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Research Article

Daily Activity and Honey Production Patterns of *Tetragonula laeviceps* Smith (Hymenoptera: Apidae) During the Wet and Dry Seasons

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ABSTRACT
Honey production by stingless bees is closely related to the foraging activities of worker bees, particularly nectar foraging. The urgency of this study stems from the community's need for adequate understanding and information on honey production which can vary due to various factors, such as forage and season. The purpose of this study was to determine the extent to which foraging activity affects the amount of honey produced by *T. laeviceps* during the rainy and dry seasons. The focal sampling method was used to observe foraging behaviour and the acetolysis method was used to observe pollen. The abiotic factors were measured through direct observation. Honey production was observed as a result of foraging behaviour and variations in abiotic factors. In SPSS v27 software, data from foraging behaviour observations were analysed using one-way ANOVA followed by Tukey's test with 95% confidence level and interpreted in tables and figures. Multiple linear regressions and Pearson's correlations were used to test the relationship between abiotic variables and bee return to hive behaviour. All honey volume data were collected, averaged, and evaluated using bar charts. Based on these results, the amount of honey produced by *T. laeviceps* in the dry season was significantly higher than that produced during the wet season. This was because the number of bees actively foraging was also higher in the dry season (June-August) which was strongly influenced by temperature and light intensity ($p > 0.05$). In addition, the number of flowering plants available for harvest during the dry season was higher than that during the rainy season. The results of this study can be used as a reference by beekeepers to determine the appropriate time to harvest honey. Information on the types of forage plants identified in this study can provide information on bee preferences in making choices related to forage plants.

INTRODUCTION

Tetragonula laeviceps Smith is a type of social insect that only produces very small amounts of honey. Its small body size (2-3 mm) and honeypot diameter (1-2 mm) are some of the reasons why it produces very little honey. However, other factors, such as the availability of food sources, flowering season, and abiotic factors, also influence stingless bee honey production. Stingless bees organise their lives into colonies and build their nests in the nooks and crannies of walls, logs, and stones (Gadhiya & Pastagia 2019). The utilisation of *T. laeviceps* has also been widely reported as a pollinator for various plants (Chuttong et al. 2022). *Tetragonula laeviceps* has the ability to visit a wide variety of flowers such as vegetable crops, fruit crops, oilseed crops, crops, fodder forage crops, weed crops, also ornamental and flower crops. In Indonesia, *T. laeviceps* has been widely recognised and cultivated for honey production and pollination. Various plant species such as *Citrus reticulata*, Family Bromeliaceae and Cyperaceae are favorite plants of *T. laeviceps* (Riendriasari & Rahayu 2022; Aldi et al. 2023). In general, beekeepers cultivate *T. laeviceps* around agroecosystem areas due to the compatibility between the morphology of agricultural crop flowers and the morphology of *T. laeviceps*. Both internal and external factors have been shown to affect the behaviour of bees during flight. It is possible that the flying activity that occurs during the intrinsic phase is related to the colony's reproductive phase. In the species *Plebeia* distant, worker bees gather more pollen in the summer and more nectar in the winter (Nunes-Silva et al. 2010; Nunes-Silva et al. 2013).

It has also been found that the activity level of stingless bees while they are in flight is related to the overall production of the colony. Honey production is an example of something that is heavily influenced by abiotic factors such as temperature, humidity, rainfall, light intensity, and wind speed. Honey production is also heavily influenced by the number of flowers that are available, as well as the amount of nectar that is collected by the worker bees (Agussalim et al. 2017; Agussalim et al. 2020). In addition to foraging activity, hive shape and size can also affect honey production, although it does not significantly affect it (Agussalim et al. 2023). The urgency of this research is the need for adequate understanding and information to the community regarding honey production that can change due to several factors, including feed and season. Because bees respond to climatic variables, studies of flight activity and resource collecting aid in understanding the ecological are niche of species. Temperature, relative humidity, light intensity, wind, and air pressure can all influence social insect flight activity. Foraging patterns can change seasonally in response to biological factors such as blossoming (Polatto et al. 2014). Most bee habitats have distinct seasons with variable climatic conditions; nonetheless, changing weather conditions can have a significant impact on bee activity. The objective of this study is to investigate the effect that the flying activity of *T. laeviceps* has on the amount of honey that is produced during both the wet and dry seasons. This research is expected to be a reference for the beekeepers of stingless bee *T. laeviceps* in determining the appropriate feed and treatment to obtain optimal honey production throughout the year.

MATERIALS AND METHODS

Materials

This research was conducted in the Indonesian provinces of Bantul, Purworejo, and Magelang during the wet season (December 2021–February 2022) and the dry season (June 2022–August 2022). At each study site, seven colonies were used in this study. The colonies employed during wet season observations were the same colonies used during the dry season.

Methods

Bee identification

First, collection, preservation, and species identification were carried out to confirm the observed bee species. Stingless bees were soaked in hot water for a few minutes to help them relax. This allows for better organisation of bee body sections. The stingless bees were then punctured with an insect size 00 needle (0.30 mm in diameter). The needle was then inserted in either the dorsal or lateral thorax. As a result, embedding has occurred. The puncturing method is designed to make it easier to observe under a microscope. Morphological features were studied and photographed with a stereomicroscope and Optilab 2.2. Observed morphological characteristics include the hind tibia, basitarsus, malar space, mandible, head, clypeus, propodeum, mesoscutum, mesoscutellum, antennae, eyes, gena, forewing, wing venation, hamuli, and body color (head, clypeus, thorax, abdomen, tegula, and wings). (Azizi et al. 2020). The identification was based on (Sakagami 1978).

Foraging behaviour

Foraging behaviour of *T. laeviceps* as well as honey production were the variables that were investigated for this study. Observation of foraging behaviour using focal sampling method (Martin & Bateson 1986). Ten days per month were spent at each site to observe the behavior of *T. laeviceps* returning to the nest. The foraging behavior of *T. laeviceps* was recorded by a CCTV camera installed at one meter from the nest. For fifteen minutes, at ten-minute intervals, a counter was used to count the number of worker bees that brought pollen back to the hive as well as the number of worker bees that did not. Observations continued until *T. laeviceps* stopped foraging.

