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RESEARCH PAPER

The contribution of pollinators varies among soybean cultivar traits

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ABSTRACT

Soybean is one of the most widely cultivated species worldwide. Empirical studies have shown that animal pollination can contribute between 0 and 50 % to soybean yields. However, the role of animal pollination in soybean production is often overlooked in management decisions. Understanding the factors driving variability in pollinator contribution can aid in developing effective management strategies. In this study, we experimentally assessed the contribution of both autonomous and animal pollination across nine widely cultivated soybean cultivars in the Chaco region of Argentina. Additionally, we explored whether specific traits of these cultivars could explain the observed variability in pollination contribution. We used field exclosure experiments to study cultivars that differed in flower color, genetic modifications, and maturity groups, and analyzed the variability in pollinators' contributions across years and locations. We found that the overall reduction in production between open and bagged plants was, on average, 40 % (CI 25–51 %). The contribution of pollinators varied depending on flower color, maturity groups, and locations, but not across different years or genetic modifications. Cultivars with purple flowers showed greater differences between open and bagged plants compared to those with white flowers, indicating that flower color may influence the attractiveness of flowers to pollinators. Additionally, pollinators' contribution varied across maturity groups, potentially due to the differential timing of the flowering affecting the local abundance of pollinators within the crop. Notably, the variable used to estimate pollinators' contribution (i.e., seeds, pods, or yield) conditioned the results. Pollinators' contribution can be highly variable, and traits associated with cultivars can help improve our understanding of such heterogeneity. Our results showed that the contribution of pollinators to soybean cultivars in the Chaco region of Argentina ranges from modest to high. This suggests that conserving pollinators and managing agricultural fields at plot and landscape scales can significantly impact soybean production.

Introduction

Crop pollination represents a nature contribution to human welfare, as it plays a crucial role in plant reproduction. It is estimated that 75 % of the crops consumed by humans benefit from animal pollination, and 35 % of global agricultural production comes from animal-pollinated crops (Klein et al., 2007; Pascual et al., 2017). This benefit represents increased quantity and/or quality of crop production. Several studies have estimated the relative importance of animal pollination in different crops and used the term "pollinator dependence" (Klein et al., 2007; Giannini et al., 2015). More recently, the term "pollinators' contribution" has been introduced to specifically refer to the difference in reproductive variables between "bagged" and "exposed" flowers (Siopa et al., 2024). Pollinators' contribution is categorized it as 'little' (<10% reduction in production in the absence of pollinators), 'modest' (10 to <40%), 'high' (40 to <90%), or 'essential' (>90% reduction) (Klein et al., 2007). The different contribution levels are tacitly considered an invariant property of the species; however, recent publications have reported within-crop variability (Garratt et al., 2021; Hudewenz et al., 2014; Bishop et al., 2020).

The variability in pollinators' contribution within a crop can be context-dependent (Bishop & Nakagawa, 2021; Perrot et al., 2019; Tamburini et al., 2019). For instance, may rely on pollinators' identity, richness, and abundance within the site (Woodcock et al., 2019) and may vary according to the available resources within the farm (Tamburini et al., 2019; e Silva, Ramos, Mertens, & Carvalheiro, 2023 and with crop genotypes and traits (Bishop & Nakagawa, 2021). Furthermore, experimental approaches to estimate pollinators'

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contribution may also result in variable conclusions; for example, estimations of pollen grain deposition can differ from those based on fruit or seed set (Bishop & Nakagawa, 2021; Chacoff & Aizen, 2007). A better understanding of intrinsic, environmental, and experimental factors that can cause variability in the contribution of pollinators to crops is crucial for robustly estimating the importance of pollinators in agriculture and making better-informed management decisions that impact both pollinators and crop production.

Soybean (*Glycine* max (L.) Merrill) is currently one of the world's dominant crops; it is cultivated in many regions and has become an important commodity, representing 25% of global edible oil and about two-thirds of protein concentrate for livestock feeding (Masuda & Goldsmith, 2009). Soybean is autogamous and self-compatible and can produce seeds without pollinators. However, remarkably, its flowers have nectar, scent, and nectar guides; all these characteristics indicate that soybean can be pollinated by insects (Erickson, 1984). Klein et al. (2007) reported that soybean has a modest dependence on pollination (i. e., the absence of pollinators can reduce production by 10–40 %). Studies from Brazil, the USA, and Argentina reported a wide variation in the benefit that animal pollination confers to soybean production. In the absence of visitors, production decreases between 6 and 50 % (Chiari et al., 2013; Cunningham-Minnick et al., 2019; de O. Milfont et al., 2013; Monasterolo et al., 2015; Garibaldi et al., 2021). Moreover, recently, da Cunha et al. (2023) revealed that pollinators' contribution to soybean is latitudinally structured, with an increasing level of contribution towards lower latitudes. Still, a large amount of variability is not explained by latitude.

The expansion of soybean cultivation entailed the development of numerous cultivars adapted to different environments and conditions. It has also been found that pollinators' contribution may be related to environmental factors that shape plant resource availability (Tamburini et al., 2019). For instance, the attractiveness of honeybees to soybean flowers was related to soil nitrogen, phosphorus, and temperature, all of which affected flower production, size, openness, and nectar production (Robacker et al., 1983). In addition, when studies were conducted over multiple years, the results on the importance of pollinators varied (Ahrent & Caviness, 1994; Blettler et al., 2018; Ortiz-Perez et al., 2006). Thus, environmental factors such as resources, weather, and management practices that vary between locations and years have been reported to affect aspects of pollination in this species.

Moreover, the development of soybean cultivars with different traits can affect their level of autogamy and their attractiveness to insect pollinators (Suso et al., 2016). The traits that confer distinctiveness to cultivars can be morphological, chemical, or physiological, and some of the most useful traits to describe a soybean cultivar are flower color, maturity group, growth habit, and genetic modifications (Singh, 2010). Erickson (1975) found that the activity of bees resulted in greater yields in two of the three studied cultivars, and variations in the floral nectar characteristics have been documented in different cultivars, potentially leading to a differential attraction of flower visitors (Erickson & Garment, 1979; Severson et al., 1987). At the same time, transgenic soybeans (GM crops) carry one or two genetic modifications. Most of the presently cultivated soybean is RR (i.e., resistant to herbicides) and some are Bt cultivars, which have an additional mutation that confers resistance to pests (i.e. resistance to lepidopteran and coleopteran pests). The use of Bt cultivars is supposed to reduce the use of insecticides (Blanco et al., 2016; Coupe & Capel, 2016), which is expected to positively impact beneficial insects, such as pollinators; however, evidence about this potential effect is scarce (Blanco et al., 2016; Oliveira & Fernandes, 2020; Yu et al., 2014). Lastly, the maturity group is a widely used agronomic characteristic of each cultivar, represented by a number that varies between 000 and X. This number considers the photoperiod length and temperature to which the cultivar is best adapted; hence, maturity groups are characteristic of each geographic location. Maturity groups with lower numbers are used in higher latitudes, while maturity groups with higher numbers are used in lower latitudes (Mourtzinis & Conley, 2017). In Argentina, common maturity groups range from II (Pampa region at 39° South) to IX (Chaco region at 23° South) (Dardanelli et al., 2006). The differential sensitivity of maturity groups to temperature and photoperiod conditions, their flowering period, the nectar characteristics, and the timing of the flowering period (maturity groups that flower sooner or later in the summer), impact their interactions with different assemblages of insects. Ahrent and Caviness (1994) reported that the level of cross-pollination in different cultivars greatly varies between years and maturity groups. They interpreted that outcrossing may occur in some cultivars under the presence of pollinators and favorable conditions, indicating a need to better understand those cultivar traits associated with cross-pollination.

