

Impact of environmental factors and sowing date on flowering phenology, pollinator behavior, and buckwheat yield

Impacto dos fatores ambientais e da data de semeadura na fenologia da floração, no comportamento dos polinizadores e na produtividade do trigo-sarraceno

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ABSTRACT

Climate change threatens pollinator-flowering synchrony, disrupting reproduction in entomophilous crops like buckwheat (*Fagopyrum esculentum*). Understanding this and promoting pollinators conservation are vital for crop productivity. This study aimed to evaluate floral visitor behavior, flowering phenology, and the effects of insect pollination and sowing date on yield components. Three independent field experiments addressed: (1) visitation frequency during the day and its relationship with environmental factors; (2) flowering phenology throughout the crop cycle; and (3) the impact of insect pollination on yield and seed quality. *Trigona spinipes* and Africanized *Apis mellifera* were identified as the main pollinators, exhibiting simultaneous nectar and pollen foraging behavior. Peak visitation rates occurred between 9:00 and 11:00 AM, when temperatures ranged from 23.2 to 25.8°C and relative humidity decreased from 83.6% to 74.6%. Visitation declined in the afternoon, and temperature, humidity, wind speed, and time of day significantly influenced pollinator activity patterns. Flowering phenology showed a generally synchronous progression influenced mainly by humidity and inflorescence traits, promoting flower production. Floral emission declined naturally over time, while seed set was more strongly associated with inflorescence size and precipitation than with temperature or humidity. Free pollination significantly increased grain yield and grain mass across sowing dates. Sowing timed to coincide with favorable environmental conditions and pollinator activity optimized plant development and seed quality. These results highlight the essential roles of morphological traits, environmental factors, pollinator activity, and sowing timing in buckwheat reproductive success. Caution is advised when extrapolating these findings to other agricultural systems and climatic zones.

Index terms: Agricultural production; *Fagopyrum esculentum*; insect activity; phenological stages; pollinators.

RESUMO

As mudanças climáticas ameaçam a sincronização entre polinizadores e florescimento, afetando culturas entomófilas como o trigo sarraceno (*Fagopyrum esculentum*). Este estudo avaliou o comportamento dos visitantes florais, a fenologia da floração e os efeitos da polinização por insetos e da data de semeadura nos componentes de produtividade. *Trigona spinipes* e *Apis mellifera* africanizada foram as principais polinizadoras, exibindo comportamento simultâneo de coleta de néctar e pólen. O pico de visitação ocorreu entre 9h e 11h, com temperaturas variando de 23,2 a 25,8°C e umidade relativa diminuindo de 83,6% para 74,6%. As taxas de visitação declinaram à tarde, sendo temperatura, umidade, velocidade do vento e horário do dia fatores que influenciaram significativamente os padrões de atividade dos polinizadores. A floração apresentou um progresso geralmente síncrono, influenciado principalmente pela umidade e características da inflorescência, o que promoveu a produção de flores. A emissão floral declinou naturalmente ao longo do tempo, enquanto a formação de sementes esteve mais fortemente associada ao tamanho da inflorescência e à precipitação do que à temperatura ou umidade. A polinização livre aumentou significativamente o rendimento e a massa de grãos em todas as datas de semeadura. A semeadura com condições ambientais favoráveis e a atividade dos polinizadores otimizou o desenvolvimento da planta e a qualidade das sementes. Esses resultados destacam o papel essencial das características morfológicas, dos fatores ambientais, da atividade dos polinizadores e do momento da semeadura no sucesso reprodutivo do trigo sarraceno. Recomenda-se cautela ao extrapolar esses achados para outros sistemas agrícolas e zonas climáticas.

Termos para indexação: Produção agrícola; *Fagopyrum esculentum*; atividade de insetos; estágios fenológicos; polinizadores.

Agricultural Sciences

Ciênc. Agrotec., 49:e010725, 2025
<http://dx.doi.org/10.1590/1413-7054202549010725>

Editor: Renato Paiva

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Received in May 19, 2025 and approved in July 28, 2025

Introduction

The global expansion of pollinator-dependent crops has intensified the demand for pollination services (Aizen et al., 2019). According to Klein et al. (2007), pollinators are essential to varying degrees for 75% of the world's leading agricultural crops, with the estimated economic value of pollination services reaching €153 billion annually (Gallai et al., 2009). Insect pollination, particularly by bees, significantly enhances both the yield and quality of agricultural production (Chambó et al., 2011; Chambó et al., 2014; Mulwa et al., 2022), contributing substantially to approximately one-third of global food production (Khalifa et al., 2021; Perez-Lopes et al. 2024). Turo (2024) highlights that the

majority of flowering plants depend on animal pollinators, with an estimated 87.5% of angiosperms relying on them, underscoring the vital role of pollinators in maintaining ecosystem integrity. However, the expansion of monocultures, which are detrimental to pollinators, has led to pollination deficits due to biodiversity loss. Monocultures provide limited and homogeneous floral resources and increase pollinator exposure to pesticides and habitat loss (Cunha et al., 2024).

Therefore, it is crucial to promote and preserve pollinator diversity and abundance within agricultural systems. Buckwheat (*Fagopyrum esculentum*) cultivation alone does not replace conventional monocultures, but its integration into crop rotations offers a promising diversification approach. In addition to diversifying cropping systems, buckwheat is a short-cycle crop that can be grown during off-seasons, enabling the adoption of no-tillage farming practices. It is also highly dependent on insect pollination, particularly by bees, for successful grain development (Mainali et al., 2020; Nagano & Miyashita, 2025). Thus, buckwheat cultivation may help mitigate the negative effects of monocultures by providing more diverse floral resources and improving agroecosystem health.

Synchronization between crop flowering and pollinator activity is vital for ensuring efficient pollen transfer and maximizing the productivity of pollination-dependent crops (Sritongchuay et al., 2020; Sáez et al., 2023). However, climatic variations, including ongoing climate change, can affect flowering phenology (Sritongchuay et al., 2020) and pollinator behavior (Chambó et al., 2017), potentially disrupting this synchronization.

Efficient pollination also depends on floral visitors, as not all flower-visiting insects act as pollinators. The timing and frequency of visits, as well as the size of the insect relative to the flower, determine pollination effectiveness (Neto, Vieira, & Schlindwein, 2021). Therefore, research on plant–pollinator interactions is critical for evaluating the efficiency of these insects, especially in light of globalization and climate change, which have impacted food production and quality. The decline in pollinators, driven by agricultural practices, the introduction of exotic species, habitat destruction, and the indiscriminate use of agrochemicals, has caused significant economic losses in fruit and seed production (Hipólito, Viana, & Garibaldi, 2016; BPBES/REBIPP, 2019). These losses are exacerbated by climate change, which affects pollinator species distribution (Elias et al., 2017).