Measurement of abiotic factors

Parameters measured included air temperature and humidity using a thermohygrometre, wind speed using an anemometre, light intensity using a luxmeter, elevation using an altimetre, and rainfall using an ombrometre. Measurement of abiotic factors was carried out during the bees' foraging activities. Measurement of these abiotic factors was carried out in conjunction with observations of foraging activities for ten days per month.

Measurement of honey volume

A measurement of honey production quantity was conducted to determine the fluctuation of honey volume produced by *T. laeviceps* during the rainy and dry seasons. The measurement of honey volume is carried out in accordance with the honey harvesting schedule by local farmers. At the time of honey volume measurement, the last honey harvest by the farmer was two months before the study took place.

Pollen collection, preservation, and identification

Pollen collection on *T. laeviceps* limbs was conducted in the morning for 3 days at each site. Pollen collection was done at 10-minute intervals during nest entry and exit observations. Pollen-carrying bees were captured at the nest entrance using an insect net. Pollen found on the limbs of worker bees was taken using tweezers and then put into an Eppendorf tube in dry conditions. After the pollen was collected, the bees were released again. The collected pollen was separated based on the location and time of sampling. Preparation and observation of pollen morphology were carried out on all collected pollen samples. Pollen preparation is done using the acetolysis method (Erdtman 1972). The acetolysis stage is when the pollen is put into a 1.5-ml Eppendorf tube and acetyl anhydride is added: H2SO4 in a ratio of 9:1. The tube containing the pollen was heated using a water bath at a temperature of 80–90°C for 5 minutes. Next, the supernatant was discarded, 1 ml of distilled water was added, and the mixture was centrifuged at 12000 rpm for 2 minutes, then the supernatant was discarded and distilled water was added until the supernatant was clear. The supernatant was discarded, and then the sample was put into an oven at 60°C for one night with the Eppendorf lid open. After overnight, the samples were removed from the oven, and pollen preparations were made. On the dried samples, each was dripped with 1 ml of glycerine and then stirred. A drop of sample was taken using a drop pipette and dripped on a glass slide, then covered with a cover glass. The pollen preparations were observed using a compound microscope and an Optilab connected to a computer. The identification of pollen refers to the Pollen Flora of Taiwan (Huang 1972) and the Australian Pollen and Spores Atlas at http://apsa.anu.edu.au/ (APSA 2017).

Data analysis

Data from observations of foraging behaviour were analysed using a one-way ANOVA followed by a Tukey test with a 95% confidence level on SPSS v27 software and interpreted in tables and figures. Correlations between abiotic factors and bee return to hive behaviour were analysed using multiple linear regression and Pearson correlation. All honey volume data were combined, averaged, and interpreted in the form of bar charts.

RESULTS AND DISCUSSION

Foraging activity of *T. laeviceps*

Between the months of December and February, there was a decrease in the number of worker bees that are out collecting food in their natural habitat and older surroundings (the wet season). Nonetheless, there was a rise in foraging activity between June and August (the dry season).

In December 2021, the average number of bees carrying out foraging activities in Bantul was 257,991±60,902 individuals; in Purworejo there was 517,681±87,513 individuals; in Magelang there was 335,215±63,608 individuals (Figure 1).

In January 2022, the average number of bees carrying out foraging activities in Bantul was 519,742±87,531 individuals; in Purworejo there was 489,913±87,523 individuals; in Magelang there was 525,761±69,811 individuals (Figure 2).

In February 2022, the average number of bees carrying out foraging activities in Bantul was 506,827±69,235 individuals; in Purworejo there was 508,1193±62,113 individuals; in Magelang there was 477,138±54,108 individuals (Figure 3).

In June 2022, the average number of bees carrying out foraging activities in Bantul was 486,714±53,125 individuals; in Purworejo there was 586,991±56,218 individuals; in Magelang there was 708,423±49,106 individuals (Figure 4).

In July 2022, the average number of bees carrying out foraging activities in Bantul was 723,918±45,112 individuals; in Purworejo there was 612,68±45,521 individuals; in Magelang there was 603,308±39,515 individuals (Figure 5).

In July 2022, the average number of bees carrying out foraging activities in Bantul was 604,122±40,715 individuals; in Purworejo there was 765,131±39,143 individuals; in Magelang there was 695,724±36,311 individuals (Figure 6).

