Environ Chem Lett (2013) 11:335–341
DOI 10.1007/s10311-013-0412-8

ORIGINAL PAPER

# Higher soybean production using honeybee and wild pollinators, a sustainable alternative to pesticides and autopollination

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Received: 20 March 2013 / Accepted: 3 April 2013 / Published online: 13 April 2013


**Abstract** This is the first report showing that using honeybee (*Apis mellifera*) and wild pollinators complementary pollination can enhance soybean productivity (*Glycine max*). Current industrial production of soybean involves autopollination and high loads of pesticides. Therefore, growers have neglected possible biotic pollination despite suggestions that soybean benefit from insect pollinators. Reports advocating possible biotic pollination are based on experiments where bees are caged with flowering plants and the absence of pesticides, thus not in field conditions. Therefore, here we compared in field conditions soybean yield produced (1) independently of biotic pollinators, (2) with wild pollinators and (3) with honeybee colonies. Results showed an increase of +6.34 % of soybean yield in areas where wild pollinators had free access to flowers. The introduction of honeybee colonies further raised the yield of +18.09 %. Our findings therefore show that, though soybean is autogamous, allowing pollination by wild pollinators leads to higher yields. Moreover, adding honeybee mitigates pollination deficits and improves yield compared to current practices.

**Keywords** *Apis mellifera* · Biotic pollination · Pesticides · Crop pollination · Crop productivity · *Glycine max* · Native pollinators · Pollination deficit · Supplementary pollination · Yield increment

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## Introduction

Soybean (*Glycine max* (L.) Merr.) is a legume native to East Asia (Fig. 1), but presently cultivated worldwide for its bean which has a variety of uses from animal to human feed to industrial application of its oil and biofuel production (USSEC 2008).

Nowadays, soybean is one of the most traded commodities and important revenues for exporter countries as well as food security of importer countries, and the world production reached 264.9 million tons in 2010 from a harvested area of 102.5 million hectares (FAO 2012).

The United States (35 %), Argentina (27 %), Brazil (19 %), China (6 %) and India (4 %) are the world's largest soybean producers and represent more than 90 % of global soybean production. In 2010, the average worldwide yield for soybean crops was 2.5 tonnes per hectare, but the three top producers had an average nationwide for soybean crop yields of about 3 tonnes per hectare (FAO 2012).

The world's ever-growing demand for soybean has produced a constant expansion of the cultivated area worldwide at the expenses of huge areas of native vegetation and is encouraging further deforestation, instead of increments in crop productivity (Fargione et al. 2008; Freitas et al. 2009). According to Masuda and Goldsmith (2009), the world soybean production increased 36 % since 2000 but 81 % of this increment was due to expansion of the cropped land. Efforts to increase soybean productivity have concentrated in developing varieties adapted to warmer climates and/or less demanding in fertilizers or resistant to pest and diseases and GMO cultivars (Embrapa 2005; Monsanto 2011). But yield increased only six percent since 2000 and contributed only 19 % to the world soybean production augment (Masuda and Goldsmith 2009). However, despite soybean is an autogamous,

**Fig. 1** *Apis mellifera* visiting a flower of *Glycine max* var. BRS Carnaúba

cleistogamous plant which flowers self-pollinate, there are evidences that it can benefit from animal-mediated pollination and increase yield when insect pollinated (Robacker et al. 1983; Free 1993).

Soybean inflorescence is a raceme bearing 5–35 flowers, and a single plant may produce up to 800 flowers during its lifetime, but each flower lasts only 1 day (Delaplane and Mayer 2000). The zygomorphic flowers are white, pink or purple, hermaphrodite and self-fertile. The corolla bears five petals, the upper one named standard, the two median petals called wings and the two lower ones forming the keel, which conceals the sexual organs (McGregor 1976; Carlson and Lersten 1987). The gynoecium is made by a single pubescent and superior ovary, containing from one to five ovules that develop simultaneously, and a curved style ending in a bifid stigma covered by papilla. The fruit grows in clusters of 3–5 and is a 3–8 cm long hairy pod that usually contains 2–4 seeds (McGregor 1976; Free 1993). The stigma becomes receptive 1 or 2 days before anthesis and anthers release pollen immediately before the flower opening, favoring autopollination (Fehr 1980; Delaplane and Mayer 2000). Wind pollination is negligible because there is little airborne pollen in and around the fields, restricted pollen dispersal and pollen grains have short life (Yoshimura 2011).

