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A Manipulable Entrance Honey Bee Hive System Allowing Bees to Forage Outside Part of the Day Improved Zucchini Pollination in Greenhouses and Reduced Damage to the Colonies

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Abstract: Honey bee colonies rapidly decline when confined to greenhouses, increasing pollination rental costs as they need to be replaced frequently. We tested a hive system with entrances that can be manipulated to direct bees to inside or outside greenhouses containing a zucchini crop. In one greenhouse, the bees could only forage inside for 15 days; in another, bees were directed to the inside from 5 to 9 AM, after which they foraged outside. This procedure was repeated two more times in each greenhouse with new hives. Data was collected on how the number of bee flower visits affected fruit production, the frequency of flower visits, and the amount of bee brood and food in the hives. Flowers visited by bees four times or more set more and larger fruit. The frequency of flower visits by bees from the hives confined to the greenhouse was reduced after eight days; it was not reduced in the greenhouse with bees that could forage outside. Bee brood area was reduced in the colonies that were confined to the greenhouse, while it was maintained in the semiconfined hives. The hive system with controllable entrances proved effective for pollination, while causing less damage to the bees.

Keywords: brood; honey; pollen; fruit set; protected cultivation

1. Introduction

Among the various options for food production, greenhouse agriculture offers various advantages that can compensate for the greater investment necessary. It allows for cultivation throughout the year, reduces plant exposure to some negative effects of ultraviolet light, improves the efficiency of natural resource utilization, especially water, and it mitigates environmental impacts due to a reduced need for pesticides. Greenhouse farming is less vulnerable to risks from varying weather conditions and pest attacks, making it more reliable compared to open-field agriculture (Koukounaras, 2020).

The use of greenhouses for food production has been growing worldwide, a trend expected to continue given the increasing challenges faced by open-field production, particularly considering climate change effects. Additionally, the reduced carbon footprint, high efficiency in the use of chemical and biological inputs, and the ease of implementing intelligent systems add value to the products obtained in this closed cultivation system, economically benefiting farmers (Nemali, 2022).

In comparison with open-field production, one of the few disadvantages of closed cultivation is the absence of biotic pollinators when plants that depend on these pollinating agents are cultivated in such environments. Introducing bee colonies into these systems can enhance fruit set and improve fruit quality (Zhang et al., 2022).

Among bees, bumble bees, *Bombus terrestris*, have been widely used to pollinate various crops in greenhouses, especially tomatoes, due to their efficient buzz-pollination behavior. However, this species is not native to many countries, and its introduction has been considered a potentially harmful alternative due to concerns about disease and pest introductions and competition with local bees (Evans, 2017). Moreover, even for plants with poricidal anthers, such as solanaceous species, honey bees, the most well-known and widespread bees globally, can act as pollinators (Cribb et al., 2015; Vinícius-Silva et al., 2017) for these plants and many other crops (Free, 1993; Iwasaki and Hogendoorn, 2021). Among cultivated crops, honey bees account for approximately 50% of all bee-mediated pollination services, which is significant considering the existence of approximately 20,000 bee species, including hundreds of social bee species (Kleijn et al., 2015; Osterman et al., 2021).

Given their efficiency in pollinating many crops, environmental adaptability, generalist habits, and well-known management systems, honey bees are often used for pollination services, even in closed environments. Their use has already proven valuable economically for pollinating various crops grown in greenhouses, including strawberries (Cao et al., 2023), sweet peppers (Dag et al., 2008), melons (Dag and Eisikowitch, 1999), cucumbers (Nicodemo et al., 2013), watermelons (Lee et al., 2018), tomatoes (Cribb et al., 2015), and squash (Rodrigues et al., 2021). However, maintaining hives in a closed environment is stressful for honey bees, many of which die trying to get out, and the limited food resources for the bees in such environments often weakens the colony, compromising colony pollination efficiency and increasing the cost to the beekeeper (Vyas and Plunkett, 2018).

