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Pollination by native bees increases fruit mass without changing protein content in snap beans

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ABSTRACT: Insects play a vital role in agricultural production by enhancing both the quantity and quality of fruits and seeds in a wide range of allogamous and autogamous crops. The common bean (*Phaseolus vulgaris*) exemplifies an autogamous crop that can benefit from insect pollination; however, the extent of this influence varies significantly among cultivars. The snap bean, a *P. vulgaris* cultivar, is cultivated and consumed globally, yet the impact of pollination on its production remains largely unexplored. This study aims to identify the pollinators of snap beans and evaluate their influence on the quantity and quality of crop production. We hypothesize that bees are the primary pollinators and that their activity enhances production compared to self-pollination. To investigate this, we conducted focal observations on pollinators and applied treatments that included open pollination (OP), spontaneous self-pollination (SS), and the pollination efficiency of *Xylocopa* sp. 1 (XP). Although SS was capable of producing fruits, both XP and OP yielded enhancements across all fruit quality parameters, including pod mass, ultimately increasing production profit. We recommend exploring strategies for managing *Xylocopa* species in agricultural crops, along with efforts to conserve habitat diversity within the landscape, as this can bolster the resilience of pollinators.

Keywords: autogamy, crop pollination, ecosystem service, wild bees

Pollination is a crucial ecosystem service that plays a vital role in the regeneration of natural ecosystems and agricultural production worldwide (Klein et al., 2007; IPBES, 2016; Klein et al., 2018). However, environmental changes caused by anthropogenic activities have drastically reduced pollinator populations, leading to a decline in the productivity of numerous crops (Potts et al., 2010; IPBES, 2016) and posing a threat to global food security (IPBES, 2016). Even autogamous crops, such as the common bean (*Phaseolus vulgaris* L.) and its various cultivars (Kingha et al., 2012; Masiga et al., 2014; Paulino et al., 2023), can experience notable enhancements in the commercial and nutritional quality of fruits and seeds due to animal pollination (Klein et al., 2007; Klatt et al., 2014; IPBES, 2016). For instance, in species of lupin (*Lupinus* L.), protein crop yields increased by 3-11 % depending on crop species and cultivar, with bumblebee visitation contributing to a protein yield increase of up to 40 % (Tijs et al., 2021).

Phaseolus vulgaris cultivars are among the most significant crops in the Fabaceae family globally, serving as the primary source of income and plant-based protein for smallholder farmers (Mesquita et al., 2007). Pollinators, particularly bee species, such as *Apis* and *Xylocopa*, as well as *Bombus* and *Trigona*, play a crucial role in this process (Ibarra-Perez et al., 1999; Masiga et al., 2014; Widhiono et al., 2017). Although *P. vulgaris* is primarily autogamous, studies have shown that insect pollination enhances the production of these cultivars. Research indicates a wide variation in cross-fertilization rates, ranging from 1 to 85 % (Pereira Filho and Cavariani, 1984; Wells et al., 1988; Paulino et al., 2023), highlighting differing levels of reliance on pollinators. Therefore, examining the impact of pollination in lesser-studied cultivars is essential for reaching a consensus on the role of pollinators in crop production, particularly when evaluating parameters like protein concentration, which is vital for human populations. Protein concentration in seeds is particularly noteworthy, as it serves as a significant nutritional source. In certain cultivated legumes, such as species of lupin (*Lupinus* L.), bee pollination can enhance seed protein concentration by 3 % to 11 %, depending on the species and cultivar (Fijen et al., 2021). Furthermore, the production of plant-based proteins, such as legumes, has a substantially lower environmental impact compared to animal-based production (Willett et al., 2019; Fijen et al., 2021). It is crucial to recognize that understanding and promoting the relevance of pollinators for agricultural production foster the adoption of sustainable agricultural practices. This knowledge guides the management of pollinators and contributes to addressing the global pollinator crisis in crop production (van der Sluijs, 2020).

The snap bean, a *P. vulgaris* cultivar, is one of the most economically significant vegetables worldwide, with an annual production of approximately 23 million tons (FAOSTAT, 2024). Thus, given the economic importance of the crop and the limited information regarding the impact of pollination on its production, this study aims to identify the primary pollinators of the snap bean and assess their influence on both the quantity (number of pods and seeds per pod) and quality (dimensions of pods and seeds, as well as seed protein content) on the yield. We propose the following hypotheses: 1) Bees from the Apidae family are the primary pollinators, and 2) Pollinators enhance snap bean production in terms of both quantity and quality.