This study aimed to evaluate the level of pollinators' contribution to soybean cultivars commonly cultivated in the dry Chaco region of NW Argentina. We asked the following specific questions: What is the level of pollinators' contribution to soybean cultivars in this region? Does this level of contribution vary in different contexts, specifically among years or locations? Does the level of pollinators' contribution change according to the metric used to evaluate yield? Do intrinsic/morphological traits of cultivars (i.e., flower color, maturity group, genetically modified genes) help to predict pollinators' contribution? To answer these questions, we conducted field tests to evaluate the yield of soybean crops from flowers that were either bagged or open. Our experiment included nine different cultivars that were grown on various farms, some of them measured over two or three years. The implications of this study include a better understanding of the role of pollinators in this widespread crop with potential for improved management practices.

Materials and methods

Argentina is the third soybean producer in the world (Innes, 2006) and 1057 cultivars, adapted to different biomes are registered in the country (www.inase.gob.ar).

In the Chaco region, soybean cultivars are adapted to arid environments, with maturity groups typically ranging from V to VIII. In recent years, Bt cultivars have seen significant expansion in this area; however, both types of genetically modified (GM) seeds Bt and RR currently coexist. Soybean flowers are small, hermaphrodite, and self-fertile; each flower produces one pod, which commonly has between one to three seeds (Kantolic & Slafer, 2007).

Experimental design

We worked at nine locations in the Chaco region (Fig. 1), in four growing seasons and surveyed nine cultivars. Cultivars differed in three morphological traits: flower colors (five white and four purple, Fig. 2), genetic modifications (i.e., four RR and five Bt), and maturity groups (i. e., V, VI, VII). Numbers of cultivars, locations and traits of cultivars sampled each growing season are provided in Table 1 (and see Appendix A: Table A.1).

In the study area, soybean seeds are sown every year in late spring (mid-December). Flowering occurs nearly 40 days after planting. Thus our experiments ran from December until the plants were harvested, nearly 110 days after planting. These experiments were conducted in 2015, 2017, 2018, and 2019.

We set exclosure experiments that consisted in comparing two pollination treatments: "open" flowers exposed to free pollination (control group, in which flowers could receive visits from pollinators) and "bagged" flowers, isolated from animal pollinators (allowing autonomous self-pollination and wind pollination) (see Appendix A: Fig. A.1). Nearby each "bagged" treatment, we established the "open" plot of an identical area. Each pair of open and bagged treatments was considered a sampling unit (plot) and was at least 100 m apart from the next one (see Zelaya et al., 2018) for further methodological details). The number of sampling units per cultivar varied between two and 39; some cultivars were only studied in one location/year while others were

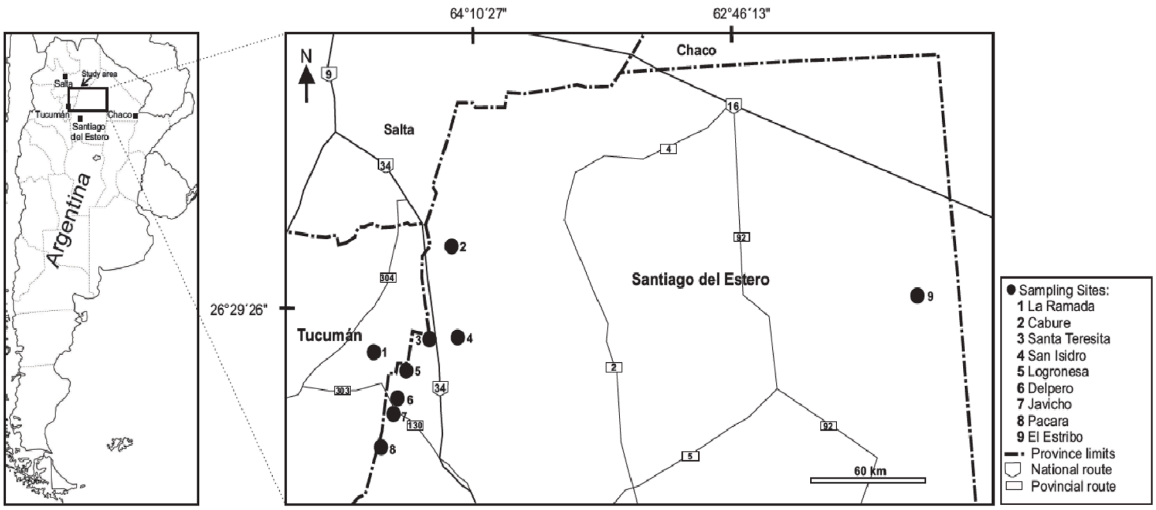

Fig. 1. Study area within Argentina (left panel) and locations (1 to 9) of the soybean crops sampled within the Chaco region, in the provinces of Tucumán and Santiago del Estero (right panel).

Fig. 2. Soybean (Glycine max) cultivars differ in traits, one of them is flower color, that can be white or purple. One of the findings in our study is that the contribution of pollinators' differ between cultivars with different colors. In cultivars with purple flowers the contribution was greater than on cultivars with white flowers. Photo credits: White flowers with *Apis mellifera* is from Julieta Carrasco and others from Silvio E. Castillo.

studied in many locations and thus had a better replication (Table 1). Soybean cultivars used in the experiment were those decided by each producer at each location and year (Table 1 and see Appendix A: Table A.1).

Animal pollination was prevented in the "bagged" flowers by using a nylon mesh, the standard method in soybean pollination studies (studies reviewed in da Cunha et al., 2023). The use of this type of mesh exclosure showed no effect on soybean plants (Santone et al., 2022). Due to the small size of the flowers and also the position of many of the flowers along the principal axis of the plant, it is practically impossible to isolate a single flower or a group of flowers from animal pollinators. For that reason, we used large mesh tents that covered roughly 12 plants (0.5 m x

Table 1

Soybean traits and design details for the cultivars studied in the Chaco region. The nine cultivars differed in three morphological traits: flower color (i.e., white or purple), type of transgenic event (i.e., RR: resistant to herbicides, Bt: resistant to herbicides and to lepidopteran and coleopteran pests), and maturity groups (i.e., V to VII). The number of replicates includes the number of plots with open treatment vs. bagged treatment. The study was conducted in the growing seasons during four years (i.e., sampling years), at nine locations.