A strategy that can mitigate the impact on colonies is a system that allows bees to enter the greenhouse according to the pollination needs of the crop, and direct them to the outside when bees in the greenhouse could interfere with other activities, such as pruning, harvesting, and pest control activities. Adopting such a system would also benefit the bees by allowing them to forage outside the greenhouse later in the day when flowers of many greenhouse crops are no longer available. To evaluate a controllable entrance hive system (Nicodemo et al., 2018) that allows farmers to direct bees to inside or outside the greenhouse, we tested it for zucchini squash production, to determine the impact on pollination, fruit formation, and honey bee colony wellbeing.

2. Material and methods

2.1. Experimental Site and Cultivation of Zucchini

This study was conducted in the municipality of Dracena, São Paulo State, Brazil (Latitude: 21° 29′ 0′′ S, Longitude: 51° 32′ 0′′ W, altitude: 392 meters), from August to December 2022. Seeds of zucchini (*Cucurbita pepo*) plants, cultivar Alicia, provided by Sakata Seed Sudamerica, were sown in trays with 128 cells filled with Carolina Soil planting substrate. After 15 days, the seedlings were transplanted into beds (1.2 x 0.8 m) in two arched greenhouses, measuring 15 x 7 m, with a ceiling height of 3.2 m, covered with agricultural film, and laterally lined with a 50% aphid-proof monofilament screen. Irrigation was provided through drip tubes along the planting lines, and fertilizers were applied at planting and as top-dressing according to Cantarella et al. (2022).

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2.2. Management of the Bee Colonies

Africanized honey bee colonies (*Apis mellifera*) were used, housed in standard depth Langstroth five-frame hives, maintained in a university apiary. Before introduction into the greenhouses, the colonies were standardized with three frames of brood and two frames of food, with a population of approximately 7,000 adult bees. In each hive, a shallow Langstroth frame was used to make room for two screen funnels to the inside of the hives in the dual entrance honey bee hive system (Nicodemo et al., 2018).

In both greenhouses, an opening was made in the aphid-proof screen at 1.4 m from the ground, providing space for inserting the hives, which were placed on wooden supports. In the greenhouse with the manipulable entrances, one double entrance of the hive was directed inside and the other outside the greenhouse. Each end of this hive had two openings, the main one for bees to enter and exit, and a secondary one (circular) for bee entry only, using a screen funnel inside the hive. Modifications to the original design of the entrances were made (Rodrigues et al., 2021), to allow the farmer/operator to control the access of the bees to the greenhouse without a need for a smoker or protective clothing (Figure 1). In the other greenhouse, the hive had only one open entrance, allowing bees access only to inside the greenhouse.