The study was conducted between November 2019 and January 2020 in Garanhuns, a municipality located in Pernambuco State, which is characterized as a semi-arid region of Northeast Brazil (8°54′25.3″ S 36°29′36.8″ W, altitude 823 m). The climate is classified as rainy tropical, with dry summers and average annual rainfall around 20° C. A total of 60 seedlings were obtained from certified seeds of the "macarrão-trepador" variety from the Topseed brand and were planted following technical guidelines. Unlike dry beans, snap beans are a pole-type cultivar that requires training for optimal growth. The planting was arranged with a spacing of 50 cm between plants and 1 m between rows. Fertilization included the use of cattle manure, and the plants were watered in the early morning and late afternoon.

While the floral biology of *P. vulgaris* is generally well understood, it may vary among different cultivars (Paulino et al., 2023), highlighting the need for verification prior to conducting reproductive experiments. The anthesis period was monitored in ten pre-anthesis floral buds from ten individuals until their senescence. To determine the presence of regions emitting odor, ten fresh flowers from ten individuals were immersed in a 1 % neutral red solution for 10 min. Odor classification was assessed by inhaling the fragrance produced by ten flowers from ten individuals that were enclosed in a sterile container for 15 min. The timing of pollen availability was evaluated in fully open flowers (n = 10), and stigmatic receptivity was measured every hour from the start to the end of anthesis (from 05h00 to 17h00). All methods were carried out using standard floral biology techniques (Dafni et al., 2005). We assessed the ultraviolet reflectance of the flowers using the ammonium hydroxide vapor method for 30 s (Scogin et al., 1977).

The behavior and frequency of floral visitors were recorded through 56 h of focal observations, conducted on homogeneously distributed non-consecutive sunny days from 05h00 to 17h00 during the peak flowering period. Floral visitors were collected and identified to the lowest possible taxonomic level by specialists.

To investigate the impact of pollination on production, we conducted three pollination treatments in 20 randomly selected plants. Each plant had three flowers marked from the pre-anthesis stage and received one of the treatments, resulting in a total of 20 flowers per treatment: natural pollination (NP), where flowers were marked and made available to floral visitors; spontaneous self-pollination (SS), where flowers were bagged from the pre-anthesis stage until senescence, and *Xylocopa* sp. 1 pollination (XP), where flowers were bagged from pre-anthesis, exposed to a single visit from the bee *Xylocopa* sp. 1 on the first day of anthesis, and then remained bagged until flower senescence. *Xylocopa* sp. 1 was selected as the most common pollinator. Twenty days post-anthesis (the average time for commercial harvest), we counted the number of pods across all treatments and recorded the following parameters: pod mass, length, width, and number of seeds, total seed mass, and seed content of soluble proteins. To measure the last parameter, 0.1 g of fresh seeds from each fruit was homogenized (using a mortar and a pestle) with tris-HCl buffer (0.2 M, pH 7.2), cooled with liquid N_2, and centrifuged at 10,000 rpm for 10 min at 4° C. Absorbance measurements were taken to determine the total soluble proteins in the supernatant at 595 nm. The medium served as the blank, while bovine serum albumin (Sigma Chemical Company) was used as the standard. The analyses were performed in triplicate, and the results were expressed in mg g^{-1} (Bradford, 1976).

Fruit set was evaluated across treatments using the Chi-square test. The normal distribution of fruit traits was assessed with the Kolmogorov-Smirnov test, revealing that all traits, except for the number of seeds, conformed to normality at a 5 % probability level. To compare fruit attributes among treatments, an Analysis of Variance (ANOVA) was performed, followed by a Tukey *a posteriori* test. All analyses were conducted in the R environment version 3.6.1.