Cultivar	Flower color	Type of transgenic event	Maturity group	Replicates (open / bagged)	Sampling Year	Locations
DM 5.8i	White	RR	V	36 (18/18)	2016	Cabure, Santa Teresita
DM 6.8i	Purple	RR	VI	16 (8/8)	2018	Santa Teresita
DM-6563 ipro	White	Bt	VI	15 (10/5)	2018	Santa Teresita
DM 60I62	Purple	Bt	VI	22 (11/11)	2019	El Estribo
DM-7.8	White	RR	VII	48 (24/24)	2016, 2018	Cabure, Javicho
DM-7870-Intacta	White	Bt	VII	15 (10/5)	2018	Cabure
M-6410-ipro	Purple	Bt	VI	90 (51/39)	2017	Cabure, Delpero, Javicho, Logronesa, Pacara, San Isidro
NS-6483	Purple	RR	VI	5 (3/2)	2015	La Ramada
NS-7209	White	Bt	VII	5 (3/2)	2015	La Ramada

1.5 m, and 1.5 m in height), as were used in many other experiments with the same crop species (da Cunha et al., 2023). The nylon mesh tents were set between two and three weeks before flowering and were maintained until soybean harvest. When harvested, we counted pods and seeds per pollination treatment and divided them by 12 (the mode of the number of plants per plot). Thus, we calculated a mean value of pods and seeds per plant under each treatment. We also weighted the dry seeds per pollination treatment (0.5 m x 1.5 m) to have an estimation of the yield (kg ha^{-1}).

Data analysis

To determine the effect size of the pollination treatment, we used as a response variable the log of bagged/open treatment per plot (as in Bishop & Nakagawa, 2021 and da Cunha et al., 2023). The estimation of the pollinators' contribution was then calculated as 1-exp (effect size), and this variable was used for visualization in the graphs (as in Bishop & Nakagawa, 2021). We used a multilevel model approach with a comparison between models using the AICc criterion.

To estimate the overall pollinators' contribution and to explain its heterogeneity, we first built models with random effects only (GLMM) and compared them with a null model with an intercept only (GLM). By using this approach, we characterized the range of variation associated with pollinators' contribution within cultivars, years, and locations (random factors). We selected the best random structure by using information criteria (i.e., the model with the lowest AICc).

After we found the random structure that better fitted our observations, we added the following set of variables as fixed factors to the models: 1) type of yield metric used to estimate pollinators' contribution (yield, seeds/plant, and pods/plant); 2) flower color (purple or white); 3) genetic modifications (RR or Bt), and 4) maturity group (varied between V and VII) (Table 2). We ran separate models for each fixed factor and we did not test interactions between variables because of an incomplete set of possible combinations. By using LRT (Likelihood ratio test), we assessed the significance of the fixed factors, comparing each model, including a fixed variable, with a model having optimal random structure. We computed the conditional R^2, which can be interpreted as a variance explained by the entire model, including both fixed and random effects (Nakagawa & Schielzeth, 2013).

The analysis was performed using *R* (R Development Core Team, 2014), with the *lme4* package to fit the models (Bates et al., 2015), the *MuMIn* package (Barton & Barton, 2015) for the conditional R2, and the *ggplot2* package for visualization and graphs (Wickham, 2016).

Results

We harvested 189 m^2, which included 3036 soybean plants. Plants from the open-pollinated treatments set on average 76 (± 21 SD) pods per plant, while plants from bagged treatments set 51 (± 15 SD) pods per plant. The number of seeds per plant was 143 (±52) in the open-pollinated flowers and 90 (±35) in the bagged flowers. Finally, the mean yield was 3342 kg.haˆ−1 (± 1215) and 1911 kg.haˆ−1 (±850) for open and bagged treatment, respectively.

Variability in pollinators' contribution across cultivars, locations, and years

The overall reduction in production in the absence of pollinators was, on average, 40-% (Confidence Interval: CI 25–51, Fig. 3, Table 3 Model R_s, and see Appendix A: Fig. A.2). There was a high heterogeneity in the effect of pollinators' exclusion on soybean production. When comparing our models using the AICc, the random structure that better fitted our observations included location. The model that included the variability across locations explained 34.8% of the variance (conditional R^2, Table 3, model R_s). The heterogeneity in the effect of pollination treatment among cultivars was similar to the variability across years (conditional R^2, 10 % and 13% of the variability, see Appendix A: Fig. A.3).

Table 2

Summary of statistical models used to evaluate pollinators' contribution on soybean cultivars in the Chaco region. The tested effect, model name, and fixed and random effects are detailed. Fixed effects included yield (three levels), flower color (two levels), maturity group (three levels), and transgenic event (two levels), while random effects included plots, cultivars, years, locations, and additive interactions.

Tested effect	Model	Fixed effects	Random Effects
M_Null	M_0		Plots (residuals)
M_Var	R_v		cultivar + Plots
M_Years	R_y		Years + Plots
M_Locations	R_s		Locations + Plots
Yield measurement	M_Yields	Yield metric (3 levels: pods/plant, seeds/plant, and yield (kg/ha))	Locations + Plots
Flower Color	M_C	Flower color (2 levels: purple, white)	Locations + Plots
Maturity group	M_MG	Maturity group (3 levels: V, VI, VII)	Locations + Plots
Genetic modifications	M_T	Transgenic event (2 levels: RR, BT)	Locations + Plots

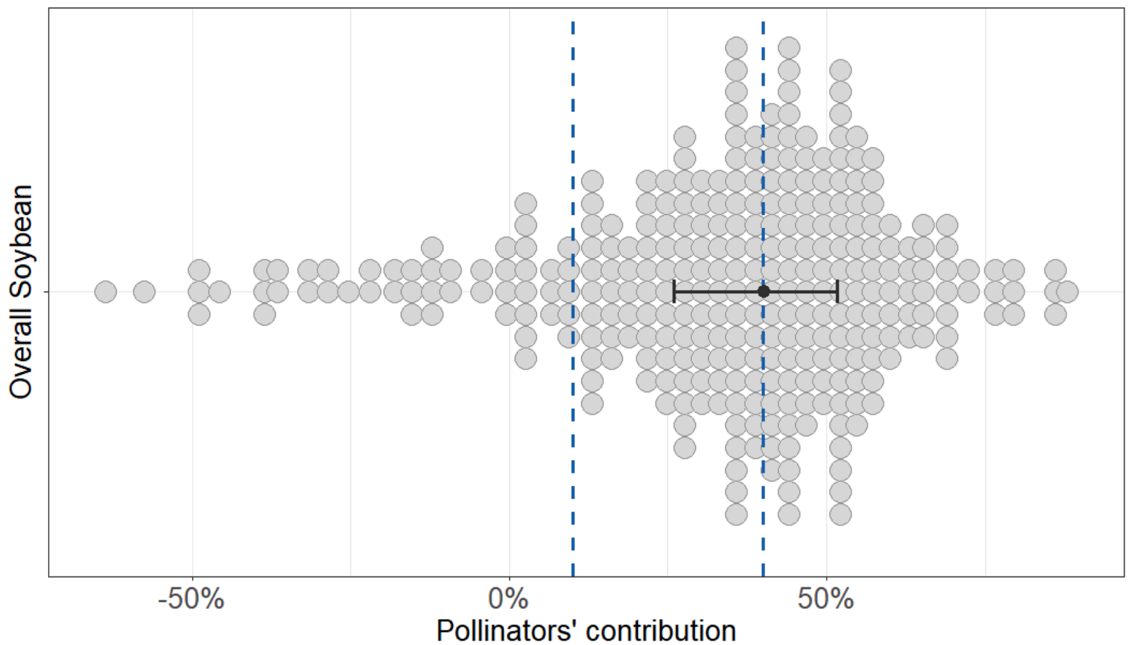

Fig. 3. Pollinators' contribution in soybean for common cultivars in the Chaco region. Each gray dot shows the proportional difference for one open/bagged replicate, and the vertical dashed blue lines indicate the limits for a crop with modest dependency on pollinators, according to Klein et al. (2007). In black, the mean value (dot) and the 95 % confidence interval (line) of pollination dependence obtained in this study are shown.

