Article

EFFECT OF HONEY BEES (*APIS MELLIFERA*) AND WILD INSECT POLLINATION ON RAPESEED (*BRASSICA NAPUS*) YIELD IN THE ARGENTINE PAMPAS: RESULTS OF A CAGING EXPERIMENT

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Abstract—Rapeseed (*Brassica napus*) can benefit from insect pollination to maximize yield. The Argentine Pampas represent one of the most intensively cultivated regions of the world, characterized by landscape homogenization and a reduced availability of natural habitats for wild pollinators. These conditions provide a unique context to explore the contribution of managed and wild pollinators to rapeseed (*Brassica napus*) yield under highly intensified agricultural systems. The objective of this study was to evaluate the influence of *Apis mellifera* and wild insect pollinators on quantitative and qualitative components of rapeseed seed yield in fields of the Argentine Pampas, using a caging experiment. Three pollination treatments were applied: open pollination, pollinator exclusion, and *A. mellifera*-only. Compared to the exclusion treatment, pollination exclusive by *A. mellifera* increased crop yield by 33%, whereas open pollination resulted in a 20% increase. These differences in total yield were mainly explained by increases in quantitative components, such as number of seeds *per* fruit and fruits *per* plant. Seed physiological quality (ripeness, germination power and vigor) followed the same pattern among treatments. Our findings emphasize the importance of adjusting agricultural practices to promote the conservation of wild pollinators in rapeseed crops, and suggest that supplemental pollination with *A. mellifera* could serve as a useful tool for enhancing crop production under intensive management systems.

Keywords—Insect pollination, canola, apiculture, crop production, sustainability

INTRODUCTION

Insect pollinators provide an essential ecosystem service in both natural and agricultural ecosystems. Many plant species rely on insect pollination for successful reproduction (Garibaldi et al. 2013; Aizen et al. 2020), and it is estimated that over 70% of the cultivated crops depend on insect pollinators (Klein et al. 2007). However, over the last decades, the diversity and abundance of insect pollinators have declined across many agricultural environments (Sarkar & Ganguly 2024). It remains unclear whether these declines lead to reduced crop yields or if they are compensated by managed pollinators, such as honey bees (Garibaldi et al. 2013).

Rapeseed (*Brassica napus* L.) is the second most important oilseed crop worldwide (Araneda et al. 2010). The oil is regarded as a high-quality product for human consumption because of its low erucic acid content (Mandal et al. 2002; Aytac et al. 2006). Additionally, it is used as a feedstock for biofuel production (Mazzilli et al. 2016). Although the rapeseed is self-fertile, its flower is very attractive to insects, achieving cross-pollination rates of up to 30% (Chambó et al. 2014; Mazzilli et al. 2016; Ouvrard et al. 2017; Hevia et al. 2018). Studies conducted in different regions of the world have shown that insect pollination can increase rapeseed yield by up to 50% both in terms of quantity and quality (Rosa et al. 2011; Bommarco et al. 2012; Stanley et al. 2013; Chambó et al. 2014; Ouvrard et al. 2017). In regard to the yield components of rapeseed, a meta-analysis pointed to the number of seeds and fruits *per* plant as the most positively affected by insect pollination, though there is no consensus on its impact on seed weight, ripeness, germination power or seed vigor (Yang et al. 2024).

In South America, the area cultivated with rapeseed exceeded 300,000 ha in 2022 and is expected to expand over the next decade (D'Angelo et al. 2023). Despite this tendency, studies assessing the effect of wild insect pollinators and *A. mellifera* on rapeseed yield remain scarce (Chambó et al. 2014; Mazzei et al. 2021). In Brazil, Chambó et al. (2014) compared the effects of open pollination by wild insects with exclusive pollination by *A. mellifera* and found that both treatments have positively influenced quantitative (e.g. seeds *per* fruit) and qualitative (e.g. germination power) rapeseed yield components. In Argentina, Mazzei et al. (2021) analyzed the effect of open pollination on quantitative yield components and reported significant increases compared with an insect-exclusion treatment. However, these authors did not assess the qualitative components of the seeds. To date, no studies in South America have simultaneously evaluated the effects of open pollination and exclusive *A. mellifera* pollination on both quantitative and qualitative components of rapeseed yield. This information is of particular interest as *Brassica* crops (e.g. *B. rapa*, *B. campestris*, *B. carinata*, *B. napus*) often achieve higher yields and seed quality under conditions of high pollinator abundance (Atmowidi et al. 2007; Stanley et al. 2013; Rijal et al. 2018; Stiles et al. 2021). Furthermore, exploring whether supplementation with *A. mellifera* can enhance rapeseed yield in this agricultural region of the Argentine Pampas may serve to adjust beekeeping and agricultural practices for maximizing rapeseed yield through pollination services.