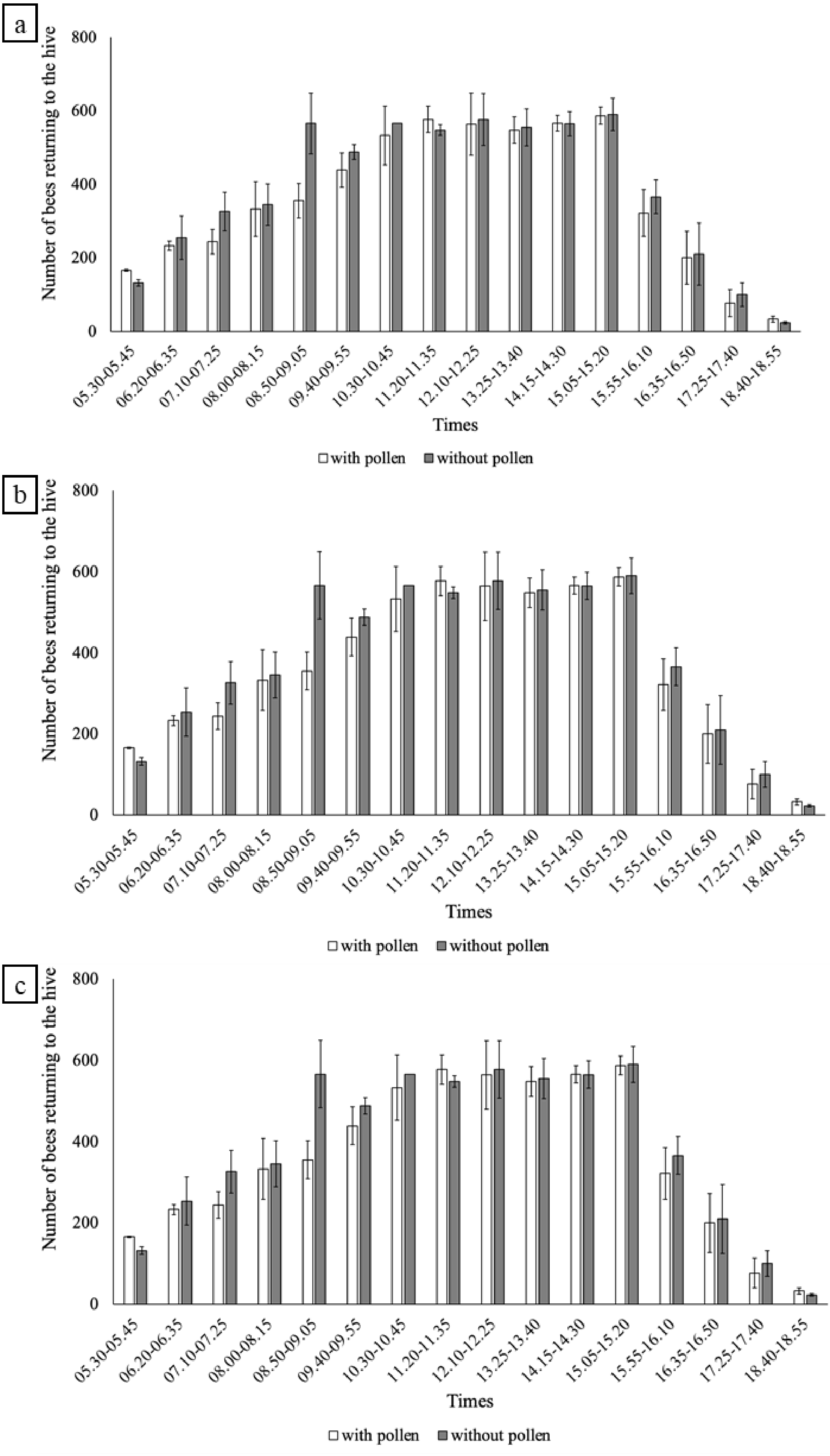


Figure 1. The number of *T. laeviceps* bees returning to the hive in December 2021 (wet season): a) Bantul, b) Purworejo, c) Magelang. Bars = standard deviation.

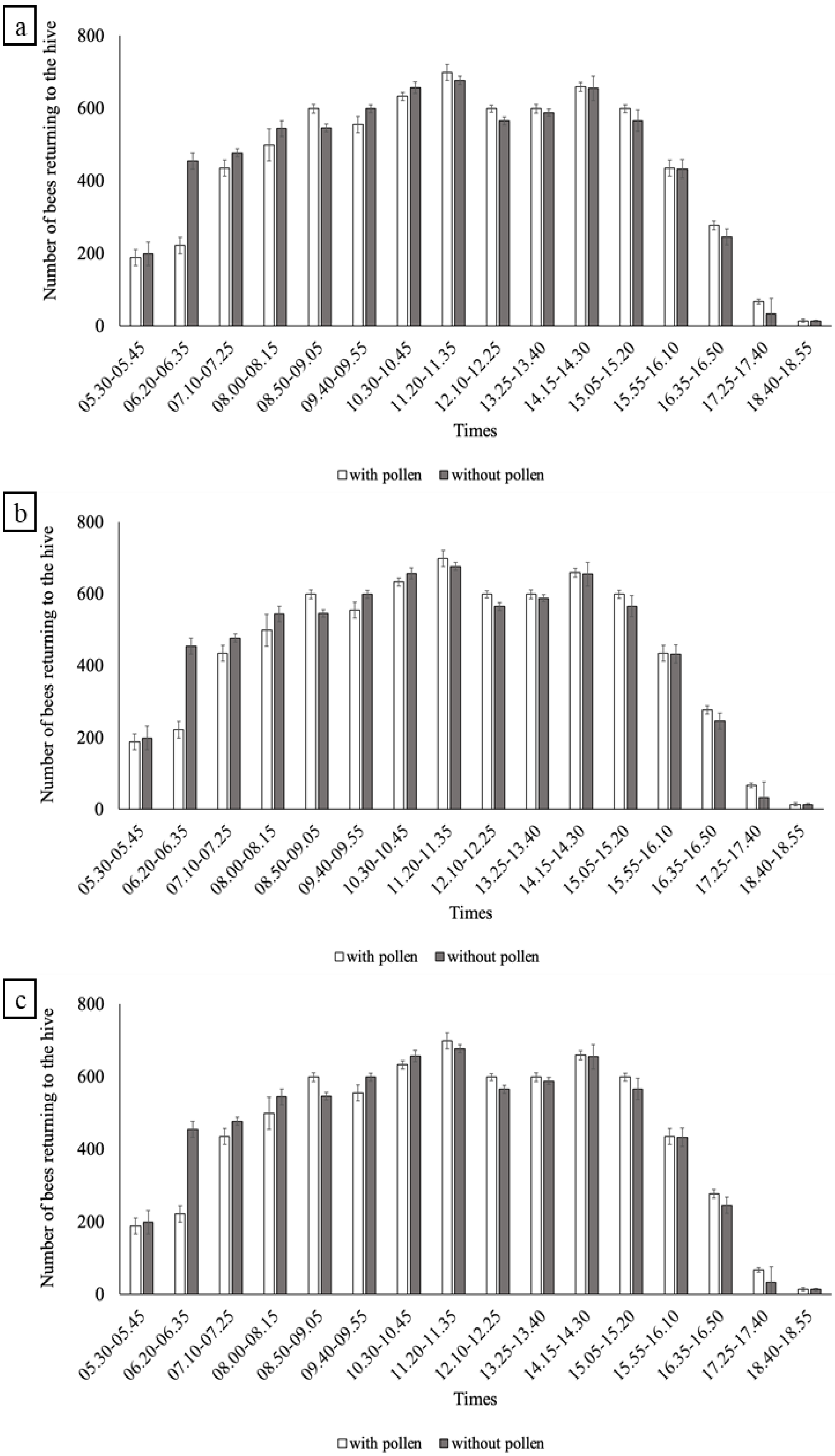


Figure 2. The number of *T. laeviceps* bees returning to the hive in January 2022 (wet season): a) Bantul, b) Purworejo, c) Magelang. Bars = standard deviation.

