**Original article**

# Contribution of honeybees to soybean yield

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**Abstract** – Despite the economic importance of soybean [*Glycine max* (L.) Merr.], knowledge on the contribution of entomological pollination on seed yield is scarce. This study estimates the production of soybean resulting from pollination by honeybees (*Apis mellifera* L.) in two consecutive growing seasons in Paraná (Argentina). Experiments had two treatments: excluded flower-visiting insects (EV) and non-excluded flower-visiting insects (NEV). The abundance of honeybees was similar in both years, although soybean production differed significantly ($P < 0.05$) between years. The NEV treatment out-yielded ($P < 0.001$) the EV treatment by 18% (5224 vs. 4415 kg ha$^{-1}$) in year 1, which was associated with an increase in the seeds per unit area but not with individual seed weight. In contrast, seed yield (on average 3830 kg ha$^{-1}$) and seeds per unit area did not differ between treatments in year 2. Individual seed weight was 3–5% ($P < 0.05$) higher in EV than in NEV in both years. The mechanisms involved in the seed yield increase could be related with pollen sterility in relegated flowers in secondary racemes or in distal locations of primary racemes under favorable conditions, as recorded in year 1. Thus, the action of honeybees carrying pollen from fertile flowers to relegated flowers may have increased the pod and seed set in treatment NEV in year 1.

pollination / foraging hours / seed yield / soybean / honeybees

## 1. INTRODUCTION

Although soybean is essentially an autogamous species (McGregor 1976; Carlson and Lersten 1987; Ahrent and Caviness 1994), it is also cross-pollinated mainly by flower-visitor insects (Erickson et al. 1978). Insect pollination, under certain conditions, has contributed to increasing seed yield (Juliano 1976; Moreti et al. 1993; Chiari et al. 2005, Chiari et al. 2008; Milfont et al. 2013; Santos et al. 2013). The increases in soybean seed yield were associated with genotypes that are attractive to bees and with respect to distance from colonies (Erickson et al. 1978). It is, however, still unclear why insect pollination sometimes results in higher seed yields. The movements of the insect for promoting the auto pollination, i.e., self-pollination mediated by insects, or carrying pollen to improve the cross-pollination have been proposed as possible mechanisms involved in the seed yield improvements (Free 1993). Moreover, a study has shown that the action of insects visiting flowers to maximize the auto pollination seems to increase seed or fruit production, even in obligatory autogamous species (Aizen 2008). Likewise, the action of insects carrying pollen from fertile to androsterile plants, which can be in a variable proportion within a crop, has been proposed as an additional mechanism involved in the increase in the number of reproductive structures in autogamous plants (Ortiz-Pérez et al. 2006).

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Honeybees are the most abundant and frequent flower-visiting insects of soybean (Delaplane and Mayer 2000), although other hymenoptera (Milfont et al. 2013; Fujita et al. 1997; Monasterolo et al. 2015; Santos et al. 2013), as well as diptera and coleoptera are also regular flower-visiting insects (Santos et al. 2013; Fagúndez et al. 2016). Accordingly, several palynological studies have demonstrated that soybean is an important nectariferous resource for production of honey by honeybees (Fagúndez and Caccavari 2003; Fagúndez et al. 2016; Gallez et al. 2005; Fagúndez 2016), even resulting in a monofloral honey of soybean in some cases (Fagúndez 2016). Moreover, soybean has been reported as the most important source of pollen for honeybee colonies during peak flowering (Fagúndez and Caccavari 2003). These results suggest that soybean is an important resource for the nutrition of honeybees and for honey production, which is especially true in the agriculture of Argentina, where ca. 70% of the cultivated area is planted with soybean (SIIA 2015). Some soybean genotypes, however, are not attractive enough to honeybees, possibly due to low sugar concentration in their nectar, among other factors (Alves et al. 2010).

Despite the economic importance of soybean, knowledge is scarce on the effect of entomological pollination on seed yield. This could be critical, since the density and diversity of wild pollinators has declined (Garibaldi et al. 2013), with the reduction of habitats providing nesting and foraging resources outside the crop flowering period (Aizen et al. 2009; Garibaldi et al. 2011). In this context, managed honeybees may be a valuable alternative to the replacement of wild pollinators and to improve soybean yields.