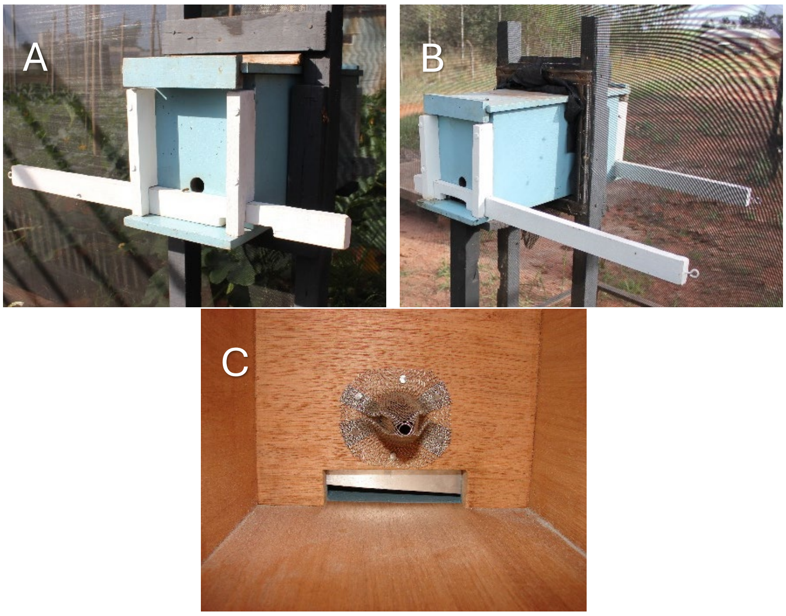

Figure 1. A. Hive with two entrances observed from outside the greenhouse with the main entrance closed. B. Detail of the open internal main entrance, allowing bees access inside the greenhouse. C. Internal view of the hive without the frames, highlighting the screen funnel; bees can enter the hive but cannot leave through this opening; an open bottom entrance and a similar screen funnel to the inside was at the other end of the hive.

Bees in the hive with entrances to inside and outside the greenhouse had access to the zucchini flowers daily between 5 and 9 AM. During the rest of the day, foraging bees were directed to outside the greenhouse by opening the external main entrance and closing the internal main entrance at 9 AM. We denominated this type of hive management semi-confinement. Bees returning to the hive from the greenhouse with the main entrance closed could only enter through the secondary entrance, through the screen funnel, preventing them from returning to the greenhouse (Figure 1). Bees could exit the hive only through the side where the main entrance was open. Each day, the internal main entrance was opened, and the external one was closed at 5 a.m.

In the other greenhouse, there was no access to the external environment; the foraging bees remained completely confined to the greenhouse. Each honey bee colony, for both confinement systems (partial or total), had access to the zucchini crop for 15 days during the flowering period. For

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each greenhouse, three colonies were used sequentially, with each colony being used only once, totaling 45 days of pollination activity.

2.3. Behavior of the Bees on the Zucchini Flowers

Observations of bees visiting the zucchini flowers were made on the 2nd, 8th, and 14th day after the placement of the hives attached to the greenhouses. On each evaluation day, three pistillate flowers and three staminate flowers were selected for observation in each greenhouse, recording the number of visits per flower and the type of floral resource collected. Observations of each flower were made for five minutes each hour during zucchini flower anthesis, which began around 5 AM and lasted until approximately 9 AM.

2.4. Zucchini Fruit Production

To study the effect of bee visits to flowers on zucchini fruit production, tulle bags covering pistillate flowers were removed until they received 2, 4, or 8 bee visits during anthesis. The positive control consisted of open bee access to pistillate flowers, while the negative control flowers were kept covered with tulle bags during the entire period of anthesis. Bee visit control was achieved by restricting access to flowers from the day before anthesis by covering them with tulle bags. Flowers continuously open to bee visits were marked but were never covered. In each greenhouse, 25 pistillate flowers were marked before anthesis for this trial, with five repetitions for each treatment.

One week after anthesis of the marked flowers, fruit set was checked. When the fruits reached approximately 20 cm in length, after a development period of up to two weeks, they were harvested and weighed using a semi-analytical balance.

2.5. Colony Conditions

The day before the introduction of the bee hives into the greenhouses, all the combs were mapped, recording the areas occupied by honey, pollen, open and sealed brood (Al-Tikrity et al., 1971). After 15 days of using the hives in the greenhouses, the combs were mapped again to record the comb area occupied by bee brood and food in the colonies.

2.6. Statistical Analysis

A factorial analysis of 2 x 3 was performed, with the first factor being the honey bee colony confinement management option and the second factor being the number of days elapsed after the introduction of the colonies into the greenhouses for observing bee visits to flowers. Comparisons between management systems, as a qualitative factor, were made using the Tukey test at a 5% probability level. Evaluation of the effect of days, as a quantitative factor, was conducted through polynomial regression.

The percentage of fruit set as a function of the number of visits received by pistillate flowers was compared using a chi-square test for non-parametric frequency comparisons. Fruit weight was studied in a 2 x 4 factorial scheme, with the two types of confinement (total and semi-confinement) and four levels of pollination activity (2, 4, 8 bee visits per flower, and freely visited flowers). Flowers not visited by bees did not set fruit; these data were not included in the analyses of fruit weight. When significant effects were found, adjusted means were compared using the Tukey test at a significance level of 5%.