To mitigate these effects, it is essential to implement pollinator conservation strategies, as pollination services are indispensable for global food security. Modeling flowering phenology and studying pollinator behavior in response to climatic variables are fundamental for understanding how these factors influence crop productivity (Chambó et al., 2017). These aspects are particularly relevant in crops such as buckwheat, due to their high dependence on pollinators.

The objective of this study was to evaluate the impact of entomophilous pollination on the yield components of

buckwheat (*Fagopyrum esculentum* Moench) in two sowing periods. Additionally, we aimed to analyze pollinator behavior and its relationship with environmental variables (temperature, relative humidity, and wind speed). We also sought to characterize flowering phenology and inflorescence morphology to provide insights into the crop's reproductive cycle.

Material and Methods

Study area and site description

The experiment was conducted with buckwheat (*Fagopyrum esculentum*) during the 2023 growing season at the experimental station of the Federal University of Recôncavo da Bahia, located in Cruz das Almas, Bahia, Brazil (12°40'S, 39°06'W; 226 m a.s.l.). The soil in the experimental area was classified as Red-Yellow Argisol. The climate in the region, according to the Köppen classification, is a transition between Am and Aw zones, with average annual rainfall of 1,143 mm, average temperature of 24°C, and relative humidity of 60%. Sunflower (*Helianthus annuus*) and common bean (*Phaseolus vulgaris*) crops were also present within a 1 km radius of the experimental area but flowered at different times than buckwheat. An apiary with six colonies of Africanized honeybees was located approximately 1 km from the site. Meteorological data (air temperature, relative humidity, wind speed, and precipitation) were recorded every 10 minutes during the experiment by the National Institute of Meteorology (INMET). Observations of flower visitation, flowering phenology, and crop yield were conducted on days with favorable weather conditions for pollinator activity (i.e., sunny days).

The buckwheat cultivar used was IPR 92 Altar, known for its drought tolerance and low soil fertility requirements. The experimental area measured 50 m × 10 m (500 m²) and was sown using a no-tillage (direct-seeding) system. Basal fertilization was performed with NPK 4-30-16 fertilizer at a rate of 400 kg ha⁻¹. Sowing occurred on May 20 and July 17, 2023, in 16 plots, each measuring 24 m². Each sowing date was assigned to eight separate plots. Thus, the two sowing dates were established simultaneously in different plots, not sequentially. Within each plot, plants were arranged in 10 rows spaced 0.34 m apart, with 0.05 m between plants and a sowing depth of 0.01 m. Fifteen days after emergence, nitrogen fertilization was applied (20 kg N ha⁻¹ as ammonium sulfate), and thinning was performed to maintain a uniform plant density of 20 plants m⁻² (Silva et al., 2022).

Floral visitation frequency

A completely randomized block design with repeated measures was used to investigate pollinator visitation patterns at different times of the day. Five blocks were established, each representing a distinct observation day. Within each block, ten plants were randomly selected from the experimental area. This design

controlled for day-to-day variability and minimized plant-level variation, allowing the focus to remain on the effects of interest.

Data were collected during the buckwheat flowering period (July 21, 22, 25, 26, and 29, 2023) from plants sown on May 20, 2023. Different plants were selected for each observation day. Plants were chosen from the central four meters of the two middle rows in each plot, representing a usable experimental area of 3.6 m², to avoid edge effects. Visitation frequency, defined as the number of insect visits per plant per minute, was recorded by a single observer during the first ten minutes of each hour from 7:00 AM to 4:00 PM. This count included all floral visitors. The observer documented the floral resources, including pollen and nectar, collected by *Apis mellifera* (Africanized honeybees) and *Trigona spinipes*. Bees and other flowers visitors were not captured to prevent interference with their behaviour; therefore, some insects were identified only to the order level.

Additionally, for *Apis mellifera* and *T. spinipes*, the observer recorded: (1) the number of inflorescences visited by each individual bee in a single plant within one minute, and (2) the time each individual spent on each inflorescence. Five individuals per species were randomly selected during each observation interval for behavioral recording. This detailed procedure was applied only to *A. mellifera* and *T. spinipes* due to their high visitation frequency and the limited time available between observation intervals.

Flowering phenology and inflorescence morphology

Phenological and morphological monitoring of buckwheat was conducted from July 21 to August 21, 2023. Ten plants were randomly selected and tagged for daily observation, covering the entire cycle from floral bud emergence to seed formation. The phenological variables recorded were the number of floral buds (NB), number of flowers (NF), and total number of seeds (NS). Additionally, inflorescence length (IL, in mm) and diameter (ID, in mm) were measured as morphological traits. Meteorological data (temperature, relative humidity, and precipitation) were also recorded during each observation period.

Impact of pollination on yield

This experiment was conducted in the same experimental field as the floral visitation and phenology studies. A completely randomized block design was used in a 2 × 2 factorial scheme with four replicates. Treatments consisted of two pollination conditions: free pollination (open-pollinated control) and restricted pollination (insect-exclusion cages), and two sowing dates (May 20 and June 17, 2023).

Pollination cages were constructed using 2 × 2 mm nylon mesh supported by ¾-inch PVC tubing, forming cages measuring 4 m × 6 m × 2 m (at peak height) and covering an area of 24 m². The cages were installed five days before flowering and removed after flowering to allow full plant development (Chambó et al., 2014).

Harvesting was performed manually after plants reached physiological grain maturity (13% moisture). The May 20 sowing cohort was harvested at 97 days (August 25, 2023), and the June 17 cohort at 113 days (October 10, 2023). Grain yield (GY, kg ha⁻¹) was estimated after cleaning, grading, and weighing grains collected from the central four meters of the two middle rows per plot (3.6 m²).

To estimate agronomic components, 30 plants were randomly harvested from each plot, and the following averages were calculated: (a) grain mass per plant (GMP, g plant⁻¹); (b) thousand-grain weight (TGW, g); (c) number of grains per inflorescence (NGI); (d) number of inflorescences per plant (NIP); (e) number of branches per plant (NBP); (f) stem diameter (SD, mm), measured 5 cm above ground level; (g) plant height (PH, cm); (h) inflorescence length (IL, mm); and (i) inflorescence diameter (ID, mm).

Statistical analysis

Floral visitation frequency: A descriptive analysis was performed to characterize pollinator visitation patterns throughout the day and across different observation days. Subsequently, a generalized linear mixed model (GLMM) was applied to investigate the effects of temporal and climatic variables on floral visitation frequency, with hour, temperature, relative humidity, and wind speed as fixed factors, and day and plant as random factors. Given that the response variable was count data (number of visits), a zero-inflated negative binomial distribution was used via the *glmmTMB* function from the *glmmTMB* package (Brooks et al., 2024). Residual normality was assessed with the Shapiro-Wilk test. Model parameters were estimated using maximum likelihood estimation. Model selection was based on the lowest Akaike Information Criterion (AIC). The significance of temporal effects, temperature, relative humidity, and wind speed was evaluated using Wald's χ^2 test. Model performance was assessed through error metrics—mean absolute error (MAE), mean squared error (MSE), and root mean squared error (RMSE)—and validated by 5-fold cross-validation.