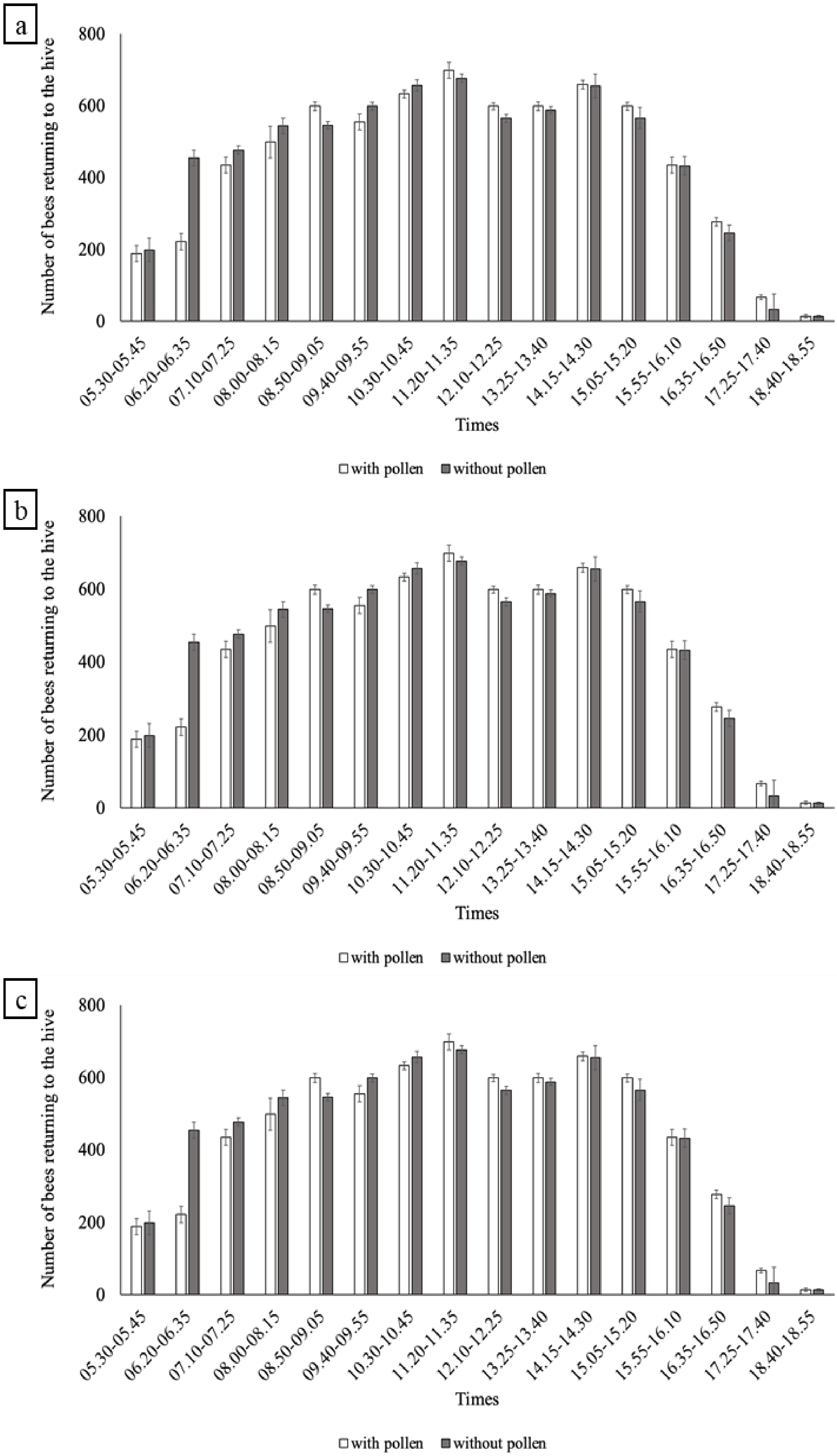


Figure 3. The number of *T. laeviceps* bees returning to the hive in February 2022 (wet season): a) Bantul, b) Purworejo, c) Magelang. Bars = standard deviation.

