of contamination are mentioned; the use of defensive crop products of biological origin is encouraged as well as further research in this topic.

Additional key words: honeybee; *Glycine max*; pesticides; repellent effect; foraging

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Correspondence should be addressed to Guillermina A. Fagúndez: guillermina_fagundez@yahoo.com.ar

Introduction

In the Pampa region over the last two decades there has been an increasing and profound transformation in the agro-productive matrix. This transformation is due, mainly, to an increment of the area allocated to soybean [*Glycine max* (L.) Merr.] farming and the technology package associated with this crop. According to Aizen *et al*. (2009), considering that the current dominance of soybean is determined by the total area cultivated which is the largest in history, it could be concluded that no other crop has been as relevant in Argentina agriculture as soybean is. Highlights of this transformation are a cluster of technological innovations such as: direct sowing, genetically modified seeds, double cropping, large-scale usage of agrochemicals, among others (Satorre, 2005). Nowadays, the growth rate of this activity, far from slowing down, is fast increasing and strengthened.

It is not yet known to what extent soybean expansion is an indicator of environmental degradation where not only diversity of crops but also of species and ecological processes associate with heterogeneous landscape are adversely affected (Altieri, 1999; Thrupp, 2000; Weyland *et al*., 2008). This process is regarded with particular concern by producers, researchers and institutions connected to beekeeping as most of the time apiaries and honey bees (*Apis mellifera* L.*)* foraging areas are near one or several soybean fields. Honey bees make use of this floral resources for nectar and/or pollen especially when the supply of alternative flowers is low or discontinuous (Basilio *et al*., 2002;

Fagúndez & Caccavari, 2003; 2006; Malacalza *et al.,* 2005; Caccavari & Fagúndez, 2010; Fagúndez, 2011; Fagúndez *et al.*, 2011).

Frequent crop spraying with pesticides would be connected to the loss of hives and/or their depopulation. Although the Colony Collapse Disorder is a multifactorial phenomenon, one of the factors involved could be pesticide contamination (Gill *et al.*, 2012; Henry *et al*., 2012). Also, low dosages of pesticide and/or reduced number of applications can also affect the behaviour of foraging honey bees (sub lethal effect) (Bortolotti *et al.*, 2003; Freitas & Pinheiro, 2010).

Considering this possibility, it would be desirable that pesticides had some bee repellent action to keep honey bees away from recently sprayed crops. Although there are some research works where repellency has been tested, most of them have been carried out under semi-field experimental conditions and, therefore, repellency has not been assessed in the field (Naumann *et al*., 1994; Mayer & Lunden, 1999).

As part of an evaluation project on the effect of entomophily pollination (especially associated with *A. mellifera*) on soybean performance and the development and production of hives during crop flowering, the present essay evaluates the visit of honey bees to crops after the application of agrochemicals aiming to check for repellency of them and estimate the possible impact on the pollination of the crop.

Material and methods

Study site

The study was carried out on an agricultural plot (23 ha) of soybean located in Oro Verde (Entre Rios, Argentina). Sowing time, cultural work and harvesting were done according to usual management practices for the region (recommended by state agencies). The variety of seed used was Nidera 4990 RG (widely used in the region), which has the following characteristics: indeterminate growth habit, highly branched, high yield potential and white corolla, glyphosate tolerant. Sowing of 11 rows 52 cm apart was done on 2 November 2010 with a commercial planter. As fertilizer, monoammonium phosphate was used at 50 kg/ha. An apiary consisting of 36 standard hives was placed near the study site. The apiary is Category I (Figini, 2006), with 6-7 frames for brood, 8-10 frames with 20000 honey bees, new materials of the hive (supers, frames, beewax, etc.), new laying queens and in excellent health.

Treatments

Six treatments and one control were established; products used and their dosages are shown on Table 1. The choice of products used for each treatment and their combination was the result of a survey carried out among agricultural producers from the area. Finally, the most commonly used products for the protection of soybeans were used, covering a broad spectrum of active ingredients and different degrees of acute toxicity (label) (Table 2). The use of glyphosate during flowering crop while it is not common practice, often is used in cases of poorly implemented or sparse crops. Also this agrochemical may be in contact with the crop in flowering as a result of drift lots nearby, because the region has very broad sowing period of this crops, allowing coexist of lots with different growth stages, so it is decided to incorporate it into the experience. The dosage used for each product is the one suggested on the label (approved by CASAFE: Argentinian Chamber of Agricultural Health and Fertilizers) adapting the quantity to plot size.