We hypothesized that (i) honeybee pollination improves soybean seed production, and (ii) increased honeybee flower visitation rate improves soybean seed production. The objective of this study was to estimate the impact of pollination by honeybees on soybean seed yield.

## 2. MATERIALS AND METHODS

### 2.1. Experiment and husbandry conditions

The study was carried out during two consecutive growing seasons (2010–2011 and 2011–2012, hereafter year 1 and year 2, respectively) on soybean fields in Paraná, Argentina (31° 50 ′S and 60° 31 ′W, 91 m above sea level). The fields, which are 1000 m apart, had 23 ha in year 1 and 20 ha in year 2 and both had oat (*Avena sativa* L.) as the previous crop. Soybean crops were sown in rows, 0.53 m apart, on 5 November in year 1 and on 2 November in year 2. Seeding rate was 27 seeds per meter and the genotype used was Nidera A 4990 RG (maturity group IV, glyphosate resistant, indeterminate growing habit). This genotype is one of the most widely used in the region.

The population of phytophagous insects was monitored weekly, and it was always below the economic injury threshold (Baigorri 2004) during the flowering and seed set period, i.e., from R1 (beginning bloom) to R6 (full seed) (Fehr and Caviness 1977). Insects were collected and counted each week from 15 samples of 0.5 $m^2$ of crop canopy.

The experiment had a completely randomized design and included two treatments with ten replicates each. One treatment excluded visitor insects, hereafter EV (excluded flower-visiting insects), by using a net (brand Claril, mesh 4 × 1 mm with shading < 15%) assembled on a metallic structure (see Fig. 1a). In the other treatment, although flower-visiting insects had free access to the crop (non-excluded flower-visiting insects (NEV)), plots were also covered with the net but only at the top of the structure, in order to account for the shading effect, but not at the side of plots in order to allow the freedom of action of the honeybees (see Fig. 1b). Each plot, defined by the metallic structure, was 2 m long and three rows wide (1.58 m). Plots were established on 21 December in year 1 and 14 December in year 2.

To ensure an adequate population of honeybees (*Apis mellifera ligustica*), 36 colonies were placed in the middle line of each field at R2 (full bloom) stage. Plots were established in regular alternance along the sides of the line of colonies (see Fig. 1c), in order to increase the action of honeybees and to reduce the influence of competitive sources of flowers, i.e., a dilution effect.

Colonies were standardized in their size and management. Young queens of *Apis mellifera ligustica* (Italian bees) were used and sanitary treatments were performed to prevent varroasis
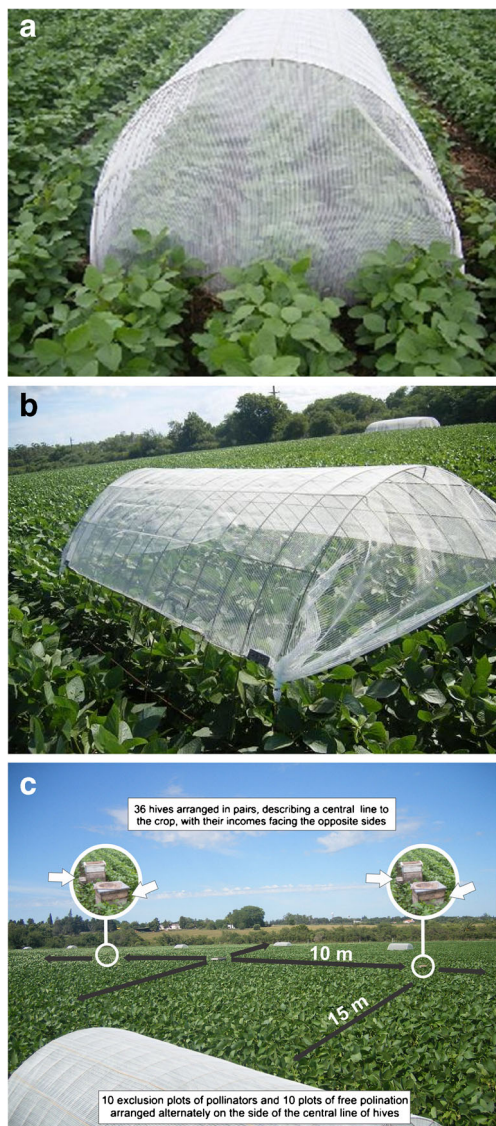
**Fig. 1.** Metallic structures supporting the net in: **a** excluded flower-visiting insects (EV) and **b** non-excluded flower-visiting insects (NEV) plots **c** shows the location of several plots location at Paraná, Argentina. NEV plots allow insect visits while maintaining identical shading conditions to EV plots.