The present study aimed to evaluate the individual and combined effects of *A. mellifera* and wild insect pollinators on reproductive parameters and seed quality of rapeseed in the Argentine Pampas. Understanding these effects is critical for maximizing crop productivity while supporting ecosystem stability through pollination services.

MATERIALS AND METHODS

STUDY AREA

This study was conducted over a two-year period (2021–2022) in Buenos Aires Province (34°15'00" S 59°28'00" W; 34°24'00" S 59°48'00" W), Argentina. Rapeseed (*Brassica napus* L. var. oleifera) crops were located in two fields separated by a distance of 8 km, one placed in an agricultural habitat with pesticide application, and the other in a semi-natural habitat without pesticide application (Concejo Delibertante de San Antonio de Areco 2014) (Supplementary Fig. 1). The soil of both fields was classified as Typic Argiudolls (INTA 2023). Weather conditions data were registered during the progress of the crop cycle at the nearby Experimental Station of San Antonio de Areco.

EXPERIMENTAL DESIGN

In each field, twelve 3 x 2-m plots were sown with the variety of *Brassica napus* L. 'Nuvette 2286' (Nuseed). Sowing was carried out in May at a seed density of 4 kg ha^{-1}. The plots were separated by 1 m and the rows within each plot by 0.2 m. Thinning was carried out at the two- to four-leaf stage to obtain a uniform density of 60 plants m^{-2} (Tomm et al. 2009).

Flowering began between August 5 and 15 and ended between September 15 and 25. Prior to this period, plots were randomly assigned to three pollination treatments (N = 4 each): (1) open, consisting of plots freely visited by insects; (2) exclusion, consisting of plots with insect exclusion cages; and (3) *A. mellifera* only, consisting of plots with insect exclusion cages containing a mini-nucleus of *A. mellifera* (Supplementary Fig. 2).

The exclusion cages (2 x 3 x 2 m) were made of 1.4 x 1.4-mm nylon mesh supported by 20-mm wide metal tubes. They were set up 5 d before flowering and removed at its end to allow the complete development of the plants (Chambó et al. 2014). At the beginning of flowering, the plots assigned to the *A. mellifera*-exclusive pollination treatment ("*A. mellifera* only") were each provided with a mini-nucleus colony containing three frames -two with brood and one with pollen and honey- and one feeder. Considering that each mini-nucleus frame represents approximately one quarter of a standard Langstroth deep frame, which typically holds 2,000–2,500 adult bees (Delaplane et al. 2013), the three-frame mini-colonies used in this study contained an estimated 2,100 adult bees. During the flowering period, mini-nuclei were supplied with water and 0.5 L of 50% sugar syrup (Free 1993).

Plots were manually harvested between October 10 and 15.

Estimated Parameters

Quantitative yield

We measured several quantitative components: fruit set, fruits *per* plant, seeds *per* fruit and 1000 seeds weight, for each pollination treatment (open, exclusion, *A. mellifera* only). Briefly, to estimate the fruit set (percentage of flower buds that develop into fruits), six plants *per* plot were randomly selected. From each plant, one inflorescence with 10 opening flowers were identified prior to flowering, and the number of fruits was recorded at maturity (Montaldo et al. 1996).

To measure the rest of the yield components, ten plants were randomly harvested from each plot. The mean values of: (1) fruits *per* plant; (2) seeds *per* fruit; and (3) 1000 seeds weight, were estimated following the methodology described by Chambó et al. (2014). Seed moisture was determined using the stove method (SAGPyA 1994; Stamm et al. 2013) and varied between 8 and 10%.

