Ecología Austral 34:470-476 Diciembre 2024
Asociación Argentina de Ecología

Effects of increasing honeybee densities on sunflower yield components

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ABSTRACT. Domesticated honeybee management (*Apis mellifera*) could be a useful tool to supplement wild pollinators and increase crop productivity. However, it is necessary to know the optimal hive density for each particular crop. Sunflower (*Helianthus annuus*) is the third most produced oil seed crop in the world and is dependent on insect pollination to increase the number of seeds and oil content. We proposed quantifying the *H. annuus* dependency to pollinators and assessing the optimal hive density of honeybees to increase sunflower productivity in 2 ha plots immersed in an extensive soybean matrix in Argentina. We set up a field experiment of five plots with a different number of hives each one (0, 5, 10, 15, 20), assessed fruit set and crop yield of flower heads open and excluded from pollinators and insect abundance. The set and crop yield increased in the heads of open flowers. The crop yield did not present a linear response to the density of the hive, although there could be a positive effect of the intermediate densities. Crop yields were not explained by the abundance of the two most frequently pollinator species, *Astylus atromaculatus* (Coleoptera) and *Apis mellifera*, suggesting that sunflower productivity was limited by resources other than pollen (e.g., the low abundance of rains). Drought conditions could have limited the response of the crop to hive addition and also influenced the high *A. atromaculatus* abundance, which could play a pollinator role in this system. More repetitions (plots and years) are needed to get these results more robust.

[Keywords: *Apis mellifera*, *Astylus atromaculatus*, crop productivity, exclusion of pollinators, fruit set, exposure to pollinators, pan traps, pollination]

RESUMEN. El manejo de abejas melíferas (*Apis mellifera*) podría complementar a los polinizadores silvestres y aumentar la productividad de cultivos. Sin embargo, es necesario conocer la densidad óptima de colmenas para cada cultivo. El girasol (*Helianthus annuus*) es el tercer cultivo oleaginoso más producido en el mundo, y la polinización entomófila aumenta su productividad y su contenido de aceite. Cuantificamos la dependencia de *H. annuus* de polinizadores y evaluamos la densidad de colmena óptima para incrementar su productividad en parcelas de 2 ha, en una matriz extensiva de soja en la Argentina. Establecimos un experimento de campo en 5 parcelas con diferente número de colmenas cada una (0, 5, 10, 15, 20), evaluamos el cuaje de frutos y el rinde en inflorescencias expuestas y excluidas de polinizadores y la abundancia absoluta de insectos. El cuaje y el rinde aumentaron en inflorescencias expuestas. El rinde no respondió linealmente a la densidad de parcelas, aunque podría haber un efecto positivo de densidades intermedias. El rinde no estuvo explicado por la abundancia absoluta de las especies más frecuentes, *Astylus atromaculatus* (Coleoptera) y *Apis mellifera*, lo que sugiere que la productividad está limitada por recursos distintos al polen, como la baja abundancia de precipitaciones. La sequía pudo haber limitado la respuesta del cultivo al agregado de colmenas y también promovido la alta abundancia de *A. atromaculatus*. Es necesario aumentar el número de repeticiones para obtener una mayor robustez de los resultados.

[Palabras clave: *Apis mellifera*, *Astylus atromaculatus*, productividad, exclusión de polinizadores, cuaje de frutos, exposición a polinizadores, trampas plato, polinización]

Editor asociado: Diego Vázquez

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Recibido: 23 de Agosto de 2024
Aceptado: 17 de Julio de 2024

Introduction

Biotic pollination is essential to increase the production of many important fruit and seed crops (Garibaldi et al. 2013; Aizen et al. 2019). However, the intense use of agrochemicals and the loss of natural areas threaten the biodiversity of pollinators in agroecosystems (Staffen Dewenter et al. 2005; Garibaldi et al. 2019). Thus, the management of domesticated honeybee colonies represents a useful tool to supplement the pollination service and increase the management of crops, especially in large fields (Isaacs and Kirk 2010). The productivity gains due to this practice can be significant and depend on the pollinator dependency of the focal crop species or variety and the pre-existing wild pollinator diversity in the managed field. For example, after adding honeybee hives, productivity increased 63% in oil tree peony (*Paeonia ostii*) (Zhang et al. 2022), 40% in kiwifruit (*Actinidia deliciosa*) (Sáez et al. 2019), and 17% in faba bean (*Vicia faba*) crops (Cunningham and Le Feuvre 2013). Nevertheless, an excessive hive load can have detrimental effects on flowers and fruit quality (Sáez et al. 2014). To achieve the maximum efficiency in honeybee management and lowest detrimental effects, it is relevant to know the optimal hive density needed for each crop (Rollin and Garibaldi 2019).