Table 3
Models' Estimates for fixed and random effects, AIC values, and Marginal/Conditional coefficients of determination (R^2) tested in Generalized Linear Mixed Models (GLMM). Fixed effects included production metrics (i.e., pods, seeds, yield), flower color (i.e., purple, white), genetic modifications (i.e., RR, Bt), and maturity group (i.e., V to VII); intercepts, and 95 % confidence intervals (CI) are shown. Random effects were cultivars (Var), locations (Loc), and sampling years; residuals (Resid) of the models are shown.

Model		Fixed effects	Random Effects				AICc	*R^2_GLMM*
	Variable	Intercept CI	Var	Loc	Years	Res		
M_0		−0.48 (−0.53– −0.44)				0.415	346	
R_v		−0.43 (−0.55– −0.29)	0.02			0.158	335	0.10
R_y		−0.45 (−0.61– −0.30)			0.02	0.159	334	0.13
R_s		−0.51 (−0.72– −0.29)		0.07		0.141	311	0.35
M_yields	Pods	−0.41 (−0.62– −0.20)		0.07		0.134		0.03/0.36
	Seeds	−0.50 (−0.71– −0.28)						
	Yield	−0.60 (−0.82– −0.39)						
M_c	Purple	−0.55 (−0.76– −0.35)		0.06		0.139		0.02/0.34
	White	−0.41 (−0.63– −0.20)						
M_t	Bt	−0.52 (−0.71– −0.33)		0.07		0.140		0.001/0.34
	RR	−0.49 (−0.70– −0.29)						
M_MG	V	−0.31 (−0.52– −0.10)		0.07		0.14		0.05/0.36
	VI	−0.57 (−0.75– −0.38)						
	VII	−0.41 (−0.61– −0.21)						

Pollinators' contribution over different measures of soybean production and traits

The estimation of pollinators' contribution varied among the metrics used to measure soybean production (i.e., yield, seeds, and pods) (Likelihood ratio test: LRT comparing models R_s with M_Yields, $\chi2$=13.19, p = 0.001). When estimation was based on pods/plant, pollinators' contribution was 34% (CI 18.1–46.7) and increased to 45% (CI 32.4 –59.9) when estimation was based on yield; thus there was an increment from pods to yield of nearly 20%. Estimation based on seeds resulted in intermediate values (39%) (Fig. 4A, see Appendix A: Fig. A.4).

When considering the cultivars' traits, the color of flowers statistically explained pollinators' contribution. Cultivars with purple flowers had a greater difference between exposed and bagged flowers than cultivars with white flowers (Fig. 4B, Likelihood ratio test: LRT comparing models M_C with R_s, $\chi2$= 6.76, p = 0.009). The mean difference between open and bagged treatments (i.e., the pollinators' contribution) among cultivars with different flower color was 14% (Post Hoc comparison between flower colors (t=−2.58, p = 0.0101, method=Tukey). There was no difference between open and bagged flowers when comparing RR and Bt cultivars (Fig. 4C). Pollinators' contribution differed among maturity groups (LRT comparing M_MG with R_s, $\chi2$= 15.89, $p \leq 0.001$) (Fig. 4D). Contribution was higher in cultivars from the VI maturity group than in cultivars from maturity groups V.

Discussion

Biotic pollination contribution to soybean yield has traditionally been perceived as negligible or even absent. Here, we found that the difference in yield between plants exposed to pollinators and those excluded from pollinators was as high as 40 %, with plants in open treatment showing greater reproduction than those under the exclusion treatment. Additionally, pollinators' contribution varied between

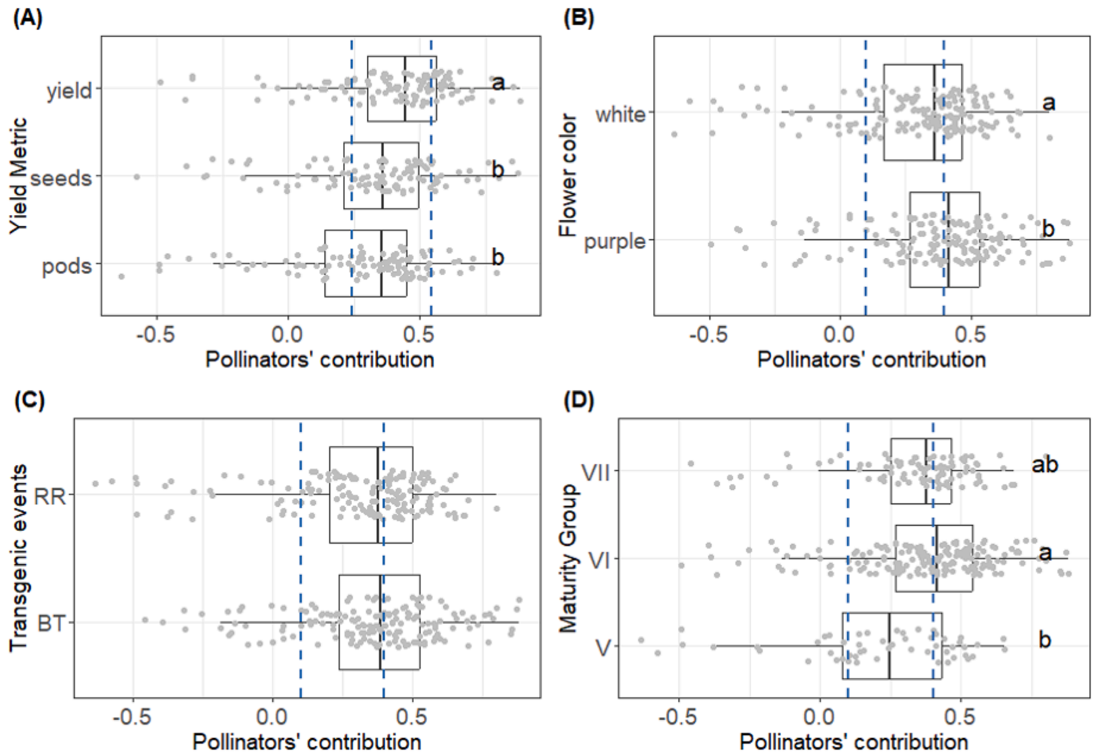

Fig. 4. Heterogeneity in the estimation of pollinators' contribution in soybean (A) using soybean pods, seeds, and yield as metrics for measuring production (i.e., Yield metric), (B) evaluating cultivars with different flower colors (i.e., Flower color), (C) evaluating different genetic modifications (i.e., Transgenic events), and (D) evaluating different maturity groups (i.e., Maturity group). Significant differences according to *post hoc* comparisons are indicated by letters (i.e., 'a', and 'b). Each box represents 50 % of the central data, the line within the box indicates the median, and the error bars are the minimum and maximum values (without outliers).

soybean traits, such as flower color and maturity group. The variability in this contribution across locations suggests that spatial context and management also affect pollinator abundance, thereby impacting production.