To estimate plants *per* m^2 two-1 m^2 samples were harvested *per* plot and the number of plants was counted. Similarly, to estimate seed yield (kg of seeds ha^{-1}), two-1 m^2 samples were harvested *per* plot, and the seeds were cleaned, sieved and weighed. The weight of the seeds was corrected by the percentage of moisture (Tomm et al. 2009). The seed yield was averaged *per* plot (kg m^{-2}) and expressed as kg ha^{-1} (Bommarco et al. 2012).

Qualitative performance

To determine the effect of the three pollination treatments (open, exclusion, *A. mellifera* only) on the qualitative components of seed yield, three plants *per* plot were used as sampling units. Percentage of ripe seeds was estimated as the number of ripe seeds relative to the total number of seeds *per* plant. Vigor (percentage of germinated seeds after three days of incubation) and germination power (percentage of germinated seeds after seven days of incubation) were calculated from 25 seeds *per* plant following the methodology described by ISTA (2018). To estimate flower longevity, the flowers selected for measuring fruit set were considered and the duration of flower life -from opening to petal fall- was registered in days (Mesquida et al. 1988).

Statistical analysis

To analyze data, Linear Mixed Models (LMM) were applied considering pollination treatment (open, exclusion, *A. mellifera* only) and year (2021, 2022) as fixed factors, and field (agricultural, seminatural) as a random factor. Variables expressed as percentages (fruit set, germination power, vigor, ripe seeds) were subjected to arcsine square-root transformation, whereas the variables expressed as integers (seeds *per* fruit, fruits *per* plants, flower longevity) were log-transformed, and analyzed under the assumption of normality. For quantitative yield variables (fruit set, seeds *per* fruit, fruits *per* plant, seed weight, seed yield), LMM were applied using the *lme* function from the *nlme* package, incorporating a varIdent structure to account for heteroscedasticity (Pinheiro et al. 2024). For qualitative yield variables (flower longevity, germination power, vigor, ripe seeds), models were fitted using the *lmer* function from the *lme4* package (Bates et al. 2015; R Core Team 2024). Model fit was evaluated both analytically and graphically. In particular, we assessed normality using QQ-plots and the Shapiro–Wilk tests, and verified homoscedasticity through residuals-versus-fitted plots and Levene's tests. Tukey's test with Bonferroni correction was applied for *post hoc* comparisons between pollination treatments. In all cases, the significance level was set at $p < 0.05$. All analyses were performed using R (R Core Team 2024).

Results

Quantitative yield

Fruit set, number of seeds *per* fruit and number of fruits *per* plant were all significantly influenced by pollination treatment (Fig. 1; Supplementary Table 1). *Post hoc* comparisons showed that exclusive pollination by *A. mellifera* (*A. mellifera* only) consistently resulted in the highest fruit set and number of seeds *per* plant, whereas the exclusion treatment produced the lowest values, with open pollination showing intermediate outcomes (Fig. 1A, B; Supplementary Table 2). For fruit production *per* plant, *A. mellifera* only and open pollination showed higher values than the exclusion treatment (Fig. 1C; Supplementary Table 2). Seed weight exhibited no significant differences among treatments (Fig. 1D; Supplementary Table 2). Differences were detected between years for all mentioned reproductive traits (Supplementary Table 1), with 2021 showing higher values compared to 2022 (Supplementary Table 3). Despite the marked interannual variation in the absolute values of these traits, the relative ranking of treatments remained consistent, confirming a stable positive contribution of insect pollination to the reproductive performance of the crop.

Seed yield varied significantly among pollination treatments (Fig. 2; Supplementary Table 1). *Post hoc* comparisons indicated that seed yield was consistently highest in plots pollinated exclusively by *A. mellifera*, intermediate under open pollination, and lowest under pollinator exclusion (Fig. 2; Supplementary Table 2). Significant differences in seed yield were detected between years (Supplementary Table 1), with 2021 exhibiting higher values compared to 2022 (Supplementary Table 3). In both analyzed years, the relative pattern among treatments remained