Flowering phenology: Descriptive statistics, including means, ranges (minimum and maximum), and 95% confidence intervals (CIs), were calculated for the response variables at each phenological stage. Linear mixed-effects models were fitted using the *lmer* function from the *lme4* package (Bates et al., 2015) to analyze the number of flowers and seeds. For both response variables, the fixed effects included day, temperature, humidity, inflorescence length, inflorescence diameter, and precipitation, with “plant” specified as a random effect. Models were fitted using restricted maximum likelihood (REML). The significance of fixed effects was evaluated through t-values and associated p-values. Multicollinearity among independent variables was assessed, and residual diagnostics were performed to confirm model adequacy.

Impact of pollination on yield: two-way analysis of variance (ANOVA) was performed to evaluate the effects of pollination (open-pollinated control and restricted pollination) and sowing date (May 20 and June 17, 2023) on agronomic components. Significant effects were interpreted directly from the ANOVA results at the 5% significance level. All analyses were conducted in R software, version 4.3.1 (R Core Team, 2023).

Results and Discussion

Floral visitation frequency

During the peak flowering period in July 2023, buckwheat attracted a diverse assemblage of insect visitors. A total of 1,499 individuals were recorded, distributed across seven taxonomic groups, comprising three identified species and four higher taxonomic categories. *Trigona spinipes* (Hymenoptera: Apidae) was the most abundant visitor, representing 49% of total visits, followed by *Apis mellifera* (Hymenoptera: Apidae) with 24%. Other recorded groups included Diptera (12%), Vespidae (8%), *Tetragonisca angustula* (5%), Coleoptera (1%), and Lepidoptera (1%).

The Shannon diversity index ($H' = 1.3921$) and Simpson index ($D = 0.6787$) indicated low evenness and high dominance in the pollinator community, despite the presence of multiple species. This pattern reflects a community structure dominated by eusocial bees, particularly *T. spinipes* and *A. mellifera*. Similar results were reported by Akter et al. (2023), who found six genera and nine pollinator species in Dhaka city, Bangladesh, with the superfamily Apoidea accounting for 44.44% of visits, followed by Coccinelloidea at 22.22%, underscoring the important role of native bees in buckwheat pollination. Campbell et al. (2016) identified *Apis mellifera* as the dominant visitor in buckwheat fields, accounting for 61% of total visits, predominantly active in the morning. In contrast, our results showed a considerably lower relative abundance of *A. mellifera* (24%) and a higher abundance of native bees, with non-*Apis* insects active throughout the day. Native wasps comprised 81.3% of this group in Campbell et al. (2016), whereas our findings revealed a lower proportion of wasps, suggesting structural differences in the pollinator community. These differences are likely driven by local environmental conditions, floral resource availability, and management practices.

Most floral visitors belonged to the order Hymenoptera. The relatively low abundance of *A. mellifera* was likely influenced by the absence of managed hives and the limited availability of floral resources in the study area. This pattern is consistent with findings by Mackell, Elsayed and Colla (2023), who reported that *Apis mellifera* abundance is generally linked to the presence of managed hives in the surrounding landscape. In contrast, *T. spinipes*, also a generalist species (Valadares, Carvalho, & Martins, 2021), exhibited a higher visitation frequency, possibly

favoured by the lower abundance of *A. mellifera* and thus reduced interspecific competition for floral resources (Mackell, Elsayed, & Colla, 2023), highlighting its potential role as a key wild pollinator under natural conditions.

Marked differences in the foraging behavior of *T. spinipes* and *A. mellifera* on buckwheat flowers were observed regarding resource collection and visitation dynamics. *A. mellifera* exhibited a higher proportion of nectar-only foraging (34%) compared to *T. spinipes* (21%), whereas *T. spinipes* performed more pollen-only collecting visits (29%) than *A. mellifera* (21%). Both species demonstrated dual foraging behavior, with 50% of *T. spinipes* workers and 45% of *A. mellifera* workers collecting both nectar and pollen during the same visit. This dual foraging likely reflects functional flexibility in resource exploitation, which may enhance pollination efficiency and contribute to colony sustainability.

Although *A. mellifera* visited more inflorescences per plant on average (3.30 ± 2.56) than *T. spinipes* (2.30 ± 1.49), the mean visit duration was similar between species, with *T. spinipes* averaging 13.8 ± 13.2 seconds and *A. mellifera* 13.1 ± 15.3 seconds. These durations are substantially longer than those reported for *A. mellifera* on rapeseed (*Brassica napus*) by Chambó et al. (2017), who recorded a visitation rate of 12.9 ± 1.40 flowers per minute, 2.96 ± 1.09 flowers per plant, and an average flower visit time of 4.2 ± 1.6 seconds. The prolonged visit duration observed here may result from larger buckwheat inflorescences or differences in resource availability and competition, factors that likely influence pollinator foraging behavior. However, it is important to note that temperature and humidity also affect visitation (Chambó et al., 2017), and a higher number of visits does not always correspond to improved pollination efficiency.

Comparisons with Aker et al. (2023) revealed temporal variation in visit duration among pollinator species and times of day in buckwheat. Their study reported that *Apis cerana* spent the longest time per floral cluster at 7:00 AM (2.85 s), while *A. mellifera* exhibited peak visit durations at 1:00 PM (4.80 s). In contrast, the present study observed consistently longer average visit durations across the entire day, regardless of species. These differences may reflect variations in floral resource availability, species-specific foraging strategies, or local environmental conditions influencing pollinator behavior throughout the day (Chambó et al., 2017).

These findings underscore the species-specific foraging patterns of *T. spinipes* and *A. mellifera*, highlighting their complementary roles in buckwheat pollination. The observed dual foraging behavior and prolonged visitation times in both species likely enhance pollen transfer, thereby contributing to the crop's reproductive success. These results suggest that integrated pollination strategies, combining both native and managed bee species, could improve pollination efficiency and overall crop productivity (MacInnis & Forrest, 2020).

In our study, the highest visitation rates occurred during the morning hours, particularly between 9:00 a.m. and 11:00 a.m., when temperatures ranged from 23.2°C to 25.8°C and relative humidity gradually decreased from 83.6% to 74.6%. Wind speeds varied from 2.7 m·s⁻¹ to 3.5 m·s⁻¹ during this period. Peak activity was recorded at 10:00 a.m., with a total of 387 individuals observed, primarily composed of *T. spinipes* and *A. mellifera*. Visitation rates dropped significantly in the early afternoon, especially after 12:00 p.m., when temperatures exceeded 26°C. Figure 1a illustrates the hourly visitation patterns, while Figure 1b suggests temporal changes in pollinator community composition across different observation days, including a potential increase in diversity and evenness.