The autopollination mechanism associated with a high use of pesticides in soybean plantations has driven growers to believe that *G. max* does not benefit from insect pollination (Milfont 2012). However, despite the great number of flowers produced by a soybean plant and its autopollination mechanism, the number of pods formed is low (13–57 % depending on genetic and environmental factors), rising suspicions of pollination limitation in this crop (McGregor 1976; Free 1993; Delaplane and Mayer 2000).

Some investigations have shown greater yields in soybean crops when bees were introduced (Erickson et al. 1978; Robacker et al. 1983; Chiari et al. 2005), but these results usually are seen with reservation because they arise from artificial conditions where plants were caged with or without bees or pesticides were not used. Nevertheless, soybean is listed among the crops which show some dependence to insect pollination (Klein et al. 2007; Gallai et al. 2009), and Lautenbach et al. (2012) report that pollination benefits through soybean farming are high in some areas of Brazil, Argentina, India, China and USA. In the present work, we investigated the capability of soybean to produce commercial crops independently of biotic pollinators and the potential of native, wild pollinators and the introduction of honeybee (*Apis mellifera* L.) colonies in increasing soybean yield in open, large fields.

## Experimental

### Site and agricultural practices

The work was carried out in a soybean commercial plantation belonging to the agribusiness Faedo Sementes (05°08′72″S,37°59′14″W and 30. 22 m above sea level), in the county of Limoeiro do Norte, state of Ceará, Brazil. The weather in this region is semiarid, hot with rains peaking in the autumn, between March and May and little or no rain between July and December. Yearly averages for relative humidity, rains and temperature are 62 %, 720 mm and 28.5 °C, respectively (DNOCS 2011).

Observation was taken from July to December 2009 in a 50 ha circular area, irrigated by central pivot and cultivated with soybean cultivar BRS Carnaúba. This is a tropical cultivar originating from the crossing [E93-392 × (BR92-31879 × Sharkey)] carried out in 1994 by the Brazilian Agricultural Research Corporation (EMBRAPA) and the Irineu Alcides Bays Foundation (FAPCEN) to allow the soybean expansion toward the northern (tropical) and northeastern (semiarid) regions of Brazil (Embrapa 2005).

Main standard agricultural practices for soybean crops such as irrigation, weed and pest control were carried out during the experimental phase. Herbs and fungus were controlled spraying systemic herbicide (potassium glyphosate, 1.5 L/ha) at 15 and 30 day of the vegetative cycle and systemic fungicide (azoxystrobin and cyproconazole, 350 ml/ha) at full bloom and end of the blooming period. Pesticides used were a synthetic pyrethroid (lambda-cyahlothrin, 50 ml/ha) sprayed during blooming at 14-day intervals and a blend of lambda-cyahlothrin and the neonicotinoid thiamethoxam (250 ml/ha) at 7-day intervals by the end of blooming.