Products were sprayed using a 3-point tractor-mounted sprayer equipped with a 7 m wide boom with flat tips 80 02. This allowed a spraying of 100 L/ha at a work pressure of 2.0 kg/cm^2.

Each treatment was applied on a 25 m × 25 m plot over a total area of 625 m^2 alternating treated areas with crop-free spaces so as not to limit each treatment with the next one. The test had a plot of the same size that the treatments. The first spraying was realized on 29 December 2010 (approximately 5% of flowering) and the second was realized on 11 June 2011.

The crop was thoroughly monitored during the flowering period, which started on 16 December 2010, and lasted until 23 January 2011. During this period the

Table 1. Products and dosage involved in each treatment.

Treatments	Products	Dosage/ha	Dosage used
T1	Glyphosate + Cypermethrin	4 L + 250 cm^3	0.25 L +15 cm^3
T2	Glyphosate	4 L	0.25 L
T3	Cypermethrin	250 cm^3	15 cm^3
T4	Lambda-cyhalothrin	200 cm^3	12 cm^3
T5	Methoxyfenozide	200 cm^3	12 cm^3
T6	*Bacillus thuringiensis*	1000 cm^3	65 cm^3

Table 2. Chemical and toxicological classification of products used (CASAFE, 2007).

Products	Chemical classification	Action	Usage	Toxicological classification[1]
Glyphosate [glyphosate-mono(isopropylammonium)]	Phosphonomethyl-glycine	Systemic	Full herbicide	Class IV
Cypermethrin [(RS)-cyano(3-phenoxyphenyl)methyl(1RS)-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate]	Pyrethroid	Contact and ingestion	Wide spectrum non selective insecticide, long residual action	Class II
Lambda-cyhalothrin [1-α (S*),3α (Z)]-(±)-cyano(3-phenoxyphenyl)methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate (9CI)	Pyrethroid	Contact and ingestion	Wide spectrum non selective insecticide, adults and larvae, long residual action	Class II
Methoxyfenozide Benzoic acid, 3-methoxy-2-methyl-,2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl) hydrazide	Diacilhydrazine	Contact and ingestion	Specific insectide that acts on lepidopterans larvae	Class IV
Bacillus thuringiensis	Biological	Ingestion	Specific insectide that acts on lepidopterans larvae	Class IV

[1] Class II: moderately toxic. Class IV: probably without risks.

population of phytophagous insects was always under the level of economic damage threshold (Aragón *et al.*, 1998) being unnecessary to apply defensives on the crop.

The use of soybean as a source of nectar or pollen was confirmed by direct observation field of activity of bees (introduction of the head and clusters of pollen in the third pair of legs or corbiculae). The botanical origin of pollen in the corbiculae was confirmed under an optical microscope in the Laboratory of Modern Palynology (CONICET-CICyTTP/UADER-FCyT, Entre Ríos, Argentina).

Collecting method

The collection of bees was carried out from the day after the first spraying of agrochemicals and repeated regularly every 4 days until the second spraying (Table 3). The day after the second spraying, trapping was restarted and continued every four days up to the end of crop flowering (Table 3). Under this model, a total of four repetitions were carried out after the first spraying and three after the second one. Collecting of bees on each treated area was done using a 38 cm-diameter entomological net resulting in 20 sweeps (two sweeps = 1 m^2) on an imaginary line on the crop canopy. Seven repetitions were performed (throughout the flowering period) with three collecting times for each repetition (9:30, 12:30 and 16:00 hours). This was repeated for each treated area at the three established collecting times for each repetition. Repetition of sampling area was avoided as it disturbs the canopy. To evaluate foraging schedules, 70 sweeps were done on non treated crop areas, on the same days and hours evaluating treatments. On 16 January the 16:00 p.m. measurement was kipped due to adverse weather conditions (rainfall).

Table 3. Number of individuals trapped of *A. mellifera* by treatment and date of sampling.