In sunflower (*Helianthus annuus*), Chambó et al. (2011) reported higher densities of *A. mellifera* during peak activity, with 2.28 nectar foragers and 0.40 pollen foragers per capitulum. These patterns support our findings of *A. mellifera*'s dominance in nectar foraging. However, in buckwheat, both *A. mellifera* and

T. spinipes exhibited substantial pollen collection, underscoring the complementary and diverse roles these species play in the pollination process.

The results of the statistical models, including parameter estimates and their significance levels, are summarized in Table 1. This table highlights the effects of environmental variables such as time of day, temperature, relative humidity, and wind speed on pollinator visitation rates.

Descriptive results indicate that the highest visitation activity occurred in the morning between 9:00 a.m. and 11:00 a.m., when environmental conditions were more moderate. A similar pattern was reported by Akter et al. (2023), who observed peak activity of *Apis cerana* at 11:00 a.m. and 1:00 p.m., and *Apis mellifera* at 1:00 p.m. in Dhaka, Bangladesh. However, diurnal dynamics in pollinator activity are known to vary considerably across geographic regions and seasons. In our study, the lowest visitation occurred in the late afternoon, after 12:00 p.m., when temperatures exceeded 26 °C.

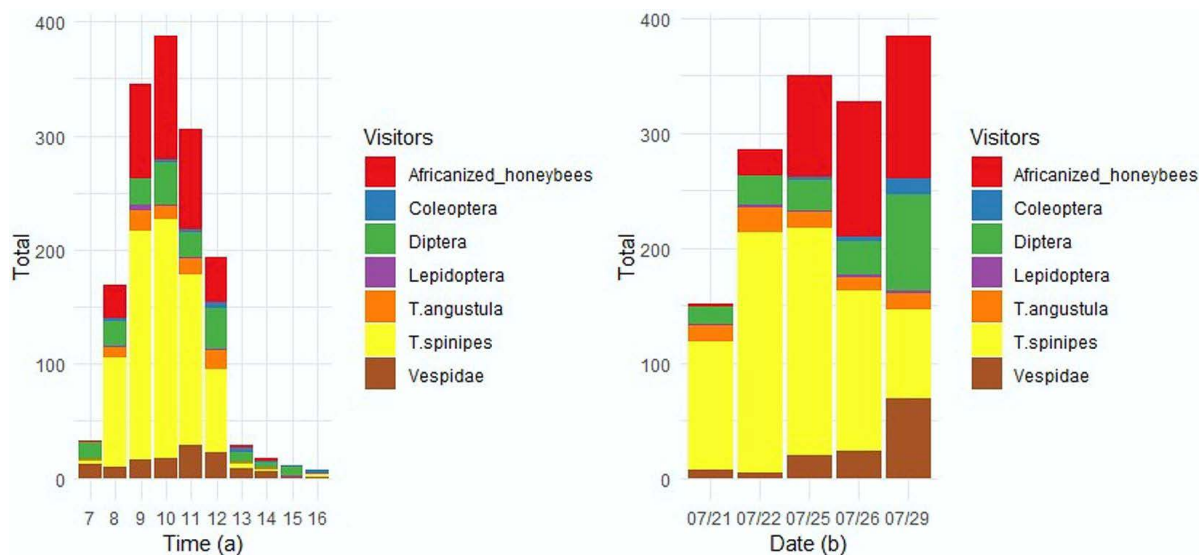


Figure 1: Variations in the number of floral visitors by hour of the day (a) and across different days (b) during the experimental period in buckwheat.

Table 1: Estimates of the parameters and significance levels for pollinators visits.

Pollinators	Intercept (β_0)	Hour (β_4)	Temp (β_1)	Humidity (β_2)	Wind (β_3)	Variance (Day / Plant)
Total visits	-0.83(ns)	-0.94***	0.57***	-0.04**	0.11***	0.02 / 0.57
<i>A. mellifera</i>	-11.9**	-1.44***	1.10***	-0.04(ns)	0.18***	5.05 / 0.004
<i>T. spinipes</i>	-4.61(ns)	-1.45***	0.68***	-0.05**	0.28***	1.24 / 0.06
<i>T. angustula</i>	-2.08(ns)	-0.96***	0.57***	-0.07(ns)	0.14*	2.87 / 1.84
Diptera	2.08(ns)	-0.97***	0.57***	-0.07(ns)	0.13*	2.87 / 1.84
Wasps	0.36(ns)	-0.57***	0.45***	-0.07**	-0.56***	9.70 / 8.41

Significance of estimates indicated by asterisks (*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant).

Our statistical models revealed that time of day had a negative and significant effect on visitation across all groups and species, confirming the decrease in activity as the day progressed. Although this pattern is apparent from descriptive results (Figure 1a), the statistical model provides a more robust assessment by quantifying the effect of time of day while controlling for climatic variables, allowing us to isolate temporal effects from environmental influences. This finding is consistent with Chambó et al. (2017), who also reported a negative effect of time of day on visitation rates. Temperature, in contrast, had a positive effect on visitation, suggesting that pollinator activity increased with temperature, likely up to a thermal optimum. Although descriptive results indicate a drop in visitation during the hottest part of the day, the statistical models offer a more nuanced interpretation of how environmental variables influence insect activity. These results corroborate those of Goodwin et al. (2021), who observed an increase in Hymenoptera activity with rising temperatures.

Relative humidity had a negative effect in most models, although it was not significant for *A. mellifera*, *T. angustula*, or Diptera. This pattern may be explained by differences in species' sensitivity to humidity, as observed by Chambó et al. (2017), who reported a positive effect of humidity on pollen-foraging pollinators but a negative effect on *A. mellifera* when collecting both nectar and pollen.

Wind speed had a positive effect on visitation for most pollinator, including *A. mellifera*, with moderate to strong effect sizes (see Table 1), but a negative effect of greater magnitude on wasps. These results contrast with those of Chambó et al. (2017), who observed a negative effect of wind speed on pollen-foraging *A. mellifera*. This suggests that pollinator responses to wind speed may vary depending on species and environmental conditions.

Additionally, random effects showed variability in visitation patterns between plants and across days, with particularly high variance for *A. mellifera*, *T. angustula*, and wasps. This suggests that factors not captured by the fixed effects, such as plant characteristics or daily fluctuations in climatic conditions, may play an important role in driving variation in visitation patterns.

Model comparisons based on Akaike Information Criterion (AIC) and deviance analysis revealed that the full models, which incorporated environmental variables and time of day, had lower AIC values and significantly better fits than the null models for all response variables. For total visitation, the AIC decreased from 3014.2 to 1952.0, with a significant improvement according to the likelihood ratio test ($\chi^2 = 1070.1$; $df = 4$; $P < 0.0001$). Similar results were found for *Apis mellifera* ($\Delta AIC = 305.3$; $\chi^2 = 313.3$), *Trigona spinipes* ($\Delta AIC = 672.7$; $\chi^2 = 680.7$), *Tetragonisca angustula* ($\Delta AIC = 40.3$; $\chi^2 = 48.3$), Diptera ($\Delta AIC = 59.7$; $\chi^2 = 67.7$), and Vespidae ($\Delta AIC = 49.2$; $\chi^2 = 57.2$), all with P values below 0.0001.