## Experimental design

Three treatments were used to assess soybean yield in the absence and presence of biotic pollinators:

1. Pollinator restricted plants (caged treatment): five areas measuring 3.0 × 6.0 m (18 $m^2$) were marked 40–60 m to the border of the cultivated area and enclosed in 3.0 × 6.0 × 1.5 m clear nylon screen (mesh 1 $mm^2$) cages 7 days before plants initiate blooming (Figs. 2, 3). The nylon screen was removed only after the plants have finished bloom (blooming period of 38 days), but the four corner posts of each cage were left in place to demark the enclosed area for harvesting.
2. Open plants to wild pollinators (open treatment): five areas measuring 3.0 × 6.0 m (18 $m^2$) and contiguous to the caged areas were demarked by four corner posts each to determine the plants that would be exposed to native pollinators (Fig. 2). During the experiment, wild flower visitors were sampled at random from non-demarked open areas at five times of the day (07:00, 09:00, 11:00, 13:00 and 15:00 h) using sweep nets to determine their identity.
3. Open plants to wild pollinators + honeybee introduction (honeybee treatment): five areas measuring 3.0 × 6.0 m (18 $m^2$) were demarked in the opposite side of the field to that where the caged and native pollinator treatment were set, c.a. 700 m away in a straight line (Fig. 2). Each area was demarked using four corner posts, and they were 40–60 m to the border of the side of the cultivated area, where eight honeybee colonies were placed to provide potential visitors to soybean flowers (Fig. 2). The colonies conformed to the health and strength recommended for pollination services and were placed by the crop area when 10–20 % of flowers were open (Free 1993).

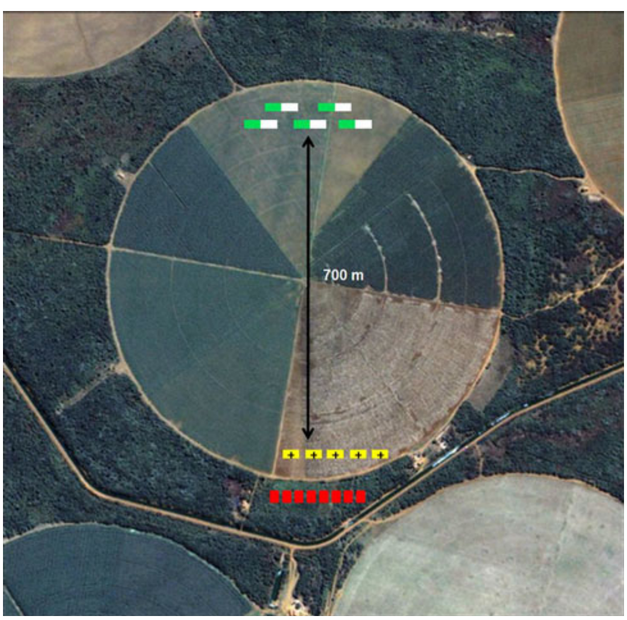


**Fig. 2** Satellite image of the central pivot area cultivated with soybean showing an illustrative scheme of the three treatments employed: caged plants (*white blocks*), open plants to native pollinators (*green blocks*) and open plants with honeybee (*Apis mellifera* L.) introduction (*yellow blocks*). The *red blocks* represent the place where eight honeybee colonies were placed. (Color figure online)

**Fig. 3** Clear nylon cages placed near the border of a soybean plantation to prevent flower visits by potential pollinators. Native vegetation appears in the background

Soybean yields for the three treatments were obtained at harvesting. In each of the 15 plots (5 per treatment), plants in the border of the 18 $m^2$ plots were discarded to avoid any border effect and only plants in the central 10 $m^2$ were considered for yield analyses. Ten of these plants were randomly harvested apart to the others of that plot (total of 50 plants per treatment) for counting the number of pods set per plant, number of pods containing one, two or three seeds and total number of seeds produced per plant. After that, these seeds were added to remaining plot harvest, and the yield of each plot was determined. Later, the average yield from the 10 $m^2$ area was extrapolated to 10.000 $m^2$ to obtain yield/ha.

## Data analyses

The three soybean yield treatments were compared by analysis of variance and means compared a posteriori by Tukey's test (5 %). Data regarding number of seeds per pod, number of pods per plant and total seeds were analyzed by a chi-square test.

# Results and discussion

## Total yield increment and pollination deficit

There were significant differences ($p < 0.05$) between treatments (Table 1). Areas with honeybee colonies produced significantly ($p < 0.05$) greater yield than both the areas open but with no honeybee introduction and the caged areas where flowers were prevented of insect visitation. Open areas also differed ($p < 0.05$) to the caged areas yielding 6.34 % more seeds (kg).