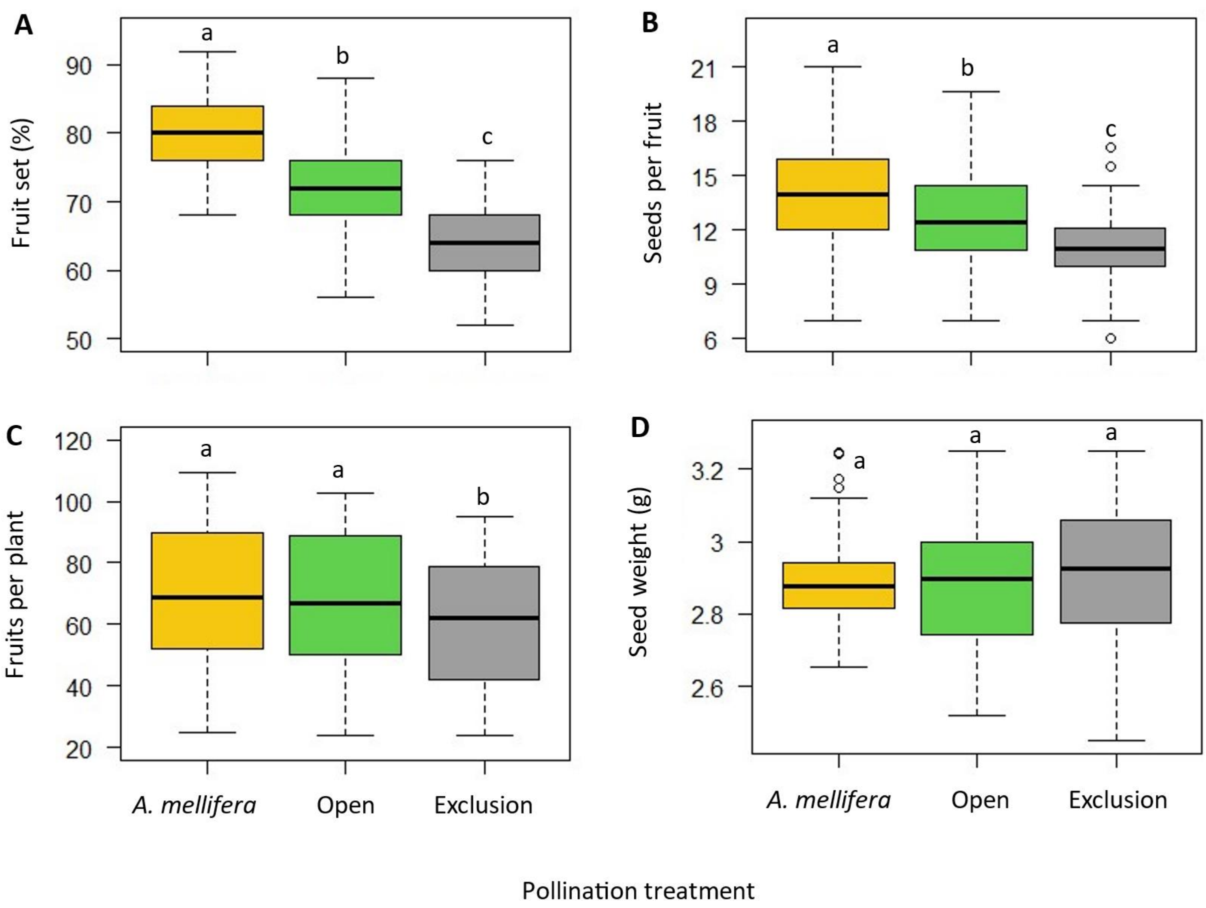

Figure 1. Box plots showing quantitative components of rapeseed yield across pollination treatments (*A. mellifera* only, Open, and Exclusion): (A) fruit set, (B) seeds *per* fruit, (C) fruits *per* plant, and (D) 1000 seed weight. Values represent means pooled across both study years. Different lowercase letters indicate significant differences among treatments according to Tukey's multiple comparisons test.