The model performance metrics indicate that all models provided excellent predictive power, with cross-validation error rates remaining low for all species. In addition, the zero dispersion index suggests that the models were well calibrated, as observed counts closely matched expected visitation rates. These results confirm the strong influence of environmental factors on visitation patterns and suggest that optimal pollination can be achieved by synchronizing flowering with favorable environmental conditions, a strategy that becomes increasingly relevant under climate change scenarios (Kehrberger & Holzschuh, 2019).

Table 2 summarizes model performance metrics and results from 5-fold cross-validation, highlighting both model fit and generalization capability. The results indicate that the models fit the data well. Additionally, the overdispersion index was close to 1 and non-significant ($P > 0.05$), indicating that the model distributions were appropriate. Cross-validation metrics reflect consistent model performance on unseen data. These findings highlight the critical role of environmental variables and time of day in shaping pollinator visitation patterns. They also provide valuable insights for the management and conservation of pollinators in agricultural ecosystems. Furthermore, the results underscore the importance of practices that support natural pollinators, as suggested by Klein et al. (2007) and Mulwa (2022), who emphasized that expanding pollinator habitats near crops could enhance food production in tropical regions.

Table 2: Performance metrics (fitted and cross-validated) and dispersion index of GLMMs for the response variables

Pollinators	MAE (fit / CV)	MSE (fit / CV)	RMSE (fit / CV)	Dispersion Index
Visitation total	1.60 / 1.70	5.27 / 6.20	2.30 / 2.48	0
<i>A. mellifera</i>	0.63 / 0.66	1.32 / 1.47	1.14 / 1.19	0
<i>T. spinipes</i>	0.99 / 1.07	2.54 / 3.26	1.59 / 1.76	0
<i>T. angustula</i>	0.23 / 0.23	0.15 / 0.16	0.38 / 0.39	0
Diptera	0.40 / 0.42	0.33 / 0.36	0.58 / 0.60	0
Vespidae	0.28 / 0.29	0.23 / 0.25	0.48 / 0.49	0

*MAE = Mean Absolute Error; MSE = Mean Squared Error; RMSE = Root Mean Squared Error; CV = Cross-validation (5-fold).

Flowering phenology and inflorescence morphology

Throughout the inflorescence development cycle of buckwheat, multiple phenological stages were monitored, and descriptive statistics with 95% confidence intervals (CIs) were calculated for the response variables. Climatic conditions corresponding to each stage were also recorded (Table 3).

During the inflorescence development cycle of buckwheat, phenological stages progressed with a generally synchronous pattern (Table 3). The initial phase (first floral buds) showed uniform development across plants, indicating coordinated reproductive timing. The start of flowering occurred under relatively humid conditions, which are conducive to floral induction. The full flowering phase was marked by intense floral emission and occurred during a period of variable climatic conditions, including fluctuations in temperature, humidity, and precipitation (Table 3). These environmental factors likely influenced flower abundance and development dynamics.

Mixed-effects models (Table 4) revealed that flower production decreased significantly over time, suggesting a gradual reduction in floral emission as plants progressed through the reproductive phase. Additionally, flower production was positively influenced by relative humidity, inflorescence length, and inflorescence diameter. Temperature and precipitation showed no significant effects on flower production in the final model. These results suggest that favorable morphological traits and increased humidity promote floral development, while floral emission declines naturally over time as part of the plant’s phenological cycle.

These mixed-effects models allow for the simultaneous consideration of fixed effects (climatic and temporal) and random effects (plant), providing more robust and precise estimates (Madden & Ojiambo, 2024). Random effects are essential for capturing the inherent variability among individual plants and observation days, ensuring a more realistic analysis of the interactions among variables.

Similar results in buckwheat were reported by Plažek et al. (2023), who found that increased floral production in certain genotypes was accompanied by higher rates of floral abortion and the formation of empty seeds. This highlights potential physiological trade-offs in reproductive allocation, especially under variable environmental conditions.

During the late flowering phase (August 16th to 20th), flower abundance markedly declined, indicating the transition to seed formation (Table 3). Climatic conditions during this period, including moderate temperatures, humidity, and low precipitation, likely influenced this phenological shift. Due to the continuous flowering pattern of buckwheat, flowering and seed formation phases overlapped, as observed by simultaneous presence of flowers and developing seeds.

In the early seed formation stage (July 24th and 25th), seed numbers were low but initiated under stable climatic conditions (Table 3). During peak seed production (July 25th to August 21st), seed set increased significantly and was positively associated with inflorescence size and precipitation, suggesting that larger floral structures and adequate water availability enhance reproductive success. These findings emphasize the importance of morphological traits and environmental factors in driving reproductive outcomes in buckwheat.

Notably, temperature and humidity did not have significant effects on seed formation ($P > 0.05$), suggesting that after flowering, these abiotic factors exert less direct influence on seed maturation. This pattern was also observed by Plažek et al. (2023), who demonstrated that traits such as inflorescence morphology and nectar characteristics, rather than isolated environmental variation, significantly affected seed weight and yield. For instance, genotypes PA13 and PA16 produced higher seed numbers, while PA15 yielded heavier seeds—likely due to specific genotypic traits and nectar availability during flowering.

Table 3: Phenological monitoring, descriptive statistics, confidence intervals, and climatic data summarized per period during the inflorescence development cycle of buckwheat.

Date	Phenological Stage	Min - Max	Mean	95% C. I. (μ)	Temp (°C)	RH (%)	Prec (mm)
21/07 - 23/07	FB	13-28	20	[18.48; 21.79]	21.4 - 22.0	77 - 89	2.4 - 9.7
22/07	SF	0-2	1	[0.64-1.76]	21.4	89	9.7
24/07 - 15/08	FF	0-19	8	[7.12; 8.18]	20.8 - 22.5	69 - 96	0 - 18.2
16/08 - 20/08	EF	0-7	1	[0.42; 1.42]	21.5 - 23.5	71 - 85	0 - 5.2
24/07 - 25/07	SSF	0-4	1	[0.48; 1.72]	21	87 - 88	1.1 - 3.6
25/07 - 21/08	PP	0-10	5	[4.31; 4.93]	20.8 - 24.3	69 - 96	0 - 18.2

FB = First Floral Buds (floral buds visible in 60% of the plants); SF = Start of Flowering (first flowers open on the inflorescence); FF = Full Flowering ($\geq 70\%$ of the inflorescences with open flowers); EF = End of Flowering (last visible flowers, with $\leq 60\%$ of the inflorescences still flowering); SSF = Start of Seed Formation (first seeds formed in the inflorescences); PP = Peak Production ($\geq 70\%$ of the inflorescences with seeds). Temp = Temperature (°C); RH = Relative Humidity (%); Prec = Precipitation (mm).