In this study, pollinators' contribution to most soybean cultivars was 'modest'; specifically, the absence of pollinators decreased production between 10 and 40 %. The variability between cultivars has been attributed to differences in the genetic background of the lines (Ortiz-Perez et al., 2006), nectar composition (Severson et al., 1987), and/or flower characteristics related to their attractiveness to flower visitors (Erickson, 1975; Robacker et al., 1983). Moreover, intra-specific variation in the capacity of self-pollination has been recently described for crops such as beans (Franceschinelli et al., 2022) and soybean cultivated over a wide latitudinal gradient (da Cunha et al., 2023). Our study, shows that pollinators' contribution within soybean is variable. Interestingly, we found that this variability differs among traits.

Soybean cultivars have white or purple flowers. Flower color is a signal of attractiveness that can increase male or female function (Chittka & Raine, 2006; Malerba & Nattero, 2012) and the differential preference of pollinators for some colors in crops such as alfalfa and radish has been previously demonstrated (e.g. Clement, 1965; Lee & Snow, 1998). In soybean, Erickson (1975) found that cultivars with white flowers were more attractive to honeybees than purple ones. Severson & Erickson (1984) studied the preference of foraging honey bee on 17 cultivars from Missouri and found that nectar characteristics vary widely between cultivars, but this variability was not related to the flower color. In this study, we found that the difference in the number of seeds between open and bagged flowers in purple flower cultivars was higher than in cultivars with white flowers, suggesting that purple flowers may be more attractive to insects than white ones. Nowadays, the development of new cultivars and the widespread cultivation necessitate an updated review. Thus, our results need to be confirmed with studies on nectar composition and flower visitation by pollinators.

The classification of soybean cultivars into maturity groups considers the photoperiod and temperature sensitivity of this species and thus reflects the environmental adaptability of a given cultivar. Song et al. (2019) found that maturity groups might differently interact with insect pollinators. Differences in the attraction of other insects according to the maturity groups have been found for insect defoliators, stink bugs, and predators, which are differently affected by the use of early vs. late maturity groups (Baur et al., 2000; Gore et al., 2006; McPherson et al., 1998, 1996). For instance, in the southern region of the United States, maturity groups differentially affected the density of the beetle *Epilachna varivestis* and *Anticarsia gemmatalis* caterpillars, resulting in a lower infestation in early maturity groups (McPherson et al. 1996). Thus, the differences in phenology and timing of maturity groups can affect the seasonal abundance of insects considered pests within the crop (McPherson et al., 1996), but there are no studies on the attractiveness of different soybean maturity groups to pollinators. Nectar production, a trait related to the attractiveness to pollinators, was found to differ between maturity groups, as the quantity of sugar per flower differed between early and later maturity group cultivars (Smith 2018). Regarding maturity groups, Erickson (1984) concluded that soybeans grown under the most optimal conditions produce higher quality and quantity of nectar as compared to poorer growing conditions. Thus maturity group seems to be a trait of soybean cultivars that captures differences in nectar sugar production along with the timing of flowering, which can affect both the attractiveness and the overlap between the flowering of the cultivar and pollinators' availability.

Cultivars with the Bt technology are designed to require fewer pesticides than RR (Coupe & Capel, 2016; Grossi-de-Sa et al., 2011). Therefore, we anticipated that fields cultivated with Bt cultivars would exhibit higher pollinator abundance and a more pronounced effect of pollinators on production. However, we found that, in Bt cultivars, the differences between pollination treatments were not greater than those observed in RR cultivars. Comparisons on the impact of Bt crops vs.

non-Bt crops on the abundance and diversity of non-target arthropods in cotton (Cattaneo et al., 2006) and soybean (Marques et al., 2018; Yu et al., 2014) have shown minimal differences. However, the evidence remains limited, and there have been no assessments regarding pollinator communities. In contrast, the effects of herbicides, which are used in Bt or RR cultivars, on pollinator fauna are clearer, showing a negative impact on pollinators' abundance and richness (Cattaneo et al., 2006; Morandin & Winston, 2005). Recently, Oliveira and Fernandes (2020) compared the pollination deficit for a RR and a Bt soybean cultivar and found that the RR cultivar had a higher pollination deficit than the Bt cultivar, and they interpreted that this difference was due to the higher presence of caterpillars in the RR cultivar. Our results, which included more cultivars, did not show differences in pollination deficit between RR and Bt. More detailed studies considering agrochemical levels and identity in the fields are needed to disentangle this issue.

Our estimation of pollinators' contribution varied significantly depending on the metrics used to estimate production, and notably, it was higher when yield was considered. Similar variations in pollinators' contribution associated with different production measures have been reported for other species showing a similar pattern (e.g., bean in Bishop and Nakagawa, 2021), as well as within soybean (Huais et al. 2020; da Cunha et al., 2023). Our higher estimated pollinators' contribution when we considered yield may indicate that pollination not only affects the number of seeds, but also their weight. Seed weight is also an indicative of enhanced pollination quality (e.g. Bommarco et al. 2012) and undoubtedly has relevance for the production perspective.

We acknowledge that our study had some experimental limitations. Firstly, our results are based on the assumption that our 'bagged' treatment only restricted the access of flower visitors without affecting any other variable (e.g., light availability, air temperature) that could have influenced the number of pods or seeds in soybean plants. However, we would like to emphasize that we followed standard procedures (Kearns & Inouye, 1993) that are widely used to estimate pollination deficits in crops (Klein et al., 2007). Moreover, Santone et al. (2022) reported that shading experiments in soybean do not affect plant performance. Secondly, in this study we did not record insect visits to flowers, but they were frequently seen on the flowers (see Appendix A: Fig. A.1). Lastly, we did not achieve a completely balanced design between cultivars, for instance, not all combinations of traits were found in the field (i.e. there were three cultivars in the maturity group VII, one of them RR and two Bt, and they all had white flowers, Table 1). However, it was not possible to select farms based on their cultivars' traits, primarily because the choice of which cultivar to plant depends on many factors, especially market dynamics. With over a thousand cultivars available, a varying subset of them are planted in different years and localities. In this sense, we made a significant effort to sample a diverse range of cultivars each year. Probably a greenhouse experiment could help to disentangle these differences.

The increment in soybean production that might be achieved with insect pollination seems to rely on complex interactions between the abiotic conditions of a given region, landscape configuration, and the cultivated cultivars, probably acting in a hierarchical manner. The impact of insect visits to soybean flowers has been indirectly assessed by using beehives, which increased the yield (de O. Milfont et al. 2013) or by adding pollinators habitats (Levenson et al. 2022), which in both cases increased production. However, their direct impact through measures on pollen deposition on flowers is still missing. Some studies reported differences in pollinators' contribution across years and locations (Blettler et al., 2018; Ortiz-Perez et al., 2008), indicating that the effect of insect visitors on soybean flowers is context-dependent. In our study, the variability in pollinators' contribution between years was lower than between localities. Erickson (1984) suggested that in cool climates soybean flowers are mostly cleistogamous, with little or no amount of nectar, and Blettler et al. (2018) stated that the effect of honeybees on seed set is increased under favorable weather conditions. The most parsimonious hypothesis is that context-dependent variability in pollinators' contribution can be attributed to differences in the availability of pollinators in the surrounding landscape, local management, differences in nectar quantity and quality of cultivars, and variability in the level of autogamy of the cultivars.