(*Varroa destructor*), American (*Paenibacillus larvae*) and European foulbrood disease (*Melissococcus pluton*) in the colonies. Each colony had six frames in the deep super and all hives were new each year. The estimated population was 20,000 bees per hive.

## 2.2. Measurements

Crop phenology, from planting to physiological maturity, was recorded weekly within the plots using the scale of Fehr and Caviness (1977) on ten consecutive plants. Seed yield was determined after physiological maturity (R8) by cutting all plants at the ground level on an area of 1.6 m$^2$, and then manually threshing. Individual seed weight was determined from a sample of the threshed seeds by counting 1000 seeds. Soybean yield was expressed as seed yield per ha at 0.135 kg$^{-1}$ moisture.

During the flowering period, i.e., from R2 (full bloom) to R4 (full pod), the activity of honeybees was monitored every 4 days at 09h30, 12h30, and 16h00 around each plot by sweeping 50 times an entomological net of 0.11 m$^2$. The time for the monitoring was chosen in following several authors who agree that the period for bee foraging in soybean is 6 h before or after noon (Erickson et al. 1978; Mahfouz et al. 2012; Blettler et al. 2016). The captured honeybees were bagged until counting. Only the number of honeybees (*Apis mellifera* L.) was recorded and other flower-visiting insects were neither identified nor quantified. Meteorological data were obtained from the agrometeorological station of INTA EEA Paraná located at ca. 1.000 m.

## 2.3. Estimation of seed yield

As is usual in studies of soybean seed yield determination (Egli 1998; Sadras and Calviño 2001), we studied the two main seed yield components, i.e., the seed number per unit area (seed m$^{-2}$) and the individual seed weight (mg seed$^{-1}$) (Egli 2004; Egli and Zhen-wen 1991). This approach allows to infer the role of honeybees in the set of seed number per unit area, which is more related with the number of flowers converted in pods than with the seed yield itself. Moreover, the seed yield in soybean may be strongly affected by the individual seed weight, irrespective of the seed number (Borrás et al. 2004).

Seed number per unit area, i.e., m$^2$, was calculated as the quotient between seed yield and individual seed weight. The abundance of honeybees was expressed as the average number of insects captured in a sweep.

## 2.4. Estimation of flower visitation rate

We estimated the flower visitation rate following Goodwin et al. (2011) for white sweet clover (*Trifolium repens* L.). Firstly, we estimated the number of flowers per unit area based on the seed number per unit area. Based on our field observations, we assumed that each pod, i.e., a fertilized flower converted into a fruit, had 2.5 seeds. Thus, the number of fertile flowers per unit area in each year was estimated as the quotient between the seed number per unit area and 2.5. Then, we assumed a flower abortion rate of 60% (van Schaik and Probst 1958; Hansen and Shibles 1978; Jiang and Egli 1993) to estimate the number of flowers per unit area, i.e., the quotient between the number of fertile flowers per unit area and 0.6. Taking into account the duration of the flowering period, i.e., 31 days in year 1 and 28 days in year 2, we estimated the daily flowering average as the quotient of the total number of flowers per unit area and the duration of the flowering period. This estimation yielded 110 flowers per $m^{-2}$ $d^{-1}$ in year 1 and 102 flowers per $m^{-2}$ $d^{-1}$, which are consistent with previous reports (McGregor 1976). Considering the size of the used entomological sweep net, we estimated 25 $m^3$ of total volume of interception in the crop canopy, which was considered equivalent to 50 $m^2$ because flowers were displayed in only ca. 0.5 m plant height (from 0.15 to 0.65 m height). The number of honeybees per unit area was calculated from the ratio between the total number of captured honeybees and the area swept with the net.