The flowers are organized in inflorescences and possess the characteristic structure of Faboidae subfamily. They are zygomorphic, featuring five petals: a standard (upper) petal, two lateral wings, and a keel formed by two petals that enclose the reproductive organs. These diurnal flowers open between 04h50 and 06h00. When the petals are white, the stigma is receptive, and the pollen is available. The stigma remains receptive until 14h00, and by around 18h00, the petals turn cream. Flower senescence occurs in the afternoon of the second day of anthesis. Osmophores – regions that emit floral odor – are situated throughout the petals, predominantly at the apex, and the odor is slightly sweet. Ultraviolet reflectance is noted across the petals, similar to findings reported by Scogin et al. (1977), who observed that 54 % of the Fabaceae species studied exhibited varying levels of ultraviolet light absorption. The flower morphology and biology documented in this study are closely linked to bee pollination and have also been observed in other *P. vulgaris* cultivars (Paulino et al., 2023 and references therein).

Bees were observed visiting flowers, including three *Xylocopa* Latreille (1802), *Apis mellifera* Linnaeus (1758), and an Augochlorini Beebe (1925) (Halictidae Thomson, 1869) (Figure 1). *Xylocopa* species emerged as the most frequent pollinators (Figure 1), particularly active around 11h00 (Figure 2). Notably, only *Xylocopa* species and *A. mellifera* exhibited true pollinator behavior, as they contacted both the anthers and stigma while collecting nectar and pollen. Conversely, the Augochlorini bee landed on the opposite side of the keel, inserting its proboscis at the base of the flower, between the corolla and the calyx, to access the nectar without interacting with the reproductive structures. This behavior resembles that observed for lepidopterans visiting the 'mulatinho' cultivar in Northeast Brazil (Paulino et al., 2023). The structural design of Faboidae flowers restricts legitimate visits by those animals strong enough to navigate the petals and reach the nectar.

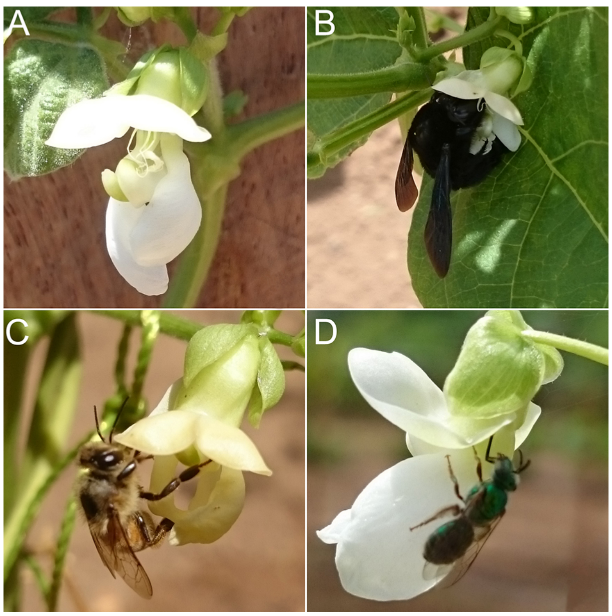

Figure 1 – Flower (A) and floral visitors of snap bean (*Phaseolus vulgaris* L., Fabaceae) in a semi-arid region of Northeast Brazil; B) *Xylocopa* sp. 1; C) *Apis mellifera* L.; and D) Augochlorini (Halictidae) bee.

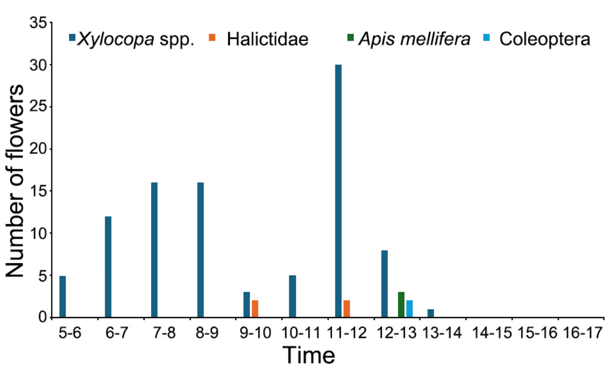

Figure 2 – Frequency of floral visitors of snap bean (*Phaseolus vulgaris* L., Fabaceae) along the day in a semi-arid region of Northeast Brazil.