Concluding remarks

Crop cultivars exhibit significant variability, with the same species adapted to different climates, soil, and biotic conditions. In our study, we found that pollinators' contribution varies across locations, and, interestingly, certain cultivar traits associated with climate adaptation and insect attraction explained part of this heterogeneity.

More than half of the global soybean production comes from South America, with Brazil and Argentina being the second and third global producers, respectively (Song et al. 2021). Our results suggest that insects can contribute to the production of soybean cultivars commonly cultivated in the Chaco region of Argentina. Pollination could be enhanced through management practices that encourage pollinators. For instance, managing soybeans with honeybees has been shown to increase yield in many cultivars and regions (e.g. Blettler, Fagúndez, & Caviglia, 2018; Chiari et al., 2005). Additionally, other insects can also improve soybean yield (Gill & O'Neal, 2015; Monasterolo et al., 2015; Santone et al. 2022).

The conservation of native forest fragments within the agricultural fields as mandated by national and provincial laws, may help maintain native pollinators that can enhance crop pollination (Zelaya et al., 2018). This practice can also support the pollination of native species from the Chaco forest, contributing to the maintenance of both crop and forest.

Availability of data and materials

The datasets used during the current study are available from the corresponding author upon request.

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CRediT authorship contribution statement

Natacha P. Chacoff: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Julieta Carrasco:** Writing – review & editing, Methodology, Investigation. **Silvio E. Castillo:** Writing – review & editing, Methodology, Investigation, Formal analysis. **A. Carolina Monmany Garzia:** Writing – review & editing, Investigation, Conceptualization. **Lucía Zarbá:** Writing – review & editing, Data curation, Conceptualization. **Roxana Aragón:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.baae.2024.10.002.

References

Ahrent, D. K., & Caviness, C. E. (1994). Natural cross-pollination of twelve soybean cultivars in Arkansas. *Crop Science, 34*(2), 376–378. https://doi.org/10.2135/cropsci1994.0011183X003400020013x

Barton, K., & Barton, M. K. (2015). Package 'mumin'. *Version, 1*(18), 439.

Bates, D., Mächler, M., Bolker, B., & Walker, S (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Baur, M. E., Boethel, D. J., Boyd, M. L., Bowers, G. R., Way, M. O., Heatherly, L. G., Rabb, J., & Ashlock, L. (2000). Arthropod Populations in Early Soybean Production Systems in the Mid-South. *Environmental Entomology, 29*(2), 312–328. https://doi.org/10.1093/EE/29.2.312

Bishop, J., Garratt, M. P. D., & Breeze, T. D. (2020). Yield benefits of additional pollination to faba bean vary with cultivar, scale, yield parameter and experimental method. *Scientific Reports, 10*(1), 1–11.

Bishop, J., & Nakagawa, S. (2021). Quantifying crop pollinator dependence and its heterogeneity using multi-level meta-analysis. *Journal of Applied Ecology, 58*(5), 1030–1042. https://doi.org/10.1111/1365-2664.13830

Blanco, C. A., Chiaravalle, W., Dalla-Rizza, M., Farias, J. R., García-Degano, M. F., Gastaminza, G., Mota-Sánchez, D., Murúa, M. G., Omoto, C., Pieralisi, B. K., Rodríguez, J., Rodríguez-Maciel, J. C., Terán-Santofimio, H., Terán-Vargas, A. P., Valencia, S. J., & Willink, E. (2016). Current situation of pests targeted by Bt crops in Latin America. *Current Opinion in Insect Science, 15*, 131–138. https://doi.org/10.1016/j.cois.2016.04.012

Blettler, D. C., Fagúndez, G. A., & Caviglia, O. P. (2018). Contribution of honeybees to soybean yield. *Apidologie, 49*(1), 101–111. https://doi.org/10.1007/s13592-017-0532-4

Bommarco, R., Marini, L., & Vaissière, B. E. (2012). Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia 2012 169:4, 169*(4), 1025–1032. https://doi.org/10.1007/S00442-012-2271-6

Cattaneo, M. G., Yafuso, C., Schmidt, C., Huang, C., Rahman, M., Olson, C., Ellers-Kirk, C., Orr, B. J., Marsh, S. E., Antilla, L., Dutilleul, P., Carriere, Y., & Carrière, Y. (2006). Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. *Proceedings of the National Academy of Sciences of the United States of America, 103*(20), 7571–7576. https://doi.org/10.1073/pnas.0508312103

Chacoff, N. P., & Aizen, M. A. (2007). Pollination requirements of pigmented grapefruit (Macf.) from Northwestern Argentina. *Crop Science, 47*(3), 1143. https://doi.org/10.2135/cropsci2006.09.0586

Chiari, W. C., Hoffmann-Campo, C. B., Arias, C. A., Lopes, T., da, S., Arnaut De Toledo, T., Chambó, E., Ruvolo- Takasusuki, M. C., & Arnaut de Toledo, V. (2013). Floral Biology and Africanized Honeybee Behaviour in Transgenic (Roundup ReadyTM var. BR-245 RR) and Conventional (var. BRS-133) Soybean (Glycine max L. Merrill) Flowers. *Herbicides - Advances in Research*, 277–298. https://doi.org/10.5772/55847

Chiari, W. C., Toledo, V. D. A. A. D., Ruvolo-Takasusuki, M. C. C., Oliveira, A. J. B. D., Sakaguti, E. S., Attencia, V. M., … Mitsui, M. H. (2005). Pollination of soybean (Glycine max L. Merril) by honeybees (Apis mellifera L.). *Brazilian archives of biology and technology, 48*, 31–36. https://doi.org/10.1590/S1516-89132005000100005

Chittka, L., & Raine, N. E. (2006). Recognition of flowers by pollinators. *Current Opinion in Plant Biology, 9*(4), 428–435. https://doi.org/10.1016/j.pbi.2006.05.002. Elsevier Current Trends.

Clement, W. M. (1965). Flower color, a factor in attractiveness of alfalfa clones for honey bees. *Crop Science, 5*(3), 267–268. https://doi.org/10.2135/cropsci1965.0011183x000500030023x

Coupe, R. H., & Capel, P. D. (2016). Trends in pesticide use on soybean, corn and cotton since the introduction of major genetically modified crops in the United States. *Pest Management Science, 72*(5), 1013–1022. https://doi.org/10.1002/ps.4082

da Cunha, N. L., Chacoff, N. P., Sáez, A., Schmucki, R., Galetto, L., Devoto, M., ..., & Aizen, M. A. (2023). Soybean dependence on biotic pollination decreases with latitude. *Agriculture, Ecosystems & Environment, 347*, Article 108376.