Honeybee flower visitation rate for soybean has been reported at 12 flowers per min (Chiari et al. 2005); therefore, the estimated flower visitation rate was 130 visits $m^{-2}$ $d^{-1}$. This rate was higher than the estimated flowering rate for the considered period, which suggests that the number of honeybees in our experiment was enough to ensure the visitation of the available flowers.

## 2.5. Environmental conditions

A water balance was performed for each year, at a daily step, from crop evapotranspiration (ET), rainfall, and plant available water (PAW). The ET was calculated as the product between reference ET and phenology-dependent crop coefficients (Allen et al. 1998). Actual evapotranspiration was assumed to be equal to ET when PAW > 0.5, and to decline linearly with PAW between 0 and 0.5 (Sadras and Milroy 1996). Further details of water budget calculations can be found in Sadras and Calviño (2001). The initial PAW was assumed to be 0.5 as a fraction for each year.

Mean temperature during the critical period for seed set, i.e., from R3 (beginning pod) to R6 (full seed), was similar in both years. However, it was higher during seed filling, i.e., the period between R6 and physiological maturity, in year 1 (Fig. 2a). Likewise, global solar radiation was similar between years during the critical period but it was higher during seed filling in year 1 (Fig. 2b).

Despite the higher total rainfall during the growing seasons in year 2 (563 mm) than in year 1 (441 mm), the fraction of plant available water was below the threshold of 30% during the critical period for seed set, i.e., from R3 to R6, during year 2 (Fig. 2c), which was associated to the higher rainfall during this period (193 mm for year 1 vs. 100 mm for year 2).

## 2.6. Statistical analyses

We used a general linear mixed-model (GLMM-ANOVA) to evaluate the effects of treatments and years on soybean seed yield, seed number per unit area, and individual seed weight. The linear model included year and replicates as random effects, whereas treatment was included as a fixed effect. We compared flower visitation rate and activity of honeybees between years using a one-tailed Student's $t$ test. Statistical analysis of this study was performed by INFOSTAT (Di Rienzo et al. 2011). When the ANOVA indicated significant differences ($P < 0.05$), we compared the treatment means using the Tukey test ($\alpha = 0.05$).

## 3. RESULTS

### 3.1. Activity of honeybees and flower visitation rate

The activity of honeybees at different hours of the day did not differed between years ($P > 0.05$). The most intense activity was recorded at 12h30
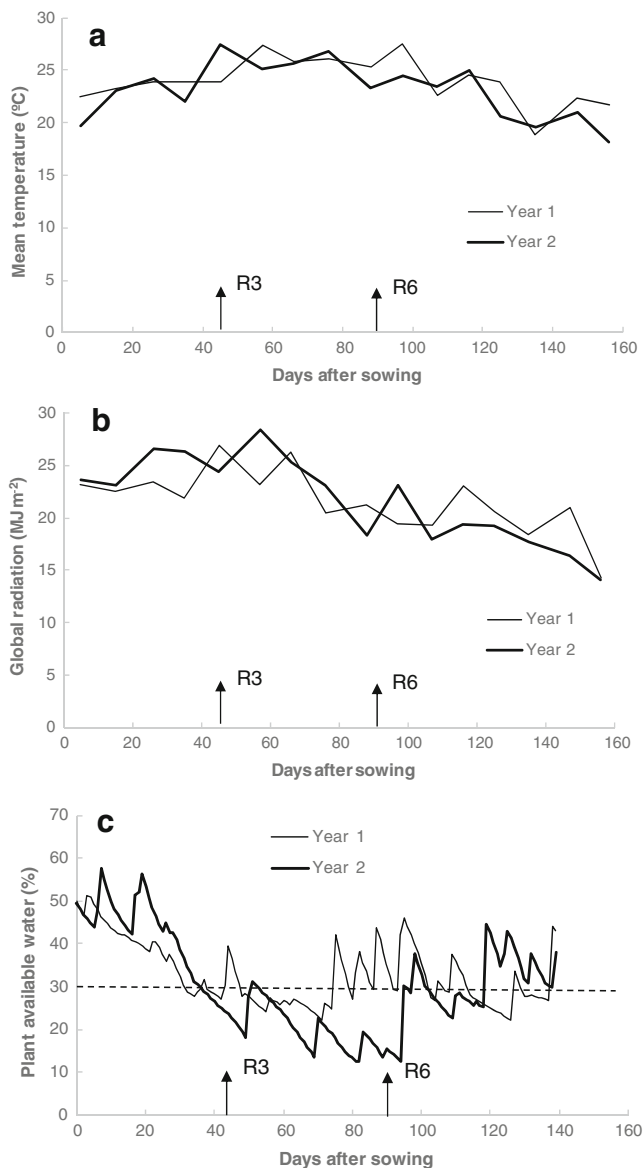
**Fig. 2.** Mean temperature (**a**), global radiation (**b**) and plant available water (**c**) during growing seasons 2010/2011 (year 1) and 2011/2012 (year 2) at Paraná, Argentina. *Arrows* indicate R3 (onset of pod formation) and R5 (onset of seed filling period), two key phenological stages of the soybean crop. *Horizontal broken line* in (**c**) indicates the threshold of 30% plant available water.