The caged treatment confirmed that soybean var. BRS Carnaúba is an autogamous autopollinating crop capable of harvesting a commercial yield over 2,800 kg/ha independently of biotic pollinators. Such a result, associate with heavy use of pesticides, can explain why pollination has never been a concern to soybean growers. However, a significant average increment of 6.34 % in the areas where native floral visitors had free access to flowers showed that this crop benefits from biotic pollinators. Indeed, recent studies had shown that self-fertile plants also seem to have seed and fruit yields augmented by improved levels of fertilization due to animal-improved self-pollination (Klein et al. 2003; Rizzardo et al. 2012).

The introduction of honeybee colonies to the soybean plantation raising yield 18.09 % in relation to caged plots not only represents almost the same yield increment in soybean productivity over the past 10 years (Masuda and Goldsmith 2009), but also confirms the role of biotic pollinators in the pollination of this crop. It also demonstrates a 11.04 % pollination deficit, as defined by Vaissière et al. (2011), in the overall soybean plantation represented here by the open plots. Both figures are within the estimate pollination reduction range of 10–40 % for soybean suggested by Klein et al. (2007) and Gallai et al. (2009).

## Number of pods and seed per pod

The average number of pods produced per plant under each treatment differed significantly ($p < 0.05$). Plants in the areas with honeybees bore significantly ($p < 0.05$) more pods than those from the caged areas (Table 2). However, no significant difference ($p > 0.05$) was observed between the average number of pods in plants of the honeybee areas to the open areas and between the open areas and the caged ones (Table 2). Considering the number of seeds set per pod, there were significant differences ($p < 0.05$) in the number of pods with one, two and three seeds within each treatment (Table 2). In all three treatments, the number of pods with two seeds was significantly ($p < 0.05$) greater than those of one and three seeds, and the number of pods with three seeds was also greater ($p < 0.05$) than that of one (Table 2).

Comparing pods with the same number of seeds between treatments, it was observed significant differences ($p < 0.05$) to one- and three-seeded pods, but not ($p > 0.05$) to pods with two seeds (Table 2). The honeybee areas produced significantly ($p < 0.05$) more one-seeded pods than the caged areas, but no significant difference ($p > 0.05$) was observed between the number of these pods in plants of the honeybee areas to the open areas and between the open areas and the caged ones (Table 2). However, the number of three-seeded pods produced was significantly greater in the honeybee treatment than in the other two treatments which did not differ ($p > 0.05$) to each other (Table 2).

This experiment showed that two-thirds to three quarters of the pods set by a soybean plant bears two seeds and is not dependent on floral visitors, but plants open to floral visitors or receiving honeybee visits produced significantly more than those not visited. Plants in the area with honeybee colonies bore significantly more one-seeded pods than those of the caged area, and this can explain partially the augment in yield observed between these two treatments. However, it cannot explain alone a gap over 18 % in yield, especially because plants in the open area that also produced a significantly lower harvest than that of the area with honeybees did not differ to it in number of one-seeded pods.

The main difference between the treatments was observed in the number of three-seeded pods set per plant. It seems that the honeybees were able to set a larger number of three-seeded pods than the native, feral visitors or the autopollinating soybean flowers. As the soybean anthers shed pollen while the flower is still closed, this pollen falls randomly over the pistil and stigma pollinating the flower (Free 1993). In some occasions, however, this autopollinating mechanism may fail in delivering enough alive, viable pollen grains on the receptive surface of the stigma and that flower is not set at all or the pod sets only one or two seeds. It seems that the latter case happens most of time because two-seeded pods represent over 66 % of all pods produced per plant.