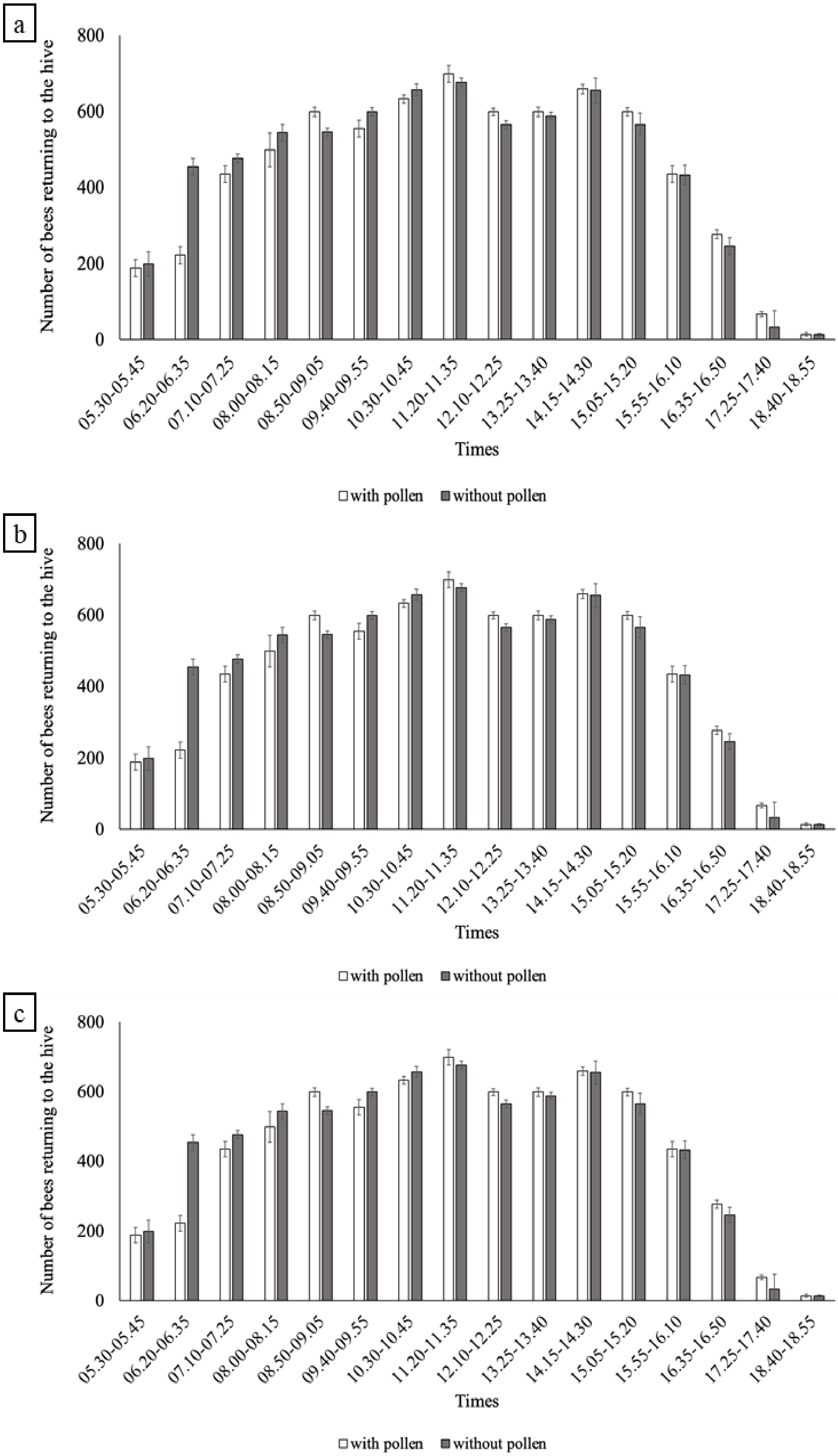


Figure 4. The number of *T. laeviceps* bees returning to the hive in June 2022 (dry season): a) Bantul, b) Purworejo, c) Magelang. Bars = standard deviation.