No significant differences were observed in fruit set rates among natural pollination (NP 95 %), spontaneous self-pollination (SS 70 %), and *Xylocopa* sp. 1 pollination (XP 85 %; NP, $p > 0.05$). However, both NP and the XP resulted in pods approximately 30 % longer, 58 % heavier, contained around 30 % more seeds, and produced seeds that were 80 % heavier compared to those resulting from self-pollination (ANOVA, degrees of freedom (df) = 2/27; Tukey's test, $p < 0.001$; Figure 3). This superior performance of insect pollination relative to self-pollination has also been observed in common bean cultivars in Cameroon (Kingha et al., 2012), Kenya (Kasina et al., 2009; Masiga et al., 2014), as well as in the United Kingdom and the United States (Free, 1966; McGregor, 1976; Delaplane and Mayer, 2000). Bean cultivars exhibit a wide range of pollination dependence levels. While many cultivars show positive effects from pollination on morphometric attributes (Kasina et al., 2009; Kingha et al., 2012; Masiga et al., 2014), some are influenced in terms of seed chemical composition (Fijen et al., 2021) and others show no impact on either morphometric traits or seed germination (Paulino et al., 2023).

Our findings support the notion that, although *P. vulgaris* cultivars are primarily autogamous, insect pollination can enhance production depending on the specific cultivar and geographic region. This pattern was similarly noted in oilseed rape (*Brassica napus* L., Brassicaceae), where some varieties exhibited varying degrees of dependency on pollinators (Ouvrard and Jacquemart, 2019), while others showed no such dependency (Ouvrard et al., 2017). Additionally, soybean cultivars may also vary in their reliance on pollinators for production (Chacoff et al., 2024).

Notably, a single visit from *Xylocopa* sp. 1 was enough to enhance production quality compared to self-pollination. This supports the broader notion that insect pollination, as demonstrated in various studies, positions native bees as a viable alternative for boosting production. In the case of *Vicia faba* L. (Fabaceae), bumblebees served as the primary pollinators, increasing the overall fruit set by 36 % when compared to spontaneous self- and wind pollination (Nayak et al., 2015).

Total soluble protein levels did not significantly differ among treatments (Figure 3). However, there was a noticeable trend indicating that pod protein was higher in the NP and XP treatments compared to SS. In this sense, recognizing the critical role of ecosystem pollination services in enhancing the nutritional contents in crops is vital for ensuring food and nutritional security for human populations. To date, no studies have been conducted to investigate the impact of pollinators on protein levels in pods, particularly within the snap bean cultivar. Although protein levels were comparable across the treatments, it is worth noting that animal pollination led to the production of larger and heavier pods, which can enhance farmers'