The sunflower, *Helianthus annuus*, is among the top three oilseed crops worldwide in terms of annual production that reaches 45 Mt (Pilorgé 2020), and the cultivated area encompasses over 1520000 ha (FAO 2022). Although some *H. annuus* varieties are partially self-compatible, the number of achenes with seed and the seed oil content often increase with insect pollination (Langridge and Goodman 1974). *H. annuus* flowers offer abundant pollen and nectar as rewards and are visited by wild and managed bees (Chambó et al. 2011; Perrot et al. 2019). Supplementation with managed honeybees could be a good alternative to intensify production, but its benefits can vary between regions and genotypes (Bartual et al. 2018; Mallinger et al. 2019). Therefore, quantifying the contributions of domestic and wild pollinators to this crop can assist farmers in optimizing the use of bee hives. And just as diversity of flower visitors enhanced honeybee movement, being the main factor influencing productivity (Gigante Carvalheiro et al. 2011), we wondered if honeybee hive addition could have an effect on activity of preexisting pollinators.

In a field experiment, we quantified *H. annuus* dependence on pollinators and evaluated the possible effect of the addition of honeybee hive on sunflower productivity and pollinator abundance in an extensive monoculture in Argentina. We compared the productivity of flower heads that were open to vs. excluded from pollinators across five levels of honeybee hives density. Through this work, we hope to contribute with useful knowledge to the recommendation of hive density management in sunflower extensive crops.

Materials and Methods

Study site

We conducted a field experiment in the southern region of the province of Córdoba, Argentina, where the most widespread crops are soybean, wheat, sunflower and maize. The annual mean temperature is 16.0±0.3 °C, with maximum daily mean of 30.7±0.5 °C in summer and minimum of 3.9±0.7 °C in winter. The average annual rainfall is 886.8±52.1 mm, with a monsoonal water regime, and more frequent precipitation during the summer months (December to March, 2001-2021) (SMN Argentina 2023). The year of the experiment, precipitation was 25% lower than average (i.e., 659.0 mm).

Experimental design and field data collection

We selected five 2 ha plots with similar cultivation management, separated by at least 4 km. All plots were sown with sunflower (*Helianthus annuus* var. TOB 3045) in early spring (between middle to late October). This variety is self-compatible and requires 69-72 days from sowing to flowering and 119 days to harvest.

We defined a gradient of hives (i.e., 0, 5, 10, 15 and 20 hives) and randomly allocated each hive level to one of the plots, at the edge of the plot. On one 100 m transect, we bagged four flower heads every 10 m using 1 mm mesh bags of 30x40 cm to exclude pollinators from the stage of reproductive bud until fruit harvest (Supplementary Material-Figure S1). During full bloom (January 2021), two pan trap stations of white, blue and yellow plastic cups containing soapy water were placed at 10, 50 and 100 m from hives for six days in two dates. At harvest time, we collected the four excluded flower heads and other four nearby open flower heads every 10 m, from 10 m to 100 m from hives. In total, we collected 400 flower heads (40 per treatment, 80 per plot), but lost 35% of them due to post-harvest rot.

At the laboratory, we classified the specimens collected by pan traps into morphospecies or species (when possible) and registered its absolute abundance. For each flower head, we calculated fruit set as the number of full achenes (achene with seed) over the total number of achenes (full+vain achenes) and unfertilized flowers (Supplementary Material-Figure S1d). We weighted the total of full achenes per flower head to estimate yield.

Statistical analyses

To assesses pollinator dependency, we applied a generalized linear mixed model (GLMM) with binomial distribution. The response variable was fruit set, the fixed effect was the inflorescence condition and the random effect was the transect sampling points nested on field. We assessed the effect of the linear and quadratic form of hive density and inflorescence condition (open vs. excluded) on yield applying a linear mixed model (LMM). Also, by a LMM, we evaluated the effect of *Apis mellifera* abundance, *Astylus atromaculatus* abundance, and their interaction on yield, as these two species were the flower visitors most frequently captured in pan traps. Both models included as random effect the transect sampling points nested on field. We constructed two separate models because we suspected that hive density influences the abundance of flower visitor species.