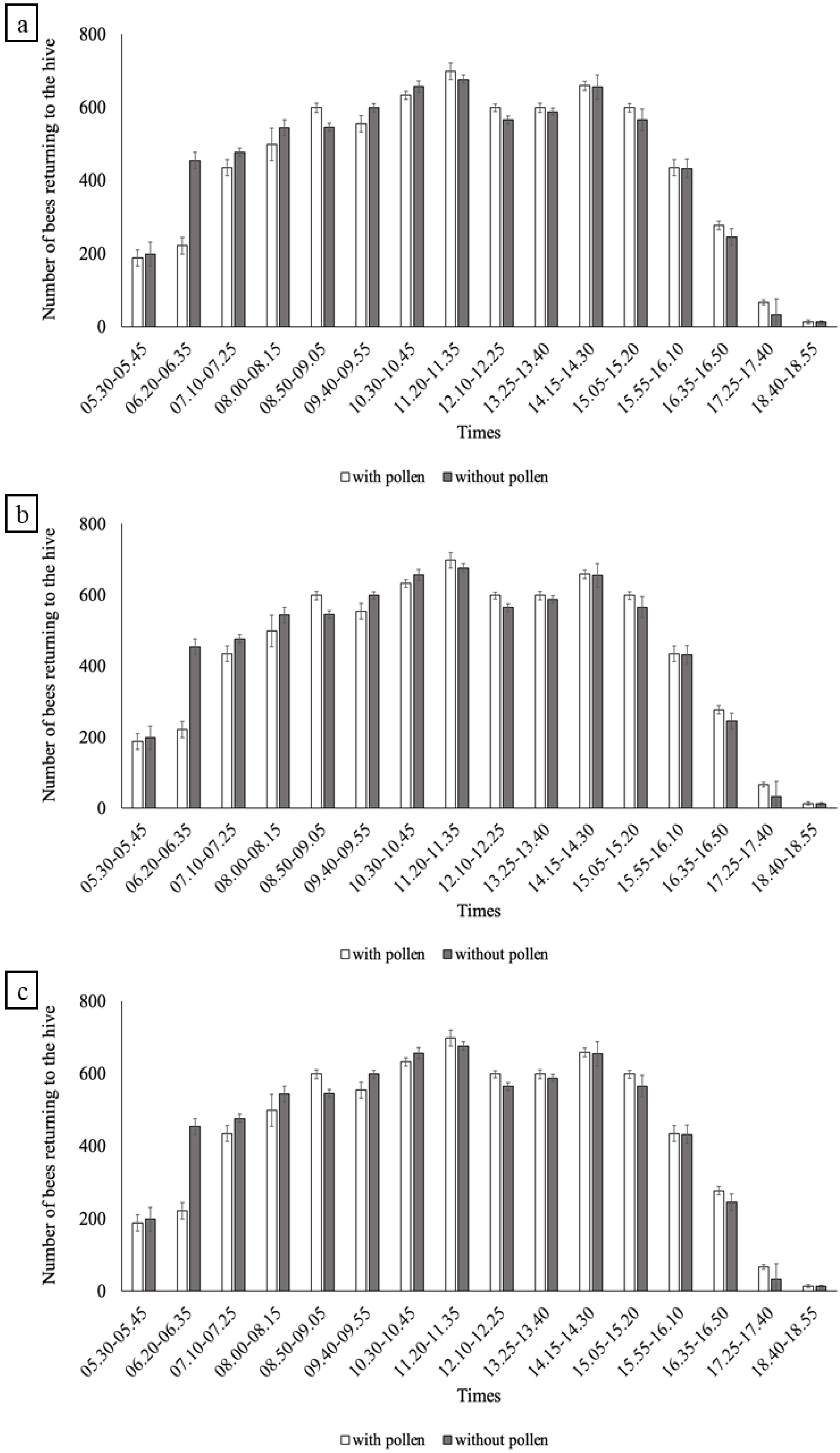


Figure 5. The number of *T. laeviceps* bees returning to the hive in July 2022 (dry season): a) Bantul, b) Purworejo, c) Magelang. Bars = standard deviation.

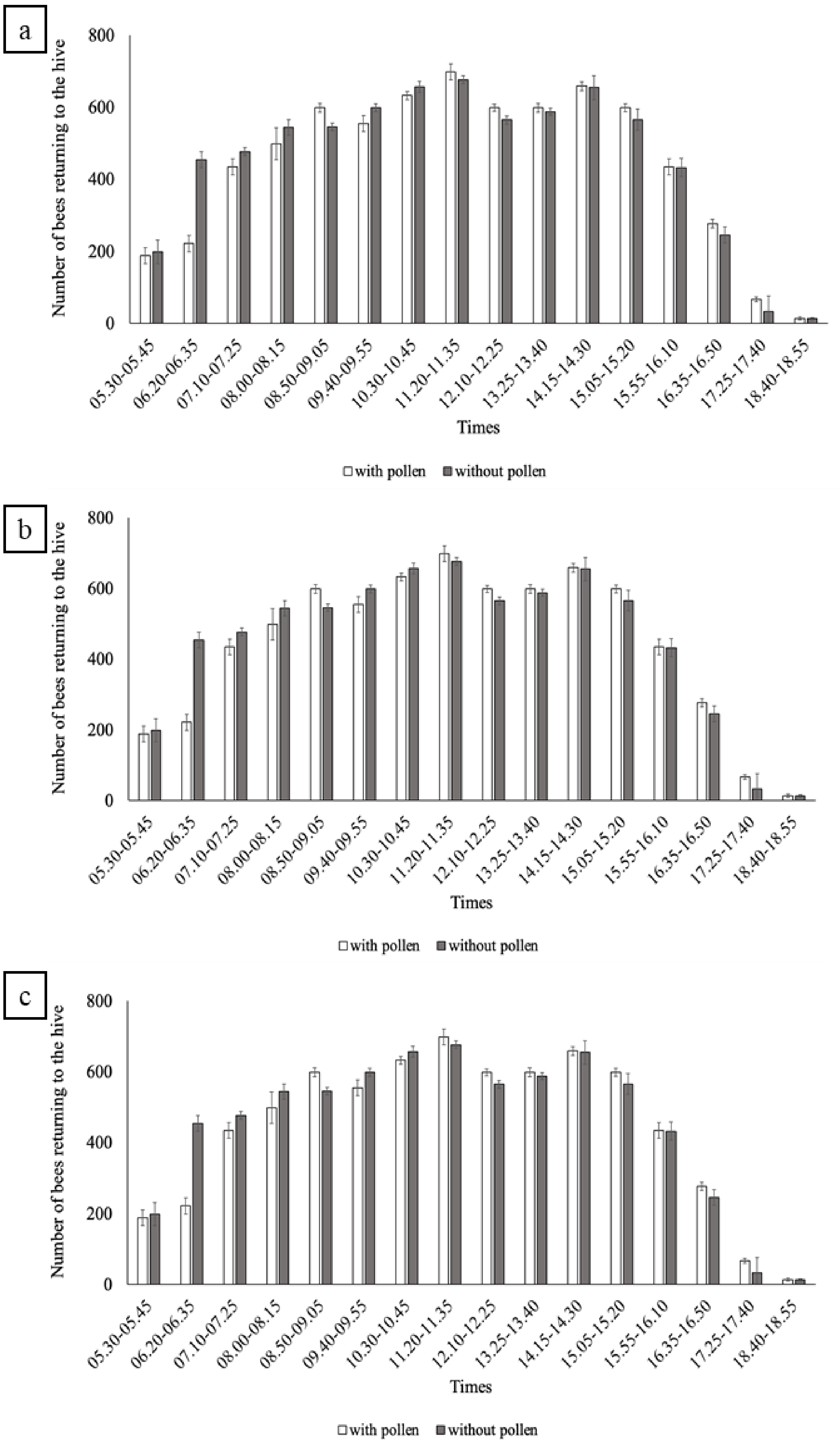


Figure 6. The number of *T. laeviceps* bees returning to the hive in August 2022 (dry season): a) Bantul, b) Purworejo, c) Magelang. Bars = standard deviation.

There was a possibility that the distance travelled by air was not directly proportionate to the distance covered by natural forage. The orientation of the feeding source could be the cause of the capacity to return to the nest after being displaced over a great distance. This extremely large gap between these bees' potential flight distance and their actual flight distance can be attributable to the quality of the resources and the spatial distribution of those resources (Westphal et al. 2006). Pollen is often the only resource that bees are choosy over, as opposed to nectar, hence foraging regions for these two resources may be different (Biesmeijer & Slaa 2004).