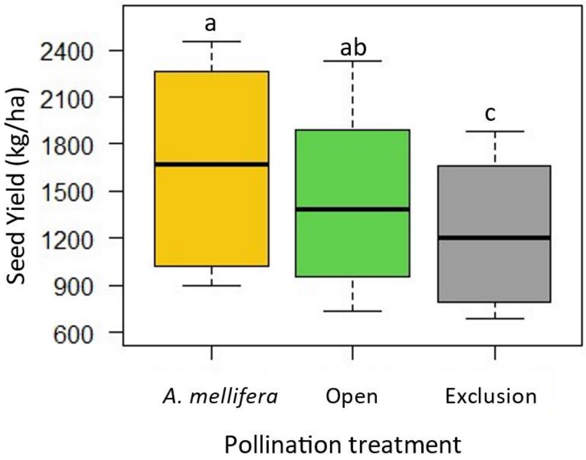

Fig. 2. Seed yield (kg ha^{-1}) under different pollination treatments (*A. mellifera* only, Open, and Exclusion). Values represent means pooled across both study years. Different letters indicate significant differences among treatments according to Tukey's multiple comparisons test.

consistent (Supplementary Table 3), suggesting that the effects of pollination treatments on crop productivity were stable and repeatable despite interannual variability.

QUALITATIVE YIELD

Flower longevity, germination power, seed vigor, and the proportion of ripe seeds were all significantly influenced by pollination treatment (Fig. 3; Supplementary Table 1). Flowers lasted longer under exclusion compared to open pollination or *A. mellifera* treatments (Fig. 3A; Supplementary Table 2). Seed quality traits showed the opposite trend: germination power, vigor, and the percentage of ripe seeds were consistently highest under *A. mellifera* pollination, intermediate under open pollination, and lowest under exclusion treatment (Fig. 3B–D; Supplementary Table 2). Significant differences in all the qualitative yield traits were detected between years (Supplementary Table 1). Although absolute values of these traits varied between years (Supplementary Table 3), the relative ranking of treatments remained unchanged, suggesting that pollination treatments exert a stable and reproducible effect on reproductive success across years.

DISCUSSION

In this study, we explore the effects of *A. mellifera* and wild insect pollinators on reproductive parameters and seed quality of rapeseed through a caging experiment at the Argentine Pampas. We found that yields from both open pollination and exclusive pollination by *A. mellifera* consistently exceeded those from the pollinator-exclusion treatment. These findings demonstrate that insect pollination plays a key role in enhancing rapeseed yield, and highlight that supplementation with *A. mellifera* colonies can compensate for the limited abundance of wild pollinators in agricultural landscapes, thereby maximizing *B. napus* productivity.

Compared to the exclusion treatment, pollination exclusively by *A. mellifera* increased crop yield by 33%, while open pollination increased yield by 20%. Present results are consistent with recent studies conducted in Argentina (Mazzei et al. 2021) and other countries (Araneda et al. 2010; Bommarco et al. 2012; Chambó et al. 2014), and demonstrate that insect pollination is efficient at increasing rapeseed productivity. Differences in total yield is likely explained by an increase in the number of seeds *per* fruit and fruits *per* plant, but not in seed weight, in agreement with a recent meta-analysis presented by Yang et al. (2024). The lower rapeseed production in our exclusion treatment compared to both the pollination exclusively by *A. mellifera* and the open pollination treatment was probably due to an insufficient deposition of pollen on the stigma to fertilize the ovules or to a lack of synchronization between stigma receptivity and viable pollen availability (Degrandi-Hoffman & Chambers 2006).

Our results showed that the pollination treatment consistently accounted for a substantial proportion of the variance in seed yield, even though absolute values differed markedly between years. This interannual consistency underscores that pollination services are not secondary contributors to productivity but rather stable and reproducible drivers of crop performance under variable environmental conditions. These findings align with pioneer studies showing that the benefits of animal pollination tend to be robust across environments and production systems, even if the magnitude of yield gains varies (Klein et al. 2007; Garibaldi et al. 2013). In the Argentine Pampas, where agricultural intensification and monoculture have reduced