Applying GLMM with Poisson distribution, we evaluated the influence of the linear and quadratic form of hive density on *A. mellifera* and *A. atromaculatus* abundance, separately. The response variables were the sum of each species abundance in the three cup colors per sampling point. The random effects, in both models, were the transect nested on sampling date and sampling point nested on transect.

To run the models, we used the lmer and glmer functions of lme4 package for RStudio software (v.4.2.0). In all models with more than one fixed effect, we compared all possible combinations of them, through a multi-model inference approach, and choose the best models based on the corrected Akaike information criterion (AICc) (Harrison et al. 2018) using the dredge function of the MuMin package (Barton 2019). The estimated effects that were at least twice their standard error were considered as significant effects.

Results

The set for open pollination condition was 64% higher than the excluded condition (Figure 1, Supplementary Material-Table S1). For the first model tested for yield, the best ranked model (the lowest AICc) included the linear and quadratic form of hive density, and inflorescence condition as explanatory variables (Supplementary Material-Table S2). The quadratic form of hive density affected yield (negative estimate), but not the linear form. The inflorescence condition also had an effect. Yield was 2.04 times greater in flower heads open to pollinators than excluded (Figure 2, Supplementary Material-Table S1). For the second model tested for yield, the best model did not include the evaluated predictors, which were the abundance of *A. mellifera* and *A. atromaculatus* and their interaction (Supplementary Material-Table S2).

Pan-trap captures were mainly represented by *A. atromaculatus*, a wild Coleoptera

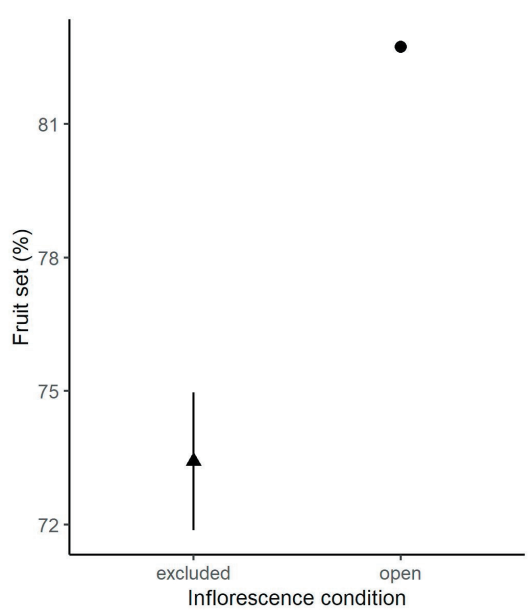

Figure 1. Fruit set (proportion of full fruits in relation with total full and vain fruits and unfertilized flowers) produced by flower heads excluded and open to pollinators. Points indicate mean values and bars standard error (SE).

Figura 1. Cuaje de frutos (proporción de frutos completos en relación con frutos completos y vanos y flores no fertilizadas) producidos por capítulos excluidos y abiertos a polinizadores. Los puntos indican valores medios y las barras los errores estándar (SE).

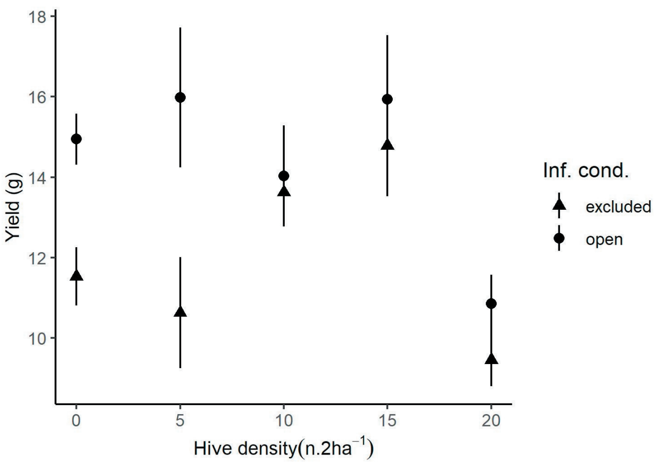

Figure 2. Yield (full achenes total weight per flower head) produced by flower heads excluded and open to pollinators, in relation with number of hive: 0, 5, 10, 15, 20 in each 2 ha plot. Points indicate mean values and bars standard error (SE).