	Control	T1	T2	T3	T4	T5	T6	Total
1st spraying								
30 Dec 2010	1	1	2	1	6	3	3	17
03 Jan 2011	1	1	3	5	3	0	2	15
07 Jan 2011	4	4	0	0	2	2	1	13
11 Jan 2011	1	1	3	1	1	1	0	8
2nd spraying								
12 Jan 2011	0	0	0	0	1	1	0	2
16 Jan 2011	0	0	1	1	2	0	1	5
20 Jan 2011	0	0	0	0	0	0	1	1
Total	7	7	9	8	15	7	8	

Statistical analysis

The number of *A. mellifera* individuals collected from different treated areas did not present a normal distribution, for this reason and only for this analysis non parametrical statistics were used (Kruskal & Wallis, 1952; Sokal & Rohlf, 1995). For the rest of the analysis all ANOVA estimates were tested and successfully overcome so parametrical statistics were used. The homogeneity of variance was tested by means of the Levene test (Sokal & Rohlf, 1995). To determine if there were significant differences among the arithmetic mean of the number of individuals collected belonging to the two agrochemical applications a one way ANOVA test was carried out, showing marked differences ($p<0.05$), the same method was used to determine the presence of honey bees at the different times of sampling. Post-hoc Tukey tests were carried out to find out among which of the different ANOVA treatments there were significant differences. Box plot graphs were made in order to show the mean density values of the number of individuals collected among the different sprayings. Descriptive statistics were also taken into consideration in the same graph (error and standard deviation) and/or expressed as a table. In all cases Minitab 16 software was used.

Results

Behaviour of *A. mellifera* towards different treatments

The results of the collection of *A. mellifera* are shown on Table 3 by treatment and date of sampling. The statistical analysis carried out on these data showed no significant statistical differences among the treatments (p=0.798). However, when grouping data from each application significant statistical differences (p=0.008) between the two applications of phytosanitary products (Fig. 1) can be observed.

Foraging behaviour of *A. mellifera* throughout the day

From the results obtained from the collecting carried out during the whole soybean flowering period at three different times of the day (Table 4), it can be observed that there were significant statistical differences (p=0.000) regarding the time of foraging of *A. mellifera*. The minimum, medium and maximum values of individuals of honey bee trapped in each time, in all the samples are shown as well as the average and the first and third quartiles (Q1 and Q3). It can also be observed that the presence of honey bees was higher around noon (Fig. 2).

Discussion

From the results, it can be inferred a foraging behaviour of honey bees independent from the different applications of pesticide tested. That is to say, honey bees seem to visit soybean crops regardless the proximity in time after sprayings and the kind of pesticide used.

Of the 6 treatments tested, 3 of them are of toxicity class IV (probably without risk): glyphosate, methoxyfenozide and *Bacillus thuringiensis* (Table 2). Thus, in the first instance; acute toxicity effect (death) in honey bees is not expected. Moreover, due to its low toxicity none of these products presents a direct repellent effect. However, glyphosate produces some sublethal effects; Herbert *et al.* (2014) mentions that traces of this herbicide affect associative learning of honey bees. The fact that the ability of honey bees to learn and become familiar to scents based on signals might be affected can impact on the colony by reducing the ability of foragers to detect the floral scent and thus nectar sources (Thompson, 2003) and become an indirect repellent. Nonetheless, this is not shown in our experience. Thompson (2003) mentions that the performance of the individual olfactory learning under lab conditions has not been fully correlated with the colonies of treated honey bees. On the other hand, Carrasco & Mendoza (2013) mentioned that the commercial formula of a pesticide active ingredient might influence beneficial insects such as honey bees, as the toxicity of a mixture of substances is not the sum of the toxicity of its parts but a new and unknown result. These authors outline an example for five different

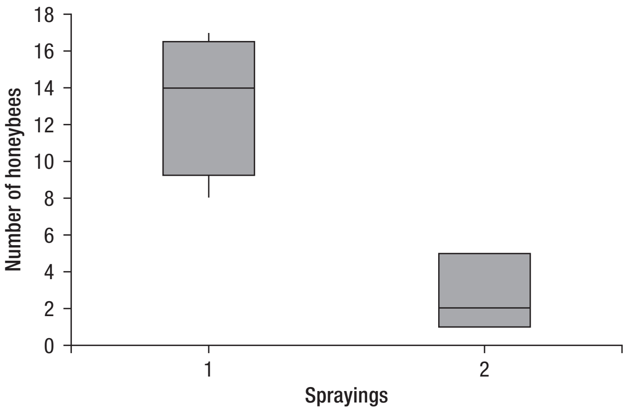

Figure 1. Total honey bee trapping after each of the two applications of pesticides to the crop.