The differences in the bee comb area with food and brood in colonies subjected to total and semi-confinement over a period of 15 days were analyzed using a t-test, considering the mapping done before and after the colonies were used for pollination services. Statistical Analysis System software (SAS Institute, 2020) was used for all data processing.

3. Results

3.1. Behavior of Bees on the Zucchini Flowers

The flowering period with concurrent occurrence of staminate and pistillate flowers began in October and lasted for 67 days. In both greenhouses, bees began visiting the zucchini flowers around 5 AM, with peak visitation observed between 6 and 7 AM. Bee visit frequency decreased after 8 AM; by 10 AM, flower visitation ceased as the flowers were already wilted.

Based on the factorial scheme analysis between confinement system and days of bee visits to flowers after the introduction of colonies into the greenhouses, a significant interaction of these two factors for the number of visits was observed, both for pistillate flowers (P = 0.001, SE = 1.604) and staminate flowers (P = 0.016, SE = 1.821), indicating that the number of bee visits to flowers differed over time when comparing the two hive confinement options. Though there were no significant differences on the 2nd day, on the 8th and 14th days, there were significantly more visits to pistillate flowers by bees from hives with entrances to inside and outside the greenhouse than from hives with bees entirely confined to the greenhouse. The number of bee visits to pistillate flowers at 14 days from hives that were completely confined was reduced more than 70% compared to day 2, while there was no reduction in floral visits for bees from hives that could forage outside during part of the day (Table 1).

Table 1. Mean effects of the hive confinement system on the number of honey bee visits to pistillate and staminate zucchini flowers at days 2, 8, and 14 after introducing the hives to the greenhouse (data for three hives sequentially placed in each greenhouse).

	Pistillate, days			Staminate, days		
Hives	2	8	14	2	8	14
Entirely confined	31.67a	15.67b	10.33b	41.00a	24.00b	14.67b
Semi-confined	26.00a	34.33a	30.33a	33.73a	40.00a	28.80a

Different letters in the columns indicate significant differences between confinement systems at each day of bee visit observation, by the Tukey test at 5% probability.

Regression analysis of honey bee visitation data from entirely confined colonies showed a decline in the number of visits over time, both in pistillate and staminate zucchini flowers, represented respectively by the equations: $Y = 33.44-1.78X$ and $Y=44.11-2.19X$, where Y is the number of visits and X is the number of days. For both types of flowers, the P value was less than 0.001.

With partial confinement, no significant difference was observed for the day factor (chi-square test, P>0.20), indicating a constant level of visitation throughout the 15-day evaluation. For this period, the equations obtained were $Y=27.33+0.36X$ and $Y=38.67-0.56X$, for visits to pistillate and staminate flowers, respectively.

3.2. Fruit Set and Fruit Weight

The fruit set rate was not influenced by confinement management (Table 2). However, for both management types, the fruit set rate was directly favored by increases in the number of visits to pistillate flowers. With eight bee visits to pistillate zucchini flowers, the fruit set rate was greater than 90%.

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Table 2. - Fruit set rate of zucchini cultivated in a greenhouse, from pistillate flowers that received 2, 4, or 8 bee visits or were freely visited by honey bees (indeterminate number of flower visits) from hives that were either entirely or semi-confined. Thirty flowers were assayed for each number of visits in each greenhouse. Flowers that were always covered with tulle bags did not set fruit.

Number of bee visits	Fruit set (%) Colony management				Probability
	Entirely confined		Semi-confined		
	aF	rF	aF	rF	
2	9.0	60.0	9.0	60.0	0.439
4	11.0	73.3	11.0	73.3	0.439
8	14.0	93.3	15.0	100.0	0.309
Indeterminate	15.0	100.0	15.0	100.0	-

aF: Absolute frequency; rF: relative frequency.