Figura 2. Rendimiento (peso del total de aquenios completos por inflorescencia) producidos por capítulos excluidos y abiertos a polinizadores, en relación con el número de colmenas: 0, 5, 10, 15 y 20 en cada parcela de 2 ha. Los puntos indican valores medios y las barras los errores estándar (SE).

(Melyridae; 56%) and *A. mellifera* (Hymenoptera, Apidae; 40%). The less common insects (~4%) were mainly 'black fly' (Diptera, Simuliidae), *Cyclocephala signaticollis* (Coleoptera, Scarabaeidae), *Agapostemon* sp. (Hymenoptera, Halictidae), *Spintherophyta* sp. (Coleoptera, Chrysomelidae) and *Nysius simulans* (Hemiptera, Lygaeidae), among others even less represented, as Lepidoptera, Neuroptera and Blattodea (Figure 2S). The best models (i.e., lowest AICc) that explained the abundance of *A. mellifera* and *A. atromacultaus* included the linear and quadratic form of the density of the hive (Supplementary Material-Table S2). The abundance of *A. mellifera* and *A. atromaculatus* were affected by the linear and quadratic form of hive density (Figures 3, Supplementary Material-Table S1). The abundance of *A. mellifera* linearly increased by 19% with hive density, and the quadratic effect was slightly concave. *A. atromaculatus* abundance linearly decreased by 8.5% with hive density, and the quadratic effect was convex.

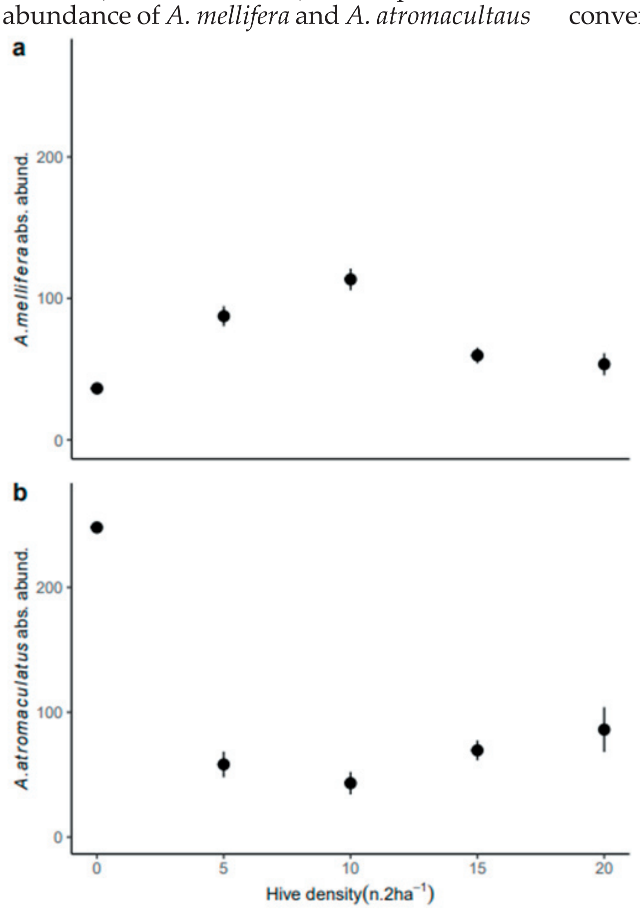

Figure 3. Abundance of a) *Apis mellifera* and b) *Astylus atromaculatus* found in pan traps in relation with number of bee-hives: 0, 5, 10, 15, 20, in 2 ha plots. Points indicate mean values and bars SE.

Figura 3. Abundancia de a) *Apis mellifera* y b) *Astylus atromaculatus* encontradas en trampas de plato en relación con el número de colmena: 0, 5, 10, 15, 20, en lotes de 2 ha. Los puntos indican valores medios y las barras SE.