Correlation between abiotic factors and foraging activity of *T. laeviceps*

Foraging behaviour in social bees can be influenced not only by the demands of the colony but also by abiotic elements such as temperature, the amount of light present, and the speed of the wind. The geographic distribution of insects and other ectotherms can be partially explained by temperature, making temperature a significant aspect in this explanation (Wallace & Lee 2010).

Table 1. Correlation of abiotic factors with the activity of *T. laeviceps* in the new environment.

Abiotic factors	Significance value	Pearson correlation value	Adjusted R square	R square
Temperature	0,071	0,522		
Humidity	0,045	0,694		
Light intensity	0,054	0,528	0,227	0,810
Wind velocity	0,102	-0,421		
Rainfall	0,045	0,533		

There was a substantial connection between abiotic conditions and bees' ability to engage in foraging behaviour as shown by the fact that the number of bees that return to the hive has a correlation value of 0.81 with abiotic factors (Table 1). The coefficient of determination was 0.227 which indicated that abiotic factors impacted 22% of the foraging activity of *T. laeviceps* in the new environment, whereas biotic factors influenced 78% of the foraging activity. The significant effect of biotic factors on the number of bees that returned to the nest of *T. laeviceps* was known at the same time as the significance value of each abiotic factor was determined. There was no effect of any biotic component on the foraging behaviour of *T. laeviceps* in the new environment ($p > 0.05$).

There was an increase in the number of bees going in and out of the hive during the day with the light intensity varying from 1789 x 10 lux to 3282 x 100 lux, according to the observations that were made. Bees use the light to guide them as they look for food. The forager's visual acuity is improved, and the bee's body temperature is raised because of the increased light intensity (Heard & Hendrikz 1993; Polatto et al. 2014). On the other hand, activity dropped off significantly in the afternoon or when the weather was cloudy, and the light intensity was 1000 lux. This condition arises because of low levels of light intensity, which reduce the insects' capacity to see well enough to fly. In the morning, the temperature has a significant impact on the number of bees that leave the nest, whereas in the afternoon, the intensity of the light has the greatest bearing on this behaviour. In Batusangkar, West Sumatera, a study found that environmental factors such as temperature, light intensity, and humidity collectively influenced the activity of *T. laeviceps* worker bees by 78%. The remaining 22% was believed to be influenced by unknown factors. These stingless bees were observed to be active from early morning when the temperature was 26°C, humidity was 87%, and light intensity was 720 Lux. The activity of the hive tended to increase as temperature and light intensity rose, while humidity decreased. Specifically, 11:00 am was recorded as the time with the highest average number of workers entering the nest (Puteri et al. 2022). The study conducted by Salatnaya et al. (2019) on *T. laviceps* in monoculture and polyculture gardens showed that the environment plays a crucial role in influencing the entry and exit activities of bees, which in turn affects propolis production. The highest level of bee activity was recorded when the temperature reached 26-28°C, humidity was between 55-71%, and light intensity was 46,875-91,347 lux. The environment also had an impact on the amount of propolis produced, with 27.79 g being produced in the monoculture garden and 48.80 g in the polyculture garden. The significant difference in propolis production was mainly attributed to environmental factors (Salatnaya et al. 2020).

It is possible for each species or group to have a unique reaction to the abiotic elements present, even when they are in the same environment. In addition, the same species can have very warious responses depending on the habitat in which it is living. A variety of abiotic elements, such as temperature, light intensity, humidity, wind speed, and rainfall, can influence the bee activity in a particular region. Because the bee's thoracic muscle needs to attain a particular temperature before it can fly, one of these factors can influence the outcome of the situation (Woods et al. 2005).

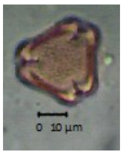
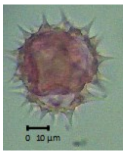
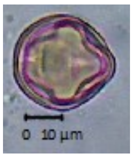
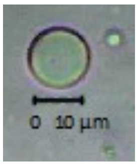
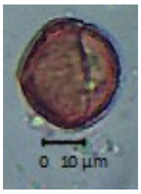
Pollen collected by *T. laeviceps* during the dry and wet seasons.

Tetragonula laeviceps bees collect more diverse pollen during the dry season than during the wet season. Pollen types identified based on identification results include *Xanthostemon chrysantus* (Myrtaceae), *Chromolaena odorata* (Asteraceae), *Picria felterrae* (Linderniaceae), *Zea mays* (Poaceae), *Portulaca oleracea* (Portulacaceae), *Antogonon leptopus* (Polygonaceae), *Euphorbia pulcherrima* (Euphorbiaceae), *Punica granatum* (Punicaceae), *Carica papaya* (Caricaceae) (Table 2).

Because illnesses are more prevalent in the environment during the wet season, there are less flowering plants than there are during the dry season. This is due to the fact that plants are more susceptible to attacks during the rainy season. In addition, precipitation of sufficient intensity causes pollen to be washed away and deteriorate, so it prevents reaching the stage of pollination (McLaren & McDonald 2005; Wijesinghe et al. 2020). Plant phenology is the outcome of the combination of biotic and climatic conditions which through the process of natural selection defines the optimal times for growth and reproduction. Because of the influence that seasonality has on patterns of seed generation, germination, seedling survival, and growth, the timing and duration of rainfall are two of the most important factors to consider when attempting to comprehend the dynamics of tropical dry forests (Khurana & Singh 2001; Borchert et al. 2004)

Honeybees collect two types of food besides water: nectar which is converted into honey and serves as a source of carbohydrates, and pollen which fulfilled the colony's need for proteins, minerals, and fats. Pollen is essential not only for the production of brood food but also for the development of tissues in newly emerged workers, such as the hypopharyngeal glands and fat bodies, which are necessary for brood rearing and winter-

Table 2. Types of pollen collected by *T. laeviceps* in the dry and wet seasons.