(Fig. 3). The amount of captured individuals at 12h30 was, on average, 2.4-fold higher than at 09h30 and 13.7-fold higher than at 16h00 (Fig. 3). We estimated 0.03 honeybees $m^{-2}$ and an average daily visitation rate of 130 visits $m^{-2} d^{-1}$; both variables did not differ significantly between years ($P > 0.05$).

### 3.2. Soybean seed yield

There were significant effects of treatment ($P = 0.003$), year ($P < 0.0001$), and the interaction treatment ×year ($P < 0.0001$) on seed yield (Table I). In year 1, treatment non-excluded flower-visiting insects (NEV) out-yielded treatment

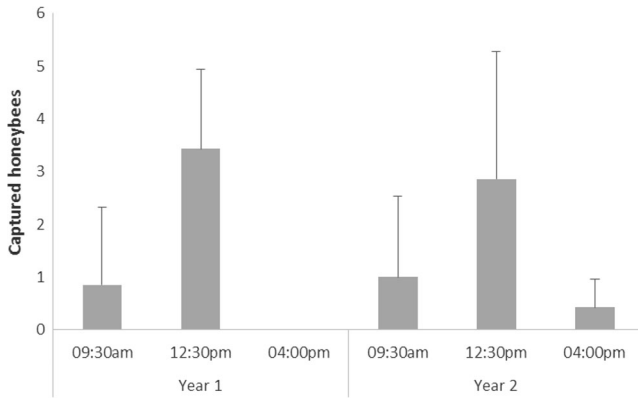**Fig. 3.** Average number of honeybees captured per sweep, over soybean flowering period of each year. *Vertical segments* over *bars* indicate the standard deviation of the mean for the flowering period.

(EV) by 18% (Table II) in contrast with year 2, where the seed yield was similar between both treatments. On average, seed yield was 26% higher in year 1 than in year 2. Similarly, seed number per unit area was 12% higher in NEV than in EV in year 1 (Table II), without significant differences in year 2. Seed weight was significantly increased by EV treatment in both years (Table I). Irrespective of year, seed weight was 3.5% higher ($P < 0.0001$) when flower-visiting insects were excluded in comparison with NEV treatment. Seed weight was 14% higher in year 1 than in year 2 (Table II).

## 4. DISCUSSION

In agreement with our first hypothesis, seed yield increase in NEV treatment as compared with EV treatment in year 1, which is coincident with what was reported by several previous studies (Table III). However, the results of the year 2 did not show the same effect of NEV on seed yield (Table I), i.e., seed yield was unaffected by treatments.