Table 4: Coefficients of the linear mixed models for the number of flowers and seeds

Variable	Estimate (β)	Standard Error	t	P
Number of flowers				
Intercept	-20.40	8.48	-2.41	0.016
Day	-0.50	0.04	-12.85	<0.001
Temperature	-0.13	0.30	-0.44	0.66
Humidity	0.07	0.03	2.13	0.034
Inflorescence Length (IL)	0.58	0.14	4.24	<0.001
Inflorescence Diameter (ID)	1.15	0.23	5.03	<0.001
Precipitation	-0.04	0.04	-0.88	0.38
Number of seeds				
Intercept	-10.76	3.05	-3.53	<0.001
Day	0.02	0.01	1.29	0.20
Temperature	0.10	0.11	0.95	0.34
Humidity	-0.01	0.01	-0.69	0.49
Inflorescence Length (IL)	0.39	0.05	7.99	<0.001
Inflorescence Diameter (ID)	0.55	0.08	6.65	<0.001
Precipitation	0.04	0.02	2.42	0.017

Although this study did not evaluate nectar composition, Plažek et al. (2023) reported that higher nectar mass was positively correlated with seed weight and negatively correlated with floral abortion, emphasizing the role of resource allocation and pollinator attraction. These findings underscore the complex interactions among floral structure, environmental conditions, and genotypic variation in shaping reproductive outcomes.

Impact of pollination on production components

For the May 20 sowing, flowering began on June 9, peaked on July 20, and ended on August 25, 2023. For the June 17 sowing, flowering began on July 6, peaked on August 28, and ended on October 7, 2023.

Grain yield (GY) and grain mass per plant (GMP) were significantly affected by the interaction between pollination treatments and sowing date. In both sowing dates, GY and GMP were higher in areas with free pollination compared to areas with restricted pollination. Regarding GY, on sowing date 1 (May 20, 2023), there was an increase of 94.00% (GY – free pollination: sowing date 1; $F = 44.70$, $df = 1$; $P < 0.0001$), and on sowing date 2 (June 17, 2023), an increase of 20.45% (GY – free pollination: sowing date 2; $F = 5.24$, $df = 1$; $P < 0.04$). For GMP, there was an increase of 126.99% on sowing date 1 (GMP – free pollination: sowing date 1; $F = 76.16$, $df = 1$; $P < 0.0001$), and an increase of 44.14% on sowing date 2 (GMP – free pollination: sowing date 2; $F = 21.80$, $df = 1$; $P < 0.04$) (Table 5). These results underscore the crucial role of insect pollinators in improving crop productivity.

The significant increases in GY and GMP under free pollination conditions align with the findings of Chambó et al. (2011), who reported a 43% higher grain yield in sunflowers visited by pollinators. Similarly, Akter et al. (2023) observed greater yield and seed weight in buckwheat under open pollination conditions. Furthermore, Chambó et al. (2014) emphasized that pollination by Africanized honeybees is especially important in adverse climatic conditions, and the yield benefits observed here across both sowing dates are consistent with their assertion that pollination can mitigate climatic limitations.

There was no significant difference in GY and GMP between sowing dates under free pollination (GY: $F = 0.10$, $P = 0.76$; GMP: $F = 0.07$, $P = 0.80$), suggesting that sowing date had a limited influence on yield when pollination conditions were adequate. However, under restricted pollination, both GY and GMP increased on sowing date 2 (June 17, 2023) (GY by 57.45%, $F = 16.70$, $P = 0.003$; GMP by 54.87%, $F = 14.43$, $P = 0.004$). The lack of significant differences between sowing dates under free pollination suggests that adequate pollination may mitigate the effects of sowing time on yield. This result is consistent with the findings of Chambó et al. (2014), who reported that cross-pollination can reduce productivity losses under unfavorable climatic conditions. The increase in yield observed for the June 17 under restricted pollination could reflect more favorable environmental conditions for self-pollination or compensatory mechanisms, but the overall lower yields highlight the benefits of maintaining active pollinator communities.

There was an effect of pollination treatments on NGI ($F = 24$, $df = 1$, $P = 0.0009$), IC ($F = 22.27$, $df = 1$, $P = 0.001$), and ID ($F = 6.36$, $df = 1$, $P = 0.03$). In the free pollination treatment, NGI was 2.75, with an IC of 15.1 mm and an ID of 6.98 mm, while in the restricted pollination treatment, NGI was 1.75, IC was 19.74 mm, and ID was 8 mm (Figure 2). Our results suggest that, in the absence of pollinators, plants may allocate surplus resources to inflorescence growth as a compensatory reproductive strategy. The greater number of grains per inflorescence (NGI) and more compact inflorescences (lower IC and ID) in the free pollination treatment further demonstrate the morphological and reproductive benefits of biotic pollination. Similar trends were observed by Akter et al. (2023), who recorded higher seed production per plant and more efficient pollination in open-pollinated buckwheat. The findings also correspond with those of Sritongchuay et al. (2020), who showed that the absence of wild bees reduced pollination efficiency, leading to incomplete fertilization and the abortion of reproductive structures, directly affecting yield.

There was a significant effect of sowing dates on TGW ($F = 12.48$, $df = 1$, $P = 0.006$), NIP ($F = 12.32$, $df = 1$, $P = 0.006$), NGI

($F = 6.00$, $df = 1$, $P = 0.04$), PH ($F = 13.58$, $df = 1$, $P = 0.005$), and ID ($F = 8.12$, $df = 1$, $P = 0.02$). On sowing date 1, TGW was 31.43 g, with an NIP of 24.38 and an NGI of 2.00, while on sowing date 2, TGW was 29.23 g, with an NIP of 40.88 and an NGI of 2.5. Notably, NIP and NGI were higher on sowing date 2, while PH and ID were greater on sowing date 1, with values of 106.63 mm and 8.00 mm, respectively, compared to 96.13 mm and 6.91 mm on sowing date 2 (Figures 3 and 4).

These results suggest that sowing date 1 provided more favorable conditions for crop development, leading to better seed quality, as indicated by the higher TGW. Although sowing date 2 resulted in greater reproductive output (NIP and NGI), the lower TGW indicates reduced seed quality, which may have negatively affected final productivity (see Figure 3). In both sowing dates, free pollination resulted in higher GY and GMP compared to restricted pollination (see Table 5). The proportional increase in these variables was greater at sowing date 1, reinforcing the idea that this period was more favorable for crop development and pollination efficiency. Similar findings were reported by Chambó et al. (2014) in rapeseed, where earlier sowing favored seed development and yield components.