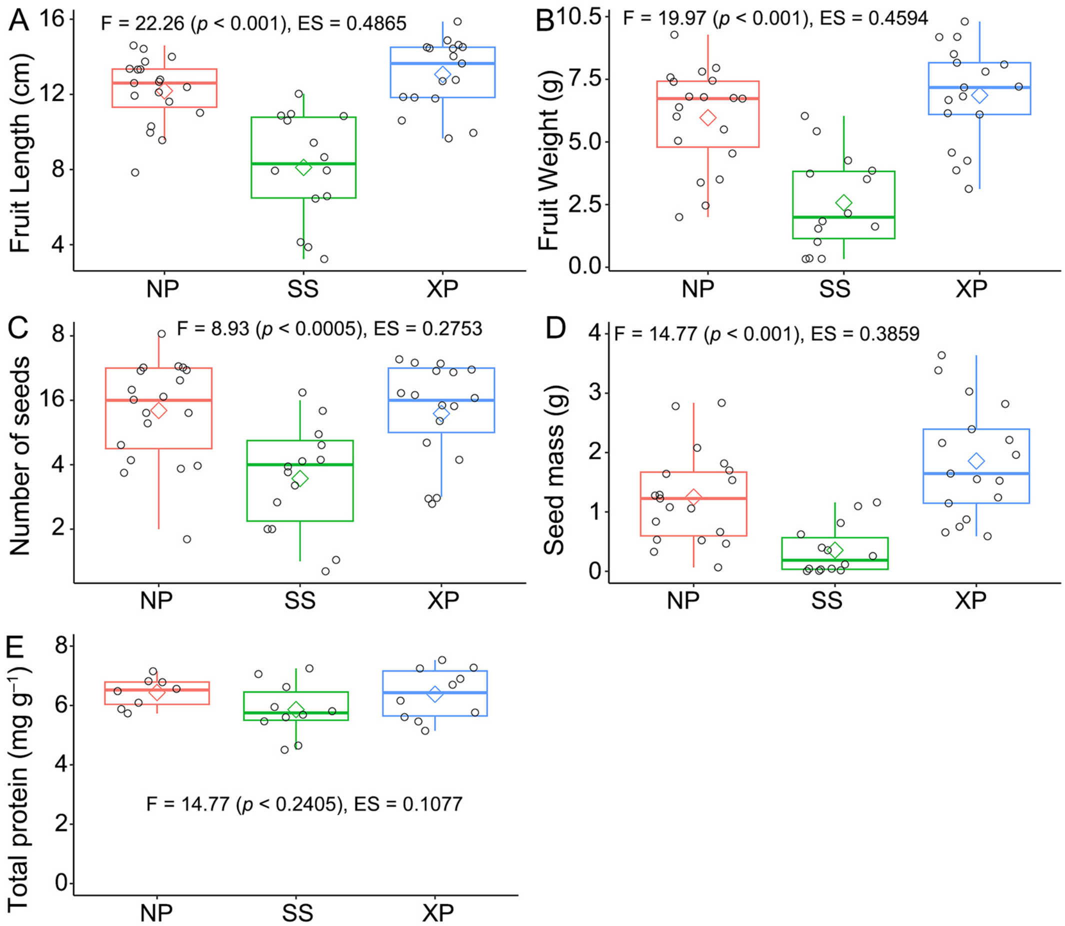

Figure 3 – Comparison of pod length (A), mass (B), number of seeds (C), total seed mass (D) and seed soluble protein content (E; mg g^{-1} of dry matter) after natural pollination (NP), spontaneous self-pollination (SS), and *Xylocopa* sp. 1 (XP) pollination of snap beans (*Phaseolus vulgaris* L., Fabaceae) in a semi-arid region of Northeast Brazil. Comparisons were conducted using one-way Analysis of Variance (df = 2) with Tukey's test ($p < 0.05$). F = F-value; ES = effect size; df = degrees of freedom.

profits. Additionally, this resulted in a greater number of heavier seeds, thereby increasing the protein content per pod and improving its overall nutritional value.

Fruits produced with the assistance of pollinators (NP and XP) tend to be heavier than those that developed without pollination (SS), suggesting that the involvement of pollinators increased producers' profits, as production is typically sold by weight. Consequently, research focused on the management of *Xylocopa* species in pod crops is highly desirable. While species of this genus are vital pollinators for both crops and native plants, effectively breeding them for crop pollination presents challenges, given that most species create their nests by excavating galleries in dead wood trunks (Freitas et al., 2017). Nevertheless, recent successful strategies for artificial hive management demonstrate that overseeing *Xylocopa* species can lead to increased production (Freitas et al., 2017). Furthermore, preserving native vegetation and surrounding areas is crucial, as these environments serve as essential refuges, feeding grounds, and reproduction sites for these bees (Silva et al., 2019; Kral-O'Brien et al., 2021), which also pollinate various legume crops (Paulino et al., 2021). Therefore, regional studies that explore pollination and its effects on production are important, as they lay the groundwork for effective management and conservation strategies for pollinators. It is important to highlight that the findings of this research may be specific for the region where it was conducted and may depend on the local fauna and flora.

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Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support this study will be shared upon reasonable request to the corresponding author.

Declaration of use of AI Technologies

The authors did not use AI Technologies.

Authors' Contributions

Conceptualization: Castro CC. **Data curation:** Tenório-Barros C. **Formal analysis:** Santoro KR. **Funding acquisition:** Castro CC. **Methodology:** Castro CC. **Project administration:** Castro CC. **Resources:** Castro CC. **Supervision:** Castro CC. **Investigation:** Tenório-Barros C, Silva RM, Campos GPA, Silva FF. **Writing-original draft:** Tenório-Barros C, Silva RM. **Writing-review & editing:** Tenório-Barros C, Silva RM, Campos GPA, Silva FF, Santoro KR, Castro CC.

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