Pollen types	Family	Species	Seasons	
			Wet season	Dry season
	Myrtaceae	*Xanthostemon chrysantus*	√	√
	Asteraceae	*Chromolaena odorata*	√	√
	Linderniaceae	*Picria felterrae*		√
	Poaceae	*Zea mays*		√
	Portulacaceae	*Portulaca oleracea*	√	√
	Polygonaceae	*Antogonon leptopus*	√	√
	Euphorbiaceae	*Euphorbia pulcherrima*		√
	Punicaceae	*Punica granatum*		√
	Caricaceae	*Carica papaya*		√
	Passifloraceae	*Turnera subulata*	√	
	Asteraceae	*Ageratum conyzoides*	√	

ing (Hoover & Ovinge 2018). The consumption of pollen by adult workers depends on their age and the tasks they perform with nurse bees that are actively involved in brood rearing and feeding the queen consuming relatively large amounts of pollen. Although no direct correlation was observed between pollen and honey production, the type of pollen collected by bees may indicate the type of plants visited by worker bees. This is significant because it is difficult to identify the plants that bees visit to collect nectar.

Honey production in all locations during the wet and dry seasons

In Indonesia, beekeeping plays a significant role in the way of life of the country's rural population. Because of this, it is necessary to ensure that an adequate quantity of honey is produced. During the dry season, several different bee species, including *T. laeviceps*, can produce enormous quantities of honey. On the other hand, honey production drops significantly during the wet season. Honey production is affected by several factors, including the size of the colony, the kind and quantity of feed sources, and foraging activity (Abou-Shaara et al. 2017). Each worker bee in a colony of stingless bees is only capable of transporting a single food item when it goes out to forage (Fikru 2015; Hoover & Ovinge 2018). Honey production is directly proportional to the number of nectar-producing plants and foragers present in the area surrounding the hive.

Honey production in *T. laeviceps* was greater during the dry season which lasted from June to August, as opposed to the wet season which lasted from December to February (Figure 7). This was because there is a larger quantity of flowers available during the dry season, as well as a greater variety of those flowers, in comparison to the wet season. The decrease in the number of bees that returned to the nest during the rainy season was the indication that the foraging rate of *T. laeviceps* dropped during this time as well. Honey production by *T. laeviceps* rose across the board at all the research sites as a direct result of increased foraging activity during the dry season. The results of the pollen identification of *T. laeviceps* showed that the number of flowering plants that were available was lower during the rainy season. This was a factor that contributed to the reduction in honey production that took place between December and February. Research results related to *T. laeviceps* honey in Subang and Cileunyi Wetan showed that the color of honey could range from clear yellow to cloudy brown, which was influenced by the location where it was cultivated. Its taste can be sour, as indicated in many studies, due to a combination of honey, pollen, and fermentation that occurs during storage in the honey pot (Abduh et al. 2020). The flavour of honey was also influenced by the types of food and abiotic factors that were available to bees during their foraging activities.

The amount of honey produced by *T. laeviceps* in the wet and dry seasons was proportional to the number of pollen species collected. In the rainy season, there were fewer flowering plants, resulting in less honey production. During the dry season, the number and types of flowering plants were greater, resulting in increased honey production. Honey production by bees was the result of a series of interconnected factors. The conditions of abiotic factors were influenced by the season, and vice versa. Abiotic factors also influenced the foraging activities of worker bees. Foraging activity ultimately determined the quality and quantity of the material brought back to the hive.

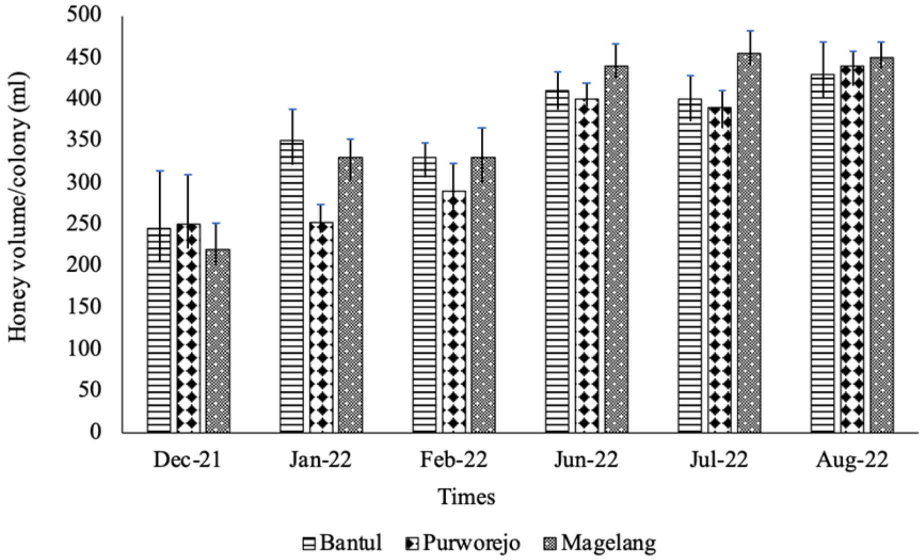


Figure 7. Honey volume per colony on *T. laeviceps* in the wet season (December -February) and dry season (June-August). Bars = standard deviation.

CONCLUSION

The variables that had a significant impact on the foraging activity of *T. laeviceps* were temperature and light intensity, whereas humidity and wind speed did not. Foraging activity of *T. laeviceps* was higher during the dry season than it was during the wet season. This was likely due to the possibility of a greater number of flowers being present during the dry season. As a result, there was a greater availability of pollen and nectar during the dry season. The foraging activity of worker bees influenced the amount of honey produced by the *T. laeviceps* species. *Tetragonula laeviceps* was seen to be more active in its nectar collection activities during the dry season as opposed to the wet season.

AUTHOR CONTRIBUTION

A.G.M.I. collected and analysed the data and wrote the manuscript. Y.M. analysed the data and wrote the manuscript. B.R. and S.K. designed the research and supervised all the process.

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CONFLICT OF INTEREST

There is no conflict of interest regarding the research or the research funding.

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