Table 5: Mean values and standard deviations for grain yield and grain mass per plant in buckwheat, based on the combination of pollination tests and sowing date

Sowing date	Pollination tests	Variable (mean $\pm$ standard deviation) ^a	
		GY (kg ha ⁻¹)	GMP (g planta ⁻¹)
20/05	Free	2018.14 Aa $\pm$ 278.84	10.24 Aa $\pm$ 0.72
	Restricted	1040.19 Bb $\pm$ 31.66	4.52 Bb $\pm$ 0.59
17/06	Free	1972.55 Aa $\pm$ 150.50	10.07 Aa $\pm$ 1.12
	Restricted	1637.75 Ab $\pm$ 283.01	7.00 Ab $\pm$ 0.68

Means in the same column followed by different lowercase letters differ between pollination tests within each sowing date, according to the F-test at the 5% significance level. Means in the same column followed by different uppercase letters differ between sowing dates for each pollination test, according to the F-test at the 5% significance level. ^aMeans calculated from four observations. GY - grain yield; GMP - grain mass per plant.

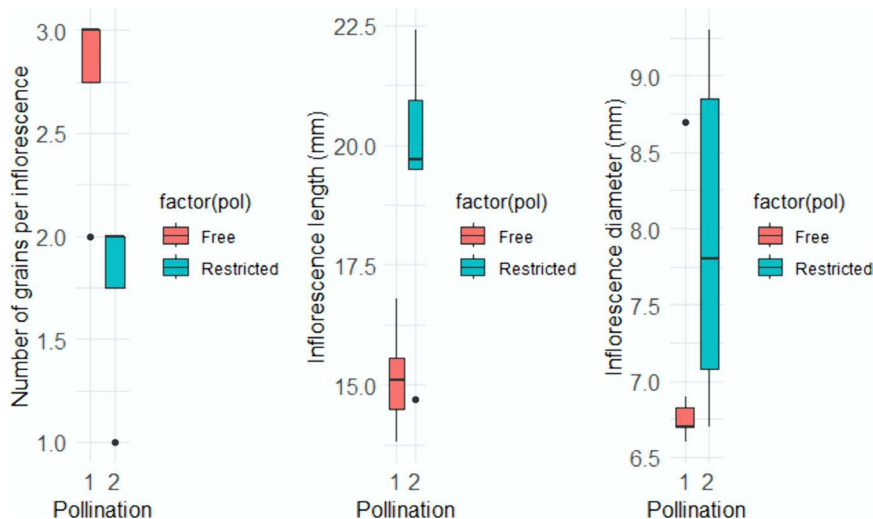


Figure 2: Effect of pollination tests on the number of grains per inflorescence (NGI), inflorescence length (IC), and inflorescence diameter (ID) in buckwheat plants, independent of sowing dates. Pollination treatments: 1 = Free pollination; 2 = Restricted pollination.

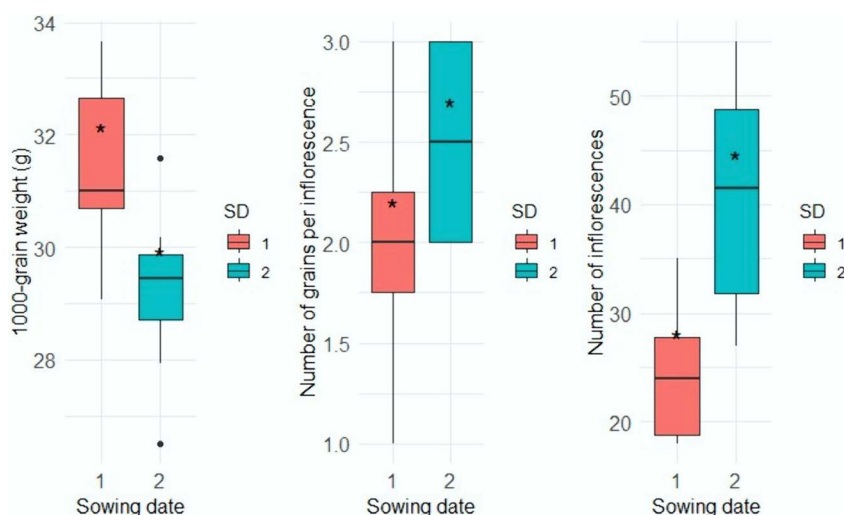


Figure 3: Effect of sowing date on 1000-grain weight (TGW), number of grains per inflorescence (NGI), and number of inflorescences per plant (NIP) in buckwheat, independent of pollination test (free or restricted).

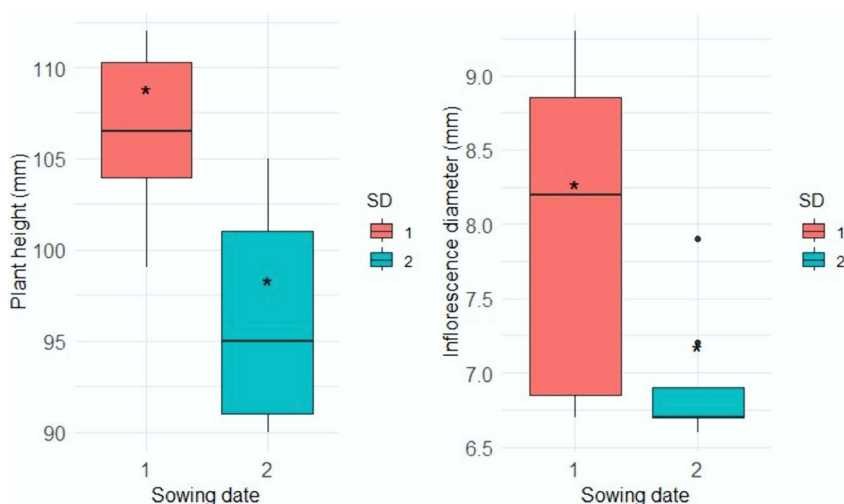


Figure 4: Effect of sowing date on plant height (PH) and inflorescence diameter (ID) in buckwheat, independent of pollination test (free or restricted).

These findings are consistent with Mainali et al. (2020), who highlighted the importance of genotype-environment interactions, including environmental factors that influence pollination, in determining reproductive success in buckwheat. Additionally, Goodwin et al. (2021) emphasized that abiotic factors such as temperature and precipitation can affect pollinator performance and, consequently, crop productivity. Based on the present findings, sowing around May 20 (sowing date 1) is recommended, as it provided more favorable conditions for plant development and pollination efficiency, leading to better seed quality and higher productivity. Maintaining active pollinator communities also proved essential, especially under suboptimal climatic conditions, reinforcing the need for integrated crop and pollinator management.