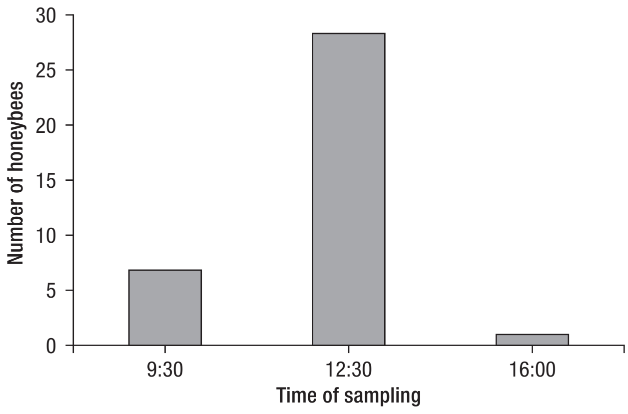

Figure 2. Total number of individuals of *A. mellifera* trapped on soybean crop at different times of sampling.

Table 4. Descriptive statistics of trappings of *A. mellifera* at different times. (N: number of samples; Q1 and Q3: first and third quartiles, respectively)

Collecting time	N	Average	Minimum	Q1	Medium	Q3	Maximum
09:30	7	1.0	0.00	0.0	1.0	1.0	4.0
12:30	7	4.1	1.00	3.0	4.0	6.0	6.0
16:00	6	0.17	0.00	0.0	0.0	0.25	1.0

glyphosate formulae and state that although they do not show acute toxic responses, there are differences among the commercial formulae. They also emphasized the fact that acute toxicity does not relate the whole situation of all possible toxic effects or the outcome of their use in the field. A problem that could only be estimated after long term and on field condition experiments are carried out.

The three remaining treatments are two pyrethroid pesticides of toxicity class II (moderately toxic) and a mixture resulting from the combination of one of these pesticides and an herbicide. Although the repellency of pyrethroids is known to limit the exposition of honey bees to this group of toxic pesticides (Thompson, 2003; Ojeda, 2012) this was not evident in the present experience for none of the pesticides used or their mixture. These results agree with Naumann *et al.* (1994) and Mayer & Lunden (1999) who stated that in most cases, the effective repellency shown have been tested under semi-field conditions. Environmental stimuli such as flower attractiveness towards pollinators that might surpass the adverse effects of pesticides have not been taken into account.

Results similar to the ones in the present work were obtained in other researches on different crops and under field conditions. Shires *et al.* (1984) tested the repellency against pollinators on canola crop sprayed with cypermethrin during the high foraging period and a slight decline in the foraging honey bees was only affected during the first 3-4 hours after spraying but was fully recovered by the following day. There could have been similar effects in the present research but they were not detected as sampling only started the day after spraying.

Moreover, there could have also been a decrease in the number of foragers on the crop as result of sublethal toxicity (repellency) (Thompson, 2003). Taylor *et al.* (1987) and Mamood & Waller (1990) attribute the reduction of the foraging capacity of *A. mellifera* exposed to pyrethroids of worldwide usage (for example, cypermethrin) to sublethal toxic effects more than to direct repellency effects. Nevertheless, this reduction was not proven in the present study. Carrasco *et al.* (2012) evaluated the toxicity (DL_{50}) of three commercial insecticides and found that the sensibility values differed considerably from the reference values of the European Union (EU). In the case of cypermethrin, a product also used in this research, the value was 6.2 times lower in the SW region of Uruguay than the reference values mentioned in the Pesticide Properties Database (PPDB, 2010) suggesting a higher tolerance of honey bees from this region than the indicated in the reference values of the EU. According to these authors, these differences could be explained by means of two factors, namely polihybridism of honey bees in the SW region of Uruguay and the specific chemical mixture. Honey bees in the SW region of Uruguay are predominantly a polyhybrid subspecies different to the one used to set the international reference. Polyhybridism gives rise to new genetic conditions that may lead to different tolerance range in the SW region of Uruguay honey bee, as suggested by Suchail *et al.* (2000). The polyhybrid origin of the Uruguayan honeybee colonies corroborated by morphometric and genetic analyses carried out by Carrasco *et al.* (2012) has also been mentioned in earlier studies curried out in that country (Burgett *et al.*, 1995; Diniz *et al.*, 2003) as well as in neighbouring countries such as Argentina and Brazil (Sheppard *et al.*, 1991a,b; Diniz *et al.*, 2003). Moreover, Carrasco *et al.* (2012) propose to consider Uruguay, Argentina and Brazil soybean areas as one region, as their honey bees are of similar genetic origin and the insecticides used and their suggested dosages are practically the same. In some cases, the only difference is their brand names, but the insecticides belong to the same international companies.