Fruit weight was influenced by the number of bee visits to flowers (Table 3) and not by the type of colony confinement management. Fruits from flowers visited by four or more bees were significantly heavier than fruits obtained from flowers visited by only two bees. Although there was a trend of increasing fruit weight with an increase in the number of visits to flowers, no significant difference was observed between fruits from flowers visited by 4, 8, or an indeterminate number of bees, regardless of hive confinement management (Table 3).

Table 3. Mean weight of zucchini fruits obtained in a greenhouse from flowers visited by 2, 4, or 8 honey bees, or freely visited by these bees that were kept in hives that were completely confined or not to the greenhouses.

Number of bee visits	Fruit weight (g)	Probability
2	185.2 b	0.001
4	265.3 a	
8	257.9 a	
Free access	270.7 a	

Means followed by the same letter do not differ significantly (Tukey test at 5%).

3.3. Effect on Comb Areas with Food and Brood in the Honey Bee Colonies

A 25% reduction in the comb area containing honey was observed in the partially confined colonies and a 50% reduction in entirely confined colonies after 15 days in the greenhouse (Table 4). The comb area containing stored pollen was reduced more than 70% in both completely confined and semiconfined colonies (Table 4).

The comb area with young brood in entirely confined colonies was reduced by 70% in 15 days. For colonies that could access the external environment for part of the day, the reduction in brood area (7%) was not significant (Table 4). A significant reduction in the area with older, sealed brood was also observed in entirely confined colonies (79%). In semi-confined colonies, the reduction of 15% in sealed brood area was not significant (Table 4).

Table 4. Comb area (cm²) of honey, pollen, open (eggs and larvae) and sealed brood in honey bee colonies entirely or semi-confined measured the day before placement in the greenhouses for zucchini pollination and after 15 days.

Colony management	Food (cm²)					
	Honey			Pollen		
	Before	After	P	Before	After	P
Entirely confined	1456.0	728.0	0.0868	249.3	89.3	0.041
Semi-confined	1116.0	842.7	0.0628	252.7	92.0	0.037
	Bee brood (cm²)					
	Open brood			Sealed brood		

	Before	After	P	Before	After	P
Entirely confined	1057.3	317.3	0.0290	1622.7	349.3	0.005
Semi-confined	1960.0	1818.0	0.8578	2229.3	1906.7	0.608

Changes in the brood and food areas in the combs were evaluated by the t-test for dependent samples; each hive contained four standard-deep Langstroth frames and one shallow Langstroth frame.

4. Discussion

The relationship between humans and bees began thousands of years ago and intensified from the 19th century onwards with the development of beehives that optimized colony management. This has made it possible to efficiently produce honey, wax, and other bee products, and to use honey bee colonies to provide pollination services for agricultural crops (VanEngelsdorp and Meixner, 2010). However, bee domestication did not affect honey bees as much as it did other species, as bees maintained in hives still closely resemble those found in the wild (Oldroyd, 2012).

Evidence that reinforces this incomplete domestication is the inherent difficulty in confining bees in greenhouses. In addition to the significant reduction in flight area, which is stressful for these insects, confinement also results in a reduction in the availability of nectar and pollen, leading to a rapid population decline of the colonies (Vyas and Plunkett, 2018). Given that the demand for food is increasing due to the growth of the world population, management of honey bee colonies in greenhouses needs to be improved, given the inherent advantages of crop production in these closed systems. The development of honey bee colony management techniques for use in greenhouses is especially urgent, because the number of hives has not kept pace with the expansion of crops that depend on these insects for pollination (Aizen and Harder, 2009).

In this context, we have found that partial confinement of honey bee colonies, using a manipulable entrance honey bee hive system (Nicodemo et al., 2018), results in increased fruit production levels, with reduced population losses in colonies. Since the number of bee visits to flowers did not change significantly during a confinement period of 15 days, colonies subjected to this flight management system could be maintained in the greenhouse for longer periods without detrimental effects on fruit production and colony condition (Table 1).