Conclusions

Floral visitor behavior to buckwheat (*Fagopyrum esculentum*) was influenced by environmental conditions. *Tetragonisca spinipes* and *Apis mellifera* were the main pollinators. Flowering phenology was synchronized and driven by humidity and inflorescence traits. Free pollination increased grain yield and mass. Sowing timed to coincide with favorable environmental conditions and pollinator activity optimized plant development and seed quality, improving yield. However, caution is advised when applying these results to different agricultural systems or climatic zones.

Author contribution

Conceptual idea: Oliveira, J.R.; Chambó, E.D.; Carvalho, C.A.L.; Methodology design: Chambó, E.D.; Data collection: Oliveira, J.R.; Silva, D.G.; Santos, N.B.; Cerqueira, L.C.; Santos, E.B.; Data analysis and interpretation: Chambó, E.D.; Oliveira, J.R.; Writing and editing: Oliveira, J.R.; Chambó, E.D.; Carvalho, C.A.L.

Acknowledgments

To the Graduate Program in Agricultural Sciences at the Federal University of Recôncavo da Bahia and to the Coordination for the Improvement of Higher Education Personnel (CAPES) for financial support.

Data Availability Statement

Data available upon request to authors.

References

- Aizen, M. A. et al. (2019). Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. *Global Change Biology*, 25(10):3516-3527.
- Akter, M. F. et al. (2023). Insect diversity and pollination effect on buckwheat (*Fagopyrum esculentum*) yield. *Journal of Biodiversity Conservation and Bioresource Management*, 9(1): 109-118.
- BPBES / REBIPP. (2019). *Relatório temático sobre polinização, polinizadores e produção de alimentos no Brasil*. São Carlos, Cubo. 184p. Available in: <https://www.bpb.es.net/wp-content/uploads/2019/03/BPBES_CompletoPolinizacao-2.pdf>.
- Bates, D. et al. (2015). Linear mixed-effects model using lme4. *Journal of Statistical Software*, 67(1):1- 48.
- Campbell, J. W. et al. (2016). Insect visitors to flowering buckwheat, *Fagopyrum esculentum* (Polygonales: Polygonaceae), in North-Central Florida. *Florida Entomologist*, 99(2):264-268.
- Chambó, E. D. et al (2011). Honey bee visitation to sunflower: Effects on pollination and plant genotype. *Scientia Agricola*, 68(6):647-651.
- Chambó, E. D. et al. (2014). Pollination of rapeseed (*Brassica napus*) by africanized honeybees (*Hymenoptera: Apidae*) on two sowing dates. *Anais Academia Brasileira Ciências*, 86(4):2087-2100.
- Chambó, E. D. et al. (2017). Statistical modeling of insect behavioral response to changes in weather conditions in *Brassica napus* L. *Arthropod-Plant Interactions*, 11:613-621.
- Cunha, L. M. et al. (2024). Land use and occupation and pesticide contamination assessment in honey from Africanized honey bees (*Hymenoptera: Apidae*) using gas chromatography-mass spectrometry. *Anais da Academia Brasileira de Ciências*, 96(4):1-16.
- Elias, M. A. S. et al. (2017). Climate change threatens pollination services in tomato crops in Brazil. *Agriculture, Ecosystems & Environment*, 239:257-264.
- Gallai, N. et al. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, 68(3):810-821.
- Goodwin, E. K. et al. (2021). Weather conditions affect the visitation frequency, richness and detectability of insect flower visitors in the Australian alpine zone. *Environmental Entomology*, 50(2):348-358.
- Hipólito, J., Viana, B. F., & Garibaldi, L. A. (2016). The value of pollinator-friendly practices: Synergies between natural and anthropogenic assets. *Basic and Applied Ecology*, 17(8):659-667.
- Kehrberger, S. & Holzschuh, A. (2019). How does timing of flowering affect competition for pollinators, flower visitation and seed set in an early spring grassland plant? *Scientific Reports*, 9:15593.
- Khalifa, S. A. M. et al. (2021). Overview of bee pollination and its economic value for crop production. *Insects*, 12(8):688.
- Klein, A. M. et al. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274:303-313.
- MacInnis, G., & Forrest, J. R. K. (2020). Field design can affect cross-pollination and crop yield in strawberry (*Fragaria x ananassa* D.). *Agriculture, Ecosystems & Environment*, 289:106738-106745.
- Mackell, S., Elsayed, H., & Colla, S. (2023). Assessing the impacts of urban beehives on wild bees using individual, community, and population-level metrics. *Urban Ecosystems*, 26:1209-1223.
- Madden, L. V., & Ojiambo, P. O. (2024). The value of generalized linear mixed models for data analysis in the plant sciences. *Frontiers in Horticulture*, 3:1423462.
- Mainali, R. P. et al. (2020). Role of intra-specific varietal mixture in pollination services: A case study in buckwheat (*Fagopyrum esculentum* Moench). *International Journal of Environment*, 9(1):115-126.
- Mulwa, R. et al. (2022). Influence of proximity to and type of foraging habitat on value of insect pollination in the tropics, with applications to Kenya. *African Journal of Agricultural and Resource Economics*, 17(2):171-191.
- Nagano, Y., & Miyashita, T. (2025). Contribution of nocturnal moth pollination to buckwheat seed set. *Arthropod-Plant Interactions*, 19:11.
- Neto, J. N. M., Vieira, R., & Schlindwein, C. (2021). Minimum size threshold of visiting bees of a buzz-pollinated plant species: Consequences for pollination efficiency. *American Journal of Botany*, 108(6):1006-1015.

- Pérez-López, L. et al. (2024). Water regime variability during the flowering phenophase of white and yellow grain maize hybrids and the relation with grain yield. *Ciência e Agrotecnologia*, 48:e005724.
- Plažek, A. et al. (2023). The yield of common buckwheat (*Fagopyrum esculentum* Moench) depends on the genotype but not on the Pin-to-Thrum ratio. *Scientific Reports*, 13:16022.
- R Core Team. (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Available in: <<https://www.R-project.org/>>.
- Sáez, A. et al. (2023). Phenological overlap between crop and pollinators: Contrasting influence of native and non-native bees on raspberry fruits over the flowering season. *Journal of Applied Ecology*, 60(12):2540-2549.
- Sritongchuay, T. et al. (2020). Insufficient native pollinators during artificially induced early flowering decrease yield and long-term economic viability of a tropical fruit crop. *Journal of Applied Ecology*, 58(1):80-91.
- Silva, E. M. S. et al. (2022). Composition and frequency of flower visitors in some varieties of melon under different crop conditions. *Revista Caatinga*, 34(4):976-984.
- Turo, K. J. et al. (2024). Insufficient pollinator visitation often limits yield in crop systems worldwide. *Nature Ecology & Evolution*, 8:1612-1622.
- Valadares, E. F., Carvalho, A. T., & Martins, C. F. (2021). Nest density, spatial distribution, and bionomy of *Trigona spinipes* (Apidae: Meliponini). *Journal of Apicultural Research*, 62(4): 680-691.