When analyzing the daily total of honey bees collected (Table 3), the results seem to indicate that there is a greater influence of the ontogenetic cycle of the crop (flowering rate reduced from approximately January 10 coinciding with a reduction in the number of captures) than the supposedly repellent effect of agrochemicals.

In the present experience, it was observed a foraging behaviour of bees independent of pesticides applications thus it could be expected little or no effect on pollination. Nevertheless, an assessment of the effect on honey bees (lethal or sub-lethal) cannot be made as this would exceed the objectives of the present study. Nor can it be said (based on the results of this work) that honey bees foraging on sprayed plots do actually return to the hives with their loads. The number of raids by repetition should be expanded to increase the capture of bees, in order to strengthen the conclusions, because soybean is a crop with little attraction.

These observations become significant when considering that in the agro-productive national context the presence of apiaries near soybean plots is preponderant and increasing rapidly as greater areas of land are allocated to soybean crops. Bearing in mind local experiences (Fagúndez & Caccavari, 2003; Fagúndez, 2011; Fagúndez *et al.*, 2011) and international ones (Borrel & Vandame, 2012), it is assumed that if honey bees have access to soybean crops, they will use them as pollen and/or nectar resource and it is also clear that these crops are repeatedly sprayed with pesticides. According to the Environment and Sustainable Development National Office, Argentina (SAyDS, 2013; www.ambiente.gov.ar) during the 2011 campaign 335.3 million of kilograms or litres of pesticides were used in Argentina alone and spraying was mainly done during the flowering period. One of the measures that can be taken to alleviate the problem is to encourage further research to assess and strengthen the inclusion of repellent agents into the current formulae (including field test to confirm repellency) and in doing so, to ensure the absence of honey bees on recently sprayed crops. On the other hand, it would also be advisable to encourage the usage of biological insecticide agents for the treatment of soybean some of which are available on the market at reasonable prices. Using these strategies it could be possible to reduce to a minimum the chances of contaminating this valuable bee product. Perhaps, with the recent introduction to the Argentine market of a soybean variety resistant to the main lepidopterans that attack the crop, the problem will be reduced.

Fortunately, soybean physiology conditions the flowers´ opening and closing cycle in a period of time that never exceeds one day (Delaplane & Mayer, 2000). Bearing in mind the flowering cycle of soybean and the foraging time chosen by *A. mellifera,* it is suggested the spraying of the phytotherapy products in the evenings avoiding a direct impact on foraging honey bees (which no longer will be above the crop) and finding the flowers (that the next day will attract bees and/or other insects) closed reducing the possibilities of nectar and pollen contamination.

These considerations show the need for further studies on the presence of pesticide residues in nectar and honey in order to clarify whether they are found in the matrices and if they persist there. Although studies carried out in Brazil (Milfont, 2012) showed no pollutants in honeys produced from soy, it would not be accurate to extrapolate these results to the Pampa region as the setting where the research was carried out, especially the weather that conditions the degradability of products, is different.

On the other hand, it is also evident the need for larger studies under field condition related to pesticides repellency as well as the evaluation of the effects of the different chemical formulations available on the market and used in the region for the protection of soybeans where the subspecies *A. mellifera* can be found.

As society discusses the development model to follow, in the agricultural field the option of a soybean specialization model of low technical complexity and maybe short termed and high environmental costs is encountered with one more diversified, of higher technological complexity but more sustainable in the long term and probably with acceptable economic returns (Aizen *et al.*, 2009). This contributes to the debate and, above all, alerts to some "not desirable externalities" of the prevailing agro productive model suggesting possible actions to mitigate the so called "negative externalities" of the system.

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