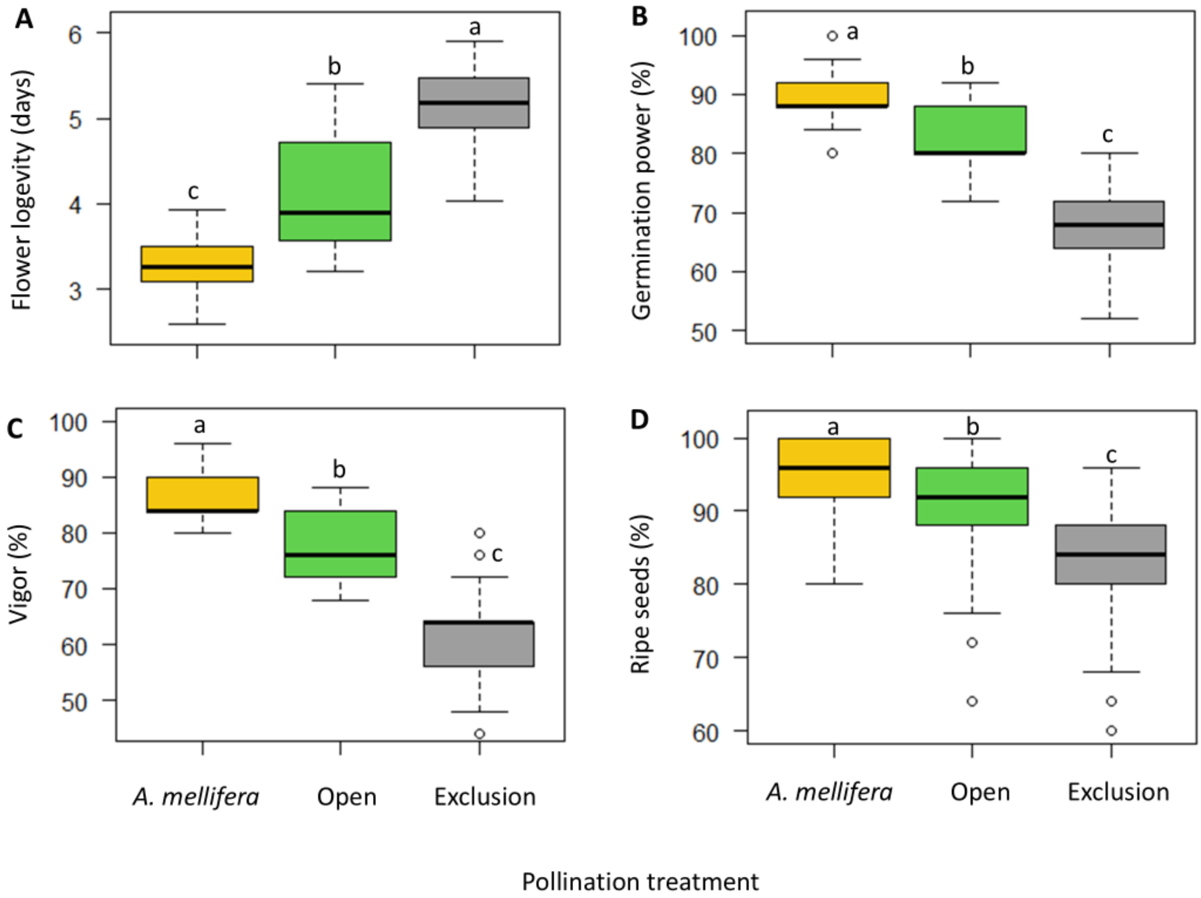

Figure 3. Box plots showing qualitative components of rapeseed yield across pollination treatments (*A. mellifera* only, Open, and Exclusion): (A) flower longevity, (B) seed germination power, (C) seed vigor, and (D) ripe seeds. Values represent means pooled across both study years. Different lowercase letters indicate significant differences among treatments according to Tukey's multiple comparisons test.

habitat availability for wild pollinators, and crop rotations are often dominated by cereals and soybeans, such robustness is particularly relevant. In this setting, the inclusion of rapeseed provides not only agronomic advantages but also ecological opportunities by diversifying landscapes and supporting pollinator activity during periods of limited floral resources. The consistency observed in our study strengthens the evidence that pollination services are a cornerstone for ensuring both yield stability and sustainability in intensified agroecosystems.