Apparently, some floral visitors like the honeybee help to place or distribute better the pollen grains over the style and stigmatic surface when forcing their ventral abdomen against the stigma, contributing mainly to set the third seed

**Table 1** Seed yield (kg/ha) of soybean (*Glycine max* (L.) Merril) cv. BRS Carnaúba under three pollination treatments in NE Brazil (s.e.m = standard error of mean)

| Treatment | Replicates | Seed yield ± s.e.m. (kg/ha) | % Increment | |
|---|---|---|---|---|
| | | | Caged | Open |
| Area with honeybee colonies | 5 | 3,333.2 ± 142.7a | 18.09 | 11.04 |
| Open area | 5 | 3,001.6 ± 97.1b | 6.34 | – |
| Caged area | 5 | 2,822.4 ± 52.6c | – | −5.97 |

Means followed by different lower case letters differ at $p < 0.05$

**Table 2** Total pod production and number of pods with 1, 2 or 3 seeds in a soybean (*Glycine max* (L.) Merril) cv. BRS Carnaúba plantation, under three pollination treatments in NE Brazil (s.e.m = standard error of mean)

| Treatments | # Of plants | Total pods | Pods with 1 seed | | Pods with 2 seeds | | Pods with 3 seeds | | % Total |
|---|---|---|---|---|---|---|---|---|---|
| | | $\bar{X}$ ± s.e.m. | $\bar{X}$ ± s.e.m. | (%) | $\bar{X}$ ± s.e.m. | (%) | $\bar{X}$ ± s.e.m. | (%) | |
| Honeybee + wild pollinators | 50 | 59.6 ± 2.71a | 5.92 ± 0.51aC | 9.93 | 39.54 ± 1.95aA | 66.34 | 14.14 ± 1.10aB | 23.72 | 100.00 |
| Wild pollinators | 50 | 57.16 ± 1.87ab | 4.54 ± 0.47abC | 7.94 | 40.94 ± 1.54aA | 71.62 | 11.68 ± 0.85bB | 20.43 | 100.00 |
| Pollinator restricted | 50 | 49.64 ± 2.64b | 3.86 ± 0.40bC | 7.78 | 36.52 ± 2.29aA | 73.57 | 9.26 ± 0.51bB | 18.65 | 100.00 |

Means followed by different lower case letters in columns and upper case letters in rows differ at $p < 0.05$

in some pods, but also a first seed in some flowers that otherwise would fail to set a fruit. The role of flower visitors in setting more or better quality fruits or seeds in autopollinated flowers has already been demonstrated to other plant species (Barret et al. 1994; Cruz et al. 2005), and recently, Garibaldi et al. (2013) found positive associations of fruit set with flower visitation by wild insects in 41 crop systems worldwide and showed that pollination by managed honeybees supplemented pollination by wild insects.

## Native floral visitors

Although dizzy and shaky insects, including honeybees, could be found in the field after pesticides application, in the other days, they looked healthy and foraged normally on the flowers. Honeybees represented 90 % of the flower visitors and the other 10 % were wild insects. Native floral visitors sampled with the sweep net were mainly solitary bees belonging to the families Andrenidae (*Psaenythia* sp.), Apidae (*Ancyloscelis* sp.; *Centris analis* Fabricius, 1804; *Exomalopsis analis* Spinola, 1853; *Florilegus* sp.; *Melitomella grisescens* Ducke, 1907), Halictidae (*Augochloropsis* sp. 1 and 2; *Augochlorella* sp.; *Augochlora* sp. 1 and 2; *Dialictus* sp. 1, 2 and 3) and Megachilidae (*Megachile* sp.). Some flies (mainly syrphids), Hemiptera, beetles and Lepidoptera were also present near or on the flowers. Many thrips were also seen inside and around the flowers, but we did not include them in the proportional calculation of honeybees and wild native floral visitors.

There are only a few reports of wild floral visitors on soybean plantation and despite representatives of Diptera, Hemiptera, Coleoptera and Lepidoptera have also been found in these studies, thrips and bees are considered as the most likely wild pollinators of cultivated soybean (Rust et al. 1980; Chiari et al. 2005; Yoshimura et al. 2006, 2011).