Cunningham-Minnick, M. J., Peters, V. E., & Crist, T. O. (2019). Nesting habitat enhancement for wild bees within soybean fields increases crop production. *Apidologie, 50*(6), 833–844. https://doi.org/10.1007/s13592-019-00691-y

Dardanelli, J. L., Balzarini, M., Martínez, M. J., Cuniberti, M., Resnik, S., Ramunda, S. F., ..., & Baigorri, H. (2006). Soybean maturity groups, environments, and their interaction define mega-environments for seed composition in Argentina. *Crop Science, 46*(5), 1939–1947.

de, O., Milfont, M., Rocha, E. E. M., Lima, A. O. N., & Freitas, B. M. (2013). Higher soybean production using honeybee and wild pollinators, a sustainable alternative to pesticides and autopollination. *Environmental Chemistry Letters 2013 11:4, 11*(4), 335–341. https://doi.org/10.1007/S10311-013-0412-8

Erickson, E. H. (1984). Research Notes: United States: Soybean floral ecology and insect pollination. *Soybean Genetics Newsletter*, (1), 11. https://lib.dr.iastate.edu/soybeangenetics/vol11/iss1/50.

e Silva, F. D. D. S., Ramos, D. D. L., Mertens, F., & Carvalheiro, L. G. (2023). Native pollinators improve the quality and market value of common bean. *Agriculture, Ecosystems & Environment, 349*, 108432. https://doi.org/10.1016/j.agee.2023.108432

Erickson, E. H. (1975). Effect of Honey Bees on Yield of Three Soybean Cultivars. *Crop Science, 15*(1), 84–86. https://doi.org/10.2135/cropsci1975.0011183X001500010025x

Erickson, E. H., & Garment, M. B. (1979). Soya-bean flowers: Nectary ultrastructure, nectar guides, and orientation on the flower by foraging honeybees. *Journal of Apicultural Research, 18*(1), 3–11. https://doi.org/10.1080/00218839.1979.11099935

Franceschinelli, E. V., Ribeiro, P. L. M., Mesquita-Neto, J. N., Bergamini, L. L., Madureira de Assis, I., Elias, M. A. S., Fernandes, P. M., & Carvalheiro, L. G. (2022). Importance of biotic pollination varies across common bean cultivars. *Journal of Applied Entomology, 146*(1–2), 32–43. https://doi.org/10.1111/JEN.12951

Garibaldi, L. A., Schulte, L. A., Nabaes Jordar, D. N., Gomez Carella, D. S., & Kremen, C. (2021). Time to Integrate Pollinator Science into Soybean Production. *Trends in Ecology & Evolution, April, 394*. https://doi.org/10.1016/j.tree.2021.03.013

Garratt, M. P., de Groot, G. A., Albrecht, M., Bosch, J., Breeze, T. D., Fountain, M. T., ..., & Zhusupbaeva, A. (2021). Opportunities to reduce pollination deficits and address production shortfalls in an important insect-pollinated crop. *Ecological applications, 31*(8), e02445.

Giannini, T. C., Cordeiro, G. D., Freitas, B. M., Saravia, A. M., & Imperatriz-Fonseca, V. L. (2015). The dependence of crops for pollinators and the economic value of pollination in Brazil. *Journal of Economic Entomology, 108.3*, 849–857.

Gill, K. A., & O'Neal, M. E (2015). Survey of soybean insect pollinators: Community identification and sampling method analysis. *Environmental Entomology, 44*(3), 488–498. https://doi.org/10.1093/ee/nvv001

Gore, J., Abel, C. A., Adamczyk, J. J., & Snodgrass, G. (2006). Influence of soybean planting date and maturity group on stink bug (heteroptera: pentatomidae) populations. *Environmental Entomology, 35*(2), 531–536. https://doi.org/10.1603/0046-225X-35.2.531

Grossi-de-Sa, M. F., Pelegrini, P. B., & Fragoso, R. R. (2011). Genetically modified soybean for insect-pests and disease control. *Soybean - Molecular Aspects of Breeding*, 429–452. https://doi.org/10.5772/16109

Huais, P. Y., Grilli, G., Amarilla, L. D., Torres, C., Fernández, L., & Galetto, L. (2020). Forest fragments influence pollination and yield of soybean crops in Chaco landscapes. *Basic and Applied Ecology, 48*, 61–72. https://doi.org/10.1016/j.baae.2020.09.003

Hudewenz, A., Pufal, G., Bögeholz, A. L., & Klein, A. M. (2014). Cross-pollination benefits differ among oilseed rape varieties. *The Journal of Agricultural Science, 152*(5), 770–778.

Innes, N. L. (2006). Global status of commercialized Biotech/GM Crops: 2012. *Experimental Agriculture, 42*(Issue 03).

Kantolic, A. G., & Slafer, G. A. (2007). Development and seed number in indeterminate soybean as affected by timing and duration of exposure to long photoperiods after flowering. *Annals of Botany, 99*(5), 925–933. https://doi.org/10.1093/aob/mcm033

Kearns, C. A., & Inouye, D. W. (1993). *Techniques for pollination biologists*. Colorado, USA: University Press of Colorado.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S.a, Kremen, C., Tscharntke, T., & Vaissiere, B. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society - Biological Sciences (Series B). Biological Sciences, 274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Lee, T. N., & Snow, A. A. (1998). Pollinator preferences and the persistence of crop genes in wild radish populations (Raphanus raphanistrum, Brassicaceae). *American Journal of Botany, 85*(3), 333–339. https://doi.org/10.2307/2446325

Levenson, H. K., Sharp, A. E., & Tarpy, D. R. (2022). Evaluating the impact of increased pollinator habitat on bee visitation and yield metrics in soybean crops. *Agriculture, Ecosystems & Environment, 331*, Article 107901.

Malerba, R., & Nattero, J. (2012). Pollinator response to flower color polymorphism and floral display in a plant with a single-locus floral color polymorphism: Consequences for plant reproduction. *Ecological Research, 27*(2), 377–385. https://doi.org/10.1007/s11284-011-0908-2

Marques, L. H., Santos, A. C., Castro, B. A., Storer, N. P., Babcock, J. M., Lepping, M. D., Sa, V., Moscardini, V. F., Rule, D. M., & Fernandes, O. A. (2018). Impact of transgenic soybean expressing Cry1Ac and Cry1F proteins on the non-target arthropod community associated with soybean in Brazil. *PLOS ONE, 13*(2), Article e0191567. https://doi.org/10.1371/JOURNAL.PONE.0191567

Masuda, T., & Goldsmith, P. D. (2009). World soybean production: Area harvested, yield, and long-term projections. *International Food and Agribusiness Management Review, 12*(4), 143–162. https://doi.org/10.1002/ejoc.201200111

McPherson, R. M., Layoton, R. C., McLaurin, W. J., & Mills, W. A. (1998). Influence of irrigation and maturity group on the seasonal abundance of soybean arthropods. *Journal of Entomological Science, 33*(4), 378–392. https://doi.org/10.18474/0749-8004-33.4.378

McPherson, R. M., Ruberson, J. R., Hudson, R. D., & Jones, D. C. (1996). Soybean maturity group and incidence of velvetbean caterpillars (Lepidoptera: Noctuidae) and Mexican bean beetles (Coleoptera: Coccinellidae). *Journal of Economic Entomology, 89*(6), 1601–1607. https://doi.org/10.1093/jee/89.6.1601

Monasterolo, M., Musicante, M. L., Valladares, G. R., & Salvo, A. (2015). Soybean crops may benefit from forest pollinators. *Agriculture, Ecosystems and Environment, 202* (April), 217–222. https://doi.org/10.1016/j.agee.2015.01.012

Morandin, L. A., & Winston, M. L. (2005). Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecological Applications, 15* (3), 871–881. http://www.esajournals.org/doi/pdf/10.1890/03-5271.