A wide range of the impact of honeybees on soybean seed yield has been reported (Table III) in the related body of literature, for instance, Erickson (1975, 1984) reported an increase from 5 to 20% depending on the year, whereas Chiari et al. (2005) reported a seed yield increase as high as 57%.

Although the fixed effects of our treatments were identical in both years, the meteorological

conditions during the critical period for seed set differed between years (Fig. 2). Therefore, seed yield was higher in year 2 than in year 1 due to the more favorable environmental conditions, particularly regarding PAW (Fig. 2c). Our results, in consequence, suggest a more important impact of pollination by honeybees when environmental conditions promote higher yields.

The most critical period for seed set in soybean spans from R3 to R6, where flowering, pod, and seed formation are overlapping (Board and Tan 1995; Egli and Zhen-wen 1991; Jiang and Egli 1995; Egli 1997). As a consequence, formation and abortion of flowers and pods and seeds set occurs simultaneously during this critical period, the duration of which varies depending on temperature, photoperiod, and genotype sensibility to these factors. Although the flower, pod and seed abortion processes are still not completely understood, distal flowers of primary racemes as well as secondary racemes are more likely to abort, than those which are located close to the node (Egli and Bruening 2006). The position of distal flowers of primary racemes or secondary racemes, in terms of assimilate (supply of nutrients) partitioning in the plant, determine a lower assimilate flux to the formation of flower parts (Egli 1998, 1999, 2004; Borrás et al. 2004; Egli and Bruening 2006), which can be derived in a deficient pollen formation. Thus, the action of honeybees carrying pollen from fertile flowers to relegated flowers in secondary racemes or in distal locations of

**Table I.** Analysis of variance of year, treatment, and the interaction year × treatment for soybean seed yield, seed number per unit area and individual seed weight in an experiment carried out in Paraná, Argentina during two consecutive growing seasons.

| Source of variation | P value | | |
| --- | --- | --- | --- |
| | Seed yield | Seed number | Seed weight |
| Model | < 0.0001 | < 0.0001 | < 0.0001 |
| Year | < 0.0001 | < 0.0001 | < 0.0001 |
| Treatment | 0.003 | < 0.0001 | 0.006 |
| Year × treatment | < 0.0001 | < 0.0001 | 0.965 |

primary racemes may have increased the pod and seed set in treatment NEV in year 1. Such a mechanism was not effective in year 2 (Table I) due to the low PAW-affected crop growth, which reduced the assimilate availability to seed set, i.e., even when pollen transfer could be effective in year 2, there were not enough plant assimilates to sustain the seed growth. This mechanism, however, needs to be more deeply addressed in further research, since to the best of our knowledge there are no previous studies available to confirm both: (i) the pollen sterility in relegated locations in racemes and (ii) the feasibility to fertilize these flower with fertile pollen in order to induce pod and seed formation.

Also, another possible mechanism to explain the contrasting response of our treatments could be related with the proportion of androsterile individuals within the population. In fact, some spontaneous mutations may result in some androsterile individuals in soybean, as reported by Graybosch and Palmer (1988) and Palmer et al. (2004), which has been suggested as having a higher probability of being frequent when a genotype is successively replicated after its release. These mutations may be favored by several environmental factors such as extreme temperatures, drought, and nutrient deficiency (Davis 2000), which suggest a differential proportion of individuals among years. These androsterile individuals will only set seeds when available pollen reach the stigmas, which becomes feasible only with the action of flower-visiting insects (Ortiz-Pérez et al. 2006).

Another mechanism, although less plausible than the previous mechanisms, has been

**Table II.** Average seed yield, seed number per unit area and individual seed weight in treatments having excluding (EV) and non-excluding flower-visiting insects (NEV) during two consecutive growing seasons in Paraná, Argentina

| Treatment | Seed yield (g m$^{-2}$) | | Seed number (Seeds m$^{-2}$) | | Seed weight (mg seed$^{-1}$) | |
| --- | --- | --- | --- | --- | --- | --- |
| | Year 1 | | | | | |
| NEV | 522.4 ± 41 | A | 3407 ± 144 | A | 153 ± 6 | A |
| EV | 441.5 ± 30 | B | 2795 ± 188 | B | 158 ± 5 | B |
| | Year 2 | | | | | |
| NEV | 377.6 ± 41 | A | 2842 ± 318 | A | 133 ± 6 | A |
| EV | 388.4 ± 24 | A | 2769 ± 152 | A | 140 ± 5 | B |