Probably, the high level of pesticides used in soybean plantations kills or drives off most wild floral visitors, but even so the ones observed foraging the flowers were enough to produce a significant yield increment. Our results suggest the need to identify the native pollinators of soybean and to adopt pollinator friendly practices such as reducing or using less harmful pesticides to pollinators or avoid spraying during crop blooming to ensure their presence in the plantations and improve yield in relation to pollinator-depleted areas, represented in this experiment for the caged crop.

## Economic and environmental issues

Our results demonstrate that native pollinators are playing an important role in the pollination of soybean and ensuring significantly greater harvests than if they were not present (extra 179.2 kg seeds/ha). Considering the present market price, this yield increment represents an additional income of US$ 59.7/ha and, potentially, over US$ 6.126 billion to the world soybean harvested area (102,556,310 ha). The introduction of honeybee colonies produced yield growths even more significant comparing to the open and pollinator-depleted areas (extra 331.6 and 510.8 kg seeds/ha, respectively), representing income increments of US$ 110.5 and 170.3/ha and, potentially, over US$ 11.335 and US$ 17.461 billion to the world economy, respectively, in the same area presently cultivated. To reach this figures with the present productivity of 2,800 kg seeds/ha, it will be necessary to expand the soybean area in approximately more 12.5 and 18.7 million ha, respectively. A major proportion of the recent worldwide deforestation is consequence of the increasing demand for meat in India and China which has demanded increased soybean production in those countries as well as the other major world producers, Argentina, Brazil and the USA (Freitas et al. 2009; Chacoff et al. 2010; Lautenbach et al. 2012).

Although benefits of bee pollination may vary between different soybean varieties and growing conditions and a precise global estimation needs knowledge on the degree of pollinator dependence of each variety (Delaplane and Mayer 2000; Chacoff et al. 2010), our estimates based on values ranging between 6 and 18 % yield increment and

US$ 59.7 and 170.3/ha income increments are modest comparing to the range of 10–40 % proposed by Klein et al. (2007) and Gallai et al. (2009), and US$ 490.0/ha advocated by Lautenbach et al. (2012).

The autopollination mechanism associated with a high use of pesticides in soybean plantations ensures harvesting commercial yields and has driven growers to overlook biotic pollination. Here, we show under real field conditions that soybean can benefit from pollination carried out by native, wild pollinators leading to higher yields, and the introduction of *A. mellifera* for complementary pollination can contribute to mitigate pollination deficits and improve yield significantly in this crop, and these findings may also contribute to reduce pesticide use and to prevent further clearing of new areas to increase seed production.

## Conclusion

Based on our results, biotic pollinators are capable of pollinating soybean flowers and improve crop productivity; wild pollinators are efficient pollinators and effective to increase yield despite pesticide spraying, and honeybees can be used for soybean pollination in field conditions. Although wild pollinators alone produce significant yield increment, complementary pollination carried out by *A. mellifera* sets more pods per plant and more seeds per pod, reducing pollination deficit and producing a greater total yield, making their association a more efficient practice.

Using biotic pollinators to improve yield can be a sustainable practice to further development of the soybean agriculture, when compared to current management practices based on a constant expansion of the cultivated area worldwide at the expenses of huge areas of native vegetation and intense pesticide use, instead of increments in crop productivity. Definitely, growers should make efforts to change their current management practices to accommodate and increase the number of native pollinators in areas cultivated with soybean and should use managed pollinators such as the honeybee to enhance productivity.

**Acknowledgments** Authors thank Faedo Sementes for permission to carry out this study in its soybean plantations, Altamira Apícola for lending the honeybee colonies, David R. Rocha for processing the figures of this article and the National Council for Scientific and Technological Development—CNPq, Brasília-Brazil for the D.Sc. scholarship to M.O. Milfont and the research fellowship to B.M. Freitas (proc. # #305062/2007-7).

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