Zucchini flowers, like those of other cucurbits, are essentially dependent on biotic pollinators for fruit production (Giannini et al., 2015). Fruit set and fruit weight are influenced by the number of visits received by pistillate flowers. In general, optimal production levels are achieved when there are eight or more visits to pistillate zucchini flowers (Rodrigues et al., 2021). Based on the data of the number of visits to pistillate flowers, the use of entirely confined colonies would tend to result in a decline in fruit production after 15 days (Table 1). Therefore, if colonies are entirely confined to the greenhouse, hives should be replaced every two weeks.

When using the semi-confinement management system, hives could be maintained in the greenhouse more than 15 days, since bee visitation to flowers was not significantly reduced during this period (Table 1). However, further studies evaluating this pollination system should be conducted to help determine the maximum continuous period of using a colony under partial confinement without reducing pollination efficiency and without significantly damaging the bee colonies. Since zucchini flowering lasted more than two months, the use of entirely confined colonies would require hive replacement every two weeks (at least four hives per greenhouse), leading to increased labor and hive rental costs. Additionally, hive rental prices would need to be increased to compensate for the deterioration of the bee colonies. The manipulable entrance honey bee hive system, even with a daily need for opening and closing hive entrances, has proven to be economically more viable than manual pollination services (Rodrigues et al., 2021) and would require fewer hive replacements during the flowering period of the crop in the greenhouse.

An option to prolong the number of days that semi-confined colonies could be used without replacement, taking into consideration the large number of visits required per pistillate flower (Tables 1, 2, 3), could be to reduce the foraging time within the greenhouse from four to two hours. Bees would have more time to forage outside the greenhouse during the morning when flowers are open and available, favoring the nutritional needs of the colony, resulting in less population reduction.

This could allow maintaining the hives in the greenhouse for longer periods, though the impact on fruit set and production would need to be tested.

In a study comparing periods of access to the interior of greenhouses, it was observed that the bee brood area was less impacted when bees had access to the external environment early in the day (Nicodemo et al., 2018). Unlike nectar, pollen is generally not released gradually by flowers throughout the flowering period. Thus, considering that many flowers open near dawn, it is important for managed bees to have access to the field during this period (Free, 1993). If pollen is scarce or bee access to the field occurs when flowers have already been visited by other bees, there may be a population decline in colonies due to inadequate nutrition and subsequent increased susceptibility of these insects to diseases and natural enemies (Di Pasquale et al., 2016).

The area of protein food (pollen or beebread) stored in the combs of hives of both confinement management systems was significantly reduced during the 15 days in the greenhouse. However, a significant reduction in the brood area was only observed in entirely confined colonies (Table 4). Probably because of the reduction in the amount of available beebread, the amount of brood in colonies subjected to partial confinement would also be reduced in the weeks following the first 15 days. In an experiment conducted in Canada for tomato pollination in greenhouses, honey bee colonies containing about 5,000 adult individuals were subjected to total confinement. The colonies survived confinement for three weeks, with a reduction in brood area and the number of adult bees (Sabara and Winston, 2003).

Given the lesser impact on honey bee colonies confined to greenhouses when they also can forage outside (Nicodemo et al., 2018), it is likely that this manipulable entrance honey bee hive system will be useful for other agricultural crops grown in greenhouses. The generalist foraging behavior of these bees and the ideal environmental conditions that crops have in this environment, including to produce pollen and nectar, which in turn contribute to the attractiveness of flowers to honey bees, makes bee pollination a viable agricultural input (Waser and Price, 2016).

5. Conclusions

Bee visits to zucchini flowers were maintained at constant levels for 15 days when the bees were allowed access to the external environment for part of the day, which was not the case for bees confined to the greenhouse. Directing the bees to the inside of the greenhouse for four hours daily, in the morning, ensured adequate pollination of the zucchini crop. Partial confinement of the honey bee colonies did not prevent a reduction in pollen stores, but the brood area was maintained for the 15-day period, implying that such colonies could remain in the greenhouse for longer periods, while satisfactorily performing pollination services.

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