Bracketed values beside means indicate the standard deviation. Different letters besides means within a year indicate significant differences according to the Tukey test ($\alpha$ = 0.05)

**Table III.** Documented changes in soybean seed yield attributed to the effect of honeybees and/or others flower-visiting insects

| Reference | Seed yield change (%) | Observation |
| --- | --- | --- |
| Erickson et al. (1978) | + 15 | Honeybees |
| Chiari et al. (2005) | + 57 | Honeybees and others visitors |
| Chiari et al. (2008) | + 38 | Honeybees |
| Milfont et al. (2013) | + 18 | Honeybees |
| Santos et al. (2013) | + 25 | Honeybees and others visitors |
| Kengni et al. (2015) | + 36 | Honeybees |

suggested to be important in seed yield improvement as affected by the action of flower-visiting insects in soybean. It is stated that with this mechanism, the action of honeybees in increases the seed set which is related with a better pollen distribution on the stigmatic surface, due to the intimate contact between the abdominal portion of the insect with the receptive structure of the flower (Milfont et al. 2013). This mechanism does not include the androsterility as a factor, but is focused on hypothetical failures of fertile pollen in reaching the stigma surface.

The increase of seed number per unit area promoted by honeybees' pollination was similar to the increase in seed yield (Table II), which is consistent with examples in the body of literature (Erickson 1975; Juliano 1976; Erickson et al. 1978; Chiari et al. 2005), where seed yield is always related to seed and pod number per unit yield. However, the seed weight decreased in the NEV treatments as compared with the EV treatments (Table II). Similarly, a negative influence of flower-visiting insects has been reported by other authors (Delaplane and Mayer 2000; Alencar Arnaut de Toledo et al. 2011).

Although seed weight is frequently reported as being less variable than seed number per unit area, an important variation in seed weight may be anticipated when important changes in the source/sink (assimilate availability per seed) relationship occurs (Borrás et al. 2004). In fact, when the assimilate availability per seed is reduced by a higher seed number per unit area under a similar environment for seed fill a reduction of seed

weight may be anticipated. Thus, in NEV treatments an important (year 1) or a negligible (year 2) change in seed number per unit area may have reduced the assimilate availability per seed leading to the reported seed weight reduction (Table II).

Notwithstanding, flower visitation rate was similar between years, and seed yield was not increased by NEV treatment in year 2 (Table II). Thus, honeybee activity had not necessarily improved soybean seed production, which is in contrast with our second hypothesis. The more restrictive environmental conditions for crop growth during the critical period of seed set in year 2 surely underlie this result as discussed above. Although, to the best of our knowledge, there are no previous reports on the attractiveness for honeybees of the used cultivar, our estimated flower visitation rate was higher than the flowering rate (see Materials and methods section), provided a rough indication of this genotype feature. This could be a valuable consideration for further research, since variability in attractiveness for honeybees has been reported (Chiari et al. 2005).

## 5. CONCLUSIONS

Soybean production in plots where flower-visiting insects were not excluded was higher than in plots where flower-visiting insects were excluded in year 1, when favorable environmental conditions occurred. The yield increase associated to honeybee visitation

was positively related with seed number per unit area and negatively related with individual seed weight. Collectively, our results suggest an important role of honeybees in increasing the seed number per unit area of soybean under high yielding conditions.

The mechanisms involved in the seed yield increase were not revealed, but we suggested a possible way, related with pollen sterility in relegated flowers within the racemes under favorable conditions, for a high seed set.

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**Contribution des abeilles à la production de soja**

**Pollinisation / heures de butinage / récolte de graines / abeilles / *Apis mellifera ligustica* / Argentine**

**Beitrag von Honigbienen zu den Ertrag von Soja Bestäubung**

**Foragierzeit / Samenetrag / Sojabohnen / Honigbienen / Argentinien**

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