Mourtzinis, S., & Conley, S. P. (2017). Delineating soybean maturity groups across the United States. *Agronomy Journal, 109*(4), 1397–1403. https://doi.org/10.2134/agronj2016.10.0581

Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution, 4*(2), 133–142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Oliveira, F.de, & Fernandes, M. G (2020). Additive Positive Effects of Pollination on Bt and Non-Bt Soybean Cultivars by Honey Bee and Native Flower-Visiting. *Journal of Agricultural Science, 13*(1), 157. https://doi.org/10.5539/jas.v13n1p157

Ortiz-Perez, E., Horner, H. T., Hanlin, S. J., & Palmer, R. G. (2006). Insect-mediated seed-set evaluation of 21 soybean lines segregating for male sterility at 10 different loci. *Euphytica, 152*(3), 351–360. https://doi.org/10.1007/s10681-006-9222-4

Ortiz-Perez, E., Wiley, H., Horner, H. T., Davis, W., & Palmer, R. G. (2008). Insect-mediated cross-pollination in soybean [Glycine max (L.) Merrill]: II. Phenotypic recurrent selection. *Euphytica, 162*(2), 269–280. https://doi.org/10.1007/s10681-007-9612-2

Pascual, U., Balvanera, P., Díaz, S., Pataki, G., Roth, E., Stenseke, M., Watson, R. T., Başak Dessane, E., Islar, M., Kelemen, E., Maris, V., Quaas, M., Subramanian, S. M., Wittmer, H., Adlan, A., Ahn, S., Al-Hafedh, Y. S., Amankwah, E., Asah, S. T., … Yagi, N. (2017). Valuing nature's contributions to people: The IPBES approach. *Current Opinion in Environmental Sustainability, 26-27*, 7–16. https://doi.org/10.1016/j.cosust.2016.12.006

Perrot, T., Gaba, S., Roncoroni, M., Gautier, J. L., Saintilan, A., & Bretagnolle, V. (2019). Experimental quantification of insect pollination on sunflower yield, reconciling plant and field scale estimates. *Basic and Applied Ecology, 34*, 75–84. https://doi.org/10.1016/j.baae.2018.09.005

R Development Core Team, I. (2014). R: A language and environment for statistical computing.

Robacker, D. C., Flottum, P. K., Sammataro, D., & Erickson, E. H. (1983). Effects of climatic and edaphic factors on soybean flowers and on the subsequent attractiveness of the plants to honey bees. *Field Crops Research, 6*(C), 267–278. https://doi.org/10.1016/0378-4290(83)90067-9

Santone, A., Mazzei, M. P., Vesprini, J., Torres, C., Amarilla, L. D., & Galetto, L. (2022). Pollination service and soybean yields. *Acta Oecologica, 116*, Article 103846. https://doi.org/10.1016/J.ACTAO.2022.103846

Severson, D. W. , Erickson, E. H. (1984). Quantitative and Qualitative Variation in Floral Nectar of Soybean Cultivars in Southeastern Missouri. Environmental Entomology, 13 (4), 1091–1096. doi:10.1093/ee/13.4.1091.

Severson, D. W., Nordheim, E.v., & Erickson, E. H. (1987). Variation in nectar characteristics within soyabean cultivars. *Journal of Apicultural Research, 26*(3), 156–164. https://doi.org/10.1080/00218839.1987.11100753

Singh, G. (2010). The soybean: Botany, production and uses, The Soybean: Botany, production and uses. *CABI*. https://doi.org/10.5860/choice.48-5085

Siopa, C., Carvalheiro, L. G., Castro, H., Loureiro, J., & Castro, S. (2024). Animal-pollinated crops and cultivars—A quantitative assessment of pollinator dependence values and evaluation of methodological approaches. *Journal of Applied Ecology, 61* (6), 1279–1288.

Smith, T. M. (2018). Effects of Cultural Practices on Soybean Nectar Production. *Theses and Dissertations, 1727*. https://scholarsjunction.msstate.edu/td/1727.

Song, W., Sun, S., Ibrahim, S. E., Xu, Z., Wu, H., Hu, X., Jia, H., Cheng, Y., Yang, Z., Jiang, S., Wu, T., Sinegovskii, M., Sapey, E., Nepomuceno, A., Jiang, B., Hou, W., Sinegovskaya, V., Wu, C., Gai, J., & Han, T. (2019). Standard cultivar selection and digital quantification for precise classification of maturity groups in Soybean. *Crop Science, 59*(5), 1997–2006. https://doi.org/10.2135/CROPSCI2019.02.0095

Song, P., Hansen, M. C., Potapov, P., Adusei, B., Pickering, J., Adami, M., Lima, A., Zalles, V., Stehman, S. V., Di Bella, C. M., Conde, M. C., Copati, E. J., Fernandes, L. B., Hernandez-Serna, A., Jantz, S. M., Pickens, A. H., Turubanova, S., & Tyukavina, A. (2021). Massive soybean expansion in South America since 2000 and implications for conservation. *Nature Sustainability, 2021*. https://doi.org/10.1038/s41893-021-00729-z

Suso, M. J., Bebeli, P. J., Christmann, S., Mateus, C., Negri, V., Pinheiro de Carvalho, M. A. A., Torricelli, R., & Veloso, M. M. (2016). Enhancing legume ecosystem services through an understanding of plant-pollinator interplay. *Frontiers in Plant Science, 7*(MAR2016), 333. https://doi.org/10.3389/FPLS.2016.00333/BIBTEX

Tamburini, G., Bommarco, R., Kleijn, D., van der Putten, W. H., & Marini, L. (2019). Pollination contribution to crop yield is often context-dependent: A review of experimental evidence. *Agriculture, Ecosystems and Environment, 280*(April), 16–23. https://doi.org/10.1016/j.agee.2019.04.022

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.

Woodcock, B. A., Garratt, M. P. D., Powney, G. D., Shaw, R. F., Osborne, J. L., Soroka, J., Lindström, S. A. M., Stanley, D., Ouvrard, P., Edwards, M. E., Jauker, F., McCracken, M. E., Zou, Y., Potts, S. G., Rundlöf, M., Noriega, J. A., Greenop, A., Smith, H. G., Bommarco, R., …, & Pywell, R. F. (2019). Meta-analysis reveals that pollinator functional diversity and abundance enhance crop pollination and yield. *Nature Communications, 10*(1), 1–10. https://doi.org/10.1038/s41467-019-09393-6

Yu, H., Li, Y., Li, X., & Wu, K. (2014). Arthropod abundance and diversity in transgenic Bt soybean. *Environmental Entomology, 43*(4), 1124–1134. https://doi.org/10.1603/EN13337

Zelaya, P., Chacoff, N., Aragón, R., & Blendinger, P. (2018). Soybean biotic pollination and its relationship to linear forest fragments of subtropical dry Chaco. *Basic and Applied Ecology*. https://doi.org/10.1016/J.BAAE.2018.07.004