Discussion

The management of domesticated honeybees is a valuable practice to intensify productivity, especially in large monocultures (Pitts-Singer et al. 2018). Here, we explored the pollinator dependency of sunflower, *H. annuus*, and the optimal number of *A. mellifera* hives to increase productivity. Our results showed that *H. annuus* (var. TOB 3045) is capable of producing fruits without external pollination, since 76% of excluded flowers bore fruit. However, the fruit set increased by 64% and the yield doubled when flower heads were open to pollinators. Despite the improvement in open pollinator condition, fruit set was lower than 100%, suggesting that other factors such as edaphic and climatic conditions could be limiting fruit formation (Mercau et al. 2001). In particular, the previous two years and the experiment season were drier than usual, since no rain was recorded during the months before sowing (May-August 2020) (SMN Argentina 2023). Therefore, it is expected that in better conditions the yield increase will be greater than that observed, which may represent an important impact in the economic profit of farmers.

Contrary to our expectations, we did not detect a linear effect of hive density on sunflower yield. However, we did observe a quadratic effect on yield, and the negative sign of the coefficient suggests maximum yield values at intermediate hive densities. But this trend is not very well reflected graphically, possibly because the sample size is not representative enough. This result agrees with the observed by Sáez et al. (2014), at low hive densities optimal pollination is not achieved. However, at very high hive densities the flowers can be damaged by receiving several honey-bees visits, which impairs fruit formation. Otherwise, neither the abundance of *A. mellifera* nor *A. atromaculatus* explains the sunflower yield. Measurements of floral visits could be the missing link to understanding how to improve productivity. Nevertheless, very good edaphic conditions of plots with intermediate hive densities could be explaining the higher yield. Once again, increasing the number of repetitions could shed light on these results.

One of the main consequences of agroecosystem simplification is the loss of biodiversity due to limited available resources (Tscharntke et al. 2005), and this was reflected in our study. Only two species represented 96% of pan trap captures in all sampled plots. Even in plots with no hive addition the abundance of *A. mellifera* was very high, suggesting the presence of feral individuals. In other studies, *A. mellifera* had been recorded as the most frequent sunflower visitor (Torretta et al. 2010; Zaragoza-Trello et al. 2023). The high abundance of honey-bees could be explained by both historical and experimental management of hives. On the contrary, the high predominance of *A. atromaculatus* could be attributed to adverse environmental conditions, resulting from the scarcity of rainfall, limiting the population growth of competitor species. *Astylus atromaculatus* is known to be a pollen consumer (Human and Nicolson 2003), although its role as a pollinator has not been widely disseminated, several studies had shown that it could be an unexpected but efficient pollinator. It can load as much pollen as *A. mellifera*. in cotton (Pierre and Hoffs 2010), but also in sunflowers (Schlickmann-Tank and Enciso Maldonado 2023). Similarly, our results on the abundance of the two dominant species in relation to hive supply indicate that there could be an antagonism between them. However, the high abundance of *A. atromaculatus* in the months of our experiment possibly has a direct relationship with the recorded drought. In the region, increases in cattle mortality due to intoxication from a high presence of *A. atromaculatus* in the forage were observed during population outbreak seasons (García et al. 2023). Although we did not observe any positive or negative effects of *A. atromaculatu*s on sunflower yield, studying the factors that influence population outbreaks could be very relevant for agricultural and livestock activities in the region.

Our outcomes show pollinators are key for fruit formation and increase yield in sunflower crops. The quadratic effect of hive density suggests there is an optimal of hive density, at intermediate levels, to enhance yield. However, increasing the repetitions and the levels of hive density is needed to better visualize the optimal, separate ecological results from climatic events and edaphic conditions of plots, and make recommendations to farmers and beekeepers. The high predominance of only two species captured by pan traps is very alarming, and it should be a wake-up call to care for biodiversity in extensive crops. Enhancing biodiversity is important not only for species conservation, but also for increasing productivity considering the crop's dependence on pollinators. Manage sunflower fields and their surrounding crops to maintain a high abundance of wild pollinator populations could increase sunflower productivity. Thus, fertilization (and nutrient availability), soil properties and climatic conditions may impact the development and determine the final number of flowers and fruits, indirectly influencing crop yield (Mercau et al. 2001).

Acknowledgements. We thank the owners of the fields for allowing us to carry out the experiment, to those who help setting the experiment at the field and with flower head processing at the laboratory, and Edilberto Mielgo for his hives.

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