Most of the yield components showed no differences between open pollination and the exclusive pollination by *A. mellifera* treatment, suggesting that wild pollinators in this region are ensuring pollination service and high yields. However, for 2022, fruits *per* plant and seed vigor evidenced higher values in the *A. mellifera* treatment compared to the open pollination treatment. This result suggests that the abundance and the pollination effect of wild insects on rapeseed yield may fluctuate among years, likely driven by climatic variability (Brunet and Fragoso 2024; Scally et al. 2025). In this context, pollination by managed *A. mellifera* can provide greater stability yield across years, given its high adaptability to changing climatic conditions and the possibility of adjusting colony abundance through relocation (Michener 2007; Delaplane et al. 2013; Chambó et al. 2018).

Fruit set and seeds *per* fruit were also higher for exclusive pollination by *A. mellifera* than for the open pollination treatment. Since fruit set is considered a measure of the quantity and quality of pollen deposited on the stigma (Garibaldi et al. 2013), the lower productivity and seeds *per* fruit under the open pollination treatment could be attributed to an insufficient quantity and quality of pollen deposited when the stigmas were receptive

(Rosa et al. 2011; Mazzei et al. 2021). This was probably the result of a lower abundance of pollinators in the open pollination compared to our exclusive *A. mellifera* pollination treatment (Scally et al. 2025). Differences in the abundance of pollinators between treatments account for our results of flower longevity, which was longer in the open pollination compared to the exclusive *A. mellifera* treatment. It is worth to note that a longer flower longevity may increase the risk of infection by the fungus *Sclerotinia sclerotiorum*, which causes stem rot and plant overturning (Mesquida et al. 1988; Sharma & Reddy 2020). On the other hand, the qualitative parameters seed germination power and vigor were higher in exclusive *A. mellifera* pollination than in the open pollination treatment. Although these components are mainly influenced by plant genetics and environmental factors, previous studies have reported promising results of *A. mellifera* pollination in rapeseed (Kevan & Eisikowitch 1990; Chambó et al. 2014) and other crops (Camacho & Franke 2008; Chiari et al. 2013).

Our results suggest that the yield of *B. napus* is increased by either wild or managed pollinators. In agreement with this finding, Mazzei et al. (2021) found a positive correlation between proximity to semi-natural habitats and the quantitative components of rapeseed yield in temperate zones of Argentina, a pattern also detected in other parts of the world (Atmowidi et al. 2007; Stanley et al. 2013; Rijal et al. 2018; Stiles et al. 2021) and emphasize the importance of pollinators as an input for rapeseed production in agroecosystems. Since the intensive land use for monoculture crop production in the Pampas region of Argentina leads to a reduction in the availability of shelter for pollinators at crop margins, the supplemental pollination with *A. mellifera* in rapeseed fields during the flowering period can represent a tool to achieve the potential yield of this crop. However, it should be kept in mind that a high abundance of *A. mellifera* may have a negative effect on the flowers of various crops and may also displace native pollinators (Aizen et al. 2020; Page & Williams 2023). It is important to note that our results come from a caging experiment with a high honey bee density and cannot be extrapolated to managed field conditions. Since visitation rates in our experiment are likely to differ from those observed with managed *A. mellifera* in crop pollination, it is not possible to provide recommendations that agricultural producers could implement under intensive production systems. In this context, further studies are needed to evaluate and estimate the optimal number of honey bee colonies to maximize crop yield without disrupting the surrounding agroecosystem.

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Author contribution

BS, MS and ACS conceived and designed the research. EZ contributed to the statistical analyses. MC contributed to the discussion of the results. BS, MS and ACS analyzed the data and wrote the manuscript. All authors read and reviewed drafts of the manuscript and approved its final version.

Disclosure statement

The authors declare no competing interests.

Generative AI disclosure statement

No generative artificial intelligence (AI) tools were used in the conception, analysis, or writing of this manuscript.

Data availability statement

Data will be made available upon reasonable request.

Appendices

Additional supporting information may be found in the online version of this article:

Supplementary Fig. 1. Satellite photos of the fields where the caging experiments were performed.

Supplementary Fig. 2. Pollination treatments in the field located at a semi-natural habitat.

Supplementary Table 1. Results of the statistical analysis comparing pollination treatments.

Supplementary Table 2. Results of the Tukey *post hoc* comparisons among pollination treatments.

Supplementary Table 3. Mean values of quantitative and qualitative yield components under different pollination treatments.

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